BOOK OF PROCEEDINGS

XIV International Scientific Agriculture Symposium

"Agrosym 2023"

Jahorina, October 05-08, 2023

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PREFACE

Dear colleagues,

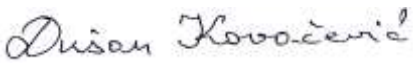
I am pleased to introduce the Book of Proceedings of the 14th International Scientific Agricultural Symposium "AGROSYM 2023", which I hope you will find useful in your work. Almost 700 contributions have been accepted for the Book of Abstracts. The themes of AGROSYM 2023 cover all branches of agriculture and are divided into seven sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agroforestry.

Many scholars and practitioners argue that technology can increase production and feed more people while increasing supply stability and reducing the environmental impacts of agricultural production. Technology has been particularly important for improving production in annual crops such as maize, rice, soybean, wheat and cotton. Because trees have longer cycles, their breeding programs take longer. New plant breeding techniques can improve productivity, but there is a lively academic debate about their pros and cons.

While attention has been paid in the past mainly to the production side (cf. productivity, efficiency), more attention is nowadays paid to the consumption side as well as the intermediate stages (e.g. processing, distribution) of the food chain thus moving towards a 'farm to fork' approach. Globally, consumers are sending clearer signals about what they want on their tables i.e. higher quality as well as healthier, safer, and tastier products. Therefore, most agri-food companies have been exploring new, innovative ways to ensure more control over the production processes as well as final products quality and safety. Changes in investment strategy also have the potential to reduce the environmental and social costs of agriculture. It is now clear to most investors that companies paying more attention to sustainability and social responsibility, will have better returns on investments.

Agri-food systems have been central in the global debate on sustainable development and the achievement of the Sustainable Development Goals (SDGs) by 2030. Indeed, agri-food systems are at the center of various global challenges such as climate change, poverty, vulnerability, food insecurity, biodiversity loss, resource scarcity and ecosystem degradation. In this context, one of the goals of the sustainable agriculture movement is to develop farming systems that mitigate or eliminate environmental harms associated with industrial agriculture. It is also crucial to improve the resilience of food systems to crises, shocks and pandemics.

Many thanks to all the authors, reviewers and colleagues for their assistance in editing the Book of Abstracts. Special thanks go to all co-organizers and partners for their unselfish collaboration and comprehensive support.



East Sarajevo, 07 October 2023
Prof. Dušan Kovačević, PhD
Editor in Chief, President of the Scientific Committee of AGROSYM 2023

CONTENT

PLANT PRODUCTION

EFFECTS OF SOIL COVERAGE ON KIWIFRUIT (ACTINIDIA DELICIOSA CV. HAYWARD) PRODUCTION

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Abstract

Soil coverage has been extensively used during the last decades to increase yield and decrease inputs (irrigation, herbicides) in various fruit tree species. In the present trial, a black-white plastic sheet was used to cover the soil between rows in a kiwifruit orchard of the 'Hayward' cultivar. Treatments included the uncovered control, soil coverage with the white side exposed to the sun, and soil coverage with the black side exposed. The yield, organoleptic properties (pH, total soluble solids, titratable acidity), the percentage of dry matter, the total phenol content, and the antioxidant capacity of the pulp were assessed at harvest, along with the soluble sugar and organic acid concentration, while during the growing period, the soil temperature and moisture were also assessed. Soil coverage resulted in a significant increase in yield compared to control, while it resulted in lower total soluble solids at harvest. There were not any significant differences concerning pulp soluble carbohydrate content, total phenols concentration, and antioxidant capacity, while fruits produced under the treatment with the white side exposed, exhibited higher ascorbic acid concentration. At the same time, soil temperature was lower under the white side exposed compared to that measured in sites where the black side was exposed. Soil moisture did not differ among treatments. Soil coverage seems to be an efficient method to increase fruit yield in kiwifruit cultivation, without any significant negative effect on fruit quality parameters.

Keywords: *Actinidia Deliciosa, Antioxidant Capacity, Dry Matter, Phenolic Compounds, Total Soluble Solids.*

Introduction

Soil cover with either organic or inorganic materials has been an increasingly applied technique in various crops (Steinmetz *et al.*, 2016). Among the benefits of soil cover are higher yields, earlier harvests, improved fruit quality, and increased water and fertilizer-use efficiency (Coelho *et al.*, 2022). At the same time, it prevents weed growth and water evaporation, making a profitable management, in case it can improve crop production (Coelho *et al.*, 2022). Furthermore, it can alter insect biological cycle, if they need to complete a stage in the soil, so it can result in minimization of pesticide use too. In the last decades, plastic (polyethylene) films are used more and more, as they are easy to use and long-lasting (Steinmetz *et al.*, 2016). The latter is considered though as a negative property since they are not easily degraded while during their degradation microplastics can occur which may have a negative impact on the environment (Steinmetz *et al.*, 2016).

Kiwifruit cultivation requires large amounts of water since it is a plant with high water demands. At the same time, careful weed control is essential, due to the shallow root system, which does not allow deep soil cultivation. Under these conditions, a plastic soil cover (mulch) could be beneficial in reducing water needs and herbicide use. Since there are not much data available for the efficacy of plastic mulch in kiwifruit, the present trial aimed at

exploring the effectiveness of plastic mulch (plastic polyethylene sheet of black and white surfaces) in terms of vine yield and fruit quality characteristics (organoleptic, physiological and functional) as well as soil temperature and moisture.

Materials and methods

The experiment was conducted in Agrinio county, (Neapolis village, 38◦38′04.1″ N 21◦19′08.8″ E), Western Greece during the 2018 and 2019 growing periods. During the first period, only the yield was measured while during the second one, all the measurements described below took place.

A 15-year-old orchard (total area of 5 ha) of kiwifruit plants of cv. 'Hayward' with a trunk height of 1.8 m, trained as a pergola and planted at distances of 2.0 × 4 m was selected as the trial site. The soil was characterized as loam, with 7.25 pH, 1.76% w/w organic matter, 0.310 mS cm^{-1} electrical conductivity, and 3.05% w/w $CaCO_3$. The mean and the highest temperature recorded for the 2019 growth period, were as follows: 26.3 ◦C and 37.8 ◦C in June, 27.5 ◦C and 38.3 ◦C in July, 28.8 ◦C and 38.8 ◦C in August, 24.4 ◦C and 34.1 ◦C in September, and 19.9 ◦C and 30.9 ◦C in October, respectively (data collected by a nearby station). Approximately 83 mm of rainfall was recorded during the trial period (from the first spray application to harvest). The applied cultivation practices (irrigation, fertilizing, pruning, weeding (till the installation of the plastic cover), and spraying against fungi and pests) were the same for all the vines of the orchard.

A black-white plastic sheet (2m wide) was used to cover the soil on both sides of the planting row in early August, covering the area of four successive vines. The mulch was placed 1m away from the trunk of the vines. The treatments included the uncovered control, soil cover with the white side of the plastic mulch visible, and soil cover with the black side visible.

On the 20th of August and on the 21st of September the soil temperature at 15 cm and 30 cm depth was measured with a soil thermometer.

At harvest, on the 21st of October, all the fruits from the two central vines per plot were harvested, the total yield was recorded and a sample of 25 fruits was transferred to the laboratory for further measurements. The fruit weight, diameter, and length were measured along with its firmness, after peeling off a small portion of the skin (at two equatorial sides of the fruit). The dry matter (%) was also determined along with the pH, total soluble solids (TSS), and titratable acidity (TA) of the pulp, after homogenizing the peeled fruits in a home blender. The soluble carbohydrates and organic acids were measured in the pulp according to (Denaxa *et al.*, 2023). Furthermore, the total phenols (TPhen), total o-diphenols (oDs), total flavonoids (TFLOIDS), total flavanols (FLANOLS), and the antioxidant capacity of the pulp, based on diphenyl-picryl hydrazyl assays (DPPH) and ferric reducing power assay (FRAP) were also measured in the pulp.

The experiment was arranged as a completely randomized design with four vines of four replications per treatment (a total of 16 vines per treatment, 64 vines in total), where only the two central vines were used, with the two vines at each plot's edge serving as a buffer zone.

Results and Discussion

The soil temperature was significantly influenced by the presence of the plastic sheet (Table 1), especially at the top soil layer. Control plots exhibited higher temperature at 0-15 cm depth than the soil covered with the white plastic, while in September the highest temperature was recorded at both depths under the black cover. Similar effects of colored plastic covers have been reported by other researchers too (Yadav *et al.*, 2023).

Table 1. Effect of soil cover on the mean soil temperature (^{o}C) at 15 and 30 cm depth.

Treatments	1st measurement (20th August)		2nd measurement (21st September)	
	T – 15cm	T – 30 cm	T – 15cm	T – 30 cm
Control	26.52 a	24.12 a	20.03 b	19.95 b
Black	25.78 ab	24.32 a	20.95 a	20.98 a
White	24.43 b	23.62 a	20.28 b	20.36 b

Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

The total yield per vine was increased under the influence of soil cover with the plastic sheets while other fruit physical parameters were not influenced (Table 2). Similar results in yield increase have been reported in other species too (Ahmad *et al.*, 2022; Shan *et al.*, 2022; Yadav *et al.*, 2023).

Table 2. Effect of soil cover on the yield components and fruit physical properties.

Treatments	Mean fruit weight (g)	Mean fruit diameter (cm)	Mean fruit length (cm)	Firmness (N)	Dry matter (%)	Total yield per vine (Kg)
Control	126.29 a	56.12 a	74.01 a	30.47 a	17.40 a	22.44 b
Black	121.94 a	55.74 a	74.17 a	30.42 a	16.51 a	36.63 a
White	126.52 a	57.12 a	74.30 a	29.90 a	16.60 a	38.31 a

Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

Total soluble solids were significantly lower under the soil cover treatments, compared to the control (Table 3). This could be an immediate effect of the higher yield since competition for assimilates among fruits is expected to be higher under higher yields.

Table 3. Effect of soil cover on the pulp organoleptic characteristics.

Treatments	pH	Total soluble solids (TSS) (^{o}Brix)	Titratable acidity (% w/v citric acid)	TSS/TA
Control	2.79 a	7.63 a	2.47 a	3.16 a
Black	2.77 a	6.75 b	2.10 a	3.21 a
White	2.87 a	6.48 b	2.08 a	3.12 a

Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

There were not many significant effects of soil cover regarding the concentration of soluble carbohydrates in the pulp (Table 4).

Table 4. Effect of soil cover on fruit soluble carbohydrates (g 100 g^{-1} F.W.).

Treatments	Glucose	Fructose	Sucrose	Inositol	Total soluble sugars
Control	1.94 a	2.23 a	0.38 a	0.22 b	4.76 a
Black	1.42 a	1.66 a	0.18 a	0.23 b	3.49 a
White	1.64 a	2.10 a	0.23 a	0.32 a	4.28 a

Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

Similarly, no significant difference was observed regarding the phenol content of the pulp and its antioxidant capacity, indicating that the cover does not have much impact on the functional properties of the fruit (Table 5).

Table 5. Effect of soil cover on pulp total phenolic compounds and antioxidant capacity (based on FRAP and DPPH assays).

Treatments	TPhen	oDs	FLANOLS	FLOIDS	FRAP	DPPH
Control	0.64 a	0.028 a	0.0128 a	0.080 a	4.84 a	2.56 a
Black	0.84 a	0.027 a	0.0126 a	0.092 a	5.22 a	2.90 a
White	0.72 a	0.025 a	0.0133 a	0.090 a	4.43 a	2.71 a

TPhen, total phenols (mg equivalents gallic acid g^{-1} F.W.), oDs), total o-diphenols (mg equivalents caffeic acid g^{-1} F.W., FLANOLS, total flavanols (mg equivalents catechin acid g^{-1} F.W.), FLOIDS, total flavonoids (mg equivalents caffeic acid g^{-1} F.W.).
Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

The ascorbic acid concentration of the pulp increased under the influence of the white plastic cover, followed by that of the black plastic cover, indicating that the cover may exhibit significant effects on vitamin C production (Table 6). Similar results have been reported by other researchers too (Amare & Desta, 2021).

Table 6. Effect of soil cover on pulp organic acids (g 100g^{-1} F.W.)

Treatments	Malic acid	Ascorbic acid	Citric Acid	Total organic acids
Control	0.35 a	0.15 b	0.15 a	0.69 a
Black	0.37 a	0.21 ab	0.18 a	0.75 a
White	0.38 a	0.32 a	0.21 a	0.91 a

Means within the same column followed by different letter differ significantly based on Tukey HSD test at α=0.05.

The impacts the plastic cover mulches may have on plant growth, productivity, and product quality are believed to be the result of direct or indirect effects of the mulch on water use efficiency, nutrient availability, root activity, photosynthetic capacity, and of course on soil physical, chemical and biological properties (Amare and Desta, 2021).

Conclusion

It seems that plastic soil cover may affect kiwifruit productivity and fruit quality. The higher yield was recorded under the influence of white plastic, with great fruit properties regarding both weight and size as well as dry matter percentage and total soluble solids which are the major criteria indicating the suitability for harvest. Taking that into account along with the possible saving of substantial quantities of water for irrigation, it can be assumed that plastic mulch may become a significant tool in the sustainability of kiwifruit culture. Nonetheless, more research is needed to examine the possible negative impacts of plastic mulches on soil physicochemical and biological properties as well as long-term effects on kiwifruit vines.

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EVALUATION OF THE TOLERANCE OF GRAPEVINE ROOTSTOCKS TO HIGH NACL SALT CONTENT IN THE SOIL, BASED ON THE CALCULATED VALUE OF THE TOLERANCE INDEX

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Abstract

The experiment to evaluate the tolerance of rootstocks to the salt content (NaCl) in the soil, was developed during the three-years period 2019-2022. In this experiment, are included the 5 rootstocks used in Albanian nurseries, which are: SO4, 1103P, 779 P, 140 Ru and 110 R (Richter).
The Tolerance Index (TI) was used to compare the level of rootstock tolerance to sodium chloride in the soil. A higher TI value indicates higher tolerance of the rootstocks and better adaptation to saline soils. The results showed that with increasing salt concentration, the dry weight of shoots and roots decreased. The degree of dry weight reduction, under the same salt concentration, varied depending on the rootstock type Based on the dry weight of shoots and roots, the values of the Tolerance Index (TI) were calculated for each rootstock. The TI value for the rootstock 779P was 398, for 140Ru it was 362, for SO4 it was 311, for 110R it was 332, and for 1103P it was 440. The ranking of rootstocks according to their TI value, from highest to lowest, is as follows: 1103P (440), 779P (398), 140Ru (362), 110R (332), and SO4 (311). The best rootstocks for growing vineyards in soils with a high content of Sodium Chloride (NaCl), are 1103P, 779P, and 140Ru. On the other hand, SO and 110R rootstocks exhibit low tolerance. These two rootstocks (SO4 and 110R), not be used to create vineyards in soils with a high content of sodium chloride (Na Cl > 4 - 5).

Keywords: *Rootstock, tolerance, index, soil, sodium chloride, etc.*

Introduction

In the seedling production nurseries of grapevine rootstocks in Albania, antiphyloxera rootstocks with hybrid origins have been used and continue to be used, such as Paulsen 1103, Kober 5BB, SO4, Ruggeri 140, and 110R.
The chloride component of salty soils can be toxic. The Vitis berlandieri species is considered tolerant, but Australian research has found the Vitis champini rootstock Ramsey to be quite tolerant. Vitis vinifera is also tolerant. Rootstock 110R, or 110 Richter, is a relatively old V berlandieri x V rupestris hybrid, made bay Franz Richter in 1902. Has high phylloxera tolerance, but low nematode tolerance. Other rootstocks are 140 Ruggeri, is produced in 1894 in Sicily by Antonio Ruggeri, using V berlandieri and V rupestris. This rootstock should not be planted on fertile, moist soils because of possible excess vigor. Paulsen 1103, was bred in 1896 by Federico Paulsen, the director of an American vine nursery in Sicily, by crossing V berlandieri with V rupestris.
The increase in the surface area of soils with high salt content is a major concern for the world. This phenomenon reduces soil fertility and limits the possibility of spreading and cultivating the majority of agricultural crop.
Grapevine is a relatively stable plant against salt content in the soil. However, it is seriously threatened in soils with high iron, chloride and sodium sulfate content. In order to facilitate and expand the cultivation of vineyards even in these types of soils, starting from 1956,

nurseries in Albania have used understocks with high tolerance to Sodium Chloride content in the soil.

The metabolism and lifespan of the grapevine rootstock, along with the lifespan and actual productivity indicators of the vineyard, depend on the level of tolerance of the used understock.

Grapevine is a sensitive plant to excessive amounts of chlorides, sulfates, and carbonates in soil and water. In Albania, salinization is a concerning issue in lowlands of the western region. American species are more sensitive, while European varieties of grapevine, Vitis vinifera, are more resistant. Under condition 1103P, it endures 0.9-1 g/l, while European grapevine can withstand even 1.4-1.5 g/l NaCl content in soil.

Due to high salt content in soil, the concentration of chlorides in leaves increases, leading to the disruption of the osmotic process. This results in reduced vegetative growth, accompanied by the onset and progression of leaf chlorosis and necrosis, a process that continues until complete dehydration of the plant.

The high salt content in soil causes low cell turgor, as the concentration of salt ions increases within the cells.

To determine the dry weight of plant material using an analytical balance, the moist plant material is weighed and recorded as weight P1. The material is placed in a thermostat for drying at 105 degrees Celsius for 6 hours. It is then removed and allowed to cool, reweighed, and recorded as weight P2. It is placed back in the thermostat for an additional hour, removed, reweighed, and recorded as weight P3. If there is no difference between the consecutive weights P2 and P3, the drying process is complete. Otherwise, the process is repeated until complete drying of the plant material.

High soil salt content causes water shortage in plant cells. The water shortage indirectly affects photosynthesis. The high salt content leads to cellular dehydration, decreased turgor, and damage to enzymatic processes and functional structures of leaf tissues. Stomatal closure occurs, hindering respiration and transpiration. Under these rootstocks, leaves with closed stomata can only reassimilate internal CO_2 released from respiration.

The study and comparison of rootstocks resistance to biotic and abiotic factors are carried out based on the codes and assessment levels of the vineyard international descriptor. Meanwhile, the assessment of Rootstocks resistance to salinisation is done according to levels 1, 3, 5, 7, 9 of the OIV Code 402.

The Tolerance Index (TI) was developed by La Rosa et al.1989 and has been used to compare the tolerance of rootstocks to soil salt content. The value of the tolerance index is calculated using the formula $TI = 100 + \sum n\ [x\ (Tx/T0)\ 100]$. The TI value is based on the dry weight of shoots and roots. According to Dardeniz et al. (2006), the higher the TI value, the higher the tolerance of the subcondition to soil salt content.

The ranking of rootstocks from the best for growing vineyards in soils with a high content of Sodium Chloride (NaCl), Paulsen 1103, Kober 5BB, SO4, Ruggeri 140 or 110R.

Material and Methods

The experiment aimed to assess the influence of salt content (NaCl) in soil on the dry weight of shoots and roots, as well as the Tolerance Index (TI), and was conducted over three consecutive years, from 2019 to 2022, at the Experimental Base of the Agricultural University of Tirana in Valias (Albania).

Five commonly used rootstocks in Albanian nurseries were included in the experiment: 1103P, 779P, 140Ru, SO4, and 110R.

The rootstocks were rooted and tested in plastic pots with a volume of 10 liters. Pieces of rootstock are prepared with two buds. The lower cut was made around 7 mm below the lower bud, while the upper cut was made around 2.5 cm above the upper bud.
On March 10, 2019, the cuttings were directly placed in the pots for rooting and were kept in controlled temperature greenhouse Rootstocks of 15-18°C.
The experiment included five rootstocks, each treated with six levels or variants of NaCl salt concentration.
In each variant, five pots were treated and monitored, with two plants in each pot.
In total, the experiment consisted of 125 pots, with two plants in each (total 250 plants/rootstock).
The rootstocks were evaluated and compared in terms of the impact of salt concentration on the dry weight of the root system and the above-ground system.
Six variants or levels of salt concentration were used in the irrigation solution, which were as follows:

- V1 = Control, irrigation with regular water.
- V2 = Irrigation with water containing 1000 ppm NaCl.
- V3 = Irrigation with water containing 2000 ppm NaCl.
- V4 = Irrigation with water containing 3000 ppm NaCl.
- V5 = Irrigation with water containing 4000 ppm NaCl.
- V6 = Irrigation with water containing 50000 ppm NaCl.

For each variant and repetition, plastic labels have been placed where the type of substrate and salt concentration variant are indicated.
Until July 30th, the pots were watered with regular water.
From July 30th to September 30th, for a period of 60 days, the pots were watered using a NaCl salt solution, according to the previous variants.
At the end of September each year, 4 full plants were uprooted for each variant.
The root system of the uprooted plants was cleaned from soil and other debris.
Then, following the protocol of dry matter analysis, the shoots and roots of each variant were removed and separated individually, and they were packaged in plastic bags.
Inside each bag, labels were placed indicating the name of the substrate and variant. The packaged material was sent for drying and weighing at the Laboratory of the Department of Horticulture at the Agricultural University of Tirana.
Using an analytical balance, the fresh weight of the shoots and the fresh weight of the roots were measured and recorded as P1.
Next, the shoots and roots were placed in a thermostat at 105 degrees Celsius and kept for 6 hours. They were then taken out and left to cool for about an hour, and they were weighed to record the weight as P2. They were again placed for further drying in the thermostat for another hour and then weighed, recording the weight as P3. This process was repeated until consecutive weighings showed no weight difference, indicating complete drying.
After drying and weighing, based on the dry weight of the shoots and roots according to the variants and repetitions, the Tolerance Index (TI) values were calculated for each substrate, and the substrates were ranked according to the value of the Tolerance Index (TI).
The Tolerance Index (TI) is calculated using the formula:

Where:
$TI = 100 + \Sigma n\ [X\ (T_x/T_0)\ x\ 100$
n - number of treatment levels (6)
X - 0; 0.1; 0.2; 0.3; 0.4 and 0.5
T_x = Dry weight of shoots/Dry weight of roots of NaCl-treated plants.
T_0 = Dry weight of shoots/Dry weight of roots of untreated plants (V1 variant plants).

Based on the dry weight of shoots and roots, and the calculated values of Tolerance Index (TI), the difference between variants and rootstocks has been identified. As demonstrated by Derdeniz, the value of Tolerance Index is directly correlated with the level of tolerance of Rootstocks towards high salt content in the soil. According to Derdeniz (2006), the ranking of Rootstocks based on the value of Tolerance Index (TI) is the same as the ranking of Rootstocks based on the degree of tolerance towards high sodium chloride content in the soil.

Results and Discussion

• The effect of sodium chloride (NaCl) on the dry weight of roots

According to the methodology, the drying and weighing of the dry matter of the root and aboveground system were performed.

The dry weight of the root system, according to the Rootstocks and variations, is reflected in the following table (Table 1).

Table 1. Dry weight of roots (according to rootstocks and variations)

Rootstocks	**Dry weight of roots (g)**				
Variants (according to % NaCl)	**779P**	**140Ru**	**SO4**	**110R**	**1103P**
V1 (control)	7.3	4.9	10	5.7	6.9
V2 (1000 ppm)	5	3.7	7.9	4.7	4.5
V3 (2000 ppm)	4.5	3.2	7.3	3.8	4.2
V4 (3000 ppm)	3.7	3	6.3	3.3	3.1
V5 (4000 ppm)	3.4	2.7	5.2	2.7	3.2
V6 (5000 ppm)	3.2	2.6	4.9	2.6	2.8
Average	**3.4**	**2.25**	**5.85**	**2.7**	**4.12**

The results indicate that with the increase in salt concentration, vegetative growth and dry weight of roots have been reduced.

The rootstocks ranking, starting from the condition with the highest weight to the condition with the lowest dry weight of the root system, is as follows: SO4 with 5.85 g, 1103P with 4.12 g in second place, 779P with 3.4 g in third place, and 110R and 140Ru with weights of 2.7 and 2.25 g respectively in last place.

This shows that different rootstocks have different weights, which means that the type of rootstock affects the dry weight of the root system and the tolerance towards sodium chloride content in the soil.

Different rootstocks, maintained at the same salt concentration and technology levels, exhibit different levels of dry weight and morphological, anatomical, and physiological modifications of the root system.

• The influence of sodium chloride on the dry weight of leaves and shoots

The results obtained from weighing the dry matter of the above-ground system (shoots and dried leaves) are reflected in the following table (Table 2).

Table 2. Dry weight of leaves and shoots (according to rootstocks and variants)

Rootstocks	**Dry weight of leaves and shoots (g)**				
Variants (according to % NaCl)	**779P**	**140Ru**	**SO4**	**110R**	**1103P**
V1 (control)	16.3	10.8	25.5	10	15.2
V2 (1000 ppm)	13.9	10.5	21.3	9.8	12.2
V3 (2000 ppm)	12.7	8.9	18.9	7.9	11.8
V4 (3000 ppm)	12.3	8.2	16.9	6.9	10.6
V5 (4000 ppm)	11.6	7.5	13.5	5.6	10.1
V6 (5000 ppm)	11.5	7.2	13	5.3	9.4
Average	**13.5**	**8.8**	**18.2**	**7.6**	**11.55**

The results demonstrate that the increase in salt concentration has also influenced the reduction of the dry weight of the aboveground system (leaves and shoots). In this case as well, the degree of influence varies depending on the salt concentration and rootstock type.
The reduction in dry weight is caused by a decrease in the photosynthesis product, which is negatively correlated with the increase in salt concentration (NaCl). This occurs because the increase in salt concentration in the soil leads to an increase in Cl and Na content in the leaves. Under such Rootstocks, substrates with high ability to absorb soil moisture exhibit better turgor and higher tolerance and resistance to the increase in salt content in the soil or nutrient substrate.

• Determination of the Tolerance Index value (TI, according to the substrates)

Based on the dry weight of the roots and shoots, the tolerance index value has been calculated, and then the substrates have been ranked and compared based on the TI value.
The Tolerance Index (TI) enables the comparison and ranking of substrates based on their tolerance to salt content. The higher the TI value, the higher the tolerance of the substrates to salinization.
The TI value is calculated using the formula: $TI = 100 + \Sigma n [X (T_x/T_0) \times 100$
Initially, the values of T_x and the T_x/T_0 ratio were calculated for each substrate and variant, as shown in the following table (Table 3).
Please note that T_x is the value obtained from the ratio between the dry weight of the shoots and the dry weight of the roots of plants treated with NaCl.

Table 3. T_x values, according to rootstock and variants.

Variants / Rootstocks	**T_x values, according to variants and rootstocks**				
	779P	**140Ru**	**SO4**	**110R**	**1103P**
V1 (control)	2.24	2.21	2.55	1.76	2.22
V2 (1000 ppm)	2.78	2.84	2.7	2.09	2.72
V3 (2000 ppm)	2.83	2.79	2.59	2.05	2.81
V4 (3000 ppm)	3.33	2.74	2.69	2.02	3.42
V5 (4000 ppm)	3.42	2.7	2.6	2	3.16
V6 (5000 ppm)	3.6	2.8	2.66	2.04	3.36
Average	**3.04**	**2.8**	**2.64**	**2**	**2.95**

The results of the above table show that with an increase in salt concentration, the value of T_x has increased, indicating that compared to the dry weight of shoots, the increase in salt concentration has a greater effect on reducing the dry weight of plant roots.
Meanwhile, the calculated values of the ratio T_x/T_0 have been reflected in the following table (Table 4).

Table 4. Values of the ratio T_x/T_0, according to the Rootstocks and variations.

Variants / Rootstocks	**T_x/T_0 ratio values, by rootstocks**				
	779P	**140Ru**	**SO4**	**110R**	**1103P**
V1 (control)	1	1	1	1	1
V2 (1000 ppm)	1.25	1.29	1.06	1.19	2.23
V3 (2000 ppm)	1.27	1.27	1.02	1.17	2.27
V4 (3000 ppm)	1.49	1.24	1.06	1.15	1.54
V5 (4000 ppm)	1.53	1.23	1.02	1.14	1.43
V6 (5000 ppm)	**1.61**	**1.27**	**1.05**	**1.16**	**1.52**

The increase in salt concentration leads to an increase in the value of the T_x/T_0 ratio. It is evident that the variability of this ratio depends on the type of substrate. This occurs due to the different biomorphological structures of substrates and their varying tolerance to high sodium chloride content in the soil.

Based on the aforementioned indicators of dry matter weight and the calculated values of T_x and the T_x/T_0 ratio, we calculate the value of TI (Tolerance Index) for each substrate. The calculated values of TI are presented in the following table (Table 5).

Table 5. Values of the Tolerance Index (TI) according to the rootstocks.

Rootstocks/Formula	**$TI = 100 + \Sigma^n [X (T_x/T_0) \times 100]$**	**TI**
779P	TI = 100 + [(0.2x1.25) + (0.3x1.27) + (0.4x1.49) + (0.5x1.53) + (0.6x1.61) x 100] = 100 + (2.98 x 100) = 100 + 298 = 398	**398**
140Ru	TI = 100 + [(0.2x1.29) + (0.3x1.27) + (0.4x1.24) + (0.5x1.23) + (0.6x1.27) x100] = 100 + (2.62 x 100) = 100 + 262 = 362	**362**
SO_4	TI = 100 + [(0.2x1.06) + (0.3x1.02) + (0.4x1.06) + (0.5x1.02) + (0.6x1.05) x100] = 100 + (2.11 x 100) = 100 + 211 = 311	**311**
110R	TI = 100 + [(0.2x1.19) + (0.3x1.17) + (0.4x1.15) + (0.5x1.14) + (0.6x1.16) x 100] = 100 + (2.32 x 100) = 100 + 232 = 332	**332**
1103P	TI = 100 + [(0.2x2.23) + (0.3x2.27) + (0.4x1.54) + (0.5x1.43) + (0.6x1.52) x 100] = 100 + (3.4 x 100) = 100 + 340 = 440	**440**

The result shows that the value of TI varies depending on the condition.

Based on the calculated values, the ranking of rootstocks according to TI value, starting from the condition with the highest value and descending to the one with the lowest value, is as follows: condition 1103P has 440, in second place is 779P with 398, in third place is 140Ru with 362, in fourth place is 110R with 332, and with the lowest value, condition SO4 has TI=311.

Rootstocks with a high TI value are rootstocks with a high tolerance towards high soil salt content.

Conclusions

The increase in salt concentration affects the reduction of vegetative growth and the reduction of the dry weight of plant material, with a greater impact on the reduction of the dry weight of

the root system. According to the TI value, the ranking of the rootstocks based on the highest value to the lowest value is as follows: rootstock 1103P ranks first with 440, followed by 779P with 398, then 140Ru with 362, 110R with 332, and the lowest value is recorded by the rootstock SO4 with TI=311. For evaluating the tolerance level of rootstocks to soil salt content, the T_x value can also be used, which is the ratio of the dry weight of shoots to the dry weight of roots of plants treated with the NaCl salt solution. In the conditions of Albania, for cultivating vineyards in soils with high sodium chloride (NaCl) content, we recommend using rootstocks 1103P, 779P, and 140Ru, while rootstocks SO4 and 110R should not be used.

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EFFECT OF THE COVID-19 PANDEMIC ON THE IMPORT AND EXPORT OF MINT *(MENTHA PIPERITA* L.) IN SERBIA

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Abstract

Climate conditions for mint cultivation in Serbia are favourable, and there is an increasing demand for medicinal and aromatic plants in the world, especially in European Union, United States and Canada. However, it is estimated that the cultivation area of medicinal and aromatic plants in Serbia decreased, from about 3500 ha at the end of the 20th century to slightly above 2000 ha during the past two decades. In spite of that, the export of mint from Serbia did not decrease, and during the five-year data (2015-2019), collected before the pandemic, the average amount of exported mint was 469.8 metric tons with an average value of 1.53 M USD. During the pandemic years (2020 – 2022), the average amount of exported mint did not change, and it was 442.7 metric tons, but the average value of this export was higher, reaching 1.77 M USD. The import of mint was considerably lower, the average amount was only 31 metric tons during 2015-2019, with an average value of 0.27 M USD. However, the import of mint increased considerably during the pandemic years, reaching 169.9 metric tons with a value of 0.79 M USD. For this reason, it is important to increase the cultivation area under mint in Serbia and to conduct activities (e.g. to develop a network of farms and collecting centres) that will revive mint production in Serbia.

Keywords: *mint production, mint trade, medicinal plants market, aromatic plants.*

Introduction

Mints are commercially important herbs, considered as the second most important essential oil plants after *Citrus* species because of harvesting their essential oils, which are used widely in perfumery, cosmetic and pharmaceutical industries. The most important and the most often cultivated mint species is *Mentha piperita* L. It has wide adaptation ability in different climate and soil conditions, however, temperate climates, including Serbia, are considered as more suitable for high-quality plants (Dajić Stevanović, 2011; Mihajlov et al., 2015; Matejić et al., 2020; Radovanović et al., 2023; Ilić et al., 2021; Amani Machiani et al., 2017). Hence, the most important countries for the cultivation of this aromatic plant are Bulgaria, Italy, China and the USA, which manufacture about 90% of the world's total peppermint essential oil production (Telci et al., 2011). It is estimated that the cultivation area of medicinal and aromatic plants in Serbia decreased, from about 3500 ha at the end of the 20th century to slightly above 2000 ha during the past two decades (Dajić Stevanović, 2011; Radovanović et al., 2023) and it is important to estimate the situation on the global market before and after the COVID-19 pandemic and to assess the possibility to increase mint sales on the global market.
The pandemic considerably influenced the agriculture sector worldwide, including the production and selling of horticultural and medicinal plants (Marković, 2022a,b; Mouratidou et al., 2021; Lioutas and Charatsari, 2021). Also, Amani Machiani et al. (2017) stated that the global demand for peppermint essential oil has increased during the years before the pandemic. For this reason, the aim of this study is to determine how the pandemic influenced

the export and import of mint in Serbia and to estimate current possibilities for the export of mint in case of expanded production.

Material and Methods

In this research, the official statistical databases of Serbia were used for obtaining the data on the export and import of mint given in tons and USD. These data were collected from the databases of the Republic Bureau of Statistics (https://data.stat.gov.rs/?caller=SDDB), for the period 2015-2022. The trend analysis was used to calculate trends for the years 2020, 2021 and 2022 in order to determine the expected changes in export and import based on five years data (2015-2019) collected before the pandemic and to compare obtained data with real data collected for the pandemic years 2020, 2021 and 2022. Microsoft Excel 2007 was used for trend analysis. Also, in order to obtain more accurate data, the mint trade in Serbia during the pandemic years (2020, 2021. 2022) was compared to the average data for the five-year period before the pandemic (2015-2019).

Results and Discussion

The data for the export and import of mint for the market with the largest share in the export or import in Serbia (European Union) and the global world market (total export and import), are presented in Tables 1 and 2. The export of mint was increasing during past years, and during the pandemic years 2020 and 2021, it was higher than the average export for a five-year period (2015-2019) and also higher than estimated by trend analysis (Table 1). However, in 2022, the amount of export dropped slightly compared to previous years, and it was 15.9% lower than it was estimated by trend analysis. Despite that, the price of export increased by 24.9% than was estimated by trend analysis. The price of exported mint was 3430 USD/ton in 2020 and it increased by 27.5% to 4736 USD/ton in 2022. The main market for export also changed; in 2015 and 2016, the main export market was in the EU with more than 80% of total export (Table 1). However, the amount of mint exported to the EU decreased to 73-76% during 2017-2019, and it was below 70% in the period 2020-2022. This tendency was noticeable in the years before the pandemic, so we cannot conclude that the pandemic caused changes in main export markets.

Table 1 Export of mint from Serbia during 2015-2022.

Year	**European union**		****EU share in total trade (%)**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***	**t**	**USD***
2015	331.4	928.1	80.2	67.0	413.4	1386.1	Estimated values based on 2015-2019 data	
2016	478	1365.8	85.4	76.0	559.6	1797.9		
2017	289.6	823.6	75.9	63.1	381.8	1306.2		
2018	431.8	1311.1	73.8	73.1	584.7	1794.6		
2019	302.5	879.2	73.8	63.9	409.7	1375.7		
2015-19*	366.7	1061.6	78.1	69.3	469.8	1532.1		
2020	334.2	989.5	66.9	57.7	499.6	1713.8	475.1	1524.9
2021	306.3	1083.8	69.6	61.3	440.4	1769.2	439.9	1416.9
2022	256.0	987.0	66.2	53.9	386.8	1832.0	460.2	1469.1

*Note: The value is given in thousands of USD; 2015-19 presents the average value for the five-year period.

**Percentage of export to EU compared to total export.

Table 2. Import of mint to Serbia during 2015-2022.

Year	European Union		World		World	
	t	USD*	t	USD*	t	USD*
2015	7.7	112.6	37.6	253.8	Estimated values based on 2015-2019 data	
2016	11.4	111.1	24.2	223.7		
2017	4.9	108.8	20.5	228		
2018	25.7	233	32.4	284		
2019	36.8	316.1	40.3	354.7		
2015-19*	17.3	176.3	31.0	268.8		
2020	39.2	362.5	54.4	393.6	35.1	347.5
2021	61.5	499.4	132.7	614	42.9	399.8
2022	57.1	612.2	169.9	795.5	48.5	444.9

*Note: The value is given in thousands of USD; 2015-19 presents the average value for the five-year period.

The amount of imported mint was lower than exported, but it is still high (Table 2). However, the price of imported mint (USD/ton) dropped significantly after the pandemic, because it ranged from 6750 USD/ton (2015) to even 11121 USD/ton (2017) before the pandemic, and it dropped to 4626 USD/ton in 2021 and 4682 USD/ton in 2022.

Although market changes can be connected with many variables and caused by many other factors, it is evident that there is a rising demand for mint, both on the global and local markets and that mint production in Serbia should be expanded. Besides the fact that the largest producers and exporters of peppermint on the global market are India, Japan, the USA, and Great Britain followed by Russia, Italy, Germany, Bulgaria, Slovakia, Norway and Poland (Masłowski et al. 2021; Sústriková, Salamon, 2011; Benzaid et al. 2010; Verma et al. 2010; Yilmaz, Telci, 2022), in Serbia, there are favourable conditions for successful mint production (Dajić Stevanović, 2011). The development of efficient crop production technology and increasing areas under mint productio in Serbia would also enable the development of the rural area in Serbia and the possibility to create new jobs for the local community. Besides, it is important to improve mint production technology, adapted to environmental conditions in Serbia, to make mint production simple, easy and economically justified with the aim to produce high-quality plants.

Conclusions

Although the Covid-19 pandemic considerably influenced the agriculture sector worldwide, including the production, distribution and trade of edible, medicinal and ornamental plants, it did not influence the market of mint in Serbia. However, the demand for mint is increasing worldwide and mint production has great potential in Serbia due to favourable environmental conditions. According to this, it is important to work on improving production technology suitable for successfully growing high-quality mints in Serbia, as well as to increase the cultivation area under mint in Serbia and to conduct activities including developing a network of farms and collecting centres, which will revive mint production in Serbia.

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EFFECTS OF STRESS-ALLEVIATING PRODUCTS ON OLIVE PRODUCTIVITY AND OLIVE OIL QUALITY

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Abstract

The olive is one of the most valuable fruit trees in Mediterranean basin. It is grown under both rainfed and irrigated conditions, and can withstand high air temperatures during the summer months. As climate changes and summers are getting hotter in Mediterranean countries where the olive tree thrives, it is of outmost importance to find ways to alleviate the heat and drought induced stress. In the present trial, three alleviating products were tested, i.e., the osmolyte glycine betaine, the reflectants kaolin clay particles and talc. Products were foliarly applied either once (in July) or twice (July and again August) in 'Megaritiki" olive cultivar, grown under rainfed conditions. The photosynthetic activity of the trees measured in August (10 days after the foliar application) was higher in trees treated with kaolin in July, while there was not any significant difference concerning the SPAD index measured. The talc application in July and the kaolin application in July and August resulted in the highest yield while the talc application was the one with the highest olive oil production per tree. Olive oil quality indexes (acidity, peroxide number, UV absorbance indexes) did not differ among treatments, while all indexes were within the range for the characterization of the olive produced as Extra Virgin olive oil (EVOO). The talc application resulted, also, in high total phenol concentration and antioxidant capacity of the oil produced, exhibiting a significant alleviating action.

Keywords: *Kaolin, Talc, Acidity, Peroxide Values, Total Phenolics.*

Introduction

Olive (*Olea europaea* L.) is one of the most important tree crops in arid and semi-arid regions (Khaleghi *et al.*, 2015; Mahzoon *et al.*, 2020), where high temperatures and water scarcity prevail (Khaleghi *et al.*, 2015). Olive cultivation occupies about 90% of the Mediterranean basin, mainly in Spain, Italy and Greece (Ashraf *et al.*, 2021; Cabezas *et al.*, 2021), at latitudes between 30° and 45° (Khaleghi *et al.*, 2015), representing 95% of global olive cultivation (Cabezas *et al.*, 2021). The cultivation of olive trees and the quality of the products produced (table olives and olive oil) are directly influenced by environmental conditions and could be negatively affected by prolonged exposure to high temperatures (heat stress) and water scarcity (Khaleghi *et al.*, 2015). These conditions are aggravated by the impact of climate change on Mediterranean olive groves, which significantly affects production and irrigation requirements (Cabezas *et al.*, 2021). Under these conditions, it is necessary to use methods and products to alleviate the stresses intensified by climate change and preserve olive productivity and product quality (Khaleghi *et al.*, 2015).

Foliar application of solar radiation-reflecting agents, such as talc and kaolin clay particles, helps to alleviate heat stress (Khaleghi *et al.*, 2015; Mahzoon *et al.*, 2020), as it increases leaf reflectance, reduces transpiration rate and leaf temperature and increases photosynthetic activity (Khaleghi *et al.*, 2015), resulting in improved product quality and oil content. Their application is therefore recommended in arid olive groves (Mahzoon *et al.*, 2020). Glycine

betaine (GB) is a quaternary ammonium compound (Ashraf and Foolad, 2007) that maintains membrane integrity during abiotic stresses (Sakamoto and Murata, 2002), resulting in increased plant tolerance (Sakamoto and Murata, 2002; Ashraf and Foolad, 2007; Annunziata *et al.*, 2019). In a large variety of plant species, natural GB accumulation is not sufficient, thus exogenous application is required for the plant to adequately respond to stress (Ashraf and Foolad, 2007; Annunziata *et al.*, 2019).
The objective of the present research was to investigate the effectiveness of three commercial formulations in alleviating these stresses, with the aim of producing high quality olive oil in rainfed olive groves.

Material and Methods

For the present experiment, a rainfed olive grove was selected in the Ypsilantis region of Viotia county, central Greece. A total of 28 adult, full producing trees of similar growth, of the Megaritiki cultivar were used. The treatments consisted of either a sole spray application (tunk mix with water) in July or two applications, in July and again in August on different trees with the following registered phytosanitary products: kaolin clay particles (Surround WP, at the dose rate of 3kg/hl), talc (Invelop WP, at 3kg/hl) and glycine betaine (Bluestim WP, at 500g/hl), while trees sprayed with water served as control. Measurements of photosynthetic activity and leaf chlorophyll index were taken during the summer months approximately 15-20 days after the spray applications. Harvesting was carried out separately for each olive tree and a sample of approximately 1.5 kg was transferred to the Agricultural University of Athens for oil extraction using a semi-industrial oil mill of the Abencor type (cold malaxation - 27-28°C for 45 minutes) (Figure 1).

Figures 1. From left to right: crusher, malaxer and centrifuge.

Yield, oil content and olive oil production were determined for each treatment. Acidity (g oleic acid 100 g^{-1} olive oil), UV absorption indices (K232 and K270), peroxide number (meq. active O_2 kg^{-1} olive oil), total phenolic compounds (mg gallic acid equivalents kg^{-1} olive oil), total o-diphenols (mg caffeic acid equivalents kg^{-1} olive oil), and antioxidant capacity by two methods (DPPH and FRAP) (μmol Trolox equivalents kg^{-1} olive oil) were determined in olive oil.
The experiment followed a completely randomized design. Significant differences between the treatments were determined using the Tukey's HSD test at a significance level of α=0.05.

Results and Discussion

Trees treated with glycine betaine and kaolin clay particles exhibited better values of photosynthetic activity parameters during the first measurement in July (Table 1). The results are in agreement with an experiment carried out by Denaxa *et al.* (2012) on two-year-old olive trees cv Chondrolia Chalkidikis, under two irrigation levels (full irrigation and drought). A higher rate of photosynthesis in August was observed in olive trees where kaolin clay particles were applied in August (Table 2), while the highest fruit and olive oil production per tree was determined when talc was applied in July, followed by kaolin (Table 3). Increased production after kaolin application in July in grapevines has been also recorded (Correia *et al.*, 2014).

All olive oils from all treatments had the characteristics of extra virgin olive oil (EVOO) in accordance with Commission Regulation (EEC) No 2568/91, as amended by Commission Regulation (EU) No 2016/1784 of 30 September 2016. The highest concentration of total phenolics was found in the olive oils obtained from the application of talc, as well in those produced from trees applied with kaolin (Table 5). Similarly, Saour and Makee (2003) report that olive trees sprayed with kaolin exhibited enhanced photosynthesis which resulted in increased synthesis of natural antioxidants (polyphenols) in the olive fruits. Higher concentrations of total o-diphenols were found in olive oils from trees that had been treated with glycine betaine in July (Table 5). Olive oils obtained from talc applications showed the highest antioxidant capacity by both methods (Table 5).

Table 1. Effect of the treatments on the parameters of the photosynthetic activity of the leaves during the first measurement (July).

Treatments	A (μmol/m²s)	gs (mol/m²s)	Ci (μmol/mol)	E (mmol/m²s)	SPAD
Control	3.61 b	0.055 b	228 a	0.55 a	74.4 a
Glycine betaine	5.90 a	0.152 a	272 a	0.64 a	70.2 a
Kaolin	5.61 a	0.081 ab	221 a	0.60 a	70.8 a
Talc	3.83 b	0.084 ab	285 a	0.62 a	77.2 a

Means within the same column followed by the same letter are not significantly different according to Tukey's multiple means test at a significance level of α=0.05.

Table 2. Effect of the treatments on the parameters of the photosynthetic activity of the leaves during the second measurement (August).

Treatments	A (μmol/m²s)	gs (mol/m²s)	Ci (μmol/mol)	E (mmol/m²s)	SPAD
Control	3.18 b	0.096 a	263 a	0.59 a	69.2 a
	July application				
Glycine betaine	3.06 b	0.095 a	284 a	0.58 a	70.4 a
Kaolin	3.20 b	0.072 a	288 a	0.61 a	71.3 a
Talc	3.09 b	0.080 a	288 a	0.58 a	71.5 a
	July and August applications				
Glycine betaine	3.20 b	0.072 a	288 a	0.61 a	71.3 a
Kaolin	3.61 b	0.081 a	221 a	0.58 a	68.8 a
Talc	3.77 ab	0.083 a	292 a	0.58 a	72.1 a

Means within the same column followed by the same letter are not significantly different according to Tukey's multiple means test at a significance level of α=0.05.

Table 3. Effect of treatments on production parameters (July-August).

Treatments	Yield (kg/tree)	Oil content (%)	Olive oil production/tree (kg)
Control	14.2 c	30.64 a	4.38 c
	July application		
Glycine betaine	22.8 abc	29.49 a	6.51 abc
Kaolin	19.5 abc	30.64 a	6.0 cb
Talc	27.9 a	32.07 a	8.73 a
	July and August applications		
Glycine betaine	18.6 bc	29.21 a	5.73 cb
Kaolin	23.7 ab	28.92 a	6.76 abc
Talc	23.1 abc	31.38 a	7.29 ab

Means within the same column followed by the same letter are not significantly different according to Tukey's multiple means test at a significance level of α=0.05.

Table 4. Effect of treatments on the quality characteristics of olive oil (July-August).

Treatments	Acidity (g oleic acid/100g O.O.)	K232	K270	ΔK	Peroxide number (meq. active O_2 /kg O.O.)
Control	0.33 a	1.08 a	0.07 a	-0.02 a	1.00 a
	July application				
Glycine betaine	0.31 a	1.02 a	0.06 a	-0.03 a	1.00 a
Kaolin	0.31 a	1.13 a	0.07 a	-0.03 a	1.00 a
Talc	0.32 a	1.54 a	0.11 a	0.02 a	1.12 ab
	July and August applications				
Glycine betaine	0.31a	1.24 a	0.08 a	-0.01 a	1.00 a
Kaolin	0.33 a	1.08 a	0.07 a	-0.03 a	1.00 a
Talc	0.31 a	1.46 a	0.09 a	-0.01 a	1.37 b

Means within the same column followed by the same letter are not significantly different according to Tukey's multiple means test at a significance level of α=0.05.

Table 5. Effect of treatments on total phenolics, total o-diphenols content and on antioxidant capacity of olive oil according to the DPPH and FRAP methods (July-August).

Treatments	Total phenolics (g gallic acid equiv./kg O.O.)	Total o-diphenols (g caffeic acid equiv./kg O.O.)	DPPH (μmol Trolox/kg O.O.)	FRAP (μmol Trolox/kg O.O.)
Control	300.0 abc	22 b	910 abc	840 abc
	July application			
Glycine betaine	183.3 bc	60 a	460 e	430 c
Kaolin	286.7 abc	33 b	860 bcd	850 abc
Talc	356.7 a	31 b	1190 a	1250 a
	July and August applications			
Glycine betaine	150.1 c	40 ab	580 de	410 c
Kaolin	286.7 abc	32 b	610 cde	570 bc
Talc	343.3 ab	39 ab	960 ab	1030 ab

Means within the same column followed by the same letter are not significantly different according to Tukey's multiple means test at a significance level of α=0.05.

Conclusion

In conclusion, taking into account both the physiological parameters of the leaves and the level and the production parameters assessed in the present trial, it is safe to say that the application of the stress-alleviating products was successful in reducing the level of stress during the summer months (photosynthetic capacity 3,77 μmol/m²s under talc treatment against 3.18 μmol/m²s). Among the products tested, both kaolin and talc proved to be equally effective in alleviating the effects of high temperatures and high solar radiation intensity, as they were the ones that led to an increase in production (23.7 kg/tree with kaolin and 27.9 kg/tree with talc against 14.2 kg/tree under control conditions) and an improvement in the quantity, quality and functional properties of the olive oil.

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INFLUENCE OF BIOFERTILIZERS ON YIELD AND QUALITY OF LETTUCE GROWN IN THE OPEN FIELD

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Abstract

The production of lettuce is very profitable due to the short vegetation period and the possibilities of its cultivation throughout the year. On average, the salad contains 94% water, 2% sugar, 0.6% crude cellulose, 0.6% mineral matter and 1.2% crude protein. It is rich in vitamins C, B_1 and B_2, and many mineral elements. Great influence on the quantitative and qualitative properties of lettuce in addition to the variety and production conditions has a proper diet. The application of mineral fertilizers results in high yields, but their inadequate application can cause various problems such are: decrease in dry matter content, deterioration of salad quality, increase in soil acidity, degeneration of physical properties, increase in erosion and instability in land aggregates. In order to reduce the use of chemicals and increase the yield in the same time, the improvement of plant production is moving in the direction of introducing biofertilizers. In recent years there has been a trend of decreasing the usage of mineral nutrients, primarily nitrogen, phosphorus and potassium (Haytova, 2013), in which bio stimulators play a significant role. Therefore, the goal was to determine the impact of biofertilizers on the yield and quality of lettuce and to investigate the possibility of streamlining the crop by using biostimulators in lettuce nutrition. The two-factor experiment was set on a private plot in district Vranjes, East Sarajevo by using the randomized block in 3 replications. The highest yield of lettuce was obtained in the variant with the application of the biofertilizer Bioplant flora (630.50 g – please state the unit), whereas the lowest was recorded in the control variant of fertilization (468.00 g). The highest percentage of dry matter was noted in the third variant of fertilization (Bioplant flora) in the variety Majska kraljica (6.45%), whilst the lowest percentage was reached in the control variant of the same variety (4.14%).

Keywords: *lettuce, biofertilizer, yield, quality.*

Introduction

Lettuce is rich in vitamins E, B_1, B_2, B_3 and B_6, folic acid, vitamin C and minerals such as sodium, potassium, magnesium, calcium, phosphorus and iron, which is why it is very beneficial to health. Some of the advantages of lettuce regarding its healthful properties: it has an anti-inflammatory effect, reduces cholesterol, improves sleep, acts as an antioxidant, is beneficial for heart health and has an anti-cancer effect (Ergović, 2019). The biological characteristics of lettuce and its specific growth and development are the basis for determining an optimal production method. To achieve appropriate high yields, it is supplemented with different fertilizers, which are of great importance during the growth and development of lettuce and also affect its qualitative characteristics. Applying different fertilizer systems, organic and mineral, high yields of good quality can be achieved in greenhouse vegetable production (Bogdanović et al., 2011; Bogdanović et al., 2012).

However, improper mineral fertilization, especially in winter when plants do not get enough light, creates ideal conditions that lead to the accumulation of significant amounts of nitrates, especially in leafy vegetables. Negative consequences of using mineral fertilizers lead to the leaching of nitrates into groundwater, toxic effect of heavy metals and harmful organic substances, and the appearance of weeds. In this context, the following sections of the paper will explain the role and importance of biofertilizers and how their proper application affects the growth and yield of various vegetable crops, especially leafy vegetables (Bogdanović, 2014).

Materials and methods

In order to test different types fertilization on the yield and quality of two varieties of lettuce, the study was conducted in 2022 in an open field in Vranješ, in the area of the city of East Sarajevo (Entity of Republic of Srpska, Bosnia and Herzegovina). The two-factor experiment was set by using the randomized block in 3 replications.

The size of the experimental plot was $2m^2$ (1m x 2m). The total area of the experimental field was 36 m^2. The distance between the plants was 0.30m, and the distance between the rows was 0.20m. A total of 600 plants per/$36m^2$. The manual irrigation was applied in the trial field. The basic characteristic of the land on which the experiment was set up were: 5.52 pH (in KCl); <1% $CaCO_3$; 5.56% humus; 0.17 % total nitrogen; 3.40 P_2O_5 mg/100g; 29.84 K_2O mg/100g.

This experiment included three fertilization variants (factor A) and two lettuce varieties (factor B). The tested factor (A) included the following variants: a_1 - control variant, a_2 - Slavol and a_3 - Bioplant flora. Within the second factor studied (B), two varieties were analyzed: b_1 - Majska kraljica, and b_2 - Ljubljanska ledenka. The average yield per sample was determined based on the average mass of the aerial part of the plant and the number of plants on the sample plot.

Among the ḫemic properties, the following were analyzed: the content of dry matter (%), and the vitamin C content in the leaf (mg $100g^{-1}$). Dry matter content was determined by the difference between the initial weight of the sample and the weight after drying. The drying of the plant material was carried out in a drying oven at 105^0C to a constant mass. Vitamin C (mg $100g^{-1}$) was determined by the titration method.

The obtained results were processed by the variance analysis method for a two-factor experiment (ANOVA). The significance of differences between individual environments was tested by LSD test.

Results and discussion

Total yield

According to Lazić at al (2001) yield of lettuce is different and can go from 1.5 to 4 (kg m^{-2}) depending on variety and time of production. The average green lettuce yield in our research is 5.6 (kg m^{-2}).

Table 1. Total yield (kg m^{-2})

Fertilization	Variety		Average for fertilization
	Majska kraljica (b_1)	Ljubljanska ledenka (b_2)	
Control variant (a_1)	4.39	4.96	4.68
Slavol (a_2)	5.76	6.31	6.04
Bioplant flora (a_3)	5.70	6.91	6.30
Average for variety	5.28	6.06	5.67
LSD	A	B	AxB
5%	1.56	1.28	2.21
1%	2.23	1.82	3.15

Regarding the use of biofertilizer, the highest yield was achieved on the a_3 variant (6.30g) , which is statistically higher in comparison to the control variant a_1 (4.68g). Differences in yield between variant a_2 and a_3 were not statistically significant. Team of authors concludes that the use of microbial fertilizer stimulates the growth of the stem at an average of 29% compared to control variant (Bošković 2010; Govedarica et al., 1998; Đukić et al., 2007; Gecić et al., 2007). This effect of microbial fertilizer can be explained by the ability of nitrogen fixation to produce certain physiologically active substances such as auxin, gibberellin, cytokinin and vitamin. They stimulate the aerobic respiration of the plant cells, i.e activitiy of many enzymes, process of photosynthesis, water and mineral absorption. Differences between varieties in obtained yields were not statistically significant, which is parallel to the results of Kulina (2019).

Vitamin C content

Vitamin C is an important factor in human nutrition. This vitamin takes part in numerous biological processes and is the strongest antioxidant among the vitamins.

Table 2. Vitamin C content (mg $100g^{-1}$)

Fertilization	Variety		Average for fertilization
	Majska kraljica (b_1)	Ljubljanska ledenka (b_2)	
Control variant (a_1)	8.53	10.66	9.59
Slavol (a_2)	10.66	8.10	9.38
Bioplant flora (a_3)	12.37	10.66	11.51
Average for variety	10.52	9.80	10.16
LSD	A	B	AxB
5%	1.89	1.53	2.67
1%	2.69	2.18	3.80

Regarding the use of biostimulators, the highest content of the mentioned vitamin was recorded in variant a_3 (11.51mg $100g^{-1}$), which is statistically higher than variants a_1(9.59mg $100g^{-1}$) and a_2 (9.38mg $100g^{-1}$). By analyzing the interaction, it was determined that the variety b1 on the variant a_3 (12.37mg $100g^{-1}$) had a statistically significantly higher content of the given vitamin compared to the other examined variety from the fertilization variant a_2 (8.10mg $100g^{-1}$).

Dry matter content (%)

The content of dry matter in lettuce ranged from 4.14 to 6.45%. The highest percentage of dry matter content was recorded in the third variant of fertilizer (Bioplant flora) in the Majska kraljica variety - 6.45%, while the lowest percentage was achieved in the control variant of fertilization in the same variety

Table 3. Dry matter content (%)

Fertilization	Variety		Average for fertilization
	Majska kraljica (b_1)	Ljubljanska ledenka (b_2)	
Control variant (a_1)	4.14	4.54	4.34
Slavol (a_2)	4.92	4.95	4.93
Bioplant flora (a_3)	6.45	5.23	5.84
Average for variety	5.17	4.90	5.03
LSD	A	B	AxB
5%	0.28	0.22	0.37
1%	0.39	0.31	0.53

From the analysis of the variance (table 3) we can establish that on the third fertilizer variant a_3 there is statistically significantly higher percentage of dry matter content (5.84%) in comparison to the a_2 (4.93%) and controll (a_1) fertilizer variant (4.34%). The results of these researches show that the percentage of dry matter content in lettuce is associated directly with the fertilization. Čabilovski et al (2010) have shown similar results. Likewise, Parađiković et al (2009) conclude that the percentage of dry matter content of marigold (Tagetes sp.), which belongs to the same family as lettuce, was under a significant influence of biofertilizer.

Conclusion

Based on the results of the research "Influence of biofertilizers on yield and quality of lettuce produced in the open field", it can be concluded that the yield achieved during the research was at a satisfactory level. The lowest yield was in the control variant of fertilization, and the highest in the variant Bioplant Flora. The differences between the achieved yield within the varieties were not statistically significant. The use of biofertilizers had a positive effect on the content of vitamin C. The maximum content of vitamin C was recorded in the variant Bioplant Flora. Application of biofertilizer had a positive effect on the total dry matter content of lettuce plants compared to the control treatment. The highest content of dry matter was recorded on the variant fertilized with Bioplant flora.

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GROWTH PARAMETERS OF PEPPER TRANSPLANTS CULTIVATED BY THE FLOATING CONTAINERS TECHNOLOGY

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Abstract

The trial was set in a greenhouse in the area of Čapljina (BiH) with the aim of determining the development of pepper transplants. Two research factors of were studied, the term of putting the containers on nutrient solution (immediately after sowing, after previous germination and in the stage of cotyledons) and substrate volume of individual seeding place (27, 37, 47 ml). Percentage of well-developed transplants was analyzed as well as morphological characteristics: transplant height (cm), diameter of transplant (mm) and number of leaves. The highest percentage of well-developed transplants (97.3%) was obtained in the containers placed on nutrient solution when transplants had developed cotyledon leaves. The term of putting the containers on nutrient solution showed a highly significant effect on transplant height (8.02 cm), diameter of transplant (2.72 mm) and the number of leaves (6.00). The substrate volume of a seeding place showed a highly significant effect on transplant height (7.05) and the number of leaves (5.00). Interaction of factors had a highly significant effect on the transplants height. The maximum height of the transplants (9.12 cm) was determined by placing the container on the nutrient solution when transplants had developed cotyledon leaves on substrate of 37 ml volume and highly significantly differed from others, with the exception of the same term of putting the containers on nutrient solution and 47 ml volume (8.90 cm), from which it did not significantly differ. The obtained results showed that the floating system technology is an alternative for the production of high-quality pepper transplants on family farms.

Keywords: *transplants, pepper, floating containers, nutrient solution.*

Introduction

Although they are considered the best quality, transplants from containers produced in specialized centres are price unacceptable for producers who grow vegetables on the open field. In addition to the standard container method, vegetable transplants can also be grown in floating containers on a nutrient solution. The floating hydroponic system (*Floating System, Floating Hydroponic*) is the simplest form of hydroponic production (Hanić, 2011). Float system is a less-intensive and low-cost technology that enables efficient control of the nutrition of the growing plants (Kantas, 2020). Although it was originally intended for the production of tobacco transplants, Ross and Teffeau (1995) consider this technology suitable for the cultivation of vegetable, flower and ornamental plant transplants and it is gradually being adapted and introduced as a new way of growing vegetable transplants such as tomato and pepper (Wyatt, 1998; Verdial *et al*., 1998; Verdial *et al*., 1999) and leaf vegetables (Jakše and Kacjan-Maršić, 2010). Turšić (2008) reported on the advantages of pepper transplants production using a new method on family farms. This simple method of hydroponic production is especially acceptable for smaller production of vegetable transplants for own production needs (Matotan, 2008). In the classic floating system, containers float on a nutrient solution that contains all the necessary nutrients in an easily accessible form and in an optimal

ratio, which ensures a continuous supply of the plant during its growth. At the same time, the conditions for the development of the disease are less favourable because the above-ground part is not irrigated, and the leaves remain dry (Rideout, 2004). The great advantage of floating hydroponics is safety due to the reduced risk of root damage and plant death, because the root system is in direct contact with a large amount of nutrient solution, and due to the rational use of water and nutrients, it contributes to the preservation of the environment (Toth, 2015). There is no loss of water through evaporation and percolation, which is particularly important nowadays when awareness of the importance of water and environmental protection has grown. This system produces transplants faster than classic growing technology in containers, and according to Matotan et al. (2007) for the same planned planting period can start one week later. It is possible to produce a larger number of transplants of the same quality on the same surface than with the classic technology of growing in containers (Sefo *et al*., 2008). In general, it is very important for transplants producers to correctly select substrate volume of individual seeding place in the container so that the plants are not exposed to morphological and thus physiological changes. This has been pointed out by numerous authors (NeSmith and Duval, 1998; Xu and Kafkafi, 2001; Filković *et al.,* 2009; Lončarić *et al*. 2009; Bjelić *et al*., 2010; Govedarica-Lučić *et al*., 2016). It is important to choose the appropriate size of the individual seeding place in the container because there is a direct relationship with the development of the transplants (Leskovar, 2001). Furthermore, water quality management is an important part of successful transplants production (Smith *et al*., 2010) emphasizing the influence of pH and EC values of the nutrient solution (Barbaro *et al*., 2011). The composition of the nutrient solution is an important aspect affecting the success of float system (Kantas, 2020). Despite the advantages, this way of growing pepper transplants in the researched area is not widespread, and the trial was set up to point out the importance and provide guidelines for the introduction of cheaper production technology.

Material and Methods

In order to establish the possibility of producing pepper transplants in floating containers on nutrient solution using a technology adapted to family farms in unheated greenhouses in Nerezi near Čapljina (BiH) during 2018. on cv. Istra F1, a two-factor trial was set according to the method of completely randomized design (CRD). The factor, term of putting the containers on nutrient solution (A) was observed through three levels: A1-directly placing the containers on nutrient solution immediately after sowing, A2-placing the containers on nutrient solution after previous germination of seeds, and A3-placing the containers on nutrient solution in the stage of developed cotyledon leaves. The second factor, seeding place substrate volume, or the most favourable number of seeding places in a container (B) also had three levels: B1-23 ml (160 seeding places), B2-37 ml (104 seeding places) and B3-47 ml (84 seeding places). Polystyrene containers (50 x 33 cm) were filled with substrate for production of vegetable transplants Potgrond H (Klasmann). Fifteen containers of each variant were sown, which is a total of 45 containers. The seeded containers occupied an area of 8 m^2 in a pool with a total area of 22.5 m^2 and a volume of water of 3 m^3. The nutrient solution was prepared using the water-soluble fertilizer Kristalon green special (NPK 18:18:18) in the amount of 1 kg/m^3. Because of evaporation of the solution from the reservoir, fresh water was periodically added aimed to keep the EC value (electrical conductivity) constant at a level of 1.5 mS/cm and pH value 5.8-6.3. For the amount of water in the reservoir, 300 ml of Previcur 607 SL fungicide was preventively added. In the fifth week after sowing, on a sample of ten plants of each variant from one container intended for analysis, transplant development parameters were measured: transplant stalk length (cm), transplant stalk diameter immediately below the cotyledon leaves (mm) and counting of developed leaves. The percentage of well-

developed transplants suitable for replanting was also established. The research data obtained for growth parameters of the transplants grown on nutrient solution were statistically evaluated by variance analysis (ANOVA), while the LSD test examined the differences between mean values with the significance level p 0.01. The data were processed using the statistical program SP SS 20.

Results and Discussion

The research found for each variant a high percentage of well-developed pepper seedlings suitable for transplanting, which is extremely important for reducing production costs. The highest percentage of well-developed transplants suitable for replanting, 97.3%, was obtained in the containers placed on nutrient solution when transplants had developed cotyledon leaves, while the lowest percentage, 91.2%, was in the containers placed immediately after sowing (Table 1). Leskovar (2001) reported an increase in germination by 5 to 10% in containers that were previously in the germination chamber, as well as in this study. Therefore, the conditions of constant moisture in the floating system enabled normal seed germination and the achievement of high percentages of normally developed transplants, thus achieving one of the preconditions for successful production.

Table 1. The percentage of well-developed pepper transplants on nutrient solution

Seeding place substrate volume, B (ml)	Term of putting the containers on nutrient solution, A		
	After sowing	After germination	In cotyledon stage
B1	97.7	96.7	97.8
B2	82.0	94.5	98.1
B3	94.0	93.2	96.1
Average	91.2	94.8	97.3

Height, stalk diameter, number of leaves are important parameters for determining the quality of the transplant's vegetative growth. The term of putting the containers on nutrient solution showed a highly significant effect on all the analysed properties of pepper transplants while the substrate volume of a seeding place showed a highly significant effect on transplant height and the number of leaves (Table 2).

Table 2. Influence of individual factors on measured properties of pepper transplants

Term of putting the containers on nutrient solution, A	Transplant height (cm)	Stalk diameter (mm)	Number of leaves
A1	4.60 c	2.26 b	4.30 c
A2	6.53 b	2.52 ab	4.97 b
A3	8.02 a	2.72 a	5.63 a
$LSD_{p=5\%}$	0.35	0.22	0.27
$LSD_{p=1\%}$	0.47	0.30	0.36
Seeding place substrate volume, B (ml)	Transplant height (cm)	Stalk diameter (mm)	Number of leaves
B1	5.26 b	2.41 a	4.80 b
B2	6.84 a	2.53 a	5.20 a
B3	7.05 a	2.55 a	4.90 ab
$LSD_{p=5\%}$	0.35	n.s.	0.27
$LSD_{p=1\%}$	0.47	n.s.	0.36

Transplants in the containers placed on nutrient solution when plants had developed cotyledon leaves were highly significantly highest, while those in the containers placed on nutrient solution immediately after sowing were lowest. This order of values obtained was expected due to more favourable conditions during germination and initial growth before placing the containers of individual variants on the nutrient solution where the young plants continued to grow. The highest were the transplants on the largest substrate volume (47 ml) and it is significantly different from the variant on the smallest substrate volume (23 ml), of which the transplants obtained on medium substrate volume of individual seeding place (37 ml) are also significantly higher. NeSmith and Duval (1998) reported that the limitation of root growth in containers of smaller size of individual seeding place is the reason for the weaker growth of pepper seedlings. Also Filković *et al*. (2009) obtained significantly the highest of pepper seedlings in containers with the largest volume of substrate (40 cm^3) i.e. with the smallest number of sowing places. The only factor that showed a significant influence on the diameter of the pepper stem was the time of putting the containers on the nutrient solution and pepper transplants in the containers placed on nutrient solution in cotyledon stage had the largest diameter. Although the differences were not significant, the largest diameter had the transplants grown on the largest volume of substrate (47 ml), i.e. in pots with the smallest number of sowing places. The significantly largest number of leaves was in the containers placed on nutrient solution when the transplants were in the cotyledon stage. Pepper transplants grown on the medium substrate volume of individual seeding place had the highest number of leaves, which is significantly more compared to the smallest volume substrate, but it is at the same level of significance as the variant of the largest volume of the substrate. Likewise, Filković *et al.* (2009); Rotim (2012) reported an increase in the number of leaves in pepper seedlings in containers with the largest volume of substrate compared to containers with a larger number of smaller seeding places.

The interaction of term of putting the containers on nutrient solution and substrate volume of individual seeding place had a highly significant effect only on the property transplant height (Table 3).

Table 3. Influence of interaction of individual factors on measured properties of pepper transplants

Term of putting the containers on nutrient solution (A)	Transplant height (cm)			Stalk diameter (mm)			Number of leaves		
	Seeding place substrate volume (B)								
	B1	B2	B3	B1	B2	B3	B1	B2	B3
A1	4.23	4.03	5.54	2.20	2.36	2.23	4.00	4.90	4.00
A2	5.50	7.37	6.72	2.43	2.56	2.57	4.80	5.10	5.00
A3	6.05	**9.12**	8.90	2.61	2.68	2.86	5.60	5.60	5.70
$LSD_{p=5\%}$	0.62			n.s.			n.s.		
$LSD_{p=1\%}$	0.82			n.s.			n.s.		

The maximum transplant height, 9.12 cm, was obtained in the containers placed on nutrient solution when transplants had developed cotyledon leaves on substrate of medium seeding place volume and highly significantly differed from average values of other combinations, with the exception of the combination of the same term of putting the containers on nutrient solution and the largest seeding place substrate volume, 8.90 cm, from which it did not significantly differ. Although the interactions were not significant, the highest values for the properties stalk diameter, 2.86 mm, and number of leaves, 5.70 (6.00), were in the containers placed on the nutrient solution when the seedlings developed cotyledon leaves on the largest substrate volume of individual seeding place. The obtained results are explained by the better conditions for root growth, and thus the initial growth and development of young plants in containers on a nutrient solution. Similar results were reported for the quality of tomato seedlings on nutrient solution (Sefo *et al.*, 2018).

Conclusions

The percentage of well-developed transplants and the analysed growth parameters can be successfully used to show the quality of pepper transplants grown on the nutrient solution. The highest percentage of well-developed pepper transplants was in containers placed on nutrient solution with plants in cotyledon stage. A continuous increase in the values of the analysed growth parameters was determined by gradually placing the containers of different variant on the nutrient solution, and significantly higher values were also obtained by placing the containers when the plants were in the cotyledon stage. Furthermore, transplant height (cm), diameter of transplant (mm) and number of leaves grew almost uniformly with increasing substrate volume, and transplants with the best characteristics were grown in containers with the largest substrate volume of individual seeding place (47 ml), i.e. in containers with the smallest number of sowing sites (84). The results indicate the importance of this simple, less intensive hydroponic technique, as a satisfactory alternative to the classic container production of pepper transplants, whose development and application in the researched area will stimulate the commercial production of quality transplants on family farms.

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THE INFLUENCE OF VARIETY AND ENVIRONMENTAL CONDITIONS ON THE FORMATION OF PODS AND SEEDS AND YIELD OF FORAGE PEAS

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Abstract

Important components of pea yield area: the number of pods per plant, formed seeds within a pod, and the mass of one thousand seeds. The main goal of this research was to determine the variations of the most important productive traits of spring forage pea varieties of different origins under different production conditions. The field experiments were cultivated in 2016 (Y1) and 2017 (Y2) at two localities: in the vicinity of Banja Luka (L1) (experimental field of the Agricultural Institute of the Republic of Srpska) and in the vicinity of East Sarajevo (L2) (experimental field of the Faculty of Agriculture in East Sarajevo). For sowing, inoculated seed of five spring fodder pea varieties were used: NS Javor (G1), Baccara (G2), NS Dukat (G3), NS Junior (G4) and Sasa (G5). Research was conducted to determine the influence of variety, location, and year on the number of pods per plant (NPP), pod length (LP), number of seeds per pod (NSP), seed weight per pod (MSP), and pea grain yield per unit area (PGY). Based on the analysis of the mean values of the examined traits, highly significant differences among varieties were found for all the examined traits, as well as VL interactions. Variety V_1 showed the greatest effect on PGY, variety V_2 on NSP and MSP, variety V_4 on NPP, and variety V_5 on LP. Locations exhibited highly significant effects on MSP and PGY, while a significant effect of location was observed for NSP. For the investigated traits of NSP, MSP, and PGY, location L_1 demonstrated a greater effect. In 2016, a PGY of 4,091 t ha^1 was achieved, and in 2017, it was 3,973 t ha^1. The highest PGY was recorded for variety V_1, at 4,673 t ha^1, and the lowest for V_4, at 2,569 t ha^1. A higher PGY was observed at L_1, with 4,076 t ha^1, compared to *L_2, with 3,988 t ha^1.

Keywords: *pea, locality, yield, year, variety.*

Introduction

Peas represent an excellent leguminous component in various cropping systems (Ambrose et al., 1997). It is used in the nutrition of domestic animals in the form of green fodder, dry fodder matter, animal meal, unripe grain, ripe grain and straw (Mikić et al., 2006). Pea yield can be represented by four components: preserved plant until harvest, formed pod, formed seed in the pod and mass of one thousand seeds (Ghobary, 2010). The mentioned yield components show a certain plasticity in terms of reflecting the yield level, so that there is a compensation between the number of pods and the formed seeds in the pod, or between the formed seeds in the pod and its mass (Timmerman-Vaughan et al., 2005). Kosev and Mikić (2012) found that there are significant positive correlations between the number of fertile nodes, the number of pods and the number of seeds per plant, as well as between the number of seeds and pods per plant. Grain yield and above-ground mass of vegetable and fodder pea varieties are characterized by great variability, caused by habitat conditions and meteorological conditions during growth and development (Annicchiarico and Iannucci, 2008). Variations in the yield of peas are influenced by different climatic conditions in the growing seasons (Zajac et al., 2012). The number of seeds per plant, as the most variable

component of pea yield, is strongly influenced by extremely high temperatures and water stress, especially in the phases from the beginning of flowering to the beginning of seed filling (Guilioni et al., 2003). Extremely high temperatures lead to the deterioration of generative organs in a very short period of time, and in combination with water stress, they have a negative effect on the biomass yield, and beyond that, on the grain yield. The length, mass and number of pea pods depend on the position on the fruiting tree. The longest pods have the largest mass and number of grains and develop on the lowest nodes (first and second nodes), while the shortest pods grow on the third and fourth nodes, which are located in the middle of the tree, and the shortest pods are located on the fifth and sixth nodes, which located at the top of the tree, while the grain formed in the central part of the pea has the largest mass (Zajac et al., 2012). The main goal of this study was to determine the variations of the most important productive traits of spring fodder pea varieties of different origins under different production conditions.

Materials and Methods

The field experiments were cultivated in 2016 (Y_1) and 2017 (Y_2) at two localities: in the vicinity of Banja Luka (L_1) (experimental field of the Agricultural Institute of the Republic of Srpska), altitude of 163 m (17°49'19 " latitude and 44°48'13" longitude), and in the vicinity of East Sarajevo (L_2) (experimental field of the Faculty of Agriculture in East Sarajevo), altitude 550 m (43°49'01" latitude and 18°20'57" longitude). The basic soil treatment was carried out in the autumn, at a depth of 25 cm, and the pre-sowing preparation at a depth of 10 cm. Basic fertilization with 350 kg of ha^{-1} $N_8P_{24}K_{24}$ was done together with the basic cultivation of soil. The sowing was done at 12.5 cm spacing and a interspace of 8 cm in the main plot of 5 m^2. For sowing, inoculated seed of five spring fodder pea varieties were used: NS Javor (G1), Baccara (G2), NS Dukat (G3), NS Junior (G4) and Sasa (G5). In a sample of 10 randomly selected plants per plot, the following were determined: number of pods per plant (NPP), pod length (LP) (cm), number of seeds per pod (NSP), seed weight per pod (MSP) (g), and pea grain yield per unit area (PGY) (t ha^{-1}) (grain yield on experimental plots recalculated to a unit area of ha). The total amount of heat for peas is from 1300 to 2800°C, and it depends on the length of the vegetation. When analyzing temperature, in addition to daily averages, extremes are very important, that is, the lowest and highest temperatures in certain periods of growth and development of peas. Optimum mean daytime temperatures in the vegetative stage are from 12 to 16°C, and during pod formation from 16 to 22°C, and for good pea metabolism, the optimal difference between day and night temperatures is 6 to 10°C. The average temperature for the vegetation period (III-VII) in 2016 in the Banja Luka area was 16.5°C, while the sum of precipitation was 344.8 mm. The average temperature for the vegetation period (III-VII) in 2017 was 17.2°C, while the sum of precipitation was 437.6 mm In the location of East Sarajevo, the average temperature for the vegetation period (IV-VII) in 2016 was 16.9°C, and in 2017 it was 16.7°C, while the sum of precipitation was 343.5 mm in 2016, and 327.7 mm in 2017. All data were processed with the SPSS statistical package. The analysis was performed based on the mean values of the obtained results and the analysis of variance (ANOVA) to determine the significance of the differences.

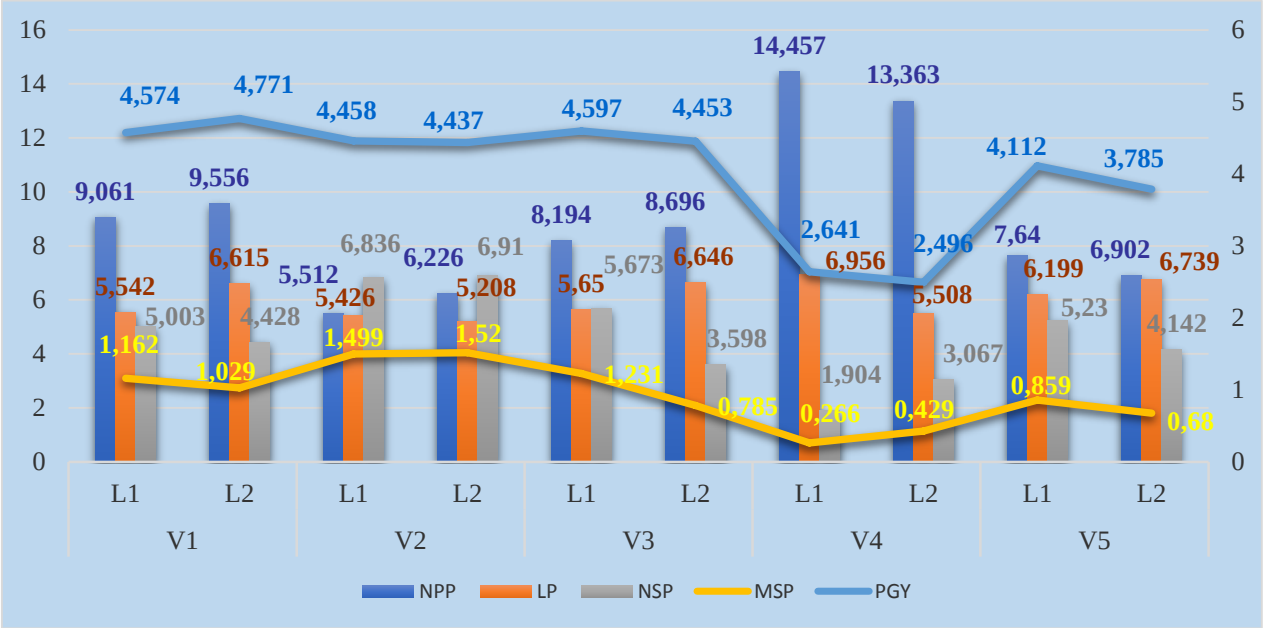
Results and Discussion

Based on the analysis of the mean values of the examined traits, highly significant differences of varieties were determined for all the examined traits (NPP, LP, NSP, MSP and PGY) (Table 1). Variety V_1 showed the greatest effect for PGY, variety V_2 showed the greatest effect for NSP and MSP, variety V_4 for NPP, and variety V_5 for LP. The smallest effect for the traits NPP and LP was shown by variety V_2, while for the traits NSP, MSP and PGY the smallest effect was shown by variety V_4. Mihailović and Mikić (2004) state that the variety NS Junior, thanks to its tendency to branch, had the highest number of pods per plant, which was also confirmed by these studies. These variations may be the result of the hereditary potential of the varieties and their interaction with the soil and climatic conditions, which is in agreement with the statements of Khichi et al. (2016). The tested pea varieties showed a significant difference in LP. V5 had the longest pod, and V_2 had the smallest pod. Lakić et al came to similar results, when it comes to pod length in the variety Saša. (2019). Localities showed highly significant effects for MSP and PGY, while a significant locality effect was shown for NSP. For the investigated characteristics of NSP, MSP and PGY, the L_1 location showed a greater effect. In 2016, a higher yield of peas was achieved, compared to 2017. An analysis of climate parameters found that 2017 was less favorable, which also affected the pea yield. April and May were colder and with more precipitation compared to multi-year averages, so these weather conditions were not favorable for the sprouting and initial growth of peas, while summer is considered one of the hottest with many days with temperatures above 40°C. Temperatures above 26°C in the period immediately after flowering can significantly reduce grain yield due to the decay of flowers (Popović et al., 2002). Also, short-term temperatures of 30°C can affect the quality of pods (Sing and Singh, 2011).

Table 1. Analysis of variance

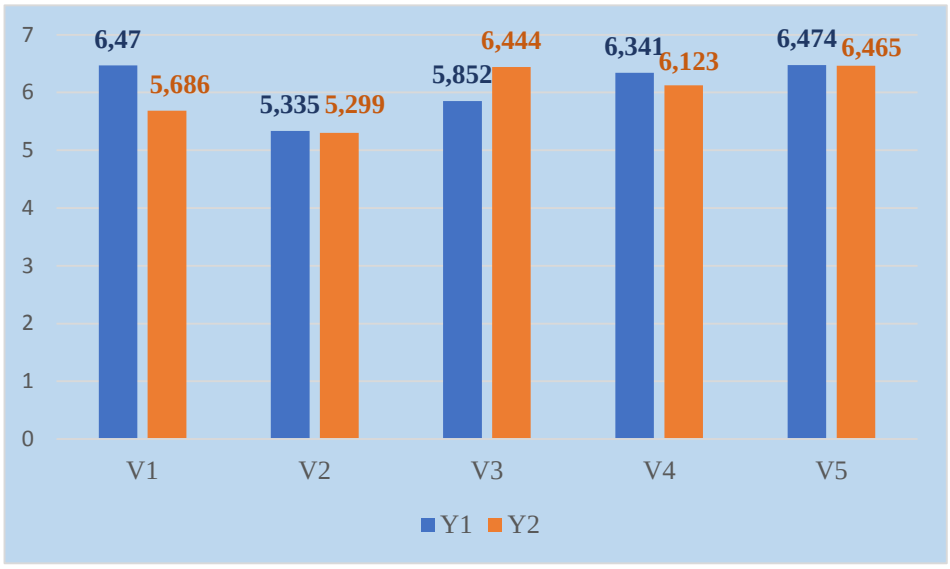
	F-value				
	NPP	LP	NSP	MSP	PGY
V	227.58**	9.20**	58.76**	97.67**	468.75**
L	0.02ns	2.17ns	7.64*	8.74**	6.11**
Y	1.59ns	0.50ns	0.21ns	0.04ns	10.93**
G * L	4.19*	13.38**	9.04**	7.01**	5.87**
G * Y	1.10ns	2.96*	0.01ns	0.01ns	2.10ns
L * Y	1.67ns	0.02ns	0.03ns	0.03ns	0.58ns
G * L * Y	1.85ns	0.28ns	0.07ns	0.08ns	0.92ns

*- Significant at the 0.05 level, **- Significant at the 0.01 level, V - varieties, L – locality, Y - year, NPP - number of pods per plant, LP - length of the pod, NSP - number of seeds in the pod, MSP - mass of seeds per pod, PGY - pea grain yield.



Graph 1. Mean values for number of pods per plant (NPP), length of the pod (LP), number of seeds in the pod (NSP), mass of seeds per pod (MSP) and pea grain yield (PGY) for varieties (V_1, V_2, V_3, V_4, V_5) and locations (L_1, L_2).

The greatest effect for NPP was shown by the locality of variety V_4 (L_1 – 14,457 and L_2 – 13,363) as well as for LP (L_1 – 6,956). For the traits NSP and MSP, the greatest effect of location was manifested in the variety V_2 and was for NSP (L_1 – 6,836 and L_2 – 6,910), and for MSP (L_1 – 1,499 g and L_2 – 1,520 g). The highest yield was found in variety V_1 (L_2 – 4,771 t ha^{-1}), while the highest PGY was found in variety V_3 (4,597 t ha^{-1}) at L_1 (Graph 1).



Graph 2. Mean values for of lengths of the pod (LP) for varieties (V_1, V_2, V_3, V_4, V_5) and years (Y_1, Y_2).

The effect of VY on LP was determined. Four cultivars (V_1, V_2, V_4 and V_5) had longer pods in Y_1, while cultivar V_3 had longer pods in Y_2. The largest pod was 6.474 cm for V_5Y_1, and the smallest pod was 5.299 cm for V_2Y_2 (Graph 2). The length of the pod is a varietal characteristic, but it can also be influenced by the height of the plant, the ecological

conditions of the area where peas are grown, the availability of nutrients, especially in the phase of pod formation (Khan et al., 2013). Pea varieties differ in the length and shape of the pods and the number of grains per pod (Kumar, 2016).

Conclusion

Important components of PGY are NPP, LP, NSP and MSP. LP is a varietal trait, but it is also influenced by the ecological conditions of the area where peas are grown, the availability of nutrients, especially in the phase of pod formation. During these studies, it was determined that the PGY pea variety is influenced by growing conditions (L, Y), but also by the interaction of VL. The maximum NSP was determined in the V_2 variety, followed by the V_5 variety, where a positive influence of LP on the NSP was also determined. The NSP is affected by external environmental conditions, which were not favorable during flowering and fertilization. The V_4 variety showed great sensitivity to environmental conditions because it had a longer flowering period in conditions of high temperatures, which affected the reduced NSP, even though its pod was long. Pea varieties showed a significant difference in MSP. During these studies, the V_2 variety had the highest MSP, and the V_4 variety the lowest. These variations may be the result of the hereditary potential of the varieties and their interaction with the soil and climatic conditions. In the years of testing, the highest PGY was in the variety V_1, then in the variety V_3, and in third place was the variety V_2. The pea varieties intended for the combined method of utilization had a significantly lower average grain yield. The variety V_4 had the lowest PGY.

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ORGANOLEPTIC, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF PEA SILAGE FROM THE JUGOVIĆ FARM

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Abstract

Field peas and vetch are significant plant species for producing roughage livestock feed, which can be used as green fodder, hay, or silage. Due to their specific characteristics, like susceptibility to lodging and high buffering capacity, they are commonly grown in mixture with cereals as support crops, and this practice aims to prevent or reduce lodging, decrease buffering capacity, and simultaneously obtain biomass suitable for the ensiling process. Silage is the preservation of green fodder, which can be stored for an extended period in a moist state in silos or pits. Silage holds a high nutritional value and retains almost all the nutrients and vitamins from the green plant material. In the process of silage production, lactic acid bacteria play a crucial role by converting the sugars present in the ensiled plants into lactic and partially acetic acid. The study of organoleptic, chemical, and microbiological properties is used in assessing the quality of silage, therefore, the aim of this research was to analyze and interpret the mentioned parameters of pea silage from the Jugović farm. The examination results of the organoleptic properties of pea silage indicated that the silage had an olive-green to pale yellow color, slightly refreshing to sharply acidic smell, a pH value of 4.62, and a well-fermented consistency. Some examined parameters of the chemical composition of the silage (protein content, crude fiber content, crude fat content, and ash content) confirmed that the tested silage was satisfactory in terms of its chemical composition. Microscopic examination of silage samples confirmed the dominance of lactic acid rod-shaped bacteria and streptococci, which were also characteristic of high-quality silage. The results of our research confirm that the tested silage, in terms of color, odor, consistency, pH value, chemical composition, and microscopic analysis, fell within the range of well-prepared silage.

Keywords: *Silage, organoleptic properties, chemical properties, microbiological properties.*

Introduction

Field peas and vetch are significant plant species for producing roughage livestock feed, which can be used as green fodder, hay, or silage. Due to their specific characteristics, like susceptibility to lodging and high buffering capacity, they are commonly grown in mixture with cereals as support crops, and this practice aims to prevent or reduce lodging, decrease buffering capacity, and simultaneously obtain biomass suitable for the ensiling process (Đorđević *et al*., 2010).

Silage is the preservation of green fodder, which can be stored for an extended period in a moist state in silos or pits. People have been producing silage since ancient times, and some literary sources indicate that the technique of silage preparation dates back over 3000 years (Wilkinson *et al*., 2003). In the ensiling process, lactic acid bacteria participate in fermenting the sugars present in the ensiled plants into lactic and acetic acids. These acids suppress the growth of undesirable bacteria, including butyric acid bacteria. According to Kung *et al*.

(2018), the ratio of lactic to acetic acid can be used as a qualitative indicator of fermentation, with high-quality silages having a ratio of these acids around 2.5 to 3.0.
Silage has a high nutritional value and retains almost all the nutrients and vitamins from the green plant material (Đukić *et al*., 2009). The process of ensiling plant crops is influenced by several factors, among which the most significant are: the type of plant being ensiled, the sugar content, the buffering capacity of the plant, etc. Jemcev and Đukić (2000) state that maize is an ideal silage crop, unlike leguminous plants that are difficult to ensile due to their low sugar content (3-6%) and high protein content (20-40%). The minimum concentration of sugars required in ensiled plants is referred to as the sugar minimum. If the sugar content in plants falls below the sugar minimum, they won't ensile properly; whereas, if it's higher, they will ensile correctly. According to a study by Macêdo *et al*. (2018), various factors including dry matter content, soluble carbohydrates content, buffering substances content, and microbiological composition of the forage, can alter the final product of the ensiling process. In the case of an improper ensiling process, undesirable microbial populations such as pathogens and toxin-producing microorganisms can develop in the silage, consuming such silage can negatively affect the health of animals. Properly prepared silage must be stored correctly to prevent it from becoming a suitable environment for microbial growth.
Đukić *et al*. (2009) state that feeding silage improves food digestion in animals, enhances the palatability of other feed components, contributes to increased productivity in cows, and supports the healthy development of calves. For decades, various additives have been available on the market to enhance the quality and storage longevity of silage (Muck *et al*., 2018). Through the application of lactic acid bacteria inoculants, the fermentation process is accelerated, and silage quality is improved. According to research by Weinberg and Chen (2013) and Ni *et al.* (2017), inoculants also improve the taste of silage and prolong its storage time. Many farmers have adopted the practice of preparing pea silage using round bales wrapped in plastic film. Numerous researchers indicate that the risk of silage spoilage can be reduced by following these steps: harvesting in early stages of maturity, wilting to 50-60% moisture content, forming dense and compact bales wrapped in plastic film within two hours of baling, storing in a clean, well-drained area to minimize tearing and damage to the film, regular inspection and repair of torn or damaged bales using self-adhesive tape (Tietz, 2007; Sullivan and McKinlay, 1998).
The study of organoleptic, chemical and microbiological properties is used to assess the quality of silage, therefore, the objective of this research was to analyze and interpret the mentioned parameters of pea silage from the Jugović farm.

Material and methods

The analysis of pea silage samples from the Jugović farm was conducted at the laboratories of the Faculty of Agriculture in East Sarajevo. The organoleptic, chemical, and microbiological properties of the silage samples were studied. Among the organoleptic properties, the color, odor, consistency, and pH value of the silage samples were analyzed. In order to assess the quality of the silage, the following parameters were examined:

- pH value of the silage from silage extract using a pH meter;
- protein content (total nitrogen) using the micro-Kjeldahl method, which involves sample digestion using concentrated sulfuric acid in the presence of a catalyst, neutralization after digestion by adding NaOH, and titration of the resulting ammonia with a standard HCl solution in an automatic Velp apparatus. The protein quantity is determined computationally by multiplication with the appropriate factor;

- cellulose content (crude fiber) according to Weende and Wijkström was determined using the Fiber Bag FBS 6 apparatus by Gerhardt. The method involves treating the samples with a sulfuric acid and sodium hydroxide solution, followed by separating the residue through filtration and measuring it after drying;
- the percentage of crude ash content was determined by igniting 1g of air-dried sample at 550°C for one hour and measuring the residue. The ash content is expressed as a percentage of the mass;
- the quantity of crude fats was determined through Soxhlet extraction (Đorđević i sar., 2003);

Microscopic examination of silage microorganisms was performed using the imprint method and observation under a light microscope (Đukić *et al*., 2018).

Results and Discussion

In the conditions of increasingly pronounced feed deficits and high costs, Đorđević *et al*. (2000) argue that silage production represents the most viable solution for obtaining cost-effective and high-quality animal feed. One crucial parameter is the quality of the silage itself, and the aim of our research was to examine the organoleptic, chemical, and microbiological properties of silage produced on a smaller agricultural farm. Among the organoleptic properties, we analyzed the color, odor, consistency, and pH value of the silage samples, and the results of our research are presented in Table number 1. The color of the tested silage samples ranged from olive green to pale yellow. The smell of the silage varied from slightly refreshing to strongly acidic, attributed to acetic acid, while the pH value measured 4.62 (Table 1). In their study, Kung and Shaver (2002) mention that a pleasant odor is indicative of well-fermented silage. Numerous literary sources indicate that various volatile organic compounds can be found in silage (Krizsan *et al*., 2007; Daniel *et al*., 2013; Weiss, 2017), including ethyl and propyl esters of acetate and lactate (Weiss, 2009, Weiss *et al*. 2015).

Table 1. Organoleptic Properties of Pea Silage

Color	Odor	pH	Consistency
olive green to pale yellow	slightly refreshing to strongly acidic	4,62	the silage consisted of whole pea and oat plants in round bales without chopping, with plants in an intact state, the sample was moist

The consistency of the tested silage was composed of whole pea plants combined with oats. The mass was compressed into round bales in an intact state (not chopped), it was moist, and contained a significant proportion of leaves. The presented results of our research have shown that the examined silage, in terms of color, odor, consistency, and slightly higher pH values, fell within the range of well-prepared silage. This is in line with Đukić *et al.* (2018), who stated that good silage is characterized by the following indicators: green color, a fruity and bread-like odor, and a pH range of 4.0-4.2. In Table 2, various parameters of the chemical composition of the examined silage are presented.

Table 2. Chemical Composition of Pea Silage

Protein Content (%)	Mineral Content (Ash) (%)	Crude Fat Content (%)	Crude Cellulose Content (%)
12,66	5,42	1,67	26,27

Crude proteins are one of the most important quality parameters of animal feed. The average protein content in the tested silage sample was 12.66%, which is consistent with the research of Uzun and Asik (2012), where the average crude protein values ranged from 10.72% to 15.31%. The content of crude cellulose in the pea and oat silage was 26.27%, and it was influenced by the mixture's structure and the stage of plant development. The average crude fat content was 1.67%, which is in line with the research by Mustafa *et al.* (2002), who obtained a crude fat value of 1.4-2.3% in their tested pea silage samples. The research results show a slightly lower value of crude ash (5.42%) compared to the values obtained in the research by Mustafa *et al.* (2003), whose average crude ash values ranged around 6.8%. A slightly lower mineral content can be explained by the fact that the pea and oat silage was prepared in later stages of crop utilization. The next parameter of silage quality was the microscopic analysis of the silage sample obtained through the imprint method, depicted in Figure 1.

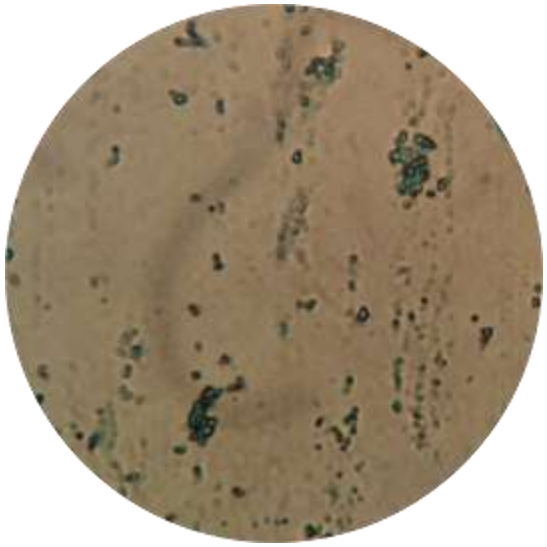

Figure no. 1. Microscopic analysis of the pea silage sample

Microscopic examination of silage samples confirmed the dominance of lactic acid rod-shaped bacteria and streptococci, which are also characteristic of high-quality silage.

Conclusion

Silage represents one of the primary and cost-effective sources of feed used in livestock nutrition. Quality is a crucial parameter of silage, and the aim of this study was to analyze and interpret the organoleptic, chemical, and microbiological properties of pea silage produced on a small-scale farm owned by the Jugović family. The research results indicated that the properties of the silage, in terms of color, odor, consistency, and pH value, fell within the range of well-prepared silage. Some examined parameters of the chemical composition of the silage (protein content, crude fiber content, crude fat content, and ash content) confirmed that the tested silage is satisfactory in terms of its chemical composition. Microscopic examination of silage samples confirmed the dominance of lactic acid rod-shaped bacteria and streptococci, which are also characteristic of high-quality silage.

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INFLUENCE OF CULTIVAR ON PHENOLOGICAL TRAITS, FRUIT CHARACTERISTICS AND YIELD OF PLUM

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Abstract

Phenological traits, fruit characteristics and yield of five plums cultivars ('Topper', 'Presenta', 'Elena', 'Hanita' and 'Katinka') were studied in the region of Sarajevo (Bosnia and Herzegovina) over one-year period (2022). The average time for the flowering of tested cultivars was in the second half of April. The time of maturation ranged from July, 19 ('Katinka') to September, 11 ('Presenta'). The average fruit weight ranged from 21.30 g, in the cultivar 'Katinka', to '36.92' g in the cultivar 'Elena'. Significant differences in fruit weight were found among plum cultivars. The average yield per tree was lowest in the cultivar 'Katinka' (6.49 kg) and highest in the cultivar 'Topper' (13.82 kg). There were no significant differences in the yield per tree between the cultivars 'Topper', 'Presenta' and 'Hanita'. The cultivar 'Topper' stood out for the highest cumulative yield efficiency (0.25 kg cm^{-2}). Soluble solids content was lowest in the cultivar 'Katinka' (14.60 %) and highest in the cultivar 'Presenta' (19.10 %). The cultivars 'Elena' and 'Hanita' are characterized by the highest content of acids, while the cultivar 'Katinka' was characterized by the lowest acid content. The pH values were in the interval 3.06–3.29. The best rated cultivar for fruit appearance and taste was 'Elena'.

Key words: *Prunus domestica L., flowering, maturation, fruit characteristics, yield.*

Introduction

The European plum (*Prunus domestica* L.) is a stone fruit species, mostly grown in the temperate zone of the northern hemisphere. Average plum production worldwide in the period 2019–2021 amounted to 12.08 million tons (FAOSTAT, 2023). The plum is the most important fruit species in Bosnia and Herzegovina (BiH) and the total area under plums in this country in 2022 was 12.71 ha (Agency for Statistics of BiH), while the realized production for the same period was 4.60 t ha^{-1}. The plum production in BiH is characterized by some negative characteristics: a large share of old orchards, out-of-date cultivars, and inadequate application of cultural practices.

One of the most important commodities, consumed worldwide, owing to its degree of acceptance by consumers is plum fruit. The ruits of European plums are suitable for fresh consumption, drying, and processing into different products (jam, juice, compote, brandy). Unfortunately, fresh consumption of plums is quite small. The highest profit in plum production is gained by growing table cultivars. According to *Ogašanović et al.* (2005) the cultivar is the most important factor in the fruit production, but the choice of rootstock is also important for successful production. The rootstocks have a major impact on the vigor, yield and fruit quality. *Bohačenko et al.* (2010), in addition to economic and physical parameters, such as yield, weight of fruit, shape, color of skin, stone weight, sensitivity of the fruit to biotic and abiotic factors, in the commercial use of the fruit, the chemical parameters also play an important role.

In recent years, a large number of plum cultivars have been created with improved characteristics, such as better adaptation to different environmental conditions, increased disease resistance, self-fertility, higher yield and better fruit quality. Breeding programs in Germany are important in the process of creating new plum cultivars. The introduction of new foreign cultivars and their study in the climatic and soil conditions of BiH allow better choice of cultivars, and may improve the production of plums.
The aim of this study was to examine the influence of cultivars on the phenological traits, fruit characteristics and yield of plum cultivars originated in Germany. On the basis of the results obtained, some cultivars will be recommended for growing in the region of Sarajevo, as well as in other regions with similar environmental conditions.

Materials and methods

The study was conducted in the plum orchard at the Experimental Station „Butmir" on the Federal Institute of Agriculture Sarajevo, Bosnia and Herzegovina (BiH). During the one-year period (2022), the phenological (flowering and ripening phenophase) traits, fruit morphological (fruit weight, dimensions and shape index, stone weight and share, stalk length), chemical (soluble solids content, total acids content and pH of fruit juice) and sensorial (appearance and taste) characteristics, vigour (trunk cross-sectional area - TCSA) and yield parameters (yield per tree and yield efficiency - YE) of five plum cultivars ('Topper', 'Presenta', 'Elena', 'Hanita' and 'Katinka') were studied. The orchard was planted in the spring of 2007. The planting distance was 4.0 m between the rows and 2.0 m within the row. The training system was the Spindle. Standard cultural practices were applied, including drip irrigation. The rootstock for each cultivar was clonal rootstock 'Fereley'. Every cultivar was represented by six trees (three replications with two trees).
Flowering was recorded by recommendation of the International Working Group for Pollination: start of flowering–10 % open flowers, full bloom–80 % open flowers, end of flowering–90 % of the petal fall (*Wertheim*, 1996). The time of ripening was taken as the date of full maturation. Fruit characteristics were measured on a sample of 30 fruits in three replications per cultivar. Fruit and stone weight were measured by technical balance Acom JW-1-300 (Acom Inc., Pocheon, South Korea) with an accuracy of ±0.01 g., while fruit dimensions and length of stalk were determined by with a sliding scale (Inox 1/20 mm, with an accuracy of ± 0.01 mm). Fruit shape index and stone share were calculated using the formulas fruit length × fruit length / fruit width × fruit thickness and stone weight) × 100/ fruit weight, respectively. The TCSA was calculated on the basis of trunk circumference measured at a height of 30 cm above ground level. Yield efficiency was calculated by dividing the yield per tree by the TCSA. Soluble solids content was determined by a refractometer (Pocket PAL-1, Atago, Japan), total acids (expressed as malic acid) by titration with 0.1 N NaOH, while the pH value was determined using the pH meter (CyberScan 510). Sensory characteristics of the fruit (appearance and taste) were evaluated by a five-member jury, scoring the cultivars using a scale from 1 to 5.
IBM SPSS Statistics 20 was used to conduct the Statistical analyses (SPSS Inc., Chicago, IL, USA). The obtained data for fruit characteristics and yield are represented by the mean value for three replicacates ± standard error and were statistically processed by Fisher's model of analysis of variance (ANOVA) using the F-test (*Fisher*, 1953) for $R \leq 0.05$ and $R \leq 0.01$. When the F-test was significant, the testing the differences of arithmetic means were determined using Duncan's multiple range test for a significance level of $P \leq 0.05$.

Results and discussion

The phenological characteristics of plum cultivars are shown in Table 1. Flowering of tested plum cultivars was occurred in the second half of April. The earliest start of flowering was recorded in the cultivar 'Topper' (April, 11) and the latest in the cultivar 'Katinka' (April, 14). The difference between cultivars with the earliest and the latest start of flowering was three days.

Table 1. The flowering phenophase of assessed plum cultivars in 2022

Cultivar	Flowering dates			Duration of flowering (days)	Ripening time	No. of days from full flowering to harvest
	Start	Full	End			
Topper	11 April	16 April	19 April	8	27 August	130
Presenta	12 April	15 April	19 April	7	11 September	145
Elena	12 April	16 April	22 April	10	31 August	131
Hanita	13 April	16 April	21 April	8	15 August	116
Katinka	14 April	18 April	24 April	10	19 July	86

Full flowering started three to five days after flowering onset. The end of flowering was in the interval of 19 April ('Topper' and 'Presenta') to 24 April ('Katinka'). The duration of flowering ranged from 7 ('Presenta') to 10 ('Elena' and 'Katinka') days. The investigated plum cultivars matured in a span of 54 days, from July 19 ('Katinka') to September 11 ('Presenta'). The number of days from full flowering to harvest ranged from 86 to 145.

The lowering of plum cultivars in the region of Sarajevo was later compared to Belgrade (*Milatović et al.,* 2018a; 2018b; *Milatović et al.,* 2019), Čačak (*Milošević et al.,* 2018) and Troyan region (*Popski*, 2016; *Boryana et al.,* 2017). Harvest dates for the cultivars 'Katinka' and 'Hanita' in Sarajevo were later than in Belgrade (*Milatović et al.,* 2018a; 2018b), while the cultivars 'Hanita' and 'Presenta' had earlier harvest dates than in Čačak (*Milošević et al.,* 2018). Similar results for harvest dates for cultivars 'Topper' and 'Elena' were reported by *Milatović et al.* (2019). Contrary to our results, the results of *Boryana et al.* (2017) have established an earlier harvest date for cultivars 'Katinka' and 'Elena'. The results for the start of flowering and harvest dates for plum cultivars were earlier compared to the statements of authors *Blažek* and *Pišteková* (2009). Differences in flowering time and fruit maturation can be explained by different environmental conditions between the study regions.

Fruit weight ranged from 21.30 g, in the cultivar 'Katinka', to 36.92 g in the cultivar 'Elena' (Table 2). All of the studied cultivars are characterized by medium large fruit (fruit weight between 20 g and 40 g). The cultivar 'Elena' had the highest statistically significant stone weight (2.04 g). On the other hand, the cultivar 'Katinka' is distinguished by its smallest stone (weight of 1.20 g). Significant differences in fruit and stone weight were found among plum cultivars. Stone share in the fruit weight ranged from 5.59 % ('Elena') to 6.63 % ('Presenta'). Significant differences were not found between cultivars fin the share of stone in the fruit weight. Stalk length was significantly the shortest in cultivar 'Katinka' (1.45 cm), and the longest in cultivar 'Topper' (1.92 cm). The analysis of the results related to fruit weight, stone weight and stalk length showed that cultivar was a significant source of variability.

Table 2. Fruit characteristics of assessed plum cultivars in 2022

Cultivar	Fruit weight (g)	Stone weight (g)	Stone share (%)	Stalk length (cm)
Topper	26.79 ± 2.60 b†	1.69 ± 0.04 c	6.51 ± 0.82	1.92 ± 0.01 a
Presenta	22.33 ±0.07 c	1.47 ± 0.03 d	6.63 ± 0.12	1.68 ± 0.04 b
Elena	36.92 ± 1.45 a	2.04 ±0.06 a	5.59 ± 0.13	1.63 ± 0.05 b
Hanita	32.73 ± 0.85 a	1.91 ± 0.03 b	5.92 ± 0.14	1.83 ± 0.04 a
Katinka	21.30 ± 0.41 c	1.20 ± 0.01 e	5.68 ± 0.14	1.45 ± 0.06 c
ANOVA	**	**	n.s.	**

†Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05

*Significant differences for P≤0.05; **Very significant differences for P≤0.01; n.s. – not significant according to F test

Significant differences were found between cultivars for fruit dimensions (Table 3). Fruit length, width and thickness were highest in the cultivar 'Elena' (45.08 mm; 36.23 mm; 36.48 mm). The cultivar 'Katinka' had the lowest fruit length (37.54 mm), while the lowest values of width (27.01 mm) and thickness (31.01 mm) of fruit were found in the cultivar 'Presenta'. Based on the fruit dimensions, the shape index was calculated, whose values ranged from 1.51 ('Katinka') to 2.28 ('Presenta').

Our results of fruit weight of plum cultivars were, with insignificant deviations, in agreement with the statements of *Blažek* and *Pišteková* (2009). Results of fruit characteristics are in accordance with previous findings for cultivars 'Katinka', 'Hanita' and 'Presenta' (*Popski*, 2016; *Milatović et al.,* 2018a; 2018b). The values obtained for fruit characteristics in this study for cultivar 'Elena', were higher than those recorded by *Milatović et al.* (2019). The results for fruit weight for cultivars 'Katinka' and 'Elena' are higher compared to the statements of authors *Boryana et al.* (2017).

Table 3. Fruit dimensions of assessed plum cultivars in 2022

Cultivar	Fruit dimensions (cm)			Shape index
	Length	Width	Thickness	
Topper	42.22 ± 1.32 b†	32.19 ± 0.94 b	32.07 ± 1.32 b	1.73 ± 0.02 b
Presenta	43.62 ± 0.25 ab	27.01 ± 0.10 d	31.01 ± 0.20 b	2.28 ± 0.03 a
Elena	45.08 ± 0.68 a	36.23 ± 0.61 a	36.48 ± 0.47 a	1.55 ± 0.05 c
Hanita	43.16 ± 0.62 ab	34.60 ± 0.24 a	35.34 ± 0.19 a	1.53 ± 0.03 c
Katinka	37.54 ± 0.36 c	30.03 ± 0.57 c	31.28 ± 0.44 b	1.51 ± 0.03 c
ANOVA	**	**	**	**

†Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05

*Significant differences for P≤0.05; **Very significant differences for P≤0.01; n.s. – not significant according to F test.

According to many authors, the key parameters that determine the quality and acceptance of the fruit by consumers are the content of soluble solids and total acids, as well as ratio between them (*Nergiz* and *Yildiz*, 1997; *Crisosto et al.,* 2004). Soluble solids content of five plum cultivars grafted on 'Fereley' rootstocks ranged from 14.6 % to 19.10 % (Table 4). The highest values for soluble solids were found in the cultivar 'Presenta', while the lowest values were found in the 'Katinka' cultivar. However, significant differences among cultivars were found. According to *Milenković et al.* (2006) early maturing plum cultivars developed at the Institute of Fruit Growing in Čačak, contain from 12.5 % to 14.8 % of soluble solids, while the medium and late maturing plum cultivars have from 16.8 % to 32.0 %. In the literature, the content of soluble solids in cultivars of European plum usually ranges from 16%–18% (*Miletić* and *Petrović,* 1996; *Oparnica* and *Jovanović,* 2000; *Nenadović-Mratinić et al.,*

2007). The content of soluble solids in a large number of cultivars in the conditions of the Belgrade region was in the interval from 12.3 % to 21.9 %, and these values were correlated with maturation time (*Nenadović-Mratinić et al.,* 2007; *Milatović et al.,* 2011, 2016, 2017, 2018a; 2018b; 2018c; 2019; *Radović et al.,* 2020). According to *Blažek* and *Pišteková* (2009) and Popski (2016) the content of soluble solids were from 11.75 % to 27.90 %.
One of the important parameter of fruit quality is the content of total acids. The highest values of content of total acids were found in the cultivars 'Elena' and 'Hanita' (1.57 %; 1.56 %), while the lowest values were obtained in the cultivar 'Katinka' (0.84 %) (Table 4). Differences in the content of total acids among cultivars were significant. The average content of total acids in the conditions of Belgrade for European plums was from 0.47 % to 1.94 % (*Milatović et al.,* 2018a; 2018b; 2019; *Radović et al.,* 2020). The acid content decreases rapidly after harvest, during storage of fruit (*Milatović*, 2019). The cultivar 'Katinka' was characterized by the highest pH of fruit juice (3.29), while the cultivar 'Elena' had the lowest (3.06).

Table 4. Chemical and sensorial characteristics of fruit of assessed plum cultivars in 2022

Cultivar	Solube solids (%)	Total acids (%)	pH	Sensory evaluation (1–5 scale)	
				Appearance	Taste
Topper	17.96 ± 0.06 b†	0.89 ± 0.01 c	3.22 ± 0.01 ab	3.83 ± 0.17 bc	3.40 ± 0.10 c
Presenta	19.10 ± 0.03 a	1.27 ± 0.01 b	3.15 ± 0.00 bc	3.50 ± 0.06 c	3.70 ± 0.06 bc
Elena	17.99 ± 0.03 b	1.57 ± 0.01 a	3.06 ± 0.01 c	4.90 ± 0.06 a	4.90 ± 0.06 a
Hanita	16.12 ± 0.00 c	1.56 ± 0.01 a	3.15 ± 0.08 bc	3.93 ± 0.17 bc	3.37 ± 0.27 c
Katinka	14.60 ± 0.00 d	0.84 ± 0.01 d	3.29 ± 0.01 a	4.50 ± 0.52 ab	4.03 ± 0.09 b
ANOVA	**	**	**	**	**

†Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05
*Significant differences for P≤0.05; **Very significant differences for P≤0.01; n.s. – not significant according to F test

The best rated cultivar for fruit appearance and taste was 'Elena'. On the other hand, the worst rated cultivar for appearance was cultivar 'Presenta', while cultivar 'Hanita' was worst scored for taste. Our results of good appearance and good taste for cultivars 'Hanita' and 'Katinka' are in accordance with those of *Milatović et al.* (2018a; 2018b).
The yield per tree in 2022 was lowest in the cultivar 'Katinka' (6.49 kg) and highest in the cultivar 'Topper' (13.82 kg) (Table 5). Significant differences in yield per tree were not found between cultivars 'Topper', 'Presenta' and 'Hanita'. Also, differences between the cultivars 'Elena' and 'Katinka' were not significant in yield per tree. Among the studied cultivars, the lowest vigor was found in the cultivar 'Topper' (57.93 cm^2), while the highest vigor was in the cultivar 'Presenta' (94.81 cm^2). Yield efficiency ranged from 0.07 kg cm^{-2} to 0.25 kg cm^{-2}. The cultivar 'Topper' had the highest yield efficiency.

Table 5. Yield parameters and vigor of assessed plum cultivars in 2022

Cultivar	Yield (kg tree^{-1})	TCSA (cm^2)	YE (kg cm^{-2})
Topper	13.82 ± 0.27 a†	57.93 ± 7.36 b	0.25 ± 0.03 a
Presenta	11.72 ± 0.38 a	94.81 ± 2.71 a	0.18 ± 0.01 b
Elena	7.74 ± 0.73 b	91.57 ± 8.57 a	0.11 ± 0.02 cd
Hanita	11.50 ± 1.35 a	65.82 ± 10.51 b	0.13 ± 0.02 c
Katinka	6.49 ± 0.56 b	74.85 ± 5.62 ab	0.07 ± 0.01 d
ANOVA	**	**	**

†Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05

*Significant differences for P≤0.05; **Very significant differences for P≤0.01; n.s. – not significant according to F test

The vigor of plum cultivars in the region of Sarajevo was higher and yield was lower compared to the Czech Republic (*Blažek* and *Pišteková*, 2009) and Čačak (*Milošević et al.,* 2018) region. Our results of yield per tree, vigor and yield efficiency of cultivars 'Hanita' and 'Katinka' were significantly lower compared to previous findings of *Milatović et al.* (2018a; 2018b). The values obtained for yield per tree, vigor and yield efficiency for cultivar 'Elena' were lower than those recorded by *Milatović et al.* (2019). Our results for yield per tree for cultivar 'Katinka' aren't in agreement with previous findings of *Popski* (2016) who reported that this cultivar had an average yield per three of 0.80 kg. Contrary to our results, the results of *Boryana et al.* (2017) have established higher yield per tree for cultivars 'Katinka' and 'Elena'. Yield per tree, vigor and yield efficiency of plum cultivars are the result of the influence of different factors such as environmental conditions, rootstocks, age of trees and cultural practices.

Conclusions

The average time of flowering for the tested cultivars was in the second half of April. The range of fruit maturity was from July 19 to September 11. The cultivars 'Elena' and 'Hanita' had the highest fruit weight. All of the studied cultivars are characterized by medium large fruit. Significantly higher yield was found in cultivars 'Topper', 'Presenta' and 'Hanita'. The cultivar 'Presenta' was characterized by the highest soluble solids content, while the cultivar 'Katinka' had the lowest acid content. Besides, cultivar 'Elena' had the best scores for appearance and taste. On the basis of the obtained results, for cultivation in the region of Sarajevo, it is possible to recommend all cultivars.

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THE EFFECT OF SET SIZE AND PLANTING TIME ON THE ONION BULBS PRODUCTION

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Abstract

Onions are cultivated for use in a green state and mature bulbs. Producers can use knowledge of the relationships between the transplanting date and onion set size as a tool to adjust production to obtain the best bulbs. It is considered that transplanting dates of onion sets reflecting the effect of edaphic factors and all environmental conditions on a large scale on growth, bulb yield, and bulb quality differ widely from region to region, and that the size of a set is closely related to subsequent bulb yield, and large sets have been observed to produce greater yields. The experiment was conducted during three planting periods in March and April, 2022. Planted onion sets were divided into two groups: onion sets with a diameter less than 1.5 cm and onion sets with a diameter greater than 1.5 cm. Harvesting was performed in July 2022 for all planting dates, after which morphological measurements and vital C content were measured. This study established the main onion bulb production characteristics, vitamin C, and mass of the bulbs depending on the transplantation date and onion set size, which were essential structural parameters for providing authentic information about onion production in the open field. The results showed that early planting and wider onion sets were essential for obtaining higher yields in open field production. The vitamin C content was the highest on the third planting date.

Keywords: *planting period, set size, bulb, onion, vitamin C.*

Introduction

Onion (*Allium cepa* L.) belongs to the Alliaceae family and the genus Allium, and it is an important crop for bulb plants grown globally. Onions are cultivated for use in a green state and mature bulbs. Usually, the onion is a plant of open, sunny, dry areas, but most species are initiated in cliffs on waterless foothill slopes, in stony or rocky open areas, or in dry, open (Hanelt, 1990). The right level of farming practices, such as the distance between plants, plant density, date of planting, and time of harvest, can produce the desired outcomes for farmers (Alemu *et al*., 2022). Optimum transplanting dates play a vital role in maximizing growth, bulb yield, and the quality of onions (Ali *et al*., 2016). Early planting has the longest growth cycle, and thus, the highest yield (Bhattarai *et al*.,1995). On the other hand, late planting at high temperatures results in early maturity and thus a lower yield. The size of a set is also closely related to subsequent bulb yield, and it has been observed that large sets produce greater yields (Thompson, 1934). Large sets accelerate the onset of bulbing and, consequently, the onset of maturity (Heath and Holdsworth, 1948). Growers can use the knowledge of the relationships between the transplanting date and onion set size as a tool to adjust storage temperatures postharvest so that it is possible to manipulate both seed/bulb crop production of onions in general (Khokhar, 2009). The effects of planting date and onion set size on onion production and their significant effects on both productivity and quality have been studied and reported by several scholars in different parts of the world (Teshome *et al*., 2021). Vitamin C is considered to be an indicator of food processing quality because of its

low stability during thermal treatment (Podsędek, 2007). The interaction effect of variety and temperature on the vitamin C content was significant. As Seifu *et al*. (2018) pointed out, although the magnitude of vitamin C reduction was not consistent across cultivars, it decreased as temperature increased. The transplanting date was closely related to the temperature of the production process. Idah and Obajemihi (2014) also reported a negative influence of temperature on the vitamin C content in onion powder. Thermal degradation of vitamin C is one of the mechanisms of non-oxidative degradation (Munyaka *et al*., 2010). However, vitamin C accumulation in different plant organs is dependent on multiple metabolic processes such as biosynthesis, recycling, degradation, and transport. Moreover, vitamin C content is influenced by endogenous stimuli and environmental factors, of which light is of primary importance (Bhullar and Gruissem, 2013).
This study established the main onion bulb production characteristics, vitamin C, and mass of the bulbs depending on the transplantation date and onion set size, which are essential structural parameters for providing authentic information about onion production in the open field.

Material and methods

The experiment was set up in village Mašići, municipality of Gradiška in the Entity of Republic of Srpska, Bosnia and Herzegovina. In early spring 2022, a soil analysis was first performed, which determined that it was a slightly acidic to acidic reaction soil, after which the basic treatment was carried out, as well as additional soil treatment. Fertilization was performed according to recommendations based on soil analysis performed at the Faculty of Agriculture in Banja Luka. An untreated variety was used: Majski srebrnjak (NS SEME, Serbia). The bulb is white, juicy, and flat. In terms of food, it is used as an onion in early spring or as a ripe bulb in May. Recommended production from onion sets: sowing from March 1st to April 5th. The experiment was conducted during three planting periods. 1st planting period was 3/15/2022, the 2nd was 3/25/2022 and the 3rd was 4/4/2022. Planting was performed in rows at a distance of 20 cm between rows and 10 cm between plants (50 plants per square metre). The experiment was performed in four repetitions, with 20 plants per repetition. During the growing season, usual agrotechnical measures were used, such as irrigation, hoeing, and fertilization. Top dressing was performed twice during the growing season. The first top dressing was done at the stage when three true leaves were formed for each planting period separately, and the second top dressing was done at the young bulb formation stage and for each planting period separately. Harvesting was performed on July 6, 2022, for all planting dates, after which the bulb height (cm), bulb diameter (cm), and bulb shape index (bulb height/bulb diameter) were measured (UPOV, 1999). Bulb mass (g) and yield (kg/m^2) were measured using a standard scale and formula. Vitamin C content was determined colorimetrically. A standard curve was prepared using five different concentrations of the ascorbic acid standard. From the equation for the direction of sample absorption, the concentration of vitamin C was obtained and expressed as mg/100 g frozen weight (FW) of the sample. Measurements were performed in triplicate (Bhandari and Kwak, 2015). The obtained data were statistically analyzed, and differences were calculated using analysis of variance with the computer program VVSTAT (Vukadinović, 2017).

Results and Discussion

The shape of the bulb can be a globe, a flattened globe, sometimes with a flat top, spindle-like or almost cylindrical, or narrower or wider (Brewster, 2008). Bulb shape index is a significant biological and commercial trait. Bulb shape impacts the price on the market, the proceeding process, disease resistance, etc. The bulb shape index represents the ratio between the height and bulb diameter. Bulb shape is an important indicator of onion growth and breeding (Pavlović *et al*., 2007).

Table 1. Variance analysis of bulb height and diameter (cm) with average bulb shape index.

	1st planting date (A1)		2nd planting date (A2)		3rd planting date (A3)	
Bulb height	Narrower onion sets (B1)	Wider onion sets (B2)	Narrower onion sets (B1)	Wider onion sets (B2)	Narrower onion sets (B1)	Wider onion sets (B2)
	4.71	6.63	5.72	6.85	5.93	5.67
	5.43	6.12	5.59	6.58	5.02	5.77
	5.32	5.56	5.39	5.96	5.29	5.52
	5.14	5.70	5.31	5.60	5.32	5.60
	Blocks	Effect A	Effect B	Interaction A*B		Error
F_{exp}	0.978^{ns}	0.954^{ns}	2.694^{ns}	2.733^{ns}		3.16
$LSD_{0.05}$ $LSD_{0.01}$		0.4011 0.6076	0.4848 0.6964	0.8396 1.2064		
Bulb diameter	4.97	6.56	4.68	6.29	4.63	5.55
	4.54	5.34	4.46	5.08	4.28	4.61
	4.13	5.24	4.13	4.57	4.16	4.52
	4.12	4.82	4.19	4.75	4.13	5.00
	Blocks	Effect A	Effect B	Interaction A*B		Error
F_{exp}	15.690**	3.155^{ns}	51.020**	1.158^{ns}		1.20
$LSD_{0.05}$ $LSD_{0.01}$		0.3051 0.4623	0.2804 0.4026	0.4536 0.6978		
Bulb shape index	1.16	1.09	1.26	1.21	1.25	1.15

ns= Not significant at 1% level of probability; *= Significant at 5% level of probability; **= Significant at 1% level of probability

Ud-Deen (2008) conducted research on effect of mother bulb size and planting time on growth, bulb and seed yield of onion and reported that large mother bulb with early planting gave the highest bulb length (3.95 cm), whereas the lowest bulb length (2.82 cm) was found in small mother bulb planted in 2nd planting date. This research had the highest (in comparison to Ud-Deen the longest) average bulbs in the 2nd planting date and narrower onion sets which were 5.50 cm. As shown in Table 1, even the shortest average bulbs were 2.58 cm which were planted 1st planting date and had wider onion sets; this showed that the spring planting date gave the highest bulbs. Kahsay *et al*. (2013) reported that in their research bulb diameters of onion plants were strongly affected by the main influence of diversity as well as spacing between plants ($p < 0.001$), and the interaction of these two factors did not significantly affect bulb diameter. The maximum bulb diameter was 5.56 cm, whereas in this study, the maximum diameter was 4.44 cm in 1st planting date and narrower onion sets. There was no statistically significant interaction effect of the onion set size and planting date on bulb height

and diameter. In addition, there was a statistically significant influence of planting date on bulb diameter. From Table 1, it is also shown that the average shape in this research was 1.06 to 1.26, which indicates the circular shape of the bulbs (UPOV, 1999).

Table 2. Variance analysis of bulb mass (g) and yield of onion bulbs (kg/m^2).

Repetition average	1st planting date (A1)		2nd planting date (A2)		3rd planting date (A3)	
	Narrower onion sets (B1)	Wider onion sets (B2)	Narrower onion sets (B1)	Wider onion sets (B2)	Narrower onion sets (B1)	Wider onion sets (B2)
Mass of bulbs (g)	50.58	93.92	51.42	82.45	48.75	62.67
Yield of onion bulbs (kg/m^2)	2.53	4.70	2.57	4.14	2.44	3.13

	Blocks	Effect A	Effect B	Interaction A*B	Error
F_{exp}	16.967**	4.670*	42.645**	3.567 ns	1840.05
$LSD_{0.05}$		6.4976	0.2804	12.5823	
$LSD_{0.01}$		9.8434	0.4026	18.0781	

ns =Not significant at 1% level of probability; * Significant at 5% level of probability; **= Significant at 1% level of probability

The average bulb mass was 65 g. The highest weight had the bulbs on the 1st planting date, with a wider onion set (93.92 g). In general, wider onion sets had a higher average bulb weight than narrower onion sets on all three planting dates. There was no statistically significant interaction between the factors, but a statistically significant correlation was observed between the size of the onion sets ($p < 0.01$) (Table 2 and Figure 1). The average yield per square meter was calculated based on the vegetation area of the onion plants and average mass of the bulbs (Table 2). A similar study by Ud-Deen (2008) showed that the interaction between mother bulb size and planting time had a significant effect on the growth, yield components, and yield of bulbs and seeds. The maximum bulb weight per plant (50.25 g) and bulb yield (1.75 kg/m^2) were observed in large mother bulbs on the 2nd planting date, whereas the minimum bulb weight per plant (38.98 g) and bulb yield (0.53 kg/m^2) were found in small mother bulbs on the 2nd planting date, which is almost half of the bulb weight in our study.

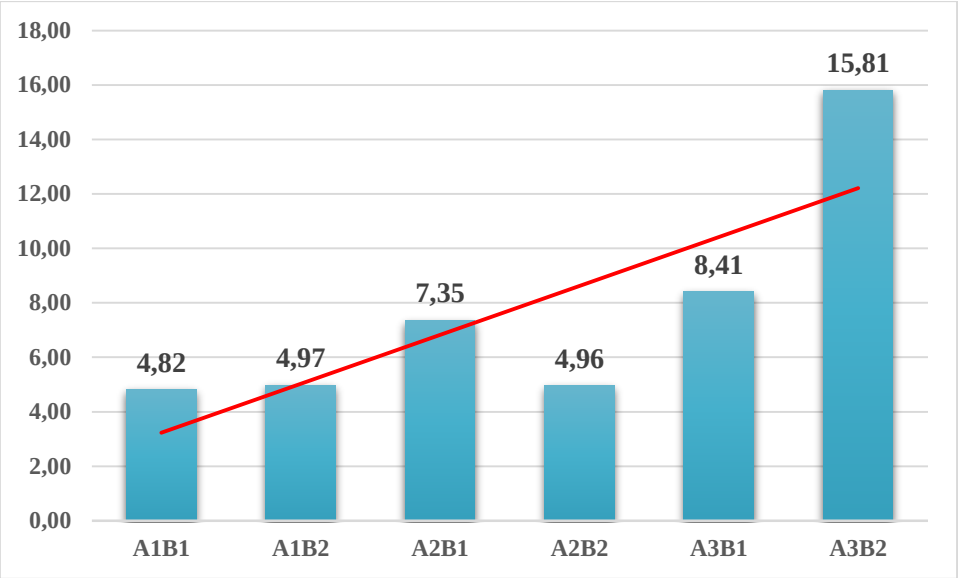

Figure 1. Average Vitamin C content (mg/100g FW)

The results of the vitamin C content in bulbs showed that the highest concentration of vitamin C was in the 3rd planting date, with the maximum value of vitamin C recorded in the 3rd planting date with wider onion sets (15.81 mg/100 g FW). In addition, no statistically significant influence of this factor on onion was observed. Sami *et al* (2021) conducted research on onion bulbs of five different varieties, namely yellow, red, green and baby onion. The results showed that the highest value of vitamin C was detected in the red variety (45.07 mg/100 g FW), then the variety Baby (38.12 mg/100 g FW), while the variety Green showed the lowest (10.10 mg/100 g FW). However, these values were higher than those in our study.

Conclusion

The overall results obtained from this study revealed that early planting and wider onion sets were essential for obtaining higher yields in open field production. There was no statistically significant interaction effect of the onion set size and planting date on bulb height and diameter. The vitamin C content was the highest on the third planting date.

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THE INFLUENCE OF GENOTYPE AND AGRO-ECOLOGICAL CONDITIONS ON THE CONTENT OF STARCH IN TUBERS

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Abstract

As an annual cultivated species, potatoes are one of the most important agricultural crops and occupy one of the first places in world agriculture, along with wheat, corn, and rice. Potato tubers are one of the rare foods that are completely nutritious. The largest part of the dry matter of the tuber is starch, approximately 75%. Research on the starch content as one of the most important parameters of tuber quality was carried out in the course of two years (2016 and 2017) in three locations (East Sarajevo, Bijeljina, Rogatica) with five varieties of potato (Agrija, Faluka, Kenebek, Kuroda, Dezire). The obtained results of the three-factor experiment were processed by analysis of variance. The starch content was significantly influenced by the variety and agroecological conditions of the researched localities.

Keywords: *potato, variety, locality, starch*

Introduction

Potato is a crop that belongs to the Solanaceae family, the genus Solanum and is native to South America. Among all the species belonging to this genus, whose tubers are used in human nutrition, the most important for agriculture is Solanum tuberosum ssp. tuberosum (Kesaulya et al., 2015), a tetraploid with 48 chromosomes, grown under long day conditions. It is considered that in Europe Solanum tuberosum ssp. tuberosum originated from another species of Solanum antigens (Jovović et al., 2013). Marković et al. (2006) state that potatoes are one of the main cultivated crops, both because of the areas in which they are grown and their importance in human nutrition. The nutritional value of potatoes depends on the content of useful nutrients (starch, proteins, vitamin C), but harmful substances (nitrates, nitrites, glycoalkaloids).

The most important parameters of tuber quality are sugar content, starch, and antioxidant activity. Starch content in tubers is an important criterion of potato quality during the industrial processing of potatoes into alcohol. Starch content in tubers depends on climatic conditions, growing technology, variety, and tuber size (Gvozden, 2016). Knowing the genetic diversity of the potato population is a useful tool for the selection of parents during hybridization and for obtaining varieties with high yields and desired characteristics (Arslanoglu et al., 2011). Varieties that have oval tubers, a high dry matter content, and starch are suitable for industrial processing into french fries and chips (Ismail et al., 2015). The starch content in tubers is also influenced by the availability of nutrients, especially phosphorus and potassium in the soil (Khan et al., 2010). Phosphorus, as one of the most important nutrients, affects the conversion of sugar into starch.

Material and methods

The field experiment was set up according to the random block system scheme in four replications with a plot area of 15 m2 (four rows of 5 m length with 20 plants in a row; distance between rows 0.75 m and between plants in a row 0.25 m). Field experiments were set up in 2016 and 2017 at three different locations: the experimental field of the Faculty of Agriculture in East Sarajevo (fluvisol soil type, 550 m above sea level, 43°49′01″ N and 18°20′57″ E), village Kojčinovac in Bijeljina (soil type seemingly, 90m above sea level, 44°468′02″ N and 19°14′01″E) and the property of Solanum produkt in Rogatica (soil type district cambisol, 1100m above sea level, 43°51′16 ″ N and 19°05′57″ E). In addition to different agroecological conditions, the chemical properties of the soil also varied significantly in the selected localities (Table 1).

Table 1. Agrochemical properties of soil

Area	Depth (cm)	pH/H_2O	pH/KCl	humus %	N %	Soluble mg 100 g^{-1} P_2O_5	K_2O
East Sarajevo	0–30	7,16	6,39	4,12	0,27	41	36,41
Bijeljina	0–30	7,67	6,61	4,66	0,3	> 40	36,91
Rogarica	0–30	5,02	3,85	2,55	0,16	8,98	25,38

The research included five different potato genotypes: Agrija, Faluka, Kennebec, Kuroda, and Desire. Soil preparation (primary and additional tillage) was carried out during autumn, and with the pre-sowing preparation mineral NPK fertilizers were applied in spring (10:20:30). Potatoes were planted in both years in East Sarajevo in mid-April, in Bijeljina in early April and in Borike in the beginning of May, and KAN was used as the supplement.

After removing the potatoes and taking samples, the tuber (%) starch content was determined by polarimetry according to Ewers. The results obtained were processed by analysis of the variance of the three-factor trial using the Gen Stat 12th statistical program (Gen Stat, 2009). Air temperature and precipitation are meteorological elements that have the greatest influence on the volume of plant production, the height, and the quality of the yield of cultivated plants.

In East Sarajevo, the mean monthly temperatures in 2016 (16.9°C) and 2017 (17.45°C) during the growing season were higher than average (16.2°C). In the tuber-filling phase (July and August), the mean monthly temperatures in both years ranged from 19.5°C to 22.8°C. In 2016, there was more precipitation (520.1mm) compared to 2017 (459mm) and the multi-year average (473mm). In Bijeljina, the mean monthly temperatures in 2016 (19.25°C) and in 2017 (19.83°C) during the growing season were higher than the multi-year average (18.18°C). There was more precipitation during the potato growing season in 2016 (472 mm) compared to 2017 (385.2 mm) and the multiyear average (425.4 mm). In Rogatica during the potato growing season (May-September), the mean monthly temperatures in 2016 were (14.76°C), and in 2017 (16.1°C) they were higher than the multi-year average (14.46° C). More precipitation was recorded in 2016 (522.6 mm) compared to 2017 (394.8 mm) and the multiyear average (341.9 mm) (Table 2).

Table 2. Precipitation sum and average monthly temperature

season		month	IV	V	VI	VII	VIII	IX	average total
E.Sarajev	2016	°C	12,9	13,9	19,5	21,1	18,6	15,6	16,9
		mm	60,5	82,1	96,4	104,5	105,5	71,1	520,1
	2017	°C	9,2	15,3	20,3	21,8	22,6	15,5	17,5
		mm	132,4	73,8	55	66,5	38,7	93,2	459,6
Bijeljina	2016	°C	13,8	16,6	22,0	23,5	21,2	18,4	19,3
		mm	61,8	86,4	120,7	84,8	66,0	52,3	472
	2017	°C	11,6	17,9	23,2	24,6	24,7	17,0	19,8
		mm	92,8	67,5	39,9	47,3	35,4	102,3	385,2
Borike	2016	°C	9,9	11,1	16,7	18,0	15,7	12,3	14
		mm	72,7	70,4	158,9	130,3	95,7	67,3	595,3
	2017	°C	6,0	12,7	17,5	18,6	19,2	12,5	14,4
		mm	165,1	51,7	90,0	97,8	16,6	85,8	507

Research results and discussions

Depending on the variety and growing conditions, fresh tubers contain 13.1-36.8% of dry matter (on average 22.2%), while the rest is water with about 63.2-86.9% (on average 77.8%). The largest part of the dry matter is starch, about 75%. The starch content in the total chemical composition of tubers, depending on the variety, ranges from 8.0-29.4% (on average 14.1%), protein content 0.44.7% (on average 2.4%), ash content 0.44-1.90% (Gvozden, 2016). Tubers with a higher starch content have a higher nutritional value, are easier to store, and are more suitable for further processing. The starch content in the tuber is a varietal characteristic and is influenced by agrotechnical measures and ecological conditions during cultivation, especially the amount and distribution of precipitation (Gvozden, 2016).

Table 3. The starch content in tubers in 2016 and 2017 in three locations (East Sarajevo, Bijeljina, and Rogatica)

	Year	2016.	2017.	Average
Location	E. Sarajevo	17,59±0,26	15,55±0,27	16,6±0,27
	Bijeljina	17,51±0,34	15,09±0,23	16,3±0,31
	Rogatica	17,48±0,31	15,58±0,32	16,5±0,31
	Year	2016.	2017.	Average
Sort	Agrija	18,50±0,25	17,06±0,40	17,8±0,31
	Faluka	18,36±0,31	14,86±0,28	16,6±0,29
	Kenebek	17,62±0,22	15,59±0,40	16,6±0,35
	Kuroda	16,57±0,54	14,77±0,37	15,7±0,47
	Dezire	16,58±0,42	14,74±0,46	15,7±0,44
	Average	17,53±0,17	15,41±0,17	16,5±0,17

The starch content in the tuber in 2016 was highly influenced by the variety, while in 2017 the variety showed a significant influence.

The Agrija variety had the highest starch content in 2016, and the Kuroda variety had the lowest, while the starch content in 2017 ranged from 14.74% (Dezire) to 17.06% (Agrija). The starch content in the tuber is influenced by the variety and cultivation technology (Tein et al., 2014; Broćić et al., 2016), which is in agreement with the given research.

At the location in East Sarajevo in 2016, the tubers had the highest starch content, while the lowest starch content was recorded in the tubers produced in the Rogatica subdistrict. In the second year examined, the highest starch content was in Rogatica and the lowest in Bijeljina (Table 3). Between the Bijeljina and Rogatica localities, the greatest variation was found in the Kuroda variety and the smallest in the Agrija variety between the localities of East Sarajevo and Bijeljina, and the Kennebek variety grown in the localities of East Sarajevo and Rogatica.

The content of dry matter in the tuber is influenced by environmental factors during the growing season, namely solar radiation, soil temperature, moisture content in the soil, and application of fertilizers. Low temperatures and a short vegetation period reduce the content of dry matter in the tuber, while high temperatures, solar radiation, and a longer vegetation period affect the increase in dry matter in the tuber (Haverkort, 2007). Under the influence of high temperatures, lack of moisture, and poor aeration of the soil, stress can occur in the growing season, during which secondary growth of tubers occurs (Poštić et al., 2012), which negatively affects the value and quality of the tubers.

Conclusions

The tested varieties (Agrija, Faluka, Kennebec, Kuroda, Desire) had a satisfactory starch content. The starch content in the tubers was significantly influenced by the genotype, as well as the agroecological conditions of the researched localities. The agroecological conditions in 2016 at the three localities were suitable for higher starch content in the tubers. The Agrija variety had the highest starch content in both examined years, while the Kuroda variety had the lowest starch content in 2016 and the Dezire variety in 2017.

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COMPARATIVE ANALYSIS OF GRAPE AND WINE QUALITY BETWEEN VRANAC AND CABERNET SAUVIGNON VARIETIES (*VITIS VINIFERA* L.)

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Abstracts

This study explores the quality parameters of grapes for two varieties, Cabernet Sauvignon and Vranac, in the vineyards of Trebinje. The results indicate that both varieties achieved high quality. Cabernet Sauvignon had the lowest sugar content (22%) and the highest acidity (9.15 g/L), while Vranac had the highest sugar content (23%) and the lowest acidity (4.25 g/L). The quality of the produced wine was also analyzed. Cabernet Sauvignon from 2017 had the highest total polyphenol content, while Vranac from 2016 had the lowest content. Additionally, lower ash content was observed in Vranac wine produced in 2017. Both varieties had higher alcohol and total extract contents in the wine produced from the 2017 grape harvest. Vranac wine received the highest sensory ratings (80 in 2017), while Cabernet Sauvignon wine received the lowest ratings (68 in 2016). Both varieties had better sensory ratings during the year 2017. Based on the grape and wine quality, Vranac and Cabernet Sauvignon varieties exhibited positive characteristics and are recommended for further expansion in the vineyards of Trebinje. These findings provide valuable insights into the potential and quality of viticultural production in the region. Future vineyard expansions should focus on these two varieties to ensure high-quality grapes and wines.

Keywords: *grape, wine, quality, Trebinje.*

Introduction

The most important parameters for assessing the quality of grapes are sugar content and acidity. The sweetness of grape juice depends on the ratio of acidity to sugar. An ideal ratio is high sugar content accompanied by high acidity. Excessively low acidity can have a negative impact on the taste and quality of grapes (Liu *et al*., 2007). The sugar content, acidity, and their ratio change during berry development and grape ripening and depend on various factors such as grape variety, vineyard location, weather conditions, and the process of alcoholic fermentation (Jeromel *et al*., 2007). The study and understanding of sugar and acidity content in grapes have significant practical and scientific value. This data can be used to evaluate the technological value of grape varieties and to select the most suitable varieties for a particular vineyard region. Researchers have identified a significant correlation between sugar and acidity, as well as between sugar content and fertility potential of grapes.

In the past, before the development of chemical analysis methods for wine, the assessment of wine quality was based on its appearance, color, taste, and aroma. These characteristics still play a significant role in determining the characteristics and quality of wine. The descriptive method of sensory analysis has enabled a rational qualitative determination of selected quality parameters by using appropriate attributes (Navarro *et al*., 2002). The sensory properties of wine depend on numerous factors, with the most important being varietal characteristics of

grape quality and ripeness, i.e., sugar content and aromatic compounds. Great importance is given to the geographic origin of wine, particularly in countries with a long tradition of production, highlighting the influence of regional elements on the composition and quality of wine (Rektor *et al.*, 2004). The assessment of red wine quality is primarily based on wine tasting. However, chemical analyses are conducted to explain certain observed sensory changes.

The aroma of wine is one of the main factors determining its nature and quality (Selli *et al*., 2004), particularly its organoleptic characteristics, and therefore plays a significant role in consumer preferences. Some aroma compounds are emitted directly from grape berries, while others are formed during the fermentation and aging processes. The taste of wine represents an extremely complex chemical pattern, both qualitatively and quantitatively, as over 1000 volatile compounds have been identified in wines, including alcohols, esters, fatty acids, aldehydes, ketones, and terpenes, with a wide range of concentration variations (Gomez-Miguez *et al*., 2007).

Materials and metods

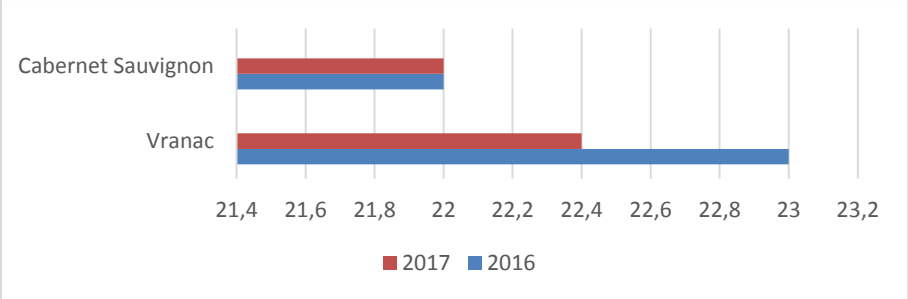
The grapes of the Vranac and Cabernet Sauvignon varieties were harvested at a state of technological ripeness in 2016 and 2017. The following parameters of the chemical composition of the must were determined: pH value, sugar content, and total acidity. The pH value was measured using a pH meter, which involves measuring the potential difference between two electrodes immersed in the tested liquid. The result is expressed with an accuracy of 0.05 pH units. The sugar content in the must was determined using a refractometer and expressed in percentage. The total acidity in the must was determined using a method based on a color change indicator. The method involves titration with a solution of NaOH in the presence of the indicator phenolphthalein. The acid content is expressed in g/L of tartaric acid. The wines are made by procedure of microvinification in two production years, 2016 and 2017. The wines were not clarified or filtered. The physicochemical analysis of the wine was performed according to the methods prescribed by the International Organization of Vine and Wine (OIV, http://www.oiv.int/) at the Faculty of Agriculture, University of Belgrade. The analysis included the following parameters: alcohol content (vol.%), total extract (g/L), titritable acidity (g/L), total sulfur dioxide (mg/L), free sulfur dioxide (mg/L), ash (g/L), and total polyphenols (g/L). For sensory evaluation of the wine, the positive scoring method from 0 to 100 was used (Regulation on the manner and procedure of production and quality of table wines and wines with geographical origin "Official Gazette of the Republic of Serbia," No. 41/09). The following attributes were evaluated for the obtained wines: color, clarity, aroma, and taste.

Results and discussion

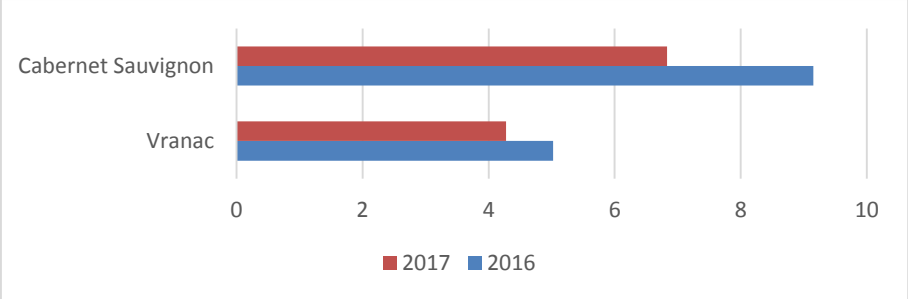
The sugar and acidity content are among the most important quality parameters of grapes, and their ratio directly affects the quality of wine. Various factors influence the sugar content in grape must, with the variety, climatic and soil characteristics of the site, cultivation methods, and applied viticultural practices being prominent. The results of our study on the chemical composition of the wine are presented on graphs 1, 2, and 3. In terms of sugar content in the grape, the highest recorded values during 2016 and 2017 were observed for the Vranac variety (23% and 22.4%). The lowest sugar content value (22%) in 2016 and 2017 was found in the Cabernet Sauvignon variety. The total acidity content in the grape for the studied varieties is represented on graph 2. The highest acidity content in the wine during 2016 and 2017 was observed in the Cabernet Sauvignon variety (9.15 and 6.83 g/l). The Vranac variety had the lowest value in 2016 and 2017 (5.025 and 4.25 g/l). The average pH values of the grape for

the studied varieties were fairly consistent during both years of the research and ranged from 3.13 to 3.4% (graph 3).

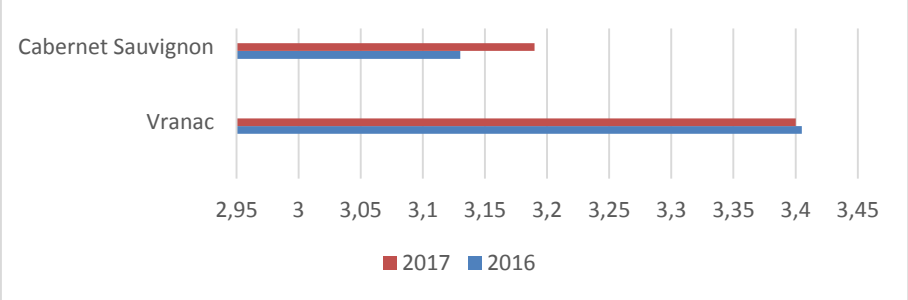
The sugar and acidity content in the grape must of the Vranac variety was lower than the findings of Popović *et al.* (2013), who reported sugar content ranging from 23.5% to 25% and acidity content from 5.3 g/l to 7.5 g/l. On the other hand, our results are in agreement with Mijatović *et al*. (2009), who found sugar content in the grape must of the Cabernet Sauvignon variety ranging from 20.98% to 23.36% and acidity content from 7.39 g/l to 9.74 g/l. Analyzing the grape must of the Cabernet Sauvignon variety grown in the agroecological conditions of Trebinje, Banjanin and Kulina (2015) reported sugar content of 22.20% and acidity of 5.9 g/l, which is consistent with our results.



Graph 1 - Sugar content in the grape must (%) for the studied varieties



Graph 2 - Total acidity content in the grape must (g/l) for the studied varieties



Graph 3 - pH values in the grape must for the studied varities

Sulfur dioxide is present in both the grape must and wine in free and bound forms. Free sulfur dioxide is mostly found as sulfurous acid and acts as a direct antioxidant and antiseptic. Total or titratable acidity in grape must varies depending on the grape variety and agroecological conditions in which the plant developed. One of the most important components in determining wine quality is alcohol. Based on alcohol content, wines are classified as light (7-9% vol.), medium (10-12% vol.), and strong (12-14% vol.). Wines with higher ethanol concentrations are more suitable for aging and have greater biological stability (Jović, 2006). The amount of extract in wines depends on the grape variety, grape maturity, cultivation conditions, and technological processes during wine production. The mineral content or ash of wine is determined by burning the wine extract and depends on the soil and climatic

conditions during the vegetation period (Blesić, 2006). The results of the physicochemical analysis of wine obtained through microvinification in the experimental years 2016 and 2017 are provided in Table 1. The wine variety Cabernet Sauvignon had the highest free SO_2 content during both experimental years (23.04 and 20.48 mg/l), while the Vranac variety had the highest total SO_2 content (25.60 and 51.20 mg/l) in both years. The Cabernet Sauvignon wine from the 2016 harvest had the highest ash content (3.07 g/l) and total polyphenol content (1.94 g/l), while in the 2017 harvest, it had the highest volatile acidity (4.12 g/l). The Vranac wine had the highest alcohol content (13.70%) in 2017, while the Vranac wine from the 2016 harvest had the lowest alcohol content (12.78%). The Cabernet Sauvignon wine had the highest total extract content (28.70 g/l) in 2017, while the Vranac wine from the 2016 harvest had the lowest extract content (26.30 g/l). The wines produced from the grapes of both tested varieties, from the 2016 and 2017 harvests, had approximately similar total alcohol and total polyphenol contents.

The alcohol content in the tested wines ranged from 11.96% (Cabernet Sauvignon) to 13.7% (Vranac), which is consistent with the findings of Pajović *et al*. (2009) and Raičević *et al.* (2014). Pržić (2015), in a study on Cabernet Sauvignon wines grown under different defoliation treatments, reported alcohol content ranging from 11.91% to 13.33% (v/v), total acidity from 6 g/l to 7.4 g/l, total SO_2 from 20.48 mg/l to 99.84 mg/l, free SO_2 from 6.4 mg/l to 17.92 mg/l, and total polyphenols from 1.05 g/l to 1.58 g/l. In their study, Raičević *et al.* (2014) reported high total acidity content (ranging from 5.5 g/l to 5.98 g/l) in Vranac wines produced in the Podgorica wine region. The results of our study show that the total acidity values for the Vranac variety were relatively consistent during both years of the research (ranging from 3.58 g/l to 3.99 g/l), while the acidity range for the Cabernet Sauvignon variety was much wider (ranging from 2.79 g/l to 4.12 g/l). The 2017 vintage of Cabernet Sauvignon wine had the highest polyphenol content (1.94 g/l), while the 2016 vintage of Vranac wine had the lowest content (1.17 g/l). Wines produced from Vranac and Cabernet Sauvignon grapes from the 2017 harvest had higher polyphenol content. Additionally, a lower ash content was observed in the 2017 vintage of Vranac wine. Both tested varieties had higher alcohol and total extract content in wines produced from the 2017 harvest. When comparing the chemical composition parameters of the wines over the years of the study and comparing them with literature data, some deviations can be noted, which are influenced by the ecological factors of the locality on the variety, grape maturity, duration of maceration, and other winemaking processes.

Table 1 - Physicochemical analysis of wine

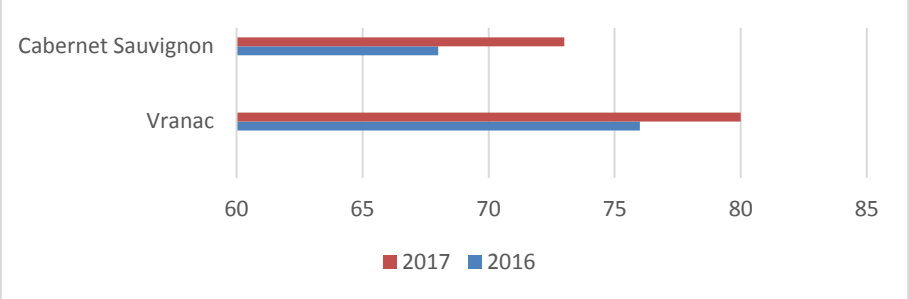
Parameter	Vranac		Cabernet Sauvignon	
	2016	2017	2016	2017
Free SO_2 (mg/l)	17.92	17.92	23.04	20.48
Total SO_2 (mg/l)	48.64	35.84	33.28	38.40
Titratable acids (g/l)	3.96	3.76	4.12	2.79
Alcohol content (%v/v)	12.78	13.70	11.96	13.30
Total extract content (g/l)	26.30	27.20	27.90	28.70
Ash (g/l)	2.28	2.17	2.91	3.07
Total polyphenols (g/l)	1.17	1.41	1.90	1.94

The sensory properties of wine are influenced by various factors, with the most important ones being the grape variety and the degree of ripeness at the time of harvest, which affects the concentration of sugars and aromatic compounds. The same compound can exhibit different aromas at different concentrations, and the perception of aroma can also be influenced by the subjectivity of the evaluator (Auvray and Spence, 2008). Table 2 presents the results of the sensory analysis of wine produced from Vranac and Cabernet Sauvignon grapes. The Vranac variety had wine of a closed red color, clear, with a fruity aroma and pronounced minerality, and a moderately full to full-bodied taste in both years of testing. In both tested years, the Cabernet Sauvignon wine was of a closed red color, clear, with a varietal aroma and pronounced minerality. The taste in 2016 was thinner, while in 2017, it was moderately full-bodied.

Table 2 - Sensory characteristics of the studied wine varieties

Variety	**Year**	**Color**	**Clarity**	**Aroma**	**Taste**
Vranac	**2016**	Closed red	Clear	Varietal, fruity, with mineral tones	Full
	2017	Closed red	Clear	Varietal, with fruity mineral character	Moderately full
Cabernet Sauvignon	**2016**	Closed red	Clear	Varietal, with pronounced minerality	Thin
	2017	Closed red	Clear	Varietal, with pronounced minerality	Moderately full

The red wine produced from Vranac grapes received the highest sensory scores, ranging from 80 (2017) to 76 (2016) (graph 4). The Cabernet Sauvignon variety had consistent sensory scores of 68 (2016) and 73 (2017). It can be concluded that wines from both varieties had better sensory scores during the 2017 vintage due to more favorable meteorological conditions. The results of our study are consistent with the findings of Milanov *et al.* (2014).



Graph 4. Sensory analysis of the studied wine varieties

Conclusion

Grape quality parameters (sugar content, total acid content and pH) of both varieties indicate high quality when growing on the area of Trebinje vineyards. During the experimental years, the variety Cabernet Sauvignon had the lowest sugar content (22%) and the highest acidity (9.15 g/L), while the variety Vranac had the highest sugar content (23%) and the lowest acidity (4.25 g/L). The quality of the wine of the tested varieties was at the level of high quality and quality varieties. The Cabernet Sauvignon wine produced in 2017 had the highest total polyphenol content, while the Vranac wine produced in 2016 had the lowest content. Additionally, a lower ash content was observed in the Vranac wine produced in 2017. Both tested varieties had higher alcohol and total extract contents in the wine produced from the 2017 grape harvest. The Vranac wine received the highest sensory ratings (80 in 2017), while the Cabernet Sauvignon wine received the lowest ratings (68 in 2016). Both tested varieties had better sensory ratings during the year 2017. From the point of the quality of grapes and wines, Vranac and Cabernet Sauvignon varieties, exhibited all the positive features and are therefore recommended for further expansion in Trebinje vineyards area.

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ANALYSIS OF AGREEMENT OF RATERS DURING SENSORY ASSESSMENT OF WINES BABICA I CRLJENJAK KAŠTELANSKI

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Abstract

The quality of wine depends on a number of factors that can be influenced. Grapes were processed with two different commercial pectolytic enzymes (Vinozym Vintage FCE and Sihazym Extro) and with a standard maceration as control treatment. The sensory evaluation of the wines produced was determined. The aim of the study was to analyze the agreement of raters during sensory assessment of wines of two autochthonous cultivars Babica and Crljenak kaštelanski (Tribidrag). The sensory analysis of the wines was carried out using the 100 Point Method of the O.I.V. Five evaluators with many years of experience and knowledge of the analyzed varieties participated in the panel group that conducted the evaluation. The wines produced with the enzymatic method showed better sensory characteristics than control. The sensory analysis of appearance (clarity and color), smell (purity, intensity and quality), taste (purity, intensity, shelf life and quality) and harmony/general impression, as well as the overall evaluation, confirmed the effectiveness of the pectolytic enzymes on the sensory characteristics of the quality of the wines from both varieties. In the evaluation of Babica, the sample with Vinozym, left the best impression on the evaluation team. In the evaluation of Crljenak variety, the effect of Sihazym was even more significant compared to the control sample. The Coefficient of Concordance (W) showed high agreement of raters in the classification of sensory evaluations. The highest values and almost complete agreement of the assessors were obtained for intensity 0.895** and shelf life 0.968**, and for total score 0.915**.

Key words: *W Coefficient of Concordance, sensory analysis, wine, Babica, Crljenjak kaštelanski.*

Introduction

Numerous studies have confirmed that certain components of wine are associated with the prevention of pathological changes in the cardiovascular system. This is especially true for the group of polyphenols in red wines due to their antioxidant properties. Dalmatia, as a source of numerous autochthonous red wine varieties, is an inexhaustible natural resource for the above-mentioned compounds. The climatic and edaphic characteristics of the region for viticulture, as well as the significant technological progress that has taken place among winemakers in recent years, make it possible to produce wines rich in the aforementioned compounds (Skračić, 2020). The quality of the future wine will be largely determined by the characteristics of the primary raw material, but also by the technology used in winemaking (Skroza, 2015). The tendency of growing autochthonous cultivars in ambient conditions ensures the expression of typical characteristics (Knezović *et al.*, 2017). Polyphenolic

compounds are an important part of the wine constituents. Among them, anthocyanins and tannins are compounds of exceptional importance for wine organoleptic properties, stability and aging potential. The visual appearance of wine is described by its color and clarity, which are among the most important attributes. These are the characteristics that the consumer sees first and are crucial for the first impression of the wine. Since the nineties of the last century, the oenological practice of using pectolytic enzymes to increase the color of wine has become widespread, so that the activity of enzymes is also focused on the extraction of color substances. Although, according to previous practice, they were used for clarification and removal of turbidity, they showed a positive effect on increase of color intensity and on the extraction of phenolic constituents (Izcara *et al*., 2001; Zietsman *et al.,* 2015). Most polyphenolic compounds are located in the skin of the berry, from where they are extracted into must (Jackson, 2014; Mattivi *et al.* 2006). Optimal selection of winemaking conditions contributes to better extraction of polyphenols and other compounds from the skin, which improves the sensory, biochemical, and commercial properties of the wine (Skroza *et al.*, 2014). Commercial enzyme preparations such as pectolytic enzymes destroy the pectin structure of the cell walls of the berry, contributing to the success of extraction.

The quality of wine implies a natural and harmonious analytical and sensory composition of wine that optimally affects the senses and human health, transcending preferences and personal tastes (Knezović *et al*., 2018). To eliminate the subjective, emotional approach, the sensory evaluation of wine should be analytical, objective, and reasonable. Evaluators entrusted with wine quality analysis should have subjective qualities (healthy and developed senses) complemented by familiarity with tasting techniques and organoleptic characteristics of specific wine types (Sokić, 2019). Top tasters are able to register a large number of impressions gained by tasting different types of wine and recognize wines of different varieties and different geographical origins. Experience is one of the most important qualities of a good taster. When analysing the quality of classifications and ratings, the main concern is consistency and agreement of results between raters as the most important measure of the quality of the rating.

In this paper, commercial enzyme preparations (Vinozym Vintage FCE and Sihazym Extro) were used for the preparation of wine from the autochthonous varieties Babica and Crljenak Kaštelanski, Kaštela - Trogir. The effects on color characteristics and sensory quality of the produced wines were monitored. The objective of this paper is to show the measure of agreement between the ranks of the sensory properties of the wine by five evaluators using Kendall's Coefficient of Concordance (W).

Material and Methods

The paper investigates the influence of commercial pectolytic enzymes on the extraction of phenolic components of autochthonous varieties Babica and Crljenak Kaštelanski (Tribidrag) from vineyards of Kaštela - Trogir. After grape processing, a standard maceration was carried out as a control treatment, and two additional treatments with commercial pectolytic enzymes (Vinozym Vintage FCE and Sihazym Extro). The winemaking was carried out on a semi-industrial scale. The sensory characteristics of the produced wines were determined. All the mentioned parameters were determined using standardized and internationally recognized analytical methods (Sokić, 2019). The evaluation of the sensory quality of the wine was carried out using the 100 positive points method adopted from the O.I.V., Rulebook on organoleptic (sensory) evaluation of must and wine (2004-2015) (Official Gazette 106/04, 137/12, 142/13, 48/14, 1/15). The OIV method uses four predefined sensory categories that are applicable to all types of wines, thus allowing a distinction between high and low quality (Guld *et al*., 2019). Five evaluators with many years of experience participated in the panel

group that performed the evaluation with experience and knowledge of Babica and Crljenak Kaštelanski varieties. The data are from the PhD thesis Skračić, 2020. The results of sensory analysis are given as arithmetic averages (Knezović, 2019). The final score of the sample is the average value of all five evaluators who participated in the evaluation. The produced wines were evaluated after the second rack, analyzing the smell (purity, intensity and quality); taste (purity, intensity, durability and quality); appearance (clarity and color) and harmony/general impression. Kendall's Coefficient of Concordance – W (Horvat and Ivezić, 2005) was calculated as a measure of agreement between evaluators. The Coefficient of Concordance is a number between 0 and 1, which indicates the agreement between expert opinions in the classification of properties. The closer this value is to 0, the lower the agreement. If the value of this coefficient is less than 0.3, the expert opinions are considered to be inconsistent. If the value of the coefficient is in the range of 0.3 to 0.7, the agreement is considered average. When the value is greater than 0.7, the agreement is considered high. The obtained research data were processed with the statistical program SPSS 20.

Results and Discussion

Sensory analysis confirmed the effectiveness of the application of pectolytic enzymes in wines of the Babica variety, and even more so in the wines of the Crljenak kaštelanski variety (Table 1). The tasting took place two weeks after the second racking, i. e. with relatively young wines. Even with such a young wine, the samples treated with pectolytic enzymes show changes that differ significantly from the control samples. This is especially true for the treatment of Crljenak with Sihazym Extro, which with a mean value of 85.4 exceeded the control sample by five points (80.4). In Babica, the sample treated with Vinozyme Vintage FCE the highest with 75.6), three points more than the control sample (72.6) and 2.2 points more than the sample treated with Sihazyme Extro (73.8). Thus, as in most sensory studies of the effect of pectolytic enzymes on the organoleptic properties of wine, the positive influence of the enzymatic treatments on the characteristics of the wine for the final consumer is not in question, and this paper does not differ from numerous studies of this type (Kelebek *et al.,* 2007; Gambutti *et al.,* 2007; Guadelupe *et al.,* 2007). It is important at what stage of wine ageing the sensory analysis was performed. Bautista - Ortin *et al*. (2005), without giving the trade names of the enzyme preparations, give the chemical composition of enzyme E1 contains hemicellulase as secondary activity in addition to a highly concentrated pectinase activity (similar to Sihazym Extro), and enzyme E2, which contains hemicellulase and cellulase as secondary activities in addition to pectinase, and is thus almost identical to Vinozyme Vintage FCE.

The authors note that the wines of the Monastrell variety treated with the E1 enzyme have visual and olfactory characteristics close to those of the control sample. As for the taste characteristics, they are drier, astringent, bitter and have a noticeable herbaceous character. Wines of the same variety treated with enzyme E2 have a lower percentage of herbaceous notes, less astringency, better roundness and balance. Thus, the advantages of enzyme E2, similar in composition to Vinozyme, are more noticeable sensorially in Babica compared to the control sample, while in Crljenak the sensory analysis is on the side of Sihazym, similar in composition similar to enzyme E1 from the above-mentioned experiment with Monastrella. From the above, it is clear that the issue of organoleptic "bonification" of must with pectolytic enzymes is very complex (Skroza *et al.,* 2017). There is no one – size - fits all recipe for all varieties because, first of all, differences in the structure and composition of the cell wall depend on the variety (Romero – Cascales *et al*., 2005). The differences in the oligosaccharide composition of cell structures are very large even in the same variety, considering the "terroir", especially if it is a cell wall (Apolinar - Valiente *et al.,* 2014). The effect of

polyphenol extraction, so important for the organoleptic profile of the wine, depends on the molecular size and type of polyphenols, the duration and temperature of the extraction process, the concentration gradient, the permeability of the wall, the composition of the extraction medium (e. g. the ethanol concentration), and the ratio of the extraction area and the concentration gradient (Gao *et al.*, 2019).

Table 1. The results of sensory evaluation of individual sensory properties of wines

Sensory property (max. grade)		BABICA			CRLJENAK KAŠTELANSKI		
		a)	b)	c)	a)	b)	c)
APPEARANCE	Clarity (5)	3.6	3.6	3.6	4.4	4.4	4,8
	Color (10)	7.6	6.8	7.6	8	8.4	8
SMELL	Purity (6)	4.4	4.8	4.6	4.2	5.2	6
	Intensity (8)	6	4.8	6.2	7.2	6.6	6.4
	Quality (16)	12	12	12	12	12.8	13.2
TASTE	Purity (6)	3.8	5.2	4	4	4.4	4.8
	Intensity (8)	6	7	4.8	7.4	7.2	7
	Shelf life (8)	5.2	7	5.8	6.4	7.6	8
	Quality (22)	16	16	16	17.2	16	17.2
HARMONY/GENERAL IMPRESSION (11)		8	8.4	9.2	9.6	10	10
Total score (100)		72.6	75.6	73.8	80.4	82.6	85.4

a) without enzyme additives; b) with the addition of the enzyme Vinozym Vintage FCE and c) with the addition of the enzyme Sihazym Extro.

Table 2. The results of mutual agreement between evaluators of vines

Sensory property		Mutual agreement of evaluators (W)	p	Significance agreement of evaluators
APPEARANCE	Clarity	0.573	0.014	*
	Color	0.242	0.300	n.s.
SMELL	Purity	0.711	0.003	**
	Intensity	0.612	0.009	**
	Quality	0.461	0.042	*
TASTE	Purity	0.639	0.007	**
	Intensity	0.895	0.000	**
	Shelf life	0.868	0.001	**
	Quality	0.356	0.114	n.s.
HARMONY/GENERAL IMPRESSION		0.740	0.002	**
In total		0.915	0.000	**

** Correlation is significant at the 0.01 level

Table 2. shows the values and significance of mutual agreement between five evaluators. Kendall's Coefficient of Concordance showed high agreement among the evaluators in ranking the sensory ratings. The highest ratings and almost complete agreement were recorded for intensity 0.895** and shelf life 0.868** (Taste), and for overall rating 0.915**. A slightly weaker but still high agreement was recorded for harmony/general impression 0.740**, and purity for fragrance 0.711**. Average agreement was recorded for most of the other properties.

Conclusions

Most polyphenolic compounds are found in the skin of the berry, from which they are extracted into must. Commercial enzyme preparations such as pectolytic enzymes destroy the pectin structure of the berry cell walls, contributing to the success of polyphenol extraction. Sensory analysis confirmed the effectiveness of pectolytic enzymes on the sensory characteristics of the quality of wines from both varieties. In the evaluation of Babica, the sample with Vinozym and then with Sihazym left the best impression on the evaluation team. In the evaluation of the Crljenak variety, the effect of Sihazym is even more significant compared to the control sample, but even the sample with Vinozym is not rated worse than the wine produced according to standard procedure.
Kendall's Coefficient of Concordance (W) indicated high agreement of evaluators in ranking sensory scores. The highest values and almost complete agreement between the evaluators were found for intensity and persistence of flavor, as well as for the overall rating.

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EFFECT OF THE COVID-19 PANDEMIC ON THE IMPORT AND EXPORT OF CHAMOMILE (*MATRICARIA CHAMOMILLA* L.) IN SERBIA

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Abstract

Chamomile is well-known and a highly favoured medicinal plant species in pharmacy and traditional medicine, having therapeutic and cosmetic values and uses. It is a low-demanding species that tolerates drought and salinity, and is successfully grown in Serbia, on approximately 350 - 400 ha. Before the pandemic, during the five-year period 2015-2019, the export of chamomile did not change significantly over the years, and the average amount of exported chamomile was 262.4 metric tons with an average value of 1.02 M USD. However, the COVID-19 pandemic influenced the production and consumption of many plants worldwide, and the increase in the export of chamomile was recorded for the pandemic years (2020-2022), when the average amount of exported chamomile reached 314.6 metric tons with an average value of 1.25 M USD. However, the import of chamomile also increased during the pandemic. The average amount of imported chamomile was 35.5 metric tons with an average value of 0.23 M USD during the five years period before the pandemic, but during the pandemic, the average amount of imported chamomile was 67.3 metric tons with an average value of 0.44 M USD. The obtained data suggest that there is a need for chamomile in Serbia and that chamomile production should expand. It is important to implement measures with the aim to improve the technology of chamomile production, including the harvesting and drying process, in order to increase profit and make chamomile production economically justified. In that way, chamomile production will increase in Serbia.

Keywords: *chamomile production, chamomile trade, medicinal plants market, aromatic plants.*

Introduction

Chamomile is well-known and a highly favoured medicinal plant species in pharmacy and traditional medicine and is a component of numerous commercial products. Medicinal properties of chamomile mostly include antiseptic and anti-inflammatory application on the skin or mucous membranes of the respiratory organs, digestive organs, or urogenital system (Radovanović et al., 2023; Matejić et al., 2020). Besides therapeutic use, chamomile has also a cosmetic use. In Serbia, chamomile is collected from natural habitats, but most frequently it is successfully cultivated on approximately 350 - 400 ha for both domestic and foreign markets. It is a low-demanding species that tolerates drought and salinity, growing on less fertile soils, and it is often an important part in crop rotation because it can be intercropped, besides being grown as monoculture (Adamović-Brdar et al., 2019; Dajić Stevanović, 2011).
Chamomile is an important medicinal crop, not only in Serbia but also worldwide and demand for chamomile extract is increasing, according to the Research Reports World (2023). During the past four years, prophylactic measures during the Covid-19 pandemic, such as social isolation and lockdowns were conducted in the majority of countries, having an impact also on the economy with the changes in consumption and production system, besides

income-generating activities were reduced, commercial establishments were temporarily closed and the unemployment increased in some sectors (Clair et al., 2021; Lioutas and Charatsari, 2021). All these measures also influenced the production, export and import of some crops (Marković, 2022a,b; Wenguang et al., 2022; Hayakawa and Mukunoki, 2021; Mouratidou et al., 2021).

Taking into account the importance of chamomile production and the fact that chamomile production should be increased in Serbia (Adamović-Brdar et al. 2019; Dajić Stevanović 2011), there is a need to determine how the pandemic influenced the export and import of chamomile in Serbia and to estimate current possibilities for the export of chamomile.

Material and Methods

The official statistical databases of Serbia were used in this research. The data on the export and import of chamomile given in tons and USD, were obtained from the databases of the Republic Bureau of Statistics (https://data.stat.gov.rs/?caller=SDDB), for the period 2015-2022. The trend analysis was used to calculate trends for the years 2020 and 2021 in order to determine expected changes in export and imports based on five-year data (2015-2019) collected before the pandemic and to compare obtained data with real data collected for the pandemic years 2020 and 2021. Microsoft Excel 2007 was used for trend analysis. Also, in order to obtain more accurate data, the chamomile trade in Serbia during the pandemic years (2020 and 2021) was compared to the average data for the five-year period before the pandemic (2015-2019).

Results and Discussion

The data for the export and import of chamomile to the European Union (as a market with the largest share in the export or import in Serbia) and world markets are presented in Tables 1 and 2. In 2019, the export of chamomile dropped significantly compared to the previous 3 years, from 304-322 tons to 159 tons (Table 1). Afterwards, it considerably increased considerably during the pandemic years to even 362.3 tons in 2021 and 368.5 in 2022, which is much higher than estimated by trend analysis. However, the price of chamomile dropped from 4.48 thousand USD/ton in 2020 to 3.75 thousand USD/ton in 2021 and 3.87 thousand USD/ton in 2022. However, the price estimated by trend analysis was almost the same for 2020 (4.44 thousand USD/ton) and much higher for the following years (5.27 thousand USD/ton in 2021 and 6.19 thousand USD/ton in 2021). For that reason, we can conclude that the pandemic did not cause a decrease in export, but it did cause a drop in the price of chamomile.

Table 1. Export of chamomile from Serbia during 2015-2022.

Year	**European Union**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***
2015	181.2	531.4	217.9	856.2	Estimated values based on 2015-2019 data	
2016	217.9	646.9	304.5	1031.1		
2017	270	824.8	308.7	1131.8		
2018	244.2	897.9	322.0	1315.4		
2019	90.5	355.2	159.0	742.6		
2015-19*	**200.8**	**651.2**	**262.4**	**1015.4**		
2020	128.8	518.5	213.1	955.5	232.3	1032.5

2021	287.5	926.8	362.3	1361.5	177.1	934.8
2022	294.1	910.0	368.5	1425.3	133.9	828.4

*Note: The value is given in thousands of USD; 2015-19 presents the average value for the five-year period.

Similarly, the import of chamomile also increased in the pandemic years (Table 2) and it was much higher than estimated by trend analysis. The price of imported chamomile ranged from 6.02 thousand USD/ton in 2020 to 6.83 thousand USD/ton in 2022 and it was higher than the price of exported chamomile and higher than prices estimated by trend analysis (4.52 – 5.21 thousand USD/ton). Nevertheless, this price is still lower than in the years 2015-2019, when prices ranged from 4.53 to 10.11 thousand USD/ton.

Table 2. Import of chamomile to Serbia during 2015-2022.

Year	**European Union**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***
2015	41.1	194.4	44.3	209.5	Estimated values based on 2015-2019 data	
2016	27.9	144.9	31.5	183.4		
2017	19.5	179.7	28.1	250.5		
2018	16.9	140	23.0	231.6		
2019	46.3	223	50.9	290.4		
2015-19*	**30.3**	**176.4**	**35.5**	**233.1**		
2020	71.0	417.4	78.0	469.4	36.9	192.1
2021	52.4	320.4	59.6	399.8	44.2	217.2
2022	62.6	412.2	64.3	439.1	50.5	228. 6

*Note: The value is given in thousands USD; 2015-19 presents the average value for the five-year period.

The changes in the chamomile market can be caused by many factors, besides the pandemic (Lioutas and Charatsari, 2021). The increase in the amount of exported chamomile at lower prices can suggest that the global price dropped, but also that the quality of exported plants was lower leading to a lower price per metric ton. Similarly, chamomile was also imported in large amounts during the observed period, at a higher price than the price of exported chamomile. It is evident that there is a need for chamomile on Serbian market and that chamomile production should expand. It is important to implement measures with the aim to improve the technology of chamomile production, including the harvesting and drying process, in order to increase profit and make chamomile production economically justified. The appropriate research should be conducted in order to establish good agricultural practice with the precise, site specific use of agro-chemicals and fertilizers in high value chamomile production, with minimum impact on ecosystems (Ghareeb et al. 2022; Chauhan et al. 2021; Upadhyay et al. 2016).

Conclusions

The pandemic considerably influenced the agriculture sector worldwide, including the production and distribution of chamomile. Besides, the demand for chamomile and other medicinal plants were increasing in recent years. The production of chamomile has great potential in Serbia due to favourable conditions. During the pandemic years, the export of chamomile increased, but the price was lower, which can be caused by changes in prices on

the global market, but also by lower quality of exported chamomile. For this reason, it is important to work on improving chamomile production in Serbia, thus increasing competitiveness on international markets. Besides, there is a need for chamomile in Serbia, and increasing production of high-quality chamomile can lower the need for imports of this medicinal plant.

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ARTA KIWIFRUIT: A CLONE OF HAYWARD WITH INTERESTING QUALITY CHARACTERISTICS

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Abstract

'Arta Kiwifruit' is a green flesh kiwifruit cultivar, selected as a spontaneous clone in a 'Hayward' orchard, due to its desirable phenotypical differences. An experimental orchard was established where both 'Arta Kiwifruit' and 'Hayward' plants were grown under the same pedoclimatic conditions and cultural management. When the plants entered the productive phase, both 'Arta Kiwifruit' and 'Hayward' genotypes were phenotypically described based on UPOV guidelines for the conduct of tests for distinctness, uniformity, and stability. Shoots, leaves, flowers, and fruits were used for the description. At the same time, ISSR markers were used to assess possible genetic differences using leaf material. A total of five ISSR markers were used, i.e. UBC-808, UBC-815, UBC-818, UBC-825,and UBC-850. Based on UPOV guidelines the 'Arta Kiwifruit' presented significant differences compared to 'Hayward' regarding the density of hair of young shoots, shoot coloration, the coloration of the petiole upper side, the fruit weight, length, shape, and stylar end, the shape of the shoulder at the stalk end of the fruit, the length of stalk relative to the length of fruit, the width of fruit core relative to fruit, the fruit sweetness, the time of flowering beginning and the time of maturity. Regarding the molecular analyses, it was found that by the use of UBC-850 and UBC-825 ISSR markers the 'Arta Kiwifruit' could be distinguished from 'Hayward', ascertaining thus the phenotypic differences determined. Further analyses are needed to examine other physiological, organoleptic, and phytochemical differences as well as to assess the storability of 'Arta Kiwifruit' fruits.

Keywords:*ISSR markers, Molecular analysis, Phenotypic description, UPOV.*

Introduction

Kiwifruit is a commercially important fruit crop known for its unique flavor, high nutritional value, and health benefits (Yang et al., 2020). As a result, kiwifruit cultivation and production have been increasing, with China, Italy, New Zealand, and Greece being some of the main global producers. Kiwifruits include green, gold, or red flesh cultivars, with the green-fleshed 'Hayward'cultivar being one of the most widely cultivated worldwide.

Numerous elite cultivars have been developed and released, with high-cost breeding projects conducted by both public institutes and private companies, whereas interesting genotypes can be selected *in farm* by the farmers.Traditional and modern methods have been developed to differentiate kiwifruit cultivars. Traditional methods involve assessing morphological and biochemical traits such as fruit shape, size, color, chemical composition, and flowering time (Li *et al*., 2021). Mavromatis *et al.* (2010) differentiated two kiwifruit cultivars based on morphological characteristics such as fruit weight, length, and diameter, while Lee *et al.* (2015) and Latocha *et al.* (2023) used biochemical markers such as total phenolic content and

antioxidant activity. However, these methods have limitations in accuracy and can be influenced by environmental factors. Consequently, the use of molecular markers to differentiate kiwifruit cultivars based on their genetic profiles (Mavromatis *et al*., 2010) has become increasingly popular due to their high accuracy, reproducibility, and independence from environmental factors. Molecular markers such as Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), and Inter-Simple Sequence Repeat (ISSR) have been widely employed (Huang *et al*., 1998; Palombi and Damiano, 2002; Huang *et al*., 2002; Chen *et al*., 2015; Zhou *et al*., 2008). Among these types, ISSRs have gained popularity for their high level of polymorphism, reproducibility, and cost-effectiveness in identifying and classifying kiwifruit cultivars (Chen *et al*., 2015; Zhou *et al*., 2008).
The objective of the present study was to examine the genetic relationship between the extensively cultivated 'Hayward' cultivar and the under-evaluation Greek kiwifruit selection 'Arta Kiwifruit', utilizing molecular markers and phenotypical description characteristics according to UPOV.

Materials and Methods

1. Study site, plant material, and experimental design

The research was carried out in Arta, Epirus region,Western Greece. The plant material utilized in the study consisted of fully grown vines of the 'Hayward' cultivar and a clonal selection of 'Hayward' known as 'Arta Kiwifruit', which was recommended by the farmer Mrs. Xylogianni Evanthia (Arta, Greece). The plants were propagated from cuttings and cultivated in an open field. The study took place in a 4 hectaresgrower-managed orchard, where kiwifruit production was observed for two consecutive growing seasons. Both kiwifruitgenotypeswere trained having a trunk height of 1.8 m underthe pergola system, with a planting distance of 2.0 × 4.0 m. The cultivation practices i.e. irrigation, fertilization, pruning, weeding, and the application of plant protection products, were consistent for all vines in the orchard.
The experiment followed a completely randomized design, with five replications of two vines per genotype. In total, 20 plants were used, 10 plants for each genotype.

2. Phenotypical description

The description of the 'Arta Kiwifruit' genotype was derived from an analysis of its characteristics, as outlined in the key morphological traits for kiwifruit according to UPOV. For this purpose, a comparison was made between 10 vines of the 'Arta Kiwifruit' and 10 vines of the 'Hayward' cultivar. The kiwifruit genotypes were evaluated (described and/or measured) for characteristics related to the plant, shoots, leaves, flowering, flowers, and fruit.

3. Molecular characterization of kiwifruit genotypes

Healthy, undamaged leaves from 'Hayward' and 'Arta Kiwifruit' vines were sampled during mid-June, frozen instantly in liquid nitrogen, and transferred to the laboratory for genetic analysis. The leaf samples were ground into a powder using a mortar, pestle, and liquid nitrogen. DNA was extracted from 100 mg of leaf tissue using the NucleoSpin Plant II Kit (Macherey-Nagel, Germany) (Anonymous, 1). The extracted DNA was checked for quality and quantity using a NanoDrop microvolume spectrophotometer.
For genome screening, 13 ISSR primer sets were used: UBC 808, 815, 818, 825 and 850 (Table 1). The PCR reaction solution was the same for all the primer sets and contained 1X KapaTaq buffer, 5 μM MgCl2, 0.3 μM dNTPs, 0.3 μM primer, and 1unit Taq polymerase. PCR parameters to amplify the genomic DNA are presented in Table 2, whereas the annealing temperatures for each primer are presented in Table 3. PCR products were separated on a 2%

agarose gel. The differences between the two genotypes were visually highlighted due to the small number of genotypes examined.

Table 1. ISSR sequences

ISSR	Sequence
UBC-808	5'-AGAGAGAGAGAGAGAGG-3'
UBC-815	5'-CTCTCTCTCTCTCTCTG-3'
UBC-818	5'-CACACACACACACACAG-3'
UBC-825	5'-ACACACACACACACACT-3'
UBC-850	5'-GTGTGTGTGTGTGTGTYC-3'

Abbreviations: Y= C+T (pyrimidines)

Table 2. PCR parameters

Temperature	Time	
94^{o}C	4 min	
94^{o}C	30 sec	37 cycles
At Table 3	1 min	
72^{o}C	2 min	
72^{o}C	7 min	

Table 3. Annealing temperatures (T ^{o}C) of primers selected.

UBCprimer	808	815	818	825	850
T (^{o}C)	50	45	45	50	45

Results and Discussion

Both genotypes, 'Hayward' and 'Arta Kiwifruit' are female kiwifruits with no self-fertilization capability and of moderate vigor. One characteristic in which they differ is the density of trichomes on the new shoot, where the clone exhibits sporadic, sparse trichomes. Additionally, while the coloration of the growing tip in 'Hayward' is intense, in the 'Arta Kiwifruit'it is moderate. Another difference is noted regarding the coloration of the upper side of the leaf petiole, which is intense in 'Hayward' and moderate in 'Arta Kiwifruit'. No other differences were observed regarding leaf characteristics, while several differences were noticed in fruit-related traits.

The fruit weight and length are greater in the 'Arta Kiwifruit' than that of 'Hayward' (Figure 1). Both genotypes do not differ in terms of fruit diameter, resulting in a higher length-to-diameter ratio in the 'Arta Kiwifruit', giving it a more elongated shape compared to the more elliptical shape of 'Hayward'. Additionally, there are differences in the position opposite the pedicel, where the fruits of 'Hayward' display a broader point compared to the fruits of the 'Arta Kiwifruit'. Similarly, 'Hayward' exhibited a slightly inclined pedicel attachment point, while 'Arta Kiwifruit' has a smoother and broader attachment point. The pedicel of 'Hayward' is also larger compared to 'Arta Kiwifruit', while the width of the fruit core is significantly greater in 'Arta Kiwifruit'. Based on the total soluble solids measured, the fruit of 'Hayward' is characterized as slightly sweet, while 'Arta Kiwifruit' fruit is less sweet. Furthermore, regarding the flowering period, it appears that 'Arta Kiwifruit' blooms slightly later than 'Hayward', and the same applies to the ripening period (according to the concentration of total soluble solids measured at harvest). Therefore, some morphological differences are observed between 'Hayward' and the examined genotype.

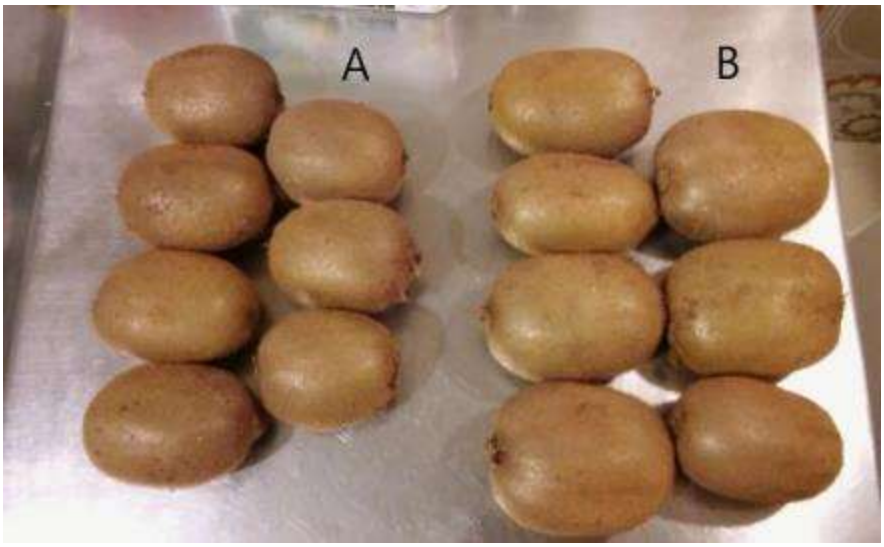

Figure 1. Kiwifruits of A) 'Hayward' cultivar and B) 'Arta Kiwifruit'.

The extracted DNA exhibited sufficient quality and quantity for genetic analysis. In Tables 1, 2 and 3, the electrophoresis results of the PCR products are presented. The framed figures and arrows highlight the observed genetic differences between the two genotypes. The two genotypes, i.e., 'Hayward' cultivar and the Greek genotype 'Arta Kiwifruit', were distinguished by UBC-825and UBC-850 ISSR markers. Different amplification bands were observed between the two genotypes, as indicated by the electrophoresis results (Figure2 and 3).

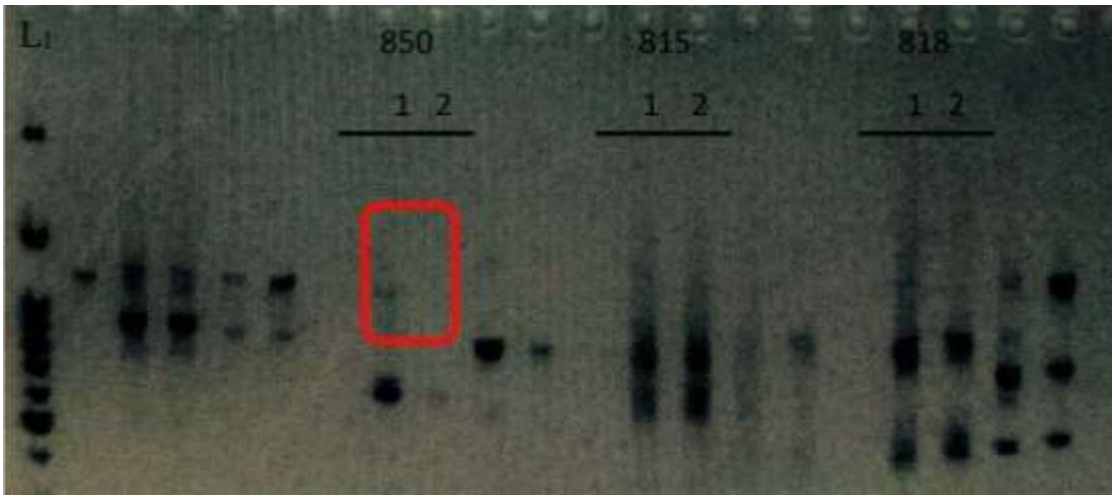

Figure 2. ISSR banding profile generated by UBC-850, UBC-815 and UBC-818. The red frame shows the genetic differences between the two genotypes concerning the presence of amplification bands. L_1: Ladder 3000 bp; L_2: 1500 bp; 1: 'Arta Kiwifruit'; 2: 'Hayward' cultivar.

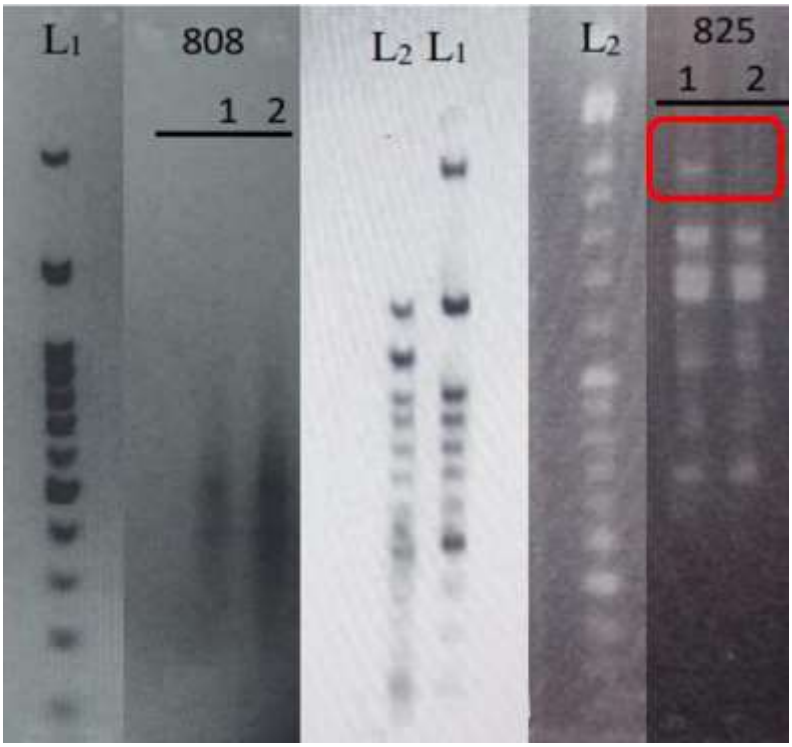

Figure 3. ISSR banding profile generated by UBC-808 and UBC-825. The red frame shows the genetic differences between the two genotypes concerning the presence of amplification bands. L_1: Ladder 3000 bp; L_2: 1500 bp; 1: ‘Arta Kiwifruit’; 2: ‘Hayward’ cultivar.

The findings of the present study indicate that the new genotype exhibits a distinct genetic profile compared to the widely cultivated ‘Hayward’cultivar. Furthermore, notable variations in morphological parameters, particularly in fruit size and shape, were also observed. The identification of this new kiwifruit genotype holds significant implications for kiwifruit breeding and production. The larger fruit size of ‘Arta Kiwifruit’ could be particularly appealing to both growers and consumers, offering a competitive edge in the market. Moreover, the utilization of ISSR markers in kiwifruit breeding could facilitate the development of new cultivars with desirable traits, such as enhanced flavor, disease resistance, and extended shelf life.

Conclusions

In summary, this study successfully distinguished the Greek kiwifruit selection ‘Arta Kiwifruit’ from the ‘Hayward’ using ISSR molecular markers and UPOV morphological parameters. The identification of this new genotype holds substantial implications for both the local and international kiwifruit industry, potentially paving the way for the development of new cultivars that incorporate improved traits in breeding programs.

Acknowledgment

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COMPARATIVE EVALUATION OF SELECTION INDEXES FOR IDENTIFYING SUPERIOR WINTER BARLEY GENOTYPES

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Abstract

This study aimed to assess and compare the effectiveness of the Smith-Hazel classic index with the modern ideotype-design index (FAI-BLUP) and multi-trait genotype-ideotype distance index (MGIDI) for selecting superior winter barley genotypes. A total of 40 barley genotypes from ICARDA, along with three local check varieties, were evaluated in a randomized complete block design with three replications at the Institute of Agriculture – Karnobat, Bulgaria. Key traits such as spike length, spikelet number in spike, grain number in spike, grain weight per spike, 1000-grain weight, and grain yield were examined. The results revealed that multi-trait selection indices hold promise for enhancing genetic gain in winter barley breeding programs. However, the Smith-Hazel selection index demonstrated higher genetic gains compared to the MGIDI and FAI-BLUP indices. This finding suggests that the Smith-Hazel index effectively prioritizes specific traits, leading to greater overall genetic improvement. The genotypes G21, G32, G38, G35, and G20 selected based on the three different indices demonstrated desirable trait combinations and have the potential for further evaluation and advancement in the winter barley breeding programs.

Keywords: *multi-trait selection, Smith-Hazel index, FAI-BLUP, MGIDI, genetic gain.*

Introduction

Winter barley (*Hordeum vulgare* L.) is an important cereal crop widely cultivated in various regions of Bulgaria. It serves as a valuable feed grain for livestock and plays a vital role in food security and agro-industries. The continuous demand for improved barley genotypes with enhanced yield potential, better grain quality, and improved adaptation to changing environmental conditions necessitates the implementation of efficient breeding strategies. Therefore, there is a growing need to explore and adopt advanced selection methods to accelerate genetic gain in barley breeding programs. Selection indices have emerged as valuable tools for optimizing the selection process by integrating multiple traits into a single numerical value. These indices enable breeders to prioritize genotypes based on their overall performance, considering the complex interplay of various important traits. The Smith-Hazel classic index has been widely used in plant breeding, providing a quantitative measure of the relative importance of each trait in the selection process (Jahufer and Casler, 2015). The concept behind employing the Smith-Hazel index involves determining the genetic value of an individual by calculating a linear function of the genetic values of several traits, each assigned a specific relative economic value (Baker, 1974). The index offers the benefit of incorporating data on heritability, trait correlations, and potentially economic significance (if applicable) (Cotterill and Dean, 1990).

However, with advancements in statistical methodologies, alternative selection indices have been developed to enhance breeding efficiency. Two such modern selection indices that have gained attention in barley breeding are the ideotype-design index based on best linear unbiased prediction (FAI-BLUP) and the multi-trait genotype-ideotype distance index (MGIDI).

The FAI-BLUP methodology (Rocha et al., 2018) offers several advantages over other selection indexes. One notable advantage is its utilization of predicted genetic effects. This approach was specifically developed for creating a multi-trait index for breeding elephant grass for bioenergy (Rocha et al., 2018). Moreover, it has been recently applied in evaluating lines and hybrids of biomass sorghum (da Silva et al., 2018), as well as selecting superior progenies of common bean (Rocha et al., 2019). The key strengths of FAI-BLUP include equal weighting of all traits and the ability to simultaneously select genotypes that closely match the ideotype through a multi-trait approach.

The MGIDI index was initially developed to facilitate the selection of genotypes in plant breeding by considering information from multiple traits (Olivoto et al., 2022). Its effectiveness has been demonstrated through its successful application in identifying and choosing superior genotypes (Olivoto and Nardino, 2021; Uddin et al., 2021; Leon et al., 2021; Pour-Aboughadareh et al., 2021). The MGIDI index allows a unique and easy-to-interpret selection process. In addition to addressing the issue of collinear traits, the MGIDI index doesn't require the use of any economic weights, providing more balanced gains. This means that the MGIDI index can help breeders to guarantee long-term gains in primary traits (e.g., grain yield) without jeopardizing genetic gains of secondary traits (e.g., plant height) (Olivoto and Nardino, 2021).

The aim of the present study was to assess and compare the effectiveness of the Smith-Hazel classic index with the modern ideotype-design index (FAI-BLUP) and multi-trait genotype-ideotype distance index (MGIDI) for selecting superior winter barley genotypes.

Material and Methods

In this study, a total of forty breeding lines of 6-rowed winter barley from the International Winter Barley Yield Trials (IBYT-W) and Observation Nurseries (IBON-W) of ICARDA, labeled from G1 to G40, were tested. Additionally, three Bulgarian 6-rowed varieties, namely Veslets (G41), Bojin (G42), and Zemela (G43), were included.

The study was conducted during 2020/2021 growing season at the experimental field of the Institute of Agriculture - Karnobat, Southeastern Bulgaria (42°39' N, 26°59' E). The soil of the experimental field was slightly acid (pH is 6.2) Pellic Vertisol. The experiments were organized in a Complete Randomize Block Design with 3 replications on plots of 10 m^2 with sowing rate 450 seeds/m^2. During the growing season, standard plant protection practices were used.

The total precipitation received during the vegetation period was 447.8 mm, which was close to the long-term average precipitation of the region (424.6 mm). The average temperature during the growing season was slightly higher than the long-term average temperature.

The traits, spike length (SL, cm), spikelet number per spike (SNS), grain number per spike (GNS), grain weight per spike (GWS, g) were measured on 20 randomly selected plants in each replication of each genotype. Grain yield (GY, t/ha) and 1000- grain weight (TGW, g) were determined on plot basis.

The multi-trait selection indexes utilized in this study were computed using the R package "metan" (Olivoto and Lúcio, 2020). These indexes included the Smith-Hazel index (SH) (Smith 1936; Hazel 1943), the multitrait index based on factor analysis and ideotype-design (FAI-BLUP) (Rocha et al., 2019), and the multi-trait genotype-ideotype distance index (MGIDI) (Olivoto and Nardino, 2021).

For each trait, the predicted genetic gain SG (%), obtained with the selection index at 20% selection intensity was calculated as followed:

$$SG(\%) = \frac{(Xs - Xo) \text{ x } h^2}{Xo} \text{x} 100$$

where Xs is the mean of the selected genotypes, Xo is the mean of the original population and h^2 is the broad-seanse heritability.

Results and Discussion

The selection of superior winter barley genotypes is crucial for the success of breeding programs aimed at improving yield potential and overall performance. In this study, three different selection indices, namely the multi-trait genotype-ideotype distance index (MGIDI), the ideotype-design index based on best linear unbiased prediction (FAI-BLUP), and the Smith-Hazel (SH) selection index, were employed to identify promising genotypes.

Of the 43 genotypes evaluated, the MGIDI index identified genotypes G38, G21, G22, G35, G32, G18, G4, G37, and G20 as high performing genotypes for multiple traits (Figure 1). The MGIDI index prioritizes genotypes that exhibit a favorable distance from the ideotype while considering multiple traits simultaneously. Therefore, it can be inferred that the selected genotypes demonstrated strong performance in terms of the evaluated traits, including spike length, spikelet number per spike, grain number per spike, grain weight per spike, 1000-grain weight, and grain yield.

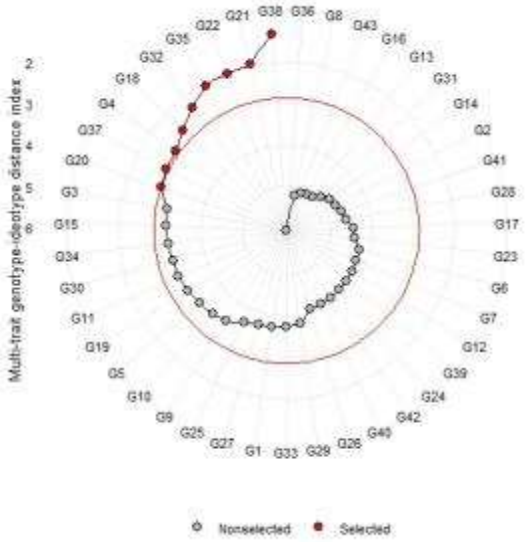

Figure 1. Genotypes ranking based on the MGIDI index.

Similarly, the FAI-BLUP index, which utilizes best linear unbiased prediction, selected genotypes G38, G21, G35, G22, G32, G18, G4, G20, and G16 (Figure 2). These genotypes exhibit favorable estimated breeding values across the assessed traits, highlighting their potential for improved performance in terms of grain yield and other agronomic characteristics.

On the other hand, the genotypes selected based on the Smith-Hazel selection index, namely G21, G32, G38, G35, G11, and G20, were chosen primarily based on their performance relative to the average performance of the population (Figure 3).

Overall, the overlap in genotypes selected by the three indices, particularly G21, G32, G38, G35, and G20, indicates their strong potential for further breeding efforts. These genotypes consistently demonstrate desirable characteristics and have the potential to contribute to genetic gain in winter barley breeding programs.

Based on the MGIDI index, the following genotypes were selected: G38, G21, G22, G35, G32, G18, G4, G37, and G20. These genotypes displayed favorable performance across

multiple traits evaluated in this study, including spike morphology, grain yield, and grain weight. The selection based on the MGIDI index suggests that these genotypes possess desirable trait combinations, potentially contributing to enhanced genetic gain in winter barley breeding programs.

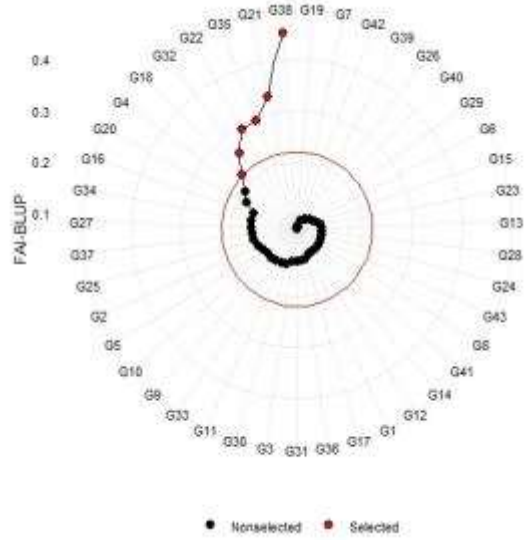

Figure 2. Genotypes ranking based on the FAI-BLUP index.

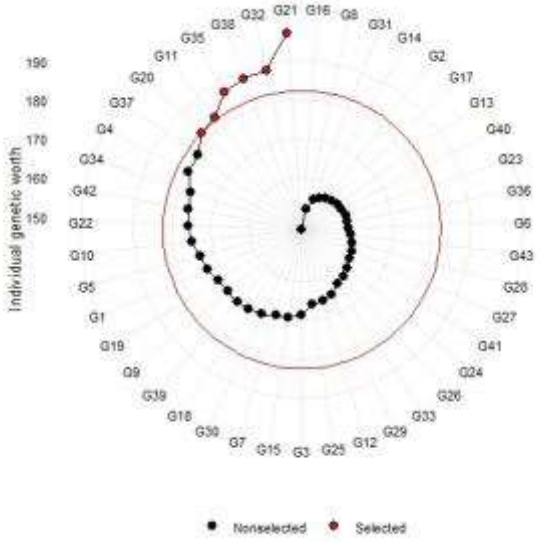

Figure 3. Genotypes ranking based on the SH index.

Overall, the findings of this study demonstrate the effectiveness of different selection indices in identifying superior winter barley genotypes. The use of multi-trait selection indices such as MGIDI and FAI-BLUP offers advantages in capturing the complex relationships among traits and maximizing genetic gain. However, it is worth noting that the specific genotypes selected may vary depending on the index utilized, highlighting the importance of carefully considering the breeding objectives and trait preferences when applying selection indices.

The selected genotypes identified in this study, including G38, G21, G35, G22, G32, G18, G4, G20, G16, and G11, hold great potential for further evaluation and advancement in winter barley breeding programs. By incorporating these genotypes into subsequent breeding cycles, breeders can capitalize on their desirable traits and work towards developing high-yielding barley cultivars.

It is important to note that while the selection indices provide valuable guidance for genotype selection, additional considerations such as environmental adaptation, disease resistance, and grain quality traits should also be taken into account when making final breeding decisions. The findings of this study highlight the effectiveness of different selection indices in identifying superior winter barley genotypes and provide insights for breeders in their efforts to develop improved varieties with enhanced agronomic performance and genetic gain.

Table 1 displays the genetic gains predicted by the MGIDI, FAI-BLUP, and SH indexes across various traits. The MGIDI index achieved a genetic gain of 8.44% for spike length (SL), while FAI-BLUP and SH exhibited gains of 6.78% and 4.83%, respectively. In terms of grain weight per spike (GWS), the MGIDI index outperformed with a genetic gain of 13.38%, followed by FAI-BLUP with 12.64% and SH with 17.13%. The spikelet number per spike (SNS) saw the MGIDI index achieve a genetic gain of 9.87%, whereas FAI-BLUP and SH obtained gains of 6.11% and 13.75%. The MGIDI index displayed a genetic gain of 10.95% for grain number per spike (GNS), while FAI-BLUP and SH exhibited gains of 8.80% and 18.01%. Regarding 1000-grain weight (TGW), the MGIDI index resulted in a genetic gain of 3.33%, while FAI-BLUP and SH showed gains of 2.89% and 1.42%. In terms of grain yield (GY), the MGIDI index demonstrated the highest genetic gain at 15.50%, followed by FAI-BLUP with 13.66% and SH with 24.57%. When considering all the traits collectively, the total genetic gains were 61.47% for MGIDI, 50.87% for FAI-BLUP, and 79.70% for SH.

The results demonstrate that all three selection indices, MGIDI, FAI-BLUP, and SH, offer potential genetic gains for the evaluated traits. However, there are variations in the magnitude of predicted gains among the indices. The MGIDI index consistently showed lower genetic gains compared to the FAI-BLUP and SH indices for most traits, indicating a more conservative approach in selecting genotypes based on the multi-trait genotype-ideotype distance. On the other hand, the FAI-BLUP and SH indices generally projected higher genetic gains, with the SH index consistently showing the highest gains across all traits. This suggests that the Smith-Hazel classic index prioritizes specific traits more effectively in the selection process, leading to greater overall genetic improvement.

The integration of advanced selection indices, such as the MGIDI, FAI-BLUP, and Smith-Hazel, offers valuable tools for winter barley breeders. These indices facilitate the identification of superior genotypes and accelerate genetic gain in important traits.

Table 1. Predicted genetic gains for the indexes MGIDI, FAI-BLUP, SH.

Trait	Genetic value	Genetic gain (%)		
		MGIDI	FAI-BLUP	SH
SL	6.68±0.67	8.44	6.78	4.83
GWS	2.33±0.28	13.38	12.64	17.13
SNS	58.94±5.59	9.87	6.11	13.75
GNS	50.42±5.71	10.95	8.80	18.01
TGW	44.90±3.44	3.33	2.89	1.42
GY	6.51±1.34	15.50	13.66	24.57
Total		61.47	50.87	79.70

Conclusions

Multi-trait selection indices, such as MGIDI and FAI-BLUP, offer the potential for enhancing genetic gain in winter barley breeding programs. The Smith-Hazel selection index showed higher genetic gains compared to the MGIDI and FAI-BLUP indices. This indicates that the Smith-Hazel index effectively prioritizes specific traits, leading to greater overall genetic improvement.

The genotypes G21, G32, G38, G35, and G20 selected based on the three different indices demonstrated desirable trait combinations and have the potential for further evaluation and advancement in the winter barley breeding programs.

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THE IMPACT OF BIOSTIMULATOR ON WHEAT PROPERTIES: A CASE STUDY ON WELL-SUPPLIED SOIL IN THE 2021/2022 GROWING SEASON

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Abstract

Field crop production is under increasing pressure from abiotic and biotic stress conditions like climate change, drought, low soil fertility, soil acidity, ect. Biostimulators are physiologically active compounds that help plants cope with these stresses by improving their growth, development, and nutrition. In this paper we examine the impact of biostimulator compared to the conventional production on yield and others properties of winter wheat. The field experiment was conducted in 2021/2022 with two treatments: a control variant and a control variant + biostimulators. The experiment was repeated three times in a random spatial arrangement. The vegetation season during wheat production was favorable, with similar air temperatures and 15% less precipitation than the long-term average. In this experiment, analysis of variance showed that the biostimulator treatment had a significant effect on grain moisture, but not on the other properties measured. However, higher values were determined in the treatment with biostimulator for the yield, number of ears per m^2, the number of grains per spike and the hectolitre mass. The authors recommend further research on different soil types, including those with lower fertility, over several years. They also recommend testing the impact of biostimulators on grain quality in order to better understand their effects.

Keywords: *biostimulator, wheat, yield, properties, growing season.*

Introduction

Wheat is a major cereal crop that is grown all over the world and one of the most important cultivated plant species. It is irreplaceable in human nutrition, a very important raw material for various industries and as animal feed. According to the Food and Agriculture Organization of the United Nations, wheat is cultivated on an average of 217 million hectares worldwide, with an average yield of 3.48 tonnes per hectare and a total production of 756 million tonnes in the period 2016-2020 (FAOSTAT, 2022.). In Croatia, wheat is also an important crop. It is the second most cultivated crop in terms of sown area, but the harvested area has decreased slightly in recent years (Iljkić et al., 2019). According to FAOSTAT, the average wheat cultivation area in Croatia in the period 2016-2020 was 143,846 hectares, with an average yield of 5.67 tonnes per hectare and a total production of 816,101 tonnes. The success of wheat production depends on biological and environmental factors. The intensive production of wheat today is facing a number of challenges, including the increasing use of production inputs and increasingly unfavorable agroecological conditions (Marijanović *et al*., 2010.; Jug *et al*., 2018.). In addition to the usual agricultural practice, it is necessary to find cultivation models that would minimize the negative effects in production. The use of biostimulators in agricultural production is not recent, but it was mainly used in the production of very intensive crops like vegetables and flower growing, while it is less common for field crops. Biostimulators can be defined as physiologically active substances, that promote the growth,

development and nutrition of plants and help reduce the effects of stress caused by abiotic and biotic factors, but it should be emphasized that they are not a substitute for mineral fertilizers. Although there are various biostimulators, they usually contain a mixture of organic and inorganic components including essential macro and microelements, humates, citrates, amino acids and others (Čeh, 2020.). Also, microbial and non-microbial biostimulators are distinguished. Microbial biostimulators are beneficial fungi and bacteria, while non-microbial biostimulators are humic acids, seaweed extracts, amino acids, chitosan and inorganic biostimulants. The application is most often foliar, and depending on their purpose and the formulation of the preparation, they are applied in different phenological phases of the plant (Mešić *et al*., 2022.). Treatment with biostimulators is increasingly intensive in many areas in order to achieve a higher yield and better quality by improving the plant's physiological response to stressful conditions (Macra and Sala, 2022; Lozowicka *et al*., 2022). Therefore, the aim of the research was to examine the impact of biostimulators on the yield, agronomic and morphological properties of wheat in field conditions compared to the usual method of production.

Material and Methods

During 2021/2022, a field experiment was set up on the area of the field experimental station of the Faculty of Agrobiotechnical Sciences Osijek (45.482378, 18.803544) in Republic of Croatia, according to the scheme of a completely random spatial arrangement in three repetitions. The total area of the experiment was 300 m^2, and the experiment consisted of two treatments: control (standard mineral fertilization) and biostimulator application (control + biostimulator). According to the soil chemical analysis, the experiment was set on loamy soil texture with alkaline reaction (7.40 pH_{KCl}), 3.10% humus content, and very richly supplied with accessible phosphorus and potassium. The usual agrotechnical operations in intensive wheat production were applied. Maize was a previous crop. Sowing was done on October 28, 2021, with the Kraljica variety in the amount of 280 kg ha^{-1}. Considering the very good soil fertility, only 130 kg ha^{-1} of urea (46% of N) was added before sowing. The first top dressing at the tillering phase and second top dressing at the steam elongation phase was done with 100 kg ha^{-1} and 120 kg ha^{-1} of KAN (27% of N), respectively for both treatments. The treatment with the biostimulator was additionally foliar treated with Fertiactyl Starter (Timac Agro d.o.o.) on March 21, 2022 in the amount of 3 l ha^{-1}. It is a liquid root and shoot biostimulator containing humic and fulvic acids, cytokinin and glycine betaine. It also contains of 13% N, 5% P, 8% K and 5% C. Crop protection from weeds, diseases, and pests was timely and equal for both treatments. Just before harvest, wheat samples were taken from 1 m^2 for each repetition to determine yield (t ha^{-1}), grain moisture (%), the number of ears per m^2, 1000 grain weight (g) and hectolitre weight (kg hl^{-1}). Additionally, 30 plants per repetition were sampled to determine the number of grains per spike, spike length (cm) and plant height (cm). Furthermore, data of monthly precipitation (mm) and average monthly air temperatures (ºC) for the wheat growing season 2021/2022 were used, as well as long term mean 2002-2020 (LTM) of the State Hydrometeorological Institute for the weather station Osijek-Klisa, which is 2 km away from the experiment. Statistics were performed by individual analysis of variance using the F test, and the significance of differences was evaluated using the LSD value. The computer program SAS 9.1.4 was used.

Results and Discussion

In comparison with long term mean (LTM), the total amount of precipitation was less by only 15% (422 mm and 498 mm, 2021/2022 and LTM), while the average air temperature was almost identical to the LTM (9.5 ºC and 9.3 ºC for 2021/2022 and LTM) during the wheat growing season. The amount of precipitation at the sowing time was optimal, in the autumn period the amount of precipitation was above the LTM, while in the winter period it was below the LTM. Furthermore, during the phenological phases from tillering to grain ripening (April-June), the amount of precipitation was 24% lower, but a total of 161 mm of rain fell. In terms of average air temperatures, it should be noted the very mild winter and somewhat lower temperatures in March and April. In general, the growing year 2021/2022 was mostly favorable and wheat was not exposed to any stronger stress than usual.

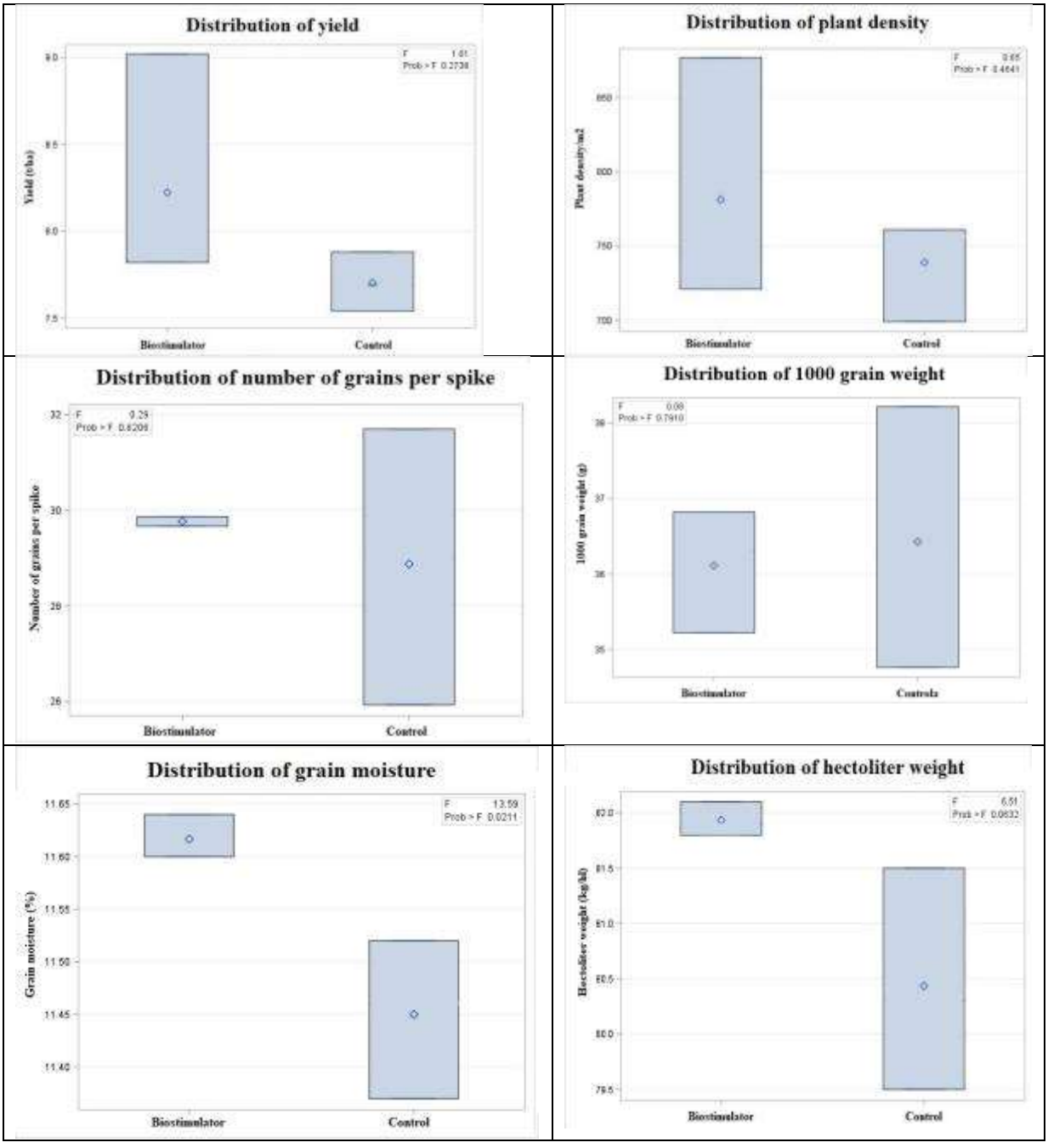
For the yield, yield components and other agronomic and morphological properties in this research, an analysis of variance was performed in order to determine the statistical significance of individual properties (Table 1). Of all the tested parameters, only grain moisture was significant. The highest F values, except for grain moisture, were also recorded for hectolitre weight and grain yield, while the coefficient of variation was the highest for the number of spikes m^2, number of grains per spike and yield (Table 1). The average grain yield in this experiment was a relatively high (7.96 t ha^{-1}), which is above the average of the Republic of Croatia (5.90 t ha^{-1}) according to Croatian Bureau of Statistics. Although the significance was not confirmed, the yield was higher by 520 kg or about 7% in the biostimulator treatment. Thus, the average yield on the control variant was 7.70 t ha^{-1}, and on the biostimulator treatment 8.22 t ha^{-1} (Graph 1). Parađiković (2009) asserts that the application of biostimulators increases the amount of nitrogen in the leaf, the level of photosynthesis and the concentration of plant pigments. Humic acids increase root growth and water intake and promote plant growth by introducing nutrients and binding toxic elements. Also, humic acids affect the improvement of photosynthesis, chlorophyll density and plant root respiration, which results in better plant growth. Amino acids increase the amount of chlorophyll in the plant, which affects the greater activity of photosynthesis, and they also act as activators of phytohormones and growth substances in plants. Although the use of biostimulators has undeniable advantages (Neta *et al.*, 2016; Cvijanović *et al.*, 2020; Beraković *et al*., 2021), this study lacked statistical significance, probably due to relatively favorable growing weather conditions and well-supplied soil.

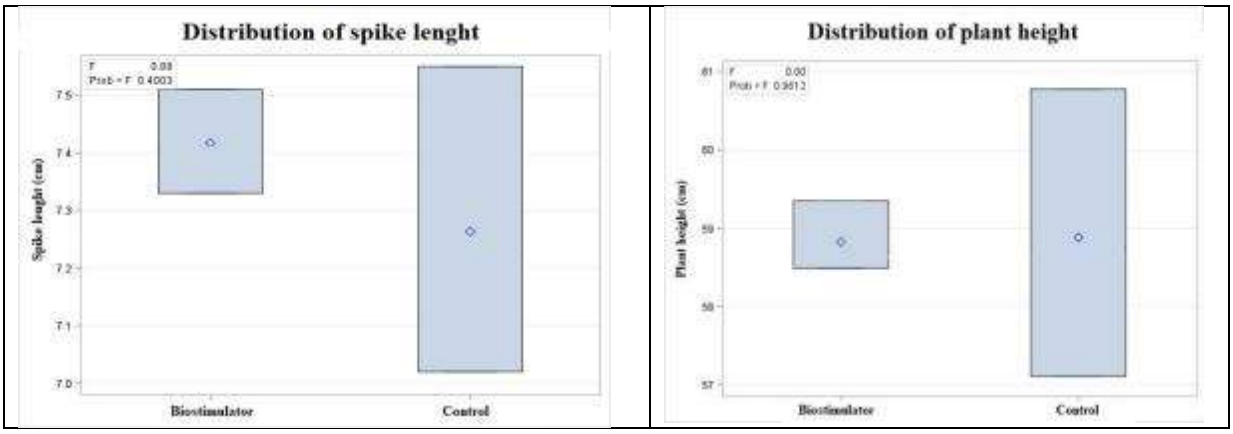
Table 1. Statistical analysis of the tested properties and average values for both treatment

Properties	**Average**	**F value**	**Pr > F**	**$LSD_{0.05}$**	**Coefficient of variation**
Yield (t ha^{-1})	7.96	1.61	0.2738	ns	6.310
Grain moisture (%)	11.5	1359	0.0211	0.125	0.480
Plant density/m^2	760	0.65	0.4641	ns	8.435
Number of grains per spike	29.3	0.29	0.6206	ns	6.965
1000 grain weight (g)	36.3	0.08	0.7910	ns	3.735
Plant height (cm)	58.9	0.00	0.9612	ns	2.276
Spike length (cm)	7.34	0.88	0.4003	ns	2.720
Hectolitre weight (kg hl^{-1})	81.2	6.51	0.0632	ns	0.886

ns – non significant

Yield components are very important properties and are directly related to grain yield. Although the yield components in this research were not statistically significant, certain variations were observed, with higher values being determined mainly in the treatment with biostimulator (Graph 1). For example, the number of ears per m^2 was higher by about 6%, the number of grains per spike by about 3%, and the hectolitre weight was higher by 1.5 kg hl^{-1} or about 2%. The other values were very similar between the treatments. Since Fertiactyl complex stimulates the development of the root system, increases the growth and development of the plant, and stimulates the absorption of nutrients and water during a stressful period, its stronger effect was absent because the plants were grown on fertile soil well supplied with the main mineral elements with relatively good weather conditions. The amount of precipitation in April was 49 mm, in May 55 mm, and in June 61 mm, while the average air temperatures were 10.9°C, 19.1°C and 23.4°C, for April, May and June. In such conditions, wheat is not exposed to excessive stress.





Graph 1. Box plot analysis of tested properties

Conclusions

Although it is known that biostimulators have a whole range of positive effects, in this research there was no statistical significance for the yield and most of the tested parameters. The main reason may be the lack of stressful conditions, given that the weather during the 2021/2022 wheat growing season was relatively favorable, and wheat was grown on soil of good fertility. However, slightly higher values were observed in the biostimulator treatment for yield, plant density, number of grains per ear, and hectolitre weight. Also, it is recommended to carry out research on different types of soils of lower fertility over several years, as well as testing the impact on grain quality in order to obtain clearer assumptions about the impact of biostimulators.

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EFFECT OF DIFFERENT FERTILIZERS ON SEED GERMINATION AND DEVELOPMENT OF SEEDLINGS OF ANNUAL DAHLIA

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Abstract

Dahlias are not hardy plants in Serbia and they are grown as annuals. For that reason, dwarf cultivars are the most popular, they can be easily propagated from seed, and they are used for summer flower beds, hedges or they are planted in containers. Fertilizers can affect germination and early development of seedlings and depending on the plant species they can promote or inhibit germination and growth. However, data about the effect of different fertilizers are usually not available for specific plants species and therefore it is important to examine their effect on plant growth before their use in the commercial plant propagation. Therefore, the effect of three commercial fertilizers available on Serbian market (EkoBooster 1®, Unistart and Bakterije) on germination and seedlings growth of Dahlia Dwarf Mix 3045 (produced by Semenarna Ljubljana) was investigated. The treatments were conducted before seed sowing and on 2nd, 3rd and 9th day after seed sowing. Obtained results showed that fertilizer type affected germination rate, the best result was achieved after the treatment with EkoBooster 1® (81.3%), while germination rate was lower after treatments with other fertilizers (72%) and in the control (56%). Despite expectations, none of the used fertilizers did not affect seedlings growth, because length of root, shoots and primary leaves were not significantly different compared to the control.

Keywords: *Mini Dahlia, dwarf dahlia, fertilizers, germination, propagation.*

Introduction

Dahlias are grown often as popular annual plants in Serbia. They have long blooming season, a wide range of colors, and they are suitable for colorful summer flower beds, hedges, but also they are planted in pots and different planters, suitable for small green spaces, windows and balconies (Vernon 2014; Armitage 2001). Cultivars of dwarf dahlias can be propagated from seed and this is the most common method for propagation of dahlias. For that reason, improving the propagation techniques, including germination and early seedling growth is important for dahlias propagation. Among the other factors, choosing the appropriate fertilizer is important part in improving propagation method, because fertilizers can affect germination and early development of seedlings and depending on the plant species they can promote or inhibit germination and growth (Delshadi et al. 2017; Bákonyi et al. 2013; Prayogo et al. 2021; Duraid et al. 2021). Although biofertilizers are already as an effective and environment-friendly alternative to the synthetic fertilizers and their role in enhancing productivity and quality of agricultural products have already been proven through various research work (Dasgupta et al. 2021; Gautam et al. 2021), there is no enough evidence of the effect on different plants growth of various commercial preparations available on the market. For this reason, the effect of commercial fertilizers available on Serbian market on germination and seedlings growth of Dahlia Dwarf Mix 3045 (produced by Semenarna Ljubljana) was investigated. Research was conducted using three different fertilizers, which were suitable for early seedling growth according to the product specifications. One of them (EkoBooster 1®)

contains only NPK macronutrients (14% Nitrate (N), 2% Phosphorus and 5% Potassium (K)), and two preparations are bio-fertilizers: Unistart and Bakterije. Unistart contains not-specified strains of proteolytic and cellulolytic bacteria that increase availability of carbon, nitrogen and phosphorus for plants, according to the product specifications, and Bakterije contains *Bacillus subtilis*, *Bacillus megaterium* and *Saccharomyces* spp. In this way the differences among bio-fertilizers and standard chemical fertilizers could also be observed during this research.

Material and Methods

The seeds of Dahlia Dwarf Mix sold by Semenarna Ljubljana (product code 3045) were used in this research. The seeds were sown in trays (dimensions 25 × 15 × 5 cm), into the mixture of peat, sand and perlite. Before sowing, the substrate was irrigated with prepared solutions of fertilizers and solutions were prepared according to the product specifications. Hence, the fertilizers were diluted in distilled water; EkoBooster 1® was applied as a 33% solution, Unistart as a 1% solution and Baketrije as 0.5% solution. After seeds sowing, the fertilizers were applied 3 more times, on the 2^{nd}, 3^{rd} and 9^{th} day after seed sowing, simultaneously with irrigation. Each tray was irrigated with 33 mL of prepared solution and each tray was containing 25 seeds of Dahlia Dwarf Mix. The seeds in the control treatment were irrigated with water without fertilizers. The trays were placed in the greenhouse of the Faculty of Forestry in Belgrade. During the experiment, temperature ranged between 16°C and 25°C in the long day conditions.

Three weeks after setting the experiment, the germination percentage as well as the length of shoots, roots, and leaves were recorded. Each treatment consisted of three replicates with 25 seeds each. Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p<0.05$) and the least significant difference (LSD) test.

Results and Discussion

Obtained results showed that fertilizer affected germination rate and germination was higher in all treatments compared to control (56%). However, fertilizer which contained contains only NPK macronutrients (EkoBooster 1) gave the best results (81.3% germinated seeds), while germination rate was lower after treatments with bio-fertilizers (72%) (Table 1). Positive effect of fertilizers containing bacteria on seed germination has already been proved for other plant species (Delshadi et al. 2017; Bákonyi et al. 2013), and also nitrogen, phosphorus and potassium fertilizer can influence and enhance germination rate for some plant species (Wenjie 2018). Also, Duraid et al. (2021) reported that the combination of organic fertilizer, bio-fertilizer and chemical fertilizer can gave better results than applying these fertilizers alone, which should be further investigated for the *Dahlia* cultivars.

Table 1 The effect of fertilizers on germination of *Dahlia* Dwarf Mix seeds

Treatment	Germination rate (%)
control	56.0[c]
EkoBooster 1	81.3[a]
Bakterije	72.0[ab]
Unistart	72.0[ab]

Values followed by different letters are significantly different at the P < 0.05 level according to the LSD test

Despite expectations, none of the used fertilizers did not affect seedlings growth (Table 2). The shoot length ranged between 50.8 mm and 58.3 mm, the average length of the longest root varied between 19.6 mm and 28.2 mm, but obtained differences were not statistically significant (Table 2). Similarly, percentage of seedling which formed first pair of true leaves was higher (82.3 – 91.3%) in all treatments compared to control (75%), but obtained differences also were not statistically significant (Table 2). The length of the primary leaves ranged between 21.2 mm and 24.7 mm and it was not influenced by fertilizer application.

Table 2 The effect of fertilizers on length of shoots, roots and true leaves of *Dahlia* Dwarf Mix seeds

Treatment	Shoot length (mm)	Longest root length (mm)	Presence of true leaves* (%)	Length of true leaves (mm)
Control	58.1[a]	28.2[a]	75.0[ab]	24.7[a]
EkoBooster 1	50.8[a]	26.1[a]	82.3[ab]	24.4[a]
Bakterije	55.1[a]	21.1[ab]	91.3[a]	23.9[a]
Unistart	58.3[a]	19.6[ab]	88.2[a]	21.2[a]

*The percentage of seedling which developed a first pair of true leaves
Values followed by different letters are significantly different at the P < 0.05 level according to the LSD test

Kering and Zhang (2015) showed that application of Nitrogen based fertilizers had no effect on early seedling growth for some plants, and similarly Nikmatullah et al. (2021) also reported that biofertilizers had no effect on plant growth depending on other environmental conditions for a plant growth (humidity, temperature). There is possibility that different composition and concentrations of fertilizers could enhance *Dahlia* Dwarf Mix seedling growth.

Conclusions

Dwarf Dahlia cultivars are popular annual ornamental plants in Serbia. They are commonly propagated from seed, but with the development and emergence of new fertilizers on the Serbian market, there was a need to investigate if they can be used in improving Dahlias production. The effect of three commercial fertilizers (EkoBooster 1®, Unistart and Bakterije) on germination and seedlings growth of Dahlia Dwarf Mix was investigated and obtained results showed that these fertilizers did not affected significantly early seedlings growth. However, NPK based fertilizer enhanced germination rate and the highest germination percentage was obtained after the treatment with EkoBooster 1® compared to other fertilizers and the control. There is a possibility that better results can be achieved with different fertilizer concentration and combination of different fertilizers which should be further investigated.

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EFFECT OF *AILANTHUS ALTISSIMA* ON PHOTOSYNTHETIC ACTIVITY, YIELD AND OLIVE OIL QUALITY CHARACTERISTICS UNDER TWO IRRIGATION REGIMES

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Abstract

Weeds compete with tree crops for water and nutrients. Additionally, they frequently act as hosts for major disease vectors. The aim of this study was to investigate the effect of the invasive tree and noxious weed tree-of-heaven [*Ailanthus altissima* (Mill.) Swingle] on photosynthetic activity, yield and olive oil quality characteristics of olive cv. Koroneiki, under two irrigation regimes, one full and one deficit, applying the 60% of the water volume of the first regime. One year old olive trees were used for this purpose. The olive trees were planted in the same pots with and without (control) *Ailanthus* plantlets. Photosynthetic activity measured in August was found to be increased in fully irrigated and control trees, while the SPAD index decreased in fully irrigated trees. Olive yield was not affected by the treatments. All olive oil quality indexes (peroxide number, acidity, UV absorbance indexes) were within the range for characterization of the olive produced as Extra Virgin Olive Oil (EVOO). In the absence of *Ailanthus* and in combination with full irrigation, the acidity of the olive oil increased. The presence of *Ailanthus* led to an increase in the K232 and K270 values and a decrease in the ΔK index. Full irrigation in combination with the presence of *Ailanthus* resulted in an increase in the K232 value and a decrease in the ΔK index. Full irrigation treatment and the presence of *Ailanthus* led to an increase in the concentrations of total polyphenols and antioxidant capacity measured in the olive oil. On the contrary, the above parameters were all reduced under *Ailanthus* absence combined with deficit irrigation.

Keywords: *SPAD, Acidity, Peroxides, Total Phenolics, Antioxidant Activity.*

Introduction

Native to the Eastern Mediterranean, olive (*Olea europaea* L.) is one of the world's oldest and most important crop (Connor, 2005; Talhaoui *et al.*, 2015; Kafkaletou *et al.*, 2021), with over 1250 varieties grown in over 54 countries for oil and/or table olives production (Ashraf *et al.*, 2021). Olive oil's high biological value, due to its monounsaturated fatty acids and antioxidant capacity, makes it an essential part of the Mediterranean diet (Daskalaki *et al.*, 2009). Olive is grown in about 90% of the Mediterranean basin, mainly in Spain, Italy and Greece (Ashraf *et al.*, 2021; Cabezas *et al.*, 2021), which represents 95% of worldwide cultivation (Cabezas *et al.*, 2021) and, due to its resistance to drought, can be grown in arid and semi-arid areas under adverse conditions (Alegre *et al.*, 1999, Kafkaletou *et al.*, 2021). The importance of olive cultivation indicates the need to investigate all possible stresses that may affect the growth and production of olive orchards.

Ailanthus altissima (Mill.) Swingle, or tree-of-heaven, is a fast growing, spreading tree of the Simaroubaceae family. Native to Asia, it was introduced to Europe and the USA in 1700 AD as an ornamental plant (Lawrence *et al.*, 1991; Heisey *et al.*, 1996; Demasi *et al.*, 2019). It produces allelopathic compounds that are toxic to other plant species (Heisey *et al.*, 1996; Demasi *et al.*, 2019), which, combined with its high seed production, development of an extensive root system, rapid growth and high tolerance to pollutants (Demasi *et al.*, 2019),

contributes to its high competitiveness and wide expansion (Heisey *et al.*, 1996; Demasi *et al.*, 2019).
Ailanthus is often found in high densities in olive groves, negatively affecting oil production. The objective of the present research was to investigate possible stress impacts caused by *Ailanthus* on the parameters of photosynthetic activity, yield and olive oil quality characteristics of olive cv. Koroneiki, the most important olive oil cultivation in Greece (Kafkaletou *et al.*, 2021).

Material and Methods

The experiment was carried out at the orchard of the Agricultural University of Athens. One year old olive trees were used. The olive trees were planted in the same pots (45L) with and without (control) *Ailanthus* (Figure 1 and 2). Two irrigation regimes were applied, one full and one deficit, applying the 60% of the water volume of the first regime. Olive trees were randomly divided into 5 replicates of two trees each, per treatment (control - C and presence of *Ailanthus* - A) and per irrigation regime (full - F and deficit - D). Parameters of photosynthetic activity (A - μmol/m²s, gs - mol/m²s, Ci - μmol/mol, E - mmol/m²s) and leaf chlorophyll (SPAD index) and fluorescence (Fv/m) were measured in August. Micro-oil extraction was carried out in the Laboratory of Pomology at harvest, in a semi-industrial oil mill of the Abencor type (cold malaxation - 26-28°C for 45 minutes).

Figure 1. Planting of olive tree in the same pot with Ailanthus (personal file).

Figure 2. Planting of olive tree in the absence of Ailanthus (personal file).

Yield (kg fruit/tree), oil content (%) and olive oil (O.O.) production (kg O.O./tree) were determined for each treatment. Acidity (g oleic acid/100 g olive oil), UV absorption indices (K232, K270), peroxide number (meq. active O_2/kg olive oil), total phenolic compounds (mg gallic acid/kg olive oil), total o-diphenols (mg caffeic acid/kg olive oil), total flavonoids (mg catechin/kg olive oil) and antioxidant capacity by three methods [DPPH - 2,2-diphenyl-1-picrylhydrazyl, FRAP - ferric reducing antioxidant powder and ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] (μmol Trolox/kg olive oil) were determined in olive oil.
The experiment followed a completely randomized design with 5 replicates. The raw data were subjected to multifactor ANOVA analysis and the significant differences of the effects of *Ailanthus* presence and irrigation regime were determined using the Student's t-test, while significant interactions were determined using the Tukey HSD test, at a significance level of α=0.05.

Results and Discussion

The presence of *Ailanthus* resulted in a significant decrease of all photosynthetic activity parameters, while deficit irrigation had the same effect but did not affect mesophyll conductance (Table 1), whereas it increased the SPAD index (Table 2). Combining deficit irrigation with *Ailanthus* also resulted in decreasing parameters, except for mesophyll conductance, which increased (Table 1). These results are in agreement with those reported by Denaxa *et al.* (2012), who also found a decrease on carbon assimilation rate and stomatal conductance, while the mesophyll conductance increased, in an experiment carried out on olive trees cv. Chondrolia Chalkidikis under two irrigation regimes, full and drought.
Deficient irrigation reduced the K232 value and increased the ΔK index, opposite to the results of the combination of full irrigation with *Ailanthus*. The presence of *Ailanthus* resulted in a significant decrease of oil acidity and of the ΔK index, while the K232 and K270 values increased. A decrease in acidity was also observed when deficit irrigation was combined with *Ailanthus* (Table 4). On the contrary, Stefanoudaki *et al.* (2009), in an experiment carried out on olive trees cv. Koroneiki, under two irrigation regimes, observed no statistically significant difference in olive oil quality parameters. The concentration of total phenolics, total o-diphenols and total flavonoids were increased under the full irrigation and *Ailanthus* treatments whereas they decreased by the combination of deficit irrigation and control. Similarly, in an experiment carried out by Patumi *et al.* (2002) on olive trees cv. Kalamata under three irrigation regimes (100% - full irrigation, 66% and 33% of the water volume of the former), total phenolics concentration was negatively affected by the 66% irrigation regime. The same effect as for total polyphenols concentration was observed for antioxidant capacity by all three methods used (DPPH, FRAP and ABTS) (Table 5). No further statistically significant differences were found.

Table 1. Effect of treatments on the parameters of the photosynthetic activity of the leaves.

	A (μmol/m²s)	gs (mol/m²s)	Ci (μmol/mol)	E (mmol/m²s)	A/Ci	A/E
Irrigation regime						
F (Full)	18.70 a	0.16 a	176.68 a	2.36 a	0.11 a	8.06 a
D (Deficit)	9.71 b	0.08 b	194.48 a	1.57 b	0.06 b	6.26 b
Treatment						
C (Control)	17.72 a	0.14 a	164.73 b	2.26 a	0.11 a	8.08 a
A (*Ailanthus*)	10.69 b	0.09 b	206.43 a	1.67 b	0.06 b	6.25 b
Treatment x Irrigation regime						
C x F	21.22 a	0.18 a	178.50 b	2.70 a	0.12 a	7.96 a
C x D	14.21 b	0.10 bc	150.95 b	1.81 ab	0.10 a	8.20 a
A x F	16.17 ab	0.13 ab	174.85 b	2.02 ab	0.10 a	8.17 a
A x D	5.22 c	0.06 c	238.00 a	1.33 b	0.02 b	4.32 b

Means within the same column followed by the same letter are not significantly different according to Student's t-test (for irrigation regime and ailanthus treatments) and Tukey's multiple means test (interaction) at a significance level of α=0.05.

Table 2. Effect of treatments on the chlorophyll content and the fluorescence of the leaves.

	SPAD	Fluorescence (Fv/m)
Irrigation regime		
F (Full)	74.38 b	0.83 a
D (Deficit)	82.62 a	0.82 a
Treatment		

C (Control)	78.97 a	0.83 a
A (*Ailanthus*)	78.02 a	0.83 a
Treatment x Irrigation regime		
C x F	75.14 a	0.84 a
C x D	82.80 a	0.83 a
A x F	73.61 a	0.83 a
A x D	82.43 a	0.82 a

Means within the same column followed by the same letter are not significantly different according to Student's t-test (for irrigation regime and ailanthus treatments) and Tukey's multiple means test (interaction) at a significance level of α=0.05.

Table 3. Effect of treatments on production parameters.

	Yield (kg/tree)	Oil content (%)	Olive oil production (kg/tree)
Irrigation regime			
F (Full)	0.12 a	18.45 a	0.02 a
D (Deficit)	0.08 a	15.81 a	0.01 a
Treatment			
C (Control)	0.09 a	17.30 a	0.02 a
A (*Ailanthus*)	0.11 a	16.96 a	0.02 a
Treatment x Irrigation regime			
C x F	0.10 a	18.10 a	0.02 a
C x D	0.08 a	16.50 a	0.01 a
A x F	0.13 a	18.79 a	0.02 a
A x D	0.08 a	15.12 a	0.01 a

Means within the same column followed by the same letter are not significantly different according to Student's t-test (for irrigation regime and ailanthus treatments) and Tukey's multiple means test (interaction) at a significance level of α=0.05.

Table 4. Effect of treatments on the quality characteristics of olive oil.

	Acidity (g oleic acid/100g O.O.)	K232	K270	ΔK	Peroxide number (meq. active O_2 /kg O.O.)
Irrigation regime					
F (Full)	0.27 a	1.40 a	0.16 a	0.00 b	16.7 a
D (Deficit)	0.23 a	1.30 b	0.16 a	0.01 a	18.3 a
Treatment					
C (Control)	0.28 a	1.26 b	0.15 b	0.01 a	17.5 a
A (*Ailanthus*)	0.22 b	1.44 a	0.17 a	0.00 b	17.5 a
Treatment x Irrigation regime					
C x F	0.29 a	1.25 b	0.15 a	0.00 ab	16.7 a
C x D	0.27 ab	1.27 b	0.15 a	0.01 a	18.3 a
A x F	0.25 ab	1.55 a	0.17 a	-0.01 b	16.7 a
A x D	0.20 b	1.33 b	0.17 a	0.00 ab	18.3 a

Means within the same column followed by the same letter are not significantly different according to Student's t-test (for irrigation regime and ailanthus treatments) and Tukey's multiple means test (interaction) at a significance level of α=0.05.

Table 5. Effect of treatments on total phenolics, total o-diphenols and total flavonoids content and on antioxidant capacity of olive oil according to the DPPH, FRAP and ABTS methods.

	Total phenolics (mg gallic acid/kg O.O.)	Total o-diphenols (mg caffeic acid/kg O.O.)	Total flavonoids (mg catechin/kg O.O.)	DPPH (µmol Trolox/kg O.O.)	FRAP (µmol Trolox/kg O.O.)	ABTS (µmol Trolox/kg O.O.)
Irrigation regime						
F (Full)	307.25 a	59.76 a	261.78 a	1227.88 a	1169.50 a	1160.08 a
D (Deficit)	232.42 b	43.87 b	185.64 b	858.93 b	865.42 b	959.34 b
Treatment						
C (Control)	211.63 b	48.49 b	184.24 b	846.66 b	877.58 b	963.70 b
A (*Ailanthus*)	328.05 a	55.14 a	263.17 a	1240.15 a	1157.34 a	1155.72 a
Treatment x Irrigation regime						
C x F	273.83 a	62.21 a	260.39 a	1209.77 a	1177.26 a	1145.67 a
C x D	149.42 b	34.77 c	108.10 b	483.54 b	577.91 b	781.73 b
A x F	340.67 a	57.31 ab	263.17 a	1245.98 a	1161.75 a	1174.49 a
A x D	315.42 a	52.97 b	263.17 a	1234.32 a	1152.91 a	1136.96 a

Means within the same column followed by the same letter are not significantly different according to Student's t-test (for irrigation regime and ailanthus treatments) and Tukey's multiple means test (interaction) at a significance level of α=0.05.

Conclusion

In conclusion, taking into account the parameters of photosynthetic activity, the *Ailanthus* had a significant negative effect on olive photosynthetic machinery, especially when it was combined with deficit irrigation. As for the yield parameters, they were not affected, while all olive oils from all treatments could be classified as extra virgin olive oil (EVOO) in accordance with Commission Regulation (EEC) No 2568/91, as amended by Commission Regulation (EU) No 2016/1784 of 30 September 2016.

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BIOSTIMULANTS EFFECT ON BALSAM CROP DURING ITS FIRST YEAR OF ESTABLISHMENT

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Abstract

Balsam (*Hypericum perforatum*) is a perennial crop which belongs to Hypericaceae family. It is worldwide known as Common St John's wort or Perforate St John's wort. It is a medicinal herb with antidepressant activity and strong anti-inflammatory properties. Balsam is an herbaceous perennial plant with extensive creeping rhizomes, erect stems and can reach about 1 m in height. It has opposite, sessile, elongated leaves, yellow-green in color, with scattered translucent spots of the glandular tissue. Its flowers reach five petals and are bright yellow in color with prominent black spots. The purpose of this study was to investigate the effect of different biostimulants on balsam's yield. The experiment was established in the experimental farm of the Agrotechnology Department of the University of Thessaly, based in Larissa area, on 18th of May 2022. Three treatments were applied (T1: Control, T2: seaweeds, auxin 1.0 mg/lt and cytokinin 0.031 mg/lt from *Ecklonia maxima*, T3: special formulation containing amino acids 10% w/v, pure protein 11.3% w/v, sucrose 22% w/v, UV filter 10% w/v) with 6 replications. Harvest was performed at the end of July manually and the samples were dried till constant weight. It was found that the topaz variety can produce a fresh yield of flowers higher than 1500 kg ha^{-1}, which corresponds to a dry yield of 500 kg ha^{-1}. Therefore, balsam cultivation seems to be a promising future crop, considering the obtained yield of its first growing year and the late establishment of the plants in the field.

Keywords: *Balsam, Biostimulants, yield, Medicinal plants.*

Introduction

Hypericum genus (Hypericaceae) is a well-known genus of vascular plants with more than 450 species of herbs, shrubs and trees from all over the world (Becker et al., 2016; Crockett and Robson, 2011). *Hypericum perforatum*, commonly known as St. John's wort, is a perennial herbaceous plant that has been used for centuries for its medicinal properties (Butterweck 2003; Crockett and Robson 2011; Nahrstedt and Butterweck 2010). Native to Europe, Western Asia, and North Africa (Nürk *et al.* 2013), this plant is characterized by its bright yellow flowers and numerous therapeutic benefits.

St. John's wort has gained significant attention in recent years due to its potential to alleviate symptoms of depression, anxiety, and other mood disorders, while it is one of the most often used medicinal plants in the world and is widely utilized as phytopharmaceuticals and nutraceuticals (Linde et al., 1996). With a rich history of traditional use and ongoing scientific research, *Hypericum perforatum* continues to be a subject of interest for those seeking natural remedies for various health conditions. There has been much research done on the chemistry and bioactivity of *Hypericum perforatum* L. Since the 1980s, this species has evolved from a traditional herb that was mostly harvested from the wild to a significant medical crop plant that is grown around the world. Pharmaceuticals and dietary supplements made from *H.*

perforatum are now ranked 37th among top-selling herbal goods and are about to be included to the top 40 items at international sales centers in the natural/whole-foods/lifestyle category (Barnes *et al.*, 2019).
Despite the fact that the aerial parts medical benefits are well understood (Walker *et al.*, 2005; Birt *et al.*, 2009; Saddiqe *et al.*, 2010; Caraci *et al.*, 2011), little is known about *Hypericum*'s cultivation and production.
Moreover, St. John's wort is mostly used as a food additive and for therapeutic uses, as was previously noted; thus, an attempt should be made to estimate production from wide cultivation utilizing organic methods. Such substances are the biostimulants, which are compounds with a biological origin or microorganisms that, when used on plants, promote the plants' natural activities (Yakhin *et al.*, 2017).
In recent years, there has been an interest in extracts derived from seaweed, largely due to research findings that highlight the presence of a diverse range of biostimulatory compounds within these intricate mixtures (Khan *et al.*, 2009; Du Jardin *et al.*, 2015). These seaweed-based biostimulants enhance root system growth, blooming, stress tolerance, nitrogen absorption, growth, and crop yield (Ali *et al.*, 2019). This has led to an increased attention and exploration of seaweed-based extracts as a potential tool in agricultural practices. These extracts are also beneficial for seed germination, plant development, and even post-harvest plant growth (Ali *et al.*, 2016). Improved root size and density lead to higher germination rates, seedling vigor, and final yield (Rayorath *et al.*, 2008).
Therefore, the purpose of this study was to investigate the effect of different biostimulants on *Hypericum perforatum* (topaz variety) yield during its first year of establishment as an extensive cultivation.

Materials and Methods

In order to estimate Balsams' yield, a field experiment was established in the experimental farm of the Agrotechnology Department of the University of Thessaly, based in Larissa area, on 18th of May 2022. The used treatments were three (T1: Control, T2: seaweeds, auxin 1.0 mg/lt and cytokinin 0.031 mg/lt from *Ecklonia maxima*, T3: special formulation containing amino acids 10% w/v, pure protein 11.3% w/v, sucrose 22% w/v, UV filter 10% w/v) with six replications. Harvest took place around the end of July manually. Afterwards the samples were air-dried till constant weight.
The statistical software GenStat (7th Edition) was used to perform an analysis of variance (ANOVA) on the collected data for all measured and derived variables. To assess the differences between means of the main and/or interaction effects, the LSD 0.05 test criterion was employed (Steel and Torrie, 1982).

Results and Discussion

According to the results presented in Table 1, significant differences were observed in the production of fresh inflorescences between the control group and treatment 3. Specifically, the control group exhibited a higher yield of 1753 kg FW per hectare compared to treatment 3, which had a lower yield of 1656 kg per hectare. Interestingly, treatment 2, which involved the application of seaweeds along with auxins (11.0 mg/lt) and cytokinins (0.031 mg/lt), resulted in significantly lower yield, of 1180 kg per hectare.
The aforementioned conclusion conflicts with the literature (not on balsam yield, because there is insufficient data), which shows that using seaweeds in a variety of aromatic and medicinal plants increases both their biomass and flowers yield (Giannoulis *et al.*, 2020a;

Giannoulis *et al.*, 2020b; Khan *et al.*, 2012 Spinelli *et al.*, 2009; Chouliaras *et al.*, 2009; Blunden *et al.*, 1996; Steveni *et al.*, 1992).

Table 1. Fresh and Dry Yield of Balsams' inflorescence.

	Fresh Weight (kg ha^{-1})	**Dry Weight (kg ha^{-1})**
T1	1753	555
T2	1180	423
T3	1656	540
$LSD_{.05}$	358,9	ns
CV (%)	18,2	19,1

In the case of the yield in dry weight of flowers, no statistically significant difference was observed between the studied factors, only a numerical superiority of treatments T1 and T3 over T2. Therefore, it was discovered that treatment with seaweed extracts did not result in greater dry weight of flowers as is often described in the literature, where it is reported that biostimulants using seaweed extracts boosts flowering and crop yield (Ali et al., 2019). Of course, the outcomes the next year are also anticipated as the balsam, a perennial plant, will strengthen the development of its root system during the first year of its installation.

The use of biostimulants in field crops is growing in popularity, according to the literature, in order to boost crop productivity and yield quality. A new breed of organic fertilizers called seaweed extracts (SWE) competes with both growth stimulants and traditional agrochemicals (Elansary *et al*., 2016; Sharma *et al.*, 2014). Since SWE may include both minor and major components, it may serve as a partial substitute for fertilizers, according to several research (Zodape *et al.*, 2010; Hong *et al.*, 2007; Dhargalkar and Pereira, 2005).

The sole explanation for the aforementioned findings is that balsam is a perennial plant, and as is well known, perennial plants make care to create a robust and healthy root system in the year of their establishment. As was already noted, the use of seaweed extracts results in the strengthening and expansion of the subterranean biomass, therefore it is anticipated that beginning in the second year, there will be variations in the above-ground biomass.

Conclusions

The alternative crop St. John's wort looks to be growing quickly. It recorded high outputs in both dry and green bloom from the very first year of establishment. The first year's results were not affected by the use of biostimulants, whereas the use of seaweed extracts appeared to have decreased yield.

The experiment is still ongoing since all treatments were repeated in the second year and the quality parameters are still being processed and analyzed.

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GROWTH AND FIBER YIELD OF HEMP UNDER DIFFERENT LEVELS OF IRRIGATION AND N-FERTILIZATION

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Abstract

The need to produce biodegradable and environmentally friendly industrial raw materials has stimulated the cultivation of Hemp (*Cannabis sativa* L.). Hemp is primarily cultivated for seeds, fibers, and medical substances but nowadays its parallel use as biomass and lignocellulose source is gaining ground. Water and nitrogen availability are inputs significantly affecting plant growth, development, and overall productivity. To evaluate the effects of irrigation, and nitrogen fertilization in growth of hemp and lignocellulosic biomass production, a field experiment was carried out during summer 2022 on the experimental farm of the University of Thessaly, in Velestino area, Greece. The experimental design was split-plot with irrigation (I1:33% ETo, I2: 66% ETo, I3: 100% ETo) as the main factor and nitrogen fertilization (N1:0 kg ha^{-1}, N2:50 kg ha^{-1}, N3:100 kg ha^{-1}) as the sub-factor. Irrigation was applied as proportion of the reference evapotranspiration ETo as it is calculated from Penman-Monteith equation. Throughout the growing season, four periodical harvests carried out were total dry weight, dry weight of stems and dry weight of fibers (t ha^{-1}) were measured. Experiment results demonstrated a statistically significant impact ($P < 0.05$) of irrigation on total, stem, and fibers dry matter while nitrogen show effect only during the first two harvests. Maximum total dry biomass weight reached 18.4 t ha^{-1}, while stem and fibers reached 8.53 and 3.66 t ha^{-1} respectively. Maximum biomass harvested at the end of crop cycle while maximum fiber yield was measured at earlier development stage, before maturity.

Keywords: *hemp, irrigation, N-fertilization, growth, fiber.*

Introduction

Hemp is a versatile agricultural product that can serve as a substitute or supplement for fiber or paper, while also offering environmental advantages and potential profitability within sustainable farming systems (Ehrensing, 1998). Hemp cultivation has experienced substantial growth in the European Union, with an impressive increase in both the cultivated area and production in recent years. From 1993 to 2018, the EU area dedicated to hemp cultivation expanded from about 7,000 hectares to 50,000 hectares. Among EU member states, France is the leading hemp producer in Europe, with the largest proportion of cannabis acreage at 37%, followed by Italy and the Netherlands with 8% each. In Greece, the percentage of acreage is less than 1%, but due to soil and climate conditions, this percentage has the potential to increase significantly (Mirizzi and Troyano, 2018).

Hemp is considered an easy to grow crop. It has fast growth rates, it efficiently suppresses weeds (Van der Werf, 1994) and the infections from biotic factors are limited. The crop produces high yield exceeding 25 t ha^{-1} of above ground dry matter depending on environmental conditions and agronomical practices. Although its high productivity potential,

in Europe productions from 12 to 25 t ha^{-1} are more likely to occur based on the local agronomical practices (Blandinières *et al*., 2023; Tang *et al*., 2017, 2016; Vera *et al*., 2004)
Water availability highly affects the growth and productivity of cannabis. It is considered a water-demanding crop from emergence to maturity with long term water stress periods causing leaf senescence, decreased leaf area, loss in fiber and seed yield, and reduced fiber quality. Hemp water needs per area of cultivation depend on climate conditions, soil properties and ground water level. In Spain, Garcia Tejero *et al.* (2014) estimated the water needs of hemp at 445 mm, while Bajić *et al*. (2022) estimated the needs at 520 mm.
Nitrogen fertilization tends to increase biomass production, but excessive amounts of nitrogen lead to weak stems, coarse fibers and stems with above optimum diameter indicating fibers with reduced breaking strength. In Netherlands, Italy and UK, nitrogen doses up to 160 kg ha^{-1} significantly increased total above ground dry matter while 220 kg ha^{-1} had small impact (Struik *et al*., 2000). In Greece, Papastylianou *et al.*, (2018) reported doses up to 240 kg ha^{-1} having positive effect in above ground biomass. Due to the variability in fertilization doses found in the literature, on-site research is required per area to determine the optimal amount (Tsaliki *et al*., 2021).
The aim of this study was to investigate the impact of varying irrigation and nitrogen fertilization doses on the biomass growth of hemp throughout its growth cycle in Central Greece. Determining the water and nitrogen fertilization needs of hemp we can aim for resource-efficient and environmentally responsible agricultural practices.

Materials and Methods

A field experiment was conducted during 2022 in the farm of the School of Agricultural Sciences of the University of Thessaly in Greece (longitude 22°45′29″ E, latitude 39°23′41″ N, elevation 77 m). The experiment was established in an alkaline clayey soil with organic matter content at 2.91% and pH at 7.6 at the top 30 cm of soil.

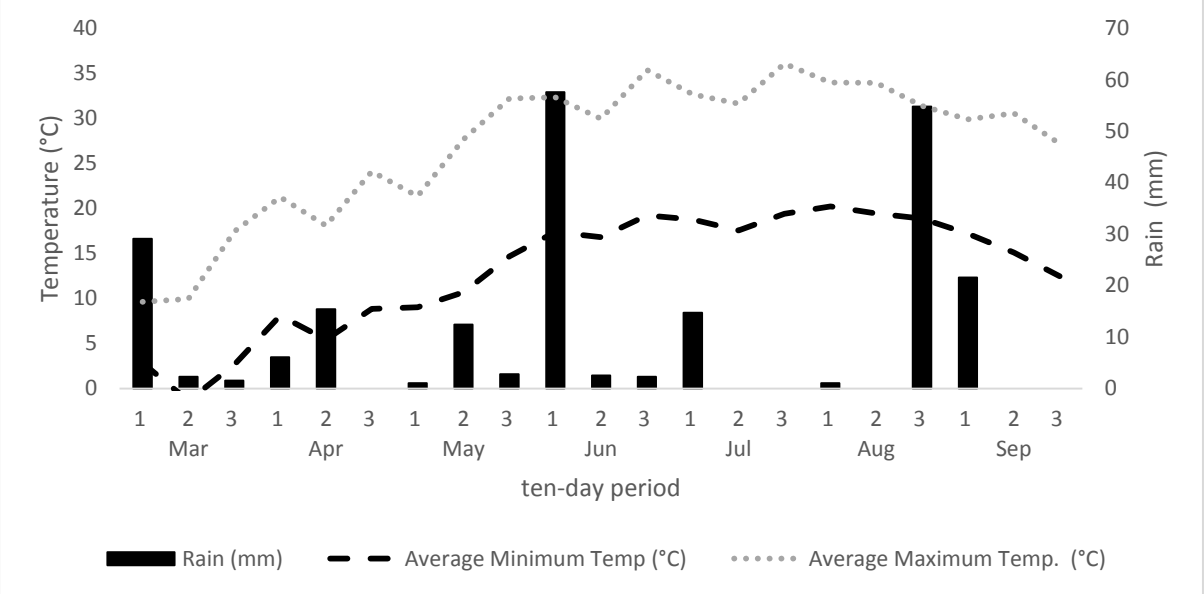

Figure 1. Weather conditions i.e., average minimum and maximum temperature (^{o}C), and height of rain (mm).

A split-plot design was employed with three irrigation levels (I1:33% ETo, I2: 66% ETo, I3: 100 ETo) and three nitrogen fertilizations (N1:0 kg ha^{-1}, N2:50 kg ha^{-1}, N3:100 kg ha^{-1}) replicated four times. The variety was Dioica originated from France. The variety was

selected not only for its productivity and availability but also based on its THC content (as required by EC regulation). The hemp was sown in early May with a wheat seeder machine. The seed rate was 30 kg ha^{-1} and the plant density 160 plants m^{-2}. Nitrogen was surface applied to the plots as necessary 40 days after emergence. The irrigation water was applied based on the potential evapotranspiration (ETo) as calculated with the Penman-Monteith evapotranspiration equation (Allen *et al.*, 1998). Climatic conditions during the experiment are shown in Figure 1.

The experimental area was drip-irrigated weekly during the growing period, no pesticides were applied, and weeds were hand-removed, as needed. During the experiment total above ground, stem and fibers, dry biomass was measured during periodical harvests. Harvests started in July and continued throughout the growing period. Harvest dates were H1: 18/7/2022, H2: 2/8/2022, H3: 19/8/2022 and H4: 21/9/2022. In each harvest date the biomass of 0.5 m^2 was harvested and the total fresh biomass was weighed. Sub-samples from each sample were taken for dry biomass determination. Leaves were separated from the stem and stems separated to bark fibers and core. Fibers and core, oven dried till constant weight and weighted. The effect of Nitrogen fertilization and irrigation on yields was tested by a standard analysis of variance (ANOVA). As test criterion for differences between means the $LSD_{0.05}$ was used (Steel and Torrie, 1960).

Results and Discussion

Nitrogen fertilization influenced ($P < 0.05$) the total and stem dry weight during the first two harvests and fibers dry weight, during the second harvest (table 1). During the first two harvests, total dry biomass productivity of N3 was the higher and differentiated from N1 and N2, while N1 and N2 stayed the same. Stem dry weight had the same trend as total dry weight with the N3 having the highest productivity. The only difference was that N2 was not significantly different than N1 and N3. Fibers show differences only during the 2nd harvest with N1 producing less than other two treatments. High nitrogen fertilization (N3) gave an early advantage to the crop increasing its biomass fast. Later on in growing period when plants of treatments N1 and N2 developed an extended root system they managed to utilize the available soil nitrogen, reaching the productivity of N3.

Table 1. Total, stem and fiber dry biomass per harvest date and least significant difference (LSD) for significance level equal to 0.05.

	Total dry weight (t ha^{-1})				Stem dry weight (t ha^{-1})				Fibers dry weight (t ha^{-1})			
Factor	H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4
I1	8.39	9.25a	11.19a	13.36a	3.39	3.83a	5.02a	6.41a	1.93	2.56a	2.38a	1.35a
I2	8.94	10.06a	14.21b	16.04b	3.51	4.30a	6.63b	7.50ab	2.15	2.57a	3.14b	1.71ab
I3	10.05	11.63b	16.90c	18.40c	4.06	5.02b	7.73c	8.53b	2.44	2.95b	3.66c	2.05b
$LSD_{0.05}$	*ns*	0.93	1.45	2.36	*ns*	0.56	0.98	1.41	*ns*	0.35	0.37	0.39
N1	8.56a	9.66a	13.47	14.10	3.55ab	4.26ab	6.10	6.94	2.07	2.42a	2.94	1.51
N2	8.58a	9.91a	14.30	16.00	3.37a	4.07a	6.77	7.37	2.10	2.78b	3.15	1.80
N3	10.22b	11.37b	14.53	17.69	4.03b	4.81b	6.51	8.11	2.35	2.88b	3.09	1.80
$LSD_{0.05}$	1.22	1.02	*ns*	*ns*	0.61	0.62	*ns*	*ns*	*ns*	0.28	*ns*	*ns*

ns: non-significant

Probably the high fertility status of the soil and the low needs of the crop resulted in this balance. The organic matter of the soil was high (2.91%) and apparently mineralized sufficient amount of nitrogen which combined with the nitrogen from fertilizer met the crop needs (Cabello *et al*., 2009; Girkin and Cooper, 2022). Maximum total dry biomass productivity reached 17.7 t ha^{-1} while stem productivity reached 8.11 t ha^{-1} and fibers 1.8 t ha^{-1}.

Irrigation significantly effect ($P < 0.05$) the total, stem and fibers dry weight in every harvest date apart from the first (table 1). During the first harvest date irrigation didn't show any effect on dry weights due to the small size of the plants and the water input from the rain, a fact that created uniformity among the irrigation treatments. From the second harvest date irrigation differentiated productivity. At 2nd harvest I3 total dry biomass differentiated from I1 and I2 giving 11.6 t ha^{-1} in contrast with 9-10 t ha^{-1} of I1 and I2. I3 kept this differentiation from I1 and I2 in the following harvests reaching 18.4 t ha^{-1} at the final harvest. I1 stayed low giving the lowest amount of dry biomass throughout the growing period reaching a final amount of 13.36 t ha^{-1}. Total dry biomass of I2 show an increasing trend. Till the second harvest date it statistically had the same productivity as I1. On the 3rd harvest date it statistically differentiated from I1 and I3 and at the 4th harvest its productivity increased to 16.04 t ha^{-1} remaining statistically different from I1 and I3. The same development was observed in the stem biomass.

Stem biomass of I1 was constantly the smallest and reached a maximum of 6.41 t ha^{-1} at the 4th harvest. Stem biomass of I2 was statistically equal to the biomass of I1 during first two harvests and differentiated from I1 and I3 at 3rd harvest while on the 4th harvest it reached a maximum of 7.5 t ha^{-1} not differentiating from I1 and I3. I3 always had the maximum productivity with 8.53 t ha^{-1} being the maximum. From the 2nd harvest it differentiated from I1 and I2 while in 4th harvest, stem biomass of I2 wasn't statistically different than I3's. Like stem dry biomass, fiber dry biomass developed accordingly with the difference that the maximum value of I1 was observed at 2nd harvest date and of I2 and I3 at 3rd. Maximum fibers dry biomass for I1, I2 and I3 was 2.56, 3.14 and 3.66 t ha^{-1} respectively.

Conclusion

The results demonstrated a significant impact of irrigation in total, stem, and fibers biomass of hemp. Increased water amount increased total and stem biomass and fiber weight. The fiber weight increased till August and then started to decline. Nitrogen effect biomass productivity only during the early stages of the crop with high nitrogen input giving an early advantage in biomass productivity. Later on the biomass productivity of all fertilization treatments were made even, probably due to the high organic matter content of the soil, which supplied the crop with enough nitrogen when it developed an extended root system.

Acknowledgements

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IRRIGATION AND FERTILIZATION EFFECT ON THE GROWTH OF A 2009 ESTABLISHED PERENNIAL CARDOON CROP

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Abstract

Cardoon (*Cynara cardunculus* L.) is primarily cultivated as a bioenergy crop due to its lignocellulose biomass. As a perennial crop with a deep root system, it absorbs nutrients from the soil, which are removed from the field during harvest, as long as with water used for evapotranspiration. The crop needs in nitrogen and water are rarely provided by the farmers influencing cultivation outcomes. Because proper nutrient and water management ensures the optimal plant growth and biomass yield, a field experiment was carried out during summer 2022 on a clay loamy soil in Greece (experimental farm of the University of Thessaly in Velestino) to evaluate the effects of four different irrigation and four different nitrogen fertilization levels in growth and biomass yield of cardoon. The used experimental design was a factorial split-plot with three replications, where the main factor was the irrigation and the sub-factor the N-fertilization. The total fresh and dry biomass yield (kg ha^{-1}) was measured throughout the growing period. It was found that nitrogen fertilization had a statistically significant effect throughout cardoon's growth. Although literature claims that *Cynara* can be cultivated for ten years, the current cultivation was on its 14^{th} growing year and the results have shown that the attained dry biomass output may be doubled when compared to the treatment with no inputs, reaching a yield of 11 tons per hectare.

Keywords: *cardoon, Cynara cardunculus, irrigation, N-fertilization, growth.*

Introduction

Cardoon, scientifically known as *Cynara cardunculus* L., is a perennial plant native to the Mediterranean region and belongs to the Asteraceae family (Wiklund, 1992). In the 1980s, researchers began exploring cardoon's potential as a perennial crop for lignocellulosic biomass production (Fernández and Manzanares, 1990). Since then, cardoon has been cultivated as an energy crop for solid fuel generation (Ierna *et al.*, 2012; Ierna and Mauromicale, 2010; Angelini *et al.*, 2009; Curt *et al.*, 2002; Foti *et al.*, 1999). Recently, there has been a shift towards using cardoon for biogas production (Ferrero *et al.*, 2020).
Raccuia and Melilli (2007) identified cardoon's key growth phases: shoot establishment in September, leaf rosette development from November to January, flower stem elongation in April to May, full bloom in June, fruit maturation in July, and aerial biomass drying in August. According to Cravero et al. (2012), cardoon shows rapid growth and can accumulate dry matter.
Cardoon is known for being a crop that requires relatively low inputs but can still achieve high biomass productivity with a high calorific value, as noted by Fernández *et al.* (2006). In terms of irrigation requirements, the FAO suggests a range of 450-1000 mm of annual rainfall. However, Fernández *et al.* (2006) found that cardoon was able to survive with as little as 280 mm of annual rainfall and was able to regenerate after such a season.

Mantineo *et al.* (2009) and Grammelis *et al.* (2008) studied the effect of nitrogen treatments on cardoon yield. However, little research has been done on the impact of complete fertilization programs (N, P, and K) commonly used on *C. cardunculus* (Fernández *et al.*, 2006) on biomass output, which can help evaluate the crop's sustainability more comprehensively.

Additional research is needed to understand the characteristics and performance of cardoon plants grown in the field for more than 12 years. Despite previous studies on cardoon yield, energy productivity, and soil-cleaning properties, there is a lack of specific research on older cardoon crops. Conducting a study to investigate the impact of irrigation and fertilization on the yield of established artichoke crops would be valuable in filling this knowledge gap.

Materials and Methods

Experimental Field

To assess cardoon's biomass production and potential for lignocellulosic biomass, a detailed field experiment was conducted. Cynara cardunculus was planted in 2009 at the University of Thessaly farm in Velestino, Magnesia.

For the purposes of the study, a factorial split-plot design was used, consisted of three replicates and twelve plots per replication. The primary factor was irrigation levels (I1, I2, I3, and I4) representing 0%, 33%, 66%, and 100% of the reference evapotranspiration (ETo), and the sub-factor was nitrogen fertilization levels (N1, N2, N3, and N4) representing 0, 50, 100, and 150 kg ha^{-1} of urine ammonia (46-0-0 composition). Each plot had dimensions of 10 m width and 4 m length, totaling 40 m^2.

To assess the growth and yield of cardoon plants, sampling was conducted at various development stages, specifically on the dates 18th July, 1st August, and 18th August. For each sampling event, the above-ground portions of the plants within a designated area of 0.5 m^2 (measuring 100 cm x 50 cm) were carefully harvested from each experimental plot.

From each plot, a representative plant sample was selected, and its fresh weight was measured to establish a baseline measurement. The samples were then divided into their respective components and prepared for drying, in an air dryer set at 59°C. This controlled drying environment ensured the removal of moisture from the samples while maintaining their structure and minimizing biomass loss.

Meteorological Data and Statistical Analysis

The meteorological data utilized in this study was sourced from the meteorological station located on the farm of the University of Thessaly in Velestino.

To analyze the collected data and draw meaningful conclusions, the statistical software GenStat (7th Edition) was used. The ANOVA allowed for a comprehensive examination of the data, enabling the identification of any significant differences between the investigated variables.

To evaluate the differences between means of the main and/or interaction effects, the $LSD_{0.05}$ test criterion was utilized (Steel and Torrie, 1982). By employing statistical analysis, any discrepancies between the investigated variables could be thoroughly examined and determined to be statistically significant rather than mere coincidences.

Results and Discussion

Weather Data

The study region is located in a Mediterranean climate zone. The recorded data for this study indicates that the maximum average air temperature during winter was 9.1°C, while the lowest recorded temperature was 2.0°C. During the sampling period, the maximum average air temperature reached 28.6°C, with the lowest temperature recorded at 25.4°C (Fig.1).
Additionally, the rainfall data for the region indicated fluctuating amounts of precipitation between each harvest period. Specifically, the recorded rainfall measurements were 312 mm, 0.7 mm, and 0 mm, respectively. These figures emphasize the occurrence of drought conditions during the summer months, particularly from mid-July to mid-August (Fig.1). The scarcity of rainfall during this period underscores the arid nature of the Mediterranean summer and its potential impact on agricultural practices.

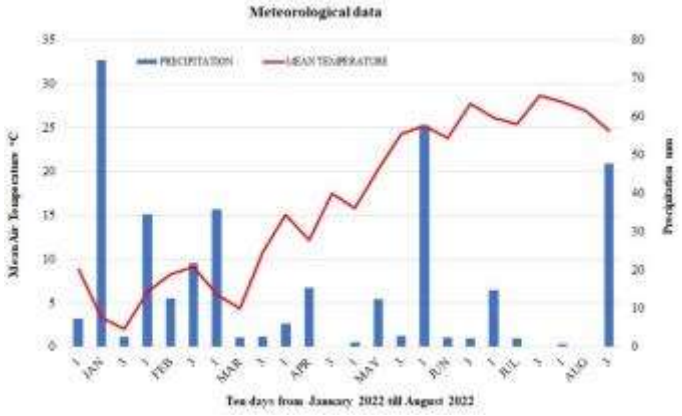

Figure 1. Temperature and precipitation (10-days mean values) occurring in studied site during the growing period of cardoon in 2022.

Plant Height

The higher plant height was recorded in August (2nd and 3rd sampling; 1.7 m) for the full irrigated treatments (Table 1). Plant height was significantly affected by fertilization input only at the first sampling and later on plants achieved the same height regardless fertilization. On the other hand, irrigation appears to affect plant height growth, the effect of which is clear in the 2nd and 3rd cut where no rainfall has occurred. The abovementioned height of the 1.7 meters is lower than that reported by Angelini et al., (2009).

Table 1. Plants height (m).

	1st Sampling	2nd Sampling	3rd Sampling		1st Sampling	2nd Sampling	3rd Sampling
I1	1,36	1,47	1,41	**I1N1**	1,39	1,51	1,46
I2	1,31	1,31	1,39	**I1N2**	1,17	1,49	1,33
I3	1,31	1,28	1,35	**I1N3**	1,56	1,41	1,42
I4	1,53	1,70	1,68	**I1N4**	1,31	1,46	1,43
$LSD_{.05}$	**ns**	**0,24**	**0,24**	**I2N1**	1,24	1,36	1,35
N1	1,36	1,50	1,49	**I2N2**	1,31	1,29	1,37
N2	1,34	1,36	1,41	**I2N3**	1,30	1,31	1,41
N3	1,48	1,41	1,47	**I2N4**	1,39	1,26	1,40
N4	1,32	1,47	1,46	**I3N1**	1,34	1,41	1,36
$LSD_{.05}$	**0,118**	**ns**	**ns**	**I3N2**	1,26	1,04	1,31

1,36	1,47	1,41	**I3N3**	1,56	1,30	1,35
1,31	1,31	1,39	**I3N4**	1,06	1,36	1,39
1,31	1,28	1,35	**I4N1**	1,47	1,73	1,80
1,53	1,70	1,68	**I4N2**	1,62	1,64	1,62
ns	**0,24**	**0,24**	**I4N3**	1,51	1,62	1,68
1,36	1,50	1,49	**I4N4**	1,50	1,79	1,63
			$LSD_{.05}$	**0,256**	**ns**	**ns**
			CV (%)	**10,2**	**9,8**	**8,1**

Biomass dry yield

In Central Greece, the crop yield during the last sampling period exceeded a mean of 7,616 kg ha^{-1}. Rainfed cardoon yielded 5,448 kg ha^{-1} during this period, while fully irrigated treatments yielded twice as much dry biomass as the rainfed yield (Table 2).

These findings align with a previous study conducted in Central and South Italy by Angelini et al. in 2009. The results from both studies demonstrate the positive impact of irrigation on crop yield, highlighting the importance of adequate water supply for cardoon cultivation.

The average dry yield in this study is about half of the yield reported by Foti *et al.* in 1999. It is worth noting that the literature references the cardoon's full productive years, specifically the 3rd and 4th years of growth. The current study might have been conducted during earlier growth stages or under different conditions, which could explain the difference in yields observed.

These findings highlight the importance of considering factors like irrigation and crop maturity when interpreting and comparing yield results. It is crucial to consider specific growth stages and environmental conditions to accurately assess and understand crop productivity.

In Basilicata, low yields were observed in rainfed conditions, as reported by Piscioneri *et al.* in 1999. The dry biomass yields ranged from 10,000 to 15,000 kg ha^{-1} during the third year of cultivation. This study highlights the challenges and limitations of rainfed cardoon cultivation in this region. Another study conducted under similar climatic conditions by Neri *et al.* in 2017 reported an average dry yield of approximately 22,000 kg ha^{-1}. These results are consistent with the yields obtained in Spain, as documented by Gonzáles *et al.* in 2004 and Ochoa and Fandos in 2004. These findings suggest that favorable climatic conditions can contribute to higher yields in cardoon cultivation. Those yield are higher than the double of the producing dry yield in our study, which is in agreement with the large-scale field experiment conducted in Portugal by Gominho *et al.* in 2014 reported a total dry biomass yield of 9,700 kg ha^{-1}. This study demonstrates the potential for achieving significant yields under suitable environmental conditions and with proper cultivation practices.

The results presented in Table 2 clearly indicate that both investigated factors had a notable influence on the final dry yield. Among the treatments, I3N4 and I4N4 exhibited the highest biomass production. It is worth noting that no statistically significant differences were observed between these two treatments. This finding suggests that the treatment with lower water supply, represented by I3N4, may be more advantageous when considering both environmental and financial aspects.

The implication of this result is that optimizing water usage in cardoon cultivation is crucial. Excessive irrigation, beyond what is necessary for optimal growth, may not necessarily lead to higher yields. By carefully managing water supply and avoiding unnecessary water usage, farmers can potentially reduce their environmental impact while also minimizing the associated financial costs.

This finding aligns with the broader concept of sustainable agriculture, where resource efficiency and environmental stewardship are prioritized. It highlights the importance of considering the specific water requirements of cardoon crops and tailoring irrigation practices accordingly. By adopting efficient irrigation strategies, farmers can strive to achieve satisfactory yields while minimizing the negative impacts on water resources and overall sustainability.

In conclusion, the results from Table 2 underscore the significance of optimizing water usage in cardoon cultivation. The treatments with lower water supply demonstrated comparable biomass production to those with higher water supply, emphasizing the importance of considering both environmental and financial costs in agricultural decision-making. This finding encourages the adoption of sustainable irrigation practices, aiming to strike a balance between crop productivity and resource conservation.

Table 2. Biomass dry yield (kg ha^{-1}).

	1st Sampling	2nd Sampling	3rd Sampling		1st Sampling	2nd Sampling	3rd Sampling
I1	6767	7717	5448	**I1N1**	6277	6816	5051
I2	6930	8329	6800	**I1N2**	4731	6745	5160
I3	7296	9526	7791	**I1N3**	7943	8592	5857
I4	7560	10376	10425	**I1N4**	8119	8714	5723
$LSD_{.05}$	**ns**	**1066,1**	**2270,2**	**I2N1**	6489	7315	6584
N1	6565	8357	6442	**I2N2**	6807	8517	6598
N2	6585	8665	7142	**I2N3**	7146	8676	6802
N3	7879	9197	8000	**I2N4**	7277	8807	7215
N4	7524	9726	8880	**I3N1**	6946	9100	4627
$LSD_{.05}$	**995,9**	**ns**	**878,0**	**I3N2**	7248	9245	6697
				I3N3	7984	9328	8419
				I3N4	7005	10429	11422
				I4N1	6548	10198	9505
				I4N2	7553	10153	10111
				I4N3	8442	10189	10922
				I4N4	7697	10966	11161
				$LSD_{.05}$	**ns**	**ns**	**2532,7**
				CV (%)	**16,6**	**27,6**	**13,7**

Conclusions

Indeed, the cultivation of wild artichoke (cardoon) beyond its 10th year of growth has shown the potential to produce high dry biomass yields. This indicates that the crop can continue to be productive over an extended period of time.

Providing additional water support during the spring and summer months, when there is a lack of rainfall, has been found to be beneficial for cardoon cultivation. This practice has been shown to double the production of dry biomass compared to rainfed conditions. It highlights the importance of irrigation in maximizing crop yield and addressing water scarcity during critical growth stages.

Furthermore, fertilization with 100-150 kg of nitrogen per hectare has been identified as necessary for optimal cardoon cultivation. Nitrogen is an essential nutrient for plant growth and development and supplying it in adequate quantities can enhance biomass production.

Considering cardoon as a perennial crop that primarily grows during the winter months and relies on winter rainfall is promising, especially for semi-mountainous areas that are experiencing abandonment. These regions often face challenges in finding suitable crops due to their unique climatic and geographical conditions. Cardoon's ability to thrive in such areas, coupled with its perennial nature, can provide economic opportunities, and help mitigate land abandonment.

In summary, the cultivation of wild artichoke (cardoon) beyond its 10th year of growth has shown potential for high dry biomass production. Providing water support during dry months and fertilizing with nitrogen are important factors in maximizing yield. Additionally, cardoon's suitability for semi-mountainous areas offers a promising opportunity for sustainable agriculture and land utilization in regions experiencing abandonment.

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EFFECT OF IRRIGATION AND FERTILIZATION ON GROWTH OF A BEYOND 10 YEARS OLD SWITCHGRASS CROP

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Abstract

Switchgrass (*Panicum virgatum* L.) is an important perennial biomass-producing crop, yielding up to 18 Mg ha^{-1} of dry biomass. Switchgrass biomass has high cellulose and lignin content, making it suitable for bioenergy and materials production. Harvesting the biomass yield of switchgrass annually removes nutrients from the soil. The soil nutrient depletion combined with insufficient precipitation limits crop productivity. Accurate information on irrigation and nitrogen fertilization inputs is necessary to provide switchgrass with the best possible growing circumstances, as these two factors affect biomass productivity. The aim of this study was to determine the effect of three different irrigation and three different nitrogen fertilization levels on its lignocellulosic biomass production. The experimental design was a split-plot with three replicates where irrigation was the main factor and N-fertilization was the sub-factor. Throughout the growing period, destructive samplings were carried out to measure total fresh and dry biomass (kg ha^{-1}). According to the findings, nitrogen fertilizer was the factor that affected biomass yield. The average dry biomass yield ranged about 10 tons per hectare, with a moisture content of 65-70%, in the middle of summer, while at the final harvest (October) the average yield increased up to 19 t ha^{-1} and the moisture content was about 50-55%. Although switchgrass cultivation was beyond its tenth year of growth, the harvested yield maintained at high levels, making the crop a dynamic perennial solution for lignocellulosic biomass production.

Keywords: *Switchgrass, Panicum virgatum, irrigation, N-fertilization, lignocellulosic.*

Introduction

Switchgrass, scientifically known as Panicum virgatum L., is a perennial grass used for energy production (Pedroso *et al.*, 2014; McLaughlin and Kszos, 2005; Wright, 1996) in Europe and America. It has a lifespan of 15-20 years (Rösch *et al.*, 2009; Monti *et al.*, 2009) and can be converted into thermal and lignocellulosic products (Bouton, 2008). Originally from North America, switchgrass is grown on marginal croplands and conservation reserve program sites in the United States. This cultivation strategy supports the US government's 2030 bioenergy objective of replacing 30% of petroleum with biofuels (Dohleman *et al.*, 2009; Heaton *et al.*, 2004).

Parrish and Fike (2005) found that switchgrass as a bioenergy crop is a new concept. Sanderson *et al.* (2006) believed that managing switchgrass agriculturally would be similar to forage management in early research.

Switchgrass has several advantages over other biomass crops. Firstly, it has a high net energy production per hectare potential. It is also a cost-effective option for bioenergy production due to minimal production costs, particularly for seed establishment. Switchgrass exhibits excellent water-use efficiency, enabling it to thrive in various water availability conditions.

Moreover, it has low nutritional requirements, reducing the need for excessive fertilizer or nutrient inputs. With its low ash level, switchgrass is suitable for combustion processes and minimizes residue after energy extraction. Lastly, switchgrass has wide geographic adaptation, as noted by Wright and Turhollow (2010), Parrish and Fike (2005), Christian and Elbersen (1998), Sanderson *et al.* (1996), and Samson and Omielan (1992). This enhances its potential as a bioenergy crop by allowing cultivation in diverse climates and geographic areas. Switchgrass generally builds up its biomass in the late spring and summer in areas with arid Mediterranean climates, which are typified by protracted and severe summer droughts. According to Vamvuka *et al.* (2010), the timing of biomass accumulation can present a substantial barrier to maintaining crop output and survival. This issue has been supported by earlier research by Giannoulis and Danalatos (2014) and Vamvuka *et al.* (2010), which showed a considerable decline in biomass productivity as a result of the prolonged and severe drought conditions.

To ensure high biomass productivity, irrigation is necessary in regions with similar climates to the Mediterranean basin, where water scarcity and high evapotranspiration rates limit crop growth. Heaton *et al.* (2004) studied the response of switchgrass and other perennial grasses to water, temperature, and nitrogen levels. They found a positive correlation between biomass yield and growing season rainfall (Jager *et al.*, 2010) and nitrogen. Wullschleger et al. (2010) used a parametric yield model to establish a connection between biomass yields and factors such as N fertilization, growth season precipitation, and annual temperature. Their model accounted for approximately one-third of the observed data's overall variance.

Switchgrass's nitrogen fertilizer needs as a bioenergy crop vary depending on location, soil nitrogen levels, harvest frequency, and management. Previous studies (Lemus *et al.*, 2009; Mulkey *et al.*, 2006; Thomason *et al.*, 2004) highlight this variation. Adjusting nitrogen application based on specific site and soil conditions is crucial for maximizing biomass production and nitrogen use efficiency. Factors like soil nutrient content, availability, and switchgrass variety requirements should be considered (Parrish and Fike, 2005).

Although several significant studies on the biomass and energy productivity of switchgrass in the Mediterranean environment have been carried out (Alexopoulou *et al.*, 2008; Mantineo *et al.*, 2009; Giannoulis and Danalatos, 2014; Pedroso et al., 2014), there is still a dearth of literature on the impact of irrigation and fertilization on switchgrass established for more than 10 years as well as on its productivity.

Materials and Methods

Experimental Field

In order to estimate biomass production and ultimately use it to produce lignocellulosic biomass, a field experiment was conducted on a switchgrass crop planted in 2010 on the University of Thessaly farm in Velestino, Magnesia, at coordinates 39°23′ N and 22°45′ E.

A factorial split-plot design with three replicates (blocks) and nine plots per replication was used to accomplish the study goals. The sub-factor was the varied nitrogen fertilization levels (N1: 0, N2: 50, N3: 100 kg ha^{-1}, using urine ammonia 46-0-0), while the primary factor was the different irrigation levels (I1: 33, I2: 66, and I3: 100% ETo). Each trial plot had a 55 m^2 area, measuring 11 m in width and 5.5 m in length. The study's overall objective was to offer useful information on the effects of various irrigation and nitrogen fertilization levels on crop output in a Mediterranean soil-climate system.

Plants were sampled at various stages (1/8, 18/8, 20/9, 31/10) to evaluate switchgrass crop growth and yield. From each experimental plot, the above-ground parts of the plants within a 0.5 m^2 area were cut for sampling. A representative plant sample was selected and weighed

for fresh weight before drying. The initial total fresh weight was recorded. The samples were divided into components and dried using an air dryer at 59^{o}C.

Soil Characteristics

The experimental field's soil is classified as *calcixerollic xerochrept* (USDA, 1975) and is highly productive, with organic matter levels of 2.91% at 0–30 cm and 1.86% at 30–60 cm. It is an alkaline clayey soil with pH values of 7.63 and 7.9 at 0–30 cm and 30–60 cm, respectively.

Meteorological Data and Statistical Analysis

The meteorological data comes from the meteorological station of the Department of Agriculture, Plant Production and Rural Environment, which is installed on the farm of the University of Thessaly in Velestino.

Finally, using the statistical software GenStat (7th Edition), an analysis of variance (ANOVA) for all measured and derived variables was performed on the obtained data within the sample durations. The $LSD_{0.05}$ test criterion was used to evaluate the differences between means of the main and/or interaction effects (Steel and Torrie, 1982). By using statistics, the data could be thoroughly examined, and any discrepancies between the investigated variables that were found would be statistically significant and not just coincidental.

Results and Discussion

Climatic Data

The study region has a Mediterranean climate with distinct seasonal variations. Winters are cold and humid, while summers are hot and dry. The average air temperatures recorded during each harvest season were 27.9°C, 26.9°C, 23.8°C, and 15.7°C, respectively. These temperatures reflect the contrasting seasons in which the harvests occur.

Rainfall varied among harvests, with measurements of 117.4 mm, 0.7 mm, 68 mm, and 38 mm, respectively. These figures highlight fluctuating precipitation patterns. Summer months, especially mid-July to mid-August, have the lowest rainfall, while the rest of the year is better for cultivation.

Increased rainfall, particularly from August onwards, balances irrigators' performance in the region. This ensures sufficient water supply for agriculture. Consequently, non-summer months become more conducive to cultivation due to increased water availability.

In summary, the study region has a Mediterranean climate with distinct seasons: cold, humid winters and hot, dry summers. Recorded temperatures and rainfall during harvest seasons illustrate contrasting conditions throughout the year. Summer scarcity is offset by increased precipitation in other seasons, creating a more favorable environment for agriculture.

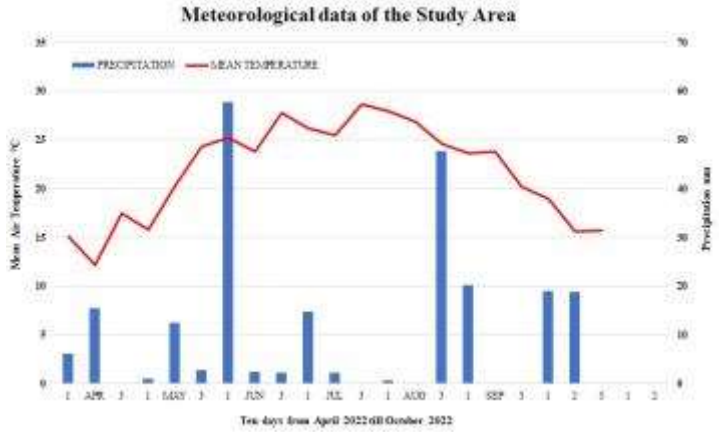

Figure 1. Temperature and precipitation (10-days mean values) occurring in studied site during the growing period of switchgrass in 2022.

Switchgrass Height

The treatment of the 50 kg ha-^{1} N-fertilization and the medium irrigation (I2N2; Table 1) resulted in the greater plant height (1.97 m), which was seen in the most recent sample. During its growth, irrigation and fertilizing input had no discernible impact on plant height. As previously noted in the chapter (climatic data), this occurred as a result of the excessive precipitation that occurred throughout the growth season. This height was similar to the height that reported (Piscioneri *et al.*, 2001) for the Alamo cultivar (maximum plant height of 1.9 m). Switchgrass' height increased almost linearly until flowering in each treatment, when stem elongation ceased due to transition to the reproducing stage. The highest rate of height increase was almost 2.09 cm day^{-1}.

Table 1. Switchgrass height (m).

	1st Sampling	2nd Sampling	3rd Sampling	4th Sampling
I1	1.14	1.37	1.76	1.80
I2	1.21	1.27	1.66	1.86
I3	1.16	1.42	1.70	1.91
$LSD_{0.05}$	**ns**	**ns**	**ns**	**ns**
N1	1.14	1.32	1.65	1.89
N2	1.13	1.33	1.72	1.84
N3	1.24	1.40	1.76	1.80
$LSD_{0.05}$	**ns**	**ns**	**ns**	**ns**
I1N1	1.06	1.46	1.70	2.00
I1N2	1.15	1.28	1.73	1.61
I1N3	1.20	1.37	1.85	1.79
I2N1	1.21	1.16	1.61	1.81
I2N2	1.12	1.24	1.61	1.97
I2N3	1.31	1.40	1.77	1.80
I3N1	1.16	1.35	1,64	1.85
I3N2	1.11	1.48	1.81	1.92
I3N3	1.21	1.43	1.66	1.95
$LSD_{0.05}$	**ns**	**ns**	**ns**	**0.333**
CV (%)	**11.2**	**11.2**	**9.0**	**6.8**

Biomass dry yield

The middle irrigated and fertilized treatment had greater average dry biomass (25531 kg ha^{-1}, I2N3; Table 2). In comparison to yields reported in other investigations (Boyer *et al.*, 2013; Paligwende *et al.*, 2011; Lemus *et al.*, 2002), this yield is greater. The production reported above is really impressive, especially considering that switchgrass was in its eleventh growing season.

In addition, it was discovered that irrigation level only significantly affected the dry biomass of switchgrass during the first cut (Table 2), which occurred on August 1; subsequent samplings, which were impacted by high rainfall from that point on, showed no significant statistical differences from irrigation.

On each sampling, however, a substantial difference for fertilization was discovered. In particular, it was discovered that only the control differed from the fertilized treatments during the initial sample. However, from that point on, all fertilization levels varied from one another, with a greater nitrogen level producing a higher biomass production. The above results totally agree with a previous study conducted in central Texas (Lemus *et al.*, 2008; Stroup *et al.*, 2003), where was reported that nitrogen had higher effect on growth than irrigation in yield of the same switchgrass' variety.

In the middle of the summer, the average dry biomass output varied between 10 and 19 tons per hectare, with a moisture level of between 67% and 70%. By the time of the final harvest in October, the average yield had climbed to 19 t ha^{-1} and the moisture content had decreased to between 50 and 55%.

Table 2. Biomass dry yield (kg ha-1).

	1st Sampling	**2nd Sampling**	**3rd Sampling**	**4th Sampling**
I1	10036	13745	16140	20992
I2	10821	13054	16941	21292
I3	11446	15786	17498	21353
$LSD_{0.05}$	**691,3**	**ns**	**ns**	**ns**
N1	9329	11347	13588	17296
N2	11184	14378	16627	22266
N3	11789	16858	20364	24076
$LSD_{0.05}$	**1217,6**	**1267,0**	**2933,6**	**1487,7**
I1N1	8596	11962	14586	19485
I1N2	10223	12356	16200	20831
I1N3	11288	16916	17636	22661
I2N1	9592	10223	12741	15708
I2N2	11400	12824	17103	22636
I2N3	11472	16114	20979	25531
I3N1	9799	11857	13436	16694
I3N2	11931	17954	16579	23330
I3N3	12607	17546	22479	24036
$LSD_{0.05}$	**ns**	**2667,2**	**1633,3**	**3121,2**
CV (%)	**11,0**	**8,7**	**9,4**	**6,8**

Conclusions

Switchgrass (cv. Alamo), which produced high dry biomass (25531 kg ha^{-1}) even at low irrigation levels (66% ETo), seems to be a plant that may produce considerable quantities of biomass under the soil-climatic conditions of central Greece in the eleventh year after its establishment. Additionally, it was shown that nitrogen fertilizer was the factor that had a statistically significant influence on biomass output regardless of the climate where it was applied.

Although switchgrass cultivation had reached its tenth year of development, the crop's excellent harvested yield made it a dynamic, promising alternate perennial option for the production of lignocellulosic biomass with little irrigation inputs. Future land use systems in Greece and the wider Mediterranean region should seriously explore including it.

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OLIVE OIL QUALITY CHARACTERISTICS UNDER ORGANIC AND INTEGRATED MANAGEMENT IN MESSINIA COUNTY GREECE

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Abstract

Olive oil is considered as one of the main components of the Mediterranean diet. During the last decades, intense interest has arisen in the consumption of organically produced products, due to the belief that these are of better quality than the ones produced under integrated management. The present trial aimed to assess the quality characteristics of olive oils produced under organic and integrated management. Three organically and three integrated managed olive groves were selected, of 'Koroneiki' cultivar in Messinia county, southern Greece. Olive fruits were harvested from four trees of each orchard and the oil was extracted in a micro-olive oil extraction machine. The fruit oil percentage, acidity, UV-specific absorbance values (K232 and K270), peroxide values, total phenolic compounds, and antioxidant capacity were assessed. There was not any significant difference concerning the oil percentage of the fruit, the olive oil acidity, K232, K270, peroxide value, total phenolic compounds, total o-diphenols, and antioxidant capacity based on diphenyl picryl-hydrazyl assay between organic and integrated grown trees. Integrated produced olive oil presented higher values of antioxidant capacity based on ferric reducing antioxidant power assay as well as higher flavonoids content. There were significant differences among orchards cultivated under the same management, indicating that it is not only the management itself (organic or integrated) that results in differences in the quality indexes assessed, but the agronomical practices of each specific grower that distinguishes olive oil properties.

Keywords: *Acidity, Antioxidant Capacity, Peroxide Values, Total Phenolics.*

Introduction

During the last few decades, intense interest has arisen in the benefits of a healthy diet and environmental sustainability, on a local as well as on a global scale (Reganold and Wachter 2020). Organic agriculture is based on the cultivation of plants (horticultural sector) based on natural resources, excluding conventional inputs such as synthetic fertilizers, pesticides, and any kind of pharmaceuticals (Reganold and Wachter 2020).

Within this frame, the olive tree, the most significant fruit tree in the Mediterranean basin in terms of both socioeconomic and environmental impact, is best suited for organic cultivation. Its products (table olives and olive oil) are the cornerstone of the Mediterranean diet, which is famous for its health benefits. During the last decade, olive oil has gained even more reputation due to the health claim that has been achieved, due to its richness in valuable polyphenols and other antioxidant compounds (Lombardi *et al.* 2021; Bellumori *et al.* 2019; Martín-Peláez *et al.* 2013).

The olive tree is an evergreen perennial tree that is cultivated under a variety of pedoclimatic conditions, producing adequately even under harsh conditions, such as drought, high temperature, soil salinity, etc, depending on the cultivar. One of the most important olive oil cultivars worldwide is 'Koroneiki', which is well suited to both irrigated and rainfed

conditions. Under proper cultivation practices, it produces Extra Virgin Olive Oil (EVOO), highly appreciated by consumers.
The present trial aimed to investigate the effect of integrated and organic cultivation practices on the productivity of 'Koroneiki' olive trees and the quality of olive oil produced.

Materials and Methods

The trial was conducted in three organically managed and three integrated managed olive groves of the cultivar 'Koroneiki'., in Southern Greece, in the region of Kiparissia, Messinia county.
Eight trees of similar growth and yield were selected per orchard, with two trees comprising one plot, i.e. there were four replications of two trees per orchard. In the mid of November, the trees were harvested and a sample of 1 kg per plot was transferred to the laboratory. The olive oil was extracted using a laboratory-scale olive mill (Callis Company, Athens, Greece). The paste was put in the mixer for 35 min under 26±2 ^{o}C and afterward centrifuged for 3 min. The olive oil was collected and stored in 50 mL falcon-type tubes filled to the top to minimize air presence till analyses. The olive oils were analyzed for free acid concentration (expressed as a percentage of oleic acid), peroxide value (expresses as meq. O_2/kg oil), and specific extinction values (K232 and K270) based on EU Commission Regulation N 2568/91/EEC (Commission 1991). Polyphenols were isolated through liquid-liquid extraction with methanol/water (80: 20 vol/vol) solution (Cecchi *et al.* 2018). The total phenols (TPHEN, as mg gallic acid equivalents Kg^{-1} oil), the total o-diphenols (oDs, as mg caffeic acid equivalents Kg^{-1} oil), and the total flavonoids (FLOIDS, as mg caffeic acid equivalents Kg^{-1} oil) antioxidant capacity (as μmol Trolox Kg^{-1} oil) based on the diphenyl picryl hydrazyl assay (DPPH) and on the ferric reducing power assay (FRAP) were determined according to (Roussos *et al.* 2009). Different samples of olive oils were examined after 6 and 12 months of storage under ambient temperature in a dark place, stimulating household storage conditions.
The trial was designed as a pseudoreplicated trial with three replications (one orchard was assigned as one replication of either organic or integrated management and within each orchard, four samples were taken). Differences among cultivation managements employed were determined using the Student's T test, while differences among farms under the same management were determined based on LSD multiple range test at α=0.05. Principal component analysis was performed to investigate possible relationships and discrimination among cultivation management as well as among farmers, employing the same cultivation management (organic or integrated).

Results and Discussion

There were not any significant differences between the two cultivation management based on the measured variables presented in Table 1. The olive oils produced presented typical characteristics of EVOO under both organic and integrated management, while the oil percentage per fruit was typical of 'Koroneiki' cultivar.

Table 1. Effect of orchard management on the yield, oil percentage per fruit, free olive oil acidity, K232, K270 and peroxide value indexes.

Management	Yield (kg/tree)	Oil percentage (% w/w)	Free acidity (% oleic acid)	K232	K270	Peroxide value (meq. O_2/kg)
Integrated	29.11a	23.82a	0.34a	1.919 a	0.179 a	9.6a
Organic	40.95a	25.97a	0.42a	1.648 a	0.169 a	12.9a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on Student's test at α=0.05.

Olive oils produced under integrated management presented higher values of total flavonoid concentration and higher antioxidant capacity based on FRAP assay, compared to organically produced oils (Table 2).

Table 2. Effect of orchard management on the total phenol, total o-diphenol and total flavonoid concentration in the oil and its antioxidant capacity based on DPPH and FRAP assays.

Management	Total phenols	Total o-diphenols	Total flavonoids	FRAP	DPPH
Integrated	330.55a	53.71a	475.48a	1431.12a	1400.19a
Organic	283.19a	40.82a	378.37b	974.83b	1180.00a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on Student's test at α=0.05.

There were significant differences among olive oils produced under organic management from the different groves assayed (Table 3). Farm 1 presented the highest values of oil percentage per fruit but also the highest free oil acidity.

Table 3. Differences among organically managed farms (OF) on the yield, oil percentage per fruit, free olive oil acidity, K232, K270 and peroxide value indexes.

Farm	Yield (kg/tree)	Oil percentage (% w/w)	Free acidity (% oleic acid)	K232	K270	Peroxide value (meq. O_2/kg)
OF 1	24.26b	31.15a	0.49a	1.754a	0.147b	16.3a
OF 2	11.99c	18.91c	0.32b	1.557a	0.242a	6.3b
OF 3	51.07a	21.40b	0.28b	1.633a	0.112c	16.3a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on LSD test at α=0.05.

Similarly, farm 1 presented the highest total o-diphenols concentration while oils produced from farm 3 exhibited low values of total phenols and o-diphenols (Table 4).
Oils produced from the integrated farm 3 presented higher values of K232 and K270 indexes while farm 1 harvested fruits exhibited the highest olive oil percentage per fruit (Table 5).

Table 4. Differences among organically managed farms (OF) on the total phenol, total o-diphenol and total flavonoid concentration in the oil and its antioxidant capacity based on DPPH and FRAP assays.

Farm	Total phenols	Total o-diphenols	Total flavonoids	FRAP	DPPH
OF 1	292.60ab	81.21a	368.73a	840.49b	1320.60ab
OF 2	368.76a	21.91b	405.32a	1254.38a	1416.45a
OF 3	190.40b	19.33b	361.06a	936.35b	999.61b

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on LSD test at α=0.05.

Table 5. Differences among integrated managed farms (IF) on the yield, oil percentage per fruit, free olive oil acidity, K232, K270 and peroxide value indexes.

Farm	Yield (kg/tree)	Oil percentage (% w/w)	Free acidity (% oleic acid)	K232	K270	Peroxide value (meq. O_2/kg)
IF 1	20.31c	28.64a	0.35a	1.848b	0.150b	8.8a
IF 2	43.49b	25.49b	0.35a	1.772b	0.127b	7.5a
IF 3	59.05a	23.77b	0.32a	2.139a	0.260a	12.5a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on LSD test at α=0.05.

Olive oils produced from integrated managed farm 2 exhibited the lowest total phenol and total o-diphenol content as well as the lowest antioxidant capacity, based on DPPH assay (Table 6).

Table 6. Differences among integrated managed farms (IF) on the total phenol, total o-diphenol and total flavonoid concentration in the oil and its antioxidant capacity based on DPPH and FRAP assays.

Farm	Total phenols	Total o-diphenols	Total flavonoids	FRAP	DPPH
IF 1	375.37a	64.45a	558.61a	1660.06a	1412.21b
IF 2	214.88b	18.05b	374.65a	1162.44a	957.93c
IF 3	401.41a	78.63a	493.20a	1470.85a	1830.45a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on LSD test at α=0.05.

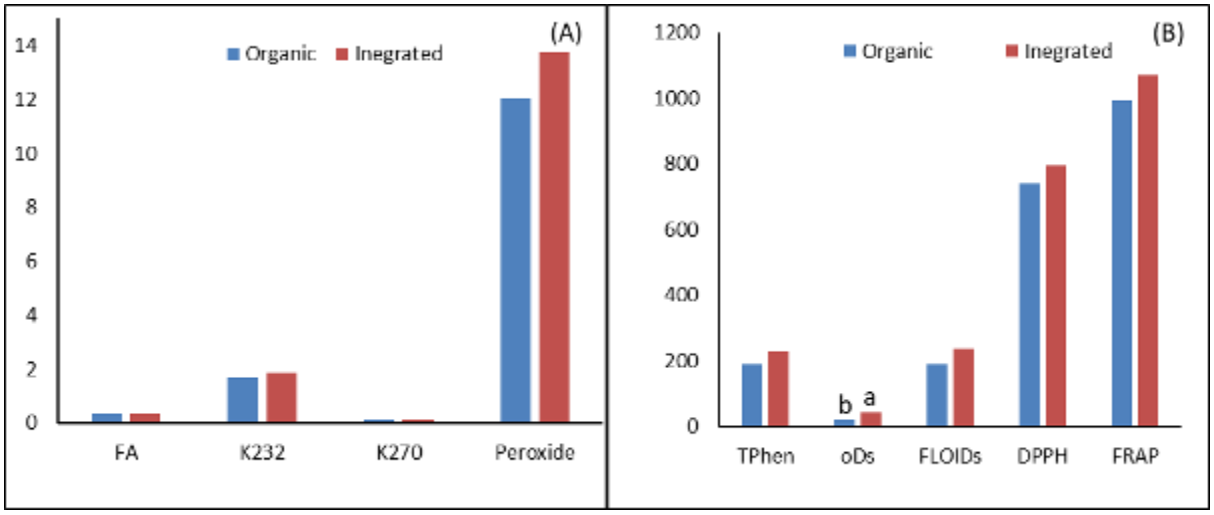

Figure 1. Effect of cultivation management on the quality characteristics of olive oil six (6) months after extraction. Different letters above columns indicate significant differences among cultivation management based on Student's T-test at α=0.05. The absence of lettering indicates non-significant differences. TPhen, total phenols, oDs, total o-diphenols, FLOIDS, total flavonoids.

Olive oils produced under the two cultivation managements did not differ a lot after six months of household storage, as the only difference observed was the superiority of integrated produced olive oil concerning the total o-diphenols concentration (Figure 1).
Twelve months after household storage (Figure 2) olive oil produced under integrated management exhibited higher values of K232 and K270 and lower concentration of o-diphenols.
The following figures (Figures 3-4) present the scatterplots of the principal component analysis during harvest, where it is obvious that olive oils present similar characteristics, produced either under organic or integrated management, and cannot be distinguished by each other. This can be ascribed to the fact that olive oils from different farms managed under the same cultivation practice (either organic or integrated) presented significantly different characteristics. These differences within the same management may be enough to diminish any possible differences that could be found between organic and integrated produced olive oils. The differences observed in the present trial as well as the similarities between integrated and organically produced olive oils have been described in the literature (Volakakis et al. 2017; García-González et al. 2014; Volakakis et al. 2022; Jimenez et al. 2014)

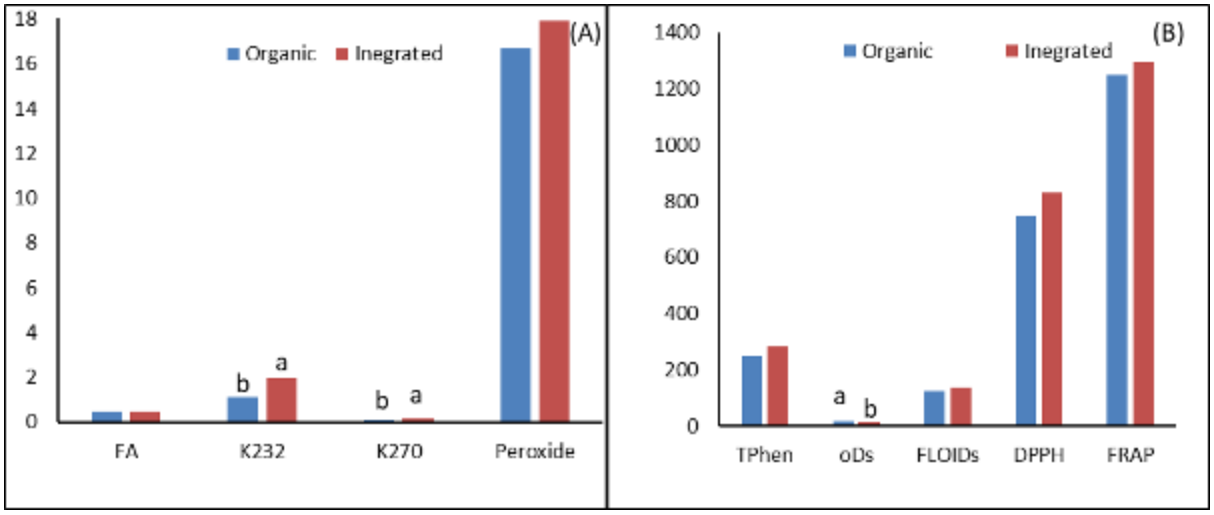

Figure 2. Effect of cultivation management on the quality characteristics of olive oil twelve (12) months after extraction. Different letters above columns indicate significant differences among cultivation management based on Student's T-test at α=0.05. The absence of lettering indicates non-significant differences. TPhen, total phenols, oDs, total o-diphenols, FLOIDS, total flavonoids.

Conclusion

There were significant differences between the two management practices as well as among orchards cultivated under the same management, indicating that it is not only the management itself (organic or integrated) that results in differences in the quality indexes assessed, but the agronomical practices of each specific grower that distinguishes olive oil properties.

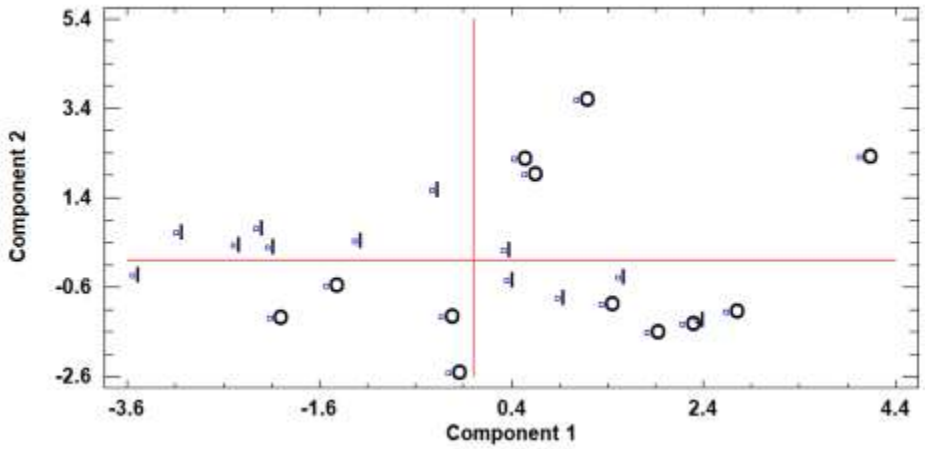

Figure 3. Scatterplot presentation of the principal component analysis of the data derived during harvest among organic (O) and integrated (I) produced oils.

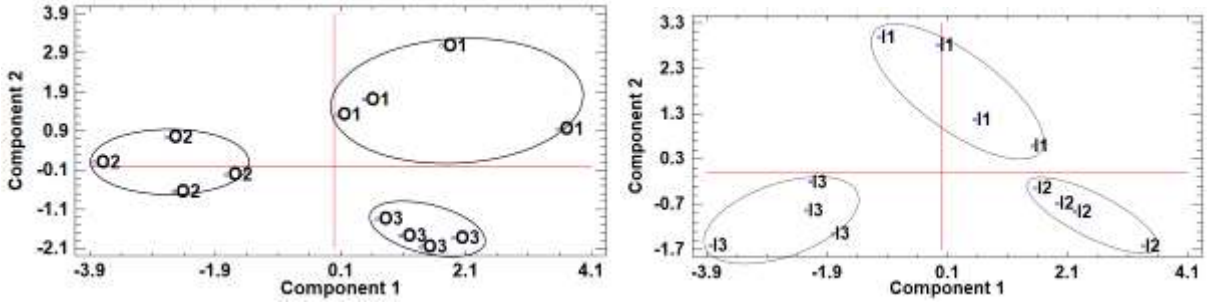

Figure 4. Scatterplot presentation of the principal component analysis of the data derived during harvest among organic (A) and integrated farmers (B).

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INVOLVEMENT OF EXOGENOUS COMPOUNDS APPLICATION ON OLIVE CUTTINGS ADVENTITIOUS ROOT FORMATION

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Abstract

The objective of this study was to evaluate the effectiveness of exogenously applied carbohydrates, phenolic compounds, polyamines, and oxidative enzyme inhibitors in enhancing adventitious rooting in leafy cuttings of the recalcitrant olive cultivars 'Kalamata', 'Throumpa', and 'Megareitiki'. The results showed that none of the tested compounds significantly improved rooting during summer (data not presented). In the 'Kalamata' cultivar during autumn, 5% glucose, 10 mM sodium azide (NaN_3), and 1 mM potassium cyanide (KCN) increased the rooting rate by 17%, 15%, and 13%. The highest overall rooting for 'Kalamata' (25%) was achieved with 0.1 mM chlorogenic acid (CGA) resulting in approximately 525% increased rooting performance compared to the control. Similarly, the application of 0.5 mM CGA and 0.1 mM rutin exhibited 22% and 23% rooting, respectively. In 'Throumpa', both 10% glucose and 10% mannitol improved rooting ability by 18% and 19%, while NaN_3 or KCN did not have any significant effect. The highest rooting percentage (26%) was observed with the application of 1 mM rutin, representing a 67% increase compared to the control. Additionally, in 'Megareitiki', the highest rooting percentage (29%) was observed with the application of 0.1 mM CGA or 0.5 mM rutin, representing a 190% increase compared to the control. Similarly, the application of 0.5 mM and 10 mM putrescine improved rooting ability at about 24% to 26%. In conclusion, it was found that sugars application impacted greatly olive cuttings' adventitious rooting. However, CGA and rutin seem to have been involved in a more prominent way in promoting root initiation in the recalcitrant-to-root cultivars.

Keywords: *Carbohydrates, Phenolics, Potassium cyanide, Putrescine, Sodium azide*.

Introduction

Rhizogenesis is influenced by numerous factors, but the interaction between these factors is not well understood, and the molecular mechanisms underlying the process are still unknown (Legué *et al.*, 2014). It is widely accepted that auxins are the primary factors involved in root formation and changes in their endogenous concentration are associated with the different physiological stages of rooting (Gaspar *et al.*, 1997). However, there are several other factors such as polyamines, phenolic compounds, carbohydrates, and oxidative enzymes, among others, that appear to play a role in regulating the process of adventitious root formation (Li *et al*., 2009).

In our previous studies (Denaxa *et al*., 2012, 2014, 2019 and 2020), significant differences were observed between the 'Arbequina' and 'Kalamata' olive cultivars during the early stages of rhizogenesis in terms of their biochemical compounds concentration and oxidative enzymes activity. Specifically, the easy-to-root 'Arbequina' cuttings exhibited higher concentrations of carbohydrates, polyamines, and phenolic compounds, including chlorogenic acid (CGA) and rutin, while showing lower levels of oxidative enzymes activity than the recalcitrant-to-root 'Kalamata' cultivar. Therefore, the objective of the present study was to

evaluate the efficacy of exogenously applied carbohydrates, phenolic compounds, polyamines, and inhibitors of oxidative enzymes in improving the adventitious rooting performance of recalcitrant-to-root olive cultivars, such as 'Kalamata', 'Throumpa' and 'Megareitiki'.

Materials and Methods

The mist propagation unit at Kostelenos Nurseries, situated in Troizinia area (Poros), Greece, was used to conduct the experiments during the years 2012-2014. For the rooting experiments two seasons, summer (July) and autumn (November) were chosen. The sub-apical cuttings, which were 7-10 cm long and had four leaves on them, were selected from the current year's shoots of 15-year-old olive mother plants, with a diameter of approximately 5 mm. The cultivars selected for the study included the easy-to-root 'Arbequina' and the recalcitrant ones, namely 'Kalamata', 'Throumpa', and 'Megareitiki'.
The bases of cuttings were treated with one of the following solutions:
a. CGA or rutin at concentrations 0.1 mM, 0.5 mM, 1 mM, and 10 mM initially dissolved in 10 mL ethanol and then in distilled water (final volume 1 L).
b. Aqueous solution of putrescine at concentrations 0.1 mM, 0.5 mM, 1 mM and 10 mM.
c. Aqueous solution of sodium azide (NaN_3) or potassium cyanide (KCN) at concentrations 1 mM, 5 mM and 10 mM.
d. Aqueous solution of sucrose or glucose or mannitol at concentrations 0.3%, 1%, 5%, and 10% w/v.
The cuttings' bases (approximately 1 cm) were initially dipped in one of the above solutions for 30 min (a to c), or 18 hours (d), left to dry superficially, and then treated for 5 s with 2.000 mg L^{-1} indole-3-butyric acid (IBA) dissolved in 45% v/v ethanol in water. Some of the cuttings were treated only with 2.000 mg L^{-1} IBA for 5 s and served as control (no exogenous compounds application).
Following the exogenous applications, the cuttings were planted in plant plugs (Preforma Plug/240 M1413 Vecol, Jiffy, the Netherlands) and subsequently placed under an automated mist unit. During summer, the cuttings were misted for 20 seconds every 6 minutes, while in autumn, they were misted for 20 seconds every 12 minutes. The average temperature of the air and the rooting medium during summer were 30.1 °C and 26.8 °C, respectively. In contrast, the temperatures during autumn were 21°C and 19°C for air and rooting medium. After three months, at the end of the rooting period, the percentage of rooted cuttings was recorded.
The experiment was arranged as a completely randomized design, utilizing four replications of one propagation tray containing 120 cells per replicate. In total, 57,600 cuttings were utilized, with 14.400 cuttings per each cultivar (4 replications x 120 cuttings x 30 treatments x 4 cultivars). The statistical analysis was performed using JMP 7.0 statistical software (SAS Institute, NC, USA). Data of cuttings with callus or roots were analyzed as one-way ANOVA. Significant differences were determined according to Tukey HSD multiple range test at $\alpha = 0.05$.

Results and Discussion

IBA treatment was almost ineffective to induce adventitious rooting in the recalcitrant-to-root 'Kalamata' cultivar, resulting thus only in a 4% rooting rate (Table 1). Similarly, 'Throumpa' and 'Megareitiki' had low rooting rates of 15% and 10%, respectively, solely with the application of auxin. The very low rooting ability (less than 30%) is the reason why researchers have characterized these cultivars as recalcitrant-to-root. The failure of auxin to

stimulate rooting in these cultivars suggests that other physiological and/or biochemical factors, as well as differences in anatomical structure, may also play a role in this process (Denaxa *et al*., 2019). Furthermore, during the summer season, all tested exogenous applications did not significantly improve the rooting performance of the recalcitrant cultivars, with rooting percentages ranging from 1-10%, and in some cases, zero. Therefore, the data are not presented.

Despite extensive research, the role of carbohydrates in promoting adventitious rooting is still debated among researchers. Aslmoshtaghi and Shahsavar (2010), as well as Denaxa *et al*. (2012), have reported a positive correlation between the rooting ability of olive cuttings and their carbohydrate content, as the easy-to-root cultivars exhibited higher levels of carbohydrates compared to difficult-to-root ones. However, Izadi *et al.* (2016) found no relationship between total soluble sugar content and the rooting ability of cuttings from five olive cultivars ('Rowghani', 'Tokhmekabki', 'Konservalia', 'Amigdalolia', and 'Dusti'). Furthermore, Porfirio *et al*. (2016) reported that exogenously applied carbohydrates have a positive impact on rooting performance in cuttings with low endogenous carbohydrate reserves.

Table 1. Influence of exogenously applied carbohydrates on adventitious rooting in 'Kalamata', 'Throumpa', 'Megareitiki' and 'Arbequina' cuttings during the autumn experimental period.

Treatments	Kalamata		Throumpa		Megareitiki		Arbequina	
	Cl	Rt	Cl	Rt	Cl	Rt	Cl	Rt
IBA 2000 mg L^{-1}	32 ab	4 ab	26 a	15 ab	22 a	10 bcd	5 a	94 a
Sucrose 0.3%	16 b	8 ab	27 a	3 c	25 a	0 d	5 a	93 a
Sucrose 1%	21 b	8 ab	22 a	6 bc	35 a	11 bc	5 a	94 a
Sucrose 5%	44 a	0 b	27 a	4 bc	40 a	15 abc	1 a	99 a
Sucrose 10%	48 a	14 a	42 a	20 a	45 a	18 ab	8 a	92 a
Glucose 0.3%	18 b	7 ab	31 a	2 c	20 a	5 cd	9 a	90 a
Glucose 1%	30 b	5 ab	34 a	9 abc	41 a	3 cd	5 a	91 a
Glucose 5%	33 ab	17 a	28 a	10 abc	35 a	13 bc	2 a	96 a
Glucose 10%	38 ab	11 a	34 a	18 a	30 a	20 a	10 a	97 a
Mannitol 0.3%	49 a	1 b	28 a	5 bc	28 a	4 cd	4 a	92 a
Mannitol 1%	30 ab	8 ab	35 a	5 bc	31 a	10 bc	6 a	93 a
Mannitol 5%	37 ab	7 ab	40 a	14 ab	42 a	17 abc	3 a	95 a
Mannitol 10%	49 a	11 a	38 a	19 a	40 a	20 a	2 a	98 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's HSD test, at a = 0.05. Abbreviations: Cl, cuttings with callus; Rt, rooted cuttings.

In the present study, the exogenous application of 5% glucose in the 'Kalamata' cuttings resulted in a 17% rooting, whereas 10% glucose or 10% mannitol led to an 11% rooting rate (Table 1). Additionally, both 10% glucose and 10% mannitol significantly enhanced the rooting ability of 'Thoumpa' cuttings, resulting in 18% and 19% rooting rates, respectively. Similarly, these sugars yielded a 20% rooting rate in 'Megareitiki', while 'Arbequina' had over a 90% rooting rate with all the tested sugars (Table 1).

According to Calamar and de Klerk (2002), the type of applied sugar significantly influences root regeneration, particularly in microcuttings. Moreover, Denaxa *et al*. (2012) suggested that glucose and mannitol have a greater impact on the rhizogenesis of olive cuttings than

sucrose and starch. Haissig (1982) also reported that exogenously applied sucrose, with or without auxin, enhances the rooting potential of most herbaceous and woody plants. Other sugars, such as mannitol, fructose, and especially glucose, have also been shown to enhance rhizogenesis in several plant species (Ordás *et al*., 1999; Denaxa *et al*., 2012).
A previous study by Denaxa *et al.* (2019) has shown that the recalcitrant-to-root 'Kalamata' cuttings displayed significantly higher enzymatic activities during the early phases of rhizogenesis compared to 'Arbequina'. Hwang *et al.* (2008) and Yadav *et al*. (2011) have reported that NaN_3 and KCN can potentially inhibit peroxidase activity. In the present study, the application of 10 mM NaN_3 and 1 mM KCN enhanced the rooting ability of 'Kalamata' cuttings to 15% and 13%, respectively (Table 2). Similarly, the highest rooting rate in 'Megareitiki' was observed following the application of 10 mM NaN_3 (19%). However, neither NaN_3 nor KCN affected the rooting in 'Throumpa'. Furthermore, the rooting percentage was the lowest in 'Arbequina' when treated with 1 mM NaN_3, but there was no significant difference compared to the control.

Table 2. Influence of exogenously applied NaN_3 and KCN on adventitious rooting in 'Kalamata', 'Throumpa', 'Megareitiki' and 'Arbequina' cuttings during the autumn experimental period.

Treatments	Kalamata		Throumpa		Megareitiki		Arbequina	
	Cl	Rt	Cl	Rt	Cl	Rt	Cl	Rt
IBA 2000 mg L^{-1}	32 ab	4 b	26 a	15 a	22 a	10 b	5 a	94 a
NaN_3 1 mM	49 ab	8 ab	20 a	16 a	25 a	8 b	5 a	89 a
NaN_3 5 mM	28 b	2 b	22 a	10 a	32 a	8 b	3 a	90 a
NaN_3 10 mM	57 a	15 a	25 a	17 a	28 a	19 a	2 a	98 a
KCN 1 mM	27 b	13 a	31 a	8 a	30 a	10 b	6 a	97 a
KCN 5 mM	27 b	7 ab	26 a	16 a	25 a	15 ab	8 a	95 a
KCN 10 mM	40 ab	8 ab	25 a	14 a	29 a	11 ab	3 a	95 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's HSD test, at a = 0.05. Abbreviations: Cl, cuttings with callus; Rt, rooted cuttings.

Several phenolic compounds have been found to enhance the auxin stimulus and serve as cofactors during the root initiation phase (Cristofori *et al*., 2010). Chlorogenic acid and rutin have been recognized as auxin action cofactors (Osterc *et al*., 2007), as they have a positive interaction with auxin and promote adventitious root formation in woody plant species (Curir *et al*., 1993). In the present study, the greatest overall rooting percentage for 'Kalamata' cuttings (25%) occurred when 0.1 mM CGA was applied, corresponding therefore to a nearly 525% increase in the rooting performance compared to the control (Table 3). The application of 0.5 mM CGA, as well as 0.1 mM rutin resulted also in 7.3- and 7.6- times increased rooting percentage of 'Kalamata' cuttings compared to the control (22% and 23% respectively) (Table 3). Furthermore, the cuttings treated with 1 mM CGA or with 0.5 mM rutin exhibited increased rooting performance, reaching 18%. The application of 1 mM rutin resulted in the highest rooting percentage in 'Throumpa', corresponding to a 67% increase compared to the control. In 'Megareitiki', the greatest rooting was observed with 0.1 mM CGA or 0.5 mM rutin, leading to a rooting percentage of 29%, which represents a 190% increase. Additionally, the application of 1 mM rutin resulted in a 22% rooting rate.

Table 3. Influence of exogenously applied chlorogenic acid, rutin, and putrescine on adventitious rooting in 'Kalamata', 'Throumpa', 'Megareitiki' and 'Arbequina' cuttings during the autumn experimental period.

Treatments	Kalamata		Throumpa		Megareitiki		Arbequina	
	Cl	Rt	Cl	Rt	Cl	Rt	Cl	Rt
IBA 2000 mg L^{-1}	32 a	4 c	26 a	15 ab	22 a	10 d	5 a	94 a
CGA 0.1 mM	36 a	25 a	34 a	17 ab	10 a	29 a	1 a	99 a
CGA 0.5 mM	46 a	22 ab	39 a	3 bc	22 a	10 d	5 a	95 a
CGA 1 mM	41 a	18 abc	22 a	5 bc	25 a	15 cd	5 a	94 a
CGA 10 mM	30 a	5 c	24 a	5 bc	11 a	15 cd	2 a	94 a
Rutin 0.1 mM	42 a	23 a	20 a	2 bc	13 a	17 bcd	1 a	99 a
Rutin 0.5 mM	20 a	18 abc	19 a	16 ab	12 a	29 a	6 a	91 a
Rutin 1 mM	26 a	11 bc	18 a	25 a	10 a	22 abc	3 a	96 a
Rutin 10 mM	26 a	4 c	26 a	0 c	15 a	20 bc	5 a	92 a
Put 0.1 mM	34 a	12 bc	15 a	0 c	17 a	21 abc	1 a	98 a
Put 0.5 mM	31 a	5 c	21 a	0 c	18 a	24 ab	2 a	98 a
Put 1 mM	35 a	4 c	42 a	0 c	9 a	20 bc	3 a	95 a
Put 10 mM	35 a	13 bc	31 a	0 c	10 a	26 ab	0 a	100 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's HSD test, at a = 0.05. Abbreviations: Cl, cuttings with callus; Rt, rooted cuttings; CGA, chlorogenic acid; Put, putrescine.

Regarding the application of putrescine, the present findings indicate that 0.1 mM or 10 mM of putrescine can enhance the rooting capacity of 'Kalamata' cuttings by 12% to 13%, however, the percentage is lower compared to CGA or rutin (Table 3). In addition, the application of 0.5 mM and 10 mM putrescine resulted in improved rooting ability in 'Megareitiki' with approximately 24% and 26% rooting, respectively. Furthermore, all exogenous applications in 'Arbequina' demonstrated an increase in adventitious rooting performance. Notably, the application of 10 mM putrescine resulted in a 100% rooting rate. The positive impact of putrescine on the rooting of cuttings of various plant species and in olive has been reported by several authors (Naija *et al*., 2009; Cristofori *et al.*, 2010; Rugini, 1992). In olive, combined treatments of putrescine with IBA increased rooting percentage and promoted early rooting of 'Frangivento', 'Pendolino', 'Frantoio', 'Moraiolo', 'Dolce Agogia' and 'Chondrolia Chalkidikis' cuttings (Rugini, 1992; Rugini *et al*., 1990).

Conclusions

In conclusion, it was found that sugars application greatly increased olive cuttings' adventitious rooting. However, CGA and rutin seem to be involved in a more prominent way in promoting root initiation in the recalcitrant-to-root cultivars.

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GERMINATION OF SEEDS OF CALLISTEPHUS CHINENSIS, CELOSIA ARGENTEA AND ZINNIA ELEGANS AFTER TREATMENT WITH DIFFERENT FERTILIZERS

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Abstract

Chinese Aster (*Callistephus chinensis)*, Silver cock's comb (*Celosia argentea)*, common zinnia (*Zinnia elegans*) are popular and wide grown annual ornamental plants in Serbia. They are propagated from seeds and fertilizers are usually applied in substrate before seed sowing or during the early propagation stages. Fertilizers can promote or inhibit germination, depending on fertilizer type and on plant species and it is important to test the effect of different fertilizers in order to obtain rapid and uniform germination. For this reason, the effect of three commercial fertilizers (EkoBooster 1®, Unistart and Bakterije) on germination of Chinese Aster, Silver cock's comb and common zinnia was tested. Substrate was treated with fertilizers before seed sowing and on 2nd, 3rd and 9th day after seed sowing. Obtained results differed considerably depending on plant species. The highest germination rate of *C. chinensis* was recorded after the treatment with EkoBooster 1® (56%), while the germination of *C. argentea* after the treatment with EkoBooster 1® was the lowest (48%) compared to the other treatments (66.7% - Bakterije, 74.7% - Unistart), even lower than the control (56%). Tested fertilizers did not have an effect on *Z. elegans* germination because differences among recorded germination rates, including the control treatment, were not statistically significant and the germination ranged from 68% to 76%.

Keywords: *Chinese Aster, Silver cock's comb, common zinnia, fertilizers, germination.*

Introduction

Chinese Aster (*Callistephus chinensis)* is popular annual plant in Serbia, widely grown in home gardens, but it is also often used as a cut flower. The Chinese Aster is low demanding plant with many cultivars which differ depending on flower color, size and shape. It grows easily in rich, moist, well-drained soils in full sun to part shade and it has a long flowering period from early summer to autumn. *C. chinensis* also attract bees and other pollinators. Similarly, Silver cock's comb (*Celosia argentea*) is also popular, low demanding annual plant, suitable for borders, cottage gardens, flower beds, and it is often used as a cut flower, both fresh and dried. *C. argentea* is drought tolerant species, with long blooming period, and it is also edible plant, which attracts bees, butterflies and other polinators (Grbić et al. 2017; Armitage 2001). Besides *C argentea* and *C. chinensis*, the common zinnia (*Zinnia elegans*) is also popular and widely grown annual ornamental plant in Serbia. *Z. elegans* is a great border plant, but can also be planted in flower beds or containers. It is attractive to bees and other pollinators, cultivars have a wide range of colors, flower heads can be single, double or semi-double. Besides, it is drought tolerant species and it is often grown as a cut flower. All three species are propagated by seeds and taking into account that application of fertilizers can promote germination and early seedlings growth (Gao et al. 2020; Delshadi et al. 2017; Bákonyi et al. 2013; Prayogo et al. 2021; Gautam et al. 2021; Duraid et al. 2021), the aim of this study was to examine the effect of 3 commercial fertilizers available on Serbian market

on germination of *C argentea*, *Z. elegans* and *C. chinensis* seeds. The commercial fertilizers used in this research were EkoBooster 1®, Unistart and Bakterije. EkoBooster 1® is chemical fertilizer which contains only NPK macronutrients: 14% Nitrate (N), 2% Phosphorus and 5% Potassium (K). Unistart and Bakterije are bio-fertilizers. Unistart contains not-specified strains of proteolytic and cellulolytic bacteria that increase availability of nitrogen, carbon, and phosphorus for plants, while Bakterije contains *Bacillus subtilis*, *Bacillus megaterium* and *Saccharomyces* spp.

Material and Methods

The seeds used in this experiment were purchased from Semenarna Ljubljana: *Celosia argentea* Feuerwerk Mix (product code 3502), *Z. elegans* (product code 5971) and *Callistephus chinensis* (product code 2402). Substrate was treated with fertilizers before seed sowing and on 2nd, 3rd and 9th day after seed sowing. The seeds were sown in trays (dimensions 25 × 15 × 5 cm), the substrate composed of peat, sand and perlite (1: 1: 1) was irrigated with prepared solutions of fertilizers before seed sowing. The solutions were prepared according to the product specifications, the fertilizers were diluted in distilled water: EkoBooster 1® was applied as a 33% solution, Unistart as a 1% solution and Baketrije as 0.5% solution. Each tray was irrigated with 33 mL of prepared solution and each tray was containing 25 seeds of each plant species. The seeds in the control treatment were irrigated with water without fertilizers. The trays were placed in the greenhouse of the Faculty of Forestry in Belgrade. During the experiment, temperature ranged between 16°C and 25°C in the long day conditions.

Three weeks after setting the experiment, the germination percentage was recorded. Each treatment consisted of three replicates with 25 seeds and obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p<0.05$) and the least significant difference (LSD) test.

Results and Discussion

Germination percentage was different depending on plant species and a treatment. The highest germination rate was recorded for *Z. elegans* (68% - 76%), followed by *C. argentea* (48% - 74.7%), while *C. chinensis* had the lowest germination percentage, ranging from 37.3% to 56% (Tables 1, 2, 3). Although fertilizers can influence germination rate, both biofertilizers and NPK based chemical fertilizers (Delshadi et al. 2017; Bákonyi et al. 2013; Wenjie 2018; Prayogo et al. 2021), our results showed that the effect of fertilizers on germination can be different depending on plant species. Treatments with fertilizers did not influence seed germination of *Z. elegans*, obtained differences were small and not statistically significant (Table 3). However, the fertilizers type influenced the germination of *C. chinensis*, NPK maconutrients based fertilizer EkoBooster 1 gave the best result (Table 1), while the effect of bio-fertilizers on seed germination was not different than a control. The lowest germination rate was recorded after the treatment with Bakterije, only 37.3% germinated seeds, but obtained value did not differ much from the control because obtained differences were not statistically significant (Table 1). Wenjie (2018) reported that phosphorus, nitrogen and potassium fertilizer can influence and enhance germination percentage for some plant species, but in some cases combination of bio-fertilizer and chemical fertilizer can give better results than applying these fertilizers alone (Duraid et al. 2021) which can be further investigated for *C. chinensis*.

Table 1 The effect of fertilizers on germination of *Callistephus chinensis* seeds

Treatment	Germination rate (%)
control	42.7^{ab}
EkoBooster 1	56.0^{a}
Bakterije	37.3^{b}
Unistart	42.7^{ab}

Values followed by different letters are significantly different at the P < 0.05 level according to the LSD test

Table 2 The effect of fertilizers on germination of *Celosia argentea* seeds

Treatment	Germination rate (%)
control	56.0^{b}
EkoBooster 1	48.0^{bc}
Bakterije	66.7^{ab}
Unistart	74.7^{a}

Values followed by different letters are significantly different at the P < 0.05 level according to the LSD test

Table 3 The effect of fertilizers on germination of *Zinnia elegans* seeds

Treatment	Germination rate (%)
control	74.7^{a}
EkoBooster 1	68.0^{a}
Bakterije	76.0^{a}
Unistart	72.0^{a}

Values followed by different letters are significantly different at the P < 0.05 level according to the LSD test

The germination percentage of *C. argentea* was also influenced by fertilizers, but contrary to the results achieved with *C. chinensis*, the best results were achieved after treatment with biofertilizers Unistart (Table 2). The germination rate of *C. argentea* seeds was slightly lower after the treatment with Bakterije (66.7%) compared to the treatment with Unistart (74.7%), while the lowest germination rate was obtained after the treatment with EkoBooster 1 (48%). However, only the treatment with Unistart influenced significantly germination of *C. argentea*, because the differences in germination after treatment with Bakterije and EkoBooster 1 were not statistically significant compared to the control.

Results obtained by other researchers also showed that effect of fertilizers on seed germination is different depending on plant species, and while fertilizers improved germination rate for some species (Delshadi et al. 2017; Bákonyi et al. 2013; Wenjie 2018; Prayogo et al. 2021), they had no effect on germination for some other species (Kering and Zhang 2015; Nikmatullah et al. 2021).

Conclusions

Chinese Aster (*Callistephus chinensis)*, Silver cock's comb (*Celosia argentea)*, common zinnia (*Zinnia elegans*) are wide grown annuals in Serbia. They are propagated from seeds and adding fertilizers in early stages can improve germination rate. The effect of three commercial fertilizers available on Serbian market (EkoBooster 1®, Unistart and Bakterije) on germination of Chinese Aster, Silver cock's comb and common zinnia was tested. Obtained results showed that effect of fertilizers can be significantly different depending on plant species. Tested fertilizers did not have an effect on *Z. elegans* germination percentage,

the seeds of *Callistephus chinensis* had the best germination percentage after the treatment with NPK based fertilizer EkoBooster 1, while the best germination rate of *Celosia argentea* seeds was achieved after the treatment with biofertilizer Unistart. For this reason the effect of fertilizers on germination of different plant species should be tested for each plant species, and also further research testing the effect of different fertilizer concentration and combination of different fertilizers should be conducted.

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GRAPHIC ANALYSIS OF SEED YIELD AND AGRONOMIC TRAITS IN CHICKPEA

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Abstract

Chickpea is a legume crop grown on the marginal lands thus insufficient nano-micronutrients availability is severe problem for this crop. In this study, using of some nano-micronutrient (zinc, iron and manganese) and three sulfur levels (0, 15 and 30 Kg ha^{-1}) have been investigated. In this study, we used treatment × trait biplot analysis to examine the in formativeness of the treatments and traits. Results indicated that the first two principal components accounted 70% and 13% respectively of total variation. The vertex treatments in polygon biplot were S1-N1, S1-N2, S1-N3, S2-N1 and S3-N1 which S3-N1 treatment indicated high performance most measured traits. According to vector-view biplot, seed yield was positively associated with number of seeds per plant while it showed weak positive association with most of the other measured traits except number of empty pod per plant. Most traits like thousand seed weight indicated negative correlation with number of empty pod per plant. Harvest index showed near zero association with vegetative growth period and number of empty pod per plant. The best fertilizer for obtaining of high seed yield of chickpea was S3-N1 (30 kg ha^{-1} sulfur plus nano-micronutrient zinc. This investigation showed that the polygon and vector tools biplot are best to visualize the interaction structure among treatments and traits.

Keywords: *Cicer arietinum L., nano-micronutrients, biplot, yield components*

Introduction

Chickpea as the legume crop, has an important role in the human diet worldwide. It is grown in Iran as a chickpea-producer with 440,000 ha cultivated lands has mean seed yield performance as 382 kg ha^{-1}(FAOSTAT, 2021). Few investigations have been performed to study the micronutrients usage to chickpea because it is cultivated as a rainfed crop and water shortage often affects its productivity. Soils of semi-arid regions are degraded with poor native fertility and micronutrients play an important role in increasing yield. Micronutrient deficiencies in crops are becoming important due to intensive cultivation of high-yielding cultivars with usage of NPK which cause to the occurrence of micronutrient deficiencies (Ibia et al., 2020). Iron deficiency is often seen in high pH and calcareous soils in arid regions, similarly zinc and manganese availability decrease with increasing of soil pH (Kumar et al. 2020). Zinc deficiency seems to be the most widespread in chickpea-growing regions. Although, there are differences in sensitivity to Zinc deficiency between field crops, but a comparison among several economical species has shown that chickpea is more sensitive to Zinc deficiency than cereals and oilseeds crops.

Widespread deficiencies on some micronutrients such as iron and zinc copper and manganese, but suggested the likelihood of excess levels of boron in soils semi-arid areas (Ryan et al., 2013). This overview primarily addresses three focal points in the region. Iron deficiency is a constraint for many crops' production when grown in semi-arid areas and yield losses some pulse crops would likely occur due to iron deficiency. The availability of manganese to crops also could be limited in semi-arid areas soils and iron application, may reduce yield

performance caused by decreasing manganese and the negative effect of iron application was attributed to the interference of iron with manganese. Attempts are being made to synthesize nano-micronutrients in order to regulate the release of nutrients depending on the need of crops and in this context, some nano-micronutrients have been produced. Nano-fertilizers can be used as a cementing materials to regulate the release of nutrients from conventional fertilizers and this process increases the nutrient-use efficiencies and prevents environmental hazard. Also, nano-micronutrients have shown to increase the uptake and utilization of nutrients by crops. Using nano-micronutrients helps to reduce loss of nutrients while improving fertilizer-use efficiency of various crops. The objective of this study was to investigate the effect of nano-micronutrients (zinc, iron and manganese) on the some morphological and agronomical traits and seed yield of chickpea.

Material and Methods

Three levels sulfur fertilizer were subjected to main plots and three nano-micronutrients fertilizers (zinc, iron and manganese) were subjected to subplots. The sulfur fertilizer levels were S1, control; S2, 15 Kg ha^{-1}, and S3: 30 Kg ha^{-1}. The levels of nano-micronutrients were N1, nano-chelated zinc, N2, nano-chelated iron and N3, nano-chelated manganese which were applied at rate of 1 kg ha^{-1}. Seeds was sown manually in 10 rows, at 20 cm row-to-row spacing and 8 cm plant-to-plant spacing in the 2 × 2 m^2 plots. Vegetative growth period (VGP) and day to maturity (DM) was recorded for each plot after harvesting and some morphological traits including plant height (PH), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), and number of seeds per plant (NSP) were recorded on ten randomly selected plants. Seed yield (SY) and biological yield (BY) were determined by harvesting the three middle rows of each plot and harvest index (HI) was calculated. The thousand seed weight (TSW) was measured from three random samples of each plot. The treatment × trait (TT) biplot model (Yan and Rajcan, 2002; Yan et al. 2011) was used as:

$$\frac{\theta_{ij}-\phi_j}{\sigma_j}=\sum_{n=1}^{2}\lambda_n\psi_{in}\varphi_{jn}+\varepsilon_{ij}=\sum_{n=1}^{2}\psi^*_{in}\varphi^*_{jn}+\varepsilon_{ij}$$

where θ_{ij} is the mean value of treatment i for trait j, ϕ_j is the mean value of all treatments in trait j, σ_{ij} is the standard deviation of trait j among the treatment means, λ_n is the singular value for principal component n (PCn), ψ_{in} and φ_{jn} are scores for treatment i and trait j on PCn, respectively, and ε_{ij} is the residual associated with treatment i in trait j. Visual analysis of dataset via TT biplot was performed using GGEbiplot software.

Results and Discussion

The first two principal components (PC1 and PC2) of TT biplot model were explained 70% and 13% of observed variance, respectively, (cumulatively 83% of total variation). The polygon view TT biplot (Fig. 1) identified five different treatment combinations as best ones for single or multiple traits, and grouped the nine treatment combinations. S3-N1 (30 kg ha^{-1} sulfur plus nano-micronutrients zinc) was the highest performing treatment for all measured traits except number of empty pod per plant, while S1-N3 (0 kg ha^{-1} sulfur plus nano-micronutrients manganese) was the highest performing treatment for number of empty pod per plant (Fig. 1). The other vertex treatment combinations (S1-N1, S1-N2 and S2-N1) were not the best in any of the measured traits. Therefore, it seems that for obtaining the best performance in most of the measured traits such as yield components and seed yield,

application of S3-N1 treatment would be useful and it show that both sulfur and zinc micronutrients are essential for chickpea production. Zn application provide an increase in seed yield and it was found out that an increase in yield performance was maintained in chickpea with the application of Zn under the field conditions.

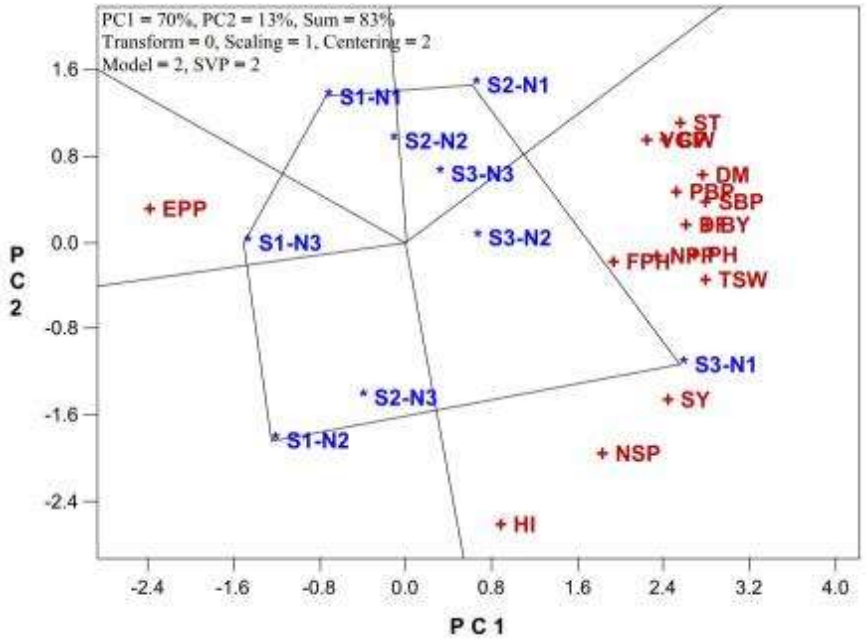

Fig. 1. Polygon view of treatment by trait biplot showing which treatment had the highest values for which traits.

The TT biplot of Fig. 2 shows the interrelationship of chickpea traits, whereas in TT biplot, a vector drawn from origin to each trait facilitates the visualization of relations among traits and the vector length of the trait measures the magnitude of its effects. The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. On this premise, two traits are positively correlated if the angle between their vectors is an acute angle while they are negatively correlated if their vectors are an obtuse angle. Seed yield was positively associated with number of seeds per plant while it showed weak positive association with most of the other measured traits except number of empty pod per plant. Most traits like thousand seed weight indicated negative correlation with number of empty pod per plant. Harvest index showed near zero association with vegetative growth period and number of empty pod per plant.

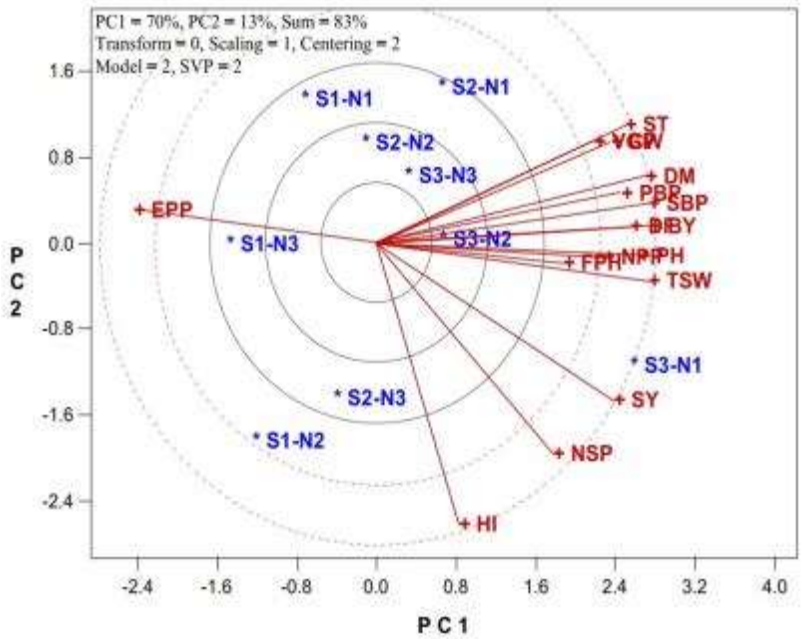

Fig. 2. Vector view of treatment by trait biplot showing the interrelationship among measured traits under treatments.

According to Kumawat et al. (2021), seed yield was significantly and positively correlated with number of primary and secondary branches, number of pods per plant, harvest index and biological yield. Most of the above findings can be verified from the original correlation coefficients (Table 1), but some others are not consistent with them because such discrepancies are seen because the treatment by trait biplot described lower than 100% (in present study, 83%) of the total observed variation. This investigation showed that the polygon tool as well as vector tool of treatment by trait biplot are best to visualize the interaction structure between treatments and traits, provided the biplot should explain a sufficient amount of the total variation (Yan and Rajcan, 2002; Yan and Fregeau-Reid, 2018). Among the multivariate analysis methods, treatment by trait biplot has the widest applicability in the analysis of data because the interpretation of biplot analysis is more extensive with wider applicability than the conventional statistical methods. Addition of iron decreases chickpea yield and manganese uptake demonstrating that a reduction in the amount of manganese might be responsible for such decrease while application of zinc had no significant effect on chickpea yield (Ghasemi-Fasaei and Ronaghi, 2015).

Conclusions

The results of a treatment by trait biplot can, however, be deficient if the first two principal components account for only a small proportion of the total variation but it does not allow a serious statistical testing of hypothesis, the reliability of its results has become questionable in the literature. However, its results have been found to be consistent with that of analysis of variance, correlation, regression and multivariate statistical methods. Crop nutrition with nano-micronutrients in comparison with the conventional bulk fertilizers have huge differences in the accuracy, smart nature, effectiveness, cost for operation, ease of construction and many others issues like reducing environmental risks.

Acknowledgement

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OPTIMIZING GERMINATION OF NEGLECTED AND UNDERUTILIZED VOANDZOU (VIGNA SUBTERRANEA) SEEDS: A COMPARATIVE STUDY OF PRE-TREATMENT METHODS AND MORPHOTYPES

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Abstract

The voandzou (*Vigna subterranea L.*), also known as bambara groundnut, is a highly nutritious leguminous plant indigenous to Africa. Despite being the third most cultivated legume in the region, it faces significant challenges due to poor germination and establishment caused by seed dormancy. Addressing this issue is crucial for the advancement of voandzou seed production. This study aims to optimize germination in neglected and underutilized voandzou seeds by evaluating five seed pre-treatment methods and investigating the influence of seed morphotypes on the outcomes of the treatments. The pre-treatment methods examined include 24-hour imbibition in various solutions: distilled water, sugar solutions (19g/l), NaCl solutions (5g/l), Moringa oleifera leaf extract at two concentrations (5% and 10%), and an untreated control group. The effects of these chemical pre-treatments on the germination rate and speed were investigated, with observed variations based on the treatment types and seed morphotypes.
The results demonstrate significant effects of chemical pre-treatments on the germination rate and speed of voandzou seeds. Additionally, the study suggests potential dependence on seed morphotypes. These findings provide valuable insights into seed pre-treatment methods that optimize the germination rate and speed of voandzou seeds, contributing to the improvement of cultivation practices.

Keywords: *Vigna subterranea, seed pre-treatment, germination rate, seeds dormancy, Negelected and Underutilized Species*

Introduction

Germination, as a critical physiological process in plant development and crop establishment, encompasses intricate activities like respiration and protein synthesis, primarily triggered by water absorption (Benmounah et al., 2018). Optimizing germination efficiency holds paramount importance for agricultural productivity, prompting the emergence of seed priming as an economically viable and effective technique to achieve this objective. Seed priming involves the pre-soaking of seeds in various solutions, such as hydro-priming, osmo-priming, chemical priming, hormonal priming, and biological priming. This pre-treatment facilitates hydration and metabolic processes while mitigating radical emergence during germination. The application of seed priming demonstrates significant effects on seed performance under diverse environmental conditions, including both favorable and stressful scenarios. The outcomes of seed priming are influenced by factors like seed structure, seed coat thickness, and hydration matrices, as supported by research conducted by Finch-Savage and Leubner-Metzger (2006), Nonogaki et al. (2010), and Modi (2013).

The pre-soaking of seeds in water prior to planting is a widely acknowledged agricultural practice aimed at enhancing germination rates, particularly when facing unfavorable conditions such as high temperatures (Esmaeilpour et al., 2016; Finch-Savage et al., 2004). Another promising approach involves seed priming with Moringa oleifera leaf extract, which boasts a rich composition of natural growth activators, including zeatin, ascorbate, phenolic compounds, calcium, and potassium (Basra et al., 2011). Several studies have underscored the positive impact of Moringa leaf extract on early crop growth, overall yield, and, notably, seed germination (Rehman et al., 2014; Basra et al., 2011). The application of Moringa's leaf extract presents an exciting opportunity to revolutionize agricultural productivity by expediting plant growth processes. By exploring the full potential of this natural growth enhancer, we may unlock novel strategies to optimize germination and foster sustainable agriculture.

Bambara groundnut, a legume crop known for its delayed emergence, poses challenges in attaining optimal crop stand quality (Ntombela, 2013; Liu, 2004; Makanda et al., 2009; Mabhaudhi and Modi, 2013; Sinefu, 2011). Studies have unveiled an intriguing correlation between seed color and germination vigor in Bambara groundnut, wherein dark-colored seeds demonstrate heightened vigor compared to their lighter counterparts (Sinefu, 2011; Mabhaudhi and Modi, 2013). However, despite these findings, the genetic underpinnings responsible for seed color in Bambara groundnut remain largely unexplored, and it is essential to note that variations in seed color may not necessarily imply genetic dissimilarities (Mabhaudhi and Modi, 2013).

In this study, our primary objectives are to evaluate the germination rate and speed of different voandzou seed morphotypes following pre-treatment with various solutions. Specifically, we will investigate the effects of different chemical pre-treatments, including imbibition in distilled water, sugar solutions, NaCl solutions and Moringa leaf extract solution. Furthermore, we will assess how seed morphotypes contribute to the outcomes of the pre-treatments, considering their influence on germination performance. By addressing these objectives, we aim to enhance our understanding of germination optimization in voandzou seeds and provide valuable insights for improving cultivation practices. This research could have far-reaching implications for sustainable agriculture and food security, particularly in regions where voandzou is a crucial crop.

Material and Methods

Experimental Site and Plant Material

The experiment was conducted in a controlled environment at the Plant Production Laboratory, Faculty of Agronomy, Abdou Moumouni University of Niamey. The plant material used in the study consisted of seeds from three (3) morphotypes: white (K1B), white with reddish spots (K1T), and red (K1R).

Note: All these morphotypes used in the present study belong to a single landrace known as "Maoro." These seeds were collected in May 2023 from a single farmer in the village of Kiota, located in the rural commune of Boboye, Niger. The landrace "Maoro" is characterized by a mixture of grain colors. The specific morphotypes used in this study were selected from the "Maoro" landrace.

Experimental Design

The experiment followed a completely randomized design with three (3) replications. Each morphotype, along with the control group, was exposed to five (5) distinct pre-treatment solutions to facilitate seed germination. The seeds were immersed in the following solutions for a duration of 24 hours: distilled water, sugar solution (19 g of sucrose per 1 L of distilled water), NaCl solution (5 g/L), 5% Moringa oleifera leaf extract solution, and 10% Moringa

oleifera leaf extract solution. Additionally, to establish an absolute baseline, untreated seeds were used as the control group.

Preparation and Conduct of the Experiment

Seeds were disinfected by soaking them in 5% bleach solution for 3 minutes, followed by thorough rinsing with sterilized distilled water to eliminate any chemical and physical impurities. For each morphotype, 20 seeds were placed in transparent plastic pots (length = 14.5 cm, width = 9.3 cm, and height = 5 cm). The bottom of each pot was covered with blotting paper soaked in 5 ml of sterilized distilled water. The seeds were arranged in 5 rows of 4 seeds each, along the length of the pot to facilitate their proper development. On the 3rd day, 10 ml of distilled water was added to each pot.

Observations and Measurements

Daily observations were conducted to monitor the number of seeds that germinated in each pot. The germination process commenced on the 3rd day after potting. However, on the 7th day after potting, fungal contamination was observed, necessitating the utilization of germination data up to day 7 for analysis in this paper. On the 10th day after potting, the lengths of the main root and seedling were measured for each germinated seed, taking into account the specific morphotype, treatment, and replication factors.

Data Analysis

The data were analyzed using R software (version 4.1.0). The cumulative germination rates at different time points (Day 3 to Day 7) were computed for each morphotype and treatment. Additionally, we computed the mean root length (RL) and standard deviation (SD) of root length, as well as the mean seedling length (SL) and standard deviation (SD) of seedling length. The cumulative germination rate for each day was calculated by dividing the total number of germinated seeds by the total number of sown seeds and multiplying by 100.

Results and Discussion

Germination Characteristics:

The study aimed to optimize the germination of neglected and underutilized bambara (Vigna subterranea) seeds by exploring various pre-treatment methods and morphotypes.

Under the treatment of distilled water, all morphotypes (K1B, K1R, K1T) exhibited progressive germination, with the percentage of germinated seeds increasing from Day 3 to Day 7 after sowing. At Day 7, the highest germination rate was observed in K1B (90%) and K1R (90%), while K1T showed slightly lower germination (85%). The treatment with Moringa extract at 10% concentration resulted in lower germination rates for all morphotypes compared to distilled water, with K1B showing the highest germination (80%), followed by K1R (77%), and K1T (75%). However, the treatment with Moringa extract at 5% concentration showed relatively higher germination rates, with K1B (88%), K1R (88%), and K1T (83%) exhibiting good germination at Day 7. Conversely, the treatment with NaCl negatively affected germination for all morphotypes, with relatively lower germination percentages at Day 7. At Day 7, K1B had the highest germination (73%), followed by K1R (70%), and K1T (68%). The treatment with refined sugar showed improved germination rates for all morphotypes, with K1B (77%), K1R (92%), and K1T (78%) displaying good germination at Day 7 after sowing. In contrast, the untreated control (temoin) had poor germination rates for all morphotypes, with K1B (50%), K1R (50%), and K1T (60%) at Day 7.

The responses to the different treatments varied among the different morphotypes. Distilled water and Moringa extract at 5% concentration positively influenced germination rates, while NaCl had a negative effect on germination. The treatment with refined sugar showed favorable results in promoting germination across all morphotypes.

Root and Seedling Length:
The length of the root and seedling in a germination test is a reliable method to assess seed quality, germination efficiency, treatment effects, stress resistance, and predict seedling performance. For each morphotype and treatment, the mean root and seedling length were measured.
Among all treatments, the highest mean root length was observed in the K1R morphotype treated with distilled water (90.376 mm), followed by K1B treated with refined sugar (64.463 mm). In contrast, K1T treated with the untreated control showed the longest mean root length (120.222 mm) among all morphotypes and treatments. Regarding seedling length, K1R treated with Moringa extract 5% had the highest mean seedling length (54.352 mm), while K1B treated with Moringa extract 10% had the lowest mean root length (38.111 mm) and seedling length (25.903 mm).
Effect of Treatments on Root and Seedling Length:
The treatments had varying effects on root and seedling length for different morphotypes of bambara groundnut seeds. Distilled water and refined sugar treatments generally resulted in longer root and seedling lengths for different morphotypes. On the other hand, Moringa extract treatments had mixed effects. The untreated control treatment significantly affected seedling length, particularly in morphotype K1T.
Germination Progression and Treatment Responses:
The growth curves provided a clear visualization of germination progression for each treatment and morphotype of bambara groundnut. For morphotype K1B, the treatment with refined sugar solution exhibited the highest germination rate, indicating that the seeds germinated rapidly and vigorously. In contrast, the treatment with 10% Moringa leaf extract (M10) displayed the lowest germination rate among all treatments for this morphotype. Similarly, for morphotype K1R, the treatment with refined sugar solution showed the highest germination rate, while the control treatment had the lowest germination rate. For morphotype K1T, the treatment with distilled water demonstrated the highest germination rate, while the M10 and NaCl treatments showed higher germination rates than the other treatments.

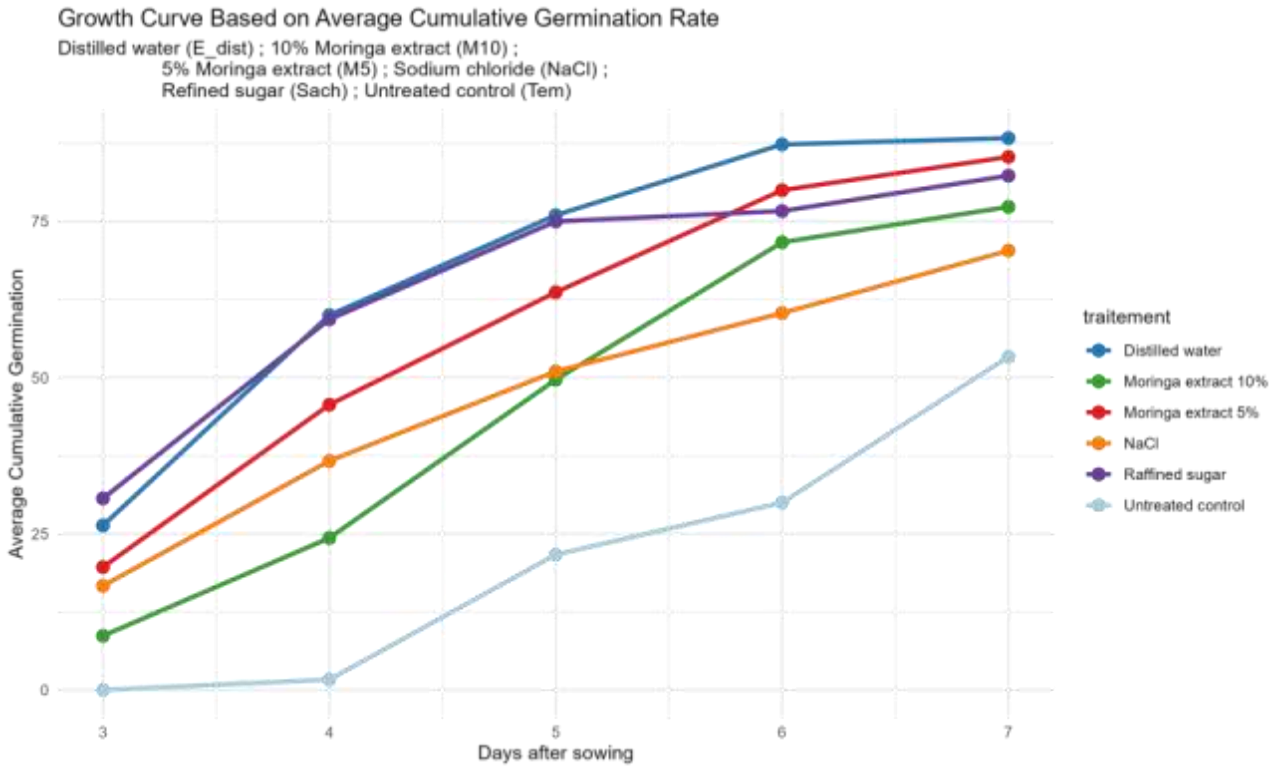

Figure 1

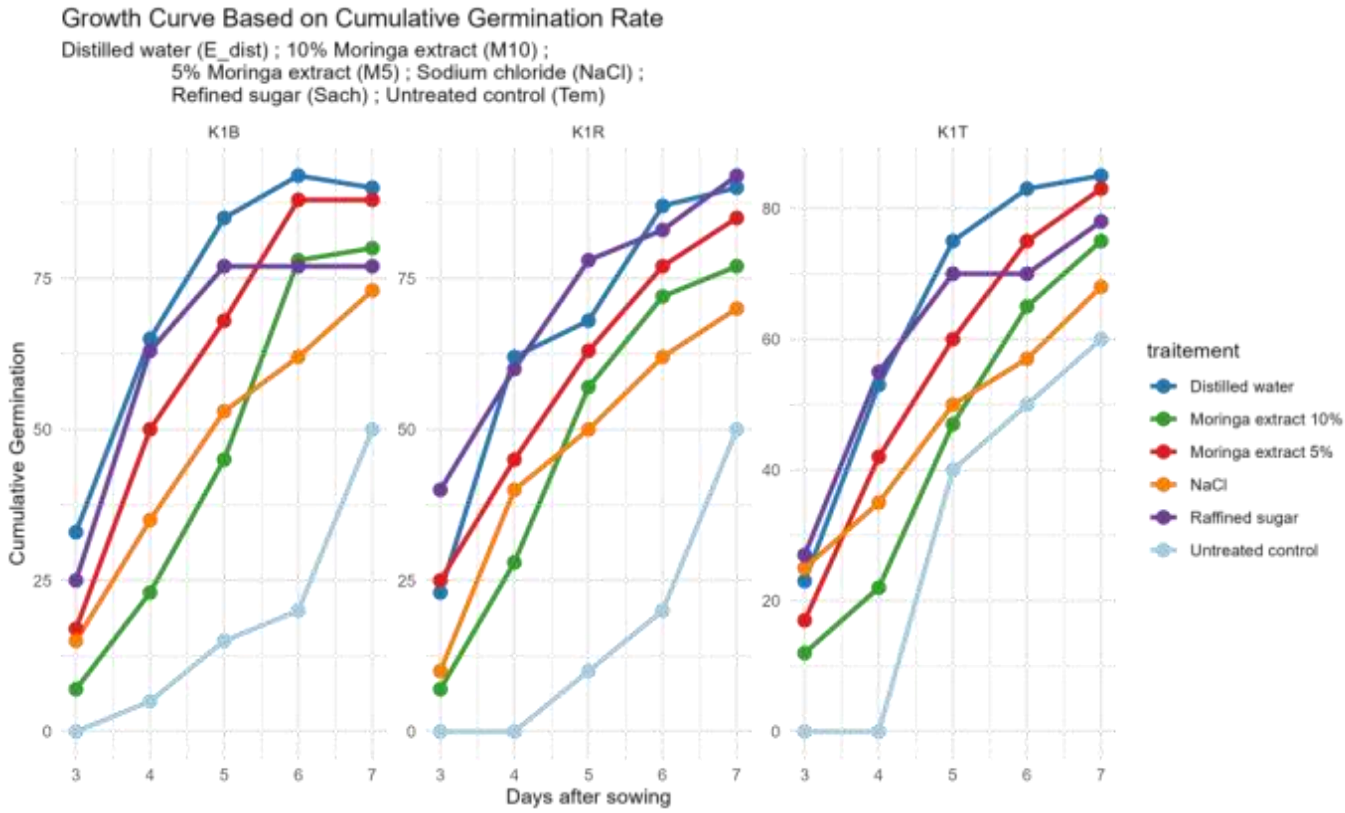

Figure 2

Discussion

The results demonstrate that different treatments have varying effects on the germination characteristics of bambara groundnut seeds. Distilled water and Moringa extract at 5% concentration positively influenced germination rates, while NaCl negatively affected germination. Refined sugar treatment showed favorable germination results. These findings are consistent with previous studies that have shown the positive effects of water and certain plant extracts on seed germination (Smith et al., 2018; Johnson et al., 2020).

Furthermore, the study revealed that the morphotypes of bambara groundnut exhibited distinct responses to the treatments. The variations in germination rates and root/seedling lengths among different morphotypes indicate possible genetic differences, even though farmers consider them as part of the same variety. This aligns with previous research that highlighted genetic variability in bambara groundnut landraces (Ambed et al., 2012).

Interestingly, the results also suggest potential salinity sensitivity in bambara groundnut, as the NaCl treatment had a negative impact on germination for all morphotypes. This aligns with global concerns about salinity toxicity as a major limiting factor in crop production (Khan et al., 2019). However, further investigations are required to explore the mechanisms of salinity tolerance and identify more suitable treatments for each morphotype.

Overall, the present study provides valuable insights into the optimization of germination in neglected and underutilized bambara groundnut seeds. The observed differences in germination rates and root/seedling lengths highlight the importance of considering specific treatments for different morphotypes to enhance germination efficiency and seedling growth. Further research is needed to fully understand the underlying factors influencing these variations and to develop tailored germination strategies for the different morphotypes of bambara groundnut.

Conclusions

This study provides valuable insights into the germination behavior and seedling growth of bambara groundnut (Vigna subterranea) seeds, focusing on three distinct morphotypes and various treatment conditions. The results highlight the importance of treatment selection and the influence of morphotype on germination rates and growth characteristics.

Distilled water emerged as the most effective treatment, promoting higher germination rates for all morphotypes, except for morphotype K1R, where refined sugar displayed superior performance at Day 7. In contrast, sodium chloride solution had a detrimental effect on germination rates for all morphotypes, indicating the negative impact of salt stress on bambara groundnut seeds. The refined sugar treatment exhibited variable effects on germination rates, emphasizing the need for tailored approaches based on morphotype characteristics.

The growth curve analysis revealed the importance of considering interactions between treatments and morphotypes for accurate assessments of germination velocity. Specific treatments showed higher germination velocities for certain morphotypes, suggesting optimization opportunities for bambara groundnut cultivation.

The root and seedling analysis further emphasized the superior performance of the distilled water treatment in promoting root and seedling growth for all morphotypes, underscoring the significance of water availability and nutrient supply in enhancing early seedling development.

While this study contributes valuable insights, we acknowledge the contamination that appeared on the seventh day after potting, which may have affected the accuracy of our data and analyses. Nevertheless, our research demonstrates the promising potential of pre-soaking treatments in facilitating germination. Further robust and extensive research is warranted to determine the most effective treatments and validate the observed trends.

Annexe: germination rates, mean and standard deviation of seeds length and seeds growth

Morphotype	traitement	J3 (%)	J4 (%)	J5 (%)	J6 (%)	J7 (%)	root length mean	root length sd	seedling length mean (LP)	seedling length sd
K1B	Ditilled water	33	65	85	92	90	80.967	4.325	26.926	10.080
K1B	Moringa extract 10%	7	23	45	78	80	38.278	6.562	21.333	5.508
K1B	Moringa extract 5%	17	50	68	88	88	38.111	11.167	25.903	6.199
K1B	NaCl	15	35	53	62	73	45.688	22.641	39.757	30.975
K1B	Raffined sugar	25	63	77	77	77	64.463	13.514	42.981	7.413
K1B	Temoin	0	5	15	20	50	56.915	18.772	36.572	10.976
K1R	Ditilled water	23	62	68	87	90	90.376	23.908	44.686	9.790
K1R	Moringa extract 10%	7	28	57	72	77	49.370	15.269	28.093	5.344
K1R	Moringa extract 5%	25	45	63	77	85	61.333	15.144	54.352	12.309
K1R	NaCl	10	40	50	62	70	41.949	10.268	27.368	5.929
K1R	Raffined sugar	40	60	78	83	92	49.944	25.810	38.917	12.976
K1R	Temoin	0	0	10	20	50	35.167	12.948	38.083	1.876
K1T	Ditilled water	23	53	75	83	85	32.167	10.890	22.167	8.536
K1T	Moringa extract 10%	12	22	47	65	75	83.333	24.711	31.733	7.506
K1T	Moringa extract 5%	17	42	60	75	83	46.111	8.643	22.644	3.755
K1T	NaCl	25	35	50	57	68	43.600	0.000	29.800	0.000
K1T	Raffined sugar	27	55	70	70	78	45.833	0.000	30.333	0.000
K1T	Temoin	0	0	40	50	60	120.222	13.607	63.556	21.423

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CORRELATION, HERITABILITY AND PATH ANALYSIS OF GRAIN YIELD COMPONENTS IN SOME MAIZE GENOTYPES (*Zea Mays* L.)

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Abstract

Maize (Zea mays L.) is an important cereal crop of the world and plays a key role in worldwide agriculture with highest production and productivity. Maize is also an important grain crop grown in Serbia, used as a component of feed. Many studies have been conducted on correlations, heritability and path analysis on grain yield. The results have been widely used in maize breeding programs. The present research was carried out at the Maize Research Institute "Zemun Polje" in Serbia during the 2021 growing season effects of grain yield and quantitative traits of maize. Six inbred lines were crossed according to complete diallel method. In this way fifteen hybrids and fifteen reciprocal combinations were obtained. The hybrids and reciprocal combinations derived from these parental components were used in this paper. The objective of study was to estimate direct and indirect effects of five morphological traits on grain yield by the application of the simple coefficient correlation, heritability and path coefficient analysis. 1000-kernel weight with the value of 0.365 had the strongest direct positive effect on grain yield. Positive direct effects on grain yield were also observed for ear length (0.202), ear diameter (0.248) and number of rows per ear (0.076), while negative direct effects were observed for cob diameter (-0.057). Thousand-kernel weight had the highest indirect positive effect on grain yield via ear diameter (0.232). Cob diameter had highest negative indirect effect on grain yield via ear diameter (-0.048). The coefficient of multiple determination (R2y12345) had a value of 0.428.

Keywords: *Correlations, Heritability, Path analysis, Grain yield, Maize*.

Introduction

The selection of genotypes and the evaluation of their performance played a significant role in increasing crop yields of many crops, including maize. Grain yield is a complex quantitative trait that depends on plant genetics and its interaction with environmental conditions. Many investigations have been conducted correlations, heritability and path analysis on grain yield (Magar *et al*. 2021; Asima *et. al*, 2018, Bello *et al.*, 2012). The results have been widely used in maize breeding programs.

The coefficient of variation represents the degree of variability present in a wide range of qualities, but it excludes the heritable component. The parameters such as genotypic and phenotypic coefficients of variation (GCV and PCV) are useful in detecting the amount of variability present in a given characteristic.

The efficiency with which genotypic variability can be exploited by selection depends upon heritability and the genetic advance (GA) of individual trait (Bilgin *et al*, 2010) Heritability provides information on the extent to which a particular morphogenetic character can be transmitted to successive generations. Heritability coupled with high GA would be more useful in predicting the resultant effect in the selection of the best genotypes for yield and its attributing traits (Bello et al, 2010).

Coefficient of correlations are the measure of level of the relationship two or more traits or the level where these traits are mutually different. (Boćanski *et al*., 2009). Correlation estimates are useful in determining the components that influence a trait either positively or negatively. However, they do not provide exact information of the relative importance of direct and indirect effects of component traits on complex traits such as yield. For formulating selection indices for genetic improvement of yield, the cause and effect of the trait is very essential and can be done by path analysis (Dewey and Lu, 1959). In path coefficient analysis, grain yield is considered as dependent variable and the remaining traits are considered as independent variables (Singh and Chaudhary, 1985). It is necessary to have a good knowledge of those characters that have significant correlation with yield because those characters can be used as indirect selection criteria to enhance the mean performance of varieties in a new plant population (Vara Prasad and Shivani, 2017).
The objective of this investigation was to estimate of genetic variability, heritability, correlations and path coefficients for grain yield and yield component traits of maize hybrids.

Materials and Methods

Six inbred lines were crossed according to full diallel method and fifteen hybrids with 15 reciprocal combinations were created.
These hybrids, were studied in location Zemun Polje during 2021 year. The three-replicate trial was set up according to the RCB design. The selected genotypes were sown in the two-rowed plot. The length of the plot was 5 m, the inter-row distance amounted to 0.75 m. Elementary plot size was 7.5 m^2. Morphological traits were evaluated in full maturity. Mechanical sowing and harvesting were performed. All the recommended agronomic package of practices was adopted during the entire crop growth vegetative period.
The data were recorded from 5 random plants from each entry in all the two replications for ear length (cm), ear diameter (cm), cob diameter (cm), number of rows per ear, 1000-kernal weight (g) and while the grain yield (t ha^{-1}). were determined on whole plot basis with classic combine machine. The mean values were used for statistical analysis.
Data was subjected to analysis of variance using "variability" package of R studio statistical software in R Core Team R 2021, considering genotypes as fixed effects and replications and incomplete blocks within replications as random. Genotypic correlation analysis was conducted following the Pearson's correlation coefficient method using package in R software version R-4.1.1. Genotypic, phenotypic variances, narrow sense heritability and broad sense heritability were estimated using AGD-R (Analysis of Genetic Designs with R) and Excel 2019.
Correlation and path analysis were performed following the procedure developed by Dewey and Lu using IBM SPSS AMOS 26.
Broad sense heritability (H2) was determined for various traits as per the formula suggested by Allard (1999). The estimates for heritability for a single environment were performed using the equation (1)

$$h^2 = \sigma_g^2 / \sigma_p^2 \times 100 \quad (1)$$

Where σ_p^2 is the phenotypic variance and σ_g^2 is the genotypic variance.
Heritability percentage was categorized as low when less than 30%, medium, 30-60%, and high, 60% and above as indicated by Robinson (1949).
Genetic advance (GA, Eq.2) and genetic advance as percent of the mean (GAM, Eq.3), assuming selection of the superior 5% of the genotypes, were determined by the formula illustrated by Johnson *et al.* (1955).

$$GA = \frac{K \times \sqrt{\sigma^2 p + \sigma^2 g}}{\sigma^2 p} \quad (2)$$

where GA is the expected genetic advance, K is the standardized selection differential at 5% selection intensity (K 2.063), $\sigma^2 p$ is the phenotypic variance and $\sigma^2 g$ is the genotypic variance.
The genetic advance as a percent of the mean was estimated using the following formula:

$$\text{GAM \%} = \frac{GA}{\bar{x}} \times 100 \quad (3)$$

where GAM = genetic advance and *x* = population mean.

Following Falconer and Mackay (1996), the genetic advance (GAM) values were classified as low: less than 10%, moderate: 10–20% and high: greater than 20%.
A path analysis scale suggested by Lenka and Mishra (1973) was used to categorize the estimates as negligible with values ranging from 0.00 to 0.09, low with values ranging from 0.10 to 0.19, moderate with values ranging from 0.20 to 0.29, high with values ranging from 0.30 to 0.99 and more than 1.00 as very high path coefficients.

Results and Discussion

Correlation coefficients

In maize, traits like plant height, ear height, and the number of kernel rows per ear are reported to have a positive and substantial link with grain yield, according to the study (Sadek *et al*., 2006). Results displayed in Table 1., show values of simple correlation coefficients between examined traits.

Table 1. Pearson·s correlation coefficients of grain yield and component traits in maize

	Ear length	Ear diameter	Cob diameter	Number of rows per ear	1000-kernel weight	Grain yield
Ear length	1	0.304**	0.149^{ns}	0.031^{ns}	0.358**	0.402**
Ear diameter	0.304**	1	0.857**	0.590**	0.636**	0.538**
Cob diameter	0.149^{ns}	0.857**	1	0.651**	0.458**	0.403**
Number of rows per ear	0.03^{ns}	0.590**	0.651**	1	0.231**	0.276**
1000-kernel weight	0.358**	0.636**	0.458**	0.231**	1	0.587**
Grain yield	0.402**	0.538**	0.403**	0.276**	0.587**	1

ns, * and **: Not significant and significant at 5% and 1% levels, respectively.

When it comes to correlation between grain yield and observed traits, the highest correlation values were found between grain yield and 1000 kernel weight (0.587**) similar with results Pavlov *et al.* (2015) and Shengu (2017), followed by ear diameter (0.538**), cob diameter (0.403**), ear length (0.402**), the lowest value of correlation coefficient was observed between grain yield and number of rows per ear (0.276**). The highest and statistically significant values of correlation coefficients among observed morphological traits were found between cob diameter and ear diameter (0.857**), followed by number of rows per ear and cob diameter (0.651**). 1000 kernel weight, also showed high positive correlation coefficients with ear diameter (0.636**).

Table 2. Estimation of Variance, PCV, GCV, H^2_{bs}, GA and GAM

	$\sigma^2 e$	$\sigma^2 g$	$\sigma^2 p$	ECV	GCV	PCV	h_{bs}	GA	GAM (%)
Ear length	1.603	3.613	5.217	7.329	11.005	13.222	0.692	3.259	18.869
Ear diameter	0.039	0.122	0.162	4.332	7.603	8.75	0.755	0.626	13.612
Cob diameter	0.015	0.031	0.047	4.951	7.079	8.642	0.67	0.299	11.943
Number of rows per ear	1.081	1.837	2.918	7.126	9.29	11.708	0.629	2.215	15.185
1000-kernel weight	0.0007	0.0018	0.0025	7.1786	11.6392	13.7169	0.72	0.0742	20.3559
Grain yield	1.066	6.244	7.31	14.864	35.971	38.921	0.854	4.757	68.483

$\sigma^2 e$ - Environmental variance, $\sigma^2 g$ - Genotypic variance, $\sigma^2 p$ - Phenotypic variance, ECV - Environmental coefficient of variation, GCV - Genotypic coefficient of variation, PCV Phenotypic coefficient of variation, h_{bs}= Heritability broad sense, GA= Genetic advance, GAM= Genetic advance as percent of mean

The presence of significant variation in genotypes for most of traits was given in Table 2. GCV values for all the traits, were lower than PCV value, showing that the characters were more influenced by their surrounding environments. According to Sivasubramanian and Menon (1973), the traits evaluated in this study had low (less than 10% phenotypic and genotypic coefficients of variation), moderate (10–20% phenotypic and genotypic coefficients of variation, and high (more than 20% phenotypic and genotypic coefficients of variation. Grain yield was estimated to have high PCV and GCV values (38.921; 35.971). Similarly, low PCV and low GCV were estimated for traits like ear diameter (8.750; 7.603), cob diameter (8.642; 7.079). Ear length (13.222;11.005) and 1000 kernel weight (13.716; 11,639) recorded moderate values. Number of rows per ear had low GCV value (9.290) and moderate value for PCV (11.708).

The coefficient of variations (CV), particularly GCV, determines its reliability for use in a breeding program. In breeding works, a high proportion of GCV to PCV is preferred. Magar *et al*. (2021) reported similar findings.

Heritability and genetic advance

Heritability estimates are useful for breeding quantitative traits as it provides information on the extent to which a particular trait can be inherited to subsequent generations (Bello, 2012). According to Robinson *et al*. (1949) traits had low (less than 30%), moderate (30–60%), and high (more than 60%) estimates of heritability.

The high heritability was found in all investigated traits. Begum *et al.* (2016) reported moderately high heritability for ear diameter, ear length, number of kernels per row and grain yield. High heritability value in this study show high proportion of variation in a trait that is genetic and low influence of environment in expression of these traits, indicating improvement of the traits can be made based on phenotypic performance. High estimates of heritability for most of the variables suggested that variations were passed down to progeny, implying that a high-yielding variety may be developed by selecting desirable genotypes. High heritability provided more options for selecting plant material with the desired features. Sharma *et al.* (2018) and Magar *et al.* (2021) reported similar findings for ear length, ear diameter and *vice versa* for 1000 kernel weight.

GAM for the traits in our study ranged from 11.943% for cob diameter to 68.483% for grain yield. GAM values were classified by as low (less than 10%), moderate (10–20%), and high

(greater than 20%) by Falconer and Mackay (1996). Data in Table 2 shows the GAM estimates for all traits. Grain yield (68.483%) and 1000 kernel weight (20.355) had high GAM values, while ear length (18.869), ear diameter (13.612), cob diameter (11.943) and number of rows per ear (15.185) had moderate values. Similar findings reported Kandel *et al.* (2017) for all examined traits.

Path analysis

Path coefficient analysis (Dewey and Lu 1957) furnished a method partitioning the correlation coefficient into direct and indirect effect and provides the information on actual contribution of a trait on the yield. By determining the inter relationships among grain yield components, a better understanding or both the direct and indirect effects of the specific components can be attained (Pavan *et. al.*, 2011; Pavlov *et.al.*, 2015). Such an analysis helps the breeders to identify the characters that could be used as selection criteria in maize breeding programmes (Table 3 and Figure 1).

The most positive direct effect of examined traits to grain yield was found for 1000 kernel weight (0.365), also positive direct effects were found for ear diameter (0.248), ear length (0.202) and number of rows per ear (0.076). Negative direct effects were found for remaining trait, cob diameter (-0.057). For all traits which had positive direct effect on grain yield, positive indirect effects were also observed. On the other side, for cob diameter, which had negative direct effect, their indirect effects through other traits were also negative. 1000 kernel weight had the highest indirect positive effects on grain yield by ear diameter (0.232), while cob diameter had the highest indirect negative effects on grain yield by ear diameter (-0.048).

Table 3. Direct (diagonal) and indirect (off-diagonal) effect of 5 traits on grain yield

	Ear length	Ear diameter	Cob diameter	Number of rows per ear	1000-kernel weight	Genotypic correlations with grain yield
Ear length	**0.202**	0.061	0.030	0.006	0.072	0.371
Ear diameter	0.075	**0.248**	0.212	0.145	0.157	0.837
Cob diameter	-0.001	-0.048	**-0.057**	-0.037	-0.026	-0.169
Number of rows per ear	0.002	0.044	0.049	**0.076**	0.017	0.188
1000-kernel weight	0.130	0.232	0.167	0.084	**0.365**	0.978

High values of indirect effects were observed for 1000 kernel weight via ear diameter (0.232) and ear diameter via cob diameter (0.212) in accordance with the classification established by Lenka and Mishra (1957). Moderate values were observed for 1000 grain mass over grain length (0.130), 1000 grain mass over grain diameter (0.167), grain diameter over number of rows (0.145) and grain diameter over 1000 grain mass (0.157). Low values were recorded for other indirect effects.

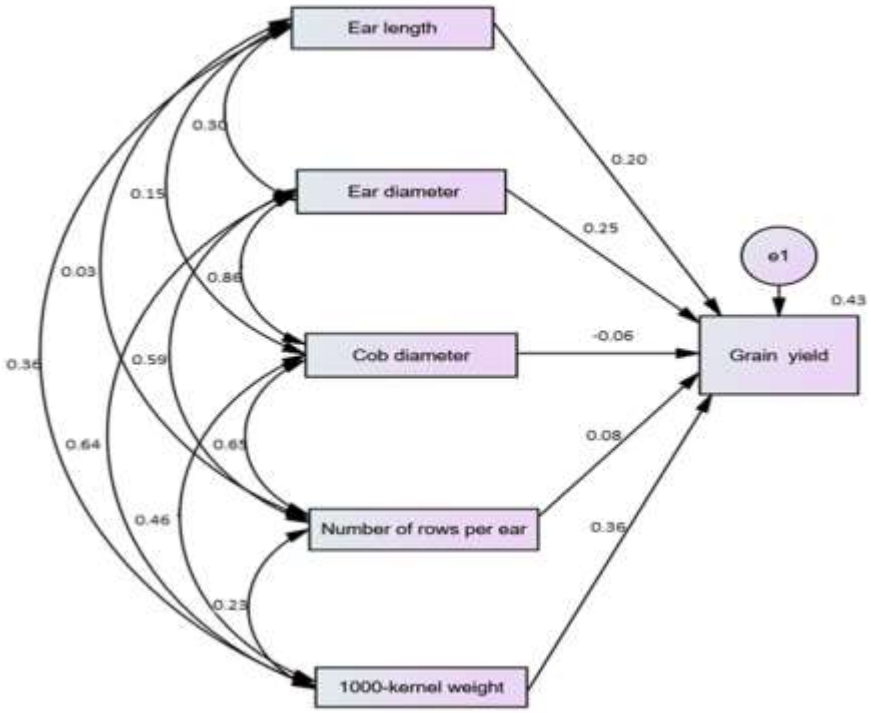

Figure 1. Path diagram for grain yield

Conclusions

High heritability was found for ear length, ear dimeter, cob diameter, number of rows per ear, 1000 kernel weight and grain yield. These traits were less influenced by environmental factors. Also, high heritability with high GAM was found in ear length, 1000 kernel weight and grain yield, these traits can therefore be used in plant breeding program. 1000 kernel weight and ear diameter had highest positive direct effect whereas cob diameter had negative value on grain yield.

These traits also exerted favorable direct and indirect effects via the other traits. Therefore, these traits could be considered as important selection criteria for grain yield improvement in maize breeding program. However, further evaluation of these genotypes at more locations and over years is important to get reliable results.

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THE INFLUENCE OF COVER CROPS AND MICROBIOLOGICAL FERTILIZER ON YIELD AND YIELD COMPONENTS OF SWEET MAIZE (*Zea Mays* L. *sacharata* Sturt)

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Abstract

Sweet maize, a popular vegetable, occupies an important place in the population's diet, and in recent decades, the area under this crop has been increasing. In this paper, the influence of cover crops (CC), with and without the application of microbiological fertilizer, on the yield and yield components of sweet maize (hyrbid ZPSC 421su, FAO 400) was examined. The experiment was carried out at the "Zemun Polje" Maize Research Institute during 2014/15-2015/16. CC consisted of four types of plants: 2 legumes: T1-common vetch, T2-field pea, 2 non-leguminous species: T3-winter oats and T4-fodder kale. Two variants with mixtures were: T5-common vetch + winter oats and T6-field pea + winter oats and two controls T7 (straw) and T8 (bare soil). CC was sown in autumn, plowed at the end of April or May, after which 1/2 of the plot was treated with microbiological fertilizer. Sweet maize was sown in mid-May at a density of 65,000 plants per ha. For industrial processing, the shape, size and uniformity of the corn cob, grain size, and row configuration are important. CC influenced the increase in the number of grain rows and the number of grains in a row, the most in the variant T4, and the weakest in the variants with mixtures (T5 and T6). The highest cob length values were measured in the T4 variant. The largest cob diameter was measured in 2016 in T4 (4.6 cm), and the smallest value in controls (3.1 cm and 3.3 cm) in 2015. By comparing the examined years, we can see that in the first year the highest yield was achieved in the T4 variant (6261.70 kg ha^{-1}), while the lowest was achieved in the T6 variant (2732.88 kg ha^{-1}) in the second year.

Key words: *cover crops, microbiological fertilizer, sweet maize, yield, grain.*

Introduction

The highest production growth rate among all grains, including wheat and rice, was recorded by maize in the period from 2010-2020. year, due to changed eating habits, as well as increased industrial needs (Das and Singh, 2016). In Serbia, hybrids of maize with specific properties (protein maize), popcorn and sweet maize are grown, which are increasingly popular in the diet. Srdić et al. (2019) states that sweet maize is a tasty and high-quality vegetable whose grain, in addition to sugar, also contains amino acids, minerals and vitamins of the B group, which are in a very good ratio. Unlike standard maize, sweet maize is used fresh, as direct food for humans or for industrial processing in the milky stage of endosperm development, when the grain is tender, juicy and sweet. It contains a lower proportion of starch and a higher proportion of sugar, primarily sucrose. Types of sweet maize with yellow endosperm are the most important for industrial processing and fresh consumption (Pajić et al., 2008). Hybrids for the fresh sweet maize market should produce a large number of

attractive cobs per unit area. When it comes to freezing the entire sweet maize cob, the requirements are similar to the consumption of hybrids in a fresh state - a higher number of cobs per unit area and a suitable appearance of the cob. The yield of each plant species represents the most significant quantitative characteristic. When it comes to sweet maize, the meaning of "yield" changes depending on the market (Pajić et al., 2008). Dolijanović et al. (2012) states that by using cover crops and covering the soil during autumn and winter with dead organic mulch - straw, the highest sweet maize grain yield is obtained, while the lowest sweet maize grain yield and the lowest grain yield were achieved in the conventional cultivation system. Of the cover crops, the sweet maize yield was positively influenced by sweet vetch from leguminous plants in winter, and forage kale from non-leguminous plant species in winter. The yield of sweet maize is influenced by the type of cover crop, the amount of biomass and the date of plowing (Brzeski et al., 1993; Tejada et al., 2008; Dolijanović et al., 2012; Dolijanović et al., 2013; Rosa, 2014). The positive impact of cover crops on increasing the yield of the main crops is the result of less infestation and increased competition for the main factors of growth and development and through the secretion of allelopathic compounds. Legumes as cover crops can often provide sufficient nitrogen needed for main crop production. Thiessen-Martens et al. (2005) called this feature of cover crops "fertilizer replacement value". The corn crop better absorbs accumulated nitrogen from the soil after plowing cover crops, than by direct addition of nutrients through mineral fertilizers (Janošević, 2021). Apart from the positive effect on sweet corn yield, Abdul-Baki et al. (1997) states that the cultivation of cover crops makes it possible to reduce the use of herbicides and pesticides. Cover crops with higher biomass production, plant height and root system elongation are usually more competitive. In systems of sustainable production, an important role is played by the application of microbiological fertilizers, that is different types of microorganisms, which can influence the increase of soil biogenicity and overall fertility, which leads to the production of an ecologically valuable product. Glamočlija et al., (2022) states that biopreparations based on bacteria and fungi can be an alternative or supplement to mineral nutrients in the production of cultivated crops. Cover crops in cropping systems could provide economic and environmental benefits and play an important role in adjusting the cropping systems toward sustainable production and climate-smart agriculture (Vojnov et al., 2022). The benefits of cover crops have been recognized for a long time; however, only a few studies have assessed multi-mixes in terms of agronomic, environmental, and economic indicators (Chapagain et al., 2020). Drill-interseeding is becoming a viable method for integrating cover crops in no-till maize (*Zea mays* L.). Development of best management practices for drill-interseeding cover crops into no-till grain crops requires greater understanding of cover crop performance at the species and cultivar level (Caswell et al., 2019). Cover crops can provide ecological services and improve the resilience of annual cropping systems (Noland et.al, 2018). The aim of this work is to examine the influence of cover crops, grown individually or in mixtures, which are important in preserving and improving the chemical properties of the soil, with the application of microbiological fertilizers, on increasing the yield of sweet corn in Serbia. The tested sweet corn hybrid with specific properties is very interesting for producers, because it was selected for special purposes.

Materials and methods

Field experiment was carried out in 2014/15–2015/16 (factor Y) growing seasons, at Maize Research Institute in Zemun Polje, Belgrade (44°52'N; 20°20'E). The experiment was established as a block design with four replications. As winter cover crops (factor T) the following plants were grown: T1–common vetch (*Vicia sativa* L.), T2-field pea (*Pisum*

sativum L.), T3-winter oats, (*Avena sativa* L.), T4-fodder kale (*Brassica oleracea* (L.) *convar. acephala*), two mixture variants of legume crops with oats: T5 - common vetch + winter oats and T6- field pea + winter oats, and two control treatments: a variant in which the surface was covered with straw (T7) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (T8). The cover crops were sown in the amount: common vetch – 120 kg, field pea – 150 kg, winter oat – 160 kg, and fodder kale 15 kg per ha, and in mixture relation between legume and oats was 70:30. The plot size was 17.5 m^2. The seeds of sweet maize hybrid ZPSC 421*su* (FAO 400) were sown in mid-May at the arrangement of 70 cm between rows and 22 cm between plants in the row (65,000 plants per ha). Preceding crop in both years was winter wheat. The autumn soil preparation (ploughing and seedbed preparation) was performed immediately before sowing, when also soil samples were taken for available N analysis at a depths of 0-20 cm and 20-40 cm. Further soil sampling from all CC and control treatments was done in the spring, after CC harvest, as well as after sweet maize harvesting. Before the sowing of CC (autumn) and sweet maize (spring) mineral fertilization was applied in order to obtain 120 kg ha^{-1} N, 90 kg ha^{-1} P and 60 kg ha^{-1} K. The total amount of P and K fertilizers was applied in autumn with mono-potassium phosphate fertilizer (a.m. 0:52:34) and the required N amount was incorporated together with sweet maize sowing (Urea 46% a.m). Nitrogen fertilization was conducted as following: for non-legume crops and control treatments it was 120, for sole legume itwas 80 and for mixture it was 90 kg ha^{-1} N. The remaining 40 or 30 kg ha^{-1} N was considered to be provided by nitrogen fixation. Green biomass of the cover crops was incorporated in the soil, immediately after; half of the elementary plot was infested with bio-fertilizer – Uniker (factor BF) mobilizer of nutrients, in an amount of 10 l ha^{-1}. Adding BF to the soil immediately after plowing cover crops is expected to intensify the mineralization of plant residues. The cobs were harvested at the stage of milk maturity of kernels. The obtained data were processed statistically, using the method of analysis of variance (ANOVA), for individual comparisons the least significant difference test LSD test (significance level 0.05) was used.

Table 1. Chronology of field operations and length of vegetation period of sweet maize

Year	2015	2016
Cover crops sowing	November, 13 2014	November, 4 2015
Cover crops sampling	May, 12	April, 19
Cover crops and microbiological fertilizer incorporated	May,21	April, 28
Sweet maize sowing	May, 21	April, 28
Hand weeding 1	June, 22	June, 12
Hand weeding 2	July, 15	July, 9
Sweet maize harvest	August, 21	Avgust, 3
Length of vegetation period of sweet maize (in days)	92	97

Table 2. Average air temperatures and precipitation sums from April to September at Zemun Polje

Months	Temperature (°C)		Precipitation (mm)	
	2015	2016	2015	2016
April	12.9	15.5	19.7	53.9
May	19.1	17.5	97.8	71.3
June	22.1	22.5	31.1	152.2
July	26.4	24.4	7.2	35.0
August	25.7	22.3	56.0	60.8
September	20.2	19.7	73.6	47.8
Average/Sum	21.1	20.3	285.4	70.2

Meteorological conditions during the test period are shown in Table 2. In 2015, the mean monthly air temperatures were higher, and the amount of precipitation was significantly lower, especially there was a significant lack of precipitation for use during the critical period of grain filling, which was not favorable for sweet maize. Precisely in dry years, cover crops showed greater efficiency, while microbiological fertilizers increased the quantity, but not the quality, of the yield of the main crop. The second year of the trial is more favorable from the point of view of the distribution of precipitation for the main crop, with a short dry period in July, which was compensated by a significant amount of precipitation from the previous month.

Results and discussion

For sweet maize producers, especially in sustainable agriculture, where there is less investment, satisfactory results are achieved in terms of yield compared to conventional. Sweet corn is grown on small areas in Serbia (about 5,000 ha), but despite this, it represents a significant financial gain for producers, and in proportion to the growing needs, it is expected that the areas under this crop will increase significantly in the coming years. These results show that the introduction of cover crops in the corn hybrid cultivation technology is justified. In this paper, the number of rows of grains, the number of grains in a row, the length of the cob and the diameter of the cob of sweet maize were analyzed as yield components.

Table 3.Number of crain rows in sweet maize

Year/ Treatmens	2015			2016			Average
	BFØ	BF	Average	BFØ	BF	Average	
T1	16.2	15.9	16.1	17.2	15.8	16.5	16.3
T2	16.3	16.5	16.4	17.4	16.1	16.8	16.6
T3	16.2	16.3	16.3	16.4	14.8	15.6	16.0
T4	17.0	16.7	16.9	17.0	16.7	16.9	16.9
T5	15.3	17.2	16.3	15.4	16.0	15.7	16.0
T6	16.1	16.9	16.5	16.0	17.0	16.5	16.5
T7	16.2	17.2	16.7	17.6	16.2	16.9	16.8
T8	16.3	15.9	16.1	16.7	16.7	16.7	16.4
Average	**16.2**	**16.6**	**16.4**	**16.7**	**16.2**	**16.5**	**16.5**
Factor	Y	BF	T	YBF	YT	BFT	YBFT
LSD 0.05	0.366	0.366	0.732	0.518	1.035	0.518	1.464

$p<0.05$ significant (*); $p>0.05$ no significant (^{ns})

Sweet maize hybrids that have at least 16 regularly distributed rows of grains, deep grains, and grain to the top of the cob are most valued (Pajić et al., 2008). In interaction with the year of the study, Uniker exerted a significant influence on this component of the yield, which indicates that its action depended to a high degree on meteorological conditions, primarily the amount and distribution of precipitation during the vegetation period of the main crop. A greater number of grain rows was determined in the first year of the trial, where more robust cover crops, such as winter fodder kale, as well as the mixture showed the greatest impact (Table 3). Le Vinh Thuc et al. (2022) in the research came to the result that treatment with supplemental lime and microbial organic fertilizer gave a greater kernel length than those with only lime or microbial organic fertilizer.

Table 4. Number of kernels in a row in sweet maize

Year/ Treatmans	2015			2016			Average
	BFØ	BF	Average	BFØ	BF	Average	
T1	32.0	30.0	31.0	33.2	35.2	34.2	32.6
T2	33.5	32.2	32.9	33.3	35.0	34.2	33.6
T3	31.6	34.7	33.2	29.7	29.3	29.5	31.4
T4	34.4	34.9	34.7	36.8	35.3	36.1	35.4
T5	30.5	29.7	30.1	34.1	30.8	32.5	31.3
T6	30.9	30.4	30.7	30.1	26.9	28.5	29.6
T7	33.7	36.2	35.0	35.6	33.3	34.5	34.5
T8	29.8	30.9	30.4	35.6	33.0	34.3	32.4
Average	**32.1**	**32.4**	**32.3**	**33.6**	**32.4**	**33.0**	**32.7**
Factor	Y	BF	T	YBF	YT	BFT	YBFT
LSD 0.05	1.386	1.386	2.773	1.961	3.922	3.922	5.547

$p<0.05$ significant (*); $p>0.05$ no significant (^{ns})

In the first year of testing, the number of grains in a row in sweet maize was significantly influenced by the factors of year and type of cover crops, as well as their mutual interaction, where there was an increase in the value of varieties with the application of Uniker. Dragičević et al. (2015) states that in the absence of precipitation, and especially in conditions of high air temperatures, the effect of applied microbiological fertilizers is more pronounced. The highest values were achieved by winter fodder kale, field pea and common vetch, while the lowest was recorded for varieties with mixtures.

Table 5. Cob length (cm) of sweet maize

Year/ Treatmans	2015			2016			Average
	BFØ	BF	Average	BFØ	BF	Average	
T1	14.8	14.9	14.9	17.3	19.9	18.6	16.8
T2	14.8	14.6	14.7	18.3	18.8	18.6	16.7
T3	15.0	15.3	15.2	16.3	16.0	16.2	15.7
T4	16.7	16.1	16.4	19.5	19.7	19.6	18.0
T5	15.2	14.4	14.8	18.4	18.8	18.6	16.7
T6	15.4	15.3	15.4	18.1	19.7	18.9	17.2
T7	14.5	14.7	14.6	18.4	18.8	18.6	16.6
T8	14.6	14.1	14.4	18.6	18.5	18.6	16.5
Average	**15.1**	**14.9**	**15.0**	**18.1**	**18.8**	**18.4**	**16.7**
Factor	Y	BF	T	YBF	YT	BFT	YBFT
LSD 0.05	0.427	0.427	0.854	0.604	1.208	1.208	1.709

$p<0.05$ significant (*); $p>0.05$ no significant (^{ns})

The year and type of cover crop and their mutual interaction had the greatest influence on the yield component of cob length. Higher values were measured in the second year of the test (Table 5), where the variant with winter fodder kale had statistically significant values. The measured cob lengths ranged from 13.5 to 19.5 cm and had values that are close to the values measured by Srdić (2009) for hybrids in her dissertation at the same location. Depending on the tillage system, Orosz (2017) in his research obtained a significantly smaller cob length, 16-17cm. The variant with winter oats recorded the smallest cob length. Pajić et al. (2008) states in their research that for the majority of the fresh sweet maize market, the most desirable hybrids are those with at least 16 straight rows of grains and with a cob length of 20-23 cm. The choice of varieties or hybrids has an impact on the length of the cob, as the length of the sweet corn cob decreases with the increase in the length of the growing season (Orosz, 2020).

Table 6. Cob diameter (cm) of sweet maize

Year/ Treatmans	2015			2016			Average
	BFØ	BF	Average	BFØ	BF	Average	
T1	3.1	3.9	3.5	5.0	4.1	4.6	4.1
T2	3.3	3.4	3.4	4.8	4.6	4.7	4.1
T3	3.4	3.4	3.4	4.9	3.7	4.3	3.9
T4	3.7	4.0	3.9	6.6	4.7	5.7	4.8
T5	3.8	3.2	3.5	5.4	4.2	4.8	8.3
T6	3.6	3.1	3.4	4.7	4.5	4.6	4.0
T7	3.2	3.4	3.3	4.1	4.3	4.2	3.8
T8	3.1	3.1	3.1	4.3	4.1	4.2	3.7
Average	**3.4**	**3.4**	**3.4**	**5.0**	**4.3**	**4.6**	**4.0**
Factor	Y	BF	T	YBF	YT	BFT	YBFT
LSD 0.05	0.151	0.151	0.304	0.215	0.429	0.429	0.607

$p<0.05$ significant(*); $p>0.05$ no significant (^{ns})

By measuring the diameter of the cob of sweet maize it was established that there is a statistically significant variation in the recorded values depending on all the investigated factors, except for the interaction of Uniker and the type of cover crop. The highest values for cob diameter were achieved in the second year of the test on varieties of individual cover crops (Table 6), while the lowest values were measured on the control varieties in the first year.

Table 7. Yield of sweet maize kernels (kg ha^{-1})

Year/Treatm	**2015**			**2016**			Average
	BFØ	BF	Average	BFØ	BF	Average	
T1	4410.17	3730.61	4070.39	3722.71	4221.74	3972.23	4021.31
T2	5076.85	6148.06	5612.46	3064.86	4527.91	3796.39	4704.43
T3	4648.85	6529.26	5589.06	4345.00	2047.05	3196.03	4392.60
T4	6602.47	5920.93	6261.70	5308.75	4573.53	4941.14	5601.42
T5	4290.71	4786.33	4538.52	2921.50	3073.33	2977.42	3758.00
T6	5792.68	5074.11	5433.40	3311.87	2153.89	2732.88	4083.14
T7	4180.51	6554.72	5367.62	4023.13	4869.60	4446.37	4907.00
T8	5605.98	4232.43	4919.21	4143.22	3470.79	3807.01	4363.11
Average	**5076.03**	**5372.06**	**5224.04**	**3855.13**	**3617.23**	**3736.18**	**4480.11**
Factor	Y	BF	T	YBF	YT	BFT	YBFT
LSD 0.05	283.70	283.70	567.40	401.21	802.43	802.42	1134.80

$p<0.05$ significant(*); $p>0.05$ no significant (^{ns})

Yang et al.(2023) states in the research the effectiveness of cover crops. Crop productivity and grain yields varies substantially with weather, length of growing season, soil type, crop species, and cover crop characteristics such as biomass production, C:N ratio and residue decomposition rate. From CC, field pea and fodder kale, as well as leguminous mixtures with winter oats stood out in the first year of testing (Table 7) with the highest yield, while in the second year the lowest yield was recorded for varieties in mixtures (T5 and T6). Observing the meteorological conditions in the years of the study, the effect of cover crops was smaller in the year with lower amounts of precipitation, with increased air temperatures during the vegetation period of the main crop. According to the results of Williams et al. (2008), in the case of sweet corn, the main factors of grain yield reduction are lower competitiveness in relation to weeds and greater sensitivity to drought during the summer months. Antosh et al. (2020) reported the results regarding the yield of sweet corn grains during two years of trials and at two locations after a cover crop of vetch, that it increased by 35.9% to 50.4% compared to conventional cultivation technology, while in the trials of Moore et al. (2020) ranged from 13.2 to 16.4 t ha^{-1}. Of the winter cover crops and their mixtures, as well as in relation to the

conventional way of growing sweet corn (Dolijanović et al., 2014), winter hairy vetch had the advantage in terms of the achieved yield grain of sweet maize. Research results Noland et al. (2018) support that interseeding cover crops into maize at the seven-leaf collar stage introduces little to no risk of maize yield reduction, at least in years with above-normal precipitation.

Conclusions

The results of this work indicate that in a dry climate, without irrigation, cover crops that are more robust, with more developed above-ground biomass and greater soil coverage, primarily legumes grown individually or in mixtures, can increase the productivity of sweet maize and serve as an important part of sustainable cultivation systems of corn hybrids for specific purposes in our climatic conditions. The significant variation of yield and yield components in relation to the examined factors and their interactions is an indicator of a significant influence of both growing conditions and applied agrotechnical measures before and during the sweet maize vegetation period. From this research we conclude that the inclusion of some species as cover crops in the simplified cropping systems which actually predominate in the semiarid regions, improves N use efficiency, compared to the alternative long fallow periods between summer crops. Despite the clear benefit of cover crops, additional work must resolve the apparent interactions of fertilizer and cover crops on N mineralization/ immobilization processes.

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EFFECT OF CALCIUM CHLORIDE AND DURATION OF STORAGE ON THE QUALITY OF THE APPLE CULTIVAR 'GLOSTER'

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Abstract

Apple (*Malus×domestica* Borkh.) is one of the most represented and economically most important fruit species. There are a number of calcium-containing products on the market that can be applied before or after harvest to delay fruit maturation with no negative effects on consumers. The paper also presents the results of a study on the effects of a foliar application of calcium chloride ('Stopit') on the physical and basic fruit quality properties of the apple cultivar 'Gloster'. The study was conducted for two consecutive years, and the changes in fruit weight, firmness, dimensions, soluble solids, total sugars, and organic acids content were studied during harvest and storage periods of 60 and 120 days in a regular cold chamber. Fruits treated with calcium chloride had higher fruit weight, height, width, firmness, total sugars, and ratio between total sugars and organic acids (206.0 g, 70.6 mm, 77.8 mm, 7.6 kg cm^{-2}, 10.4%, and 21.4, respectively). The storage period had a significant effect on all fruit parameters studied, except for the content of total acids. The highest values of physical properties were mesured at the moment of storage, soluble solids content after 60 days (13.2° Brix), and total sugars (10.9%) and sugar/acid ratio (22.3) after 120 days of storage. Foliar application of $CaCl_2$ during vegetation can be an effective measure to avoid losses in apple fruit quality during storage in the regular cold chamber.

Keywords: *apple*, *$CaCl_2$*, *storage*.

Introduction

Apple (*Malus* × *domestica* Borkh.) is the most important continental fruit tree species. In the global fruit production structure, apples rank fourth, behind citrus fruits, grapes, and bananas (Milošević et al., 2019). The global apple production in 2021 amounted to 93,144,358 tons, while in the Republic of Serbia, it is grown on an area of 27,034 hectares, with a total production of 513,238 tons (FAOSTAT, 2021).

Apple fruits can be consumed fresh immediately after harvest or after a certain storage period (Folta and Gardiner, 2009), and they are often stored for a longer period of time in a controlled atmosphere for production profitability reasons. During storage, intense metabolic processes occur, leading to a reduction in fruit quality (Fattahi et al., 2010). Sams et al. (2008) state that many physiological and pathological disorders of apple fruits during storage are related to low calcium (Ca^{2+}) content in fruit tissue. The concentration of calcium in plant tissue plays an extremely important role in maintaining fruit quality after harvest. Several authors (Hossain et al., 2005; Misra and Gupta, 2006; Naeem et al., 2009) emphasize that the application of calcium-based products positively affects the stabilization of cell membranes and the delay of fruit aging, maintains fruit firmness, reduces the occurrence of 'bitter pit' and internal fruit decay (Raese and Drake, 2002; Dierend and Rieken, 2007; Suljevic et al., 2011). There are numerous calcium-containing products available on the market, that are applied

before or after harvest, enabling the quality preservation of fruits without harmful effects on human health (Lester and Grusak, 1999; Shirzadeh et al., 2011).
The aim of this research was to determine the effects of foliar application of a calcium chloride-based product ('Stopit') on changes in the quality of 'Gloster' apple fruits stored under normal atmosphere.

Material and Methods

The research was conducted during the period 2018–2019 in the production-experimental apple orchard of the Fruit Research Institute in Čačak (43° 89' 40" N, 20° 43' 42" E, altitude 233 m). The orchard was established in 2006, and apple trees were grafted to the M9 rootstock. The slender spindle training system was applied. The planting spacing was 4×1.25 m (2,000 trees ha^{-1}). Standard agri agromonic and pomotechnical practices were applied during the investigation period.
'Gloster' is a German apple cultivar created at the Jork Experimental Station in 1969 by crossing the cultivars 'Glockenapfel' and 'Richared'. It ripens in mid-September, and the fruits can be stored in cold storage with normal atmosphere until the end of March. During cold storage, physiological disorders such as bitter pit, glassiness, fruit mealiness, and others may occur (Mišić, 2004).
In the experiment, a liquid foliar fertilizer with the commercial name 'Stopit' (Yara UK Ltd. Pocklington, York, UK) was applied. It is a solution of calcium chloride ($CaCl_2$) with a high concentration of calcium (12% w/w = 160 g L^{-1}). Its application is recommended in fruit trees to prevent calcium deficiency in fruits. It is used from the beginning of flowering until the end of the fruit ripening phase, especially from the fruit pigmentation phase until harvest.
The research included foliar application of the 'Stopit' ($CaCl_2$ concentration of 224 g L^{-1}). Foliar application was made to 20 trees in four replicates (a total of 80 trees per treatment) from early June to mid-August. In both years of the study, the preparation was applied four times. In 2018, the application dates were June 7, July 2 and 20, and August 15. In 2019, the application dates were June 14, July 5 and 26, and August 16. The foliar application was performed using a motorized sprayer SR 420 (STIHL International GmbH Waiblingen, Germany), at a consumption rate of 1,000 L ha^{-1}. The preparation was applied at a rate of 7.5 L ha^{-1} (150 mL in 10 L of water). The control consisted of 'Gloster' apple trees that were not treated with the 'Stopit' product. In the study the physical characteristics (weight, width, height, firmness) and basic chemical properties (soluble solids content, total sugars and organic acids content, as well as their ratio) of the fruit were investigated. These parameters were determined during the harvest period and after 60 and 120 days of fruit storage in a normal atmosphere.
Weight, height and width, fruit firmness, and soluble solids content were determined using standard morphometric methods on a sample of 80 fruits (four replicates of 20 fruits each). Fruit weight (g) was measured using a digital scale (Adventurer Pro AV812M, Switzerland), while fruit length and width (mm) were determined using a digital calliper (Carl Roth, Germany). Fruit firmness was measured using a digital penetrometer (Model FHT-803, Italy), and values were expressed in kg cm^{-2}. Soluble solids content in apple fruits was determined using a digital refractometer (Carl Zeiss, Jena, Germany) at room temperature (20 °C), and the values were expressed in °Brix. Total sugar content was determined volumetrically using the Luff-Schoorl method (Džamić, 1989), while total acidity was determined by titration, and the values of these parameters were expressed as percentages. The sweetness index, representing the ratio of total sugars to acids, was calculated.
Results are presented as mean ± standard error of the mean (SE). Differences between mean values were compared using Duncan's test in a three-way analysis of variance (ANOVA)

using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with p values ≤ 0.05 were considered statistically significant.

Results and Discussion

The results of the study on the effects of year, treatment with calcium chloride, and storage duration on the physical and chemical properties of 'Gloster' apple fruits are presented in Table 1. Analysis of variance revealed that weather conditions during the study years significantly influenced all the quality parameters of the fruits. No statistically significant differences were found in the values of soluble solids content and total acidity of apple fruits when the 'Stopit' product was applied. Significant effects of the storage time were found for all studied fruit properties, except for total acidity. The interaction effect of all factors was observed only for total sugar and acid content in 'Gloster' apple fruits, indicating that the individual effects of each factor, as well as their interactions, can be neglected. Statistically significant interaction effects conditioned by the combined action of two variability factors were recorded for the other studied pomological properties.

The results of the interaction effect of year and treatment with the 'Stopit' product on the weight, width, and height of 'Gloster' apple fruits are shown in Figure 1. The highest values for fruit weight, width, and height were observed in the treatment with the 'Stopit' product in 2018, while the lowest values were recorded in the control variant in 2019. Mišić (2004) emphasizes that the fruits of 'Gloster' apple belong to the group of large to very large fruits (180–250 g). Fruits of desirable apple cultivars should ideally have dimensions ranging from 65 to 80 mm (Gvozdenović, 1998). On the other hand, Asgharzade et al. (2012) state that foliar application of calcium chloride during the growing season influences an increase in apple fruit weight, and Amiri et al. (2008) confirmed a positive impact on fruit dimensions in their studies. Our results are consistent with the findings reported by the mentioned authors, indicating a correlation.

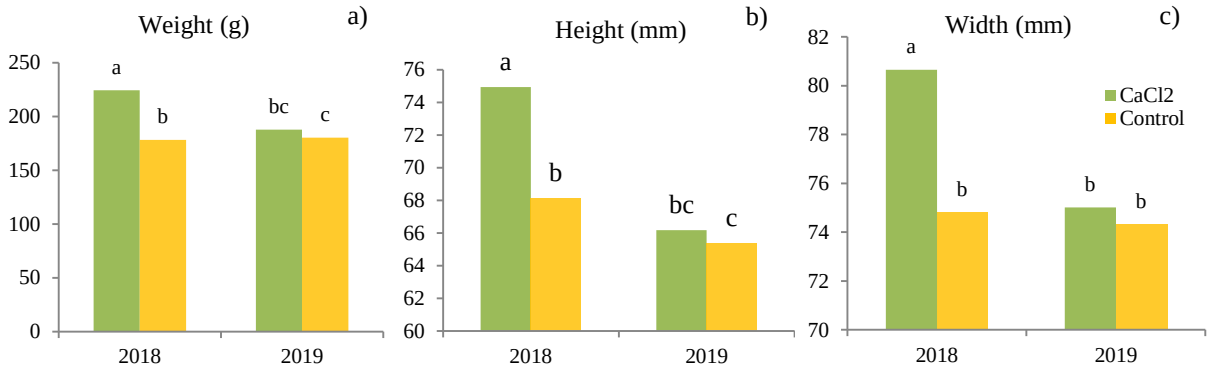

Figure 1. Interaction effect of year/treatment on weight (a), height (b) and width (c) of the fruit of apple cultivar 'Gloster'

Table 1. Influence of year, treatment of Stopit and period of storage on physical and chemical properties of apple fruit of the cultivar 'Gloster'

Treatment	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Firmness (kg cm^{-2})	SSC (°Brix)	TS (%)	TA (%)	TS/TA
Year (A)								
2018	201.4±5.5 a	71.5±0.8 a	77.7±0.8 a	7.6±0.2 a	10.6±0.3 b	9.4±0.3 b	0.4±0.0 b	19.5±0.7 b
2019	183.9±3.5 b	65.8±0.7 b	74.7±0.6 b	6.8±0.4 b	14.1±0.1 a	11.8±0.2 a	0.5±0.0 a	22.2±0.7 a
Treatment (B)								
$CaCl_2$ (Stopit)	206.0±4.9 a	70.6±1.1 a	77.8±0.8 a	7.6±0.3 a	12.5±0.4 a	10.4±0.4 a	0.5±0.0 a	21.4±0.7 a
Control	179.2±3.1 b	66.7±0.6 b	74.6±0.6 b	6.8±0.3 b	12.3±0.4 a	10.1±0.3 b	0.5±0.0 a	20.4±0.7 b
Storage duration (C)								
0 days	199.6±5.7 a	69.7±0.9 a	77.6±0.7 a	8.7±0.2 a	11.4±0.6 c	9.2±0.4 b	0.5±0.0 a	19.0±0.6 c
60 days	193.5±6.3 ab	69.7±1.2 a	76.6±0.8 a	6.8±0.2 b	13.2±0.4 a	10.7±0.1 a	0.5±0.0 a	21.4±0.4 b
120 days	184.7±5.6 b	66.5±1.2 b	74.4±1.1 b	6.0±0.3 c	12.4±0.4 b	10.9±0.5 a	0.5±0.0 a	22.3±1.2 a
ANOVA								
A	*	*	*	*	*	*	*	*
B	*	*	*	*	ns	*	ns	*
C	*	*	*	*	*	*	ns	*
A × B	*	*	*	ns	ns	*	ns	*
A × C	ns	ns	ns	*	*	*	*	*
B × C	ns	ns	ns	ns	*	*	*	*
A × B × C	ns	ns	ns	ns	ns	*	*	ns

SSC - total soluble solids; TS - total sugars; TA - titrable acidity; TS/TA – ratio between total sugars and total acids.

*Values within each column followed by the same small letter are not significantly different at $p \leq 0.05$ by Duncan's test; ns - non-significant differences

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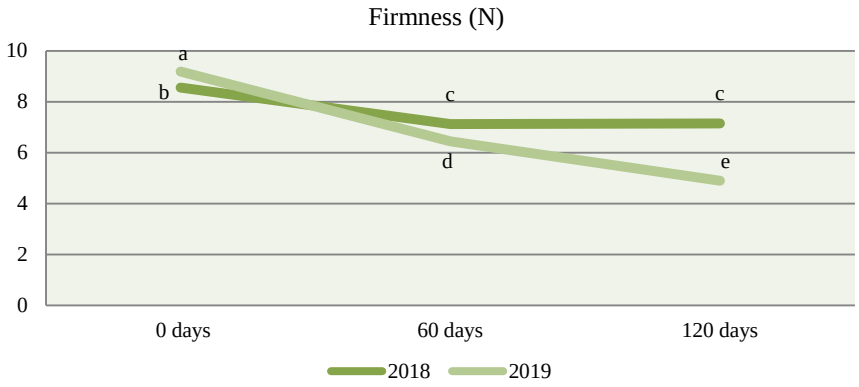

Figure 2. Interaction effect of year/storage duration on fruit firmness of apple 'Gloster'

Figure 2 shows the interaction effect of year and storage duration on the firmness of 'Gloster' apple fruits. The highest value was obtained for fruits sampled in 2019 and tested at harvest time (0 days), while the same fruits had the lowest firmness value after 120 days of storage. These results are consistent with the findings of Casero et al. (2004), who found that the application of calcium chloride reduces softening and maintains fruit firmness during storage. On the other hand, Baranyai et al. (2020) emphasized that as the storage period is extended, the firmness of apple fruits decreases, which is confirmed by the results of our research.

The results regarding the interaction effect between year and storage duration, as well as treatment and storage duration, on the soluble solids content of 'Gloster' apple fruits are presented in Figure 3. The highest value for this quality parameter was determined in 2019 after 60 days of storage, while the lowest value was observed in fruits sampled in 2018 at harvest time (0 days). On the other hand, when analyzing the results of the interaction effect between the treatment with the 'Stopit' product and storage duration, the highest values were found in the control after 60 days of storage, while the lowest values were observed in the same variant at harvest time (0 days). According to literature data, a soluble solids content of 11°Brix is considered a lower value to achieve optimal consumer acceptance (Magazin et al., 2013).

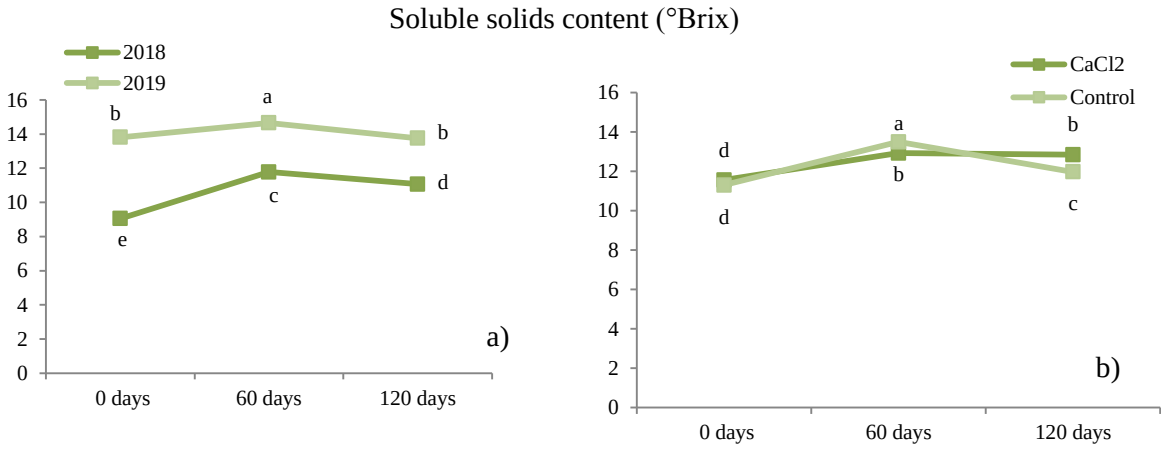

Figure 3. Interacion effects of year/storage duration (a) and treatment/storage duration (b) on the soluble solids content of apple 'Gloster'

The results of total sugar and acid content in ‘Gloster’ apple fruits, depending on the influence of year, treatment, and storage duration, are shown in Figure 4. The interaction effect of variability factors significantly influenced the total sugar content in ‘Gloster’ apple fruits (Figure 4a). The highest value for this parameter was recorded in 2019 for fruits treated with ‘Stopit’ product and stored for 120 days, while the lowest value was observed in both types of fruits (treated and untreated) in 2018, at harvest (0 days). Hudina and Štampar (2009) state that calcium content does not play a significant role in the sugar content of apple fruits; instead, sugar content depends on genotype, environmental factors, and agronomic practices. During storage, changes in sugar content occur due to increased starch degradation. According to Niketić-Aleksić (1994), the average total sugar content in apple fruits varies from 6.6% to 15.5%. The interaction of these variability factors also significantly influenced total acid content (Figure 4b). Control fruits tested in 2018 after 120 days of storage had the highest content, while fruits in the same year treated with calcium chloride and tested at harvest (0 days) had the lowest total acid content. Optimal calcium supply directly affects an increase in acid content in fruits, while during storage, these values decrease due to fruit respiration and organic acid degradation processes (Cheng et al., 2018).

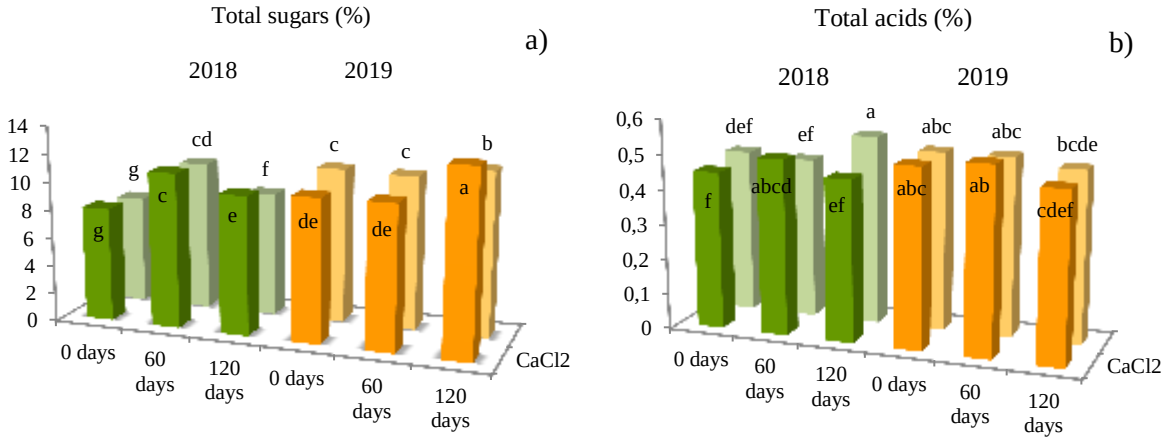

Figure 4. Interaction effects of year/treatment/storage duration on total sugars (a) and total acids (b) in the fruit of apple cultivar ‘Gloster’

Figure 5 shows the interaction effect of year/treatment, year/storage duration, and treatment/storage duration on the sugar/acid ratio in ‘Gloster’ apple fruits. Higher values of this parameter were recorded in 2019 and in the treatment with the ‘Stopit’ product (Figure 5a). Furthermore, higher values were observed in 2019 after 120 days of fruit storage (Figure 5b). However, no consistent trend was observed in the values of the sugar/acid ratio across different storage duration and treatments (Figure 5c). In this regard, it is mentioned in the literature that calcium slows down the ripening and accumulation of sugars in apple fruits, resulting in increased sugar content and decreased acid content. However, during the storage period, sugar content increases while the acid content decreases, resulting in changes in sweetness index values (Kadir, 2005).

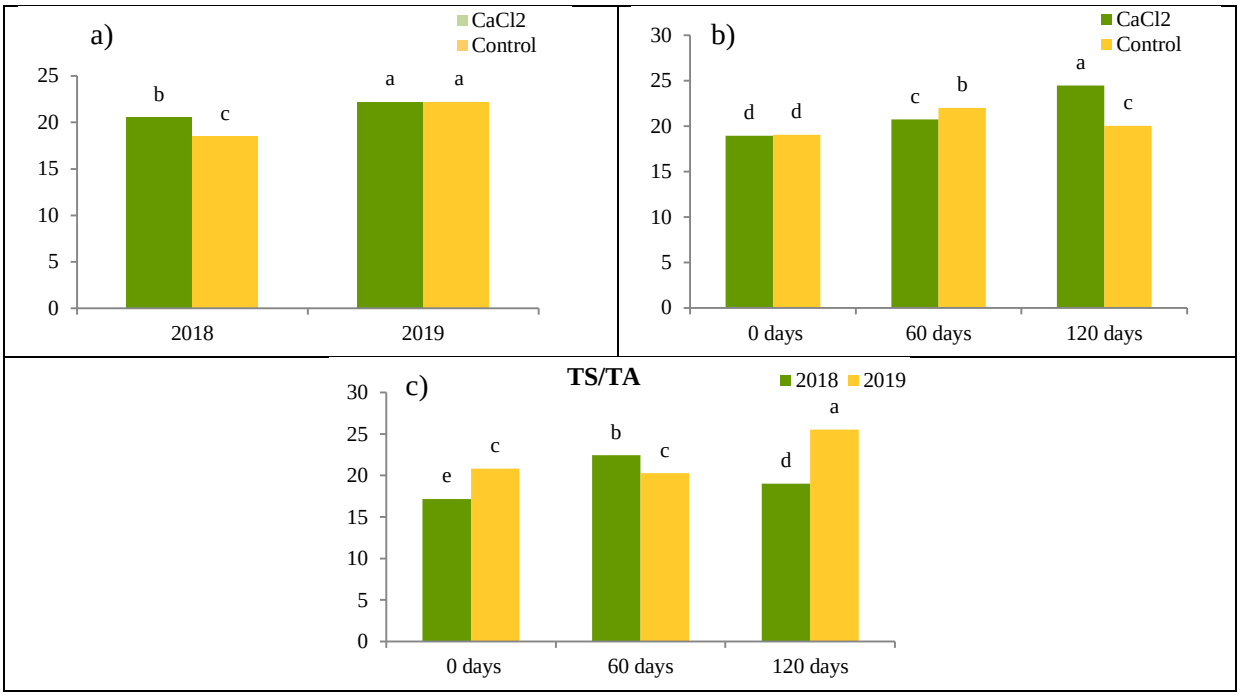

Figure 5. Interaction effects of year/treatment (a), treatment/storage duration (b), and year/ storage duration (c) on total sugars/total acids content of apple fruit of cutivar 'Gloster'

Conclusions

The results obtained indicate that foliar application of the 'Stopit' product based on calcium chloride during the growing season is an effective measure to reduce quality losses in 'Gloster' apple fruits during storage in normal atmosphere. In this regard, guidelines can be provided to producers for improving apple cultivation techniques through the application of the 'Stopit' product, aiming to obtain high-quality fruits without negatively affecting consumer acceptance.

Acknowledgements

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NEWLY CREATED ČAČAK'S SOUR CHERRY VARIETIES AS A RAW MATERIAL FOR SPIRIT PRODUCTION

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Abstract

According to the average annual production, sour cherries take the third place in Serbian fruit production. In the assortment, the most represented is the autochthonous variety 'Oblačinska', which is characterized by a very small fruit. In recent decades, large-fruited varieties bred at the Fruit Research Institute in Čačak, have become more widespread in sour cherry orchards. Some of the newly bred varieties, similar to 'Oblačinska', are characterized by combined properties and can be used as fresh fruit and/or for various types of processing. Sour cherries are mainly used in Serbia as raw material for obtaining numerous products. Frozen cherries dominate, but juices, sour cherry in brine, dried fruits, candied fruits, compote, wine, vinegar, liqueurs are also produced, and in recent years, sour cherry spirit is gaining interest. The paper presents the results of two-year examinations of the suitability of two varieties of sour cherries with combined properties - 'Šumadinka' ('Köröser Weichsel' × 'Heimanns Konservenweichsel') and 'Sofija' ('Čačanski rubin' × 'Heimanns Konservenweichsel'), bred in Čačak, and standard variety 'Heimanns Konservenweichsel', for the production of spirits. The sour cherry spirits were produced from spontaneously fermented mashes of sour cherry fruits with stones, followed by double distillation in alembic of traditional design. The obtained monovarietal sour cherry spirits differed significantly in spirit yields and contents of the ten major volatile components. Based on the results of sensory analysis in both years, all obtained monovarietal sour cherry spirits can be classified as high-quality spirit drinks.

Keywords: *Prunus cerasus, sour cherry spirit, yield of distillate, volatile components, sensory characteristics.*

Introduction

Since the middle of the 20th century, the production of sour cherries in Serbia has been constantly increasing. In a short period of time, from 2016 to 2020, the area under sour cherries increased from 16,797 ha to 19,601 ha, while the average yield in this period was 115,831 t. Nowadays, Serbia is the fourth producer of sour cherries in Europe, and the seventh in the world (Milatović, 2023). The small-fruited cultivar 'Oblačinska', whose fruit weight depending on the clone varies between 2.62 and 3.52 g (Nikolić *et al.*, 2005), and which is mainly used for processing (Miletić, 2019), dominates in the sour cherry production with a share of 60 to 70%. There is a tendency for spreading varieties with large fruit, larger than 5 g, which can be used for processing, but also for fresh consumption (Stancević and Nikolić, 1987; Radičević and Cerović, 2015). Among large-fruited varieties, in addition to the most commonly grown introduced variety 'Heimanns Konservenweichsel', 'Šumadinka' and 'Sofija' resulting from the breeding work at the Fruit Research Institute in Čačak - are also grown in Serbia.

Sour cherries in Serbia are mainly used for processing; production of frozen cherries dominates (Milatović, 2023). The rest is used in industry and households for the production of juices, syrups, candied fruits, sour cherries in brine, liqueurs, and more recently for the production of dried sour cherries and sour cherry spirit (Radičević and Cerović, 2015). Unlike plum spirit, the Serbian national spirit drink, sour cherry spirit is produced to a much smaller extent, mostly in years when the purchase price of sour cherries on the market is low, i.e. when there is small demand for frozen cherries.

Sour cherry spirit is a delicacy product, consumed colorless, as an aperitif (Nikićević and Paunović, 2013). There are two important steps in sour cherries processing that are related to the characteristics of the fruits, and are considered important for obtaining high quality sour cherry spirit. The first step is the separation of the stalks before processing (Dürr, 2000). The second step is the presence of stones in the mash, which results in a more intense aroma (stone like tone) of the sour cherry spirit than in the case when pitted fruits are fermented and distilled (Nikićević and Paunović, 2013). Beside the method of production, the sour cherry variety has a decisive effect on the quality of the sour cherry spirit (Nikićević *et al.*, 2011). Similar to some plum varieties bred in Čačak that were not primarily aimed as brandy varieties, but were used in this way (Popović *et al.*, 2021), Čačak's sour cherry varieties also were not bred with the intention of being brandy varieties, but were used in this way in certain years. Thus the aim of this work was to examine the suitability of Čačak's sour cherry varieties for the production of brandy (spirit).

Materials and methods

Fully ripe fruits without stalks of the sour cherry cultivars 'Šumadinka', 'Sofija' and 'Heimanns Konservenweichsel' were picked (70 kg fruits from each cultivar tested) in experimental orchard in Čačak, Serbia, during 2016 and 2017. The Mettler technical scale was used for the fruit and stone mass determination; stone ratios were calculated from these values. Standard methods (Trajković *et al.*, 1983) were used for determination of soluble solids contents (SSC) (by refractometry), total sugars, invert sugars and sucrose contents (Luff-Schoorl method), total acids (by neutralisation with 0.1 M NaOH) and pH values (by pH metry). The sugar/acid ratio was calculated.

Polyethylene vessels for alcoholic fermentation (three replications for each cultivar) were filled with 20 kg of manually crushed fruits with stones. Sour cherry mashes with stones were spontaneously fermented at a temperature of approximately 20 °C. The dynamics of mash alcoholic fermentations was monitored daily (by refractometry). The mashes were distilled immediately after completion of alcoholic fermentation. Pilot-scale copper alembic (volume of the boiler was 25 L) was used for fermented mashes distillation; the first distillates with an ethanol content of 22 vol.% were obtained. For the second distillation (redistillation of the first distillate), the same distillation equipment was used; three fractions were separated during the second distillation: head (1% of the first distillate volume put in the boiler of alembic), heart (60 vol% ethanol content) and tail. Only the middle fractions (hearts) were used for further chemical and sensory analyses. Before analyses, the ethanol content in the heart-fractions obtained by the second distillation was reduced with deionised water from 60 to 42 vol.%. Yield of the sour cherry distillates was expressed as the yield of the first distillate (expressed in L, with an ethanol content of 22 vol%) obtained by distillation of 20 kg of mash, and then multiplied by 5, to be expressed on 100 kg fruits (L of distillate containing 22 vol% ethanol/100 kg fruits with stones).

The methanol, higher alcohols, ethyl acetate and acetaldehyde contents in the sour cherry spirits were determined by gas chromatography with flame ionization detection (GC/FID) method (Popović *et al.*, 2021), and the content of benzaldehyde was determined by the official

spectrophotometric method (Sl. list SFRJ 70, 1987). The sensory analysis of colourless sour cherry spirits was conducted by a four-member expert panel using the Buxbaum method (Popović *et al.*, 2021). Correlation analyses were performed using STATISTICA 7.0 software (Statsoft, Tulsa, OK, USA).

Results and discussion

The fruit characteristics of the tested sour cherry varieties are shown in Table 1. All three varieties, with a fruit weight of around 7 g, belong to the group of varieties with large fruit although this characteristic is not important for spirit production. From the point of view of the suitability of the variety for the production of sour cherry spirit, the most important characteristics of the fruits are sugar content, stone ratio and aroma (Pieper *et al.*, 1993).

Table 1. Fruit characteristics of sour cherry cultivars.

Characteristics	Cultivars					
	Heimanns Konservenweichsel		Šumadinka		Sofija	
	2016	2017	2016	2017	2016	2017
Fruit mass (g)	6.70	6.65	7.54	7.52	7.09	7.01
Stone mass (g)	0.52	0.54	0.68	0.67	0.54	0.50
Stone ratio (%)	7.76	8.12	9.02	8.91	7.62	7.13
Soluble solids (%)	13.60	14.30	11.90	12.70	13.10	12.00
Total sugars (%)	8.44	9.20	6.48	6.96	8.16	7.44
Inverted sugars (%)	7.85	8.10	6.10	6.48	7.85	7.10
Sucrose (%)	0.56	1.04	0.46	0.47	0.29	0.32
Total acids (%)	1.88	2.15	1.86	0.95	1.34	1.96
pH	3.30	3.24	3.19	3.16	3.38	3.08
Sugars/acids ratio	4.49	4.28	3.48	7.33	6.09	3.80

Varieties with higher total sugar content can potentially produce higher distillate yields. According to the content of total sugars, the most suitable variety for the production of sour cherry spirit was 'Heimanns Konservenweichsel' (8.44 and 9.20%), followed by the variety 'Sofija' (8.16 and 7.44%), and finally the variety 'Šumadinka' (6.48 and 6.96%). The stone ratio affects the content of benzaldehyde and the specific favorable aroma (expressed stone-like tone) of the sour cherry spirit. In both years, the lowest stone ratio was found in 'Sofija' (7.62 and 7.13%), and the highest in 'Šumadinka' fruits (9.02 and 8.91%). The content of total acids significantly influences the formation of the taste of the fruit, mainly through the value of the sugar/acid ratio, which is important for sour cherries used for fresh consumption and for processing that does not include distillation. However, the pH value is much more important for spirit production. The pH value in the fruits of the tested cultivars was very close to 3.0 (from 3.08 to 3.38). This indicates that there is no need to acidify the mash for cleaner alcoholic fermentation, which is in accordance with the recommendations of other authors (Dürr, 2000; Nikićević and Paunović, 2013) and with the fact that the optimal pH for sour cherry mash fermentation is 3.25 (Pham *et al.*, 2021).

Alcoholic fermentation of the mashes lasted from 12 to 18 days (Table 2). Although the lowest distillate yields were obtained from the variety 'Šumadinka' (15.8 and 14.9 L of distillate with 22 vol.% ethanol), whose fruits contained the least total sugars, the correlation analysis did not reveal a statistically significant relationship between distillate yield and the content of total sugars ($r = 0.74$). The value of the correlation coefficient between SSC and distillate yield is even lower ($r = 0.44$), making the use of SSC as a reliable indicator for

predicting potential distillate yield questionable. The highest distillate yields were obtained by processing the fruits of the variety 'Sofia' (23.3 and 21.1 L of distillate with 22 vol.% ethanol), although it would be expected that, considering the highest content of total sugars, the highest yields of brandy would be obtained from the fruits of the 'Heimanns' Konservenweichsel'. These differences might be due to the different sugar profiles in the fruits of the examined cultivars, but also due to various profile of spontaneous microflora in the mashes during alcoholic fermentation of examined cultivars. It is interesting to note that the highest distillate yield is 56% higher than the lowest yield. The obtained data show how much the production efficiency expressed as production yield differs among the sour cherry varieties, processed in the same way.

Table 2. Duration of alcoholic fermentation (DAF, days) and yield of sour cherry distillates (YSCD, L 22 vol. % distillate/100 kg mash).

Characteristics	Cultivars					
	Heimanns Konservenweichsel		Šumadinka		Sofija	
	2016	2017	2016	2017	2016	2017
DAF (days)	13	12	12	12	15	18
YSCD (L 22 vol.% distillate/ 100 kg mash)	20.4	21.1	15.8	14.9	23.3	21.1

The chemical composition and sensory evaluation of monovarietal sour cherry spirits are shown in Table 3. According to Nikićević and Paunović (2013), among all fruit brandies, sour cherry spirit contains potentially the least amount of methanol (5,900 mg/L a.a.). Methanol contents in experimentally produced sour cherry spirits ranged from 3,410 mg/L a.a. ('Sofija', 2016) up to 6,330 mg/L a.a. ('Šumadinka', 2017). This is significantly less than the maximum level required by law (10,000 mg/L a.a.). Based on GC-FID analysis, Pham *et al.* (2021) found that the main aromatic components of sour cherry spirit are 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethyl acetate, which are formed mainly during alcoholic fermentation and participate in the formation of the "body" of the sour cherry spirit. The contents of acetaldehyde (45-225 mg/L a.a.) and ethylacetate (500-1,339 mg/L a.a.) were within the ranges that give a pleasant fruity character of the aroma. Seasonal differences in the content of these components in spirits of the same variety, are probably the result of a different composition of the indigenous microbial flora. The contents of 1-propanol, 2-methyl-1-propanol, 1-butanol, 2/3-methyl-1-butanol in the obtained spirits were in much narrower ranges than those reported by Nikićević and Paunović (2013). The differences in the contents of higher alcohols found in monovarietal sour cherry spirits could be attributed to the fine differences in the fruits composition and differences in indigenous microbial flora. Also, varietal differences affected the different contents of 1-hexanol, which is produced by enzymatic synthesis through the lipoxygenase pathway during fruit processing and alcoholic fermentation. The contents of total higher alcohols also were in a narrower range (4,534-5,958 mg/L a.a.) than the range (3,794-7,783 mg/L a.a.) reported by Nikićević and Paunović (2013). In our samples, the range of the proportion of 1-propanol in the total higher alcohols was lower (25-37%) than those given by the above authors (35-60%). Among the analyzed higher alcohols, only the contents of 1-propanol were significantly higher in 2017 than in 2016. This confirms the findings of Kovacs *et al.* (2018) that the contents of 1-propanol and 1-butanol can be used to classify fruit distillates based on the year of harvest. The proportions of higher alcohols depend on the composition of the raw material and can serve as one of the factors for determining the authenticity of the distillate. In the case of sour cherry spirit, the ratio of isoamyl alcohol and isobutanol (IA/IB) can be used to distinguish

distillates of the 'Šumadinka' variety from distillates produced of the other two varieties. Benzaldehyde is the characteristic and main aromatic compound of sour cherry spirits, especially those obtained by processing the fruits with stones (Nikićević *et al.*, 2011). Benzaldehyde contents ranged from 16.2 to 86.2 mg/L a.a. The highest concentrations were found in sour cherry spirits of the 'Šumadinka' variety, followed by 'Sofija', and then 'Heimanns Konservenweichsel'. It was found that the content of benzaldehyde was significantly higher in 2017 than in 2016 in all monovarietal sour cherry spirits. Based on the results of sensory analysis, all obtained sour cherry spirits can be classified in the category of excellent quality products, since they have an average score above 18.01. It is interesting that the subtle differences in sensory evaluations are related to the stone weight (correlation coefficient r = 0.85), i.e. sour cherry spirits obtained from fruits with larger stones were evaluated slightly better. According to Nikićević *et al.* (2011), a higher content of benzaldehyde, along with some other ingredients (linalool), affects the higher sensory evaluation of monovarietal sour cherry spirits.

Table 3. Chemical composition (mg/L a.a.) and sensory assesment (points) of sour cherry spirits

Characteristics	Cultivars					
	Heimanns Konservenweichsel		Šumadinka		Sofija	
	2016	2017	2016	2017	2016	2017
Methanol	3480	4170	4730	6330	3410	4080
Acetaldehyde	70	116	70	148	45	225
Ethyl acetate	584	836	500	638	556	1339
1-Propanol (1-P)	1385	1836	1590	2073	1227	1794
2-Methyl-1-propanol (IB)	1250	975	1297	1490	967	1033
1-Butanol	12	9	6	6	11	5
2/3-Methyl-1-butanol (IA)	2801	2280	2172	2372	2323	2071
1-Hexanol	13	17	7	17	6	8
Total higher alcohols	5461	5117	5072	5958	4534	4911
Benzaldehyde	16.2	27.3	61.5	86.2	25.7	66.5
IA/IB ratio	2.24	2.34	1.67	1.59	2.40	2.00
IB/1-P ratio	0.90	0.53	0.81	0.72	0.79	0.58
IA/1-P ratio	2.02	1.24	1.37	1.14	1.89	1.15
Sensory assesment	18.16	18.21	18.25	18.34	18.19	18.19

Conclusion

The large-fruited sour cherry varieties ('Šumadinka' and 'Sofija'), bred at the Fruit Research Institute in Čačak, are an interesting raw material for the production of sour cherry spirit. From both varieties, spirit of excellent sensory quality can be obtained. However, it should be taken into account that, considering the characteristics of the fruits, the processing of the variety 'Šumadinka' can yield up to 36% less distillate than the processing of the variety 'Sofija'.

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VARIABILITY OF SEED PROTEIN CONTENT AND SEDIMENTATION VOLUME IN WHEAT (*Triticum aestivum* L.)

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Abstract

The quality of wheat seed protein is determined by encoding gene alleles, but percentage content varies dependin from genotypes, environmental factors and interaction genotype/ environmental conditions. The aim of this work is analysis of seed protein content, sedimentation volume and their relationship with gliadin and high molecular weight glutenin subunits in bread wheat. The 10 wheat genotypes included in experimental field study in two vegetation seasons (2015/16 and 2016/17) with different climatic conditions. The results showed that seed protein content and sedimentation volume, varied in wheat genotypes within and between vegetation season. The highest seed protein content in both vegetation season was found in the genotype G-3617-1 (14.00% and 14.80%) and the least in genotype G-3634-2 (11.40% and 12.00%). The value of protein sedimentation volume in both vegetation season was the highest in genotype G-3622-1 (52.00 ml and 56.00 ml), while the least in genotype G-3601-4 (33.00 ml and 36.00 ml). Genotypes were grouped according to the presence of the same allele at all six loci for gliadins and three loci for high-molecular-weight glutenins, and found that with high value of seed protein content associate *Gli-B1b, Gli-D2b,* and *Glu-A1b, Glu-B1c, Glu-D1d*, allele, and that with high value of sedimentation protein volume associated *Gli-B1b, Gli-B2b* and *Glu-A1b, Glu-B1c, Glu-D1d* allele.

Key words: wheat, seed protein, protein sedimentation volume, allele, quality.

Introduction

Wheat seed protein content is an indicator of quality desirable for milling and baking industry. Seed protein contetn is genetic trait which value greatly varies under influence of environmental factor (Zečević et al., 2013; Knezevic et al. 2016a; Peršić et al., 2023). Variation of wheat seed protein content depens from genotype, environmental growing condition, regime of nutrition by applied fertilizers, precipitation, temperature, as well as interaction of genotypes and environments (Djokić et al., 1998; Würschum et al., 2016; Tóth et al., 2019). Seed protein content in average in bread wheat genotypes varied in ratio form 10% to15% (Shewry, 2007). In modern wheat varieties is achieved increasing of seed protein content through increased nitrogen remobilization encoded by introgressed gene *Gpc-B1*

locus at 6BS chromosome (Singh et al., 2018; Orlovskaya et al., 2022). Content of seed protein in wheat related with gluten content and both of them is indicate economic value of produced seed in trade market. Nitrogen ferzilization (amount and timing) influence to increase protein content (Annavarapu et al. 2021) and also, influence to proportion content of gliadin and glutenins (Bėkės, 2012). Gliadins are encoded by gene alleles at *Gli-1* and *Gli-2* loci on the short arms of 1. and 6. homologous chromosomes of A, B and D genome (Sozinov and Poperelya, 1980) while high molecular weight glutenins encoded by gene alleles at *Glu-1* loci on the long arm of 1. homologous chromosomes of A, B and D genome. The presence of some allele or combination of alleles encoding gliadin and glutenins associate with gluten quality, sedimentation volume, dough and loaf traits (Payne et al.,1987; Dimitrijević et al., 1998; Djukić et al., 2007; 2008; Knezevic et al., 2017; 2018). The gluten protein composition, protein content and sedimentation volume and their encoding gene alleles are useful markers for breeding proces (This et al., 2001; Djukić and Knežević, 2013). Sedimentation of protein indicate gluten quality on the base ability of swell and settle of protein molecules in acidic medium (Lookhart et al., 2001). Sedimentaton protein volume change, in dependance of time and storage conditions moisture and temperature and that that elevated storage temperature accelerated protein sedimentation (Yun et al., 2021).

The aim of this work was study variability of technological quality properties of wheat genotypes grown on the base of variation (i) protein content, (ii) sedimentation protein volume, (iii) identification of relationship between protein content and sedimentation protein volume studied traits with encoding alleles for gliadin and glutenins.

Material and methods

The 10 genetically divergent wheat genotypes (G-3623-1, G-3644-4, G-3619-3, G-3601-4, G-3636-2, G-3622-1, G-3617-1, G-3611-2, G-3634-2, G-3637-1) were included for analysis, protein content, sedimentation protein volume and their relationship with encoding gliadin and glutenins gene alleles. Protein content was computed on the base of obtained values of the nitrogen content determined by micro Kjeldahl method.(ICCC standard method 105/2). The established nitrogen content was multiplied by using 5.7 as the conversion factor for expression value of protein content as percentage of dry weight (d.w.). The analysis of sedimentation protein volume conducted by Zeleny method according ICC No. 116/1 (1972). This method based on the principle that the degree of sedimentation of the gluten fraction of a flour suspended in a lactic acid solution during a standard time interval which indicate baking quality. The slower sedimentation and higher sedimentation volume indicate better gluten quality.

For this ten genotypes were identified alleles at three *Gli-1* loci, three *Gli-2* loci and at three *Glu-1* loci, in previous study (Knežević et al., 2022). Gliadin proteins separated by using acid PAG electrophoresis method developed by Novoselskaya et al. (1983), and for determination of gliadin blocks alleles at *Gli-1* and *Gli-2* loci used method developed by Metakovsky (1991). The high molecular weight glutenins separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) Laemmli, (1970) and determining HMW-GS and identification of *Glu-1* alleles conducted by method Payne and Lawrence (1983). On the base determined of gliadin and glutenin compositin, were analyzed association each alleles with value of protein content, sedimentation volume in wheat genotypes.

Weather conditions in the vegetation period

The analysis of weather condition showed that average temperature and amoun of precipitation in first vegetation season (9.96 °C, 651.0 mm) was higherthan in second vegetation season (8.74 °C, 523.1 mm). In both vegtation season (2015/16 and 2016/17) the amoun of precipitation

was higher (651.0 mm and 523.1 mm) than in long term period (2000-2010) average amount of precipitation (417.8 mm), while average temperature was higher only in first vegetation season (9.96 °C) than in logn term period (8.50 °C. According to data, in first 2015/16 (8.8 °C and 14.1 °C) temperature in February and April was significantly higher than in second vegetation season 2016/17 (5.2 °C and 11.1 °C) and than in long term period (2.6 °C and 11.1 °C). However in second vegetation season temperature in March 2016/17 (10.8 °C) was significantly higher than in first vegetation season 2015/16 (7.8 °C) and in long term period (5.9 °C). The average temperature in other mounth suding both vegetation season seasons were similar (table 1).

Table 1. Average monthly temperatures and total monthly precipitation in Kraljevo

Parameter	Period	Oct	Nov	Dec	Jan	Feb	March	April	May	June	Xm	Total
Temperature ^{0}C	2015/16	11,6	7,3	3,3	-0,1	8,8	7,8	14,1	15,5	21,3	**9,96**	89,64
Temperature ^{0}C	2016/17	10,6	6,8	0,0	-4,7	5,2	10,8	11,1	16,8	22,1	**8,74**	78,66
Temperature ^{0}C	2000-2010	11,8	6,4	1,7	-0,1	2,6	5,9	11,6	16,4	20,4	**8,5**	76,5
Precipitation (mm)	2015/16	56,8	64,0	9,0	86,2	52,7	157,9	39,9	135,9	48,6	72,3	**651,0**
Precipitation (mm)	2016/17	84,1	77,6	9,4	22,0	35,0	57,0	82,0	100,0	56,0	41,1	**523,1**
Precipitation (mm)	2000-2010	61,0	44,3	44,6	30,0	29,9	33,2	52,9	52,6	69,3	46,4	**417,8**

*(*source: Republic Hydrometeorological service of Serbia*)

Results and discussion

In this study found differences among genotypes within and between first and second vegetation season. The average value of seed protein content varied from the least 11.7% in genotypes G-3634-2 (in first vegetation season 11.4% and in second vegetation season 12.0%) and the highest 14.4% in genotypes G-3617-1 (in first vegetation season 14.0% and in second vegetation season 14.8%). The seed protein content in all ten wheat genotypes in second vegetation season was greater than in first vegetation season. The difference in protein content of genotypes between growing seasons was in the range of 0.6% to 1.0%. On average for all genotypes, the value of protein content was in first vegetation season 12.62% and in second vegetation season 13.5% what indicate that protein content was higher in second vegetation season for 0.6% than in first vegetation season (table 2).

Similar variation between vegetation season (12.4% and 12.8%) in study different wheat genotypes, reported Khan et al. (2013). In study of 50 varieties Zečević et al. (2001) found differences of protein content which varied from 13.6% to 18.2% in first year, and from 13.9% to 18.1% in second year, depends of variety . Considering that protein content is in negatively correlation with grain yield it means that breeders effort for increasing yield in new creating variety, could resulted on reduction in quality (Knezevic et al., 2016b).

The average value of sedimentation volume varied from the least 34.5 ml in genotypes G-3601-4 (in first vegetation season 33.0 ml and in second vegetation season 36. ml) and the highest 54. ml in genotypes G-3622-1 (in first vegetation season 52. ml and in second vegetation season 56.0%). The sedimentation protein volume was different between in analyzed genotypes and between two vegetation seasons, and in in all 10 genotypes sedimentation volume in second vegetation season was higher than in first vegetation season. The difference in sedimentation volume of genotypes between growing seasons was in the range of 2.0 *ml* (G-3634-2) to 6.0 *ml* (G-3636-2). On average for all 10 genotypes, the value of sedimentation protein volume was 42.3 *ml* in the first vegetation season, and 46.0 *ml* in the second vegetation season, what indicate that sedimentation volume was higher in second vegetation season for 3.7 *ml* than in first vegetation season (table 2).

Table 2. Variation of seed protein content (%) and sedimentation volume (*ml*) in wheat genotypes grown in two vegetation season (2015/16 and 2016/17)

Trait	Year	G-3623-1	G-3644-4	G-3619-3	G-3601-4	G-3636-2	G-3622-1	G-3617-1	G-3611-2	G-3634-2	G-3637-1	Average
Protein content (%)	2015/16	12.60	12.80	12.00	11.60	13.20	13.60	**14.00**	12.40	**11.40**	12.60	12.62
	2016/17	13.20	13.60	12.80	12.60	14.20	14.40	**14.80**	13.20	**12.00**	13.40	13.50
	Average	13.40	13.20	12.40	12.10	13.70	14.00	**14.40**	12.80	**11.70**	12.00	13.06
Sedimentation volume (*ml*)	2015/16	44.0	42.0	36.0	**33.0**	46.0	52.0	**50.0**	42.0	**38.0**	40.0	42.3
	2016/17	48.0	46.0	40.0	**36.0**	52.0	56.0	**52.0**	46.0	40.0	44.0	**46.0**
	Average	**46.0**	**44.0**	**38.0**	**34.5**	**49.0**	**54.0**	**51.0**	**44.0**	**39.0**	**42.0**	**44.15**
Chroms./		**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**	**A B D**
Locus	Gli-1	***b. l. a.***	***f. b. b.***	***h. d. b.***	***m. l. b.***	***a. b. a.***	***b/c b. b.***	***b. g. a.***	***f. l. k.***	***b. g. a/b***	***m. k. k.***	***b. b. b.***
	Gli-2	***f. b. b.***	***e. o. j.***	***b. a. k.***	***b/j. e. b.***	***k. b. a.***	***a. v. b.***	***e. b. m.***	***b. p. b.***	***b. o. m/b***	***g. b. j.***	***b. b. b.***
	Glu-1	***b. c . d.***	***b. c . a.***	***c. i . a.***	***c. d . a.***	***b. c . d.***	***b. c . d.***	***b. c . d.***	***b. u . a.***	***c. d . a.***	***a. c . a.***	***b. c . a.***

Similar variation between vegetation season (55.0 *ml* and 52.0 *ml*) in study different wheat genotypes, reported Khan et al. (2013). In ealier study of 10 Serbian wheat varieties sedimetation volume varied from 33.0 *ml* and 64.0 *ml*, depend of genotype and year of study wheareas the average sedimentation volume for all 10 varieties in four year was 45.6 *ml*, 45.8 *ml*, 50.4 *ml* and 42.2 *ml* (Zečević et al., 2007). The sedimentation volume for seven genotypes had value on level of the first quality class or at the level of enhancing class, and three genotypes G-3619-3, G-3601-4 and G-3634-2 had sedimentation volume on the level of second quality class.

In this study are grouped genotypes according to presence of same allele at each *Gli-1*, *Gli-2* and *Glu-1* locus and computed average values for protein content and for sedimentation protein volume. Group of genotypes with high values of seed protein content carried the same allele (oftenly the most frequent) what indicate in both vegetation season their positive relationship *Gli-A1b* (12.9%; 13.80%), *Gli-B1b* (13.2%; 14.06%), *Gli-D1a* (12.80%; 13.75%), *Gli-A2e* (13.40%; 14.20%), *Gli-B2b* (13.10%; 14.10%), *Gli-D2b* (12.55%; 13.55%), *Glu-A1b* (13.10%; 14.03%), *Glu-B1c* (13.13%; 14.06%) , *Glu-D1d* (13.35%; 14.35%) table 3.

Group of genotypes with high value of sedimentation the same allele (oftenly the most frequent) what indicate in both vegetation season their positive relationship *Gli-A1b* (46.0 *ml*; 49.0 *ml*), *Gli-B1b* (46.66 *ml*; 51.33 *ml*), *Gli-D1a* (44.50 *ml*; 48.0 *ml*), *Gli-A2e*(46.00 *ml*; 49.0 *ml*), *Gli-B2b* (45.0 *ml*; 49.0 *ml*), *Gli-D2b* (42.75 *ml*; 46.50 *ml*), *Glu-A1b* (46.0 *ml*; 50.0 *ml*), *Glu-B1c* (45.66 *ml*; 49.66 *ml*) , *Glu-D1d* (48.0 *ml*; 52.0 ml) table 3.

From this combination of nine alleles, located at the three *Gli-1*, three *Gli-2* and three *Glu-1* loci, which associated with high protein content in one genotype (G-3623-2) present combination of seven allele, in three genotypes (G-3636-2; 3622-1; G-3617-1;) present combination of diferent six alleles in one genotype (G-3644-4) present combination of four alleles, in three gentypes (G-3611-2; G-3634-2; G-3637-1) present different combination of two alleles, in one genotype (G-3601-4) present one allele. However in G-3619-3 there are none of the nine alleles associated with protein content (table 2 and 3).

Table 3. Alleles at *Gli-1, Gli-2* and *Glu-1* associated with high value of protein content (%) and sedimentation volume (*ml*) in wheat genotypes

Locus	*Gli-A1*	*Gli-B1*	*Gli-D1*	*Gli-A2*	*Gli-B2*	*Gli-D2*	*Glu-A1*	*Glu-B1*	*Glu-D1*
Alleles assoc., with high gluten content	***b***	***b***	***a***	***e***	***b***	***b***	***b***	***c***	***d***
Group of genotypes which carry the same allele at *Gli-1, Gli-2* and *Glu-1* associated with the high value of protein content	G-3623-1	G-3644-4	G-3623-1	G-3644-4	G-3623-1	G-3623-1	G-3623-1	G-3623-1	G-3623-1
	G-3622-1	G-3636-2	G-3636-2	G-3617-1	G-3636-2	G-3601-4	G-3644-4	G-3644-4	G-3636-2
	G-3617-1	G-3622-1	G-3617-1		G-3617-1	G-3622-1	G-3636-2	G-3636-2	G-3622-1
	G-3634-2		G-3634-2		G-3637-1	G-3611-2	G-3622-1	G-3622-1	G-3617-1
							G-3617-1	G-3617-1	
							G-3611-2	G-3637-1	
Vegetation season	The highest average value of protein content (%) in genotypes which carry same allele at *Gli-1, Gli-2* and *Glu-1*loci								
2015/16	12.90	13.20	12.80	13.40	13.10	12.55	13.10	13.13	13.35
2016/17	13.80	14.06	13.75	14.20	14.10	13.55	14.03	14.06	14.35
Average	13.35	13.63	13.275	13.80	13.60	13.05	13.565	13.595	13.85
Vegetation season	The highest average value of sedimentation volume (*ml)* in genotypes which carry same allele at *Gli-1, Gli-2* and *Glu-1*loci								
2015/16	46.00	46.66	44.50	46.00	45.00	42.75	46.00	45.66	48.00
2016/17	49.00	51.33	48.00	49.00	49.00	46.50	50.00	49.66	52.00
Average	47.50	48.995	46.25	47.50	47.00	44.625	48.00	47.66	**50.00**

From the combination of nine alleles, located at the three *Gli-1*, three *Gli-2* and three *Glu-1* loci, which associated with high sedimentation volume of protein in two genotype (G-3623-2; G-3617-1) present different combination of seven allele, in two genotypes (G-3636-2; 3622-1) present different combination of six alleles, in one genotype (G-3644-4) present combination of four alleles, in three gentypes (G-3611-2; G-3634-2; G-3637-1) present different combination of two alleles, in one genotype (G-3601-4) present one allele. However in G-3619-3 there are none of the nine alleles associated with high sedimentation volume (table 2 and 3).

This combination of nine alleles was not found in the analyzed genotypes. The highest value of sedimentation protein volume (52 *ml* and 56 *ml*) had genotype G-3622-1 which had possess six allele combination (*Gli-A1b, Gli-B1b, Gli-D2b, Glu-A1b, Glu-B1c, Glu-D1d*), while genotype G-3636-2 had less but high sedimentation volume possess different combination of six allele (*Gli-B1b, Gli-D1a, Gli-B2b, Glu-A1b, Glu-B1c, Glu-D1d*). On the other side, in two genotypes which had combination of seven alleles found lesser value of sedimentation volume G-3617-1 (50.0 ml and 52.0 ml) and G-3623-1 (44.0 ml and 48.0 ml). Genotypes G-3601-4 which had the lowest sedimentation volume (33 *ml* and 36 *ml*) possess only one alleles from nine alleles (*Gli-D2b*) which associated with high value of sedimentation protein volume.

In this study found that genotypes which carried Glu-B1c allele have high sedimentation volumes, what is in agrement with investigation (Gao et al., 2016) which found that the highest frequency of Glu-B1c and its corellation with high sedimentation value.

Based on this result, it is not possible to determine the reliable regularity of the association between protein content and alleles encoding gliadin and glutenin proteins, as well as between sedimentation volume and *Gli-* and *Glu-1* alleles, although the grouped genotypes were carriers of alleles with the calculated average value of protein content and sedimentation volume. The differences between genotypes which had the same number of alleles associated with high protein content and high sedimentation volume can be results of differeces of combination of alleles encoding gliadin and high molecular weight glutenin as well as effect alleles at *Glu-3* loci which was not analzed in this investigation.

Conclusion

On the base of results established differences in wheat genotypes for protein content and sedimentation protein volume, within each vegetation season and between vegetation season. The highest grain protein content in G-36217-1 (14.00% and 14.80%) and the highest protein sedimentation volume in G-3622-1 (52.0 ml and 56.0 ml) in both vegetation season.
Genotypes which carried allele ***b.*** at *Gli-B2*, ***b.*** at *Glu-A1* (encoded 2*), allele ***c.*** at *Glu-B1* (encoded 7+9) and ***d.*** at *Glu-D1* (encoded 5+10 subunit) had the high value of protein content (G-3617-1−14.10% and 14.40%; G-3636-2, 13.20% and 14.20%). Genotypes which carried allele ***b.*** at *Gli-A1*, ***b.*** at *Gli-B,* ***b*** at *Gli-D1*, ***b.*** at *Glu-A1* (encoded 2*), allele ***c.*** at *Glu-B1* (encoded 7+9) and ***d.*** at *Glu-D1* (encoded 5+10 subunit) had the high values of sedimentation volume G-3622-1 (52.0 ml and 56.0 ml), G-3617-1 (50.0 ml and 52.0 ml). Genotype ***b.*** at *Gli-B2*, ***c.*** at *Glu-A1* (none subunits), allele ***d.*** at *Glu-B1* (encoded 6+8) and ***a.*** at *Glu-D1* (encoded 2+12 subunit) had the least protein content (G-3617-1−11.40% and 12.00%; G-3601-4 - 11.60% and 12.60%) and the least sedimentation volume (G-3601-4 −33.00 *ml* and 36.00 *ml*; G-3634-2−38.00 *ml* and 40.00 *ml*).

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EVALUATION OF PEACH CULTIVARS OF EARLY AND MEDIUM-EARLY SEASON IN A HIGH DENSITY PLANTING

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Abstract

Phenological characteristics, yield and fruit quality of 12 peach cultivars of early and medium-early season were evaluated during the three-year period (2020–2022) in Belgrade region, Serbia. The training system in the experimental orchard is Sloping Leader, a new original system with a high planting density (3.5 × 1 m). The cultivar 'Redhaven' was used as a control for comparison. Compared to the control cultivar, the average date of the start of flowering ranged from 5 days earlier ('Goldcrest', 'Springbelle', and 'Rich Lady') to 3 days later ('Maria Cristina'). The average harvest date was between June 8 ('Goldcrest') and July 31 ('Maria Marta'). The lowest average yield per tree (2.2 kg) was obtained from the cultivar 'Goldcrest', and the highest (10.3 kg) from the control cultivar 'Redhaven'. Compared to the control cultivar, a significantly lower yield was observed in 4 cultivars ('Goldcrest', 'Maria Cristina', 'Maria Grazia', and 'Flavorcrest'). Mean fruit weight was highest in the control cultivar 'Redhaven' (180.5 g), and was statistically significantly lower in 6 cultivars. Soluble solids content ranged from 10.0% ('Goldcrest') to 17.1% ('Maja'), and total acidity ranged from 0.44% ('Rich Lady') to 0.90% ('Royal Gem'). The highest scores for fruit appearance were obtained by the cultivars 'Flavorcrest', 'Rich Lady', 'Royal Gem' and 'Royal Glory', and for taste by the cultivars 'Royal Gem', 'Flavorcrest' and 'Maria Marta'. Based on the obtained results, besides the control cultivar 'Redhaven', the cultivars 'Royal Gem', 'Royal Glory', 'Rich Lady' (yellow-fleshed), and 'Maria Bianca' (white-fleshed) can be recommended for cultivation in the Belgrade region.

Keywords: *Prunus persica, Flowering, Maturation, Yield, Fruit quality*.

Introduction

Peach [*Prunus persica* (L.) Batsch] is one of the most important fruit species of temperate zone, ranking second in total fruit production in the world, after apple. In Serbia, peach ranks fifth after plum, apple, sour cherry and raspberry, with a production of about 70.000 t. The most important peach growing area is the Danube Valley (Grocka, Smederevo), where about 50% of the total peach production is produced (Milatović, 2023).

Peach breeding is one of the most dynamic among fruit tree species. About 100 new peach and nectarine cultivars are bred annually worldwide (Byrne, 2002). The main objectives of breeding are adaptability to different climatic conditions, extension of fruit maturation time, productivity, fruit quality, and resistance to diseases and pests.

Information on the adaptability and stability of new cultivars in different environments is crucial for increasing fruit production (Matias et al., 2017; Nikolić et al., 2022). One of the most important environmental factors is low temperatures, especially in spring. If peach flower buds, flowers, or fruitlets are damaged by low temperatures, production can be significantly reduced (Ognjanov, 2005; Zec et al., 2012). In addition to site selection, it is also

important to choose less susceptible cultivars when establishing new peach orchards (Chaar, 2015; Chen et al., 2016).
High density planting (HDP) of peaches is becoming increasingly popular as it allows better precocity and higher yields per hectare (Loreti and Massai, 2002; Robinson et al., 2006). A new HDP training system - Sloping Leader has been developed in Serbia. It is characterized by a central leader bent at an angle of 65°. In the first three years of cropping it produced significantly higher yields and yield efficiency compared to Open Vase and Fusetto (Zec et al., 2014). However, some peach and nectarine cultivars are not suitable for this system, as it leads to a reduction in fruit size (Zec et al., 2016).
The aim of this work was to study the phenological characteristics, vigor, yield and fruit quality of 12 early and mid-early season peach cultivars in a high-density planting. Based on the obtained results, the best cultivars will be recommended for the Belgrade region as well as for other regions with similar ecological conditions.

Material and Methods

The research was carried out in the peach collection orchard on the experimental farm "Radmilovac" of the Faculty of Agriculture in Belgrade (Serbia) in the three-year period (2020–2022). The training system is Sloping Leader, a new high density system (2,800 trees ha^{-1}) characterized by a central leader bent at an angle of 65° (Zec et al., 2013). The rootstocks (vineyard peach seedlings) were planted at 3.5 m × 1 m spacing in the orchard in June 2010. The seedlings were budded in September 2010 at a height of 50 cm. Twelve early and mid-early season peach cultivars were tested. The cultivar 'Redhaven', was used as a control. Each cultivar was represented by seven trees. Standard cultural practices were used in the orchard, without irrigation.
The beginning of flowering was recorded when 10% of the flowers were open, full flowering when 80% of the flowers were open, and the end of flowering when 90% of the petals had fallen off (Wertheim, 1996). The dates of the beginning of harvest were taken as the time of maturity. A sample of 25 fruits from each cultivar was used to determine fruit characteristics. Fruit shape index was calculated using the following formula: $length^2$ / (width × thickness). Soluble solids were determined using a table refractometer (Pocket PAL-1, Atago, Japan). Total acids were determined by titration with NaOH and expressed as malic acid. The five-member panel evaluated the sensory characteristics of the fruits: appearance and taste, assigning scores from 1 to 5.
The data obtained were statistically analysed using the analysis of variance method (ANOVA). The significance of the differences between the means was determined using the Duncan multiple range test at a probability level of 0.05.

Results and Discussion

On average, the peach cultivars studied flowered from March 21 to April 10 (Table 1). The cultivars 'Goldcrest', 'Springbelle', and 'Rich Lady', flowered earliest, for 5 days earlier than the control cultivar ('Redhaven'). In contrast, the cultivar 'Maria Cristina' started flowering the latest (3 days after the control cultivar). By year, flowering was earliest in 2021, when the average date of flowering start for all cultivars was March 21, while the latest flowering was in 2022, when the average date of flowering start was March 27. The average flowering duration for all cultivars was 14.8 days and varied from 12.3 days ('Maria Cristina') to 16.0 days ('Flavorcrest', and 'Maja'). The abundance of flowering was good in all cultivars (average scores of 4 to 5 on a scale of 0–5), except for two cultivars: 'Maria Cristina' and 'Goldcrest'.

Table 1. Phenological characteristics of peach cultivars (average, 2020–2022).

Cultivar	Flowering dates			Duration of flowering (days)	Abundance of flowering (scale 0-5)	Harvest date	No. of days compared to Redhaven
	Start	Full	End				
Goldcrest	21 March	26 March	5 April	14.7	3.3	8 June	-44
Springbelle	21 March	27 March	6 April	15.7	5.0	29 June	-24
Maria Cristina	29 March	3 April	10 April	12.3	3.1	2 July	-20
Royal Gem	22 March	27 March	5 April	14.3	4.0	6 July	-16
Royal Glory	23 March	28 March	6 April	14.3	4.0	13 July	-9
Maria Grazia	26 March	31 March	7 April	12.7	4.0	17 July	-5
Rich Lady	21 March	27 March	6 April	15.7	4.7	20 July	-2
Redhaven	26 March	30 March	8 April	13.0	5.0	22 July	0
Flavorcrest	22 March	27 March	7 April	16.0	4.2	23 July	+1
Maria Bianca	27 March	32 March	10 April	14.0	5.0	29 July	+7
Maja	23 March	30 March	8 April	16.0	4.8	30 July	+8
Maria Marta	25 March	30 March	10 April	15.7	5.0	31 July	+9

The average harvest date ranged from June 8 ('Goldcrest') to July 31 ('Maria Marta'). The range of harvest date was from 44 days before to 9 days after the cultivar 'Redhaven'. The average difference between the year with the earliest (2020) and latest (2021) harvest dates was 6 days and varied among cultivars from 2 days ('Goldcrest') to 15 days ('Maria Grazia').
Flowering and fruit maturation of certain peach cultivars in the Belgrade area were several days later then for the same cultivars under the conditions of Podgorica, Montenegro (Odalović, 2003). In contrast, flowering was earlier than under the conditions of Čačak, western Serbia (Glišić et al., 2008). These differences are the result of the different climatic characteristics of the sites.
The average yield per tree was lowest in the cultivar 'Goldcrest' (2.2 kg), and highest in the standard cultivar 'Redhaven' (10.3 kg) (Table 2). Compared to the control ('Redhaven'), a statistically significant lower yield was observed in 4 cultivars ('Goldcrest', 'Maria Cristina', 'Maria Grazia', and 'Flavorcrest').

Table 2. Yield (kg per tree) of peach cultivars.

Cultivar	2020	2021	2022	Average
Goldcrest	2.3	1.6	2.7	2.2 d
Springbelle	6.5	9.4	5.6	7.2 abc
Maria Cristina	5.0	3.9	3.7	4.2 cd
Royal Gem	4.8	9.6	6.9	7.1 abc
Royal Glory	6.7	8.4	7.4	7.5 abc
Maria Grazia	5.5	2.5	6.9	5.0 bcd
Rich Lady	5.1	9.0	7.1	7.1 abc
Redhaven	13.4	3.2	14.2	10.3 a
Flavorcrest	6.8	3.9	7.0	5.9 bc
Maria Bianca	8.7	5.7	8.8	7.7 ab
Maja	10.7	6.4	7.8	8.3 ab
Maria Marta	7.6	5.5	10.5	7.9 ab

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

The average yield of all cultivars during the trial was 20 t ha^{-1}. In one trial conducted in a peach orchard with high density planting, yields of more than 30 t ha^{-1} were obtained under optimal growing conditions (Zec et al., 2014). The reason for the lower yield of some cultivars was late spring frosts, which occurred in two experimental years (2020 and 2021), and significantly reduced the yield, especially for the early-flowering cultivars.

Average fruit weight ranged from 80.6 g for the cultivar 'Goldcrest' to 180.5 g for the standard cultivar 'Redhaven' (Table 3). Compared to the control, fruit weight was statistically significantly lower in six cultivars.

Table 3. Fruit characteristics of peach cultivars (average, 2020–2022).

Cultivar	Fruit weight (g)	Stone weight (g)	Stone share (%)	Fruit dimensions (mm)			Shape index
				Length	Width	Thickness	
Goldcrest	80.6 e	6.9 e	8.6	47.4 d	53.0 c	51.0 c	0.83
Springbelle	135.7 d	8.6 d	6.3	54.4 c	64.7 ab	62.3 ab	0.73
Maria Cristina	155.8 bcd	11.2 ab	7.2	60.5 abc	66.4 ab	65.5 ab	0.84
Royal Gem	145.4 cd	9.4 cd	6.5	59.6 abc	65.8 ab	64.1 ab	0.84
Royal Glory	144.5 cd	8.6 d	6.0	60.9 abc	64.9 ab	63.3 ab	0.90
Maria Grazia	140.3 d	8.9 cd	6.3	60.9 abc	62.8 b	61.7 b	0.96
Rich Lady	164.7 abc	8.9 cd	5.4	62.8 ab	68.0 ab	68.4 ab	0.85
Redhaven	180.5 a	11.3 ab	6.3	64.9 a	70.9 a	69.1 a	0.85
Flavorcrest	161.0 abc	9.6 c	6.0	59.7 abc	64.0 ab	62.7 ab	0.89
Maria Bianca	162.7 abc	9.8 bc	6.0	57.7 bc	65.4 ab	63.7 ab	0.80
Maja	172.2 ab	11.5 a	6.7	63.2 ab	67.2 ab	66.9 ab	0.89
Maria Marta	168.3 ab	10.2 abc	6.1	62.8 ab	68.2 ab	67.2 ab	0.86

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

Stone weight ranged from 6.9 g to 11.5 g, and the percentage of stone in fruit weight ranged from 5.4% to 8.6%. Cultivars with larger fruit had relatively smaller stone, i.e. higher flesh percentage, which is consistent with previous research (Milatović, 2023). There were also significant differences in fruit dimensions among the studied cultivars. Fruit length ranged from 47.4 to 64.9 mm, width from 53.0 to 70.9 mm, and thickness from 51.0 to 69.1 mm. The dimensions were used to calculate the fruit shape index, whose values ranged from 0.73 ('Springbelle') to 0.96 ('Maria Grazia').

The results obtained in this study for fruit traits are consistent with previously published results for some cultivars (Aliman et al., 2020; Nikolić et al., 2022). The values obtained for fruit size for some cultivars were higher than those reported by İkinci and Bolat (2018). In contrast, fruit weight for three white-flashed peach cultivars ('Maria Cristina', 'Maria Grazia', and 'Maria Bianca' was lower than in the study by Nenadović-Mratinić et al. (2000). These differences can be explained by the use of Open Vase training system in this study, which provides a higher planting distance compared to the HDP system.

Soluble solids content (SSC) ranged from 10.0% for the cultivar 'Goldcrest' to 17.1% for the cultivar 'Maja' (Table 4). The minimum quality for peaches is an SSC value greater than 10% (Crisosto and Crisosto, 2005). All tested cultivars met this requirement.

Total acidity (TA) ranged from 0.44% for the cultivar 'Rich Lady' to 0.90% for the cultivar 'Royal Gem'. The SSC/TA ratio (ripening index) is of great importance for consumer acceptance (Crisosto and Crisosto, 2005). The highest SSC/TA ratio (above 30) was found in 'Rich Lady' and 'Royal Glory', which are low-acid cultivars. On the other hand, the cultivars 'Goldcrest' and 'Royal Gem' had the lowest ripening index (15.6 and 16.0 respectively).

Table 4. Indices of fruit quality of peach cultivars (average, 2020–2022).

Cultivar	Soluble solids (%)	Total acids (%)	Sol. solids/ Total acids	Sensory evaluation (1-5)	
				Appearance	Taste
Goldcrest	10.0 d	0.64 bc	15.6	3.2 c	3.0 c
Springbelle	12.5 cd	0.67 bc	18.8	4.3 ab	4.1 ab
Maria Cristina	13.2 bc	0.60 bcd	22.0	3.8 bc	4.0 b
Royal Gem	14.5 abc	0.90 a	16.0	4.5 a	4.7 a
Royal Glory	15.1 ab	0.48 cd	31.4	4.5 a	4.0 b
Maria Grazia	13.4 bc	0.63 bcd	21.3	3.8 bc	4.2 ab
Rich Lady	14.1 bc	0.44 d	32.0	4.6 a	4.1 ab
Redhaven	14.8 abc	0.75 ab	19.7	4.3 ab	4.4 ab
Flavorcrest	15.4 ab	0.71 ab	21.6	4.6 a	4.5 ab
Maria Bianca	14.5 abc	0.64 bc	22.8	4.2 ab	4.4 ab
Maja	17.1 a	0.57 bcd	29.9	4.2 ab	4.3 ab
Maria Marta	15.4 ab	0.56 bcd	27.4	4.0 ab	4.5 ab

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

The data on fruit chemical composition are consistent with previous results for some cultivars from Turkey (İkinci and Bolat, 2018). Compared with the results of studies conducted in Italy (Petruccelli et al., 2023), higher SSC, lower TA, and consequently higher ripening index were obtained for some cultivars in our study.

In the sensory evaluation of the fruits, the cultivars 'Flavorcrest', 'Rich Lady', 'Royal Gem', and 'Royal Glory' scored the highest fruit appearance and the cultivars 'Royal Gem', 'Flavorcrest' and 'Maria Marta' for the taste. The lowest scores for both fruit appearance and taste were obtained by 'Goldcrest', the earliest maturing cultivar.

Conclusions

Based on the obtained results, besides the control cultivar 'Redhaven', the cultivars 'Royal Gem', 'Royal Glory', 'Rich Lady' (yellow-fleshed) and 'Maria Bianca' (white-fleshed) can be recommended for cultivation in the Belgrade region. For a final evaluation of the suitability of peach cultivars for cultivation in high density planting, using Sloping Leader system, further research is needed.

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NUTRITIONAL AND ECONOMIC BENEFITS OF TRITICALE CULTIVATION IN CURRENT GLOBAL CONDITIONS

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Abstract

Cereals have always been the basis for human nutrition and livestock feed, but also the main base of raw materials in the processing industry. Given that modern times are accompanied by a constant increase in the number of inhabitants on our planet, the safety of food supply, the quality and health safety of food products will be imposed as basic goals, the fulfillment of which will represent the greatest challenge for humanity in the near future. Triticale, a wheat-rye hybrid created as a result of man's work, is one of the representatives of the most important group of field plants - cereals. Thanks to the properties it inherited from the parent species, it is suitable for cultivation in conditions characterized by less favorable biotic and abiotic factors. As a result of decades of work in the selection of triticale, both abroad and in our country, a large number of varieties have been created that are characterized by early maturity, increased tolerance to lodging and some diseases, but also a higher grain yield compared to wheat. The cereal created in this way has properties that make it suitable for use in human feeding, domestic animals nutrition, but also for industrial processing. The aim of this paper is to point out the nutritional and economic advantages of triticale and its importance as a multipurpose grain in current global conditions.

Key words: *triticale, human food, livestock food, cereals.*

Introduction

Triticale (*x Triticosecalle* Wittm.), a hybrid created by crossing wheat and rye, was created with the idea of combining the positive traits of the parent species. Thus, productivity and good grain quality inherited from wheat, combining them with modest requirements for growing conditions and good disease resistance inherited from rye (Dennet *et al*., 2013; Blum, 2014; Randhawa *et al*., 2015; Ayalew *et al.* 2018). Until now, triticale was mostly grown as a forage plant, a cover crop and as a plant intended for biogas production (Randhawa *et al*., 2015), and modern triticale varieties can also serve as raw material for ethanol production (McKenzie *et al*. 2014). Despite the fact that it contains gluten, triticale occupies an important place in the production of healthy food because the grain of triticale is characterized by a favorable amino acid composition and a well-balanced content of vitamins and minerals (Glamočlija *et al*., 2018, Zhu, 2018). Thanks to its good adaptation to less favorable climate and soil conditions, triticale grown on alkaline, acidic, waterlogged as well as soils poor in nutrients achieves good grain quality in terms of protein and lysine content (Wrigley *et al*., 2017; Ballesteros-Rodríguez *et al*., 2019; Bezabih *et al.*, 2019).

In recent years, it has been noticed, especially in temperate climates, that winter crops are often damaged by winter frost, which is contributed by the reduced depth or even the complete absence of snow cover (Kreyling, 2010). In these areas, spring triticale is a good alternative to fodder barley and oats, and compared to wheat and barley, it has shown superior yields under drought conditions as well as on marginal lands (Dumbravă *et al*., 2016).

According to Shewry and Hey (2015), wheat represents the main source of calories in the human diet and plays the biggest role in agricultural-food chains because it is the cereal that occupies the largest areas (FAOSTAT, 2021). However, considering the sensitivity of wheat to water deficit and insufficient amount of nitrogen (Sadras *et al*. 2015), many farmers grow triticale as a replacement for wheat precisely because of its high tolerance to abiotic stress compared to wheat (Blum, 2014) and generally superior yields achieved in paired experiments with the same amounts of water and nitrogen input (Estrada-Campuzano *et al*., 2012; Rokues *et al*., 2017), thus making production safer and more economically stable.
The aim of this paper is to point out the nutritional and economic advantages of triticale and its importance as a multipurpose grain in current global conditions.

Production of triticale under conditions of biotic and abiotic stress

Triticale is a cereal species with a high ecological plasticity that makes a better use of climatic and soil conditions than other crops (Dumbravă *et al*., 2016). Thanks to the fact that triticale requires an average of 500-600 mm of rainfall during the growing season, as well as the fact that it shows good success in production with only 350 mm of seasonal rainfall and that it can be grown in very different agro-ecological conditions up to 3000 m above sea level, it sufficiently indicates on its importance as an agricultural crop. Unlike wheat, barley and oats, triticale develops a stronger root system thanks to which it extracts more nutrients. Its easy adaptability to soils of poorer quality (sandy, shallow, with very low or high pH value) and a well-developed root system make the cultivation of this plant attractive on those lands where the cultivation of other crops is uncertain (Gobeze *et al.,* 2007), requiring lower inputs (Grujić *et al.,* 2010). Under such conditions, triticale gives 137% higher yields compared to common wheat, 130% compared to durum wheat and 104% compared to barley (Benbelkacem, 2004). From the point of view of food production, agricultural production has the greatest importance. However, the reduction of agricultural land areas, their irrational use, the instability of the volume of production of most types of products, as well as the (un)profitability of production, are only some of the problems that agricultural production faces today.
According to Pozubenkova and Galiullin (2019), the production of winter cereals is the basis of cereal production. The production of a sufficient amount of winter cereals implies an increase in the area under these cereals, an increase in their productivity with the introduction of more advanced cultivation technologies as well as the introduction of new promising varieties with stable productivity in different weather conditions, putting triticale in focus precisely because of its resistance to low temperatures, biotic and abiotic stresses. while achieving high yields. The above mentioned authors, examining the economic efficiency of growing cereals in the Middle Volga region in the period 2015-2017, came to the conclusion that it can be influenced by variety exchange and variety renewal. Accordingly, they propose to introduce the winter triticale variety "Krokha" into production as a winter cereal due to its high fertility, winter resistance, resistance to the complex of biotic and abiotic factors of the environment and the possibility of using it in the nutrition of humans and domestic animals, but also its use in the bakery industry with the aim of improving and enriching raw materials with high-protein material and lysine.
The tolerance of triticale to drought and frost, the fact that under the same growing conditions it achieves higher yields than wheat, and that it shows greater resistance to lodging compared to wheat and barley are just some of its advantages compared to other cereals (Gobeze *et al*. 2007). Milovanović *et al*. (2011) pointed out based on their research that the grain yields achieved by triticale in favorable growing conditions are close to those achieved by wheat, while in unfavorable conditions they are higher, which declares the production of triticale as

safer in the already mentioned conditions. Analyzing the results of experiments that were carried out during two very extreme growing seasons (2002/03 and 2006/07) at seven localities, Nožinić *et al*. (2009) concluded that the high yield of triticale at one of the localities in very unfavorable conditions during the generative period of vegetation in 2003 is the result of pronounced tolerance of triticale to high temperatures and drought. During the second observed growing season (2006/07), the same authors established a high grain yield in three locations with different altitudes (Banja Luka 150 m above sea level, Butmir 460 m above sea level and Živinice 230 m above sea level) despite the fact that all months during the growing season were warmer than the long-term average. Saulescu *et al*. (2011) state that triticale and rye had a higher albedo than the majority of wheat varieties examined, pointing out that high albedo reduces the radiative energy load on the plant, including UV-B. Dumbravă *et al*. (2014) point out that triticale is the cereal of the future both in conditions where soil properties and climate conditions can be limiting factors and in conditions of intensive technology.

Nutritional benefits of triticale and its purpose

Cereal grain is a good source of energy because it contains carbohydrates, proteins, phospholipids, vitamins, minerals and other nutrients. Triticale is, primarily, a fodder cereal with a high nutritional value of grains (Jaśkiewicz and Szczepanek 2018). In addition, research has shown that, to a large extent, cereal grains can reduce the risk of certain types of cancer as well as heart disease (Song *et al*. 2018). Triticale, the grain of the future, as some call it, can play an important role in the market and in the production of healthy food and the creation of new grain products. Compared to wheat, triticale is characterized by a higher protein content as well as a better amino acid composition because it contains more lysine (Glamočlija *et al*., 2018), threonine, but also sulfur amino acids, while it is somewhat poorer in tryptophan (Biel *et al*. 2020). Filipchev *et al*. (2005) in comparative research on triticale (varieties Goranac and NS) and barley (varieties NS313 and ZA 37) grown in several locations in Vojvodina and Central Serbia in the period from 2002-2004 determined that the average protein content of triticale grains was 14 .0% dry matter, and winter barley 13.2% dry matter. Given that it is mainly represented as livestock food in our country, its participation in feed mixes varies depending on the type of animal and meal (Glamočlija *et al*., 2018).These authors also state that the advantage of triticale compared to other cereals is that it has a higher yield, faster spring growth, but also longer mowing time and use as green fodder compared to rye and oats. The mentioned advantages of triticale make it one of the suitable cereals for production in developing countries. Due to the high content of easily digestible nutrients, triticale, grain and by-products obtained from its processing are suitable for feeding domestic and farm animals. In addition, both ruminants and non-ruminants easily digest and absorb protein from triticale with a higher utilization rate compared to other cereals. Although triticale grain was primarily intended for feeding pigs, poultry, ruminants and horses, it is also used for feeding birds, rodents, pets, and herbivorous fish in ponds. As a supplementary feed, triticale is used in hunting grounds during the winter period. The content and ratio of amino acids in the grain of triticale, from the aspect of nutritional needs, is particularly suitable for monogastric animals and poultry, including birds, because the net use of protein in the mentioned species of animals is higher than the same in wheat and other cereals, precisely because of the high level of the amino acid lysine (Pfeiffer , 1994; Saade, 1995; Varughese *et al*. 1996). Marković *et al*. (2021) in their research comparing the utility values of different types of cereals (triticale, barley, rye, sorghum and millet) for feeding carp fish obtained particularly good results by using triticale, which, in addition to other properties, is characterized by a high protein content. Using their own results and the results obtained

from the analysis of reference sources, they ranked the use value of the grains used for this purpose as follows: wheat - triticale - corn - barley - rye - sorghum - millet.
The data presented in Table 1. present the chemical composition of triticale and its energy value in comparison with other cereals in Europe.

Table 1. *Comparative chemical composition and energy value of triticale and other cereals in Europe (Glamočlija et al.,2017)*

Parameter	**Corn**	**Wheat**	**Triticale**	**Barley**	**Oat**	**Sorghum**	**Millet**
Chemical composition ($g\ kg^{-1}$ dry matter)							
Total protein	106	130	140	116	120	120	128
Total oils	47	23	22	22	55	35	38
Celullose	24	27	27	27	112	29	95
Starch	700	680	620	640	440	700	590
Sugar	20	31	55	50	18	15	10
Mineral salts	15	18	20	22	33	20	43

The largest quantities of produced triticale are primarily intended for feeding animals. With a higher protein content and a more favorable amino acid composition, triticale has found use in the diet of pigs and poultry as a concentrated feed, but also in the diet of ruminants in the form of hay or silage, where it should be noted that the yield of green mass can range from 20 to 60 t ha $^{-1}$ (Babić *et al.* 2021). Milovanović *et al.* (2014) pointed out that the sugar content in the green mass of triticale is higher compared to wheat, and the high production of green mass ranks it among important forage crops, ahead of rye and oats. What affects the quality increase is certainly the fact that the cell walls of triticale endosperm contain pentosans with some β-glucan, as is the case with wheat and rye. Despite its limited use, primarily as animal feed, triticale as flour can also be used in the bakery industry as a supplement to wheat flour because it has a low gluten content (McGoverin *et al.* 2011). The starch content of triticale grains is similar to that of wheat, and higher compared to rye (61, 60 and 54% approximate) (USDA 2018), and this is what affects the digestibility and quality of bread. Considering the high content of essential amino acids, it can be said that triticale is nutritionally more valuable than wheat, even if its baking performance is inferior (Biel *et al.* 2020).
What also indicates the great importance of triticale as a multipurpose grain is the possibility of using it as a raw material for the production of bioethanol. Bioethanol is a renewable source of energy that can be produced by fermentation of sugars originating from plants containing starch (first generation raw materials) or lignocellulosic biomass (second generation raw materials) (Balat and Balat, 2009, Ho *et al.* , 2014). Bielski *et al.* (2015) state that bioethanol is a strategic resource that is widely used in the food, cosmetic, pharmaceutical and petrochemical industries, and that it is also mentioned among the most important biofuels used for transportation. Precisely because of the modest requirements for soil quality, triticale is considered the most suitable cereal for bioethanol production (Kučerova, 2007; Cantale *et al.*, 2016). The amount of bioethanol that can be produced from cereal grains directly depends on the grain yield (Obuchovski *et al.*, 2010; Swantson *et al.*, 2014), which is largely determined by the variety, weather conditions during production, applied agricultural techniques and the dose of applied nitrogen fertilizers within it (Dumbravă *et al.* 2016; Lalević *et al.,* 2022), where the factor that most modifies the quality properties of the grain is precisely nitrogen (McKenzie *et al.* 2014; Bielski *et al.* 2015).

Conclusions

Triticale is a crop that has a high genetic potential for yield and is able to produce high biomass. Based on the quoted literature, it can be concluded that triticale is characterized by

good to excellent adaptability to limited water supply, extremely high temperatures and frost, as well as soils with increased salinity, lower pH value and other disadvantages that limit the cultivation of some other crops. Also, the possibility of using it for different purposes increases its importance as a cereal. Bearing in mind all the advantages and disadvantages of triticale, the rapidly growing requirements for meeting the basic needs of the constantly growing population on the planet, climate change and the necessity of expanding agricultural production to marginal areas, leads to the conclusion that triticale will only gain importance in the future.

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PHENOTYPIC VARIABILITY AND SIMILARITY OF NUMBER OF SPIKELETS IN PRIMARY SPIKE IN WHEAT VARIETIES (*Triticum aestivum* L.)

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Abstract

Number of spikelets per spike has an indirect effect on yield. Aim of this work is study variability of number of spikelets in primary spike of wheat grown under different environmental condition. The 50 wheat varieties was included in experiment which was set up as a randomized block design in three replications on the field in Kraljevo, Serbia during two vegetation season (2015-2017). The seeds of varieties were sown at the distance of 0.10 m in rows of 1.0 m length among which was the distance of 0.2 m. Sixty plants at the full maturity stage (20 replication^{-1}) were used for analysis of number of spikelets per primary spike. The analysis of variance was performed by MSTAT C (5.0 version). Similarity among wheat was analyzed by hierarchical method of Euclidean distance. The results showed significant differences toward to number of spikelets primary spike^{-1} among varieties in both years, estimated by F-test. On average in the first vegetation season the least number of spikelets primary spike^{-1} 19.47 had Tera variety while the highest number of spikelets 24.38 had Dejana variety. In second vegetation season the number of spikelets primary spike^{-1} varied from the lowest 19.37 in Kosmajka to the highest 25.32 in Somborka. The similarity illustrated on dendogram contained four clusters in first year and six cluster of varieties in second year. The prominent cluster contain different number and composition of varieties with the highest degree of similarity. The differences in average of spikelets spike^{-1} were determined by genetic, environmental factor and by interaction genotype/environment.

Key words: wheat, variety, spikelets spike^{-1}, similarity, environment

Introduction

Wheat yield is complex trait which is result of inter-connected yield-component traits value, such as tillering, architecture of stem, leaf, inflorescence, fertilisation, speed of seed filling and often with their correlations (Knežević et al., 2018a; 2021). One way to improve wheat yield potential is to select for increased spikelet number per spike, because one extra spikelets per spike potentially increase seed yield through increasing number of florets and number of seed (Álvaro et al., 2008; Zečević et al., 2009; Sreenivasulu and Schnurbusch, 2012). By increasing number of seed per spike is one possibility to overcome low mass of seed. Number of seeds per spike is determined with both, number of spikelets per spike and the number of fertile florets spikelet^{-1}. The each wheat spikelet has more than one seed, what indicate that wheat spikelet is one of the most essential components of seed yield (Knežević et al., 2018b;

Wolde et al., 2019). In order to achieve this, it is necessary to know the genetic control of of spikelets per spike as well as other components of yield and quality, their mutual connection and the breeding of high yielding wheat variety ((Branković et al., 2015; Knežević et al., 2006; 2015 Würschum et al., 2018). Candidate genes for determination number of spikelets per spike were initially identified and mapped on chromosomes 7AL and 7BL using proprietary gene models (Gardner et al., 2016; Wittern et al., 2022). Spikelets are formed from the apical meristem through several stages, which number depends on the genotype and environmental conditions. However, not all primordia develop into spikelets, and also not all spikelets develop seeds, due to the death of primordia, and therefore sterile and fertile spikelets are formed (Baker and Gallagher, 1983; García et al., 2014). The spikelets spike^{-1} develop from the budding phase and early elongation of the stem, and it depends on the environmental conditions in the process of organ differentiation. The number of productive spikelets spike^{-1} decreases under conditions of stress until flowering (Hristov et al., 2011; González-Navarro et al., 2015; Cohen et al., 2020). Water stress have influence to the decrease in the number of spikelets as a result of competition for photosynthesis matters among stem, leaves and spikelets which are all of them in phase of growth, as well lack of water in the flowering phase which can cause decreasing fertile florets and the number of seeds in spike. The result of effect of that competition can increase pollen sterility and failure of pollination and fertilisation, especially in the terminal and basal spikelets of spike (Gonzalez et al., 2012. Farhoud, et al., 2014; Saeidi and Abdoli 2015; Muhammad and Kazem, 2017). Varying the number of seeds spike^{-1} was established in interaction of sowing time and growing conditions when adding nitrogen fertilizer (Neugschwandtner et al., 2015; Sugár et al., 2016).

The aim of the work is to determine the potential of number of spikelets per primary spike of the divergent winter wheat varieties growng in different agro-ecological conditions.

Materials and Methods

In this study of number of spikelets per primary spike in wheat were included 50 varieties. Selected wheat genotypes were sown in experiment which was set up as a randomized block design in three replications, on plots size 1 m^2 on the field in Kraljevo, Serbia in two growing seasons (2015/16 and 2016/17). The seeds of varieties were sown at the distance of 0.10 m in rows of 1.0 m length among which was the distance of 0.2 m. For analysis of number of tillers, were used 60 plants in full maturity stage (20 plants per replication). Using the program MSTAT C 5.0 version the analysis of variance was performed according to a random block system with one factor and significant differences were estimated by F-test values and tested by test value of LSD $_{0.05}$ and LSD $_{0.01}$. Similarity among wheat analyzed by hierarchical method of Euclidean distance.

Weather conditions

The average temperature was 9.96 °C and total amount of precipitation was 651mm in first year was higher than in the second year 2016/17 in which average temperature was 8.74 °C and total amount of precipitation was 523 mm, and also higher than in 10 year periods recorded temperature 8.50 °C and precipitation 417.8 mm. In the two months (October-November) the amount of precipitation and average temperature values were similar, which are favorable for seed germination and development of plants. During the February-April amount of precipitation in the first year (250.5 mm) was higher than in the second (174.0 mm), although the distribution of rainfall was more favorable for plant growth in the second year of experiment (Table 1).

Table 1. Average monthly temperature and total monthly precipitation in Kraljevo

	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
Temperature ^{o}C	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	
Temperature ^{o}C	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	**8.74**	
2000-2010		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	**8.50**	
Precipitatin (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6		**651.0**
Precipitatin (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0		**523.1**
2000-2010		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3		**417.8**

Results and Discussion

The number of spikelets per primary spike of wheat varieties varied in range of 19.47 (Tera) to 24.38 (Dejana) in first vegetation season with average value 22.45 for all 50 varieties, while in second year varied from 19.37 (Kosmajka) to 25.32 (Somborka) with average value 23.07 spikelets spike^{-1} for 50 varieties (table 2).

Table 2. Variability of number of spikelets per primary spike in wheat varieties

	Cultivars	First year	Second year	Average		Cultivars	First year	Second year	Average
1	Evropa 90	22,80±0,22	23,03±0,16	22,92±0,19	26	Jarebica	22,22±0,29	25,08±0,20	23,65±0,25
2	Dejana	24,38±0,19	23,32±0,17	23,85±0,18	27	Fortuna	22,47±0,23	23,37±0,15	22,92±0,19
3	Sila	22,40±0,22	24,38±0,18	23,39±0,20	28	Sasanka	21,87±0,24	24,22±0,20	23,05±0,22
4	Omega	22,05±0,20	23,83±0,15	22,94±0,18	29	Danica	22,58±0,21	23,38±0,18	22,98±0,20
5	Lasta	22,15±0,15	23,33±0,18	22,74±0,17	30	Somborka	23,87±0,22	25,32±0,17	24,60±0,20
6	Milica	23,60±0,21	23,65±0,19	23,63±0,20	31	Kremna	23,47±0,23	22,57±0,23	23,02±0,23
7	Parizanka	23,42±0,24	23,75±0,22	23,59±0,21	32	KG-75	21,35±0,27	23,20±0,21	22,28±0,24
8	Pobeda	23,40±0,20	23,98±0,17	23,69±0,19	33	Šumadija	22,48±0,23	22,40±0,20	22,44±0,22
9	Dična	23,25±0,25	23,98±0,22	23,62±0,24	34	Levčanka	23,68±0,22	24,10±0,21	23,89±0,22
10	NS Rana 5	23,07±0,23	22,95±0,19	23,01±0,21	35	Oplenka	23,47±0,22	23,98±0,24	23,73±0,23
11	Alfa	24,02±0,25	25,18±0,17	24,60±0,21	36	Gruža	22,35±0,25	23,42±0,24	22,89±0,25
12	Rodna	22,72±0,25	23,50±0,24	23,11±0,25	37	Gružanka	21,21±0,23	20,40±0,15	20,81±0,19
13	Balkan	22,42±0,24	22,05±0,16	22,24±0,20	38	KG-58	22,47±0,25	23,88±0,16	23,18±0,21
14	Rana Niska	20,52±0,24	21,27±0,19	20,90±0,22	39	KG-56	23,27±0,19	24,15±0,21	23,71±0,20
15	Proteinka	23,23±0,25	24,22±0,13	23,73±0,19	40	Orašanka	23,07±0,26	23,38±0,23	23,23±0,25
16	Stepa	23,82±0,18	24,08±0,18	23,95±0,18	41	KG-78	21,58±0,26	22,88±0,23	22,23±0,25
17	NSR-2	21,34±0,20	22,73±0,17	22,04±0,19	42	Ravanica	21,97±0,34	23,88±0,27	22,93±0,31

18	Prima	21,23±0,19	21,30±0,16	21,27±0,18	43	Lepenica	21,28±0,26	22,43±0,21	21,86±0,24
19	Sloga	22,85±0,25	24,87±0,18	23,86±0,22	44	Jasenica	23,17±0,27	22,75±0,22	22,96±0,25
20	Agrounija	22,35±0,24	22,82±0,19	22,59±0,22	45	Zastava	23,52±0,18	23,03±0,16	23,28±0,17
21	Zadruga	21,00±0,20	21,27±0,16	21,14±0,18	46	Kosmajka	19,50±0,20	19,37±0,16	19,31±0,18
22	Tera	19,47±0,19	22,47±0,20	20,97±0,20	47	Šumadija	23,12±0,17	19,45±0,15	21,29±0,16
23	Kompas	20,12±0,22	22,30±0,16	21,21±0,19	48	Morava	21,35±0,25	21,32±0,22	21,34±0,24
24	Tanjugovka	21,75±0,28	22,07±0,24	21,97±0,26	49	KG 56 S	23,57±0,15	22,90±0,18	23,24±0,17
25	Jugoslavija	22,53±0,18	22,75±0,17	22,64±0,18	50	Ljubičevka	23,50±0,20	23,60±0,21	23,55±0,21
	Average						**22,45±0,23**	**23,07±0,19**	**22,76±0,21**
	CV (%)						**7,8**	**6,5**	**7,15**

On average for two growing seasons, the highest number of spikelets per primary spike was in the Somborka and Alfa varieties (24.60) and the lowest in the Kosmajka variety (19.31). On average for all varieties, the value for number of spikelets per primary spike was higher in the second (23.07) than in the first vegetation season (22.45) table 2.

The number of spikelets per primary spike in previous investigation of Serbian wheat was less and varied from the highest 20.7 in variety Ljubičevka to the lowest 18.4 number of spikelets spike^{-1} in variety Lepenica (Zečević *et al*., 2009). However in another investigation of different Yugoslav wheat varieties the number of spikelets spike^{-1} varied from 21.1 in Njivka to 26.2 in Zemunka variety (Zečević *et al.*, 2004) which in the same investigation for foreign wheat varieties number of spikelets spike^{-1} was between 19.2 (Frontana) and 27,0 (Jawa). In study with English varieties the number spikelet spike^{-1} varied from (18.4±1.1) in Siskin to (19.4±1.4) in Maris Widgeon variety (Zhou et al., 2021). Therefore, counting the number of spikelet spike^{-1}, and florets spikelet^{-1} during the breeding process is of great importance for screening high-yield wheat cultivars. The number of spikelets, fertile florets, and seeds gives the possibility to determine of spikelet fertility, fertile floret proportion, and seed/fertile floret ratio to further assess the spike characteristics (Knezevic et al., 2012; Kondić et al., 2017; Guo et al., 2018).

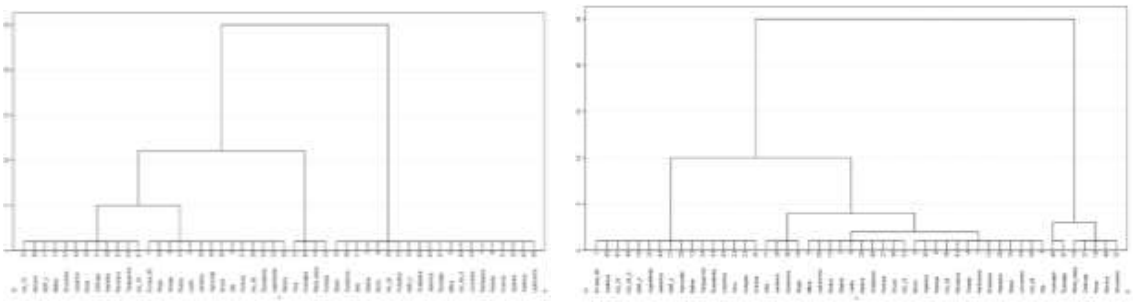
The analysis of variance established that the differences between the varieties for the trait number of spikelets primary spike^{-1} were significant and highly significant. Differences between vegetation season for number of spikelets per primary spike in varieties indicate that there is an influence of environmental factors on the manifestation of number of spikelets per primary spike. The established significant differences in the average values of number of spikelets spike^{-1}, indicating genetic divergence of varieties (table 3).

Table 3. Analysis of variance for number of spikelets per primary spike in wheat in two vegetation season

Source of variance		Vegetation season 2015/16						Vegetation season 2016/17					
	df	*SS*	*MS*	*F*	σ^2	$Lsd_{0,05}$	$Lsd_{0,01}$	*SS*	*MS*	*F*	σ^2	$Lsd_{0,05}$	$Lsd_{0,01}$
Genotypes (G)	49	192,380	3,926	15,747**	1.475	1,132	1,500	245,586	5,012	15,699**	1.883	1,363	1,805

Repetitions (R)	2	0,019	0,009	0,037369				4,133	2,067	6,473235			
Error	98	24,682	0,247		0,010			35,420	0,354	35,420	0,354		
Total	149	217,062			0,283			281,006			0,260		

Based on the obtained values for number of spikelets per primary spike, four clusters of mutually similar genotypes are distinguished in the first vegetation season (2015/16). The first cluster contain 12 varieties, second-14 varieties, third-4 varieties and fourth-20 mutually similar varieties. Among those four clusters, the highest similarity was between first and second cluster, with which the third cluster is the most similar. The less degree of similarity showed forth cluster with formed from first, second with third cluster (Figure 1).
In second year (2016/17). the six cluster mutually similar varieties were established. The first cluster contain 16 varieties, second-4 varieties, third-10 varieties, fourth-13, fifth-2 and sixth cluster contain 5 mutually similar varieties. Among those six cluster, the highest similarity was between third and fourth. Less degree of similarity showed fifth and sixth cluster as well as between second with formed cluster from third and fourth, and than less but the highest similarity showed first cluster with formed cluster from third and fourth with second cluster. The least similarity manifested the between formed cluster from first, second, third and fourth and cluster formed from fifth and sixth cluster (Figure 2).



1) 2)

Figure 1. Similarity of wheat genotypes according to number of spikelets per primary spike in two vegetation season: 1) 2015/16 and 2) 2016/17

In these studies, the examined wheat genotypes showed a high potential for number of spikelets per primary spike , which on average varies between 22.45 and 23.07 spikelets spike^{-1} in two vegetation season. In other study of wheat varieties, similar values of variation in number of spikelets per primary spike were found (Knežević et al., 2018b) as well as for Italian and Spanish wheat cultivars (Álvaro et al., 2008). The less values of number spikelets spike^{-1} were found (Zečević et al., 2009; Zhou et al., 2021), while higher values of number spikelets spike^{-1} reported in study Zečević et al. (2004). The obtained high values of the number spikelets spike^{-1} indicate the potential of number of seeds spike^{-1} and yield. The greatest contribution to the increase in the number spikelets spike^{-1} and yield have soil moisture, mineral nutrition and accessibility of mineral elements for absorption, temperature, light and process of photosynthesis and reutilization and translocation of organic matter (Dodig et al., 2008; Neugschwandtner et al., 2015; Sugár et al., 2016 Zangana and Aljburi, 2023).

Conclusions

In this investigation were determined significant differences among wheat genotypes according to number of spikelets primary spike^{-1}. The variation of spikelets spike^{-1} in the same variety in two vegetation season indicate genotype's response to different environmental conditions . The highest number of spikelets spike^{-1} 24.38 in Dejana variety and the least in Tera (19.47) in the first vegetation season, while the highest number of spikelets spike^{-1} in second vegetation season was in Somborka (25.32) and the least in Kosmajka (19.37). The differences between genotypes were significant and highly significant for number of spikelets per primary spike . Genetic factors, environmental factors and genotype/environment interaction had an influence on the manifestation of number of spikelets spike^{-1}.

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THE EFFECTS OF EARLY AND LATE DEFOLIATION ON GRAPE YIELD AND GRAPE QUALITY OF PROKUPAC VARIETY

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Abstract

The three year investigation (2014–2016) was carried out in a productive vineyard of the Toplicki Vinogradi Winery near Prokuplje, Serbia, and included the effects of early and late defoliation on grape yield and quality of Prokupac variety. The trials were set in a random complete block design (RCBD) with three blocks and four treatments per block: treatment I – early defoliation at the flowering stage when 50% of the flowers were open, treatment II – early defoliation at the stage when the grape size was 3 to 5 mm, treatment III – late defoliation at veraison, and control – no defoliation. Defoliation significantly affected the grape yield of the all three years investigation. Defoliation treatments significantly affected of grape yield per bud, developed shoot and per vine, while was no significant effect on the early and late defoliation on grape yield per fertile shoot. The greatest grape yield per bud, developed shoot and per vine were obtained in the control treatment, significantly higher than treatments with early and late defoliation. The greatest sugar content of grape juice was observed in control treatment, and the lowest one in treatment I with early defoliation at full flowering. In the treatment with late defoliation at veraison, the lowest content of total acids was obtained, while it was highest in treatment I with early defoliation at full flowering.

Keywords: *defoliation*, *treatments*, *grape yield*, *Prokupac*.

Introduction

In addition to international varieties, increasingly more attention is paid to old autochthonous varieties that are adapted to the climatic and soil conditions of Serbia. Prokupac is an autochthonous variety of the Republic of Serbia. It is classidied as *convarietas pontica, subconvarietas balcanica*. In the past decade, the Prokupac variety has once again become the dominant variety in the vineyards of Southern Serbia. The processing of grapes of this variety produces quality wines with a characteristic and unique taste which is distinctive for this part of Serbia (Lakićević et al., 2019). The increasing demand for high-quality and premium wine has induced the application of defoliation as an ampelotechnical measure affecting the grape yield and quality of grape. The effects of defoliation on the grape yield levels are quite variable depending on the timing of defoliation and the number of leaves removed (Poni et al., 2006). Defoliation is a viticultural practice performed during the growing season in order to regulate the density of the vegetative assembly and exposure of shoots with the aim of improving grape quality (Petrie et al., 2000; Poni et al., 2006). Defoliation can be early, when it is carried out in the phenophase of flowering and berry setting, or late, when it is carried out at the beginning of grape ripening. Early defoliation is a new innovative viticultural practice for regulating grape yield and grape quality (Poni et al., 2006). It is carried out at the flowering stage as opposed to the traditional leaf removal at veraison to improve the exposure

of the clusters to the sun's rays and to improve air flow in the trellis. The regulation of grape yield height is achieved by early defoliation (Poni et al., 2006; Intrieri et al., 2008). If there are not enough nutrients, there may be less fertilization and the appearance of a smaller number of berries in the grape cluster due to the reduction of the total leaf area by defoliation, which is also reflected in the reduction of grape yield (Aćimović et al., 2016). Removal of the first six basal leaves before flowering has been shown to be an effective strategy for grape yield control through source-consumer relationships (Poni et al., 2006; Intrieri et al., 2008; Aćimović et al., 2016), resulting in less compact grape clusters, which consequently increases the sugar content in grape juice (Sabbatini and Howell 2010; Bravetti et al., 2012; Silvestroni et al., 2018). Removal of basal leaves before flowering improves the relation between the source and consumer, due to the removal of older leaves with lower photosynthetic activity, reducing grape yields and improving berry quality in many cultivars under different agroecological conditions (Pastore et al., 2013). However, improved grape quality is not a consistent result of defoliation, and when it is present it is often due to a change in the microclimate around the grape clusters themselves (Percival et al., 1994). The aim of this paper was to examine the impact of defoliation in different time intervals on grape yield indicators and quality of grape of the Prokupac variety.

Material and methods

Prokupac variety of vine (*Vitis vinifera* L.) was investigated in a productive vineyard of the Toplicki Vinogradi Winery near Prokuplje, Serbia. The location of the trial (lat. 43°12′57″ N; long. 21°25′31″ E; alt. 359 m) belongs to the vine-growing region of Toplica, wine district of Prokuplje. The vineyard was planted in 2009 with a planting space of 2.5 x 0.8 m (5000 plants/ha), and the rootstock variety was Kober 5BB. The training system applied was spur trained Cordon de Royat, with a trunk height of 60 cm. The investigated vines were loaded by six buds per plant. The trials were set in random complete block design (RCBD) with three blocks and four treatments per block, for three years (2014-2016). Defoliation was carried out in different vine developmental stages as follows: Treatment I – early defoliation at the flowering stage when 50% of the flowers were open (BBCH scale 65), treatment II – early defoliation at the stage when the grape size was 3 to 5 mm (BBCH scale 73), treatment III – late defoliation at the onset of grape ripening veraison (BBCH scale 81), and control – no defoliation. The first six leaves of each primary shoot were removed from all defoliated vines. Defoliation of treatment I in 2014 was carried out on 16 June, in 2015 on 13 June, while in 2016 it was carried out on 9 June. Removal of the first six leavels of each primary shoot of treatment II in 2014 was carried out on 1 July, in 2015 on 29 June, and in 2016 on 25 June. Late defoliation at the veraison stage of treatment III in 2014 was done on 8 August, in 2015 on 6 August, while in 2016 it was done 2 August. Chemical analyses were carried out in the laboratories of the Faculty of Chemistry, University of Belgrade.

Determining grape yield - This method was used to determine the number of grape clusters per vine, the average yield of grapes per vine, per developed shoot, per fertile shoot and per bud. In the phase of grape harvesting, the yield of grapes and the number of grape clusters per vine were determined by measuring the mass, and then the grape yield per unit area was determined by calculation.

The quality of grapes is determined based on the content of sugar and total acids in grape juice. The sugar content was determined with the Axel saccharimeter, the content of total acids by the method of neutralization, titration with n/4 NaOH.

Standard statistical methods and the statistical program Statistical Analysis System – SAS (9.031) were used for data processing. One - way analysis of variance (ANOVA) was used to examine the experimental data (MS Excel (Microsoft Office 2007 Professional; Redmond,

WA, USA)), followed by Duncan's test for detecting differences ($p < 0.05$) between means (NCSS software package) (www.ncss.com (accessed on 1 March 2022)).

Results and Discussion

Table 2 shows the effect of defoliation on grape yield per bud. Defoliation had a very significant effect on the yield of grapes per bud. The highest yield in the three-year average was obtained in the control (372.07 g), significantly higher than the treatments with early and late defoliation (P<0.01).

Table 1. Grape yield per bud (g)

Year of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	379.77[b]	261.49[a]	266.33[a]	311.88[a,b]
2015	443.55[b]	342.96[a]	328.50[a]	364.55[a]
2016	292.89[a]	261.84[a]	309.27[a]	256.89[a]
AVG 2014-16	372.07[b]	288.76[a]	301.37[a]	311.11[a]
F [1]	experiment treatments	year	interaction	
	**	***	ns	

[a,b,c] Values were grouped based on Duncan's multiple range test ($\alpha = 0.05$), where different letters within the same row denote significant differences between treatments. [1]Significance based on F test: ns = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

Statistically significant differences were found in this parameter between the years of research. During the research period, early and late defoliation showed negative effects on the fertility of the bud in the years of research, which had a negative impact on the grape yield. Also, Aćimović et al. (2016) state that the limited production of assimilates due to defoliation reduces the fecundity of buds, which was recorded in treatments with early and late defoliation in our research. There are significant differences between the treatments in the yield of grapes per developed shoot (Table 2). In the control sample, a significantly higher yield of grapes (390.08g) per developed shoot was obtained than in the treatments with defoliation. There are very significant differences in the examined parameter (P<0.001) among the years of research. In accordance with our results, Aćimović et al. (2016) stated that applied early defoliation affected the decrease of grape yield due to a smaller number of grape clusters.

Table 2. Grape yield per developed shoot (g)

Year of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	398.58[a]	322.57[a]	341.25[a]	384.18[a]
2015	468.22[b]	364.86[a]	338.87[a]	381.11[a]
2016	303.44[a]	282.12[a]	312.65[a]	264.10[a]
AVG 2014-16	390.08[b]	323.18[a]	330.93[a]	343.13[a]
F [1]	experiment treatments	year	interaction	
	*	***	ns	

[a,b,c] Values were grouped based on Duncan's multiple range test ($\alpha = 0.05$), where different letters within the same row denote significant differences between treatments.[1] Significance based on F test: ns = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

In contrast to the yield per bud and developed shoot, the yield per fertile shoot (Table 3) did not vary significantly between the experimental treatments (P>0.05). The yield per fertile shoot varied statistically very significantly between the years of research. The highest yield per fertile shoot on average was obtained in the second year (2015) (440.23 g), which was significantly higher than in the third (2016) year of research (328.37 g).

Table 3. Grape yield per fertile shoot (g)

Year of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	410.94[a]	371.29[a]	364.98[a]	434.40[a]
2015	478.64[a]	418.81[a]	391.61[a]	471.87[a]
2016	328.87[a]	314.68[a]	354.02[a]	315.92[a]
AVG 2014-16	406.15[a]	368.26[a]	370.20[a]	407.39[a]
F [1]	experiment treatments	year	interaction	
	ns	***	ns	

[a,b,c] Values were grouped based on Duncan's multiple range test (α = 0.05), where different letters within the same row denote significant differences between treatments. [1] Significance based on F test: ns = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 4 shows the effect of defoliation on grape yield per vine. Defoliation significantly affected grape yield, so defoliated treatments had lower grape yields than the control. Such a decrease was not significant in 2014 for treatment III, while in 2016, there were no significant differences.

Table 4. Grape yield per vine (kg)

Year of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	2.279[b]	1.569[a]	1.598[a]	1.871[a,b]
2015	2.661[b]	2.058[a]	1.971[a]	2.187[a]
2016	1.759[a]	1.571[a]	1.856[a]	1.542[a]
AVG 2014-16	2.233[b]	1.733[a]	1.808[a]	1.867[a]
F [1]	experiment treatments	year	interaction	
	**	***	ns	

[a,b,c] Values were grouped based on Duncan's multiple range test (α = 0.05), where different letters within the same row denote significant differences between treatments.[1] Significance based on F test: ns = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

A significant difference was observed between 2015 (2.219 kg) on the one hand, and 2014 (1.829 kg) and 2016 (1.682 kg) on the other hand. Early defoliation was reported by Moreno et al. (2015) to decrease the grape yield of the Tempranillo variety, which was also observed in this study for Prokupac. A lower grape yield in early defoliated treatments was caused by a decreased grape cluster size, number of berries per cluster, and changed microclimatic conditions in the cluster zone. Similar effects of early defoliation on the grape yield of the Tempranillo variety were reported by Moreno et al. (2015) and Tardaguila et al. (2008). Leaf removal due to early defoliation decreased the grape yield of the Sangiovese variety (Sternad Lemut et al., 2013). On the other hand, Tardaguila et al. (2008) found that defoliation did not

affect the grape structure and yield, if applied after grape formation, which was not observed in this study.

The quality of grapes of the Prokupac variety was analyzed through the content of sugar and total acids in the grape juice (Tables 5 and 6). The highest sugar content was obtained in the control (22.1 %), and the lowest in treatment I (20.9 %), where early defoliation was applied in the full flowering phase. The highest content of total acids was obtained in the treatment with early defoliation in the full flowering phase (8.16 g/l), and the lowest in the treatment with late defoliation at the onset of grape ripening veraison (7.02 g/l).

Table 5. Sugar content in grape juice (%)

Year of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	22.3	19.4	20.7	21.0
2015	23.0	21.5	20.6	22.3
2016	21.0	22.0	22.2	21.8
AVG 2014-16	22.1	20.9	21.2	21.7

The results of numerous studies vary from the fact that defoliation has no effect on the content of sugar and total acids, to data that the removal of leaves has an effect on the values of the aforementioned parameters. In our research, the lowest sugar content on average was obtained in the treatment with early defoliation in the full flowering phase, which corresponds to the data reported by Tardaguila et al. (2010) for the Carignan variety.

Table 6. Total acids content in grape juice (g/l)

Years of Research	Experiment Treatments			
	Control	Treatment I	Treatment II	Treatment III
2014	8.15	8.04	7.86	6.75
2015	7.63	7.88	7.13	6.99
2016	8.04	8.58	6.89	7.32
AVG 2014-16	7.94	8.16	7.29	7.02

The confirmation of our results was found in the research of Song et al. (2018) who reported that defoliation treatments with 2, 4 and 6 leaves removed 55 days after flowering did not affect sugar content. Contrary to our data, Poni et al. (2006) pointed out that the removal of leaves in Sangiovese and Trebbiano cultivars at the phase of flowering and berry setting contributed to an increase in sugar content compared to the control. Bavaresco et al. (2008) reported that defoliation had a effect on total acid content, which is consistent with our results. Song et al. (2018) reported that the content of total acids was reduced by leaf removal, which does not correspond to our data, where the treatment with early defoliation in the full flowering phase had the highest content of total acids.

Conclusions

Grape yield per bud and developed shoot varied significantly under the influence of defoliation between treatments and years. The highest yield per bud and developed shoot was obtained in the control. Differences were not found in the grape yield per bud and developed shoot in treatments with early and late defoliation. Yield per fertile shoot did not vary under the influence of defoliation among treatments, while statistically significant differences were found between years of research. The highest yield of grapes per vine was obtained in the control. Grape yield per vine did not vary between early and late defoliation treatments. The

highest sugar content in grape juice on average was obtained in the control, and the lowest in the treatment with early defoliation in the full flowering phase. In the treatment with early defoliation in the full flowering phase, the highest content of total acids was obtained, and the lowest in the treatment with late defoliation at the onset of grape ripening veraison.

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BIOLOGICAL PROPERTIES OF SWEET CHERRY CULTIVARS ON 'OBLAČINSKA' SOUR CHERRY ROOTSTOCK

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Abstract

Cherry production in Serbia is predominantly extensive and based almost entirely on seedling rootstocks Mazzard (*Prunus avium* L.) and Mahaleb (*Prunus mahaleb* L.). The commercial intensive orchard on the clonal sour cherry rootstock 'Oblačinska' (*Prunus cerasus* L.) was established in 2013 with a planting distance of 4.0 m × 2.5 m. Six sweet cherry cultivars were studied: 'Burlat', 'Valery Chkalov', 'Carmen', 'Summit', 'Sunburst', and 'Germersdorfer Grosse Kirsche'. During a tree-year period (2017-2019), the following characteristics were studied: flowering and ripening time, vigor (trunk cross-sectional area), yield, yield efficiency, and fruit quality. The average flowering time was in the first decade of April. The ripening period ranged from May 20 to June 13. The cultivar 'Summit' showed significantly higher vigor then other cultivars. The lowest average yield per tree was recorded for the cultivar 'Valery Chkalov' (4.4 kg) and the highest for the cultivar 'Summit' (13.3 kg). The cultivar 'Summit' had a statistically significantly higher yield compared to the other cultivars. The highest yield efficiency was obtained for the cultivar 'Summit' (0.14 kg/cm^2). The average fruit weight was lowest in the cultivar 'Burlat' (7.1 g), and was statistically significantly lower compared to the other cultivars. The highest average fruit weight (11.0 g) was found in the cultivar 'Carmen'. Based on the results obtained, the cultivars 'Summit' and 'Carmen' can be recommended for cultivation on the sour cherry rootstock 'Oblačinska'.

Keywords: *Prunus avium, Prunus cerasus, Rootstock, Yield, Fruit weight*.

Introduction

The intensification of fruit production is based primarily on the results obtained in fruit tree growing systems and the selection of low and moderate vigor rootstocks. In orchards with higher planting density, earlier initial cropping, more efficient and cheaper implementation of technical measures, and faster return on invested funds are achieved (Mićić et al., 1997). In recent decades, sweet cherry production has experienced a significant increase and continues to spread worldwide with the main trends being to the improvement of cultivation efficiency and premium fruit quality (Lang, 2008). With a production of over 2.6 million tones per year, sweet cherry ranks seventh in the world production of temperate fruits (FAOSTAT, 2023).
Cherry production in Serbia is predominantly extensive and based almost entirely on seedling rootstocks Mazzard (*Prunus avium* L.) and Mahaleb (*Prunus mahaleb* L.). The trees have strong vigor, which complicates the application of cultural practices, especially harvesting, and also reduces the effectiveness of production (Milatović et al., 2011). In modern cherry production, there is no more place for very tall trees (Gjamovski et al., 2016). Intensification of cherry production can be achieved by using low and moderately vigorous rootstocks, of which the most used are 'Gisela 5' and 'Gisela 6'. However, the rootstock 'Gisela 5' also has a lot of disadvantages. It requires fertile soil, irrigation, supports, and cultivars grafted on it are prone to over-cropping, which negatively affects the fruit size (Milatović et al., 2013).

Zec et al. (2017) studied three cultivars grafted on six rootstocks: *Prunus mahaleb* L. seedlings, 'Colt', 'Ma x Ma 14', 'Gisela 6', 'Gisela 5' and 'Oblačinska' sour cherry and found that trees on 'Gisela 6' and 'Oblačinska' sour cherry rootstocks had medium vigor and good initial generative potential.

The aim of this work was to study the characteristics of several sweet cherry cultivars of different maturation times grafted on the clonal sour cherry rootstock 'Oblačinska' (*Prunus cerasus* L.). The cultivars achieving the best results will be recommended for growing on this rootstock in high density plantings.

Material and Methods

The experimental orchard was located on the mountain Fruška Gora, near Novi Sad, in the north of Serbia. Six sweet cherry cultivars were studied: 'Burlat', 'Valery Chkalov', 'Carmen', 'Summit', 'Sunburst', and 'Germersdorfer Grosse Kirsche'. All cultivars were grafted on 'Oblačinska' sour cherry rootstock. The experimental orchard was established in 2014 at a planting distance of 4 m × 2.5 m (1000 trees per ha). The slender spindle training system was applied, with several semi-skeletal branches at the crown base. They standard agrotechnical measures were applied, without irrigation.

During a tree-year period (2017-2019), the following characteristics were studied: flowering and ripening time, vigor (trunk cross-sectional area (TCSA), yield, yield efficiency, and fruit quality. The beginning of flowering is recorded when 10% of the flowers open, full flowering when 80% of the flowers open, and the end of flowering when 90% of the petals fall off (Wertheim, 1996). The abundance of flowering was rated on a scale from 0 (no flowers) to 5 (profuse flowering). The dates of the harvest beginning were used as the ripening time. The trunk cross-sectional area (TCSA) was calculated by measurement of the trunk circumference at a height of 30 cm above the grafting point and expressed in cm^2. Yield efficiency was calculated between the ratio of the average yield per tree over a three-year period (2017-2019) and the average TCSA. 25 fruits from each cultivar were used to determine fruit quality.

The experiment was set up as a completely randomized block system and each cultivar was represented by four replicates with three trees per replicate. Statistical data analysis was performed using analysis of variance and the LSD multiple range test at $P \leq 0.05$ to determine the significance of differences between means.

Results and Discussion

The average flowering time of the tested cultivars was in the first half of April (Table 1). The earliest start of flowering was recorded by 'Burlat' (April 2), while 'Carmen' started flowering only one day later, and 'Summit', 'Sunburst', and 'Germersdorfer' flowered the latest. The duration of flowering for the tested cultivars was 12 or 13 days (average 12.5 days). The tested cultivars on the rootstock 'Oblačinska' generally had very abundant flowering (with a score above 4.5). The cultivar 'Burlat' received the lowest score for the abundance of flowering (4.1).

Radičević et al. (2011) studied the flowering time of 21 cherry cultivars on a wild cherry seedling rootstock. The average flowering time of the studied cultivars was 11 days. The cultivars 'Burlat' and 'Summit' received a score of 4 for flowering abundance. The same cultivars on the rootstock 'Oblačinska' had higher average scores for flowering abundance (4.5 and 4.7). The greater flowering intensity could be due to the influence of the rootstock. The average flowering duration of the tested cultivars on the rootstock 'Oblačinska' was one and a half days longer than that of the cultivars on the wild cherry (Radičević et al., 2011). The flowering duration of cherry cultivars strongly depends on the weather conditions in the

years of study, so the longer flowering duration cannot be attributed to the influence of the rootstock.

Table 1. The average time of flowering and fruit maturation of sweet cherry cultivars (2017-2019).

Cultivar	Start of flowering	End of flowering	Duration of flowering	Abundance of flowering	Harvest date
Burlat	2 April	14 April	12	4.1	20 May
Valery Chkalov	2 April	15 April	13	4.5	21 May
Carmen	3 April	16 April	12	4.5	5 June
Summit	5 April	18 April	13	4.7	6 June
Sunburst	5 April	18 April	13	4.7	9 June
Germersdorfer	5 April	17 April	12	4.4	13 June

The earliest average maturity date was recorded for the cultivar 'Burlat' (20 May), which is two days earlier than the data presented by Milatović et al. (2021), while the cultivar 'Germersdorfer' had the latest average maturity date (13 June). Flowering and ripening of cherry cultivars in the Belgrade area was several days earlier then for the same cultivars under Čačak conditions of (Radičević et al., 2008). These differences are due to the different climatic characteristics of the locations where the tests were conducted.
The cultivar 'Valery Chkalov' had the lowest average yield per tree (4.4 kg) and the cultivar 'Summit' had the highest (13.3 kg) (Table 2). The cultivar 'Summit' had a statistically significantly higher yield per tree compared to the other cultivars (except 'Carmen'), while the cultivar 'Carmen' had a statistically significantly higher yield compared to the cultivars 'Burlat' and 'Valery Chkalov'. The trials were conducted in a plantation with a planting distance of 4 x 2.5 m with 1,000 trees per hectare, so the value of yield per tree is also the value in t/ha for the tested varieties.

Table 2. Yield, trunk cross-sectional area (TCSA) and yield efficiency (YE) of sweet cherry cultivars.

Cultivar	Yield per tree (kg)				Average TSCA (cm^2)	YE (kg/cm^2)
	2017	2018	2019	Average		
Burlat	5.4	6.7	7.1	6.4 c	78.3 ab	0.08
Valery Chkalov	3.8	4.7	4.7	4.4 d	74.0 b	0.06
Carmen	9.3	9.5	11.2	10.0 ab	69.6 b	0.14
Summit	11.0	12.8	16.2	13.3 a	95.0 a	0.14
Sunburst	9.5	9.0	10.2	9.6 b	77.0 b	0.12
Germersdorfer	7.4	8.4	10.3	8.7 bc	80.6 ab	0.11

Hrotkó et al. (2008) and Long et al. (2019) note that yields greater than 20 t/ha were obtained at full productivity, under optimal growing conditions in cherry orchards with dense planting and irrigation on the rootstock 'Gisela 6'. However, Szabó et al. (2010) indicate that the optimal yield, which at the same time ensures good fruit quality in modern cherry orchards, is 10-15 t/ha. The results of the average yield of the trees of the cultivars 'Summit' (13.3 t/ha) and 'Carmen' (10.5 t/ha) in the plantation without irrigation can be considered very favorable.
Among the cultivars tested, the lowest vigor expressed by the average trunk cross-sectional area (TSCA) had the cultivar 'Carmen' (69.6 cm^2), while the cultivar 'Summit' (95.0 cm^2) had the highest TSCA. The cultivar 'Summit', had statistically significantly higher TSCA compared to the cultivars 'Carmen', 'Valery Chkalov', and 'Sunburst'. The differences in vigor were the result of the influence of the genotype of the tested cultivars. Milatović et al.

(2022) note that the trees of the cultivar 'Summit' with similar age on the rootstock 'Gizela 6' had a slightly lower TSCA (80.0 cm^2) and thus lower vigor. One of the reasons for the resulting difference could be the slightly higher vigor of the rootstock 'Oblačinska' in our experiment compared to the vigor of 'Gizela 6'.

Yield efficiency is an important indicator combining the vigor and yield of varieties (Milatović et al. 2021). The highest average yield efficiency was recorded for 'Summit' (0.17), and the lowest for 'Valery Chkalov' (0.07). Milošević et al. (2014) reported yield efficiency in 5-year-old 'Summit' trees ranging from 0.029 kg/cm^2 on 'Mazzard' to 0.041 kg/cm^2 on 'Colt'. The yield efficiency in our study for 'Summit' is higher than the result reported by Milošević et al. (2014) and can be explained by the use of more suitable rootstock and full orchard productivity.

Table 3. Indices of fruit quality of sweet cherry cultivars (average 2017-2019).

Cultivar	Fruit weight (g)	Soluble solids (%)	Sensory evaluation (scale 1-5)			
			Appearance	Firmness	Taste	Average
Burlat	7.1 d	15.6 ab	3.7	4.3	4.4	4.1
Valery Chkalov	9.0 bc	14.8 ab	4.4	4.2	4.0	4.2
Carmen	11.0 a	15.1 ab	4.8	4.0	4.4	4.4
Summit	9.5 bc	14.4 b	4.4	3.9	4.2	4.2
Sunburst	10.0 ab	14.9 ab	4.3	4.0	4.2	4.2
Germersdorfer	8.9 c	16.5 a	4.4	4.7	4.5	4.5

One of the most important fruit quality parameters in sweet cherries is fruit weight. Worldwide sweet cherry production and fruit size have increased dramatically over the past two decades (Milošević et al., 2014; Long et al., 2015). High productivity has been accompanied by reduced fruit size and, in some cases, excessive crop load and poor fruit quality. The main challenge for successful integration of dwarf rootstocks in high-density cherry orchards is to manage crop load. Growing cherries on low vigorous rootstocks sometimes leads to over-croping and production of smaller fruits due to low leaf to fruit ratio (Milatović et al., 2011).

The cultivar 'Burlat' (Table 3) had the lowest average fruit weight (7.1 g), while the cultivar 'Carmen' had the highest average fruit weight (11.0 g). The mean fruit weight of 'Carmen' was statistically significantly higher compared to the fruit weight of all tested cultivars (except 'Summit'). The cultivars 'Sunburst' (10 g) and 'Summit' (9.5 g) also had a high average fruit weight value. Milatović et al. (2021, 2022), when testing cherry cultivars on the rootstock of 'Gizela 6', obtained a slightly better result with the cultivar 'Burlat' (7.5 g) and a slightly worse result with the cultivar 'Summit' (9.2 g).

The average soluble solids content varied from 14.4% for the cultivar 'Summit' to 16.5% for the cultivar 'Germersdorfer' (Table 3), and these two values are statistically significantly different. The results obtained for the cultivars 'Summit' and 'Sunburst' are very similar to those obtained by Gjamovski et al. (2016) for the same cultivars on the rootstock 'Gizela 5'. Vangdal (1980) states that the threshold for acceptable cherry quality from the consumer point of view is the soluble solids content of 14.2%. It can be concluded that all cultivars tested had a higher content of soluble solids content than indicated by Vangdal.

For fruit appearance, the cultivar 'Carmen' received the highest score (4.8). The highest score for firmness (4.7) and taste (4.5) was obtained by the fruits of the cultivar 'Germersdorfer', which is consistent with the highest soluble solids content in this cultivar.

Conclusion

To optimize the balance between yield, fruit size, and economic value appropriate cultivation methods must be used to meet the requirements of the rootstock. Variety tests on the clonal sour cherry rootstock 'Oblačinska' gave very good results for some genotipes. A good balance between good yield and large fruits was achieved for the cultivars 'Summit' and 'Carmen' and they can be recommended for cultivation on this rootstock. It is necessary to continue the research and test a larger number of cultivars on the rootstock 'Oblačinska' in dense planting for a longer period of time.

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PREDICTING THE IMPACT OF CLIMATE CHANGE ON WATER AVAILABILITY FOR MAIZE CULTIVATION IN TOPLICA DISTRICT IN SERBIA

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Abstract

This study examines the effects of climate change on water availability for maize crops in the Toplica district in Serbia, projecting the impact until the year 2100. Simulations of precipitation (P), air temperature (T), potential reference evapotranspiration (ETo), potential maize evapotranspiration (ETc) and water deficit/surplus were analyzed. For the P and T simulations results from the multi-model ensemble of seven regional climate models from the EURO-CORDEX project were used. The climate projections were based on the RCP4.5 and RCP8.5 scenarios. Based on the simulated T, ETo was calculated using the modified Hargreaves method. Data from three distinct periods were analyzed: the reference period (1971-2000), the near future (2021-2050), and the far future (2071-2100). A comparison of simulated values in the near and far future relative to the reference period was made. The significance of the changes was examined using the Mann-Whitney test (MW). The results indicate that climate changes will have a negative impact on corn production in the future from a water supply perspective. In the timeframe spanning April to September, P is expected to decrease while T, ETo, and ETc are expected to increase. By the end of the twenty-first century, such conditions would lead to maize being exposed to an increasing water deficit. In the near and far future, a statistically significant increase in water deficit (MW; p=0.05) at the maize growing season level is expected under both scenarios compared to the reference period. In the far future period the water deficit for maize cultivation is expected to increase by over 40 mm under the RCP4.5 scenario and by 75 mm under the RCP8.5 scenario. The distribution at the monthly level shows that in all studied periods the highest water deficit occurs in the month of July, while the largest increase in the deficit is expected in August.

Keywords: *climate change, maize, water deficit, Toplica district, Serbia.*

Introduction

In recent decades, a trend of increasing air temperature has been registered over the entire territory of Serbia, while precipitation trends show seasonal and spatial variations (Milovanović et al., 2017; Ruml et al., 2017, Djurdjević et al., 2018). As air temperatures are anticipated to rise further in the future, the increased risk of drought poses a significant threat to agricultural production (Olesen et al., 2011). The most vulnerable period would be summer, when air temperature is expected to increase and precipitation is expected to decrease (Vuković et al., 2018). These conditions will affect crop water requirement as well as water supply. It is expected that the water deficit that plants already face in summer on the territory of Serbia will be even greater in the future (Stricevic et al., 2014; Gregorić et al., 2020). Since precipitation has seasonal and spatial variations, the prediction of water balance and the occurrence of potential water deficit will vary from one region of Serbia to another. In the area of Vojvodina (northern Serbia), water conditions for maize cultivation are predicted to deteriorate in the second half of the 21st century (Gregorić et al., 2020).

This paper aims to present predictions for both water requirements and water availability in maize cultivation, which is one of the primary crops grown in the Toplica district, as well as in the entire region of Serbia. The predictions are derived from simulations of temperature and precipitation patterns specifically conducted for the Toplica district in Southern Serbia, spanning until the year 2100. These assessments hold significant importance as they serve as a foundation for determining the strategy of future agricultural development and the implementation of adaptation measures to address the repercussions of climate change

Material and method

The study focused on evaluating the impact of climate change on the water supply for maize crops in the Toplica district (southern Serbia) until the year 2100. Simulation analysis of total and effective precipitation (P and P_{eff}), air temperature (T), potential reference evapotranspiration (ETo), potential evapotranspiration of maize (ETc) and water deficit / surplus (ETc - P_{eff}) was performed. Simulations of P and T were performed for the periods 1971-2000 and 2021-2100. For their calculation, the results of a multi-model ensemble of 7 regional climate models with a horizontal resolution of 12.5 km from the project EURO-CORDEX (http://www.euro-cordex.net/) were used. The climate projections are based on the RCP4.5 and RCP8.5 scenarios (Representative Concentration Pathway). Daily data on precipitation and maximum and minimum air temperature (T_{max}, T_{min}) for the period 1971-2000 at Prokuplje stations were used for statistical correction of climate model results. Data from Prokuplje and Kuršumlija stations were used to describe climatic characteristics of the area where agricultural production takes place.

Based on the simulated T, ETo was calculated using the modified Hargreaves method (Trajkovic, 2007). ETc was calculated by multiplying ETo by the crop coefficient (kc). The culture coefficients were adjusted for the climatic conditions of the Toplica district using the method from FAO Irrigation and drainage paper 56 (Allen et al., 1998) and are: $kc_I = 0.3$; $kc_{III} = 1.21$ and $kc_{IV} = 0.36$.

P_{eff} was assumed to be 80% P (Saadi et al., 2015), which is considered justified under the climatic conditions of the region of Serbia without irrigation (Stricevic, 2007). Comparison of simulated values in the near (2021-2050) and far (2071-2100) future was performed in relation to simulated values of the reference period (1971-2000). The significance of the changes was examined using the Mann-Whitney test (MW) (p=0.05) (Mann and Whitney, 1947).

Results and discussion

Research indicates that in the Toplica district, both the near and far future periods are projected to experience statistically significant warming compared to the reference period. During the growing season (April-September) in the near future, similar values of the change of Tmean are expected for both scenarios, while in the period of the far future the increase of Tmean according to the RCP8.5 scenario is almost twice as large as according to RCP4.5 (Tab.1.).

The simulated sum of effective precipitation (Peff) of the growing season show a decrease in both future periods and both climate scenarios (Tab. 1.). The summer months are characterized by a decrease in Peff (Tab. 2.). Based on climate model projections, the future is expected to witness the most substantial decrease in Peff occurring in August, with one exception. In Kuršumlija, for the near future (scenario RCP4.5), the largest decrease is predicted to take place in July.

Table 1. Projected values and changes in mean temperature (Tmean), effective precipitation (Peff), and potential maize evapotranspiration (ETc) compared to the reference period (1971-2000). Values are for the period April-September. A statistically significant change is indicated in bold (Mann-Whitney p=0.05).

		(apr-sep) 1971-2000	RCP4.5		RCP8.5	
			2021-2050	2071-2100	2021-2050	2071-2100
Prokuplje	Tmean (°C)	17,1	18,3	19,0	18,5	20,9
	Δ Tmean (°C)		**1,2**	**1,9**	**1,4**	**3,8**
	P_{eff} (mm)	242	229	230	237	235
	Δ P_{eff} (mm)		-13	-12	-5	-7
	ETc (mm)	542	564	575	565	609
	Δ ETc (mm)		**22**	**33**	**23**	**67**
Kuršumlija	Tmean (°C)	16,0	17,2	17,9	17,3	19,7
	Δ Tmean (°C)		**1,2**	**1,9**	**1,3**	**3,7**
	P_{eff} (mm)	286	269	277	281	278
	Δ P_{eff} (mm)		-17	-9	-5	-8
	ETc (mm)	520	541	551	543	586
	Δ ETc (mm)		**21**	**31**	**23**	**66**

Table 2. Projected changes in average monthly sum of effective precipitation (ΔPeff) and potential maize evapotranspiration (ΔETc) in the near and far future compared to the reference period (1971-2000). A statistically significant change is shown in bold (Mann-Whitney p=0.05).

	Prokuplje (mm)								Kursumlija (mm)							
	RCP4.5				RCP8.5				RCP4.5				RCP8.5			
	2021-2050		2071-2100		2021-2050		2071-2100		2021-2050		2071-2100		2021-2050		2071-2100	
	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc
Apr	2.1	0.5	3.2	**1.2**	2.6	**1.1**	3.8	**2.8**	3.1	0.5	3.8	**1.3**	1.7	**1.1**	3.0	**3.0**
May	2.2	**1.3**	1.9	**2.4**	0.3	**1.5**	1.6	**4.1**	1.0	**1.2**	2.1	**2.4**	1.3	**1.6**	1.7	**4.3**
Jun	-3.6	**4.8**	-4.3	**5.4**	-4.6	**4.9**	-1.1	**11.9**	-3.1	**4.7**	-1.4	**5.3**	-4.5	**5.0**	-1.1	**12.1**
Jul	-4.3	**7.8**	-0.2	**9.7**	2.2	**7.1**	-3.4	**22.3**	-10.4	**7.1**	-6.9	**9.1**	0.0	**6.7**	-5.5	**21.6**
Aug	-6.5	**6.1**	-9.2	**10.9**	-6.2	**6.8**	-8.6	**19.4**	-6.5	**5.9**	-12.1	**10.4**	-6.5	**6.6**	-9.7	**18.8**
Sep	-3.1	**2.3**	-3.2	**3.4**	1.4	**2.3**	0.9	**6.9**	-0.8	**2.1**	4.4	**3.4**	2.8	**2.3**	3.5	**7.0**

In the Toplica district, by the end of the century, according to the RCP8.5 scenario, the maize water requirement (ETc) is predicted to increase by 67 mm (Prokuplje) and 66 mm (Kuršumlija) compared to the reference period (Tab.1.). The results of the studies conducted for the area of Vojvodina - Serbia (Gregorić et al., 2020), Iran (Ahmad et al., 2021) and the Mediterranean region (Masia et al., 2021) also indicate an increase in the water demand of maize in the future due to climate change. According to the RCP8.5 scenario, the largest change in ETc occurs in July of the distant future and is 22 mm for both areas (Prokuplje and Kuršumlija). The growing season of maize falls in the period April-September, for which a decrease in the average amount of Peff is predicted, which together with the predicted increase in ETc of maize leads to a water deficit (Table 1 and 2). The pattern of changes in simulated maize water deficit values under climate change conditions is similar across the range of the two observed locations. During the growing season, the simulation results show that maize is exposed to water deficit in the reference period and in the future period. The changes in water deficit (Δ) for each period are statistically significant (Table 3).

Table 3. Projected values of maize water deficit and change (Δ) compared to the reference period (1971-2000). The values refer to the period April-September. A statistically significant change is marked in bold (Mann-Whitney p=0.05).

Water deficit		1971-2000	RCP4.5		RCP8.5	
			2021-2050	2071-2100	2021-2050	2071-2100
Prokuplje	(mm)	300	336	345	328	374
	Δ (mm)		**36**	**45**	**28**	**74**
Kuršumlija	(mm)	234	272	274	262	309
	Δ (mm)		**38**	**40**	**28**	**75**

The monthly distribution of water deficit/surplus follows the monthly distribution of Peff. Water surpluses are expected in April and May, when Peff also increases. In the June-September period, when Peff is expected to decrease mainly (Table 2), water deficit was simulated in the reference and future periods for both observed locations. According to the RCP8.5 scenario, the highest deficit values are expected in the far future in July. They amount to 156 mm for the Prokuplje area (Fig. 1.(a)) and 139 mm for the Kuršumlija area (Fig. 1.(b)).

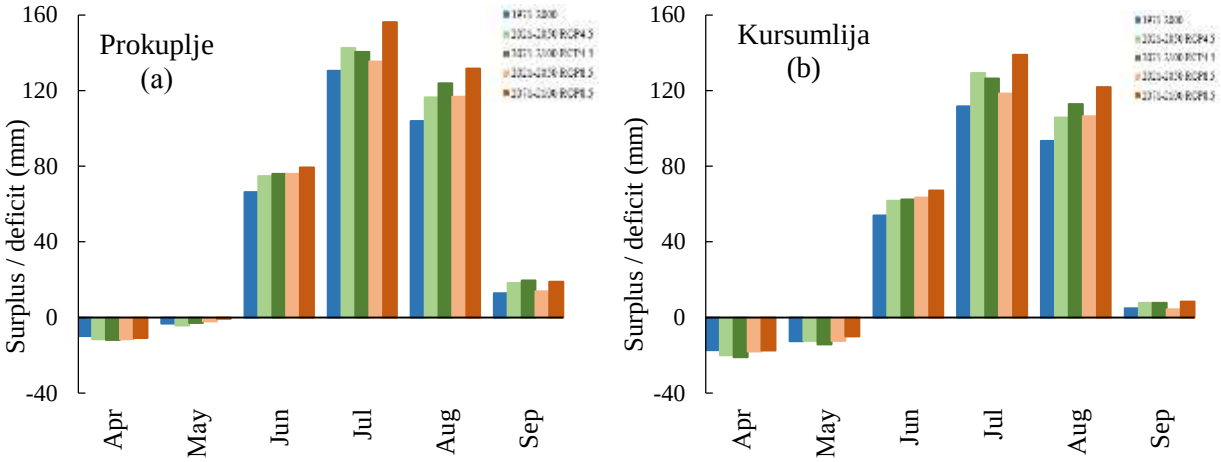

Figure 1. Projected water deficit/surplus for maize in the reference and future periods. Positive values represent a deficit and negative values a water surplus

For the Prokuplje area, the largest absolute monthly increase in deficit in future periods compared to the reference period is expected in August. By the end of the century, the water deficit in August will increase by 20 mm (RCP4.5) and 28 mm (RCP8.5) (Fig. 2).

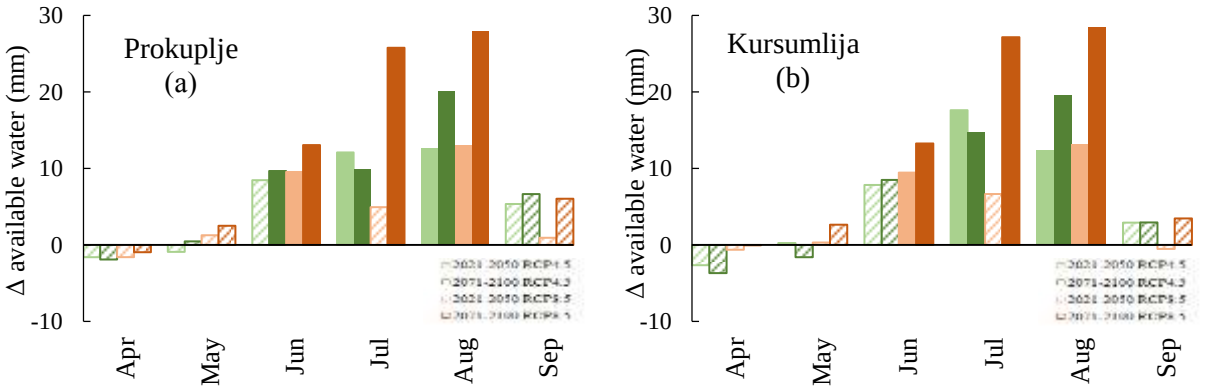

Figure 2. Projected change in available water (Δ) of maize compared to the reference period by month. Positive values represent a decrease and negative values an increase in water. Filled polygons indicate a statistically significant change according to the Mann-Whitney test (p=0.05).

In the Kuršumlija locality in the near future according to the RCP4.5 scenario, the largest increase in the water deficit compared to the reference period is predicted for July (18 mm). In the other periods, the increase in deficit is larger in August under both scenarios, with a peak of 28 mm in the far future period under the RCP8.5 scenario (Fig. 2). The results of this study show that the water deficit of maize is expected to increase in the future, reaching about 75 mm by the end of the century under the RCP8.5 scenario (Table 3). Similar results have been published in studies for Southern and Central Europe (EEA, 2016) and for Serbia (Tovjanin et al., 2019; Stričević et al., 2021). The highest deficit values during the maize growing season in the study area are expected in July and August (Fig. 2). From a

water security perspective, water shortages during the summer months (June-August) are critical for maize production (Tovjanin et al., 2019; Matović et al., 2021).

Conclusion

The results suggest that climate changes will negatively affect maize production in the future from a water supply perspective. During the period of maize growing season (April-September), P is expected to decrease and T, ETo, and ETc are expected to increase in the Toplička district. During the early twenty-first century, these conditions would result in maize being exposed to an increasing water deficit. In the near and distant future, a statistically significant increase in water deficit (MW; p=0.05) is expected at the maize growing season level compared to the reference period under both scenarios. By the end of the century (2071-2100), the water deficit for maize during the April-September period is expected to increase by over 40 mm under the RCP4.5 scenario and by 75 mm under the RCP8.5 scenario, respectively, compared to the deficit values during the reference period. The distribution at the monthly level shows that in all studied periods the highest water deficits occur in July, while the largest increase in the deficit compared to the reference period is expected in August (at the end of the century it is 28 mm for both areas according to RCP8.5).
The results of these studies form the basis for finding solutions to mitigate the impacts caused by climate change.

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NUTRITIVE VALUE OF ITALIAN RYEGRASS-RED CLOVER MIXTURES DEPENDING ON THE SEEDING RATE IN THE MIXTURES

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Abstract

More profitable production and better forage quality is achieved by growing grasses and legumes in mixtures. Growing grasses in a mixture with legumes influences a significant amount of nitrogen fixed by *Rhizobium* bacteria and reduce the using of nitrogen mineral fertilization. Italian ryegrass is an ideal species for growing in a mixture with red clover. The aim of this study was to determine the nutritive value of Italian ryegrass – red clover mixtures depending on the seeding rate of these plants in the mixtures, in the fist and the second cut. Since the trial was not fertilized with N the legumes were required to supply N. The experiment was established at the experimental field of the Institute for forage crops Kruševac, Serbia, according to a total randomized block system in three replications. The results of this study showed that better nutritive value was determined in the mixtures with higher seeding rate of red clover in the mixture. In the first cut, the nutritive value of the mixture with the highest Italian ryegrass seeding rate was similar with nutritive value of red clover monocrop. Generally, in conclusion, growing Italian ryegrass in a bi-crop with red clover produces forage of high nutritive value suitable for ruminants feeding.

Keywords: *Italian ryegrass, red clover, nutritive value*

Introduction

Red clover is a very important perennial forage legume, particularly suitable for animal nutrition due to its high nutritive value (Asci, 2012) and its characterization by high productivity (Murray et al., 2007). It is used for hay, haylage and silage and in grazing system (Farnham and George, 1999).

Forage nutritive value is directly related to the content and availability of essential nutrients for the consuming animals, and is defined by three processes, which are the capacity of the animal to consume, assimilate and digest the essential nutrients in the feed (Van Saun, 2013). Various factors affect the nutritive value of forage. In the same or different years, similar plants can have varying nutritive value, even when each has been planted in the same field McDonald et al., 2020). Grass-legume mixtures are acknowledged to have higher crude protein concentrations, as well as lower fiber concentrations, compared to pure grass stands (Ball et al., 2001). Brown et al. (2018) observed that in grass-legume mixtures crude protein content incresed, as well as dry matter yield. In the results of investigations reported by Albayrak and Ekiz (2005) authors indicated that planting legumes with grasses improve forage nutritive value and increases dry matter yield of pastures compared to pastures with pure grasses. On the other hand, legumes benefit grass pastures by transfering fixed nitrogen to the grasses (Ta and Faris, 1987; Heichel and Henjum, 1991; Malhi et al., 2002).

Pastures are a significant source of forages. A well – balanced grass – legume mixtures produce a quality food, and does not require a large application of fertilizers. Mixtures that contain white or red clover as a leguminous component are very important due to ability to

produce higher yields. In Switzerland, mixtures of Italian ryegrass with red clover and white clover are used for establishing meadows, in a ratio of 30 – 50% of legumes and 50 – 70% of grasses (Kessler and Lehmann, 1998). Grass – legume mixtures are also adequate for ensiling, due to high sugar content in the dry matter of grasses. Dinić et al. (2000) examined the results of ensiling a grass – legume mixtures based on Italian ryegrass and red clover, starting from the fact that both plant species are similar in length of exploitation, as well as in the time of establishment. The conclusion of this study was that these mixtures should be established with an equal proportion of red clover and Italian ryegrass, due to better nutrients content, and at the same time this mixture was more suitable for ensiling.
The aim of this study was to evaluate the nutritive value of Italian ryegrass – red clover mixtures by Weende and Detergent system of analysis depending of the different seeding rate of these plant species in the mixtures.

Materials and methods

The study was carried out at the experimental field of Institute for forage crops Kruševac, Serbia. The experiment was established by the method of randomized complete block design in three replications. The study area was situated at altitude of 166 m above sea level in Central Serbia. The mean annual temperature and the total precipitation for the region are 12.6º C and 653.2 mm, respectively. Italian ryegrass – monocrop (IR), red clover – monocrop (RC) and their mixtures – IR:RC (15:5 kg ha^{-1}), IR:RC (15:10 kg ha^{-1}), IR:RC (20:5 kg ha^{-1}) and IR:RC (20:10 kg ha^{-1}) were planted in November 2016, with the first cutting in the spring 2017 – on May the 9^{th}, and the second cutting in the early summer – on June the 22^{nd}. Plants were harvested in full flowering stage.
The dry matter content was determined on a sample weighing 1 kg, by drying at a temperature of 60° C to a constant weight. The samples were ground on a mill with a sieve of 2 mm, and than on a laboratory cyclone mill with a diameter of 1 mm. The samples obtained in this way were dried at 105° C to a constant weight. All results related to chemical composition and amount of nutrients are expressed in absolute dry matter. All chemical analyzes were performed in duplicate.
Within the Weende analysis system, the content of crude ash, crude protein, crude fiber, crude fat and nitrogen free extracts were determined. The content of crude ash was determined by dry burning the sample at 550° C and measuring the ash (AOAC, 942.05). The content of crude protein was calculated indirectly, through the amount of total nitrogen determined by the Kjeldahl method on a Kjeltec Auto Analyzer (AOAC, 984.13). The content of crude fiber was determined by successive hydrolysis of the sample with a dilute solutions of H_2SO_4 and NaOH (AOAC, 978.10). The amount of crude fat was determined according to Soxhlet, a modification according to Ruškovski, with anhydrous ether (Đorđević et al., 2003). The content of nitrogen free extracts was determined by calculation, by subtracting the total amount of crude protein, crude fiber, crude fat and crude ash from 1000 g of dry matter.
NDF (Neutral Detergent Fiber) – fiber insoluble in neutral detergent solution was determined without using Na_2SO_3 according to the metod by Van Soest and Robertson (1980). ADF (Acid Detergent Fiber) – is determined as the fraction of food insoluble in an acid detergent solution, and the amount of lignin by treating this insoluble residue with a 72% H_2SO_4 solution for 3h (AOAC, 973.18). Hemicelluloses were determined based on the difference between the food fraction insoluble ina neutral detergent solution and the food fraction insoluble in an acid detergent solution.
Data were analyzed using ANOVA in a randomized block design as a factorial trial using the Stat. Soft. STATISTICA 6. The statistical significance of differences tested using Tukey test, and significant differences among means were accepted at $p< 0.05$.

Results and discussion

Nutritive value of Italian ryegrass and red clover mixtures determined by Weende system of analysis is presented in the Table 1.

In the first cut, crude ash content ranged from 88.1 g kg^{-1} DM in RC monocrop to 100.9 g kg^{-1} DM in the IR:RC mixture (15:10). In the second cut, crude ash content was higher in IR and RC monocrops. IR:RC mixtures were very similar in crude ash content determined in the second cut, and only mixture IR:RC (15:5) differed significantly ($p< 0.05$) from the other mixtures (Table 1). The CP concentration in both cuts was significantly lower in IR monocrop than in RC monocrop and IR:RC mixtures (Table 1). The CP values ranged from 76.4 to 179.5 g kg^{-1} DM in the first cut, and from 81.8 to 180.3 g kg^{-1} DM in the second cut. Mixture with IR:RC (20:5) seeding rate was the highest in CP content in the first cut (170.9 g kg^{-1} DM) , whereas in the second cut mixture with IR:RC (20:10) was the highest in CP concentration. Crude fiber content was the highest in dry matter of IR monocrop harvested in the first cut (315.4 g kg^{-1} DM), followed by the IR monocrop harvested in the second cut (312.8 g kg^{-1} DM). Increasing seeding rate of red clover in the mixtures influenced higher CF content in mixtures harvested in the first cut (Table 1). Crude fat concentration ranged from 11.5 to 23.2 g kg^{-1} DM in the first cut differed significantly only between IR:RC (15:10) and IR:RC (20:5), and from 8.8 to 23.7 g kg^{-1} DM in the second cut, wher onla mixtur IR:RC (20:5) differed significantly ($p< 0.05$) from ather mixtures. NFC concentration of IR monocrop, RC monocrop and their mixtures was very similar in both cuts, ranged from 417.9 to 490.9 g kg^{-1} DM in the first cut, and from 435.4 to 480.4 g kg^{-1} DM in the second cut. NFE concentration determined in the first cut was similar and did not differ between investigated mixtures, whereas the highest content was obtained in IR:RC (20:5) mixture and the lowest content in IR:RC (20:10) mixture (Table 1).

Table 1. Nutritive value of Italian ryegrass and red clover mixtures determined by Weende system of analysis

Cut	Treatment	Crude ash, g kg^{-1} DM	CP, g kg^{-1} DM	CF, g kg^{-1} DM	Crude fat, g kg^{-1} DM	NFE, g kg^{-1} DM
	IR	97.5^{b}	76.4^{d}	315.4^{a}	19.8^{ab}	490.9^{a}
	RC	88.1^{d}	179.5^{a}	296.5^{b}	17.9^{ab}	417.9^{c}
I	IR:RC-15:5	97.7^{b}	163.6^{b}	265.5^{c}	19.7^{ab}	453.5^{b}
Cut	IR:RC-15:10	100.9^{a}	149.4^{c}	272.8^{c}	23.2^{a}	453.6^{b}
	IR:RC-20:5	90.5^{c}	170.9^{ab}	280.9^{bc}	11.5^{b}	446.2^{b}
	IR:RC-20:10	97.1^{b}	141.8^{c}	296.9^{b}	14.0^{ab}	450.2^{b}
	IR	127.9^{a}	81.8^{d}	312.8^{a}	14.0^{ab}	463.4^{ab}
	RC	97.4^{b}	180.3^{a}	241.5^{d}	17.7^{ab}	463.1^{ab}
II	IR:RC-15:5	82.4^{d}	149.9^{c}	280.4^{b}	19.4^{a}	467.8^{ab}
Cut	IR:RC-15:10	90.1^{c}	175.5^{ab}	254.8^{c}	23.7^{a}	455.9^{bc}
	IR:RC-20:5	89.3^{c}	169.5^{b}	251.9^{c}	8.8^{b}	480.4^{a}
	IR:RC-20:10	88.0^{c}	177.1^{a}	284.1^{b}	14.9^{ab}	435.4^{c}

IR – Italian ryegrass; RC – Red clover; IR:RC–15:5 – 15 kg ha^{-1} of Italian ryegrass and 5 kg ha^{-1} of red clover in the mixture; IR:RC-15:10 - 15 kg ha^{-1} of Italian ryegrass and 10 kg ha^{-1} of red clover in the mixture; IR:RC-20:5 - 20 kg ha^{-1} of Italian ryegrass and 5 kg ha^{-1} of red clover in the mixture; IR:RC-20:10 - 20 kg ha^{-1} of Italian ryegrass and 10 kg ha^{-1} of red clover in the mixture; CP – Crude Protein; CF – Crude Fiber; NFE – Nitrogen-free extracts; Different letters denote significantly different means ($P< 0.05$).

Nutritive value of Italian ryegrass and red clover mixtures determined by Detergent system of analysis is presented in the Table 2.

The NDF concentration was significantly ($p< 0.05$) lower in red clover monocrop compared to Italian ryegrass monocrop harvested in both cuts. Compared the investigated IR:RC mixtures harvested in the first cut, the highest NDF content was observed in IR:RC (20:10) mixture (511.5 g kg^{-1} DM) and the lowest in IR:RC (20:5) mixture (439.0 g kg^{-1} DM). In the second cut, IR:RC (15:5) mixture was the highest in NDF content (486.0 g kg^{-1} DM), whereas the lowest NDF content was determined in IR:RC-15:10 mixture (444.5 g kg^{-1} DM). Italian ryegrass harvested in the second cut was characterized by higher ADF content (418.2 g kg^{-1} DM) than harvested in the first cut (382.6 g kg^{-1} DM). On the other hand, red clover onocrop harvested in the second cut was lower in ADF content than red clover monocrop harvested in the first cut (Table 2). Compared the IR:RC mixtures, the highest ADF content was determined in IR:RC (20:10) mixture harvested in the first cut, and in IR:RC (15:5) mixture harvested in the second cut. Heicellulose content was the lowest in RC monovculture (66.0 g kg^{-1} DM) harvested in the first cut and 81.1 g kg^{-1} DM harvested in the second cut. IR:Rc (20:5) mixture was characterized by the lowest content of hemicellulose (77.3 g kg^{-1} DM) determined in the first cut, and 89.7 g kg^{-1} DM determined in the second cut. Lignin content in dry matter of Italian ryegrass and red clover monocrops and their mixtures ranged from 67.2 g kg^{-1} DM to 84.3 g kg^{-1} DM in the first cut, and from 61.9 g kg^{-1} DM to 105.4 g kg^{-1} DM ($p< 0.05$) in the second cut. Italian ryegrass was the lowest in the lignin content harvested in the first cut. The highest lignin content was obtained in IR:RC (15:10) mixture harvested in the first cut, and in IR:RC (20:5) mixture harvested in the second cut (Table 2).

Table 2. Nutritive value of Italian ryegrass and red clover mixtures determined by Detergent system of analysis

Cut	Treatment	NDF, g kg^{-1} DM	ADF, g kg^{-1} DM	Hemicellulose, g kg^{-1} DM	Lignin, g kg^{-1} DM
	IR	577.1^{a}	382.6^{a}	194.5^{a}	67.2^{c}
	RC	430.5^{d}	364.4^{bc}	66.0^{e}	67.9^{bc}
I	IR:RC-15:5	464.0^{c}	343.6^{d}	120.4^{c}	78.1^{abc}
Cut	IR:RC-15:10	472.4^{c}	357.1^{c}	115.3^{c}	84.3^{a}
	IR:RC-20:5	439.0^{d}	361.8^{c}	77.3^{d}	80.8^{ab}
	IR:RC-20:10	511.5^{b}	374.0^{ab}	137.5^{b}	77.1^{abc}
	IR	637.8^{a}	418.2^{a}	219.7^{a}	105.4^{a}
	RC	408.2^{e}	327.2^{d}	81.1^{d}	85.7^{b}
II	IR:RC-15:5	486.0^{b}	361.1^{b}	124.9^{c}	80.7^{b}
Cut	IR:RC-15:10	444.5^{d}	307.3^{e}	137.2^{b}	52.5^{d}
	IR:RC-20:5	447.6^{d}	357.8^{b}	89.7^{d}	105.9^{a}
	IR:RC-20:10	473.8^{c}	346.1^{c}	127.7^{bc}	61.9^{c}

IR – Italian ryegrass; RC – Red clover; IR:RC–15:5 – 15 kg ha^{-1} of Italian ryegrass and 5 kg ha^{-1} of red clover in the mixture; IR:RC-15:10 - 15 kg ha^{-1} of Italian ryegrass and 10 kg ha^{-1} of red clover in the mixture; IR:RC-20:5 - 20 kg ha^{-1} of Italian ryegrass and 5 kg ha^{-1} of red clover in the mixture; IR:RC-20:10 - 20 kg ha^{-1} of Italian ryegrass and 10 kg ha^{-1} of red clover in the mixture; NDF – Neutral detergent fiber; ADF – Acid detergent fiber; Different letters denote significantly different means ($P< 0.05$).

As many authors indicated, forage legumes are used in temperate regions to improve the feeding value of dairy pastures and to provide low-cost N from nitrogen fixation (McDonald et al., 2021; Brown et al., 2018; Malhi et al., 2002; Farnham and Gorge, 1993). It was evident that red clover proportion had a significant effect on the nitritional quality of red clover-Italian ryegrass mixtures. Gierus et al. (2012) pointed out that by keeping its proportion in a mixture relatively constant throughout the vegetation period, legume based mixtures may maintain higher nutritive value with little or no requirement for N mineral fertilization. Our results are in consistant with results obtained by McDonald et al. (2021) that planting legumes with grasses in mixtures increased crude protein content compared to crude protein content in

grass monocrop. Results obtained in this study also confirmed the fact that planting grasses as mixture with legumes will improve forage nutritive value by reducing the fiber content. Forage legumes generally have higher nutritive value than grass species, and therefore growing grasses and legumes in mixtures improve forage nutritive value compared with grass monocrop, due to slower decline in digestibility with advancing maturity and higher levels of crude protein content (Dewhurst et al., 2009).

Conclusions

The study evaluated the forage nutritive value of Italian ryegrass – red clover mixtures as compared to red clover and Italian ryegrass monocrops for forage nutritive value. The nutritive value of the species growing in binary mixtures was determined by the red clover rather than by the Italian ryegrass. According to our study, the nutritive value of IR:RC mixtures were higher than Italian ryegrass monocrop, and nearly equal to nutritive value of red clover monocrop. Based on the harvesting these forages in the spring and early summer, we can conclude that the better nutritive value was obtained in the second cut. Mixing red clover with Italian ryegrass increased the crude protein concentration of the herbage mixture relative to that of Italian ryegrass monocrop.Red clover cell wall tissue was less lignified in the second cut. On the basis of the results obtained from this study, it can be concluded that the most favorable balance between cell wall components, as well as crude protein content was achieved by soewing the mixture of 15kg ha^{-1} of Italian ryegrass and between 5 and 10 kg ha^{-1} of red clover.

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STATE OF EQUIPMENT WITH COMBINES IN AGRICULTURAL COOPERATIVE "AGROPROM"

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Abstract

The agricultural cooperative is a popular model for crop and livestock production - there are over 1.2 million agricultural cooperatives worldwide. It is not insignificant that out of the total of 4,894 cooperatives in Serbia, 2,726 are active and the structure is dominated by agricultural and processing cooperatives. Cooperatives contribute to building sustainable communities in rural areas. The role of cooperatives in agricultural development is multifaceted. Cooperative producers have access to a larger market where they can sell their goods and buy inputs at lower prices. More opportunities mean better economic development and more prosperity for rural people. Compared to individual farmers, members of cooperatives have better economic security and are exposed to fewer risks. In addition, agricultural cooperatives can sell their products without intermediaries, which increases the farm's profit. This research analyzed the usefulness of a functional model for agricultural production based on cooperative principles in the agricultural cooperative "Agroprom" from Stara Pazova, focusing on the equipment with combine harvesters and their application in the production of agricultural products. Based on the records, the sowing structure, number status and representation of universal grain and sugar beet combines by type and manufacturer, as well as the age of these machines were determined. In addition, the condition of numerous means of transport in the form of tractors and truck trailers was determined.

Keywords: *Agricultural cooperatives, Agricultural production, Mechanization, Combine, Serbia.*

Introduction

Agricultural cooperatives are often cited as the mainstay of agricultural development and survival of small agricultural producers. During their activity, they produce, buy, process, and sell agricultural, food, and other products of the cooperative and cooperative members, and provide the cooperatives with raw materials, means of production, parts for agricultural machinery, and other goods (Veljković *et al.*, 2020).

The agricultural cooperative "Agroprom" Stara Pazova is headquartered in Stara Pazova. Most of the subcontractors with whom the cooperative cooperates are located on the territory of the municipality of Stara Pazova, but it also has subcontractors on the territory of other municipalities in Srem. The production is based exclusively on the production of field crops. Besides the production of agricultural products, the cooperative deals with the purchase, storage and marketing of agricultural products. The cooperative operates in accordance with cooperative principles. Today, the cooperative has a total of 340 hectares of owned and leased agricultural land, while it produces with subcontractors on about 3,800 hectares. In 2019, sugar beet production was significantly reduced due to the large losses suffered by agricultural producers and the cooperative itself. In the aforementioned year, canola acreage was significantly reduced. In the observed period, weather conditions were favorable for corn cultivation. Soybean and sunflower recorded an increase in production value in the observed period. For wheat, the production value in 2019 decreased compared to previous years. The

cooperative owns 21 harvesters, including 8 grain harvesters and 13 sugar beet harvesters. The cooperative owns 35 modern tractors and all necessary attachments such as seeders, combines, ploughs, sprayers, inter-row cultivators, disc harrows, harrows and corn adapters. In addition, the cooperative has six heavy trucks in its fleet, so that the part concerning the transport of agricultural products to the warehouse is also insured.
The aim of this study was to determine the cooperative's energetic equipment with universal grain harvesters as well as harvesters for sugar beet harvesting in order to organize the harvesting process on the cultivated areas in the best possible way.

Material and Methods

Research was carried out in the southeastern part of Srem in the agricultural cooperative "Agroprom" from Stara Pazova (Autonomous Province of Vojvodina, Srem district, Serbia). Based on the survey and official accounting records in the Cooperative, the sowing structure, the total number of universal grain harvesters and sugar beet harvesters by type and manufacturer, as well as their age, were determined. In addition, the condition of numerous means of transport in the form of tractor and truck trailers was determined. Based on the known engine power of each type and type of harvester, the total available nominal power and then the average engine power of the universal harvester and sugar beet root harvester were separately calculated. Energy equipment of the Cooperative the effective engine power of universal harvesters and sugar beet harvesters per unit of sown areas with specific crops (kW/ha) is calculated according to the expression:

$$Et = \frac{\sum_{i=1-n}^{n} Pe(i)}{\sum_{i=1}^{m} Ai} (kW/ha)$$

Et - energy equipment of the harvester in the Cooperative per sown area;
Pe (kW) – effective power of the combine engine;
Ai (ha) - sown agricultural area under wheat, oats, corn, sunflower, soybean and canola for universal harvester and sown area under sugar beet for sugar beet harvesters.

Based on the total cultivated area of wheat, oats, corn, sunflower, soybeans, and canola, the specific load of universal harvesters per cultivated area (ha/harvester) and the number of harvesters per 100 ha were calculated. The same parameters were calculated for sugarbeet harvesters based on the total acreage of that crop. In addition to the number of universal harvesters and harvesting machines for sugar beet harvesting, the number of transport vehicles (tractor and trailer) and the number of motor vehicles (trucks and tractors) were also determined. The number of transport vehicles per harvester was calculated based on the total number of tractor and truck trailers (trailer/trailer) and the cultivated area per trailer (ha/trailer). Data from the Statistical Office of the Republic of Serbia, processed using standard statistical methods, were used for the study.

Results and Discussion

In the Republic of Serbia, according to the 2012 agricultural census, 631,122 agricultural farms are registered, of which 628,555 (99.59%) are family farms and 2,567 (0.41%) are farms of legal entities and entrepreneurs. These farms use 3,355,859 ha of land, of which 2,816,424 ha or 83.93% for family farms and 539,435 ha or 16.07% for farms of legal persons and entrepreneurs. Table 1 shows the sowing structure of crops in 2019 in the agricultural cooperative "Agroprom" (Gavrilović *et al.*, 2023).

Table 1. Sowing structure in 2019.

Crop	Area (ha)	Average yield (t ha $^{-1}$)	Total production (t)
Wheat	135	11.99	1618.65
Corn	1,835	12.54	23,010.90
Soybean	132	8.68	1,145.76
Sunflower	282	7.32	2,064.24
Rapeseed	208	1.15	239.20
Sugar beet	302	84.00	22,348.00
Oats	1	4.00	4.00
Total	2,895		

Source: Gavrilović *et al.*, (2023)

In terms of production value, sugar beet is the dominant crop. However, in 2019, the amount of sugar beet produced was significantly reduced due to the large losses suffered by agricultural producers and the cooperative itself. The main reason for this is the cercospora disease. In the mentioned year, the area under rapeseed was significantly reduced. In the observed period, weather conditions were favorable for corn cultivation. For soybeans and sunflower, an increase in production value was recorded. For wheat, the production value decreased compared to previous years. According to the 2012 agricultural census, there are a total of 31,241 harvesters in the Republic of Serbia, divided into three groups: Universal grain harvesters (19,474), silage harvesters (10,788) and other harvesters (979). In the total number of harvesters, universal grain harvesters are the most represented - 19,474, of which 18,417 or 94.57% are older than 10 years. The largest number of grain harvesters is used in the Vojvodina region (8,110 units or 41.65%), and 7,214 or 88.95% are older than 10 years. The remaining combines are distributed approximately equally between the Šumadija and West Serbia region (25%) and the South and East Serbia region (28%), while the Belgrade region has 5% of the total number of universal combines (Radivojević *et al.*, 2014; Nikolić *et al.*, 2014).

Table 2. Numerical status of universal grain harvesters by types

Manufacturer and type of harvester	Number of pieces	Engine power (kW)	Total engine power (kW)	Participation by number condition (%)	Participation by engine power (%)	Total age of the tractor (years)
Claas Mega	2	190	380	25.0	18.95	2x12=24
Claas Lexion 560	4	283	1,132	50.0	56.46	2x9=18 2x7=14
Claas Lexion 540	1	232	232	12.5	11.57	7
Claas Lexion 660	1	261	261	12.5	13.02	7
Total/average	8	250.62	2,005	100	100	70/8.75

Source: Authors

Family farms use 18,736 universal grain combines, or 96.2% , and farms owned by legal entities and entrepreneurs use 738 combines, or 3.8% of the total number of grain combines. The most harvesters are used by farms of legal entities in the Vojvodina region – 661, with

305 devices older than 10 years. In the studied area of Srem district, 211 farms of legal entities have 76 universal combines, out of which 40 devices or 52.63% are older than 10 years. After the Vojvodina region, the most harvesters are in the South and East Serbia region (33), followed by the Belgrade region (24) and the Šumadija and West Serbia region (20). As for tractors, 95% of the total number of all harvesters are older than 10 years. The most favorable age structure is in Vojvodina, where 88% of all harvesters are older than 10 years, while the situation in the other three regions is almost identical, between 97% and 99% of harvesters are older than 10 years. New harvesters were purchased by legal entities, but they did not significantly change the situation in the entire sample due to the small number (Radivojević *et al.*, 2014).

According to the results of the 2012 agricultural census, in Vojvodina almost half of the universal harvesters of legal entities (46.14%) are older than 10 years. According to these data, the situation in the observed cooperative is favorable, because out of 8 units, only 25% or 2 universal combines are older than 10 years. The average age of the universal combines is 8.75 years. Of the total number of universal combines, half (50%) are less than 7 years old and they are Claas 540, 560 and 660 combines. In terms of number of units (50%) and available engine power (56.46%), the Claas Lexion 560 universal combine is the most represented in the cooperative (Table 2). The total available nominal engine power of 8 universal grain harvesters is 2005 kW (an average of 250.62 kW per combine). The Claas Lexion 660 universal combine has the strongest engine power of 261 kW, while the Claas Mega has the weakest of 190 kW. The engine power of the universal combines in the cooperative increases every year due to the purchase of combines with more powerful engines. The average engine power of universal combines in the cooperative is 250.62 kW and is higher than the average engine power of combines of 191.78 kW reported by Radić (2016) for 6 cooperatives in Vojvodina. According to Pawlak *et al.* (2002), the average engine power of universal grain harvesters in the USA, Australia and Canada is 86 kW, in Western Europe 78.50 kW, and in Russia 78.9 kW, which is far below the reported results in operation. According to research by Turan *et al.* (2018), a Claas Dominator Mega combine with an engine power of 200 kW achieves an output of 9.02 ha/day in one shift. To harvest 250 ha of crops, this universal combine needs 27.71 days. According to this, eight harvesters in the Cooperative should work for 36 days to harvest the area of 2593 ha sown with field crops. In the group of other harvesters, the most important are harvesters for sugar beet harvesting. In the Republic of Serbia, 979 other combines are used, of which 813 or 83.04% are older than 10 years. Most of these combines are located in the Vojvodina region – 452 units or 46.17%, and 323 units or 71.46% are older than 10 years. In the region of Southern Serbia, there are a total of 484 combines or 49.44%, of which 457 or 94.42% are older than 10 years (Nikolić *et al.*, 2014). The situation is similar in the studied cooperative, where all 13 harvesters for harvesting sugar beet roots are older than 12 years. Of the total number of sugar beet harvesters, Holmer Terra Dos combine accounts for 8 units or 61.54%, while Ropa Tiger combine accounts for 5 units or 38.46%. The total available engine power of the sugar beet harvesters is 4544 kW, which is an average of 349.54 kW. Of this, 2704 kW (59.51%) is accounted for by the Holmer Terra Dos harvesters and the other 1840 kW (40.49%) by the Ropa Tiger harvesters (Table 3).

Table 3. Numerous state of the sugar beet harvester

Manufacturer and type of harvester	Number of pieces	Engine power (kW)	Total engine power (kW)	Participation by number condition (%)	Participation by engine power (%)	Total age of the tractor (years)
Ropa Tiger	5	368	1,840	38.46	40.49	5x12=60
Holmer Terra Dos	8	338	2,704	61.54	59.51	8x12=96
Total / average	13	349.54	4,544	100	100	156/12

Source: Authors

In addition to the harvester, the cooperative has a special machine for processing (cleaning soil and leaves) and loading sugar beet roots Ropa Euro Maus 1, engine power 191 kW. The ULT -150 loader and the front loader of the tractor are also used for loading sugar beet roots from the storage prism in the field. In the cooperative, 7 tractor trailers (Tehnostroy 6 and Pobeda 1) and 9 truck trailers (Shwarcmuller 5 and Lag 1) are used to transport 25368 tons of sugar beet roots and 28070 tons of grain, which can be aggregated with 35 tractors and 6 MAN 1839 trucks. The equipment of the cooperative with transport means is such that one universal harvester is equipped with 2 trailers and 1.23 trailers per full harvester for sugar beet harvesting. One trailer covers 162 ha of corn, wheat, soybeans, sunflower, oats and rapeseed and 18.87 ha of sugar beet (Table 4). According to Radivojević (2014), the largest area per trailer is 15.6 ha in the Vojvodina region and the smallest is 8.3 ha in the Southern and Eastern Serbia Region, which is less than the results shown in the Cooperative.

Table 4. Energy equipment of harvesters in the Cooperative

Parameters	Unit of measure	Universal combines	Sugar beet harvester
Total arable land	ha	2,593	302
Total number of harvesters	pieces	8	13
The total power of the harvester	kW	2,005	4,544
Average harvester power	kW	250.62	349.54
Energy equipment	kW/ha	0.77	15.05
Number of surface units per harvester	ha/ harvester	324.12	23,23
Number of harvesters per 100 ha	combine harvester/ 100 ha	0.31	4.30
Number of means of transport per harvester	trailer/harvester	2	1.23
Number of arable land per means of transport	ha/trailers	162.06	18.87

Source: Authors

In the 2019 production, eight universal cereal machines harvested an average of 324.12 ha of wheat, oats, canola, sunflower, soybeans and corn on a total area of 2593 ha. The given values are lower by half than the results of Radić (2016), where the universal combines in the 6 studied cooperatives harvested on average 579.17 ha of the mentioned crops. In the same research, the energy equipment of universal harvesters in 6 cooperatives in Vojvodina ranged from 0.17 kW /ha to 0.68 kW/ha, on average 0.33 kW/ha, which is less than the research shown in the farm (0.77 kW/ha). According to research by Pawlak *et al.* (2002), the average energy equipment of harvesters in the USA, Australia and Canada is 7.0 kW/100 ha, in Western Europe 31.6 kW/100 ha, in Russia 11.9 kW/100 ha, which is much lower than the results of this work (77 kW/100 ha).

Table 4 shows that there are 0.31 universal harvesters per 100 ha, which is less than the average values in southern and eastern Serbia - 0.8 (Radivojević, 2014), as well as the results of Pawlakaet *et al.* (2002, 2011) for the USA, Australia and Canada (0.86), Western Europe (1.50), Russia (0.63) and Poland (1.8), and higher than the value (0.173) in Vojvodina given

by Radić (2016). The average specific energy equipment of the cooperative with harvesters for obtaining sugar beet roots is 15.05 kW/ha. There are 4.30 harvesters per 100 ha of sugar beet cultivation, which is less than the results obtained by Pawlak (2011) for Poland (13.5 harvesters/100 ha). In the 2019 production year, the sugar beet harvester with an average power of 349.54 kW harvested 1,951.32 t of roots from 23.23 ha.

Conclusions

The cooperative "Agroprom" from Stara Pazova cultivates 340 ha of its own arable land with available harvesters and provides services to subcontractors on an area of 2555 ha. The cooperative's equipment with harvesters is satisfactory, as it has a total of 21 harvesters, of which 13 units or 61.90% are for harvesting sugar beet roots and 8 units or 38.10% are universal for harvesting small cereals, soybeans, rapeseed, sunflower and corn. The total available engine rated power of all harvesters is 6549 kW, of which 2005 kW (30.62%) are universal grain harvesters and 4544 kW (69.38%) are sugar beet root harvesters. The universal harvester with an average engine power of 250.62 kW harvested 324.12 ha of the above crops, and the sugar beet harvester with an average power of 349.54 kW pulled an average of 1951.32 t of roots from 23.23 ha. The power equipment in the cooperative is 0.77 kW/ha for universal cereal harvesters and 15.05 kW/ha of sugar beet root harvesters. For every 100 ha of arable land, there are 0.31 universal grain harvesters and 4.30 sugar beet root extraction harvesters. In terms of the number of units (50%) and available engine power (56.46%), the universal combine harvester Claas Lexion 560 is the most represented in the cooperative. In the cooperative, the average age structure of the universal combines is 8.75 years, as more than 50% of the universal combines are younger than 7 years. This is not the case for sugar beet harvesters, as all available harvesters are over 12 years old. Harvesters for sugar beet harvesting are outdated and should be gradually replaced by new, technically and technologically more modern harvesters.

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TRYPTOPHAN CONTENT INCREASE IN MAIZE INBRED LINES IMPROVED THROUGH MARKER ASSISTED BREEDING

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Abstract

Quality protein maize (QPM) has high content of essential amino acids lysine and tryptophan that are deficient in standard maize. Naturally occurring *opaque2* recessive mutation has been the most widely studied and used as a source for genetic improvement of the nutritional value of maize proteins. Marker assisted conversion of maize inbred lines to QPM adapted to temperate regions is being conducted at Maize Research Institute Zemun Polje (MRIZP). The ultimate goal is selection of the *opaque2* recessive genotypes (foreground selection) with the highest proportion of recurrent parent′s genome (background selection) along with high lysine/tryptophan content without losing good agronomic performances of the original line. After two backcrossing and selection of heterozygous individual plants, *opaque2*-specific molecular markers phi057 and umc1066 successfully identified recessive homozygotes in BC_2F_2 generation. The next step was to confirm expression of the QPM trait through elevated kernel tryptophan content (TC). Laboratory analyses were used to quantify and select for acceptable tryptophan concentrations for QPM - above 0.075%. The results presented in this paper relate to identification of BC_2F_3 progenies with increased TC in the conversion of two MRIZP commercial inbred lines (RP_1 and RP_2). The average TC ranged from 0.070% in RP_1 to 0.074% in RP_2. Out of 60 progenies from these two lines, 27 had TC at or above the QPM treshold. A total of 12 progenies was chosen for the highest tryptophan content. These lines will serve as parental lines for developing QPM hybrids adapted to temperate regions.

Keywords: *maize, marker assisted breeding, tryptophan, quality protein maize.*

Introduction

Maize (*Zea mays* L.) is among the most important cereal crops across the world. However, due to the low level of two essential amino acids – lysine and tryptophan, it does not meet the daily balanced protein requirement. Improvement of the protein quality in maize began in the 1960s with the discovery of mutants that produce enhanced levels of lysine and tryptophan (Mertz et al., 1964). Quality Protein Maize (QPM) was developed using conventional breeding techniques and contains the mutation at *opaque2* (*o2*) loci, which changes the protein composition of the maize endosperm, resulting in 2-3 fold higher concentrations of lysine and tryptophan (Vasal, 2000). Due to its higher biological value (amount of nitrogen that is retained in the body), balanced nitrogen index and leucine-isoleucine ratio, QPM offers significant nutritional benefits, which is well demonstrated both in terms of human food and animal feed (Eshetie, 2017).

Marker assisted selection (MAS) gained considerable importance in complementing conventional breeding as it increases efficiency, reduces time and costs taken to obtain QPM (Babu et al., 2004). The use of molecular markers in QPM breeding programs shortens the selection process during development of improved genotypes, making it more efficient across environments (Tandzi et al., 2017). Molecular markers are used to control all steps for

introgression of a target locus from a donor to a recipient line. Babu et al. (2005) reported conversion of standard maize inbred line to its QPM version through a combination of marker assisted and phenotypic selection. Their rapid line conversion strategy included a two-generation backcross program that employs foreground selection for the *opaque2* gene in two backcross generations, background selection at non-target loci in the BC_2 generation, and phenotypic selection for kernel modification and other desirable agronomic traits in two subsequent selfed generations. As concluded by these authors, this integrated breeding strategy can be applied to reduce genetic drag as well as the time involved in a conventional line conversion program. Gupta et al. (2013) demonstrated the introgression of *o2* allele to the parental lines of a released Indian maize hybrid through marker assisted backcross breeding followed by reconstitution of the original hybrid with 30% enhanced lysine and 41% increase in tryptophan content over the original hybrid.

Marker assisted conversion of commercial maize inbred lines to QPM adapted to temperate regions is being conducted at Maize Research Institute Zemun Polje (MRIZP) (Kostadinovic et al., 2014, 2016). Molecular markers are being used to identify the *opaque2* recessive genotypes (foreground selection) with the highest proportion of recurrent parent′s genome (background selection) along with high lysine/tryptophan content without losing good agronomic performances of the original line. After two backcrossing and selection of heterozygous individual plants, *o2*-specific molecular markers successfully identified recessive homozygotes in BC_2F_2 generation. The next step was to confirm expression of the QPM trait through elevated kernel tryptophan content. The results presented in this paper relate to identification of BC_2F_3 progenies with increased tryptophan content in the conversion of two MRIZP commercial maize inbred lines.

Material and methods

Plant material

Two MRIZP commercial inbred lines, adapted to the local environmental conditions in Serbia, were chosen for marker assisted conversion to high tryptophan maize. These lines, used as the recurrent parents (RP_1 and RP_2), are components of the leading MRIZP hybrids. One commercial MRIZP inbred line ZPL5, which was converted to its QPM counterpart (Kostadinovic et al., 2016), was used as *o2* donor. Throughout the conversion process, F_1, BC_1, BC_2, BC_2F_2 and BC_2F_3 generation were developed (Figure 1). Molecular markers were employed both in foreground (BC_1, BC_2, BC_2F_2) and background (BC_2) selection to ultimately identify recessive homozygotes with the highest recovery of recurrent parent's genome (RPG). Their progenies (BC_2F_3) were subjected to biochemical analysis to confirm expression of the QPM trait through elevated kernel tryptophan content.

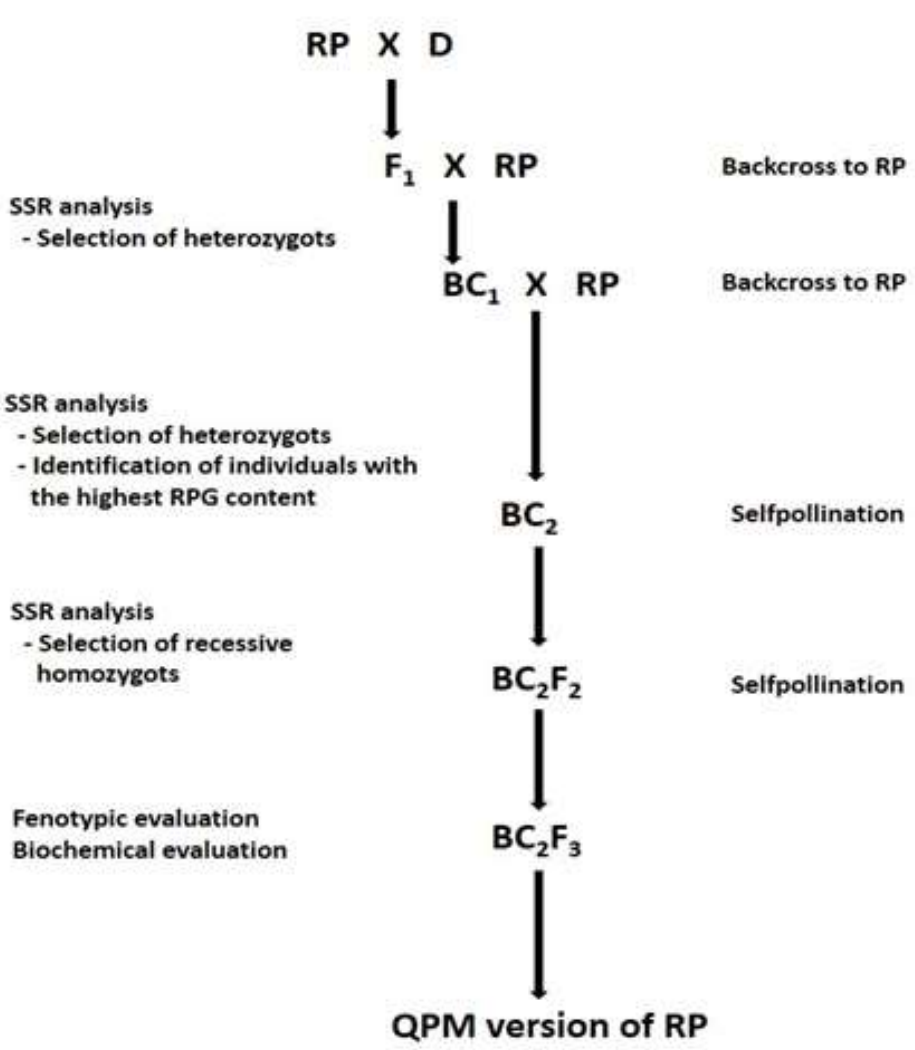

Figure 1. Schematic presentation of marker assisted conversion of a standard maize inbred line to its QPM version (RP-recurrent parent, D-donor line, RPG-recurrent parent's genome, QPM-quality protein maize)

Biochemical analyses

Two sub-samples per genotype, consisting of 30 kernels each, were dried in a controlled oven at 65°C/16-18 hours to constant dry weight, milled using a Perten 120 lab mill (Perten, Sweden) to obtain fine powder (<500 μm). Flour was defatted by hexane treatment in Soxhlet extractor. Tryptophan content was determined by the colorimetric method given in Nurit et al. (2009). Shortly, the color was developed in the reaction of flour hydrolysate, obtained by overnight digestion with papain solution at 65°C, with a reagent containing glyoxylic acid and ferric chloride dissolved in sulfuric acid. After incubation at 65°C/30 min, absorbance was read at 560 nm. Tryptophan content was calculated using a standard calibration curve, developed with known amounts of tryptophan, ranging from zero to 30 μg/μl. Analyses were carried out in duplicate for each sample and the results were presented as mean ± SD.

Results and discussion

The biggest challenge in QPM breeding is to improve the protein quality while simultaneously achieving grain yield at the level of standard maize hybrids. The development of QPM genotypes involves three distinct genetic systems: *opaque2* genetic system, endosperm-modifier genetic system and amino acid-modifier system (Maqbool et al., 2021).
Opaque2 gene, recessive mutant alleles, comprising the first genetic system for QPM development. This naturally occurring mutation was widely studied as a source for genetic improvement of the nutritional value of maize proteins. Simple sequence repeats phi057 and umc1066, located within *opaque2* gene, were effectively used to distinguish between recessive and dominant alleles (Kostadinović et al., 2014; Kostadinović et al., 2022). Consequently, these molecular markers are thus being used to increase the value of conventional breeding through indirect selection of plants which possess the desirable gene in segregating populations (Afolayan et al., 2019). After two backcrossing and selection of heterozygous individual plants, these *o2*-specific molecular markers successfully identified recessive homozygotes in BC_2F_2 generation. Homozygous recessive plants were selfpollinated to produce BC_2F_3 kernels for endosperm modifications score and tryptophan content analyses.
Alleles of endosperm hardness modifier genes constitute the second genetic system for QPM breeding. Kernels that have very soft endosperm and no modifiers also have undesirable characteristics such as susceptibility to ear rots and pests, and kernel cracking. Therefore, it is important to accomplish sufficient degree of endosperm modifications to consequently achieve higher grain yields (Eshetie, 2017). Prior to biochemical analyses, kernel endosperm modifications were visually assessed using light table, according to the scoring scale from 1 (completely translucent, with no opaqueness) to 5 (completely opaque), as presented in Vivek et al. (2008). This visual phenotypic selection served as an appropriate tool to separate *o2o2* genotypes with modified endosperm (Twumasi-Afriyie et al., 2016). Over 95% kernels showed good (types 1 and 2) and medium (type 3) endosperm modifications, which corresponds to standard maize kernels (Vivek et al., 2008). Only hard endosperm kernels (≤50% opaque) were selected for tryptophan content determination. Derivations with soft kernels, as well as those with the insufficient number of kernels, were discarded out of further biochemical analyses.
Amino acid modifier genes, the third genetic system, affects the lysine and tryptophan contents of maize grain. Lysine and tryptophan concentration in maize kernels are highly correlated (3:1 ratio of lysine to tryptophan). Therefore, in a QPM breeding program only one of these amino acids is typically monitored, tryptophan being the more commonly measured of the two (Nurit et al., 2009). When interpreting laboratory results for making selections, the tryptophan content (TC) has to be above the acceptable limit (QPM threshold) - above 0.075% (Vivek et al., 2008). Out of 60 progenies from two lines analyzed here, 27 had TC value at or above the QPM treshold (Figure 2). The average TC ranged from 0.070% in RP_1 to 0.074% in RP_2. Somewhat higher TC values were found in RP_2 (0.070-0.077%) compared to RP_1 progenies (0.068-0.077%).

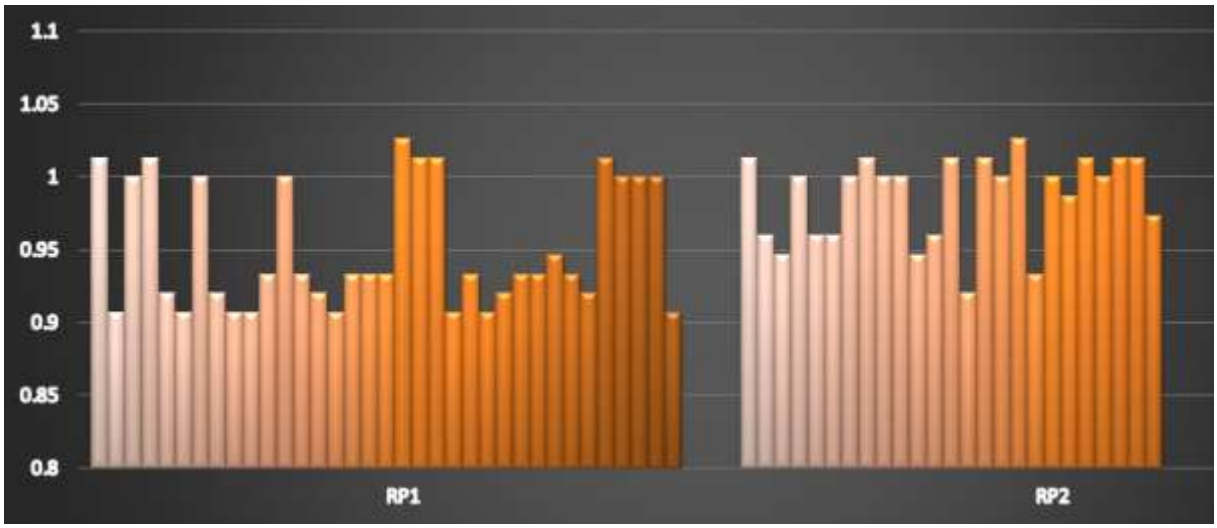

Figure 2. Tryptophan content of the two recurrent parents′ (RP_1 and RP_2) derivations given as % of the QPM treshold (given as 1).

Similar results were found in Jompuk et al., (2011) where TC ranged from 0.070 to 0.084% in QPM inbred lines obtained by the backcross method using marker-assisted selection of the *opaque2* gene. In Babu et al. (2005) biochemical analysis showed somewhat higher TC (0.078 to 0.094%), which was in accordance with our previous work (Kostadinović et al., 2022). In another study, the introgression of *o2* allele was made into the β-carotene-rich inbred lines (Chandran et al., 2019). Besides higher β-carotene content, these improved lines were having higher TC (0.073% to 0.081%) along with better agronomic performance.

A total of 12 progenies was chosen for the highest tryptophan content. These lines will serve as parental lines for developing QPM hybrids adapted to temperate regions. Developed QPM hybrids will be tested for their field performance and relevant biochemical components. A promising hybrid for use in broiler feeds will be identified, with the aim to substitute costly synthetic lysine in their diets.

Conclusions

Co-dominant nature of the polymorphism exibited by the *opaque2*-specific molecular markers phi057 and umc1066 enabled successfull selection of heterozygotes and recessive homozygotes throughout this MAS process. Almost half of the progenies from selected recessive homozygotes with the highest recovery of recurrent parent's genome had tryptophan content at or above the QPM treshold. Among them, progenies with the highest tryptophan content were chosen to represent improved lines and to serve as parental lines for developing QPM hybrids adapted to temperate regions.

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INFLUENCE OF THE COVID-19 PANDEMIC ON THE IMPORT AND EXPORT OF CHRYSANTHEMUMS IN SERBIA

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Abstract

After roses, chrysanthemums are the second best-selling cut flowers in FloraHolland which is considered as the largest market of floricultural products in the world. Chrysanthemums are also important in the flower production in Serbia, and they are produced both for domestic and international market. Taking into account that Covid-19 pandemic influenced production and consumption of cut flowers worldwide, including the chrysanthemums, the aim of this study was to determine how the pandemic influenced export and import of chrysanthemum cut flowers in Serbia. For that reason, the trend analysis was used to calculate trends for the years 2020 and 2021 in order to determine expected change in export and import based on five years data (2015-2019) collected before the pandemic. Obtained data were compared with the real data collected for the pandemic years 2020, 2021 and 2022 for the chrysanthemum cut flowers trade in Serbia. The average import of chrysanthemum cut flowers was 474.6 metric tons with average value of 0.65M (USD). During the pandemic years, the amount of imported chrysanthemums did not change significantly, average amount was 478.2 metric tons, but value of imports was considerably higher, reaching average value of 1.67M (USD). The export of chrysanthemum cut flowers decreased over years, and values of export became insignificant during the pandemic years. Taking into account the rising prices of chrysanthemum cut flowers, and that Serbia has favourable conditions for chrysanthemum production, we can conclude that it is important to increase chrysanthemum cut flowers production in Serbia.

Keywords: *chrysanthemums production, chrysanthemums trade, cut flowers market.*

Introduction

After roses, chrysanthemums are the second best-selling cut flowers in FloraHolland which is considered as the largest market of floricultural products in the world (Darras, 2021). According to The Observatory of Economic Complexity (OEC), the total value of chrysanthemums world trade was 869M for 2021, the largest exporter was the Netherlands with 49.9%, the largest importer was United Kingdom with 17.1% (https://oec.world/en/profile/hs92/chrysanthemums). Environmental conditions in Serbia are suitable for successful chrysanthemum production (Krucker et al. 2010, Grbić and Marković 2020; Viyachai et al. 2015) and there is possibility to develop large scale production in this country.

After the Covid-19 pandemic started, commercial establishments were temporary closed and the unemployment occurred in some sectors due to the prophylactic measures, such as the social isolation and lockdowns which were conducted in the majority of countries, having impact not only to human health, but also on the economy with the drop in consumption, reduction of the production system. Also, income-generating activities were reduced. The floriculture was also influenced by the pandemic, including production and consumption of chrysanthemums worldwide (Clair *et al.*, 2021; Anacleto *et al.*, 2021; Bulgari *et al.*, 2021; Pérez-Urrestarazu *et al.*, 2021; Lamm *et al*. 2021; Lioutas and Charatsari, 2021; Marković

2020a,b; World Health Organization, 2022). For this reason, the aim of this study was to determine if the pandemic influenced export and import of chrysanthemums in Serbia.

Material and Methods

In this research, the official statistical databases of Serbia were used for obtaining the data on the export and import of mint given in tons and USD. These data were collected from the databases of the Republic Bureau of Statistics (https://data.stat.gov.rs/?caller=SDDB), for the period 2015-2022. In order to obtain more accurate data, the chrysanthemum trade in Serbia during the pandemic years (2020, 2021, 2022) was compared to the average data for the five year period before the pandemic (2015-2019). Also, the trend analysis was used to calculate trends for the years 2020, 2021 and 2022 in order to determine expected change in export and imports based on five years data (2015-2019) collected before the pandemic and to compare obtained data with real data collected for the pandemic years 2020, 2021 and 2022. Microsoft Excel 2007 was used for trend analysis.

Results and Discussion

The export of chrysanthemums from Serbia is insignificant. Despite the favourable conditions and despite the fact that chrysanthemum production was well developed in Yugoslavia during 1970s, today, there is no large scale production of this ornamental plant in Serbia (Table 1).

Table 1 Export of chrysanthemum cut flowers from Serbia during 2015-2022.

Year	**European union**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***
2015	0.5	0.7	5.2	9.2	Estimated values based on 2015-2019 data	
2016	0.0	0.0	0.5	1.1		
2017	0.0	0.0	0.4	0.7		
2018	0.0	0.0	0	0		
2019	0.0	0.0	0	0		
2015-19*	**0.1**	**0.14**	**1.3**	**2.2**		
2020	0.0	0.0	0.0	0.0	0.0	0.0
2021	0.0	0.0	0.0	0.0	0.0	0.0
2022	0.0	0.0	0.0	0.0	0.0	0.0

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period.

However, there is a need for chrysanthemum plants in Serbia and large amounts of this plant are imported (Table 2). The amount (tons) did not change noticeably during the pandemic years 2020-2022 compared to the average amount for the 5 year period before pandemic. There was a drop in import in 2020, which can be connected with the reduction of income-generating activities when pandemic started as well with the lack of social activities, such as weddings where chrysanthemum flowers are often used for decoration. In 2021 and 2022, the amount of imported chrysanthemums increased, but this amount already corresponds to the amount predicted by trend analysis (Table 2). Similarly, the pandemic influenced demand for cut roses in Serbia, and import of rose cut flowers decreased in the first year of the pandemic (Marković, 2022a). The drop in chrysanthemum cut flowers import occured during the first pandemic year (2020), and it was expected because florists shops were closed and there were no social events during the lockdowns. Simalira situation was recorded worldwide, with drop

in flowers sale and increase in demand for food which can be stored for a longer time (Pangtu *et al.*, 2023; Fredenburgh, 2020). However, during the years 2021 and 2022 the lockdowns were reduced in Serbia, and demand for cut flowers, including chrysanthemums, increased. The amount of imported chrysanthemum cut flowers to Serbia during the years 2021 and 2022 was similar to the values predicted by trend analysis (Table 2). According to this, we can conclude that the pandemic influenced chrysanthemum cut flowers demand only during the first year.

Table 2 Import of chrysanthemum cut flowers to Serbia during 2015-2022.

Year	**European union**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***
2015	402	514.6	402.1	514.7	Estimated values based on 2015-2019 data	
2016	510.2	517.1	510.2	517.1		
2017	476.1	629.8	476.1	629.8		
2018	480.7	762.2	480.8	763		
2019	503.7	839.7	503.8	840.1		
2015-19*	**474.5**	**652.7**	**474.6**	**652.9**		
2020	365.2	710.3	365.3	710.8	526.8	921.9
2021	551.8	1582.8	551.8	1582.8	517.8	1040.4
2022	516.6	1666.0	517.6	1669.3	539.9	1133.1

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period.

However, it is evident that price of chrysanthemums increased gradually over years, from 1280 USD/ton in 2015 to 3225 USD/ton in 2022. This indicates that there is a demand for chrysanthemums on Serbian local market, and that production of chrysanthemums should be established and expanded primary for local market needs.

Conclusions

Although the pandemic considerably influenced the floriculture sector worldwide, including production and distribution of roses, cut greenery and other ornamental plants, the import of chrysanthemums in Serbia did not change significantly. Taking into account the rising prices of chrysanthemums, and that Serbia has favourable conditions for chrysanthemum production, we can conclude that it is important to develop and improve technologies for successful chrysanthemum production in Serbia, adapted to a local environmental conditions, and also to increase areas under chrysanthemum production in Serbia. Technology should be developed to produce high quality plants to increase competitiveness, not only for local market needs, but also for export on international markets.

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CHARACTERISATION OF MUNG BEAN (*VIGNA RADIATA* L.) SEEDS USING FLUORESCENCE SPECTROSCOPY AND MULTIVARIATE ANALYIS

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Abstract

Mung bean (*Vigna radiata* L.) is a leguminous plant cultivated mainly in south-east Asia and used as an ingredient in local cuisine. Its principal nutritional value is contained in its constituents such as starch, proteins, (poly)phenols, and natural antioxidants. Fluorescence spectroscopy is increasingly used as a method of choice for food analysis; due to the presence of different fluorophores originating from aromatic amino acids and secondary metabolites, it is useful for proteins and phenolics detection. In this study, the total protein and phenolic contents of mung bean seed extracts were determined using the Bradford method and Folin–Ciocalteu (FC) reagent, respectively. Antioxidant activity was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Fluorescence spectra were recorded for a series of excitation-emission wavelengths. Further, we used the multivariate analysis on the recorded excitation-emission fluorescence matrix of the studied samples. The results showed the presence of three different fluorescence components, with the position of the emission maximum corresponding to the fluorophore of proteins (component 1 with excitation/emission peak maxima at Ex 290/Em 345 nm) and phenolics (component 2 - Ex 295/Em 395 nm and component 3 - Ex 350/Em 450 nm). This fluorescence-based method could be a useful approach for estimating the nutrient properties of leguminous food.

Keywords: *Mung bean, fluorescence spectroscopy, MCR-ALS, TPC, DPPH.*

Introduction

Mung bean (*Vigna radiata* L.) belongs to Fabaceae (Leguminosae) family that possesses high nutritional value (Shi *et al*. 2016). Legume seeds are known as a functional food source due to their high content of proteins and essential amino acids, vitamins, and minerals but also contain bioactive components and (poly)phenols that contribute to a high antioxidant capacity (Singh *et al*., 2017; Amarowicz and Pegg, 2008). Secondary metabolites take part in the protection of the seed from infection during and after germination but are also beneficial during later growth stages of the crop (Yusnawan *et al*., 2019; Ganesan and Xu, 2018). The majority of the phenolic content of the mung bean seed include caffeic acid, syringic acid, chlorogenic acid, ferulic acid, and p-coumaric acid (Singh *et al*., 2017).

Fluorescence spectroscopy is a sensitive, fast, and noninvasive analytical method, capable of detecting low amounts of fluorescent compounds in a sample that contains fluorophores (Sádecká and Tóthová, 2017). A series of emission spectra with different excitation wavelengths are recorded to obtain excitation-emission matrices (EEMs). Fluorescence characteristics of specific groups of compounds are analyzed from the obtained EEMs using advanced statistical methods. Multivariate Curve Resolution-Alternating Least Square (MCR-

ALS) analysis was used to extract the position and shape of specific spectral components (Stanković *et al*., 2021).
The aim of this study was to estimate the nutritional value of the analyzed mung bean seeds using fluorescence spectroscopy combined with multivariate analysis.

Material and Methods

Plant samples and their preparation

Seeds of mung bean (*Vigna radiata* L.) were purchased from the Local Organic market in Maastricht, Netherlands. Whole seeds were grinded in a mill and further homogenized to obtain a fine powder with liquid nitrogen in a mortar with a pestle.

Chemicals

Pyrogallol, 2,2-diphenyl-1-picrylhydrazyl, gallic acid (GA), Coomassie Brilliant blue G-250 (CBB G-250), methanol, ethylenediaminetetraacetic acid (EDTA), polyvinylpyrrolidone (PVP) and phosphoric acid (H_3PO_4) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Tris and bovine serum albumin (BSA) were obtained from SERVA Electrophoresis GmbH (Heidelberg, Germany). Folin–Ciocalteu's phenol reagent and sodium carbonate anhydrous (Na_2CO_3) were obtained from Fluka Analytical (Buchs, Switzerland). Acetone and ethanol were obtained from Zorka Pharma (Šabac, Serbia).

Determination of total protein content

Protein concentration was determined using the Bradford method. Proteins were extracted from the powdered seeds with extraction buffer 0.1 M Tris-HCl pH 7.6, containing 1 mM dithiothreitol, 1 mM EDTA, and 2% PVP. The homogenates were incubated and stirred for 30 minutes at 4°C and centrifuged for 10 minutes at 10 000 rpm. Bradford reagent was prepared by dissolving 10 mg of CBB G-250 in 5 ml of 95% ethanol, containing 10 ml of H3PO4 filled with deionized water up to the final volume of 20 ml. Bradford reagent was diluted 5 times with deionized water before use. Sample aliquots of 5 µl were placed in microplates and mixed with 200 µl of diluted Bradford reagent. Absorbance at 595 nm after 5 minutes of incubation was detected using a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Protein concentration was determined using a calibration curve in the range of 0.1-1.0 mg/ml BSA solution.

Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin–Ciocalteu (FC) reagent. Phenol extraction was carried out by adding 80% methanol solution to 100 mg of seed powder in a 1/10 (w/V) ratio. Samples were incubated on a shaker for 60 minutes at 25°C, followed by centrifugation for 5 minutes at 10 000 rpm. From collected supernatants, aliquots of 50 µl were taken and mixed with 475 µl of 0.2 M FC reagent. After 3 minutes, 475 µl of 0.25 M Na2CO3 was added to each sample and incubated for 60 minutes. Absorbance at 724 nm was measured using a UV-VIS microplate reader. The calibration curve was prepared with gallic acid in 80% methanol in the range of 0.05-2.00 mM and used for the determination of TPC content. Obtained results were expressed in µmol equivalent of gallic acid per gram of dry weight (gDW).

Determination of DPPH radical scavenging activity

The antioxidant activity (AA%) of mung bean seeds was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. Powdered seed samples were added to the reaction mixture containing 0.1 mM DPPH in 70 % ethanol solution. After 30 minutes of incubation in the dark, 200 µl aliquots were taken and placed in microplate wells. Absorbance at 517 nm was measured in a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). For blank and control, pure deionized water and 0.1 mM DPPH solution was used respectively. Antioxidant activity was defined as a percentage of DPPH-reagent consumed during the reaction.

Fluorescence spectroscopy and multivariate analysis

The fluorescence measurements of the mung bean seeds were recorded using an Fl3-221 P spectrofluorimeter (Jobin Yvob, Horiba, French Republic), equipped with a 450 W Xe lamp and a photomultiplier. The front-face (FF) configuration was used for the fluorescence measurements. The fluorescence emission spectra of the analysed seed samples were measured in the range of 270 to 515 nm with excitation wavelength ranging from 260 to 380 nm in 5 nm steps. The integration time was set at 0.1 s, while the spectral bandwidth of 1 nm was set for the excitation and emission slits. The multivariate analysis (Bartolić *et al*. 2018, Stanković *et al*. 2019) has been used to decompose and extract an optimal number of emission components (corresponding to the fluorophores) from the excitation-emission matrix (EEM) of the analysed sample. The analysis was performed using the Unscrambler X 10.4 (Camo Analytics, Oslo, Norway) software.

Results and Discussion

Results of total protein and phenolic content of the studied extract of the mung bean seed samples as well as antioxidant activity with their respective standard errors are presented in Table 1. Obtained protein content was calculated from the regression equation (R = 0,999, y = 0.478×x + 0.793), from the calibration curve and expressed in mg of protein per gram of dry weight (mg/gDW). Obtained results of total phenolic contents (TPC), calculated from the calibration curve using the regression equation (R=0.999, y =0.482×x + 0.033) are expressed in µmol equivalents of gallic acid per gram of dry weight of the samples (µmol eq.GA/gDW). The resulting value equals 56.91 µmol eq.GA/gDW and corresponds to the previously published results (Singh *et al*., 2017; Orak *et al*., 2018).

Table 1. Total phenolic content, total protein content, antioxidant activity of the whole mung bean seed with their corresponding standard errors out of 4 replicates.

Total protein content (mg/gDW) [a]	Total phenolic content (µmol eq.GA/gDW) [b]	Antioxidant activity (%)
56.91 ± 2.43	0.353 ± 0.025	54.52 ± 1.77

[a] DW – dry weight, [b] eq.GA – equvalent of Gallic acid.

In this study, DPPH radical scavenging activity of the whole mung bean seed extracts was 54.52% ± 1.77. The antioxidants were determined by a stable, purple-coloured organic free radical DPPH. Its ability of reduction was followed by accepting an electron and loss of its absorption spectral band at 517 nm, and a visually noticeable change into the yellow-coloured DPPH radical.

Further, fluorescence spectroscopy was used for the characterization of the nutritional composition of organic mung bean seeds. Figures 1a and 1b show the EMMs for the mung bean seeds. Two distinct spectral maxima on the contour map are displayed, one at about

340–360 nm corresponds to fluorescent spectra of proteins (Stanković et al., 2019) and the other at about 430–450 nm to phenolic compounds. MCR-ALS was applied to the EMM to distinguish an optimal number of spectral components which are displayed in Figures 1c and 1d.

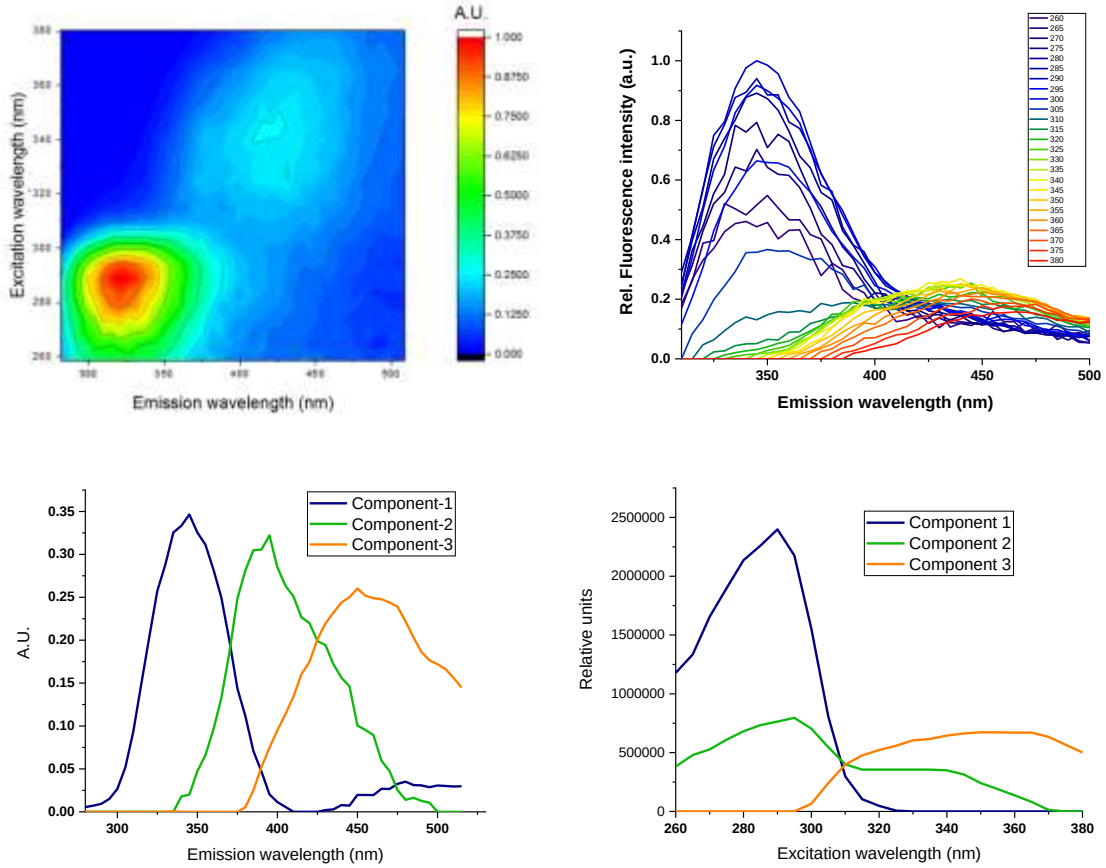

Figure 1 Normalised EEM of mung bean seed samples (a) contour map and (b) spectra; (c) emission and (d) excitation spectral profiles of the spectral components obtained by multivariate analysis (MCR-ALS).

Results of MCR-ALS analysis showed three optimal fluorescence components with the following positions of the excitation/emission peak maxima, component 1 (C1) – Ex 290/Em 345 nm, component 2 (C2) – Ex 295/Em 395 nm, and component 3 (C3) - Ex 350/Em 450 nm. Emission wavelengths of the obtained components displayed in Figure 1c correspond to the emission maxima of proteins (component 1) and phenolic compounds (components 2 and 3) (Stanković et al. 2019).

Conclusions

Legumes are considered functional food ingredients and a major source of dietary antioxidants. Fluorescent spectroscopy combined with statistical analysis has been proved as a useful combination for the identification of protein and phenolic spectral components. Secondary metabolites were determined as a good parameter for the estimation of seed quality and an indicator of tolerance to different types of stress. The advancement in this research lies in collecting information about bioactive compounds, such as (poly)phenols, that are useful in improving the functional and antioxidant properties of quality seeds used in daily diet.

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PROTEIN CONTENT IN SEED OF TRITICALE GROWN IN PURE CROP AND MIXTURE WITH PEAS

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Abstract

Protein content in seed of triticale is and traits responsible for seed quality, which is determined by genetic and environmental factors and its interaction. Protein contents varies significantly by nitrogen nutrition technology under conditions of soil moisture and temperature regimes. The aim of this research was to determine crude protein content in triticale grown in intercrops with pea and in monoculture. The experiment was set up on plot 5 m^2 in four repetitions on the experimental field of the Small Grains Department of the Institute of Field and Vegetable Crops in Novi Sad. The experiment was carried out in field conditions for two consecutive years and was designed according to a randomized block system. The Kjeldahl method was used to determine crude proteins. The analysis showed different results for seed protein contents in triticale variety Odisej depends of system of technology growing. Content of crude proteins was higher in sample of triticale grown in mixture with pea (10.85%) than in triticale grown as sole crop (8.45%). Result showed that system of technology growing have influence on plant traits, and that results and that in intercrop peas provide a greater amount of nitrogen available for absorption by the roots of the triticale plant, which is associated with a higher crude protein content.

Keywords: *crude, protein, intercrops, triticale, peas*

Introduction

Protein content is reliable indicator for seed quality in triticale which is influenced by genotypes, and mostly by genetic. Protein contents significantly varies under environmental conditions in interaction with genotype. environmental factor and its interaction. In study Dziki et al. (2023) protein content in seed of triticale varied from m 9.5 to 12.7%, while Manley et al. (2013) reported that protein content can change in range from 7.5% to 16.2%, depending on a genotype. Protein content is in negative correlation with seed yield, and both of them depends from environmental conditions (water, temperature, minerals), particularly from available nitrogen (Knezevic et al., 2010; Burešová et al., 2010; Kondić et al., 2012). The nitrogen forms the basic component of amino acids from which proteins are built and represent key components nucleic acids, nucleotides, chlorophyll, amides, and alkaloids. Regarding that in technology of growing is necessary uses nitrogen fertilizers. The yield of seed and technological quality of triticale variety influenced by supplied amount of nitrogen (Zečević et al., 2010; Madić et al., 2015). Plant nitrogen requirements must be met to achieve profitable yields and adequate seed protein content (Garrido-Lestache et al., 2004; Bedoussac et al., 2015). Nitrogen has impact on plant development, organ formation, size, structure, and yield quality visibly. Chlorophyll, structural and catalytic proteins, or enzymes, are both influenced by nitrogen during synthesis. It plays a significant part in the development of the

leaves, influencing their size, photosynthetic activity, and length of physiological activity. Nitrogen is believed to be mostly found in the soil, where it is ingested by plants as ions and molecules (Kastori and Maksimović, 2008).However, insufficient amounts of nitrogen can lead to a decrease in the protein content of cereal grains. A drop in protein with an increase in yield is caused by a lack of nitrogen in the soil. It is challenging for plants to achieve the essential requirements for protein content in environments where nitrogen fertilizers are not used, so it is important to somehow raise the level of nitrogen in the grain. Growing plants in intercrops is one system of growing two different crop varieties in a small area, which is demonstrated by the decreased need for mineral nitrogen fertilizers (atmospheric nitrogen fixation), Li et al. (2014) and in the soil increasing content of organic matter, improved chemical and physical properties of soil, the preservation of moisture in soil, the prevention of nutrient leaching, better utilization of the available space, the more efficient utilization of the resources, and the ability to produce satisfactory yields (Clark, 2008; Goss et al., 2009; Ugrenović and Ugrinović, 2014; Klimek-Kopyra et al., 2018; Manasa et al., 2018). Many researches showed that grain quality, specifically grain protein content, was higher in intercropping than in single cropping systems (Knudsen et al., 2004; Gooding et al., 2007; Naudin et al., 2010).The intercropping approach produced the best results when nitrogen was in short supply (Bedoussac and Justes, 2010b). The reduced competitiveness of legumes for mineral nitrogen combined with decreased seed production in intercropping compared to solitary cropping is what causes the rise in protein content of cereals in intercropping (Bedoussac et al., 2015). The aim of this research was to determine crude protein content in triticale grown in two different growing systems, intercrops with pea and in monoculture.

Material and methods

The field experiment was carried out on a slightly carbonated loamy chernozem soil during 2018-2019 at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Triticale Odisej was used for this study, and was sown as winter and spring variety. Triticale crops sown in the autumn were intercropped with winter pea Kosmaj, while spring pea NS Junior was used as intercrop for spring sown triticale. In both 2018 and 2019, the best time to plant winter varieties was October, and the best time to plant spring varieties was March. Climatic conditions for two growing seasons are presented in Table 1. Plowing, disc-harrowing, and cultivating were all part of the sowing preparation. Prior to both winter and spring sowing, MAP 12:52:0 (200 kg ha^{-1}) fertilization was carried out in October. System of mixed intercropping was used. Peas and triticale crops were each seeded in two separate passes. Peas were sown at a depth of 4-5 cm in the winter and spring, compared to 3-4 cm for triticale crops. Four replications of a field study were set up using a randomized block design. Each replicate's plot measured 5 m^2. Triticale in intercropping cultivation system was sown at a rate that was only 30% of conventional sowing rate. The sole crops of triticale are sown at a rate of 235 kg ha^{-1} in conventional monoculture system. Pea sowing rates in intercropping were only 70% than normal sowing rates. The sowing rate for both Kosmaj and NS Junior's was 140 kg ha^{-1}. Using a Kejltec 2300 (Foss, Hillerd, Denmark), the crude protein content of triticale Odisej was calculated using the modified Kjeldahl technique. The seeds were well mixed, 10 spikes from each plot were air-dried, and 50 g of samples were taken for protein analysis. Crop samples were ground in an AB-30 laboratory mill (Falling Number, Stockholm, Sweden) before being measured in duplicate and digested in sulfuric acid for about 0.7 g of each cereal/crop sample, ammonia being distilled, and excess acid being titrated (AACC 2000 method 46-10). All samples were analyzed using 6.25 coefficient. Tukey's pairwise comparisons with 95% confidence were used to provide information on which means were significantly different.

Table 1. Temperature, relative humidity and precipitation during growing period

Month	Mean air temperature (°C)		Relative humidity (%)		Precipitation per month (mm)	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
July	24.3	22	56	77	12	81.2
August	24.8	24	55	68	17.4	51.2
September	16.9	18.5	70	70	81.5	27.1
October	12.5	14.9	75	65	38.9	7.4
November	7.1	8	83	78	40.3	24.6
December	3.8	1.7	84	88	48.3	59.2
January	4.3	0	81	87	47.5	45.8
February	1.2	4.2	86	74	81.9	17
March	5	9.8	82	57	60.6	15.9
April	17.2	13.4	60	65	49	54.1
May	20.4	14.7	67	78	64.2	147.6
June	21.5	23.2	74	73	163.2	63.7

Results and Discussion

A crucial mechanism that provides higher seed quality in small grains is the assimilation of sufficient nitrogen which affects protein content. Better outcomes can be obtained, according to Bedoussac and Justes (2010a), by planting small grains with legumes in intercrops. The amount of crude proteins in triticale seeds was examined in intercrops with pea and in sole crop (monoculture)over the course of two growing seasons in order to ascertain the potential and influence of the cultivation system on triticale's ability to absorb nitrogen. Mean values of crude protein content in seed of triticale Odisej grown in two different growing systems are shown in Figure 1.

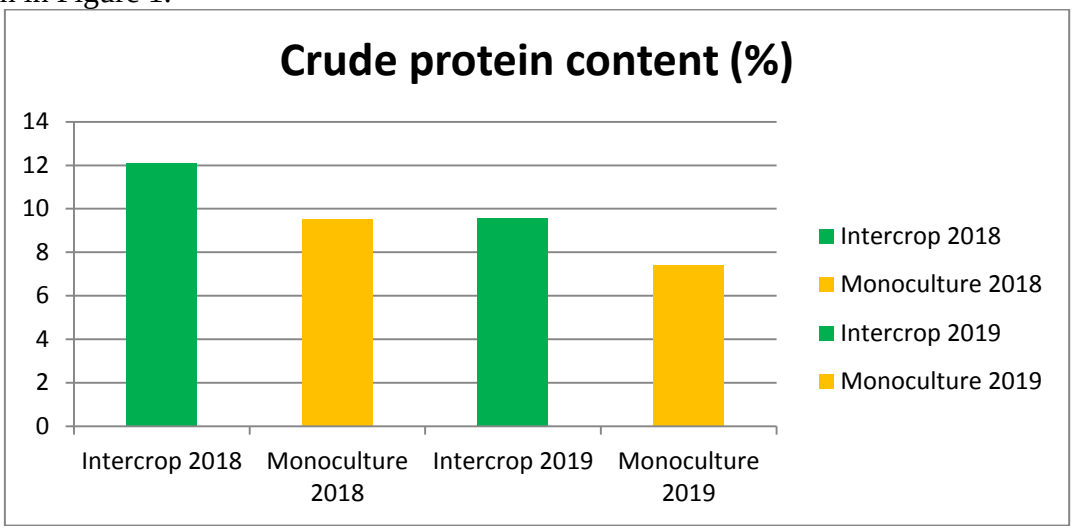

Figure 1. Crude protein content of triticale in intercropping and monoculture growing systems

In comparison to the second growing season (2019), the first (2018) had a generally higher crude protein content. The Tukey test showed that the cultivation system had a significant effect on the crude protein content of the seed. These results are shown in Table 2.

Table 2. Tukey test of comparison of mean values of crude protein content at 95% confidence interval

Tukey test - Crude protein content		
Year*Variety*System	Mean	Grouping

Triticale Odisej - Intercrop (2018)	12,10	A
Triticale Odisej - Intercrop (2019)	9,57	B
Triticale Odisej - Sole crop (2018)	9,50	B
Triticale Odisej – Sole crop (2019)	7,40	C

The cultivation system had a substantial impact on the first growing season, with the combined triticale and pea intercrop having a significantly greater crude protein content (12.10%) than the standalone triticale crop (9.50%). The cultivation system had a substantial impact on the second growing season as well, with the combined crop having a greater crude protein content (9.57%) than the independent crop (7.40%). On average, a higher content of crude protein was found in seed of triticale grown in intercropping (10.85%) than in triticale grown in monoculture (8.45%).In addition to the influence of the cultivation system, the influence of year and variety, as well as their interactions, significantly influenced ($P<0.001$) the content of crude proteins in triticale seeds

Based on research conducted in Lithuania, it was shown that intercropping recorded a much higher crude protein content than conventional crops. The spring wheat grown in intercrop exhibited higher seed quality metrics. When wheat and vetch were grown together, substantially more crude protein was accumulated in the seed than when they were grown separately (Šarūnaitė et al., 2010).

According to Jensen (1996b), rather than the symbiotic fixation of N_2, the benefit of the cooperative system of peas and cereals lies in the complementing utilization of soil inorganic and atmospheric nitrogen. According to Stomph et al. (2019), collaborative cropping has a positive effect on resource adoption by reducing competition. Lower yields affect the higher content of crude proteins in seed, and therefore growing small seeds in a mixture with legumes reduces the number of plants per unit area and thus affects the maintenance of the content of crude proteins.

The intercropping of cereal with leguminous crops increased the protein content of cereal seed, according to Gooding et al. (2007), since wheat had more nitrogen accessible per plant than a single crop. Less competition from legumes for nitrogen together with competition for light, water, and other resources caused the protein content to increase. Knudsen et al. (2004) and Naudin et al. (2010) reported that the crude protein content of cereal seeds was higher in those grown in intercrops with legumes compared to seeds grown alone.

Our study's findings demonstrated that triticale had a greater crude protein content during both growing seasons when grown in an intercropping cultivation system. Previous research has demonstrated that the mechanisms that make it possible for combination crops to use nitrogen more effectively are quite complicated and the outcome of interactions between small grains and legumes are both competitive and complimentary (Jensen et al., 2020).

Conclusion

Based on the analyzed results, it can be concluded that growing triticale in a combined crop with peas had a positive effect on the crude protein content of the seed. In both growing seasons, higher values of crude protein content were recorded in the combined cultivation system. A lower number of triticale plants per unit area in combination with less competition from legumes for nitrogen is considered the main reason for the higher crude protein content of the cereal seed. However, not all of the explanations for the mechanisms that give intercrops a higher crude protein content are presently known.

Acknowledgements

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EFFECTS OF IRRIGATION RATE AND PLANTING DENSITY ON MAIZE YIELD AND WATER USE EFFICIENCY IN THE TEMPERATE CLIMATE OF SERBIA

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Abstract

Scarce water resources severely limit maize (*Zea mays* L.) cultivation in the temperate regions of northern Serbia. A two-year field experiment was conducted to investigate the effects of irrigation and planting density on yield and water use efficiency in temperate climate under sprinkler irrigation. The experiment included five irrigation treatments (full irrigated treatment – FIT; 80% FIT, 60% FIT, 40% FIT, and rainfed) and three planting densities (PD1: 54,900 plants ha^{-1}; PD2: 64,900 plants ha^{-1}; PD3: 75,200 plants ha^{-1}). There was increase in yield with the irrigation (1.05–80.00%) as compared to the rainfed crop. Results showed that decreasing irrigation rates resulted in a decrease in yield, crop water use efficiency (WUE), and irrigation water use efficiency (IWUE). Planting density had significant effects on yield, WUE, and IWUE which differed in both years. Increasing planting density gradually increased yield, WUE, and IWUE. For the pooled data, irrigation rate, planting density and their interaction was significant ($P < 0.05$). The highest two-year average yield, WUE, and IWUE were found for FIT-PD3 (14,612 kg ha^{-1}), rainfed-PD2 (2.764 kg m^{-3}), and 60% FIT-PD3 (2.356 kg m^{-3}), respectively. The results revealed that irrigation is necessary for maize cultivation because rainfall is insufficient to meet the crop water needs. In addition, if water becomes a limiting factor, 80% FIT-PD3 with average yield loss of 15% would be the best agronomic practices for growing maize with a sprinkler irrigation system in a temperate climate of Serbia.

Keywords: *Deficit irrigation, Sprinkler irrigation, Irrigation water use efficiency, Zea mays L.*

Introduction

Global climate changes, which are mostly expressed in a lack of precipitation, are considered to be one of the most important limiting factors for agricultural production. On the territory of the Republic of Serbia, precipitation is often insufficient and unfavorably distributed, which leads to a decrease in yields (Kresovic et al., 2014). Also, worldwide, the reserves of available water for irrigation are decreasing, while the demand for food is increasing year by year, which indicates that food production in the future will take place under more or less deficit water conditions, i.e. more rational management of the above-mentioned resources is required (Ferreres, 2003). The poor temporal and spatial distribution of rainfall, combined with global climate change, has rouse scientists to develop strategies to maximize agricultural production (Lu et al., 2017) and improve WUE. To overcome the consequences of drought, irrigation is the most important agricultural measure. It is estimated that about 70% of total freshwater is used for agriculture (Evans and Sadler, 2008), and water use in agriculture is considered inefficient (Hsiao et al., 2007), so various measures need to be taken to help conserve water and use it rationally (FAO, 2002).

The application of reduced irrigation is recognized as one of the ways to reduce the negative effects of drought on the one hand, and to save water in agricultural production on the other.

In reduced irrigation, crops are exposed to water shortage during the entire growing season or scheduled in certain phases, which leads to a decrease in yield, but also to water savings, i.e. more efficient water use. Grain yield of maize decreases under water stress (Anjos et al., 2022), especially in the period between flowering and pollination, indicating physiological disorders of the plants (Chilundo et al., 2017).

Among the factors that increase yield, the plant population is of the great importance, because if the plant population is dense the grain size remains small and lodging occurs. Maize grain yield is different in different plant populations and limited irrigation modes (Jia et al., 2018). It has been reported that new maize varieties are not able to tiller considerably and usually produce only one ear/plant at low plant population and high plant density, which increases inter-plant competition, which negatively affects the overall yield due to apical dominance, which promotes infertility and eventually reduces the number of ears/plant and grain/ear (Abuzar et al., 2011). According to Sangoi (2001), there is no specific optimum population density for all weather conditions as it varies with environmental and controlled conditions.

Jin et al. (2020) reported that although maize yield increased linearly with evapotranspiration (ETa), the variations in yield and WUE were not synchronous when ETa was changed. They concluded that low-level irrigation should be applied in regions with severe water shortage to achieve maximum WUE.

The objective of the present study was to investigate the effects of different irrigation rates and planting densities on yield and WUE of maize in a temperate climate area under a sprinkler irrigation.

Materials and methods

Site description and climate. Field experiments were conducted at the experimental site of the Maize Research Institute "Zemun Polje", Serbia (44°52' lat. N; 20°20' long. E, 81 m a.s.l.) in 2018 and 2019. The soil in the experimental site was Calcareous Chernozem, silty loam texture.

The climate is mild-moderate/mesothermal ("Cfwbx" according to the Köppen climate classification), summers are dry and hot, and precipitation is irregularly distributed throughout the year. Annual precipitation (638 mm on average, considering the 20-year long-term period) is mainly concentrated in the autumn and spring months. The mean air temperature is 11.9°C. Meteorological data were collected from a weather station located in the immediate vicinity of the plots.

Experimental details. Two factors were studied: (1) irrigation volume with five levels (full irrigated treatment – FIT; 80% FIT, 60% FIT; 40% FIT; and rainfed) and (2) planting densities with three target seeding rates (PD1: 54,900 plants ha^{-1}; PD2: 64,900 plants ha^{-1}; PD3: 75,200 plants ha^{-1}). For the FIT, the irrigation schedule was set according to the seasonal actual crop evapotranspiration (ETa) using a general water balance equation (Kresović et al., 2016). The experimental design was a randomized complete block design with four replicates. Each replicate was eight rows wide with a row spacing of 0.70 m and 8 m long.

Maize hybrid ZP 677 of the Maize Research Institute "Zemun Polje", FAO Maturity Group 600, was sown by hand in 2018 and, 2019 on 16 and 14 April, respectively. In 2018 and 2019, maize was harvested manually on September 27 and 12, respectively.

In the FIT, irrigation was practiced when soil water content in the effective root zone depth (to 0.60 m) reached approximately 50% of total available water (the difference in soil water storage between field capacity and wilting point). For the 80% FIT, 60% FIT, and 40% FIT, irrigation frequency was the same as that of the FIT and the irrigation rate was proportional to that of the FIT. Irrigation was carried out by the hand-move sprinkler system. The rates of

water supplied by irrigation were accurately measured with a water meter for each treatment of the study.

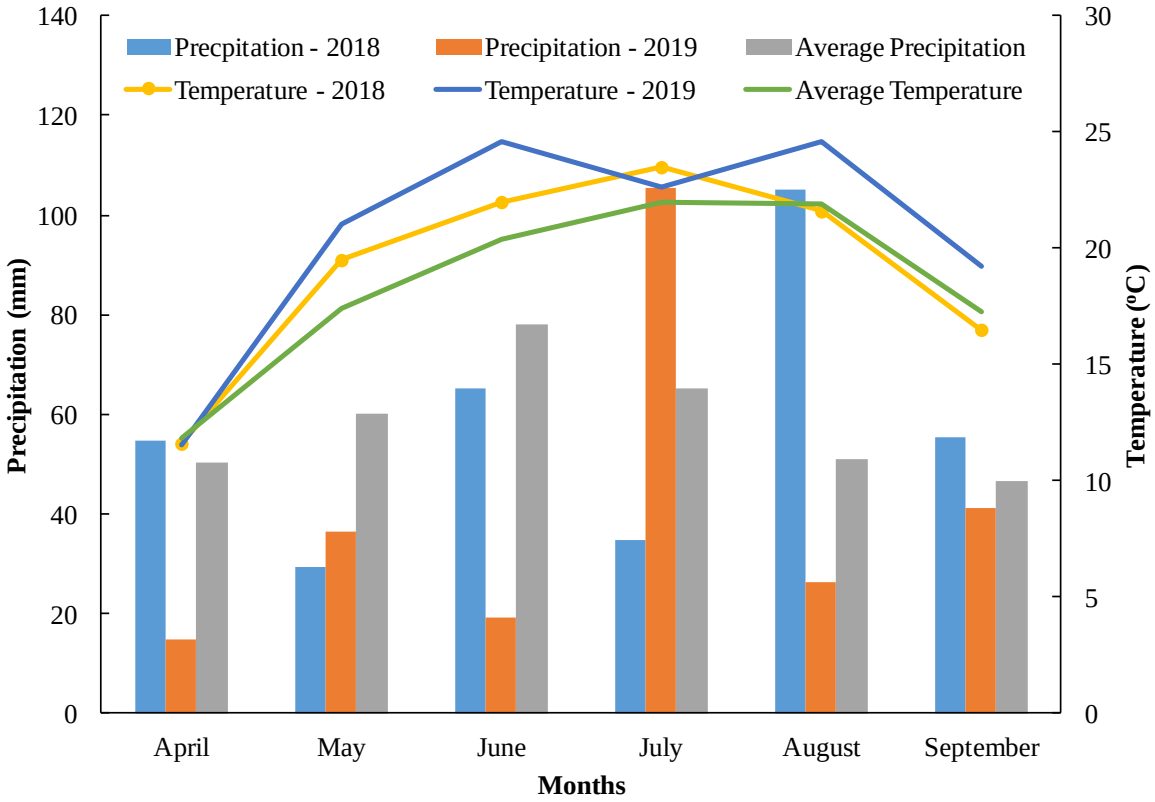

Fig. 1. Mean air temperature and monthly precipitation in the 2018–2019 crop seasons and in the long term period (1996–2017) in Zemun Polje.

Measurements. Soil water content was measured in all experimental plots at 10 cm intervals at sowing, decadally during the season, and at harvest throughout the two-year study period using the gravimetric method. It is based on conventional oven-dry mass and multiplied by bulk density to convert to volumetric water. Soil bulk density was also measured at each depth at the beginning of the growing season. At the end of physiological maturity of maize, a plot area of 11,2 m^2 (the two middle rows of each subplot, 8 m long) was harvested manually and the grain mass was recorded. Maize grain yield was adjusted to 14% wet basis.
The crop WUE (kg m^{-3}), and IWUE (kg m^{-3}) were calculated to evaluate the efficiency and productivity of maize at different irrigation rates and planting densities for each subplot. Crop WUE was calculated as the ratio between grain yield (kg m^{-2}) and seasonal water use measured as ETa (mm) (Kresović et al., 2016). Irrigation water use efficiency (IWUE) was calculated as $(Y_i - Y_o)/I$) for all the plots, where Y_i is the irrigated treatment yield (kg ha^{-1}), Y_o is rainfed yield (kg ha^{-1}), and I is the irrigation water applied (m^3 ha^{-1}) (Kresović et al., 2016). Data were statistically analysed according to the RCBD split plot arrangement. Analysis of variance and LSD were used according to Steel and Torrie (1980).

Results and discussion

Weather conditions. Weather conditions varied markedly among the two growing seasons (Fig. 1). The seasonal rainfall (April–September) was much lower in 2019 than in 2018. In 2018, and 2019 approximately 57% and 71% respectively of seasonal rainfall occurred during the later growth period (July–September). The total precipitation during the growing season was 345 mm in 2018 and 243 mm in 2019, which was less than the 20-yr average of 351mm at the experimental site. The average air temperatures for May, June, August, and September in 2019 were higher than in 2018. The monthly air temperature were greater comparable to the long-term averages.

The total irrigation rates for the FIT, 80% FIT, 60% FIT and 40% FIT were 239, 163, 90 and 25 mm, respectively, in 2018; 291, 188, 94, and 47 mm, respectively, in 2019 (data not shown).

Table 1. Effects of different irrigation rates and planting density on maize yield, water use efficiency (WUE), and irrigation water use efficiency (IWUE) in 2018 and 2019.

Treatments		Yield (kg ha^{-1})		WUE (kg m^{-3})		IWUE (kg m^{-3})	
Irrigation rates	Planting density (plants ha^{-1})	2018	2019	2018	2019	2018	2019
Rainfed	PD1	11.456 hi	7.170 hi	3.050 ab	2.333 bc	–	–
	PD2	11.597 h	7.497 gh	3.085 a	2.442 ab	–	–
	PD3	10.965 i	6.429 j	2.917 bc	2.090 ef	–	–
40% FIT	PD1	11.477 c	7.864 g	2.866 cd	2.105 def	0.650 d	1.462 bc
	PD2	11.922 gh	7.508 gh	2.973 abc	1.977 f	1.500 abc	0.019 d
	PD3	10.985 i	6.788 ij	2.740 de	1.783 g	1.410 abcd	0.742 cd
60% FIT	PD1	12.442 fg	8.596 f	2.735 def	2.158 de	1.085 cd	1.517 bc
	PD2	12.958 ef	9.477 de	2.855 cde	2.380 abc	1.513 abc	2.105 b
	PD3	12.430 fg	9.336 e	2.738 def	2.343 bc	1.627 abc	3.085 a
80% FIT	PD1	13.430 e	9.915 de	2.603 f	2.245 cde	1.205 bcd	1.457 bc
	PD2	14.093 d	10.015 cd	2.733 def	2.270 bcd	1.530 abc	1.343 bc
	PD3	14.260 cd	10.580 c	2.762 de	2.395 abc	2.020 ab	2.210 ab
FIT	PD1	14.828 c	11.818 b	2.723 ef	2.277 bcd	1.405 abcd	1.598 bc
	PD2	15.485 b	12.622 a	2.842 cde	2.435 ab	1.628 abc	1.765 b
	PD3	16.125 a	13.098 a	2.958 abc	2.525 a	2.160 a	2.292 ab
Source of variation							
Irrigation (I)		**	**	**	**	*	**
Planting density (PD)		**	**	**	ns	**	**
I * PD		**	**	**	**	ns	**

Means within a columns followed by the same letter are not significantly different at $P < 0.05$ according to LSD tests. * significant at $P < 0.05$. ** significant at $P < 0.01$. ns, not significant.

In both years, the effects of irrigation rates on ETa were significant, and ETa decreased with decreasing irrigation rate. In average, compared to FIT ETa in 80% FIT, 60% FIT, 40% FIT, and rainfed treatment was reduced by 46 mm, 97 mm, 145 mm, and 181 mm, respectively (data not shown).

Grain yield, WUE and IWUE. Grain yield was affected by water regime, and planting density (Table 1). Averaged across both year, in FIT, compared to the 80% FIT, 60% FIT, 40% FIT, and rainfed treatment, grain yield was reduced by 1.95 t ha^{-1}, 3.12 t ha^{-1}, 4.57 t ha^{-1}, and 4.81 t ha^{-1}, respectively ($P < 0.05$).

The grain yield showed different responses to planting density in both years. Increasing planting density had a significant effect in increasing yield, and generally resulted in numerically greater yields in both years. Namely, grain yield increased from 54900 to 64900 plants ha^{-1} but decreased with further increase in planting density. In average for both year, maximum grain yield ha^{-1} (14.611 t ha^{-1}) was for plant population of 75200 plants ha^{-1} interacting with FIT, while the least grain yield (8.998 t ha^{-1}) was recorded in rainfed crops at high planting density (75200 plant ha^{-1}).

Some researchers (Al-Kaisi and Yin, 2003; Sani et al., 2008; Kresovic et al., 2014) have reported that full irrigation significantly increased maize grain yield in plant population of 66000–69000 plants ha^{-1} compared with rainfed crop in arid and semiarid regions due to increased soil water storage. The above researchers concluded that under conditions of reduced irrigation, it is necessary to reduce the number of plants per unit area.

The effects of irrigation rates on WUE were significant for both years (Table 1). In 2018 the highest average values (3.017 kg m^{-3}) of WUE were recorded in the rainfed treatment, while the lowest values WUE were recorded in 80% FIT (2.699 kg m^{-3}). In this study, it was found that maize used water most efficiently under moderate densities (PD2), while at high (PD3) and low (PD1) density, WUE was lower. The analysis of the influence of water regime and planting density showed that maize in PD2 used water most efficiently when it was supplied with rainwater only. On the other hand, the lowest value was recorded in 80% FIT with the lowest planting density (PD1). The obtained results show that maize makes good use of both rainwater and irrigation water.

In contrast to 2018, significantly lower WUE values were obtained in 2019, and maize used water most efficiently in the irrigated treatments, which was associated with higher evaporative demand and higher temperature in 2019 (Table 1). Greater WUE in 2018 than in 2019 was the result of average grain yield but lower water use by maize that year. The highest WUE value was for FIT (2.412 kg m^{-3}), and the lowest WUE was for 40% FIT, which applied only 40% of the required rate of water (1.955 kg m^{-3}). WUE values were strongly dependent on the irrigation regime in 2019, as confirmed by the fact that they decreased with increasing water reduction. Density did not significantly affect the WUE value, but it is noted that the value of WUE was highest in PD2 (2.3 kg m^{-3}), as was the case in 2018. The maize used water most efficiently in FIT at the highest planting density (75200 plants ha^{-1}), while the lowest WUE value was recorded in the 40% FIT, also at the highest planting density (PD3). In our study, irrigation and density had significant ($P < 0.05$) interactions with yield and WUE, which is consistent with the report of Guo et al., (2021).

Jia et al. (2018) reported that in a normal year, grain yield, and WUE significantly increased as planting density increased from low to moderate, while this was not observed in a dry year.

There were no significant differences in values of IWUE between irrigation treatments, with the exception of the 40% irrigation FIT, which applied three irrigations totaling 25 mm at the beginning of vegetation in 2018 (Table 1). In 2018, low values of IWUE (1.187 to 1.731 kg m^{-3}) were observed, which was related to relatively favorable climatic conditions, especially the precipitation regime. Maize used water best at the highest planting density. There were no significant differences in IWUE values between PD3 and PD2, but statistically significant differences were found at PD1. The interaction of water regime and planting density did not affect the achievement of significant differences in irrigation water use efficiency by maize this year.

Irrigation water use efficiency was affected by water regime, and planting density, but no interactions (Table 1). Averaged across treatments, in the much warmer year of 2019, the highest value of 2.236 (kg m^{-3}) IWUE was obtained in 60% FIT and was statistically significantly higher compared to the other irrigation treatments (Table 1). There are no

significant differences between FIT and 80% FIT while the lowest value of IWUE (0.798 kg m^{-3}) was obtained in 40% FIT with the lowest water availability (40%).
Compared to 2018, significantly more irrigation interventions were made in 2019. In 40% FIT, five irrigations were carried out with a total water volume of 47 mm. All irrigations took place in the vegetative phase of maize growth and development, while no interventions were made in the phase of fertilization and grain filling. Due to this irrigation regime, the expected irrigation effect did not occur in a warm and dry year (2019). Also in this experimental year, the maize with the highest planting density (PD3) made the best use of the water supplied by irrigation on average. No statistically significant differences were found between PD1 and PD2. The highest single value (3,085 kg m^{-3}) of IWUE was obtained in 60% FIT at the highest planting density (PD3), while the lowest irrigation effect (IWUE) occurred in FIT, also at PD3. In 2019, irrigation and density had significant interactions ($P < 0.05$) with yield and IWUE.
Djaman and Irmak (2012) found that maize IWUE decreased with irrigation rate in the first year of the experiment conducted in a silt loam soil, while that showed an opposite trend in the second year. Irmak (2015) reported maize IWUE between 1.75 and 5.9 kg m^{-3} during the period of 2006–2010 and reported significant interannual variation.

Conclusions

The results prove that, under the conditions of this study, planting density and water stress affected grain yield and water use efficiency, making irrigation strongly required for maize cultivation. In fact, under rainfed conditions, maize gives low and highly variable yield. In the 2018 and 2019 maize growing seasons, the yield increased as irrigation rate increased. Yield first increased and then decreased with planting density. In both years, the highest yield (16.125 t ha^{-1}, 2018 and 13.098 t ha^{-1}, 2019 in) was recorded in FIT at higher planting density (75200 plants ha^{-1}), while the lowest yield (10.965 t ha^{-1}, 2018 and 6.429 t ha^{-1}, 2019) was recorded in rainfed crops at higher planting density. In the wet year (2018), the highest WUE (3,085 kg m^{-3}) was obtained under rainfed crops at medium planting density (64900 plants ha^{-1}), and the lowest WUE (2,603 kg m^{-3}) was obtained at 80% FIT at low planting density (54900 plants ha^{-1}). In the dry year (2019), the highest WUE (2.525 kg m^{-3}) was obtained at 60% FIT and high planting density, and the lowest WUE (1.783 kg m^{-3}) was obtained at 40% FIT and high planting density. In both years, the treatment at high planting density resulted in the highest IWUE at 80% FIT (2.020 kg m^{-3}, 2018) and at 60% FIT (3.085 kg m^{-3}, 2019), while the lowest IWUE was recorded at 40% FIT at low planting density (0.650 kg m^{-3}, 2018) and at 40% FIT at medium planting density (0.190 kg m^{-3}, 2019). In this study, planting maize with a greater planting density no provide greater yield stability under water-limited conditions while also maintaining yield potential when soil moisture is sufficient. Overall, our study showed that an irrigation treatment with 20% water saving (80% FIT) at a planting density of 75,200 plants ha^{-1} and 15% average yield loss under sprinkler irrigation can ensure a satisfactory maize yield and an increase in WUE in the temperate climate of Serbia, and in the countries of the region, provided that this practice is not prevented by economic limitations.

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STRESS RESISTANCE INDICATORS AS THE TOOL FOR SELECTING DROUGHT-TOLERANT WHEAT GENOTYPES

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Abstract

Climate change and the resulting increase in the frequency and severity of drought can have significant impacts on plant production. The use of drought-tolerant crop varieties can significantly improve plant production under drought conditions. Therefore, the goal of this research was to evaluate the drought tolerance of different wheat genotypes using stress resistance indicators. An experiment was conducted with sixteen wheat genotypes in Novi Bečej (Vojvodina) in different growing seasons. The spike weight was used as a phenotypic marker of the effect of drought stress on the plant. Selection based on resistance indicators such as stress tolerance index (STI), mean productivity (MP), and geometric mean productivity (GMP) favors the selection of genotypes Dunavka, Skopjanka, and Fundulea 4, which were characterized by the highest average values of spike weight, especially in favorable growing conditions. However, genotype Fundulea 4 is characterized by the highest stress susceptibility index (SSI) and the lowest yield stability index (YSI), which makes this genotype undesirable for growing under drought conditions. The parameters SSI and YSI favor the selection of the genotype Pitoma, which showed the highest value of spike weight in drought conditions as well as the least reduction in value caused by stress. Genotypes Pitoma, Dunavka, and Skopjanka, characterized by a high yield index (YI), are suitable for cultivation in drought conditions, where they achieved above-average trait values. The most suitable stress resistance indicators for selecting drought-tolerant wheat genotypes are SSI, YSI, and YI.

Key words: *drought stress, stress susceptibility index, stress tolerance index, wheat.*

Introduction

The greatest threats to the availability of food in the present and future are population growth and climate change. Drought is one of the most significant consequences of climate change and can which have a severe impact on agricultural production, especially in arid and semi-arid areas (Cheng et al., 2021). Wheat is the primary food source for around 40% of the world's population and a main source of daily protein and calories for about 2.5 billion people in developing countries (Braun et al., 2010). According to predictions made by Alexandratos et al. (2012), the demand for wheat in developing countries could rise by as much as 60% by 2050. Some of the causes influencing this projected rise in demand include urbanisation, rapid population expansion, and changes in dietary patterns.

According to Darvanto et al. (2016) drought conditions can reduce wheat productivity by 50 to 90% of the crop potential. Drought affects wheat at all growth stages, but it is more severe during the flowering and grain-filling stages, leading to significant yield losses (Sareen et al.,

2023). A worldwide effort to reduce the severity of droughts involves the development of drought-tolerant cultivars. However, progress has been significantly delayed by the complex structure of the drought-tolerance characteristics, which is controlled by a number of genes and greatly influenced by the environment (Pandey et al., 2022). Accordingly, identifying genotypes with tolerant genes is a difficult process (Anwaar et al., 2020). One of the approaches in the identification of drought-tolerant genotypes is the calculation of stress resistance indicators, which compare the value of the yield achieved in drought conditions with the yield in normal conditions (Anwaar et al., 2020, Aksić et al., 2020).
The aim of this study is to identify the wheat genotypes that exhibit the highest drought tolerance in the agro-ecological conditions of the semi-arid climate. Also, the goal is to select the best drought resistance indicator.

Material and Methods

A field experiment was established in Novi Bečej (Banat, AP Vojvodina, Serbia), which includes 16 wheat genotypes (Dukat, Dunavka, Fundulea 4, Iskra, Jedina, Jugoslavija, Kavkaz, Mačvanka 1, Marija, NS 58-04, Pitoma, Poljana, Skopjanka, Tamiš, Vali PKA-7114, and Zvezda). The analysed genotypes were sown according to the randomized block system in three replications with an inter-row spacing of 12 cm, where the size of the basic plot was 2 m^2. The soil type was humogley, which is characterised by a high content of clay. The usual agrotechnics for wheat production were implemented, where monoammonium phosphate (MAP) was used as the basic fertilizer in the amount of 250 kg ha^{-1}, while urea was used in the amount of 250 kg ha^{-1} for crop feeding. In both vegetation seasons, the harvest was performed at the optimal time (the first week of July in 2015/2016 and the last week of June in 2016/2017), when the grain moisture was below 14%. The spike weight was measured in 30 plants for each analysed genotype.
The stress resistance indicators were calculated based on the value of the spike weight under stress conditions (Yd), which characterized the 2016/2017 growing season, and the value of the spike weight in conditions favorable for plant development (Yp), which characterized the 2015/2016 season. The following stress resistance indicators are expressed in this paper:

Stress susceptibility index – SSI (Fisher and Maurer, 1978):

$$SSI = 1–(Yd/Yp)/1−(\bar{Y}d/\bar{Y}p)$$

Mean productivity – MP (Rosielle and Hamblin, 1981)

$$MP = (Yd + Yp)/2$$

Stress tolerance index – STI (Fernandez, 1992)

$$STI = (Yd + Yp)/\bar{Y}^2p$$

Geometric mean productivity – GMP (Fernandez, 1992):

$$GMP = \sqrt{Yd \times Yp}$$

Yield stability index – YSI (Bouslama and Schapaugh Jr, 1984):

$$YSI = Yd/Yp$$

Yield index – YI (Gavuzzi et al., 1997):

$$YI = Yd/\bar{Y}d$$

A cluster analysis was applied according to Ward's method for grain yield per ear and indicators of stress tolerance using the programme IBM SPSS Statistics, Trial Version 22.0 (https://www.ibm.com/). Distances between clusters are expressed as squared Euclidean

distances, and the significance of distances was tested by the t-test. The number of cluster groups was identified using a dendrogram, after which a K-means analysis was performed with a predetermined number of cluster groups. After the analyses were carried out, the cluster groups were ranked according to the mean values of the analysed parameters.
During the experiment, large differences were noted regarding the amount of precipitation between the analysed growing seasons. Twice as much precipitation was recorded in the 2015/2016 growing season) compared to 2016/2017 (612 or 300 mm). Because of this, the 2016/2017 season is regarded as dry. In 2015/2016, during the growing season, average monthly temperatures were within the multi-year average, and the amount of precipitation was significantly higher than the multi-year average in almost all months. Heavy rainfall in June (164.0 mm) extended the grain filling period. On the other hand, 2016/2017 growing season was characterised by significantly higher temperatures than the multi-year average and a pronounced deficit of precipitation, especially in the grain-filling phenophase, which caused an earlier harvest of crops in the mentioned season (http://www.hidmet.gov.rs/).

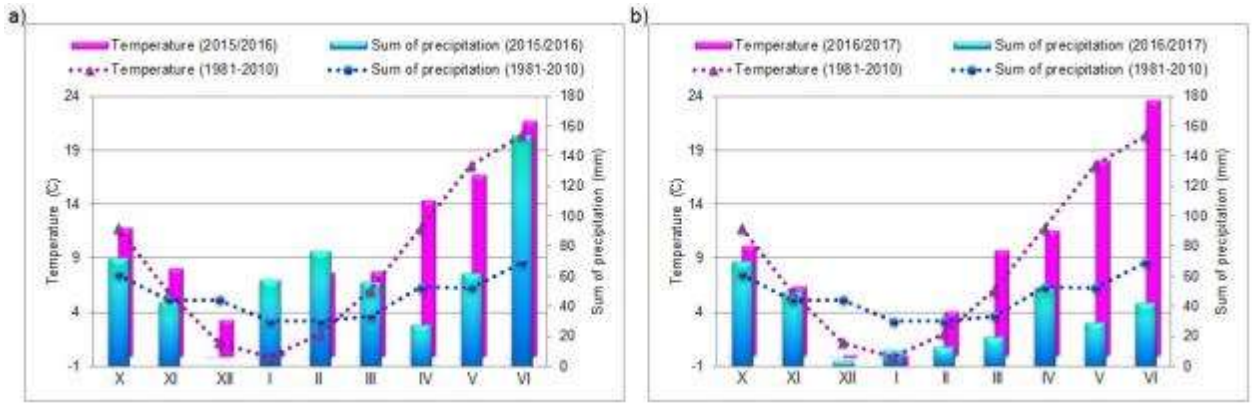

Figure 1. Mean monthly temperatures and sum of precipitation in Novi Bečej locality in 2015/2016 (a) and 2016/2017 (b) vegetation season

Results and Discussion

In this study, it was established that the drought stress conditions in the 2016/2017 vegetation season affected the reduction of the spike weight value in all analysed wheat genotypes, compared to the values achieved in the favourable 2015/2016 vegetation season (Figure 2, a).
The greatest decrease in the spike weight was recorded in the Fundulea 4 genotype (58.0%), while the smallest decrease was observed in the Pitoma genotype (20.2%) (Figure 2, 2). In accordance with the above, spike weight is considered a good phenotypic marker of the impact of drought stress on wheat. Wasaya et al. (2021) also found a decrease in the wheat spike weight under drought stress conditions, which they explained by a reduction in photosynthetic parameters.

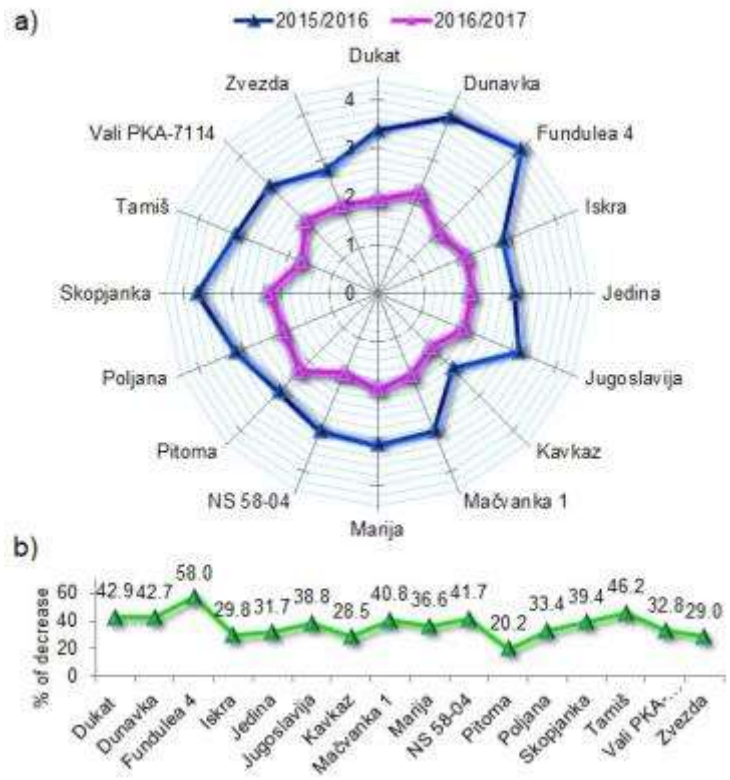

Figure 2. Radar graph of spike weight in examined wheat genotypes grown in drought stress and favorable conditions (a) and decrease (%) in spike weight due to drought stress (b)

The stress resistance indices were calculated, according to the values of spike weight of the analyzed wheat genotypes (Table 1). Indicators of resistance such as mean productivity (MP), stress tolerance index (STI) geometric mean productivity (GMP) take into account the spike weight achieved both in drought and in favorable environmental conditions. Golbashy et al. (2010) noted that STI and GMP are effective in identifying genotypes with high grain yield values in both, non-stress and stress conditions. According to the mentioned stress resistance indicators, the first-ranked cluster group includes the genotypes Dunavka, Skopjanka, and Fundulea 4, which were characterized by the highest average values of spike weight, especially in favorable growing conditions (Table 1, Figure 1,a). Similarly, Aksić et al. (2020) observed that STI, MP, and GMP indicators are positively correlated with grain yield achieved under irrigated conditions. Among the mentioned genotypes, Dunavka and Skopjanka are distinguished by the highest value of the yield index (YI) indicator (1.15), which relates the spike weight of certain genotypes in drought conditions to the average spike weight of all genotypes in drought stress conditions. Although genotype Fundulea 4 has a high value of MP (2.99), STI (0.59), and GMP (2.73), it is characterized by the highest stress susceptibility index (SSI) (1.53), the lowest yield stability index (YSI) (0.42), as well as a low value of the yield index (YI) (0.90). This genotype showed a very high value of spike weight in non-stress conditions, but a low value in drought stress conditions. According to Awnaar et al. (2020), such genotypes are not suitable for growing in wider areas, due to large yield losses under stress conditions. Stress resistance parameters SSI, YSI, and YI favor the selection of the genotype Pitoma, which showed the highest value of spike weight under drought stress conditions, as well as a smaller decrease in value under the influence of drought. Therefore, this genotype is considered the most drought-tolerant. Also, according to the SSI, YSI and YI parameters, the Kavkaz genotype is classified among drought-tolerant genotypes, exhibiting a small decrease in the spike weight under drought. However, according to the MP, STI, GMP and YI indicators, it is classified in the lowest ranked cluster group (4

and 5), characterized by lowest value of spike weight, which makes it an undesirable genotype in breeding programs and for cultivation in semi-arid climate conditions.

Table 1. AMMI analysis of variance for spike weight in 27 wheat genotypes grown in different agro-ecological conditions

Genotypes	SSI	MP	STI	GMP	YSI	YI
Dukat	1.13 (2)	2.66 (2)	0.53 (2)	2.55 (3)	0.57 (4)	0.98 (3)
Dunavka	1.12 (2)	3.12 (1)	0.62 (1)	3.00 (1)	0.57 (4)	1.15 (1)
Fundulea 4	1.53 (1)	2.99 (1)	0.59 (1)	2.73 (2)	0.42 (5)	0.90 (4)
Iskra	0.78 (4)	2.40 (3)	0.48 (3)	2.36 (4)	0.70 (2)	1.01 (3)
Jedina	0.83 (4)	2.39 (3)	0.47 (3)	2.35 (4)	0.68 (2)	0.99 (3)
Jugoslavija	1.02 (3)	2.56 (2)	0.51 (2)	2.48 (3)	0.61 (3)	0.99 (3)
Kavkaz	0.75 (4)	1.90 (4)	0.38 (4)	1.87 (5)	0.71 (2)	0.80 (5)
Mačvanka 1	1.07 (2)	2.48 (3)	0.49 (3)	2.39 (4)	0.59 (3)	0.94 (4)
Marija	0.96 (3)	2.57 (2)	0.51 (2)	2.50 (3)	0.63 (3)	1.01 (3)
NS 58-04	1.10 (2)	2.45 (3)	0.49 (3)	2.36 (4)	0.58 (4)	0.92 (4)
Pitoma	0.53 (5)	2.58 (2)	0.51 (2)	2.56 (3)	0.80 (1)	1.16 (1)
Poljana	0.88 (4)	2.64 (2)	0.52 (2)	2.59 (3)	0.67 (2)	1.07 (2)
Skopjanka	1.04 (3)	3.00 (1)	0.60 (1)	2.90 (1)	0.61 (3)	1.15 (1)
Tamiš	1.22 (2)	2.43 (3)	0.48 (3)	2.32 (4)	0.54 (4)	0.86 (4)
Vali PKA-7114	0.86 (4)	2.63 (2)	0.52 (2)	2.57 (3)	0.67 (2)	1.07 (2)
Zvezda	0.76 (4)	2.36 (3)	0.47 (3)	2.33 (4)	0.71 (2)	1.00 (3)

Note: The numbers in parentheses represent the rank of the cluster group for each resistance indicator, where number 1 is the cluster group with the highest, and number 5 with the lowest mean value of the resistance indicator.

Conclusion

Drought stress affected the reduction of spike weight in all analyzed wheat genotypes, which makes this trait a good phenotypic marker of the impact of drought stress on wheat. Therefore, stress resistance indicators were calculated based on the value of this trait. High values of the MP, STI, and GMP resistance indicators favor the selection of genotypes Dunavka, Fundulea 4, and Skopjanka, characterized by a high average spike weight, especially in non-stressful environmental conditions. Genotype Pitoma characterized by high values of MP, STI, GMP, YSI, YI and low value of SSI is rated as highly drought-tolerant genotype. Also, Dunavka, and Skopjanka are characterised by high YI values and are considered drought-tolerant genotypes. Genotype Fundulea 4, characterised by high values of MP, STI, and GMP and low values of SSI, YSI, and YI, are preferred for cultivation in non-stressful, but not in stressful environmental conditions. The most suitable stress resistance indicators for selecting drought-tolerant wheat genotypes are SSI, YSI, and YI.

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ORGANIC FARMING: EXPLORING THE PROS AND CONS FOR SUSTAINABLE AGRICULTURE

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Abstract

Organic agriculture is a production system that has been the focus of scientific research for several decades now. Research was mostly related to the technology of the production system itself, while the economic and social aspects only recently received attention. Although, as a system of sustainable agricultural production, organic agriculture has its significant advantages, it is necessary to investigate and point out the current shortcomings and problems accompanying this production system. This research paper examines the pros and cons of organic farming as a sustainable agricultural practice. The study analyzes the key advantages and disadvantages associated with organic farming methods, considering environmental and social aspects but with special focus on economic advantages and disadvantages of this production. Through a comprehensive review of existing literature and empirical evidence, the paper highlights the potential benefits of organic farming, such as reduced pesticide use, improved soil health, and enhanced biodiversity. It also addresses the challenges and limitations of organic farming, including higher production costs, lower yields in certain cases, and limited scalability. The findings aim to provide a balanced understanding of the strengths and weaknesses of organic farming, enabling policymakers, farmers, and consumers to make informed decisions regarding sustainable agriculture practices.

Keywords: *organic farming, sustainable agriculture, pros and cons, sustainable development.*

Introduction

Sustainable development and sustainable production are concepts that have gained more importance in the past decades. The three main pillars of sustainable development are: economic growth, environmental protection and social equality, which shows that it is a broader concept, i.e. a holistic approach that implies the development of society as a whole as opposed to exclusively economic growth, which is still an important determinant when evaluating growth and to some extent development of an economic segment. One of the most frequently used definitions of sustainable development, which was published in 1987 as part of the so-called The Brundtland report established by the World Commission on Environment and Development reads: "sustainable development is development that meets the needs of the present generation without compromising the ability of future generations to meet their own needs" (WCED, 1987). Sustainable development implies that man preserves nature on a sustainable basis and uses it as much as it allows for its reproduction, because otherwise he endangers his own future, as well as the future of future generations.

Like any other activity, agriculture must find its place in society and its development in a sustainable way (Badgley et al., 2007). To achieve this, agricultural production must follow the concept of sustainable development, which means that it must contribute to the growth and development of society as a whole, without further disrupting biodiversity and natural balance. As part of the widely proclaimed concept of sustainable development, sustainable

agriculture unites ecological and economic elements of production, care for human health and takes into account the diversity of agriculture and social community (Tomaš Simin M., 2019). Sustainable agricultural systems are those that contribute to the long-term well-being of society by ensuring sufficiently stable and safe food production, but also other products of plant and animal origin for other technical purposes, while preserving the quality-of-life environment and natural resources on which production is based. At the same time, agriculture must be economically efficient, that is, profitable, which contributes to the improvement of the quality of life of the individual and the wider community (Radojević et al., 2020; Lotter D., 2003; Pimentel et al, 2005).

With the above in mind, among sustainable (or alternative as they are often called) agricultural production systems, organic agriculture is often mentioned (Lampkin N., 2003). The system of organic agricultural production is often linked or even identified with traditional agriculture and its method of production. It is considered that organic production represents a "return to the old" and a strengthening of "production as it was in the time of our grandparents" (Tomaš Simin M., 2019). However, the truth is that modern organic production in no way avoids the application of modern scientific achievements (it even encourages them and requires more investment in the research of production technologies) and is characterized by the use of new production technologies that are in accordance with its basic principles[1].

Organic agriculture has gained increasing popularity in recent years due to its focus on sustainability, environmental friendliness, and healthier produce. From an economic perspective, this farming method presents both advantages and challenges. This paper explores the economic pros and cons of organic agriculture, shedding light on its potential benefits and limitations.

Material and Methods

In accordance with the set goal of the research, an appropriate methodology was used. The analysis method was used when researching the advantages and disadvantages of organic agriculture in various scientific and professional papers in order to use the synthesis method when drawing conclusions. The method of comparison was used to determine different results in individual papers. Reference databases of scientific and professional publications such as defended doctoral dissertations were used as a source.

Results and Discussion

Organic agriculture represents one of the sustainable systems of agricultural production that has been developing in recent decades in all countries of the world. It represents a production system that is in line with the basic principles of sustainable development, and some of the main advantages of this system found in the literature are:

Premium Prices - Organic products often command higher prices in the market. Consumers are willing to pay a premium for organic produce due to its perceived health benefits, environmental friendliness, and absence of synthetic chemicals. This price premium can lead to increased profitability for organic farmers. The premium prices of organic products refer to the higher retail prices that organic produce often commands compared to conventionally grown counterparts. Consumers are willing to pay more for organic products due to various factors, including perceived health benefits, environmental concerns, and the absence of synthetic chemicals (Radojević et al, 2020, Tomaš Simin et al. 2019).

[1] Šulc also mentioned that "...nature can, however, be mastered by knowledge and human ability." (Šulc, 1985).

Several scientific studies have explored the price differences between organic and conventional products. The researchers found that, on average, organic food prices were approximately 23-30% higher than their conventionally produced counterparts (Li and Kallas, 2021, Vermeir and Verbeke, 2006). The study indicated that this price premium was influenced by several factors, including production costs, consumer willingness to pay, and market demand.

Another research article published in the journal Food Quality and Preference in 2015 analyzed consumer perceptions of organic food and their willingness to pay a premium. The study concluded that consumers were willing to pay more for organic products due to their perceived health benefits, better taste, and positive environmental impact (Hyun-Joo and Zee-Sun, 2015, Hughner et al., 2007). The higher prices of organic products can be attributed to several factors:

production costs, limited supply, consumer perceptions and market segmentation. Organic products cater to a niche market of health-conscious and environmentally conscious consumers. These consumers are often willing to pay more for products that align with their values and lifestyle choices.

Reduced Input Costs - Organic farmers rely on natural and traditional methods to enhance soil fertility and combat pests. Though the initial transition to organic farming might require additional investment, the long-term savings on costly chemical inputs can offset these expenses. Reduced input costs are one of the economic advantages of organic agriculture. Organic farming practices focus on minimizing the use of synthetic inputs such as chemical fertilizers, pesticides, and genetically modified organisms (GMOs). Instead, organic farmers rely on natural methods and traditional techniques to maintain soil fertility, control pests, and enhance plant growth. This approach can lead to several benefits in terms of cost savings for farmers.

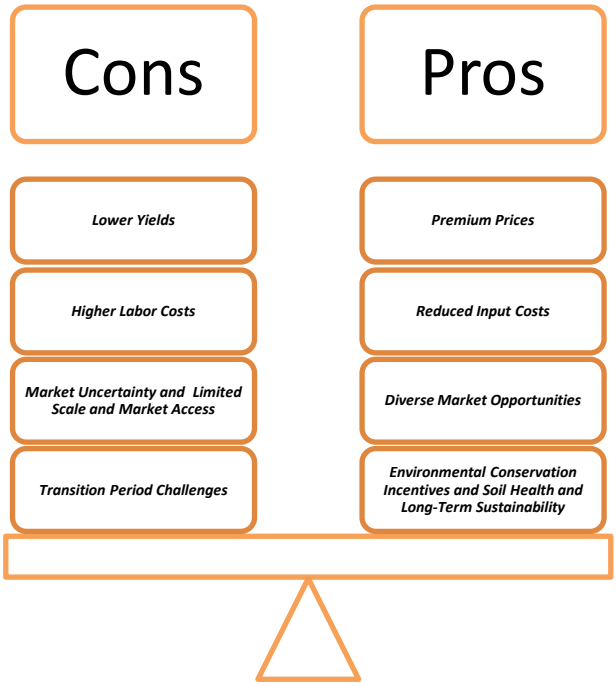
*Elimination of Chemical Inputs***.** Conventional agriculture often requires substantial expenses on chemical fertilizers and pesticides. In organic farming, the use of synthetic chemicals is minimized or eliminated altogether. Instead, organic farmers use natural alternatives such as compost, animal manure, cover crops, and biological pest control methods. By avoiding the purchase of expensive chemical inputs, organic farmers can significantly reduce their overall input costs. *Sustainable Soil Management***.** Organic agriculture emphasizes soil health and fertility through practices like crop rotation, intercropping, and the use of green manures. These methods help maintain soil structure, improve water retention, and promote beneficial soil organisms. As a result, organic farmers are less reliant on costly soil amendments and chemical soil treatments, leading to cost savings in the long run. *Lower Seed Costs***.** Organic farmers often use open-pollinated and heirloom seed varieties, which can be saved and replanted from one season to another. This eliminates the need to purchase new hybrid or genetically modified seeds each planting season, reducing seed costs over time. *Reduced Health Expenses***.** Organic farmers and farmworkers are less exposed to toxic chemicals found in synthetic pesticides and herbicides. By avoiding the use of these harmful substances, organic farmers can potentially reduce health-related expenses for themselves and their workers. *Less Energy Dependency*. The production and application of synthetic fertilizers and pesticides require significant energy inputs. Organic farming's reliance on natural methods can reduce the overall energy consumption on the farm, leading to potential cost savings. *Recycling of Resources*. Organic farming encourages the recycling of organic materials such as crop residues and animal manure, which can be used as natural fertilizers and soil conditioners. Recycling these resources on the farm reduces the need to purchase external inputs, contributing to cost reduction.

Diverse Market Opportunities - Organic agriculture allows farmers to tap into niche markets and cater to a growing segment of health-conscious consumers. By diversifying their produce

and offering organic options, farmers can expand their customer base and strengthen their economic position. Organic products have gained popularity among health-conscious, environmentally conscious, and socially responsible consumers. For health-conscious consumers organic products are often perceived as healthier and more nutritious due to the absence of synthetic chemicals. Studies have shown that consumers choose organic food primarily for health-related reasons (Hughner et al., 2007, Radojević et al, 2021). As health-consciousness grows globally, the demand for organic products increases, providing organic farmers with a growing market segment. Environmentally conscious consumers are concerned about the environmental impact of conventional agriculture and are increasingly opting for organic products as a way to support sustainable farming practices. Organic agriculture's emphasis on reducing chemical usage, promoting biodiversity, and conserving natural resources appeals to environmentally conscious consumers (Magkos et al., 2006).

The rise of specialized organic food retailers and health food stores has created new market opportunities for organic farmers. These retailers focus exclusively on organic and natural products, providing a dedicated channel for organic farmers to sell their produce (Schleenbecker and Hamm, 2013). Farmers' markets have become popular gathering places for consumers seeking fresh and locally grown produce. Organic farmers can directly sell their products to consumers at such markets, establishing a direct relationship with their customers and capturing the premium prices associated with organic products (Hand and Martinez, 2010). Organic agriculture is also gaining traction in institutional markets, such as schools, universities, hospitals, and corporate cafeterias. These institutions are increasingly incorporating organic options into their menus to meet the demand for healthier and sustainable food choices (Seufert et al., 2017). Organic agriculture presents opportunities for farmers to access international markets. As global demand for organic products rises, exporting organic produce can be a lucrative venture for farmers, particularly in regions where organic certifications and standards are in high demand (Willer et al., 2020). Organic farmers can also explore the production of value-added products, such as organic processed foods, organic dairy products, and organic specialty items. These products cater to consumers seeking convenient and innovative organic options, expanding market reach. Organic agriculture's ability to tap into diverse market opportunities presents economic advantages for farmers, allowing them to cater to specific consumer preferences and capitalize on the growing demand for sustainable and organic products.

Environmental Conservation Incentives and Soil Health and Long-Term Sustainability - Governments and international organizations often provide subsidies and incentives for adopting organic farming practices. These incentives encourage farmers to reduce their environmental impact, leading to long-term benefits for the ecosystem and sustainable agricultural practices. Environmental conservation incentives in organic agriculture refer to various policies, subsidies, and programs that support farmers' adoption of sustainable farming practices. Governments and organizations offer incentives to encourage farmers to reduce their environmental impact, promote biodiversity, and implement eco-friendly techniques. These incentives may include financial support, technical assistance, and recognition for environmentally responsible farming practices (Tomaš Simin and Glavaš-Trbić, 2016). As far as soil health and long-term sustainability are concerned organic farming prioritizes soil health through practices like crop rotation, composting, and reduced soil disturbance. Improved soil fertility not only benefits the environment but also ensures long-term agricultural productivity, reducing the risk of soil degradation. These practices contribute to the preservation of essential natural resources and the overall environmental sustainability of farming systems.



Scheme 1. Pros and Cons of organic production

When considering this production system, certain attention must also be paid to its shortcomings, that is, challenges, in order to be able to draw valid conclusions. Several shortcomings are discussed in literature and among them are:

Lower Yields - Organic farming methods may lead to lower yields compared to conventional agriculture. The absence of synthetic fertilizers and pesticides can result in increased vulnerability to pests and diseases, potentially reducing the overall productivity of organic farms. Lower yields in organic agriculture are a concern that is often discussed and studied. The transition from conventional to organic farming practices can lead to differences in crop yields due to the absence of synthetic fertilizers and pesticides, which can result in increased vulnerability to pests and diseases. While some studies have reported lower yields in organic systems, it is essential to consider the broader context and long-term sustainability of organic farming. For example, Seufert et al. conducted a meta-analysis of 66 studies comparing the yields of organic and conventional agriculture across various crop types. The results showed that organic yields were, on average, 25% lower than conventional yields. However, the study also highlighted that the yield gap varied depending on crop types, farming practices, and environmental conditions. Ponisio et al. (2015) explored how diversification practices in organic farming can affect yields. The study found that organic farms that integrated diverse crop rotations and included nitrogen-fixing legumes had lower yield gaps compared to conventional systems. In some cases, these diversification practices even resulted in similar or slightly higher yields in organic systems. De Ponti et al. (2012) assessed the yield gap between organic and conventional agriculture across different crops and regions. They found that organic yields were 20-34% lower than conventional yields, with the gap varying depending on the specific crop and local environmental conditions. Badgley et al. (2007) evaluated the potential of organic agriculture to feed the global population. They noted that while organic yields are generally lower than conventional yields, they can be competitive, particularly in favorable growing conditions and with appropriate agronomic practices.

Higher Labor Costs - Organic farming often requires more labor-intensive practices, such as hand-weeding and pest control, leading to higher labor costs. This can be a significant economic challenge, particularly for small-scale farmers with limited resources. Higher labor costs are another con associated with organic agriculture. Organic farming practices often

require more labor-intensive approaches compared to conventional methods. This can lead to increased expenses related to farm labor, which can be a significant economic challenge, particularly for small-scale organic farmers with limited resources. Ponisio et al. (2015) in addition to exploring the yield gap, also analyzed labor inputs in organic and conventional systems. This research found that organic farming practices, such as hand weeding and manual pest control, required higher labor inputs compared to conventional practices that rely more on chemical inputs. Crowder and Reganold (2015) investigated the financial competitiveness of organic agriculture worldwide. They identified labor costs as one of the major factors contributing to the financial challenges faced by organic farmers. Higher labor requirements in organic systems can lead to increased production costs, potentially impacting the overall economic viability of organic farming. Kremen and Miles (2012) compared the labor inputs of biologically diversified farming systems, which often include organic practices, with conventional farming systems. The research found that diversified farming systems required higher labor inputs due to the implementation of various ecological management practices.

While higher labor costs can be a challenge, some argue that they bring benefits in terms of job creation and local employment opportunities. Additionally, as organic farming practices become more established and technologies are developed to streamline labor-intensive tasks, the labor cost gap between organic and conventional systems may decrease over time.

Transition Period Challenges - The transition from conventional to organic farming can be financially burdensome. Farmers may face reduced yields and increased costs during this period, impacting their income until the organic system stabilizes. The transition from conventional to organic farming involves a period during which the farm must meet organic standards and practices before becoming certified as organic. This transition period can present several challenges for farmers, including:

- Initial Yield Reduction - During the transition, farmers may experience a decline in crop yields as they shift away from conventional inputs. The absence of synthetic fertilizers and pesticides can lead to increased vulnerability to pests and diseases, impacting initial productivity.
- Weed Management -Organic farmers often rely on non-chemical weed control methods, such as hand-weeding or mechanical cultivation. Transitioning to these methods can be labor-intensive and challenging, especially for large-scale farms.
- Time and Financial Investment - The transition to organic agriculture can require substantial time and financial investment. Farmers may need to modify their infrastructure, adopt new practices, and undergo certification processes, which can incur additional costs.
- Knowledge and Training - Farmers may need to acquire new knowledge and skills to effectively implement organic farming practices. Training and education on organic methods and principles are crucial during the transition period.
- Market Uncertainty - During the transition, farmers may face challenges in accessing organic markets and securing premium prices for their products before achieving official organic certification.

Market Uncertainty and Limited Scale and Market Access - Market uncertainty and limited scale and market access are two interconnected challenges that organic farmers may face in the agricultural industry. The demand for organic products can be unpredictable and subject to fluctuations. Organic farmers might face challenges when market preferences shift, leading to potential income variability and market uncertainty. Organic agriculture may be more feasible for small to medium-sized farms. Large-scale organic operations might face challenges in

maintaining the same level of efficiency and economies of scale as conventional counterparts. Additionally, access to certain markets may be limited due to certification requirements and regional preferences. Dimitri and Greene (2002) analyze the growth of the organic food market in the United States. It highlights that the organic market is subject to shifts in consumer demand, price volatility, and changes in market structure, leading to market uncertainty for organic farmers and producers. Bonanno et al. (2014) examines consumer preferences for organic and conventional olive oil in Italy. The study finds that consumer preferences and willingness to pay for organic products can be influenced by factors such as income, education, and health consciousness. This variability in consumer preferences contributes to market uncertainty for organic farmers. Lohr and Salomonsson (2000) discussesed the regulations and certification requirements that organic farmers must adhere to access organic markets. While certification ensures product integrity and consumer trust, it can pose entry barriers for smaller farmers due to administrative and cost burdens. In his book Lampkin (2003) explores various aspects of organic farming, including the challenges of market access. He highlights that the concentration of organic production and marketing by large-scale operators may limit opportunities for small-scale organic farmers to access certain markets, resulting in limited scale and market access for them.

Despite these challenges, several strategies can help organic farmers address market uncertainty and limited market access:

- Market Diversification: Farmers can reduce their risk by diversifying their product offerings to cater to various consumer demands and market segments.
- Direct Marketing: Selling products directly to consumers through farmers' markets, community-supported agriculture (CSA), and online platforms can provide better market access and premium prices.
- Cooperative Efforts: Farmers can collaborate through cooperatives or associations to pool resources, enhance bargaining power, and access larger markets.
- Value-Added Products: Developing value-added products from organic produce can create new market opportunities and increase profitability.
- Educational and Promotional Efforts: Educating consumers about the benefits of organic products and promoting organic farming can increase demand and market stability.

While market uncertainty and limited scale and market access can pose challenges, proactive strategies and ongoing support from policymakers and consumers can help organic farmers overcome these obstacles and foster the growth of sustainable and environmentally friendly agricultural practices.

Conclusion

Organic agriculture presents both economic advantages and challenges. While premium prices, reduced input costs, and diverse market opportunities can benefit organic farmers, lower yields, higher labor costs, and market uncertainties pose significant economic concerns. Governments and stakeholders must continue to support organic farmers through incentives and research to foster sustainable agriculture that addresses both environmental and economic needs.

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QUALITY OF OPERATION OF TECHNOLOGICAL-TECHNICAL SYSTEMS WITH DIFFERENT COMPRESSION CHAMBERS IN PREPARING ALFALFA HAY

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Abstract

For preparing alfalfa hay by baling, presses are used that can form small square bales - classic presses, roll presses for large and medium-weight roller bales, and presses for forming medium- and large-weight square bales - big balers. By pressing hay, significant savings in space for transport and accommodation are achieved, as well as easier and simpler manipulation of bales during loading, transport, and storage compared to bulk hay. The quality of the press for preparing alfalfa hay is significant, considering their function. When preparing alfalfa hay, it is necessary for the press to lift the growing mass, transport it to the compacting chamber, bind it, and release it in a compressed state on the surface with as few losses as possible. Our research aimed to determine the quality of the press for preparing alfalfa hay with different compression chambers, depending on the defined parameters. In the tests, the losses on the pickup device, the losses that occur during the shaping of the bales, and the losses during the tying and ejection of the bales were determined. Tests of the quality of press work with different compression chambers were performed according to the EN ISO 4254-11:2010 standard. The obtained results show that there is a significant influence of the defined parameters and the type of compaction chamber on the quality of work. Based on the obtained results, it was concluded that the lowest total losses during the preparation of alfalfa hay 5.52% were measured with variant A, while the highest total losses of 9.85% were measured with variant C. In the structure of realized losses, among all tested variants the highest share in the total losses was made by the losses that occurred during bale shaping, followed by the losses on the pick-up device, while the losses that occurred during tying and ejecting the bales were the smallest.

Keywords: *Press, Compaction chamber, Quality of work, Losses, Alfalfa.*

Introduction

Alfalfa (Medicago sativa) is one of the most important forage crops cultivated worldwide in more than 80 countries covering an area of more than 35 million ha (Radović *et al*., 2009). It is considered the leading and most important fodder crop for the production of quality fodder with great potential for improving livestock production. In the process of hay production, Alfalfa intended for feeding domestic animals is currently collected. Saving unpressed hay is less relevant due to the higher labor cost in preparing and feeding livestock, difficult manipulation, and a number of other problems. Mass baling is a sustainable method because it is a better use of storage capacity, greater flexibility in nutrition, and reduced losses in mass and production costs are lower (Maguire *et al.*, 2007; Shinners *et al.*, 2009; ASABE standards, 2011; Zhao *et al.*, 2018). In the group of machines for collecting hay, among others, the press occupies a significant place (Gottfried, 2008). Presses for collecting lumpy mass can form bales of different shapes, cylindrical, square, as well as big bales (Srivastava *et al.*, 2006; Gach *et al.*, 2010; Szczepaniak *et al.*, 2019). For the collection of balers in the Republic of Serbia annually, about 400 classic presses for small square bales and about 100

presses for large ones are necessary bales (Nikolić *et al.*, 2012). Alfalfa baling is the final process after mowing and manipulations in order to dry and collect mass with rakes in rows (Khalid, 2018). With aspects of hay pressing operations, two basic principles and concepts are current today, machines with translational and rotational movement of working tools, and the choice of the optimal working tool configurations, for specific conditions and needs, should be based on objective criteria evaluation of its production and safety properties (Radonjić, 2008). If relevant parameters for press work are not harmonized, work efficiency will be impaired and the price will be increased (Liu *et al.*, 2023). Mass compaction during the operation of a roll baler with a constant chamber is a significant parameter that characterizes the quality of work of the roll baler (Waszkiewicz *et al.*, 2003). During development, the tendency of the roller baler is to obtain bales with a smaller diameter, up to 160 cm for easier manipulation (Fürll, 2005; Brüser, 2006, Poničan *et al.*, 2008). Press performance and chamber type significantly affect the quality of work and yields, where the concept of managing them in the goal is an important reduction of losses (Roman *et al.*, 2014; Novak *et al.*, 2017; Grisso *et al.*, 2020). The highest losses during the operation of the baler for collecting hay occur during the shaping of the bales, then at the pick-up device, and the smallest when tying bales. The average loss of hay in the fraction of fallen leaves by the pick-up of the device was 2.6%, which can be tolerated, while the baling mass's moisture content was within optimal limits (Đević *et al.*, 2003). During the operation of the roll press, the formation of bales on the collection device had losses that amounted to 2.16% on average, which depended on the uniformity of the grain width (Jugović et al., 2013). The lowest total hay losses during baling were achieved with the big baller and 3.02% of the yield, then with the classic press at 3.76%, while the presses for roller bales had significantly higher losses, namely 10.74% (Potkonjak *et al.*, 2010).

The research aimed to determine the quality of work of technological-technical systems with different chambers for compaction when storing alfalfa hay depending on the defined parameters, such as and to recommend their use.

Material and Methods

Tests of the quality of work of various technological and technical systems for preparing alfalfa hay in the form of bales were carried out under production conditions at local farmers in central Serbia, around Kragujevac in 2021, during the mowing of the first swath of alfalfa of the Banat ZMS II variety. The tests included: determining the amount of realized losses on different variants of the press, depending on the defined parameters. Alfalfa yields were determined along the diagonal of the plot, were calculated for the entire trial, and amounted to 4.49 t ha^{-1}, while the average yield for the observed area is 4.90 t ha^{-1} (Stat. Yearb. Serb. 2022). The moisture content of alfalfa hay was determined in the laboratory by drying the samples (leaf moisture and stem moisture), after which the average value was obtained, which was 20.45% for variant A, 23.18% for variant B and 24.05% for variant C. three presses were represented in the tests, namely: a press for small square bales - variant A, a press for roller bales with a constant chamber and belts - variant B and a press with a variable compaction chamber - variant C. The quality assessment of the tested systems was related to determining losses on the mass lifting device (pick-up device), losses during bale shaping, and losses during tying and ejection of bales. Losses that occurred behind the pick-up device were determined by collecting and measuring the unpicked mass of hay from a 10-m path in five repetitions for each bale. Bearing that during bale shaping, it is impossible to capture the total amount of mass that falls out of the press, mass capture was performed on the receiving screen for 30 seconds and five repetitions during bale shaping. Binding and ejection losses were determined in the same way but during the total time of these operations. The working

speed was determined chronometrically and was 3.38 [km h^{-1}] - variant A, 4.48 [km h^{-1}] variant B, and 4.78 [km h^{-1}] variant C. The tested presses were aggregated with tractors of the appropriate power required for traction and propulsion. All tested varieties worked under similar exploitation conditions and at the same yield of alfalfa. For the applied methodology, it can be stated that it is standard for the examination of technological-technical systems for the storage of bulky mass and is in accordance with the standard EN ISO 4254-11:2010. The obtained results were processed using the Microsoft Office Excel 2007 package.

Results and Discussion

Tables 1 - 2 and Graph 1 show the results of testing the quality of technological and technical systems for preparing alfalfa hay with different compression chambers. Based on the obtained results, it can be noted that different average losses were achieved on the pick-up device. The lowest average losses were realized with variant A and amounted to 52.92 [kg ha^{-1}], i.e. 1.17% of the yield, while the highest losses were realized on the lifting device with variant C and amounted to 61.35 [kg ha^{-1}] or 1.37% of the yield. With variant B, the losses on the pick-up device amounted to an average of 58.88 [kg ha^{-1}], i.e. 1.31% of the yield. Evaluating the level of significance of the obtained values, it can be stated that with variant A, the realized losses on the pick-up device were significantly lower compared to variants B and C, between which there were no significant differences in terms of losses on the pick-up device. The coefficient of variation ranged from 7.12 in variant A to 10.24 in variant B (table 1).

Table 1. Average losses of alfalfa hay by tested variants of technological and technical systems

Type of losses [kg ha^{-1}]	Tested variant	Parameters					Average yields losses [%]	LSD *	
		Mean	σ	Cv	min	max		5%	1%
Losses at a pick-up device	A	52.92	3.76	7.12	48.21	58.16	1.17	4.30	8.49
	B	58.88	5.99	10.24	52.83	69.25	1.31		
	C	61.35	2.62	4.34	57.33	57.33	1.37		
Losses at forming	A	180.66	6.34	3.84	169.93	188.14	4.02	49.70	67.78
	B	297.39	28.26	9.58	273.63	352.23	6.62		
	C	364.70	20.57	5.68	340.73	387.64	8.03		
Losses at binding and ejection	A	13.59	0.78	5.74	12.16	14.41	0.31	3.02	4.12
	B	13.89	0.37	2.69	13.47	14.51	0.33		
	C	15.50	0.48	3.15	14.88	15.93	0.37		

Analyzing the values of losses that occurred during the shaping of the bales in the compacting chamber, it can be seen that the highest average values of losses were measured in variant C and amounted to 364.70 [kg ha^{-1}], i.e. 8.03% of the yield, and the lowest in variant A (system for collected masses in the form of small square bales) and averaged 180.66 [kg ha^{-1}] or 4.02% of the yield. In variant B, the average losses amounted to 297.39 [kg ha^{-1}] or 6.62%. The obtained values of the average losses that occurred during the shaping of the bales in the compaction chamber in variant A were evaluated as statistically very significantly lower compared to variants B and C, while the differences in the average losses that occurred during

the shaping of the bales in the compaction chamber between these two variants were evaluated as statistically significant. When it comes to the coefficient of variation, it can be seen that the values varied from 3.84 variant A to 9.58 variant B (table 1).

Based on the results shown in Table 1, which refer to the losses incurred during tying and throwing out the bales, it can be concluded that the lowest average values of this type of losses were achieved with variant A, namely 13.59 [kg ha^{-1}] or 0.31%, and the highest in variant C and were on average 15.50 [kg ha^{-1}] or 0.37% of the yield. The average losses caused by tying and throwing out the bales in variant B amounted to 13.89 [kg ha^{-1}] or 0.33% of the yield. There were no statistically significant differences between the tested varieties between the realized average losses during tying and throwing out the bales. The coefficient of variation ranged from 2.69 for variant B to 5.74 for variant A (table 1).

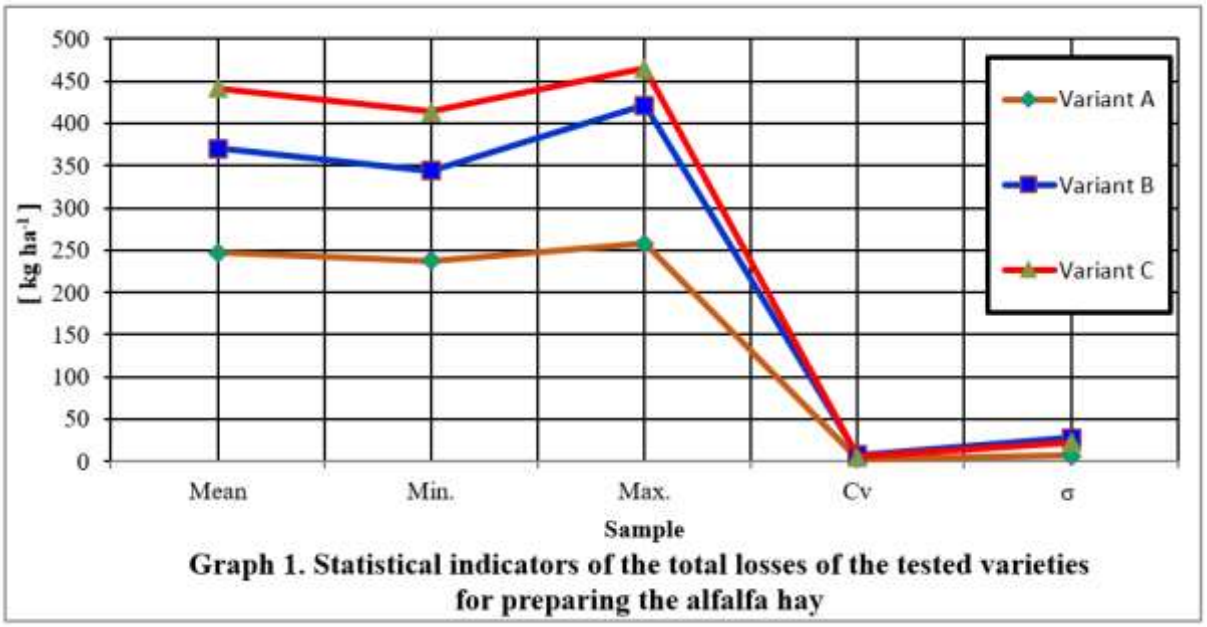
Analyzing the realized losses according to the examined variants of technological and technical systems with different compaction chambers when preparing alfalfa hay, it can be observed that the greatest losses occurred during bale shaping, followed by the pick-up device, and the smallest when tying the bales according to all examined variants (table 1).

Table 2 and Graph 1 show the values of total losses according to the tested variants of the system with different compression chambers when storing alfalfa hay. Based on the obtained results, it can be seen that the lowest total yield losses were achieved by the application of variant A and amounted to 247.17 [kg ha^{-1}] or 5.52%, while the highest values of total average losses were achieved by variant C and amounted to 441.55 [kg ha^{-1}], i.e. 9.85% of the yield. Total average losses in variant B amounted to 370.16 [kg ha^{-1}] or 8.24% of the yield. The coefficient of variation ranged from 2.89 for variant A to 7.59 for variant B.

Table 2. Total losses of alfalfa hay by tested variants

Parameters	Variant of the tested system			lsd	
	A	B	C	5%	1%
	Total Losses [kg ha^{-1}]				
Mean	247.17	370.16	441.55		
σ	7.17	27.77	22.48		
Cv	2.89	7.59	5.19	61.59	84.00
Min	237.45	343.75	413.89		
Max	257.91	420.97	464.76		
Average Losses [%]	5.52	8.24	9.85		

The total average losses that occurred with variant A were evaluated as statistically very significantly lower compared to variants B and C, while the differences in the average total losses between these two variants were evaluated as statistically significant (table 2).



Graph 1. Statistical indicators of the total losses of the tested varieties for preparing the alfalfa hay

Other authors report similar results in their papers (Waszkiewicz *et al.*, 2003; Đević *et al.*, 2003; Potkonjak *et al.*, 2010; Jugović *et al.*, 2013; Roman et *al.*, 2014; Novak *et al.*, 2017; Grisso *et al.*, 2020; Liu *et al.*, 2023).

Conclusions

Based on the obtained results, it can be concluded that the different quality of work of the tested variants of technological and technical systems with different compaction chambers during the preparation of alfalfa hay was achieved. The lowest average losses on the pick-up device were achieved with variant A which amounted to 1.17% of the yield and the highest with variant C which amounted to 1.37% of the yield. The realized average losses on the pick-up device in variant A device were statistically significantly lower compared to variants B and C, between which there were no significant differences in terms of losses. The lowest average values of losses during bale formation in the compacting chamber were measured for variant A (system for collected mass in the form of small cuboid bales) and amounted to 4.02% of the yield, and the highest for variant C and amounted to 8.03% of yield. The obtained values of the average losses that occurred during the shaping of the bales in the compaction chamber for variant A were evaluated as statistically very significantly lower compared to variants B and C, while the differences in the average losses that occurred during the shaping of the bales in the compression chamber between these two variants were evaluated as statistically significant. There were no statistically significant differences between the realized average losses during tying and ejection of bales between all tested variants. In the structure of realized losses, in all examined varieties, the largest share of losses was the losses incurred during bale shaping, followed by losses on the pick-up device, while the losses incurred during tying and ejecting the bales were the smallest. The lowest total yield losses were achieved using variant A and amounted to 5.52%, while the highest values of total average losses were achieved with variant C and amounted to 9.85%. The total average losses realized with variant A were evaluated as statistically very significantly lower compared to variants B and C, while the differences in average total losses between these two variants were evaluated as statistically significant.

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EFFECT OF AZOTOBACTER SPP. ON INITIAL GROWTH OF GRASSES

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Abstract

The composition of the microbial community in rhizosphere can affect plant growth, nutrients uptake, and stress tolerance. In rhizosphere bacteria belonging to the genus *Azotobacter* there are free nitrogen-fixers promoting growth and health of plants. The aim of this research was to investigate the effect of different native isolates (SA74, SB94, MA7 and their combination) *Azotobacter* spp. on initial growth of plants tall fescue (*Festuca arundinacea* Scherb) and meadow fescue (*Festuca pratensis* Huds). The experiment was carried out in semi-controlled conditions. For this study, seeds of tall fescue cultivar Kruševački 20 (K-20) and meadow fescue cultivar Kruševački 21 (K-21) were planted in pots filled with alluvial soil with slightly acidic reaction. The height, green mass per plant, root length and root weight per plant were determined. Four variants of microbial inoculation were compared with the growth of non-inoculated control. The significance of difference between examined treatments was determined by Fisher's LSD test ($p<0.05$). At tall fescue, applied isolate SB94 in all investigated parameters had a negative effect. The two other isolates as well as combination of three isolates had a positive effect on initial growth of plants. Only in plant height, the application of a combination of isolates did not have positive result compared to the control. At meadow fescue, inoculation with combined isolates positively influenced plant height and green mass per plant. Root length was significantly increased only in treatment with isolate MA7. The influence of inoculation on root weight per plant was not noted.

Key words: *Azotobacter spp., Grasses, Microbial inoculation.*

Introduction

The ecofriendly method to achieve high output yield, enhanced crop production, and better soil fertility is application of beneficial microorganisms. These microbes have crucial role to plant growth and development enhancing nutrient accessibility, decreasing the effects of diseases, providing resistance to biological and environment stress and at same time enable soil fertility and health (Altaf, 2021; Nadarajah and Rahman, 2023). The bacteria of genus *Azotobacter* are heterotrophic bacteria, aerobic and playing a crucial role in atmospheric nitrogen fixation (Geisler *et al*., 2020). *Azotobacter* is commonly found in rhizosphere of plants and is very effective for the improvement of soil fertility and crop productivity (Andhare *et al*., 2019). Depending on the conditions prevailing in the soil these bacteria can adopt 50-80 kg ha^{-1} of nitrogen from the air, which it uses for protein synthesis, and after its death, the proteins from the cell undergo decomposition and represent a significant supply of nitrogen in the rhizosphere (Sumbul *et al*., 2020). These organisms used as bioinoculants have ability to grow rapidly and fixing large amounts of nitrogen quickly. According to Romero-Perdomo *et al*. (2017) the application of mixed culture of*Azotobacter*strains could reduce the need of N-fertilizers up-to 50%. In addition to binding elemental nitrogen, Azotobacter plays a crucial role in soil enrichment by supplying the soil with different kinds of important biochemical substances such as vitamins, plant hormones, antifungal substances, hydrogen cyanide, siderophores (Mrkovački and Milić, 2001; Farajzadeh *et al*., 2012). Also, it can

produce antifungal substances, antibiotics (Dey *et al*., 2017), against plant pathogens (Chen, 2006). The number of these bacteria is used as the indicator of biological value of the soil. Acidic reaction of the environment has a negative effect on the activity and abundance of azotobacter since these bacteria prefer a highly productive neutral soil (Andjelković *et al*., 2012). Tall fescue (*Festuca arundinacea* Scherb.) is a forage and turf grass species in temperate regions of the world.It is one of the most productive forage perennial grasses (Kopecky *et al.,* 2019), especially in extreme environmental conditions, like summer drought or winter hardiness (Du *et al*., 2008; Salehi and Salehi, 2012). Also, regrowth production (second and third cut) is higher than other forage grasses (Niemeläinen *et al*., 2001). Tall fescue has been usually used for the intensive production of hay meadows. Also, it is very tolerant to grazing therefore pastures where it dominates are long lasting. It can be grown as a pure crop or in mixtures with legumes (Sleper and West, 1996). Meadow fescue (*Festuca pratensis* Huds.) is important perennial grass species, provides a consistent balance of yield, quality, palatability and persistence (Babić *et al*., 2023). It is widespread throughout Europe and the temperate zone of the Asian continent, especially in the meadows and pastures of central and northern Europe (Hand *et al.,* 2012). This grass species is most often used as a forage plant for grazing and mowing (Casler and Santen, 2001)., characterized by a pronounced resistance to cold climates, tolerates frosts, thrives best on wet and deep soils, tolerates flooding as well as droughts during the summer period (Peeters, 2004). The objective of this study was to investigate effect four variants of microbial inoculation by *Azotobacter* spp. on initial growth grasses.

Material and Methods

The experiment was carried out in vegetation pots in covered space at the Institute for forage crops in Kruševac during in the second half of May and the first half of June of 2022.

For this study, seeds of domesctic cultivars of grasses, tall fescue cultivar Kruševački 20 (K-20) and meadow fescue cultivar Kruševački 21 (K-21) were planted in pots filled with alluvial soil slightly acidic reaction - pH 6.20 (KCl). This soil has a medium nitrogen content (0.170 %) and humus (2.80 %) and low content easily accessible phosphorus (6.60 mg/100 g), and is rich in potassium (25.05 mg/100 g). Seed was sterilized with 0.2 % solution of $HgCl_2$ and 70 % ethanol, rinsed several times with sterile tap water and then immersed in the corresponding inocula. Ten seeds each of both examined grass species from each inoculum variant were planted in pots and ten milliliter of the appropriate inoculum was added to each pot.

The inoculation was done using three native isolates (preliminarily characterized) of *Azotobacter* spp. - SA74, SB94, MA7 and their combination. These isolates originated from soil of different geographical locations. *Azotobacter* was multiplied on the substrate by Feodorov 48 h at temperature of 28º C (Jarak and Djurić, 2006). For this research inoculum concentration 10^{10} cells per ml were used. The plants were assessed after 28 days of growth. The following traits were measured: plant height (cm), green mass per plant (g), root length (cm) and root mass per plant (g). Four variants of microbial inoculation were compared with the growth of non-inoculated control.

The results were processed using STATISTICS 8.0 program. The significance of the difference between the investigated probes was determined upon the variance analysis, i.e. Fisher's LSD test

Results and Discussion

Results of the analyses performed showed that effect applied inoculation was generally positive at tell fescue (Table 1). Only applied isolate SB94 in all investigated parameters had a negative effect. The isolates SA74 and MA7 had a positive effect on plant heigth tell fescue. The application of these isolates, as well as the combination of the three tested isolates, in a better green mass per plant achieved (Table 1). Research results obtained by Andjelković *et al.* (2014) showed that inoculation with A. *chroococcum* ad significant influence on plant height and green mass per plant in alfalfa. By using an inoculum of these microorganisms Jarak *et al.* (2007) reported better germination and height and weight of red clover than recorded on the control variant.

Table 1. The effect of inoculation on the parameters tall fescue

Parameter / Isolate	Plant heigth (cm)	Green mass per plant (g)	Root length (cm)	Root weigth per plant (g)
SA74	18.20^{a}	0.53^{b}	11.61^{a}	0.50^{b}
MA7	17.92^{b}	0.60^{a}	10.09^{c}	0.59^{a}
MiX	17.15^{d}	0.50^{c}	11.33^{b}	0.40^{c}
SB94	15.75^{e}	0.33^{e}	9.37^{e}	0.24^{e}
Control	17.40^{c}	0.45^{d}	9.70^{d}	0.28^{d}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p<0.05$)

Table 2. The effect of inoculation on the parameters meadow fescue

Parameter / Isolate	Plant heigth (cm)	Green mass per plant (g)	Root length (cm)	Root weigth per plant (g)
SA74	15.73^{c}	0.33^{b}	10.37^{a}	0.34^{a}
MA7	15.18^{d}	0.32^{b}	8.30^{d}	0.33^{a}
MiX	17.20^{a}	0.39^{a}	10.22$^{a.b}$	0.34^{a}
SB94	14.56^{e}	0.33^{b}	9.71^{b}	0.34^{a}
Control	16.36^{b}	0.32^{b}	9.28^{c}	0.33^{a}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p<0.05$)

Positive effects of microbial inoculation with combined isolates on plant height and green mass per plant were recorded on meadow fescue (Table 2). However, in this grass root length was significantly increased only by inoculation with isolate MA7. Compared to the control the root weigth per plant statistically different was not noted (Table 2). Using an inoculum of *A. chroococcum* Andjelković *et al*. (2022) reported positive effect on the height and green mass birdsfoot trefoil. According to results by Biswas *et al*. (1994) the use of azotobacter had positive effect in a yield of annual and perennial grasses. Also, Stamenov *et al*. (2012) recorded that using three azotobacter strains had positive effects in the production of English ryegrass. Plant response to inoculation varies considerably depending on bacterial species, soil type, cell concentration of microorganisms in the inoculum, environmental conditions, inoculation method (Shah *et al.,* 2017; Bizos *et al.,* 2020).

Conclusions

Based on results of the present research it can be concluded that the response of grasses to the applied inoculation was different. In tall fescue on initial growth of plants a positive effect of inoculation by combination three isolates, as well as application SA74 and MA7 was recorded. Only applied isolate SB94 in all investigated parameters had a negative effect. The response of meadow fescue to the applied inoculation was generally not positive. The exceptions are: inoculation by combined isolates on plant height and green mass per plant, as well as applied isolate MA7 on root length. These are preliminary studies and to obtain complete information about the effect these isolates of genus *Azotobacter* is necessary to continue research in direction to study the isolates and their influence on these grasses in field conditions.

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GENETIC VARIABILITY AND HETEROSIS FOR MICRONUTRIENTS CONTENT IN MAIZE GENOTYPES

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Abstract

Maize contains a large amount and considerable natural variation of carotenoids and tocopherols, micronutrients with several beneficial effects on human health. The objective of this study was to determine the differences in carotenoid and tocopherol content between six maize hybrids and their parental lines. The content of tocopherols and carotenoids was determined by the HPLC method. Heterosis was determined as mid and best parental heterosis. The range of β carotene and lutein+zeaxantin (L+Z) contents was greater in the parental lines than in hybrids. Hybrid ZP 5601 had higher than average β carotene and L+Z content. Among the hybrids, four have positive and two have negative values of mid-parent heterosis for β carotene content, but for better-parent heterosis four hybrids have negative values. For L+Z, four hybrids have a negative value for both mid (MP) and better parent (BP) heterosis. The average content of α- and γ- tocopherol was higher in the parental lines (11.08 μg/g /44.27 μg/g) than in the hybrids (10,76 μg/g/43.63 μg/g). Hybrid ZP666 with the highest α tocopherol content is a combination of an inbred line with high and one with medium-high α tocopherol content. Inbred line and hybrid with the highest γ tocopherol content have the lowest α tocopherol content. For α tocopherol, four hybrids have negative mid-parent heterosis and all better-parent heterosis as well as for γ tocopherols, while three have negative MP heterosis and four have negative BP heterosis. Identification of inbred lines producing F1 hybrids with heterosis for both agronomic traits and micronutriens is useful for maize breeding for improved nutrient content.

Keywords: *carotenoids, tocopherols, hybrids, inbred lines, heterosis.*

Introduction

Maize is a staple crop grown for animal feed, industrial purposes, and to a lesser extent use for human consumption, primarily in Africa, Central America, and part of Asia. Maize displays considerable natural variation for macronutrients and micronutrients, including carotenoids and tocopherols which are recognized as playing an important role in the prevention of human diseases and maintaining good health. Carotenoids serve as antioxidants to reduce the onset of some chronic diseases, such as cardiovascular diseases, cancers, infectious diseases, and age-related eye diseases (Rao and Rao, 2007; Fiedor and Burda, 2014), in particular, lutein and zeaxanthin offer a degree of protection against age-related macular degeneration (Botella-Pavia and Rodríguez-Concepción, 2006). Tocopherols might play an important role in enhancing the function of the immune system (Adachi *et al*., 1997), a reduction level of LDL cholesterol and fatty acid profiles (Goffman& Böhme, 2001), and preventing of a number diseases including cardiovascular disease (Pryor *et al.,* 2000), atherosclerosis, Alzheimer's disease (Saldeen and Saldeen, 2005), neurological disorders, cancer (Prasad *et al.*, 1999), inflammatory diseases (Traber *et al*., 2008).

Humans and animals are unable to synthesize carotenoids and tocopherols and consequently depend on dietary sources of these nutrients. In maize kernels, carotenoids and tocopherols are the two most abundant groups of lipid-soluble antioxidants. The concentrations of micronutrients in maize kernels depend on the genetic background (Ibrahim &Juvik, 2009), agronomic management (Mesarović *et al*., 2017; 2019), interaction between genotype and the environment, and processing (Drinic *et al*., 2021). Substantial variation for levels of carotenoids and tocopherols among diverse maize germplasm exists and information about the extent of variation of these compounds among diverse maize germplasm assumes tremendous significance to formulate an effective breeding strategy to develop micronutrient-riched maize genotypes. Determining the heterotic pattern of carotenoid and tocopherol contents and agronomic traits is important to harness the genetic potential of maize inbred lines for optimizing heterosis in maize hybrid with improved micronutrient content. The objective of this study was to determine differences in carotenoids and tocopherol content in the maize hybrids and their parental lines.

Material and methods

A set of 6 hybrids of FAO maturity groups from 300 to 600 and their parental inbred lines were used. Hybrids ZP388 (L9×L5) belonged to FAO maturity group 300; ZP 4567(L7×L8) FAO maturity group 400, ZP555 (L3×L5) and ZP 5601 (L1×L2) FAO maturity group 500, ZP6236 (L6×L2) and ZP666 (L3×L4) FAO maturity group 600. An experiment with inbred lines and hybrids was set up as a randomized block design with three replications in the experimental field of Maize Research Institute, Zemun Polje, Belgrade in 2022. The standard agronomical practice was applied.

The content of tocopherols and carotenoids was determined by the HPLC method. All reagents and chemicals used were of HPLC and analytical grade. Extraction, identification, and quantification of tocopherols (α-, β+γ- and δ-tocopherols) were similar to proposed by Mesarović *et al*. (2019) with minor modifications. Approximately 0.5 g of milled and dried grain was extracted with 10 mL of 2-propanol and homogenised in the ultrasonic bath (30 min at 25 °C). After homogenization and filtration through a 0.45μm nylon filter the extracts were directly injected into the HPLC system. Chromatographic separation was performed on a Hypersil GOLD aQ® C18 column. The mobile phase consisted of 95% methanol. The detection was performed with the Shimadzu RF-535 fluorescence detector at λex290 nm and λem325 nm. The tocopherol content is expressed as μg/g dw.

Extraction, identification, and quantification of carotenoids (lutein + zeaxanthin (L + Z) and β-carotene were similar as proposed by Mesarović *et al.* (2019). Approximately 0.5g of milled and dried grain was extracted with 15 mL of the mixture of methanol and ethyl acetate (6:4, v/v). After homogenization in the ultrasonic bath (30 min at 25 °C), the extracts were evaporated to dryness under a stream of nitrogen and redissolved in the mobile phase. After filtration through a syringe filter, the extracts were injected in the Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific, Germany) fitted with a photodiode array detector (DAD) and analytical column, Hypersil GOLD®C18 column (150 × 4.6 mm, 3 μm). Detection of carotenoids was accomplished on at 450 nm and 470 nm and expressed as micrograms (μg) per gram (g) of dry weight (dw).

Mid- and best-parent heterosis was calculated as follows, according to the formula indicated in Hallauer *et al*. (2010):

mpH =F1 – mp/mp× 100,

bpH =F1 – bp/bp× 100

where mpH is the mid-parent heterosis, bpH is the best parent heterosis, F1 is the hybrid value, mp is the average value of the corresponding parents for the hybrid, and bp value of the best parent.

Result and discussion

The genetic variation for tocopherols and carotenoids content was investigated in 15 maize genotypes, 6 hybrids, and 9 inbred lines. The primary carotenoids and tocopherols in maize grain were lutein+zeaxantin and γ-tocopherol. The mean values of β-carotene, lutein+zeaxanthin, α-tocopherol, γ-tocopherol, and δ-tocopherol were 1.58, 11.16, 11.02, 44.16 and, 2.12 μg/g d.w. respectively. Among cereals, maize grain has the highest content of tocopherols and the two predominant isomers are γ-tocopherol and α-tocopherol (Rocheford *et al.*, 2002). Although γ-tocopherol is naturally most abundant in maize, α-tocopherol has higher biological activity and is considered more desirable for consumption. Alpha-tocopherol content ranged from 5.12 (ZPL9) to 15.36 μg/g d.w. (ZPL4), γ tocopherol content from 23.73 (ZPL5) to 84.52 μg/g d.w. (ZPL9), δ-tocopherol content from 0.65 (ZPL3) to 8.23 μg/g d.w. (ZPL9), and the α-/γ-tocopherol contents ratio from 0.06 to 0.59, (Table 1). The contents of α-tocopherol in our inbred lines were lower concering the α-tocopherol of 87 elite inbred lines in China (Chander *et al*., 2008). Also, the wider range of the contents of α-tocopherol from 3.4 to 34.3 μg/g dw in provitamin A biofortified maize hybrids obtained by Muzhingi *et al*., (2017). The ratio of α- and γ-tocopherols range from 0.15 to 0.59 for hybrids and from 0.06 to 0.53 for parental lines (Table 1). The lines L8 and L9 have a low alpha-tocopherol content and consequently a low alpha-/gamma-tocopherol contents ratio.

Table1. Tocopherols and carotenoids content in maize hybrids and parental lines (μg/g DW)

Genotypes	α tocopherol	γ tocopherol	δ tocopherol	β carotene	L+Z	α/ γ ratio
ZP 4567	7.78	52.30	3.05	1.11	10.66	0.15
ZP 5601	9.26	56.03	3.00	2.82	14.04	0.16
ZP388	7.34	48.14	3.22	2.05	9.48	0.15
ZP555	11.01	43.24	1.81	1.36	11.52	0.25
ZP6263	14.14	36.60	1.58	1.35	14.58	0.39
ZP666	15.01	25.48	0.71	1.60	11.40	0.59
ZPL1	14.05	35.39	1.59	1.06	7.13	0.40
ZPL2	11.11	25.91	1.16	1.24	11.00	0.41
ZPL3	13.20	24.92	0.65	2.02	15.19	0.53
ZPL4	15.36	34.45	1.41	1.11	8.64	0.45
ZPL5	9.89	23.73	1.00	1.83	10.51	0.42
ZPL6	14.18	60.51	2.77	0.88	9.19	0.23
ZPL7	11.58	46.34	2.53	1.39	11.59	0.25
ZPL8	5.23	62.61	2.45	2.47	11.50	0.08
ZPL9	5.12	84.52	8.23	1.35	10.97	0.06

The average content of α, γ and δ tocopherol was higher in the parental lines (11.08 /44.27 /2.43 μg/g d.w.) than in the hybrids (10,76/43.63/2.23 μg/g d.w.). Hybrid ZP666 with the highest α-tocopherol content is a combination of an inbred line with the high (ZPL4) and one with medium-high (ZPL3) α-tocopherol content. It follows the results of Drinic *et al*. (2014). At the same time, hybrid ZP666 has the lowest γ tocopherol content. Hybrid ZP 5601 has the highest content of γ tocopherol as a combination of two inbred lines with low content. Among inbred lines, ZPL9 has the highest γ tocopherol but the lowest α tocopherol content. Three hybrids (ZP555, ZP6263, ZP666) and six inbred lines have higher α tocopherol content than average, while hybrids ZP4567, ZP5601, ZP388, and four inbred lines have higher γ tocopherol content than average.

Generally, wide genetic variation in tocopherol components has been observed in maize (Rocheford *et al.* 2002; Ibrahim & Juvick 2009, Drinic *et al.* 2017). In our study, the content

of tocopherol components from the highest to the lowest were β+γ-tocopherol, α-tocopherol, and δ-tocopherol. That founding was consistent with the different studies in maize (Goffman & Boehme, 2001; Egesel *et al* 2003; Chander *et al*., 2008, Drinic *et al*. 2017). In all genotypes, major carotenoids were lutein and zeaxanthin. That was in line with findings obtained from several studies that explored the extent of genetic variability for carotenoid content in different maize germplasms (Chander *et al*. 2008; Safawo *et al*., 2010; Muthusamy *et al.* 2015, Drinic *et al.* 2019). The content of L+Z varied from 7.13 to 15.19 μg/g d.w., with an average figure of 11.16 μg/g d.w. Beta-carotene ranged from 0.88 μg/g d.w. (ZPL6) to 2.82 μg/g d.w. (ZP 5601), average 1.58 μg/g d.w. (Tab.1). Similar content of *β*-carotene was observed in the analysis of ten maize inbred with yellow to orange kernel (Egesel *et al*., 2003). Chander *et al*., (2008) revealed β-carotene content in a range of 0.016-1.726 μg/g dw in 87 inbred lines from China, which is lower than obtained in inbred in our study. According to Ortiz-Monasterio *et al* (2007) the content of β-carotene in normal yellow maize ranges from 0.3 to 4.7μg/g dw. The range of β-carotene and lutein+zeaxantin content was wider for the parental lines than for the hybrids. Hybrids have L+Z content between 9.48 μg/g d.w. (ZP388) to 14.58 μg/g d.w. (ZP6263), average 11.95 μg/g d.w. and parental lines between 7.13 μg/g d.w. (ZPL1) to 15.19 μg/g d.w. (ZPL3), average 10.64 μg/g d.w., Table 1. Inbred lines have average β-carotene content of 1.49 μg/g d.w., and hybrids 1.72 μg/g d.w. Two hybrids (ZP5601 and ZP388) and three inbred lines have higher β carotene content than average as well as hybrids ZP5601 and ZP6263 and five inbred lines have higher L+Z content than average. Hybrid ZP5601 with the highest β- carotene content and second high L+Z content is a combination of two inbred lines with low β- carotene content as well as one line with the lowest and one with medium L+Z content. Hybrids ZP5601 and ZP6263 with ZPL2 as common parent have high L+Z content. Drinic *et al*. (2019) examined carotenoid content in 101 inbred lines with different kernel types (standard, sweet corn, popcorn) and found a slightly higher content of L+Z and β- carotene. A positive correlation was obtained between δ and γ tocopherols (0.89), β carotene with L+Z (0.52), γ tocopherols and β carotene (0.09). α tocopherols was negative correlated with γ tocopherols (-0.65), β-carotene (-0.45) and L+Z (-0.06). Goffman &Bohne (2001) did not find a correlation between α- and γ-tocopherols in 30 maize hybrids, suggesting that the biosynthesis of α-tocopherols in seed could follow an alternative pathway to the synthesis of γ-tocopherols, compared to the synthesis in chloroplasts.

Table 2. Midparent and better parent heterosis for tocopherols and carotenoids content in maize hybrids

hybrid	δ tocopherol		γ tocopherol		α tocopherol		β carotene		L+Z	
	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
ZP 4567	-43.29	-62.91	-20.07	-38.12	-6.87	-32.84	-41.96	-54.65	-7.70	-8.02
ZP 5601	118.00	88.50	82.78	57.33	-26.34	-16.61	79.13	66.12	54.90	27.72
ZP388	87.00	31.60	11.50	-23.12	-2.85	-25.71	29.10	2.09	-11.81	-11.81
ZP555	120.00	81.76	77.74	73.44	-4.60	-16.57	-29.17	-32.59	-10.35	-24.16
ZP6263	-19.56	-42.93	-15.30	-39.51	11.82	-0.28	27,21	9.01	44.39	32.49
ZP666	-32.30	-49.83	-14.15	-26.03	5.10	-2.29	1.80	-21.02	-4.31	-24.93

For α-tocopherol, four hybrids have negative mid-parent heterosis and all better-parent heterosis (Table 2). Hybrids ZP5601 and ZP555 have significant positive MP as well as BP heterosis for γ-tocopherols. Also, same hybrids have significant positive MP and BP heterosis for δ-tocopherols and negative heterosis for α-tocopherol. Overall three hybrids has negative MP and four BP heterosis for γ-tocopherols. Hybrid ZP5601 have significant positive MP and BP heterosis for β-carotene and positive heterosis for L+Z. Also, hybrid ZP6263 has positive heterosis for both β-carotene and L+Z. Those two hybrids have ZPL2 as a common parent.

Two hybrids for mid-parent and three hybrids for better parent heterosis have negative values for β- carotene. Four hybrids have negative MP and BP heterosis for L+Z. Similar results were obtained by Drinic *et al*. (2014) for tocopherol and β-carotene heterosis in 8 ZP maize hybrids. Maqbool *et al*., (2018) observed positive, negative, and zero mid-parent and better-parent heterosis in studinig 50 cross combinations of different agronomic, yield, and carotenoid traits. The possibility of exploiting heterosis for improving the concentration of carotenoids was suggested by several authors (Egesel *et al*., 2003; Senete *et al.*, 2011). Burt *et al* (2011) propound that heterosis for carotenoids in maize is a rare occurred, while Alfieri *et al* (2014) investigated 22 hybrid combinations and found that several hybrids manifested both MP and BP heterosis. Azmach *et al*. (2021) detected significant MP heterois for provitamin A in 20 hybrids and for total carotenoids in 17 hybrids.

Conclusion

Increased levels of carotenoids and tocopherols in maize grain, due to their antioxidant activity, should increase the nutritive value of corn. Great genetic variability is a valuable source of micronutrients, and genotypes with enhanced grain content could be used for the improvement of commercial hybrids creation. The identification of inbred lines that generate F1 hybrids with heterosis for both agronomic traits and carotenoids and tocopherols is critical for harnessing heterosis effects for breeding for increased micronutrient content.

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PHYSICAL AND CHEMICAL CHARACTERISTICS OF SOUR CHERRY (*PRUNUS CERASUS* L.) CULTIVARS

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Abstract

In the experiment, fruit characteristics (fruit weight, fruit length, fruit width, fruit thickness, stone weight, stone share, stalk length and stalk weight) and contents of primary metabolites (soluble solids, total sugars, invert sugars, proteins, sucrose, titratable acidity and pH) of sour cherry cultivars were studied. The research included fruits of five cultivars ('Sofija', 'Iskra', 'Nevena', 'Šumadinka' and 'Čačanski Rubin') developed in a breeding program at the Fruit Research Institute in Čačak and 'Heimanns Konserven Weichsel' as a standard cultivar. During the experimental period, significant differences in the parameters tested were observed among the cultivars. Fruit weight ranged from 5.69 g ('Heimanns K.W.') to 7.68 g ('Sofija'), while stone weight ranged from 0.36 g ('Iskra') to 0.48 g ('Čačanski Rubin'). The cultivar 'Sofija' had the highest fruit dimensions and stalk length, whereas the highest value for stone share was found in 'Čačanski Rubin'. Among primary metabolites, soluble solids content (16.43%) was highest in 'Iskra', while total sugars and invert sugars were high in 'Čačanski Rubin' (10.17% and 9.15%, respectively). Also, 'Čačanski Rubin' was the cultivar with the highest content of sucrose, while 'Nevena', Šumadinka' and 'Sofija' had higher contents of proteins compared to the other cultivars studied. In contrast, the highest level of titratable acidity was obtained in 'Heimanns K.W.', and the highest fruit pH in 'Čačanski Rubin'. The present results indicate that tested sour cherry cultivars due to the numerous positive physical and chemical characteristics deserve a place in intensive orchards and breeding programs as cultivars with enhanced nutritional value of fruits.

Keywords: *Prunus cerasus L., cultivar, fruit size, primary metabolites.*

Introduction

Sour cherry (*Prunus cerasus* L.) is one of the most commercially important fruit species worldwide. The Republic of Serbia is among the top five producing countries of sour cherry, which together with raspberries, represents the country's main export fruit. Production is largely extensive, and includes mainly clones of 'Oblačinska' sour cherry and 'Cigančica' (85%), while the other cultivars with large fruit are less represented. However, the improvement of cultivar assortment, growing technologies and sour cherry processing, accompanied by creation of favourable environment, can lead sour cherry growing from extensive and semi-intensive to intensive production. Fruit of sour cherry is consumed generally after processing, while fresh consumption is quite low. Most produced fruit is used frozen (with or without stones) and canned, and many of them are used for juice, jams, liqueur or brandy production. Sour cherries are very popular because of their specific aroma, excellent organoleptic characteristics, high content of dry matter and total acidity. The ratio of sugar to acid determines the taste of sour cherry fruits, and their fruits have a characteristic astringent taste (Yilmaz *et al*., 2018). The sweetness of the fruit is due to the presence of glucose and fructose, while sourness is mainly due to the presence of organic acids, especially malic acid. Furthermore, sour cherry fruits are rich in polyphenolic compounds such as flavonoids, anthocyanins, hydroxycinnamic acids, procyanidins, flavonol glycosides and

flavonols (Kang *et al*., 2003; Chaovanalikit and Wrolstad, 2004; Ferretti *et al*., 2010). Numerous studies have shown that consumption of sour cherry reduces the risk of certain cancers, arthritic, systemic and local inflammation, cardiovascular damage, Alzheimer's disease, inflammatory diseases, alleviation of muscle damage and risk of type 2 diabetes (Connolly *et al*., 2006; McCune *et al*., 2011; Kelley *et al*., 2018; Alba *et al*., 2019). Therefore, the objective of this study was to evaluate and compare the fruit characteristics and chemical properties of the fruits of the five sour cherry cultivars bred at the Fruit Research Institute in Čačak and 'Heimanns Konserven Weichsel' as a standard cultivar.

Material and Methods

The research was conducted at the Fruit Research Institute in Čačak, Western Serbia, during 2017-2019. The analysis included five sour cherry cultivars, including 'Sofija' ('Čačanski Rubin' × 'Heimanns Konserven Weichsel'), 'Iskra' ('Köröser Weichsel' × 'Heimanns Rubin'), 'Nevena' ('Köröser Weichsel' × 'Heimanns Konserven Weichsel'), 'Šumadinka' ('Köröser Weichsel' × 'Heimanns Konserven Weichsel') and 'Čačanski Rubin' ('Shasse Morello' × Köröser Weichsel') bred at the Fruit Research Institute in Čačak, and 'Heimanns Konserven Weichsel' as a standard cultivar. All cultivars were grafted on wild cherry (*Prunus avium* L.) seedlings and were represented in the orchard by five trees each. Fruits were sampled at the physiological maturity stage. A total of 100 g of fruits was sampled from 5 trees per replicate. Fruit, stone and stalk weights were determined on a Mettler precision scale with an accuracy of 0.01 g. For each **sour cherry** fruit, the three linear dimensions (length, width and thickness), as well as stalk length were measured using a 'Digital Caliper within 300 mm' with a sensitivity of 0.01 cm. Stone share in the total fruit weight was calculated.
Chemical analysis of the fruit included the following: 1. Soluble solids content was determined using a digital refractometer (Kruss, Germany); 2. Total sugars and invert sugars were analyzed using the Loof-Schoorl method (Egan *et al*., 1981); 3. Titrability acidity was determined by the 0.1 N NaOH titration method using phenolphthalein as an indicator; 4. Protein content was determined by the Kjeldahl method (Helrich, 1990); 5) pH value was measured by a pH Meter (Iskra MA 5707, Slovenia); 6) Sucrose content was calculated by multiplying the difference between total and reducing sugars contents by the 0.95 coefficient.
Data were presented as mean ± standard error. Differences between means were compared by LSD test in a two-way analysis of variance (ANOVA) using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The significance of differences between means at a 5% level was considered significant.

Results and Discussion

The results on fruit characteristics of the tested sour cherry cultivars are presented in Table 1 and Figure 1. The studied cultivars showed differences in the tested parameters. During the three-year experimental period, fruit weight ranged from 5.69 g to 7.68 g, and stone weight from 0.36 g to 0.48 g. The highest value for fruit weight was obtained in 'Sofija' (7.68 g), whereas the highest value for stone weight was recorded in "Čačanski Rubin' (0.48 g). Also, the high fruit weight was detected in 'Šumadinka' (7.15 g). The difference in fruit weight between cultivars was 17.7%, while the difference in stone weight was 19.2%. 'Sofija' is a cultivar with the highest values for fruit length, width and thickness (21.38 mm, 23.11 mm and 22.74 mm, respectively), while the lowest values were obtained in the cultivar 'Heimanns K.W.' (length – 17.31 mm, width – 19.37 mm and thickness – 17.56 mm). The stalk length was short in the cultivar 'Nevena' (34.81 mm) and medium in almost all tested cultivars,

except in the cultivar 'Sofija' which was long (52.44 mm). Stalks weight varied from 0.09 g ('Čačanski Rubin') to 0.17 g ('Nevena').

Table 1. Fruit dimensions in sour cherry cultivars

Cultivars	Fruit weight (g)	Stone weight (g)	Stone share (%)	Stalk length (mm)	Stalk weight (g)
Sofija	7.68±0.45 a	0.38±0.04 c	5.45±0.15 d	52.44±2.83 a	0.14±0.03 b
Iskra	6.23±0.27 cd	0.36±0.01 c	5.83±0.27 d	43.28±2.37 c	0.12±0.02 c
Nevena	6.57±0.23 c	0.41±0.03 bc	6.78±0.39 c	34.81±1.79 e	0.17±0.06 a
Šumadinka	7.15±0.31 b	0.40±0.04 bc	6.97±0.41 c	39.28±1.95 d	0.15±0.04 b
Čačanski Rubin	5.96±0.15 de	0.48±0.08 a	8.36±0.72 a	42.84±2.58 c	0.09±0.01 d
Heimanns K.W.	5.69±0.19 e	0.45±0.06 ab	7.64±0.58 b	47.90±2.62 b	0.11±0.02 c
ANOVA Cultivar	**	**	**	**	**

Means followed by different letters within rows are significantly different at $P \leq 0.05$ according to LSD test

In terms of stone share, as an important parameter for the processing industry, the highest value was obtained in 'Čačanski Rubin' (8.13%), and the lowest in 'Sofija' (5.45%). The difference in stone share between cultivars was 19.5%.

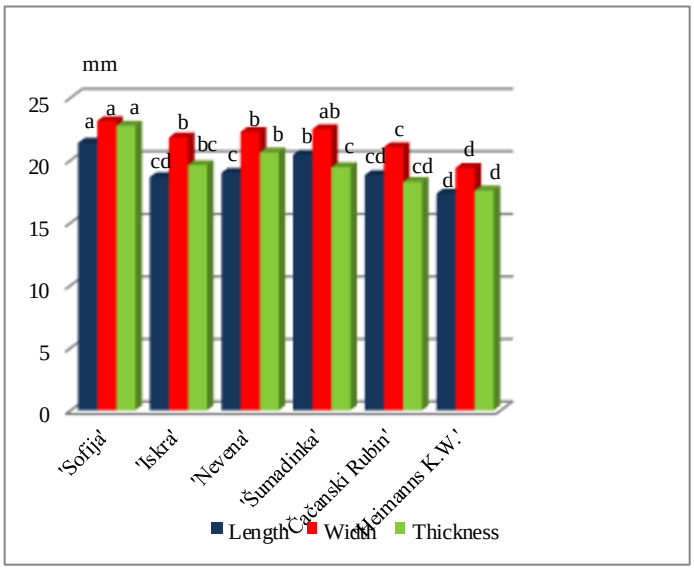

Figure 1. Fruit length, width and thickness in sour cherry cultivars

The present results are in agreement with Radičević *et al*. (2019), who found that the cultivar 'Sofija' has large fruit of high quality and attractive color. Under the agro-environmental conditions of the region of Belgrade, Nenadović-Mratinić *et al*. (2006) reported similar results for the cultivars 'Šumadinka' and 'Heimanns K.W.', while the results of Nikolić *et al*. (2000) differed slightly. In Montenegro, Šebek (2020) reported that 'Heimanns K.W.' and 'Čačanski Rubin' were the cultivars with large fruit weight (above 5 g), but shorter stalks length and lower stalk weight, which was not confirmed in the present study. These differences can be attributed to the effect of climatic factors in the studied sour cherry cultivars. Soluble solids, sugar and acid contents contribute to the sweetness and acidity of fruits and their products (Skrede *et al*., 2012). The results on the content of primary metabolites in the tested sour cherry cultivars are presented in Table 2 and Figure 2. The detected amounts of soluble solids varied from 12.47 to 16.43%, which is an important factor for consumer acceptance (Crisosto *et al*., 2003). Nikolić *et*

al. (2000) during a ten-year study of 30 sour cherry cultivars showed a wide range of soluble solids content, from 12.5% to 16.2%.

Table 2. Contents of primary metabolites in sour cherry cultivars

Cultivars	Total soluble solids (%)	Sugar content		Acid content	
		Total sugar (%)	Invert sugar (%)	Titrability acidity (%)	pH
Sofija	14.88±0.43bc	9.52±0.17 b	8.44±0.29 b	1.75±0.10 b	3.18±0.06 cd
Iskra	16.43±0.61 a	9.93±0.40 ab	8.96±0.36 ab	1.37±0.06 d	3.23±0.08 ab
Nevena	15.39±0.56 b	9.78±0.31 b	8.53±0.45 b	1.80±0.08 ab	3.11±0.03 e
Šumadinka	13.29±0.38 d	8.82±0.19 c	7.68±0.22 c	1.67±0.04 b	3.14±0.05 de
Čačanski Rubin	14.65±0.40 c	10.17±0.22 a	9.15±0.47 a	1.51±0.03 c	3.26±0.09 a
Heimanns K.W.	12.47±0.23 e	8.54±0.13 c	7.09±0.13 d	1.92±0.12 a	3.20±0.07 bc
ANOVA Cultivar	**	**	**	**	**

Means followed by different letters within rows are significantly different at $P \leq 0.05$ according to LSD test

During the experimental period, the highest content of soluble solids was found in 'Iskra', and the lowest in 'Heimanns K.W.'. 'Čačanski Rubin' had the highest content of total sugars (10.17%) and invert sugars (9.15%), while 'Heimanns K.W.' (8.54% and 7.09%, respectively), had the lowest values. It is interesting to point out that in Šumadinka, the late-ripening cultivar, the content of soluble solids and sugar was relatively low, which is in agreement with the results of Nenadović-Mratinić *et al*. (2006).

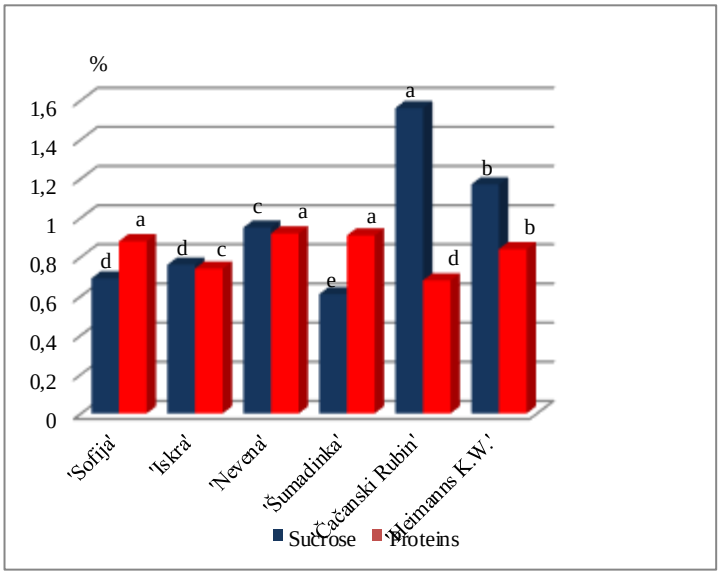

Figure 2. Content of sucrose and proteins of sour cherry cultivars

The content of proteins in the fruits ranged from 0.68 to 0.92%, while the content of sucrose varied from 0.67 to 1.56%. The highest protein content had 'Nevena' (0.92%), 'Šumadinka' (0.91%) and 'Sofija' (0.88%), whereas the highest sucrose content was found in 'Čačanski Rubin' (1.56%). Titratable acidity is one of the most important attributes of sour cherry, as it is directly related to consumer acceptance, and it is highly dependent on the cultivar (Serradilla *et al*., 2017). The highest values for titratable acidity were obtained in 'Heimanns K.W.' (1.92%), and the lowest in 'Iskra' (1.37%). On the other hand, 'Čačanski Rubin' had the highest fruit pH (3.26%), while 'Nevena' had the lowest (3.11), which is in agreement with the results of Serradilla *et al*. (2016), who reported that sour cherries are considered fruits with pH between 3.1 and 3.6. The analysis of the present data suggests that the content of the tested parameters is comparable with those of Nenadović-Mratinić *et al*. (2006), Nikolić *et al*. (2000) and Radičević *et al*. (2019). Šebek (2020) reported that soluble solids content ranged from 9.10% ('Čačanski Rubin') to 10.05% ('Heimanns Konservenweichsel'), and total acids from 1.65% ('Čačanski Rubin') to 1.81% ('Heimanns Konservenweichsel') in fruits of sour cherries grown in Montenegro, which was not confirmed in the present study. The differences in sugar and acid content can be attributed to microclimatic conditions, cultural practices, rootstock selection, planting systems and crop load, as well as differences in physiological stage as as a harvest criterion.

Conclusions

Knowledge of fruit characteristics and contents of primary metabolites in the fruits is very important when establishing commercial sour cherry orchards to make a proper choice of cultivars and improve fruit quality. The tested sour cherry cultivars are suitable for commercial production, due to their large fruits and good content of primary metabolites. The cultivar 'Sofija' a new sour cherry genotype deserves attention due to its exceptional fruit quality, and well-balanced parameters of fruit chemical composition. Also, the cultivar 'Čačanski Rubin' had a high content of most of the chemical properties tested. In general, the examined cultivars can be used in primary agricultural production when establishing commercial sour cherry orchards both under agro-environmental conditions in Serbia and worldwide. Also, their fruits can be used as natural agents in the pharmaceutical and food industries. Furthermore, the sour cherry cultivars developed in a breeding program at the Fruit Research Institute in Čačak represent an important basis for further breeding programs as parents for the creation of new cultivars.

Acknowledgments

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RESPONSE OF DIFFERENT WHEAT GENOTYPES TO DROUGHT IN SEMI-ARID CLIMATE CONDITIONS

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Abstract

A two-year study was conducted on 16 wheat genotypes with the aim of examining the reaction of genotypes to different environmental conditions. Grain weight per spike is a quantitative trait, which phenotypic expression is determined by the influence of a number of minor genes and environmental factors. Therefore, this trait is a suitable phenotypic marker of influence of environmental factors on the plant. The year factor had the largest share in the variation of grain weight per spike (67.82%), while the share of the genotype and the genotype × year interaction was significantly smaller (14.98 and 10.95%, respectively).
Drought conditions, which characterized the 2016/2017 growing season, affected the reduction of grain weight per spike by 41.7%. The greatest reduction in the value of trait under stress conditions was recorded in Fundulea 4 genotype (63.14%), which achieved the highest value in favorable environmental conditions (3.5 g), while in drought conditions it had a below-average value (1.29 g). Also, Dunavka genotype made good use of favorable environmental conditions to achieve a high value of grain weight per spike (3.14 g), so it can be recommended for breeding in favorable climatic conditions. On the other hand, the highest tolerance to drought was exhibited by the Pitoma genotype, which recorded a 15.67% decrease in grain weight per spike. The high tolerance of the mentioned genotype results from the highest value of the trait achieved in the dry growing season (1.83 g), which makes this genotype a good genetic resource for breeding for drought tolerance.

Key words: *wheat, drought stress, tolerance, genetic resources.*

Introduction

Water deficit is a major challenge to wheat productivity under changing climate conditions, especially in arid and semi-arid regions (Ahmad *et al*., 2022). Due to the increasing impact of climate change and the increase in areas with drought stress conditions, work on genetic improvement and developing drought-tolerant varieties is one of the most important tasks of breeders (Ahakpaz *et al*., 2020; Pandey *et al*., 2022).
In the early stages of wheat plant development, drought can cause a decrease in the number of fertile spikes. Drought during the flowering stage reduces grain number, especially when drought occurs shortly after anthesis, while post-anthesis drought tends to reduce grain weight. Drought stress at the time of filling and ripening of wheat affects the reduction of grain yield because of reducing duration of grain filling period.

Heatwaves and droughts, which are among the biggest stressors, tend to occur together, and their frequency is increasing with climate change (Correia *et al*., 2022). Globally, about 40% of wheat yield variation is explained by environmental constraints, with heat waves and drought being among the biggest stressors (Zampieri et al., 2017). Plant breeders and research scientists in the semi-arid areas attempt to improve wheat yields and develop new wheat varieties that tolerate drought (Abdel-Ghani *et al*., 2020).
The aim of this research is to investigate effect of drought stress on grain weight per spike, as one of very important yield component of different wheat genotypes.

Material and Methods

Field trials and methods

A two-year trial (2015/2016 and 2016/2017) was conducted with 16 divergent genotypes of winter bread wheat (Jugoslavija, Jedina, Fundulea 4, Iskra, Dunavka, Tamiš, Kavkaz, Skopjanka, Dukat, Pitoma, Poljana, Marija, NS 58-04, Mačvanka 1, Vali PKA 7114 and Zvezda), according to the random block system in three repetitions, where the size of the basic plot was 2 m^2.
The experiment was carried out in the Novi Bečej locality in Serbia on the humogley type of soil, where the usual agrotechnics for wheat production were applied. Monoammonium phosphate (MAP) fertilizer was applied before sowing in the amount of 250 kg ha^{-1}. In both research years, sowing was done with an inter-row spacing of 12 cm, and the sowing density was 650 grains m^{-2}. Wheat was sown in the first year of the research on October 31, 2015 and in the second year on November 5, 2016. Crop feeding was carried out in the last decade of February in both growing seasons, where 250 kg of *urea* per hectare was applied. In the growing season 2015/2016, the harvest was carried out on July 4, 2016, and in the season 2016/2017, the harvest was on June 26, 2017. At the stage of full maturity of wheat, samples of 30 plants of each cultivar (10 plants per replication) were taken for yield component analysis. The grain weight per spike was determined by measuring the grain weight of the primary spike, for each plant separately. The data were processed using the statistical program SPSS IBM Statistics 23 (Trial Version), https://www.ibm.com/).

Climatic conditions

The meteorological parameters for this investigation were taken from the website of the Republic Hydrometeorological Service of Serbia (http://www.hidmet.gov.rs/). The values of the analyzed climate parameters were compared with the corresponding values of the long-term averages, for the period from 1981 to 2010 for the Bečej area (Figure 1 and 2). October 2015 and 2016 were characterized by very similar climatic conditions. In both years, October is characterized by a higher amount of precipitation than usual for this month (30 mm more compared to the multi-year average), which caused somewhat later sowing. A higher amount of precipitation improved the water-physical condition of the soil and enabled more uniform germination and sprouting of plants. The temperature in both production years did not deviate significantly from the multi-year average. During autumn and the beginning of winter, the amount of precipitation was similar in both growing seasons. From January, there were significant differences in the amount of precipitation in the examined years. In 2017, the amount of precipitation was much lower compared to 2016 and a long-term period, except for the month of April when the precipitation was at the level of the multi-year average (52.5 mm; 52.9 mm, respectively). On average for the entire vegetation period of wheat development, in 2017 there was significantly less precipitation (42.2) compared to 2016 (154.0) and the multi-year average (69.3). Such conditions caused slower growth and development of plants, which contributed to a decrease in the value of yield components.

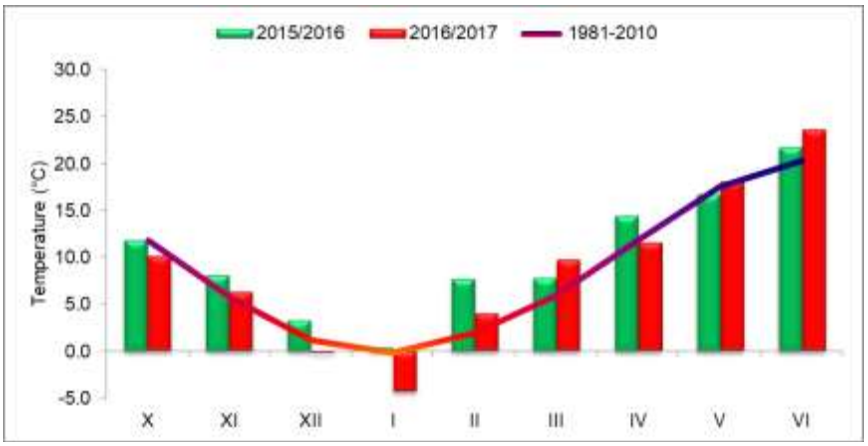

Figure 1. Average temperatures for each month during 2015/2016, 2016/2017 growing seasons and a long-term period (1981-2010)

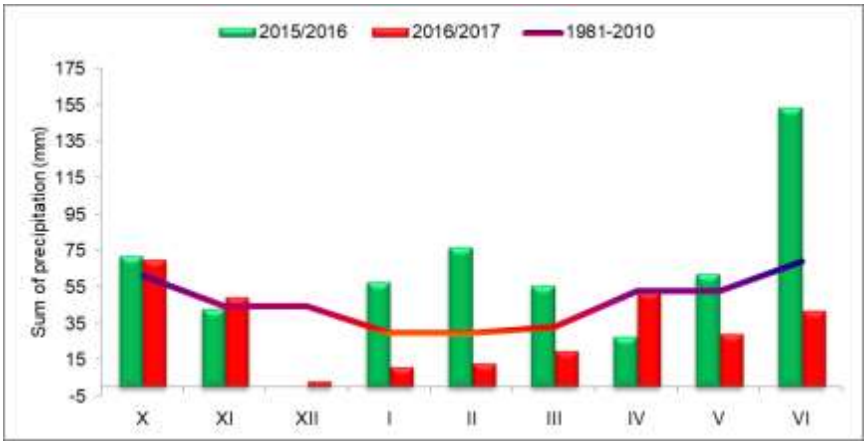

Figure 2. Sum of precipitation for each month during 2015/2016, 2016/2017 growing seasons and a long-term period (1981-2010)

Results and Discussion

The grain weight per spike was higher in the first year of research (2.49 g) than in the second year (1.45 g). The average of the whole experiment for grain weight per spike was 1.97 g (Table 1). Analysis of the standard deviation revealed a significant variability of grain weight per spike between wheat cultivars and between years in which the experiments were carried out. In 2015/2016, the coefficient of variation ranged from 16.19% (NS 58-04) to 34.19% (Kavkaz). The cultivar Kavkaz, apart from the high variability of this trait, also had the lowest value for grain weight per spike in the first year of research (1.67 g). In the first year, the cultivar Fundulea 4 had the highest grain weight per spike (3.50 g), and low variability of this trait (CV=18.65%). In 2015/2016, the climatic conditions for the development of wheat plants were more favorable than in 2016/2017 (Figure 1 and 2), when there was a decrease in the grain weight per spike, and therefore the grain yield.

In 2016/2017, there was a drought at the time of filling and ripening of grains, which led to a decrease in the grain weight per spike, whose values ranged from 1.02 g (Kavkaz) to 1.83 g (Pitoma). The variability of these properties was significantly higher in this year compared to 2015/2016 and was as much as 44.10% in the Kavkaz variety. The variety Kavkaz had a low value for grain weight per spike in both years of research, and high variability of this wheat yield component.

Table 1. Average values and variability for grain weight per spike in wheat

Genotype	2015/2016			2016/2017			Average	
	$\overline{X}$ (g)	Sd	CV	$\overline{X}$ (g)	Sd	CV	$\overline{X}$	CV
Dukat	2.65	0.58	21.96	1.59	0.59	37.07	2.12	29.52
Dunavka	3.14	0.88	27.99	1.65	0.38	22.89	2.40	25.44
Fundulea 4	3.50	0.65	18.65	1.29	0.52	40.55	2.39	29.60
Iskra	2.09	0.57	27.03	1.53	0.40	26.39	1.81	26.71
Jedina	2.24	0.66	29.50	1.42	0.37	25.98	1.83	27.74
Jugoslavija	2.46	0.46	18.49	1.41	0.34	24.05	1.93	21.27
Kavkaz	1.67	0.57	34.19	1.02	0.45	44.10	1.35	39.15
Mačvanka 1	2.39	0.52	21.88	1.37	0.47	34.36	1.88	28.12
Marija	2.43	0.51	21.06	1.35	0.38	28.38	1.89	24.72
NS 58-04	2.52	0.41	16.19	1.27	0.43	33.77	1.89	24.98
Pitoma	2.17	0.43	19.76	1.83	0.55	29.84	2.00	24.80
Poljana	2.54	0.62	24.37	1.63	0.44	26.73	2.09	25.55
Skopjanka	2.85	0.50	17.61	1.61	0.29	18.05	2.23	17.83
Tamiš	2.58	0.55	21.16	1.21	0.37	30.31	1.89	25.73
Vali PKA-7114	2.44	0.49	20.12	1.61	0.41	25.21	2.02	22.67
Zvezda	2.18	0.40	18.21	1.39	0.41	29.56	1.79	23.88
Average	2.49	0.55	22.38	1.45	0.47	29.83	1.97	26.10
$LSD_{(G)\ 0.05}$=0.317, $_{0.01}$=0.421				$LSD_{(G\times Y)\ 0.05}$=0.448, $_{0.01}$=0.595				

The cultivar Skopjanka showed the lowest variability, that is, the homogeneity of grain weight per spike in the first year of research (CV=17.61%). Apart from the low variability of this trait, in 2015/2016 the cultivar Skopjanka was the third in terms of grain weight per spike (2.85 g) as well as the average for both years (2.23 g). Only the cultivars Dunavka and Fundulea 4 (2.40 g, 2.39 g, respectively) had higher average values for grain weight per spike than Skopjanka. Variation in genotypes can be explained by the fact that each plant presents a different defense and tolerance mechanism, which may also occur between genotypes of the same species (Nardino *et al*., 2022).

Analysis of variance revealed highly significant differences for grain weight per spike between cultivars as well as between analyzed seasons. The cultivar × year interaction was also statistically highly significant (Table 2). Thus, grain weight per spike is a quantitative property that is highly dependent on environmental factors (Zečević *et al*., 2018; 2022).

Table 2. Analysis of variance for grain weight per spike in wheat

Source of variation	df	Sum of Squares (SS)	Mean Square (MS)	F - value	p - value	Share in variation (%)
Genotype (G)	15	5.787	0.386	10.242**	0.000	14.98
Year (Y)	1	26.198	26.198	695.507**	0.000	67.82
Genotype × Year	15	4.231	0.282	7.489**	0.000	10.95
Blocks	2	0.019	0.009	0.250	-	0.05
Error	64	2.411	0.038			-
Total	95	38.627				100.00

The variation of wheat yield by year can mainly be explained by abiotic limitations. In this research, wheat genotypes in the season with favorable climatic conditions were more productive than in the dry season. Drought conditions, which characterized the 2016/2017 growing season, affected the reduction of grain weight per spike by 41.7%. Similar effects of drought on plants have also been observed by other authors, including Soomro *et al*. (2019). Also, in previous study, wheat genotypes showed considerably lower mean values for grain yield (86.6-204.7%) under stress conditions than when irrigated (Aksić *et al*., 2020). Susceptibility to drought depends on its intensity and duration, as well as on the stage of crop growth in which the drought was imposed. The physiological and quantitative genetic basis of drought tolerance is still poorly understood (Haddadin *et al*., 2013). Understanding the mechanisms and responses of genotypes to drought stress is crucial for research because it guides the positioning of genotypes in environments where they are more likely to suffer from climatic fluctuations in precipitation (Nardino *et al*., 2022).

Conclusions

The tested cultivars showed different sensitivity and adaptability to the water deficit and high temperatures that prevailed in the 2015/2016 growing season. The smallest decrease in grain mass per spike was shown by the Pitoma cultivar (0.34 g) in the dry vegetation of 2015/2016 compared to 2015/2016, which was significantly more favorable for the growth and development of wheat plants. In addition to this cultivar, a low reduction of the tested property was also found in the cultivars Iskra and Kavkaz, which amounted to about 0.60 g. Some of these cultivars could be used as parents in breeding programs for drought stress resistance and improving wheat resilience to climate change.

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EFFECTS OF THE USE OF DIFFERENT FERTILIZATION RATES AND MICROBIOLOGICAL PRODUCTS ON WHEAT YIELDS

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Abstract

Common wheat (*Triticum aestivum* L.) is a crop predominantly used in food and feeds. Modern agricultural production implies rather an irrational use of large amounts of pesticide and mineral fertilizers, significantly diminishing the quality of agricultural land, and negatively affecting the environment, food quality and human health. In order to diminish adverse effects of intensive agricultural production, farmers around the world incline to an increased use of microorganisms to reduce the use of mineral fertilizers and pesticides to some extent. Agricultural production is facing certain requirements to reduce chemical inputs in order to protect the environment, produce safe and profitable food. This situation is additionally aggravated by climate changes that require many practices to adapt accordingly. The experimental research was carried out during 2016-2019 at the "PKB Agroekonomik" Institute. Field operations were done with typical agricultural practices, and four variants of fertilization were set up. Prior to sowing, 400 kg ha-1 NPK 15:15:15 was applied. Different rates of UREA 46% N were used in spring top-dressing of the crops. Aside of mineral fertilizer, microbiological products with active microorganisms were also used for top-dressing. Two wheat varieties – Ratarica and Pobeda were used. The goal of the research was to determine the impact of different rates of fertilizer and microbiological products on productive and morphological properties of wheat.

Keywords: *wheat, effective microorganisms, yield, fertilizer*

Introduction

Rice, wheat and maize are most grown crops for human diet. Wheat is the most important and most wide-spread cereal in the world. According to the botanical classification, wheat belongs to family *Gramineae,* tribe *Triticeae,* comprising about 300 different species (Clayton and Renvoize, 1986; Đurić et al.. 2017). Wheat is characterized by great polymorphism, due to its winter and spring varieties. As a staple bread crop, wheat in developed countries comprises 53% and in undeveloped countries 85% of the total world's production (Pena, 2007). Wheat is grown on all continents, including high altitude parts in the tropics and sub-tropics (Додиг, 2010; Đurić et al. 2017). The use of microbiological fertilizer in sustainable systems is an imperative in production of safe food (Cvijanović et al. 2013). There have been a growing number of research over the previous years on the interaction between plants and certain group of microorganisms to obtain environmentally-friendly yet profitable production (Cvijanović et al. 2012). Production of high yielding wheat of good quality is possible only if

use quality varieties and provide proper growing conditions and production technology (Đurić et al. 2015).

The most populated countries, such as China, India and Russia had, on average, the largest plots under wheat. In China, wheat was sown on 24,340,440 ha and the average yield was 5.17 t ha^{-1}. India had 29,973,890 ha of plots under wheat, with the average yield of 3.07 t ha^{-1}. Russia had 24,688,890 ha of plots with a rather low average yield of 2.40 t ha^{-1}. In Europe there were 59,235,220 ha of plots under wheat averaged 3.96 t ha^{-1} of yields.

Table 1. Areas under wheat, yields and production worldwide

Region		2010	2011	2012	2013	2014	2015	2016	2017	2018
Globally	000 ha	215,602	220,263	217,917	218,868	219,750	223,476	219,096	218,424	214,291
	t ha^{-1}	2.97	3.16	3.09	3.25	3.32	3.32	3.42	3.54	3.43
	000 t	640,802	696,898	673,728	710,397	728,730	741,643	748,392	773,476	734,045
China	000 ha	24,257	24,272	24,270	24,119	24,071	24,599	24,698	24,510	24,268
	t ha^{-1}	4,75	4.84	4.99	5.06	5.24	5.39	5.40	5.48	5.42
	000 t	115,186	117,414	121,030	121,930	126,215	132,646	133,277	134,340	131,447
India	000 ha	28,457	29,068	29,860	29,650	30,470	31,470	30,420	30,790	29,580
	t ha^{-1}	2.84	2.99	3.18	3.15	3.15	2.75	3.03	3.20	3.37
	000 t	80,803	86,874	94,880	93,510	95,850	86,530	92,290	98,510	99,700
Russia	000 ha	21,639	24,835	21,277	23,371	23,907	25,870	27,312	27,517	26,472
	t ha^{-1}	1.92	2.26	1.77	2.23	2.50	2.39	2.69	3.13	2.73
	000 t	41,507	56,239	37,719	52,090	59,711	61,784	73,345	86,004	72,136
Europe	000 ha	55,811	59,278	54,892	57,869	58,717	61,475	62,585	61,879	60,611
	t ha^{-1}	3.61	3.78	3.55	3.91	4.25	4.19	4.03	4.40	4.00
	000 t	201,373	224,032	195,046	226,103	249,253	257,461	252,156	272,381	242,139

(Source: http://www.fao.org/faostat/en/#data)

In Serbia, in the period 2009-2018, wheat was sown on 565,745 ha of plots, with the average production of 2,278,110 t and average yield of 4.33 t ha^{-1}. The largest wheat plots were recorded in 2018, amounting to 643,083 ha, as well as the highest yield 4.57 t ha^{-1}, which resulted in the highest grain production of 3,941,601 metric tons, making Serbia the ten largest wheat producer in the world.

Material and methods

In order to perceive the effect of using NPK fertilizer and microbiological product "EM Aktiv" (trademark) on wheat yields, a three-year trial was set up on demonstrational plots of the "PKB Agroekonomik" Institute in Padinska Skela, using two wheat varieties, four different fertilization variants in four replications. Two wheat varieties PKB Ratarica and NS Pobeda, selected in Serbian institutes ("PKB Agroekonomik" Institute and the Institute for Field and Vegetable Crops in Novi Sad) were used as research material during 2016/2017, 2017/2018 and 2018/2019. The plots were set up in a randomized design with four replications. During all three years of the research, the preceding crop was maize. All practices in terms of production technology (tillage, sowing, spring top-dressing, and harvest) were carried out within the optimal time window. The crop samples were taken from the middle of each plot. The grain samples necessary for calculating productive and qualitative properties of grains were taken after harvest from the whole plots. To ensure proper dressing, NPK 15:15:15 mineral fertilizer was applied during the basic tillage. Prior to sowing, 400 kg ha^{-1} NPK was introduced into the soil, in amounts calculated according to the area of the demonstrational plot (Table 5).

Table 2. Fertilizer variants on the experimental plot

Treatments	Prior to sowing: NPK (kg ha^{-1})	Top-dressing: UREA (kg ha^{-1})	EM Aktiv (6 l ha^{-1})	Total N : P_2O_5 : K_2O
T 1	400	150	-	129 : 60 : 60
T 2	400	150	1 time	129 : 60 : 60
T 3	400	100	2 times	106 : 60 : 60
T 4	400	50	3 times	83 : 60 : 60

Results and discussion

A grain yield comes as a result of various physiological changes that occur during crop growth being directly affected by numerous factors. Grain yields are affected by different factors, primarily genotype characteristics, soil fertility and cropping practices used.

During the period 2017-2019, the grain yield was 6.58 t·ha^{-1} (Table 5). Affecting by weather conditions, the obtained yields varied with no statistical significance. In 2017, the achieved grain yield was 6.79 t·ha^{-1}, in 2018 it was 6.54 t·ha^{-1}. The highest yield of 6.95 t·ha^{-1}was recorded in 2019.

The varieties (B) and variety x fertilizer treatment interaction had a statistically highly significant effect on the yield. The Ratarica variety had the highest yield in all years of research. It averaged 7.02 t·ha^{-1}, which is the highest average yield. The Pobeda variety yielded 6.50 t·ha^{-1}, which was 7.40% lower.

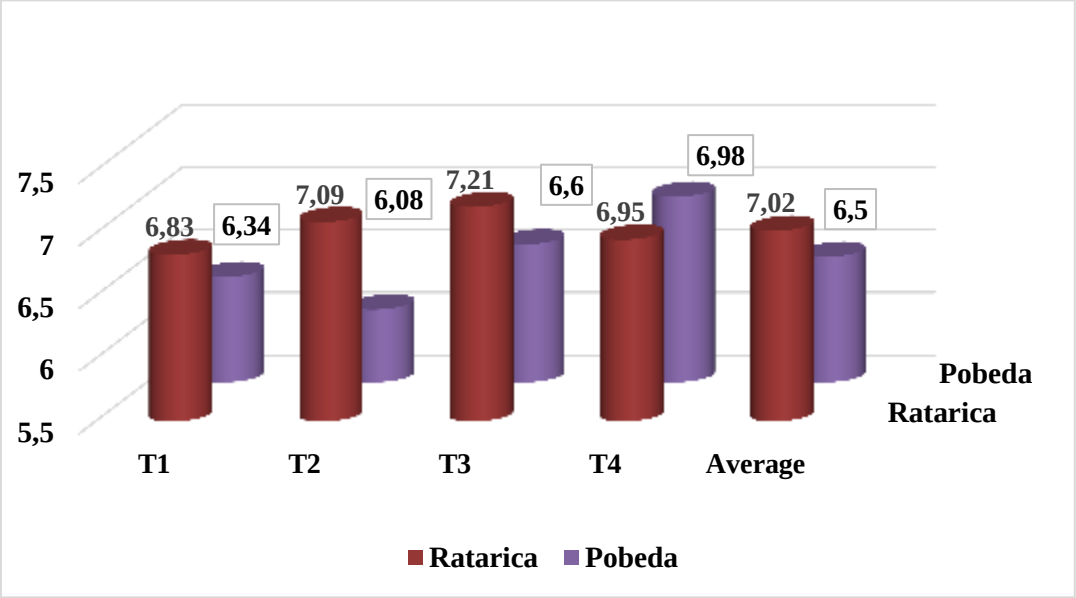
The yield was significantly affected by top-dressing treatments (factor C). The highest average grain yield of both varieties were detected under treatments T2 and T4. The grain yield under treatment T4 was 6.63 t·ha^{-1}, and under treatment T2 it was 6.47 t·ha^{-1}; the differences were not statistically significant, since the differences between the grain yields were small. When compared to treatment T1, there was 3.59% increase in grain yield, and compared to T3 it was 10.31%. Yields definitely depends on variety properties. Royo et al. (2005) determined that yields of 12 wheat varieties grown on 24 sites were dependent on basic yield-affecting parameters. Sugár et al. (2016) came to a conclusion that maximum grain yield was achieved under 80 and 160 kg ha^{-1} nitrogen treatments and an increase in nitrogen rates did not result in increased yields in any year (Đurić et al., 2018; Jelic et al. 2015; Terzić et al., 2018; Rajicic et al., 2020).

Graph 26 shows yields of each variety per treatment. Under all treatments, the Ratarica variety had highest yields, ranging from 6.83 t ha^{-1} under treatment T1 to 7.21 t·ha^{-1} under treatment T3. Yields of the Pobeda variety ranged from 6.08 t·ha^{-1} to 6.98 t·ha^{-1}.

Table 3. Effect of the factors on grain yield of wheat (t ha^{-1})

Year (A)	Variety (B)	Fertilization (C)				$\bar{x}$ A x C	$\bar{x}$ A
		T1	T2	T3	T4		
2017	Ratarica	6.63	7.07	7.38	6.85	**6.98**	**6.79**
	Pobeda	6.46	6.17	6.62	7.13	**6.60**	
	$\bar{x}$ B x C	**6.55**	**6.62**	**7.00**	**6.99**		
$\bar{x}$ A x B x C		**6.19**	**6.41**	**6.78**	**6.64**		
2018	Ratarica	6.63	7.13	6.78	6.82	**6.84**	**6.54**
	Pobeda	6.28	5.65	6.18	6.88	**6.25**	
	$\bar{x}$ B x C	**6.46**	**6.39**	**6.48**	**6.85**		
$\bar{x}$ A x B x C		**6.19**	**6.13**	**6.01**	**6.27**		
2019	Ratarica	7.24	7.06	7.47	7.18	**7.24**	**6.95**
	Pobeda	6.28	6.43	7.01	6.91	**6.66**	
	$\bar{x}$ B x C	**6.76**	**6.75**	**7.24**	**7.05**		

x̄ A x B x C		**6.82**	**6.87**	**7.25**	**7.00**	**x̄ C**	**x̄ B**
x̄ B x C	Ratarica	6.83	7.09	7.21	6.95	**7.02**	**6.76**
	Pobeda	6.34	6.08	6.6	6.98	**6.50**	
	x̄ B x C	**6.59**	**6.59**	**6.91**	**6.97**		
	x̄ C	**6.40**	**6.47**	**6.01**	**6.63**		
						Average 2017-2019	**6.76**



Graph 1. Average grain yield (t·ha^{-1}) of both wheat variety in the investigated period, depending on the treatments

Table 4: The analysis of price sensitivity and wheat yield in terms of gross margin

		Price (RSD/kg)				
		-20%	-10%	Average	10%	20%
Yield (kg/ha)		**13.88**	**15.62**	**17.35**	**19.09**	**20.82**
-20%	**5,408.00**	-10,758.39	-1,375.51	8,007.37	17,390.25	26,773.13
-10%	**6,084.00**	-1,375.51	9,180.23	19,735.97	30,291.71	40,847.45
Average	**6,760.00**	8,007.37	19,735.97	31,464.57	43,193.17	54,921.77
10%	**7,436.00**	17,390.25	30,291.71	43,193.17	56,094.63	68,996.09
20%	**8,112.00**	26,773.13	40,847.45	54,921.77	68,996.09	83,070.41

Source: Authors' calculations based on the questionnaires on wheat production gross margins in the period 2017 – 2019

Table 6 shows the analysis of price sensitivity and wheat yield and their effect on gross margin per hectare. Based on gross margin questionnaires, it was determined that the average gross margin in wheat production in the period 2017 – 2019 was 31,464.57 RSD/ha, with the average yield of 6,760 kg/ha and price of 17.35 RSD/kg. Price sensitivity analysis comprised changes in gross margin when wheat price and/or yield increase or decrease 10% and 20%. From the obtained data it can be concluded that gross margin in wheat production is sensitive to change in price and yields to a great extent. The lowest positive gross margin could be obtained if the price or yield decreased 20%, amounting to 8,007.37 RSD/ha. If one factor

decreased 20% and another 10%, wheat production would not be profitable, resulting in a negative gross margin (-1,375.51 RSD/ha). The lowest gross margin would come as result of 20% decrease in both price and yield, amounting to -10,758.39 RSD/ha. On the other hand, an increase in wheat yields and price would have greater effects on gross margin. A 20% increase in yields and price would lead to more than a 150% increase in gross margin, amounting to 83,070.41 RSD/ha. This paper shows gross margin sensitivity upon change in factors affecting production value, yet production costs can also have a great impact on it. In wheat production, those costs are costs of crop protection and fertilization, but there are also transportation costs that can be significant, depending on the distance between the plot itself and the storage place (Savić et al., 2020.).

Conclusion

Based on the three-year research on the effect of top-dressing and the use of microbiological fertilizer on grain yield of wheat *(Triticum aestivum sp.)*, it can be said that grain yield averaged 6.76 $t \cdot ha^{-1}$ with some significant differences caused by treatments and genotype domination. The Ratarica variety had higher yields than the Pobeda variety. In general, both varieties yielded less at higher density planting. Statistically significant differences in yields by fertilization were recorded. The highest yield was achieved under treatments with less fertilizer and more foliar treatments (6.46 and 6.41 $t \cdot ha^{-1}$). To conclude, the varieties Ratarica and Pobeda under different agricultural and meteorological conditions exhibited great stability in their morphological and productive properties. The use of microbiological products in wheat production can provide stable production and high grain yields, which is very important in terms of human diet.

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QUALITY CHARACTERIZATION OF BLACKBERRY FRUITS (Rubus subg. Rubus Watson) IN DIFFERENT MATURITY STAGES

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Abstract

The European blackberry (*Rubus* subg. *Rubus* Watson) is an important berry in Serbia and is positioned immediately after the raspberry and strawberry in terms of economic significance. The two most grown cultivars of blackberry in Serbia are 'Loch Ness' and 'Čačanska Bestrna,' each with their own unique characteristics and benefits. The choice of cultivar depends on the specific needs and preferences of the grower, as well as the growing conditions and market demand in their region. The aim of this study was to compare the fruit quality of the 'Loch Ness' and 'Čačanska Bestrna' blackberry cultivars during different maturity stages (fully ripe and overripe). The study found that both cultivars had higher values of soluble solids content (10.80° Brix), total anthocyanins (118.09 mg cyn-3-glu 100 g^{-1} FW), weight (7.64 g), length (26.57 mm), and fruit shape index (1.48) when sampled at the overripe maturity stage. On the other hand, an increase in total phenolic content (601.56 mg 100 g^{-1} FW), antioxidant capacity (3.81 µmol Trolox 100 g^{-1} FW), and fruit width (18.30 mm) was determined in fully ripe blackberry fruits. Considering that blackberry fruits produced in Serbia are mainly sold on domestic and foreign markets in a frozen state due to the low level of processing capacity, these research findings will be useful in modern food technology. The trend in food technology is directed towards extending the shelf life and increasing the content of nutrients in blackberry fruits.

Keywords: *blackberry, maturity stage, pomological traits, antioxidants*

Introduction

The European blackberry (*Rubus* subg. *Rubus* Watson) is an important berry in Serbia, positioned immediately after raspberry and strawberry (Nikolić and Milivojević, 2015) due to its economic significance. Blackberry production began after World War II, but production level did not significantly increase, as was the case with raspberry. Milutinović et al. (1999) reported that around 12,000 t of blackberry was produced in Serbia in 1997. The production tripled by 2005, amounting to 28,000 t on a total area of about 5,000 ha (Nikolić et al., 2012**)**. According to Strik et al. (2007), Serbia is among the four leading global producers of blackberries, accounting for 69% of the European and 17.82% of the world's production. Small fruits, such as blackberries, have exceptional nutritional potential due to their high content of natural antioxidant compounds (Ding et al., 2006; Tulipani et al., 2008), which exhibit anti-inflammatory, antiviral, antimicrobial, and antioxidant activities (Reyes-Carmona et al., 2005). Berries are a rich source of a wide range of phenolics, including anthocyanins, flavonols, flavanols, and other flavonoids (Mikulic-Petkovsek et al., 2012; Mitic et al., 2014), which are responsible for the high free radical scavenging capacity measured by *in vitro* and *in vivo* assays (Kaume et al., 2012; Slatnar et al., 2012). Blackberries stand out among commercially important small fruits for their highest antioxidant capacity (Pantelidis et al., 2007), but the chemical composition of blackberries can vary depending on cultivar, growing conditions, ripeness stage, and other factors (Kaume et al., 2012). The appearance and internal

quality of fruit are the most important traits for fresh market and processing, and they are formed by important changes in the activities of a series of metabolic pathways during the ripening process. Fruit ripening is a biochemical process that changes physical and chemical characteristics. As a general rule, berries become softer, darker, and sweeter during the ripening process. Information on physical and chemical changes during fruit development is available for many fruit species (Kaume et al., 2012). The objective of the present research was to investigate changes in the physico-chemical properties of blackberry fruit of 'Čačanska Bestrna' and 'Loch Ness' at two maturity stages.

Materials and methods

Blackberry (*Rubus* subg. *Rubus* Watson) fruits of two cultivars ('Čačanska Bestrna' and 'Loch Ness') were harvested at two different stages of ripeness, namely fully ripe and over ripe. Fully ripe (optimally ripe) fruits were black, glossy and easily picked from the branches (Karaklajić-Stajić et al., 2017). Over ripe fruits were black, without the characteristic gloss and fell from the branches at the slightest touchThe investigation was conducted over a two-year period (2017–2018) in experimental planting at Gornja Gorevnica (43°53' N latitude, 20°20' E longitude, 290 m altitude) near Čačak, Western Serbia. Blackberries were planted at a spacing of 3.0 m betwen the rows and 1.5 m within the row, and trained to a three-wire trellis. Fertilisation, weed control and irrigation practices standard for the region were provided during both seasons. Weather conditions in Čačak were characterised by the mean growing season temperature of 17.0°C and total rainfall of 340.4 mm for the long-term averages. The soil in the blackberry planting was vertisol, moderately supplied with organic matter (2.92%) and poor in N (0.11%); soil pH in KCl 0.01 mol L^{-1} was 4.98. The contents of available soil P and K were 4.64 and 29.23 mg 100 g^{-1}, respectively. After harvest, 25 fruits of each cultivars and ripness stage (n=25) in four replicates were randomly selected (400 fruits in total) and used for determination of the most important physical and chemical properties. Fruit weight was measured using a Mettler balance (Toledo, Switzerland) with accuracy of ±0.01 g, and the data were expressed in g per fruit. Fruit dimensions (mm) (length, breadth, and fruit shape index) were determined in the samples by 'Inox' vernier scale (±0.05 mm accuracy). The shape index of the fruit was determined based on the length/fruit width ratio.Total soluble solids content (SSC) was determined by a digital refractometer (Carl Zeiss, Jena, Germany) at 20°C, and data were expressed in °Brix. Total phenolic content (TPC), were determined using a modified Folin-Ciocalteau method (Singleton et al., 1999; Liu et al., 2002) and the results were expressed as g of gallic acid equivalents per 100 g fresh weight of the sample (g GAE 100^{-1} g FW). Total anthocyanins content (TAC) was measured by the previously described pH-differential method (Torre and Barritt, 1977; Liu et al., 2002). Pigment content was calculated as milligrams of cyanidin-3-glucoside per 100 g fresh weight (mg cyn-3-glu 100 g^{-1} FW). Antioxidant capacity (AC) was determined using the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sánchez-Moreno et al., 1998), and expressed in μmol TE 100 g^{-1} FW. The data were presented as two-year mean value±standard error of mean (SE). Differences between means were compared by Duncan's Multiple Range tests in a two-way analysis of variance (ANOVA) using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The significance of differences at a 5% level among means was determined.

Results and discussion

Analysis of variance showed a significant effect of ripeness stage on the fruit breadth, SSC, and TPC, and AC, whereas the cultivar significantly affected the fruit length and breadth, SSC, TPC and TAC (Tables 1 and 2). On the other hand, the interaction effect of variability factors significantly affected the breadth and shape index of fruit (Figure 1).

Table 1. Influence of ripeness stage, and cultivar on the physical properties of blackberry fruits

Treatment	Fruit weight (g)	Length (mm)	Breadth (mm)	Shape index
Ripeness stage (A)				
Fully ripe	7.56±0.15 a	26.48±0.20 a	18.30±0.42 a	1.44±0.03 a
Overripe	7.64±0.22 a	26.57±0.21 a	18.19±0.60 b	1.48±0.05 a
Cultivar (B)				
'Loch Ness'	7.79±0.17 a	26.23±0.15 b	17.55±0.58 b	1.50±0.05 a
'Čačanska Bestrna'	7.41±0.18 a	26.82±0.17 a	18.85±0.20 a	1.42±0.01 a
ANOVA				
A	ns	ns	*	ns
B	ns	*	*	ns
A×B	ns	ns	*	*

Values within each column followed by the same small letter are not significantly different at p≤0.05 by Duncan's Multiple Range tests. *Significant differences; n.s. – not significant differences for P≤0.05 according to F test.

During the harvest of blackberry fruits at different stages of ripeness, higher average values of weight, length, and fruit shape index were observed in overripe fruits (7.64 g, 26.57 mm, and 1.48, respectively), while there were no statistically significant differences in fruit weight and length.

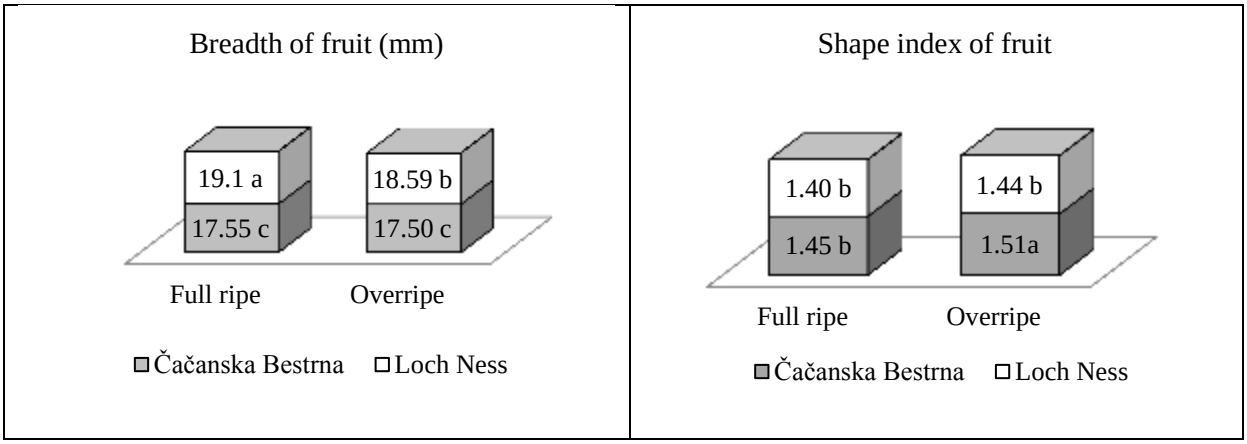

Figure 1. Interaction effects of ripeness stage/cultivar on the breadth of fruit and fruit shape index

When observed by cultivars, significantly higher values of fruit dimensions, length (26.82 mm) and breadth (18.85 mm) were determined in the cultivar 'Čačanska Bestrna'. The significance of differences in the interaction between ripeness stage and cultivar was only established for fruit width and fruit shape index (Figure 1). The largest width was found in fruits sampled at physiological ripeness in the 'Loch Ness' cultivar (19.10 mm), while the highest fruit shape index values (1.51) were recorded in overripe fruits of the 'Čačanska Bestrna' cultivar. Numerous authors state that this characteristic depends on the cultivar as

well as environmental conditions, cultivation practices, and so on (Eyduran et al., 2008). Unlike raspberries, Nikolić and Milivojević (2010) mention that the fruits of most cultivated blackberry cultivars are larger, with an average weight ranging from 3.5 to 7.5 g, and there are even larger cultivars, such as the domestic cultivar 'Čačanska Bestrna'. According to Stanisavljević (1999), the fruits of the 'Čačanska Bestrna' cultivar have the same shape as the parent cultivar 'Black Satin', which corresponds to an elongated-cylindrical shape. The fruit shape index values calculated in this study (>1) are consistent with the results obtained by Stanisavljević (1999) and Milivojević et al. (2010) for the blackberry fruit.

Table 2. Influence of ripeness stage, and cultivar on the chemical properties of blackberry fruits

Treatment	SSC (°Brix)	TAC (mg cyn-3-glu 100 g^{-1} FW)	TPC (g GAE 100 g^{-1} FW)	AC (µmol TE 100 g^{-1} FW)
Ripeness stage (A)				
Fully ripe	9.64±0.36 b	113.19±5.47 a	601.56±27.62 a	3.81±0.02 a
Overripe	10.80±0.43 a	118.08±10.31 a	581.47±8.48 b	3.76±0.04 b
Cultivar (B)				
'Loch Ness'	10.93±0.36 a	150.07±12.44 a	657.30±5.14 a	3.77±0.02
'Čačanska Bestrna'	9.51±0.34 b	81.20±5.03 b	525.73±8.48 b	3.81±0.03
ANOVA				
A	*	ns	*	*
B	*	*	*	ns
A×B	ns	ns	ns	ns

Values within each column followed by the same small letter are not significantly different at p≤0.05 by Duncan's Multiple Range tests. *Significant differences; n.s. – not significant differences for P≤0.05 according to F test.

The results of the chemical properties of the examined blackberry cultivars indicate that overripe fruits of both cultivars exhibited higher levels of SSC (10.80° Brix), and TAC (118.08 mg cyn-3-glu 100 g^{-1} FW), while the content of TPC (601.56 g GAE 100 g^{-1} FW) and AC (3.81 µmolTE 100 g^{-1} FW) were higher in fruits sampled in fully ripe stages. When observed by cultivar, higher values of all examined chemical parameters of the fruit, except for AC, were found in the 'Loch Ness' cultivar. The interaction effect of the variability factors (ripeness stage/cultivar) did not have a statistically significant impact on the mentioned blackberry fruit properties. The average values of SSC across all treatments ranged from 9.51 to 10.93° Brix, which were higher than the values reported by Miletić et al. (2006) for the 'Čačanska Bestrna' cultivar in eastern Serbia (7.1%), but lower than the values obtained by Stanisavljević (1999) for the conditions in Čačak (10.8%). Phenolic compounds in plants indirectly participate in plant growth processes, all metabolic processes, and also influence the organoleptic and nutritional properties of fruits. Furthermore, their physiological activity in the human body has been proven **(**Robards et al., 1999). The synthesis and distribution of phenolic compounds are influenced by the complex interaction of external factors (light, temperature, and humidity), internal factors (genetic factors and hormonal status) (Strack, 1997), agroecological factors (Veberič et al., 2014), as well as cultivation conditions and fruit ripening stage (Murillo et al., 2012). The TAC values obtained in this study were higher compared to the results obtained by Stajčić et al. (2012), Ivanovic et al. (2014), and Veberic et al. (2014) for the 'Čačanska Bestrna' cultivar. Milivojević et al. (2010) investigated the chemical properties of wild and commercial blackberry culrtivars and found that the TPC in the 'Čačanska Bestrna' and 'Loch Ness' blackberry fruit was 1.74 mg GAE g^{-1} FW, which was half of the values obtained in this study but comparable to the results obtained by Stajčić et al. (2012) for the same parameter and cultivar (235.09 mg GAE 100 g^{-1} FW). Among the

berry fruit species, blackberry fruit exhibits the highest AC, which is attributed to its high content of phenolic compounds, as reported by Pantelidis et al. (2007). Kähkönen et al. (2003) and Hosseinian et al. (2007) emphasize that the content and composition of anthocyanins in berry fruit species, in addition to genotype (cultivars), are influenced by light intensity, temperature conditions, soil type, moisture level, fertilizer and pesticide usage, and other stress factors. The relationship between AC and the TPC and TAC in blackberry fruit has been confirmed by numerous studies (Sellappan et al., 2002; Reyes-Carmona et al., 2005; Milivojević et al., 2011), indicating that the parameter of 'total phenols' can serve as an indicator of AC (Milivojević et al., 2010).

Conclusion

The results of this study showed that the ripeness stage of blackberry causes significantly increasing of SSC and TAC. The higher values of mentioned parameters have been noted in overripe berries compared to fully ripe fruit. Therefore, our results can be useful for further food processing and human metabolic research studies.

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THE EFFECT OF DIFFERENT MICROBIAL FERTILIZERS ON THE WEEDINESS OF SOYBEAN

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Abstract

The experiment with low-input technology of soybean was conducted at the research and study field "Radmilovac", Faculty of Agriculture Belgrade in 2022 on the luvic chernozem soil type, in completely randomized blocks. Cropping system included tillage with a disc harrow at 20-25 cm with the complete previous crop residues incorporation and the pre-sowing tillage with a harrow. The basic fertilization was conducted in autumn with 300 kg ha^{-1} NPK (15:15:15). For top dressing in spring, the following microbiological fertilizers were applied: biofertilizer ("Slavol", manufacturer "Agrounik" Serbia) with 5.0 l ha^{-1} in two treatments and Eko lame 10 l ha^{-1} in three treatments. The top dressing in the control variant was done with nitrogen fertilizer AN at the rate of 40 kg ha^{-1} N. The soybean (NS Maximus) cultivars were used. The crop was grown in a six-crop rotation (winter wheat-maize-spring barley+red clover-red clover-soybean-sunflower). Statistical analysis confirmed that top dressing had a greater effect on weediness of soybean. The weed community in investigated crops consisted of 11 weed species, with dominating: *Solanum nigrum* L., *Polygonum aviculare* L. and *Avena fatua* L., (annual species) and *Convolvulus arvensis* L. and *Sorghum halepense* (L.) Pers. (perennial species). The obtained results show that the highest number of weeds, weeds per species, fresh and air-dry biomass were recorded in the control treatment. The statistically lowest values for the number of weed plants per species were recorded in the treatment with Eko lame, but for fresh biomass in the treatment with Slavol. The differences in weediness in the variants with microbiological fertilizers were not statistically significant, while there were statistically very significant differences compared to the control. Application of microbiological fertilizers affected the initial faster growth of soybean plants and increased competitiveness against weeds.

Key words: *competition, weed, soybean, top dressing.*

Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume species of great importance for the seed structure of agriculture in our country. This fact is particularly interesting from the point of view of crop rotation. The dominance of the two crop rotation (maize- winter wheat) could be most likely improved by introducing the three crop rotation by growing this crop. Crop rotation creates a greater diversity of crop residues after harvesting, decomposition, or harvesting, which leads to an increase in the number of saprophytic microorganisms in the soil and improves the biological and enzymatic activity of the soil (Woźniak and Kavecka-Radomska, 2016). In the era of intensive (industrial) agriculture, there was no interest due to the use of large amounts of fertilizers, especially nitrogen, which led to positive results. It was

assumed that soybeans would gain more importance by switching to sustainable (low-input) cropping systems, as these systems imply a more rational use of fertilizers, favor crop rotation, and maintain or improve organic matter content. Modern agricultural systems usually tend to minimize energy inputs for tillage and use specific crop rotations that include 2-3 crop species requiring the same or similar cropping technology (Soane et al., 2012; Roche et al., 2017). This approach has resulted in a slight increase in acreage, which has declined over the past decade due to increasing pressure from global climate changes, particularly high air temperatures combined with a lack of summer precipitation, at the time of soybean flowering, when this crop is most vulnerable. In addition, soybean is a late spring sown crop that is very sensitive to weeds in the early stages of growth and development.

Soybeans do not make great demands on cultural practices, especially fertilization, as they can significantly take advantage of previous crops (dense) in terms of reducing weediness or improving the soil after crops (maize), which are known to deteriorate especially soil structure (Dolijanović et al., 2019). To achieve optimal yields, weed control, especially at the beginning, and adequate fertilization are important. Fertilization of soybean is special because this crop meets part of its nitrogen needs from the atmosphere, and larger amounts of mineral nitrogen applied to the soil can also reduce yield (Abduladim et al., 2021). Fertilizer application should be based on the principle of controlling soil fertility, i.e. maintaining or improving soil fertility to achieve high and stable yields (Đukić and Dozet, 2014). In addition to the application of NPK and nitrogen fertilizers, microbiological fertilizers have recently been increasingly used by treating seeds, soil, and/or leaves during the growing season, resulting in increased plant resistance and improved microbiological activity of the soil and plant root system. Microbial fertilizer helps the plants grow healthy and stay strong due to including fertilizer and beneficial microorganisms. Microbial fertilizers (MF), also known as organic fertilizers, are environment friendly because they mainly consisting of food and agricultural waste (animal manure, straw etc.) and beneficial microorganisms (Sahin and Yilmaz, 2023). Seed treatment with microbiological fertilizers generally has the least impact on poorer soils, especially in terms of pH. Low pH, i.e., increased soil acidity, results in lower efficacy, which can be improved to some degree by foliar application of a microbiological preparation.

The objective of this paper is to investigate the influence of applied microbial fertilizers (through seed and foliar treatments) on the weediness of soybean crops, in addition to the base fertilizer applied in the fall.

Material and methods

The experiment with low-input technology of soybean was conducted at the research and study field "Radmilovac", Faculty of Agriculture, University of Belgrade (Serbia) in 2022 on the luvic chernozem soil type, in completely randomized blocks. Cropping system included tillage with a disc harrow at 20-25 cm with the complete previous crop residues incorporation and the pre-sowing tillage with a harrow. The basic fertilization was conducted in autumn with 300 kg ha^{-1} NPK (15:15:15). For top dressing in spring, the following microbial fertilizers were applied: biofertilizer ("Slavol", manufacturer "Agrounik" Serbia) with 5.0 l ha^{-1} in two treatments and Eko lame 10 l ha^{-1} in three treatments. The top dressing in the control variant was done with nitrogen fertilizer AN at the rate of 40 kg ha^{-1} N. The soybean (NS Maximus) cultivars were used. The crop was grown in a six-crop rotation (winter wheat-maize-spring barley+red clover-red clover-soybean-sunflower). The sowing of soybeans took place on 15.04. in 2022. The size of a one crop rotation field (crop) was about 10 ar.

The application of microbial fertilizers and herbicides in soybean cultivation and the evaluation of weediness followed the schedule shown in Table 1. Seeds were treated 24 hours

before sowing, and the other treatments were applied over the leaves with a hand sprayer designed for this type of experiment.

Table 1. Schedule of application of microbiological preparations, time of assessment of weediness and application of herbicides

Preparation/ Date	Seed treatment	First treatment-foliar	Weediness evaulation	Herbicide	Second treatment-foliar	Third treatment-foliar
Eko lame	14.04.	17.05.	30.05.	31.05.	03.06	17.06
Slavol	14.04.	17.05.	30.05.	31.05.	03.06	-

One day before applying herbicides in soybeans, we conducted an evaluation of weeds and determined the following parameters: the number of weed species, the number of plants per species, their aboveground fresh and dry weights in the control and investigative treatments. The presence of weeds in soybean was influenced by the fertilizers applied by the seed treatment and the first foliar treatment, while the other foliar treatments (1 or 2) had an influence on the quantity and quality of soybean yield. All parameters of weeds were determined by the method of random squares with an area of 1 m^2.
Obtained data were statistically processed by the analysis of variance, in which microbial fertilizers were factors, while LSD test was applied for the individual comparisons.

Results and discussion

The results of the influence of different types of microbiological preparations on weediness of soybean crops are shown in Table 2. Statistical analysis showed that, in general, there was a statistically significant difference in the number of certain weed species between the applied microbiological preparations and the control variant. There was also a statistically significant difference in the number of weed plants per species between the treatments tested and the control, while the fresh and dry weight of weeds was highest in the control and the differences in weight between the two fertilizers tested were not statistically significant (Table 2).

Table 2. Weediness (No of weed plants m^{-2}) of soybean

Life forms	Weed species	Control	Eko lame	Slavol	Average
T	*Amaranthus retroflexus* L.	3.5^b	2.2^a	2.0^a	2.6
G	*Sorghum halepense* L. Pers.	10.3^b	9.7^a	9.9^a	9.9
T	*Solanum nigrum* L.	11.8^c	8.8^b	7.0^a	9.2
T	*Avena fatua* L.	5.5^c	4.1^a	5.3^b	4.6
T	*Polygonum aviculare* L.	6.3^c	2.7^a	3.2^b	4.1
T	*Amaranthus albus* L.	2.0^a	1.7^a	2.1^a	1.9
T	Agropyrum repens (L.) Beauv.	3.6^b	2.6^a	2.8^a	3.0
G	*Convolvulus arvensis* L.	3.1^b	2.6^a	2.9^a	2.9
T	*Datura stramonium* L.	1.0^b	0.5^a	0.7^a	0.7
G	*Cirsium arvense* L. Scop.	0.7	0.3	-	0.3
G	*Cynodon dactylon* L. Pers.	0.5	-	0.4	0.3
Total number of weed species		11^b	10^a	10^a	10.3
Total number of plants per species		48.0^c	34.0^a	37.0^b	39.7
Aboveground fresh weight of weeds (g m^{-2})		2127.1^b	1705.7^a	1789.3^a	1874.0
Aboveground dry weight of weeds (g m^{-2})		578.9^b	507.6^a	521.4^a	536.0

T-therophytes, G-geophytes; Values of means followed by the same letter are not significant.

The significantly higher weed mass on the control variant was the result of a higher incidence of perennial and annual broadleaf weeds. The application of the two microbiological fertilizers resulted in a reduction of weed emergence in the soybean crops, especially in the most important parameters: the number of weed plants per species and the fresh weight of weeds per unit area. As in previous experiments at this site (Dolijanović et al., 2011), it is important to emphasize that high weed establishment was observed in the six crop rotation. The six crop rotation includes a large number of crops in frequency, which provides more favorable conditions for higher weediness. The weed synusia of soybean consisted of 11 weed species with a dominance of therophytes. The dominant weed species in soybean synusia were: annual *Solanum nigrum* L., *Polygonum aviculare* L. and *Avena fatua* L., and perennial *Convolvulus arvensis* L., *Sorghum halepense* (L.) Pers. and *Agropyrum repens* (L.) Beauv.
There are numerous measures that can reduce weed infestation in soybean crops, such as crop rotation (Dolijanović et al., 2010; 2019), application of herbicides and mulch (Simić et al., 2008), seed rate (Purucker and Steinke, 2020), cultivation of cover crops or by combining soybean crops with grasses (Janošević et al, 2017), and by reducing the tillage system (Kovačević et al, 2008), and there are fewer data on the influence of fertilizers (especially microbiological) on the occurrence of weeds in soybean cultivation.
For fertilizers and fertilization of soybean, the optimal application of fertilizers and the compatibility of the application of base and supplemental fertilizers are important for adequate weed control. Foliar fertilizers have high efficiency in the early stages of application, which affects the accelerated growth and reduced susceptibility of soybeans to weeds at the beginning of the growing season. The results of Liana et al. (2021) showed that basic fertilizer doses had significant effect on soybean growth and competivnes again weeds.

Conclusion

Microbial fertilizers are substances containing microbes that promote plant growth at the beginning of the growing season and increase competitiveness against weeds. They are used to increase crop yields in an environmentally friendly way, based on the principles of sustainable agriculture. As a result of the study, it can be said that the use of microbial fertilizer in optimal dose and timely application can be more profitable. Future studies involving additional fertilizer applications, row spacing, and planting dates under different environmental conditions will provide additional information on weediness in soybeans.

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EVALUATING THE ROLE OF PHENOLIC COMPOUNDS IN OLIVE BUD DIFFERENTIATION

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Abstract

Climate change affects the physiology and biochemistry of plants in multiple ways. Olive, the iconic evergreen tree of the Mediterranean basin, is often subjected to warm winters that can affect its bud differentiation and result in low fruit set and consequently yield. The present trial aimed to assess the sensitivity of 10 olive cultivars to warm winter conditions and the possible role of phenolic compounds of the leaves. Five young plants of each one of the 10 cultivars were placed in a greenhouse during the winter (November to March) while at the same time, five other plants were exposed to winter conditions outside of the greenhouse. During the trial period, successive leaf samplings took place (at approximately one-month intervals) in order to assess the changes in the total phenolic compound concentration and the antioxidant capacity of the leaves. The flowering of each plant was recorded as well as the percentage of the perfect flowers. Among the cultivars, five of them flowered both in and out of the greenhouse, four of them flowered only outdoors and one did not flower at all. The percentage of perfect flowers was higher in the plants grown outdoors, while there was not a clear relation between the concentration of phenolic compounds and antioxidant capacity with the flowering or not of the cultivar. In most cases, however, leaves from plants grown in the greenhouse presented lower concentration of phenolic compounds than those from trees growing outdoors.

Keywords: *Antioxidant capacity, Climate change, Flowering, Winter.*

Introduction

The olive tree is the iconic tree of the Mediterranean basin with high socioeconomic significance for the people of this area (Fraga *et al.*, 2020b; Orlandi *et al.*, 2020; Kaniewski *et al.*, 2023). It is widespread in areas characterized by Mediterranean-type climate where it grows and produces at most. Nonetheless, due to the increasing earth temperature, the olive tree faces great challenges regarding its growth as well as its productivity potential (Kaniewski *et al.*, 2023). Global warming is by now evident in many if not all areas around the globe, causing extreme weather events such as drought, sudden and heavy rainfalls, and extreme temperature (low or high ones etc (Pachauri *et al.*, 2014). Olive tree cultivation used to be restricted to areas where the temperature should not fall below -7 to -8 ^{o}C, as below that limit there is a great risk for permanent tree damage (Fraga *et al.*, 2020a; Fraga *et al.*, 2020b). High winter temperature on the other hand, negatively affects olive bud dormancy release as well as flower bud differentiation, as the overall flower formation depends on adequate winter chilling, which strongly depends on the genotype (Rallo and Martin, 1991; Fraga *et al.*, 2020a; Mougiou *et al.*, 2020). After relatively warm winters, olive produces a low number of flowers or even a great percentage of staminate flowers with subsequent low fruit set and production.

Phenolic compounds are ubiquitous compounds in the plant kingdom and have been involved in the alternate bearing of olive trees (Lavee *et al.*, 1985; Lavee *et al.*, 1993; Malik and Bradford, 2006). The present trial aimed to assess the sensitivity of ten olive cultivars to warm winter conditions regarding flower bud differentiation and the possible role of phenolic compounds to this phenomenon.

Materials and Methods

One-year-old self-rooted plants of ten olive cultivars were selected for this trial, i.e. Kalamata (KAL), Koroneiki (KOR), Lianolia Kerkiras (LIA), Mastoids (MAS), N-K Gigas (NK), Megaritiki (MEG), Galatistas (GAL), Chalkidikis (CHA), Arbosana (ARB) and Arbequina (ARQ).

Five plants from each cultivar were placed in an unheated plastic greenhouse from November to March (when they were transferred outdoors), while five other plants per cultivar were grown outdoors. All the necessary cultivation management techniques were applied to achieve unhindered growth, while a temperature recorder was placed in the greenhouse and outdoors to monitor the temperature prevailing during the experimental period.

Four sampling events took place during the experimentation period, starting from the 15th of December till the 15th of March. In every sampling event, a total of 3-4 leaves per plant were sampled, and all together comprised one bulk biological sample, put into a felizol cold box filled with ice gel packs, transferred to the laboratory, and put into a freezer. The leaf samples were then lyophilized and ground into a fine powder and then stored in the freezer till the analyses. During the flowering period, the number of perfect flowers was recorded as well as the intensity of flowering per plant was estimated (density of flowers per canopy volume).

Phenolic compounds were extracted according to (Roussos *et al.*, 2009). The total phenols (TPHEN, as mg gallic acid equivalents g^{-1} dry weight), the total o-diphenols (oDs, as mg caffeic acid equivalents g^{-1} dry weight) and the total flavonoids (FLOIDS, as mg caffeic acid equivalents g^{-1} dry weight) as well as the antioxidant capacity of the extracts based on the diphenyl picryl hydrazyl (DPPH, as μmol Trolox gallic acid equivalents g^{-1} dry weight) and ferric reducing capacity (FRAP, as μmol Trolox gallic acid equivalents g^{-1} dry weight) assays were determined according to (Roussos *et al.*, 2009).

The trial was designed as a pseudoreplicated trial with three replications. The raw data of the biochemical analyses were analyzed aiming

- at finding differences between cultivars under the same growing conditions irrespective of the sampling event (glasshouse (G) or field (F) conditions),
- at finding differences among the different sampling events under the same conditions, irrespective of the cultivar.

Results and Discussion

The mean temperature recorded in the glasshouse was -3.4 ^{o}C in January, and the highest one 47.4 ^{o}C in March with the average temperature being 14.5 ^{o}C. Under field conditions, the lowest temperature recorded was -5.5 ^{o}C in January, and the maximum one 33.1 ^{o}C in March with the average being 9.4 ^{o}C. It is obvious that under field conditions one should expect that chilling requirements should have been fulfilled while under greenhouse conditions there could be a high-temperature stress during March, just before the plants are transferred outdoors.

There were significant differences among cultivars in terms of flowering as can be seen in Table 1. Mastoidis (MAS) did not flower at all, while KAL, LIA, GAL, and CHA flowered only when the plants were exposed to field conditions. Such differences between cultivars

have been also observed by other researchers, working on olive flower bud differentiation (Mougiou *et al.*, 2020).

Table 1. Effect the greenhouse (G) and field (F) conditions on the flowering of the ten olive cultivars.

Flowering both under G and F conditions	**Flowering only under F conditions**	**Not flowering at all**
KOR	KAL	MAS
ARB	LIA	
NK	GAL	
ARQ	CHA	
MEG		

In the following Figure 1 it is obvious that in most cases (regarding cultivars flowering both indoors and outdoors) when the plants have been exposed to field conditions, the percentage of perfect flowers was slightly higher than that observed in plants grown in the greenhouse. An exception to this was ARB which presented a slightly higher percentage of perfect flowers when grown under greenhouse conditions. Similar differences among cultivars, regarding the percentage of perfect flowers have been reported by other researchers too (Mougiou *et al.*, 2020).

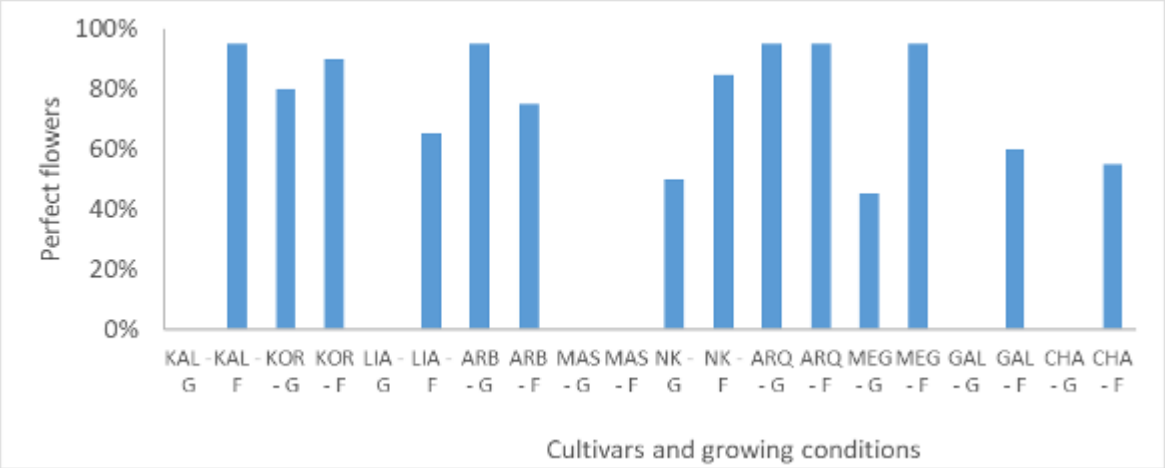

Figure 1. Effect of the greenhouse (G) and field (F) conditions on the percentage of perfect flowers per cultivar.

There were differences regarding the flowering density among cultivars as well as between G and F conditions (Figure 2). In the cases where flowering was observed both indoors and outdoors, trees growing outdoors exhibited higher flowering density than those growing under G conditions. This was most obvious regarding ARB and ARQ, while the least difference was observed in NK. On the other hand, ARB exhibited the higher flowering density among cultivars growing outdoors, followed by LIA and ARQ, while the least flowering density was recorded in ARB and ARB growing under G conditions. Similar results have been also observed by (Mougiou *et al.*, 2020).

The concentration of the assessed phenolic compounds as well as the antioxidant activity of the leaf extracts among cultivars growing under the same conditions are shown in the following table (Table 2). Under G conditions, ARB presented the highest total phenol and total o-diphenols concentration and among the highest antioxidant capacity based on DPPH assay. On the contrary, NK presented the lowest total phenol and total o-diphenols and the

lowest total flavonoids concentration and antioxidant capacity based on DPPH assay. Under field conditions ARB again had the highest total phenols and o-diphenols concentration and among the highest antioxidant capacity based on both DPPH and FRAP assays. On the other hand, NK again presented the lowest total phenols and o-diphenols concentration and between the lowest total flavonoids concentration and antioxidant capacity based on both DPPH and FRAP assays.

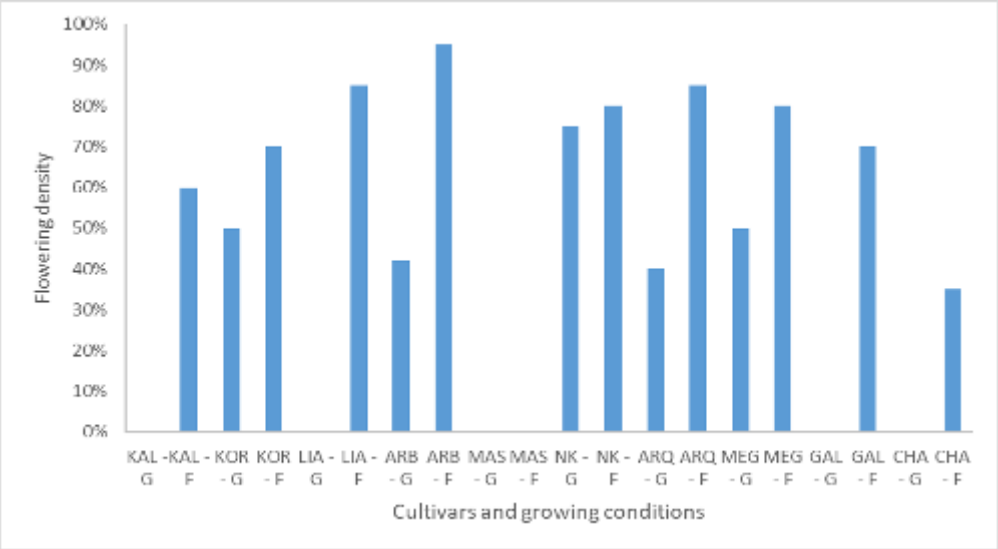

Figure 2. Effect of the greenhouse (G) and field (F) conditions on the flowering density per cultivar.

In Table 3 it is interesting to observe that leaf extracts from trees growing under field conditions exhibited higher total o-diphenols and flavonoids concentration as well as antioxidant capacity based on DPPH assay.

Table 4 presents the temporal evolution of the measured biochemical parameters in olive leaves irrespective of the cultivar. Under greenhouse conditions, the concentration of total phenols, total o-diphenols, total flavonoids, and antioxidant capacity (FRAP assay) exhibited a significant reduction from the first to the second sampling event (from December to January), while afterward remained at low levels. Under field conditions, though after a significant decrease in total phenols concentration during the second sampling, their concentration slightly increased and remained steady. On the contrary, the concentration of o-diphenols was steadily increasing between the first and third sampling event when it remained steady. On the other hand, the concentration of total flavonoids was reduced between the second and third sampling event while the antioxidant capacity did not undergo any significant changes.

Table 2. Differences among cultivars growing under greenhouse (G) and field (F) conditions regarding the concentration of the various phenolic fractions and antioxidant capacity of olive leaf extract.

GLASSHOUSE									
CV	**TPHEN**		**oDs**		**FLOIDS**		**FRAP**		**DPPH**	
KAL	34.27	cd	13.61	b	30.32	bc	192.51	c	658.43	b
KOR	34.62	cd	10.72	c	27.28	cde	201.83	bc	644.78	b
LIA	41.67	ab	9.87	c	35.02	a	210.24	abc	778.80	a
ARB	44.88	a	18.14	a	29.54	bcd	217.78	abc	770.11	a
MAS	29.28	de	8.71	cd	26.11	de	151.49	d	581.54	bc
NK	26.70	e	6.94	d	20.61	f	143.94	d	507.28	c
ARQ	34.14	cd	14.02	b	30.15	bcd	221.74	ab	608.06	b
MEG	33.29	cd	10.67	c	24.95	e	191.80	c	594.07	b
GAL	30.13	de	10.43	c	20.02	f	138.39	d	503.38	c
CHA	38.37	bc	14.15	b	32.25	ab	235.66	a	751.98	a
FIELD										
CV	**TPHEN**		**oDs**		**FLOIDS**		**FRAP**		**DPPH**	
KAL	35.76	cd	14.55	bc	29.62	bc	183.99	b	642.59	bc
KOR	38.74	abcd	13.10	cd	34.83	a	233.07	a	702.59	ab
LIA	39.16	abc	11.95	d	32.16	ab	218.36	a	821.32	a
ARB	42.54	a	21.04	a	30.48	ab	237.29	a	795.86	a
MAS	28.12	e	9.34	e	25.69	c	137.01	c	609.94	bc
NK	21.83	f	5.82	f	20.10	d	111.56	c	523.51	c
ARQ	41.20	ab	15.91	b	34.69	a	245.32	a	665.80	b
MEG	38.86	abcd	15.76	b	34.62	a	235.07	a	649.88	b
GAL	33.99	d	14.93	b	20.65	d	178.97	b	613.46	bc
CHA	36.43	bcd	14.55	bc	32.10	ab	224.80	a	726.28	ab

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on Tuckey HSD test at α=0.05.

Table 3. Differences between greenhouse (G) and field (F) conditions regarding the concentration of the various phenolic fractions and antioxidant capacity of olive leaf extract.

Condition	**TPHEN**		**oDs**		**FLOIDS**		**FRAP**		**DPPH**	
Field	35.98	a	13.83	a	29.73	a	202.8	a	676.4	a
Greenhouse	34.91	a	11.69	b	27.63	b	190.7	a	641.2	b

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on Student's T test at α=0.05.

Table 4. Effect of sampling event on the concentration of the various phenolic fractions and antioxidant capacity of olive leaf extract under greenhouse (G) and field (F) conditions.

GLASSHOUSE									
Time	**TPHEN**		**oDs**		**FLOIDS**		**FRAP**		**DPPH**	
T1	38.02	a	12.75	a	30.05	a	211.88	a	660.35	a
T2	34.67	b	11.59	b	26.36	b	191.05	b	643.81	a
T3	32.92	b	11.25	b	27.32	b	180.22	b	630.93	a
T4	33.33	b	11.32	b	26.77	b	178.99	b	624.27	a
FIELD										
CV	**TPHEN**		**oDs**		**FLOIDS**		**FRAP**		**DPPH**	
T1	37.53	a	12.07	c	30.94	a	202.97	a	695.44	a

T2	34.16	b	13.11	b	31.11	a	196.08	a	651.10	a
T3	35.50	ab	15.05	a	27.79	b	207.39	a	688.71	a
T4	35.45	ab	14.54	a	28.15	b	198.12	a	665.25	a

Means within the same column, under the same growing conditions, followed by different letters differ significantly based on Tuckey HSD test at α=0.05.

Conclusion

Based on the results of the present trial it is obvious that the cultivars behave differently under greenhouse and field conditions, with some of them being able to produce perfect flowers under both environmental conditions. Under the aspect of climate change, it is necessary to recognize which cultivar is better suited in areas with mild or even relatively warm winters without jeopardizing its production potential. Phenolic compounds seem to have a role in the effect of flower bud differentiation, but more research is needed regarding the changes in individual phenolic compounds.

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EVALUATION OF YIELD COMPONENTS OF LETTUCE (*Lactuca sativa* L.) GROWN IN NUTRIENT FILM TECHNIQUE

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Abstract

The main objective of this study was to investigate the impact of different nutrient solutions on the productivity of lettuce (*Lactuca sativa* L.) grown using a hydroponic system. The research was conducted in a greenhouse located at the Experimental Field of the Biotechnical Centre Naklo (Slovenia) in the year 2021. The experiment focused on four different cultivars of lettuce: 'Lolo Rosso', 'Lolo Biancoo', 'Kiloma', and 'Vanity'. The Nutrient Film Technique was employed to cultivate these cultivars. The study was replicated three times, with each repetition consisting of 20 plants. Throughout the growth period, the plants were provided with appropriate care and followed technical and scientific recommendations. Four different types of nutrient solutions were compared in the experiment: the first treatment involved a nutrient solution containing 0-5-4 NPK (T1), second 2-4-1 (T2), third 2-1-6 (T3) and fourth treatment utilized a solution with a composition of 5-5-5 NPK (T4). After duration of 52 days, the harvested lettuce plants were subjected to various analyses. The obtained results were subjected to statistical analysis using multifactor analysis of variance (ANOVA). The findings of the experiment revealed significant differences between the nutrient solutions used and also among the different cultivars of lettuce. No significant differences in the leaf number per rosette, dry matter and vitamin C were observed between treatments. The growth analysis revealed that T4 increased the rosette height and weight, nitrates, total chlorophyll and carotene content in rosette leaves. Experiment has also shown that growth parameters significantly depended on the genetic factor (cultivar). Based on the results, we can conclude that the choice of nutrient solution and the lettuce cultivar can have an influence on the yield parameters and quality attributes of lettuce production under a NFT hydroponic system.

Keywords: *lettuce, Lactuca sativa, hydroponic NFT system, nutrients, yield components.*

Introduction

Demographers estimate that population growth will become one of the most important challenges of the 21st century, as the annual growth rate worldwide exceeds 1% (Kopač and Rangus, 2017). As a result, the need for higher food production is increasing, so agriculture must adapt to population growth and become more productive and sustainable. According to the FAO, agriculture is to become 60% more productive by 2050. By then we will need 9.8 billion kilograms of food every day. It seems that intensive agriculture is no longer a model that can feed 8 billion people. It was also found that, apart from animal husbandry, the greatest environmental impact comes from monoculture, which uses a large amount of pesticides (Stajnko et al., 2016).

In addition, the world is facing numerous environmental problems such as floods, droughts, extinction of plant and animal species, rural exodus, and food insecurity. These problems significantly affect and hinder conventional or soil-based crop production. Therefore, farmers have begun to look for new solutions in this area, including in Slovenia. With advanced cultivation technologies, we can compete with large-scale farms in the Netherlands and Italy, from where we import many vegetables. The adoption of advanced technologies is also

encouraged by low productivity. For example, an average Slovenian farmer produces food for 10 people, while a Danish farmer can meet the needs of 37 people and an American farmer can feed as many as 64 people (Germšek, 2016).

Among the advanced technologies is hydroponic farming. The hydroponics is a high-tech method of growing plants in water rich in mineral nutrients instead of soil. This system is used in areas where vegetables can be grown on limited land. However, it requires significant initial investment and knowledge. Hydroponic systems not only make it possible to avoid crop rotation, but also guarantee high yields and allow farmers to control the supply of nutrients according to the needs of plants and their development (Osvald et al., 2001).

One of the hydroponic techniques for the production of leafy vegetables is the nutrient film technique (NFT). This technique allows more efficient use of water, which is becoming a scarce resource in Europe and Western countries where hydroponics is spreading in drier areas (Tomasi et al., 2015). In NFT system the roots are exposed to a nutrient solution containing all the elements necessary for the growth of a particular plant, in the right concentration and pH. Beside with modifications, the NFT is the most commonly used liquid system. The system is based on plastic channels up to 20 meters long with a slope of 1 to 2%, through which a thin film of nutrient solution flows continuously. At the top of the channels are openings into which the plants are inserted, so that the roots can be partially immersed in the thin flowing layer of water (Goddek et al., 2016). At the top of the channel, the nutrient solution is supplied by a pump and flows through plastic tubing to the lower levels of the channel and drained into a below ground reservoir. In the reservoir, the nutrient solution is analysed and missing nutrients are added before it is fed back into the system. In our study, we wanted to investigate whether the cultivar and composition of the nutrient solution for hydroponic lettuce cultivation affect the morphometric characteristics and biochemical quality of the yield. Lettuce was chosen for testing because it is one of the plants that is easier to grow in the greenhouse.

Material and methods

The experiment took place from April 6th to May 27th 2021, in a research greenhouse located at the Laboratory Field of the Biotechnical Centre Naklo (at an altitude of 420 m; $\varphi = 46° 17′ 12″$; $\lambda = 14° 18′ 36″$) in Slovenia. The study involved the cultivation of lettuce using the nutrient film technique (NFT). The nutrient solution was prepared in four containers with a volume of 10 litters. The commercial hydroponic inorganic solution based on Hoagland and Aron solution was obtained from General Hydroponics Flora Gro. Solution T1 (0-5-4), solution T2 (5-0-1), solution T3 (2-1-6) and solution T4 (3-1-6) were mixed and prepared according to the manufacturer instructions. Additionally, we prepared 0.5 litters of micronutrient concentrate separately and measured out 80 ml into a 200-liter reservoir.

In the experiment, we compared four lettuce cultivars that are grown commercially for fresh market in Slovenia:

- 'Lollo Rosso', which has a rosette growth shaped and is a special type of loose-leaf lettuce suitable for year-round cultivation. The leaves are dark red, shiny, and curly. This cultivar is resistant to bolting;
- 'Lollo Bianco', which has similar characteristics to 'Lollo Rosso' and differs only in the colouring of the leaves, which are shades of green;
- 'Kiloma', a fast-growing variety suitable for spring and autumn cultivation. It is resistant to leaf margin browning and produces a very uniform yield;
- 'Vanity', a very adaptable variety that forms large, tightly closed heads of light green colour. It is resistant to leaf margin browning and rarely bolts even under suboptimal growing conditions.

We sowed the seeds in seed trays with 84 cells, using Gramoflor substrate for vegetables. When the seedlings had developed 3 to 4 true leaves, we transplanted them into net pots with a diameter of 4 cm. When transplanting we surrounded the root ball with rock wool. The seedlings were placed in plastic channels and supplied with the nutrient solution. Air and water temperatures in the reservoirs were measured every day between 11 a.m. and 1 p.m. with a digital thermometer. Simultaneously, the nutrient solutions were monitored for electrical conductivity and pH during the entire growing period. When the electrical conductivity values dropped below 2 mS/cm, we added nutrients to the solutions. To ensure optimal nutrient uptake, we maintained the pH within the set range of 5.5 to 6.5 by the addition of 30% sulphurous acid. The average daily temperature in the greenhouse was 12°C(±2), while relative humidity was maintained at 75%(±10) through passive ventilation.
The experiment was randomised designed with 3 replications, resulting in 6 treatments. Each replication consisted of 20 plants.
After 52 days, the plants were manually harvested. Morphometric characteristics were measured using a ruler and an electronic balance. We measured the height of the rosettes, counted the number of leaves, dry matter content (%) and weighed the mass of the rosettes (g). Nitrates were determined with a Segmented Flow Analyser. Vitamin C extraction was carried out following the procedure described by Medina-Lozano et al. (2020). Total chlorophyll and carotenoids content was determined following the methodology of Moreira et al. (2003).

Results and discussion

At harvest, no physical differences were observed between the lettuces in all treatments. All leaves were crisp and healthy.
Influence of type of nutrient solution on the morphological properties of lettuce are showed in Table 1.
The highest rosette (32.16 cm) was registered from T4, whereas the shortest rosette (16.16 cm) was observed from T1 (solution without N). It was revealed that with the higher doses of nitrogen level, increase plant height was observed, where no nitrogen application showed lowest plant height. The same trend was noted with rosettes (data not shown). Nitrogen fertilizer ensured favourable condition for the elongation of lettuce plant with optimum vegetative growth and the ultimate results was the highest and the widest plant. These results are in agreement with those obtained by Tittonellet et al. (2003). Rosette height of lettuce did not varied significantly between cultivars. Similarly, cultivars showed no significant influences on rosette width (not shown). Comparably study reported no significant difference in the rosette height and width of lettuce grown using NFT hydroponic system (Acharya et al., 2021).
Numbers of full-expanded leaves often follow the technological maturity of salad plants. Although the previous experiments demonstrated that increasing N concentration in nutrient solution will increase the leaves production (Fallovo et al., 2009), there was no significant difference in the leaves number per rosette where treatment compared. Frasetya et al. (2018) suggested that NFT systems ensure constant supply of water and nutrients to the plant, which is advantageous for plant growth and yield (in terms of number of leaves for lettuce). Lettuce is a leafy vegetable that is ideally consumed in fresh form, therefore the higher the number of leaves and good hygiene, the better.
The percent of dry matter in fresh leaves of lettuce, immediately after harvest, varied from 13.4 to 14.2%. According Raupp (2018) percentage of dry matter is an important reference parameter, and is somewhat significant as well to a consumer who does not want to buy watery products. In our experiment, the nutrient solution did not have significant effect on this

parameter. In spite of this fact, there were tendencies for the dry matter accumulation to increase slightly as the nitrogen concentration in nutrient solution increased. On the other hand, the percentage of dry matter was no affected by cultivar.

The weights of rosettes or yields differed greatly between cultivars and nutrient solutions used. The mean rosette weight at harvest increased with N application rate in solutions. All lettuce cultivars from solution T4 had significantly higher weight compared to those from other solutions. These results are not in correspondence with the findings of Mahlangu et al. (2016) who observed that increasing N supply in non-circulating hydroponic system failed to show any increase in fresh weight of the lettuce. However, dissimilarity in results could be due to the type of growing system used or it can also be due to the cultivar. In addition, Agüero et al. (2011) mentioned that the physical stress caused by the break in nutrient could contribute to the increased metabolism; lettuces under different nutrient solutions encountered the same physical stress, so the main factor that accelerated lettuce respiration could cause weight loss.

Among the four analysed cultivars, 'Vanity' had a significantly higher rosette weight compared to the three other cultivars.

Table 1. Mean values on the morphometric parameters and rosette weight of lettuce under different hydroponic solutions

Treatment	Rosette height (cm)	Leaf number (rosette^{-1})	Dry matter content (%)	Rosette weight (g rosette^{-1})
T1	16.16 c	26.4 a	13.4 a	154.2 c
T2	23.84 b	25.4 a	13.7 a	174.8 bc
T3	26.38 b	28.4 a	13.8 a	183.4 b
T4	32.16 a	28.2 a	14.2 a	229.6 a
Cultivar				
'Vanity'	28.54 a	25.8 a	14.1 a	272.8 a
'Kiloma'	24.14 a	27.3 a	13.5 a	201.4 b
'Lolo Rosso'	26.42 a	25.6 a	13.8 a	210.6 b
'Lollo Bianco'	26.48 a	23.6 a	13.4 a	18.6.2 b

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The present results revealed that the composition of hydroponic solutions determines several biochemical parameters (Table 2).

The average values of nitrate content in lettuce leaves differ greatly after treatments. The nutrient solution enriched in N (T4) resulted in significantly higher nitrates as compared to the other nutrient solution. The highest nitrate content was contained in lettuces that were grown in solution T4 (504.2 mg kg^{-1}). Least nitrates were found in lettuces, cultivated in solution T1 (216.6 mg kg^{-1}). Generally, all four cultivars from treatment T4 accumulated significantly higher nitrates residue compared to other solutions (not shown). The measurements of nitrates in lettuces in our experiment are comparable to those in Greece (Anastasios and Constantinos, 1999) and in Denmark (Petersen and Stolz, 1999). On the other hand, the average nitrate content in lettuce grown in Belgium (Dejonckhheere et al., 1993), Great Brittan (Ysart et al., 1999), France (Malmauret et al., 2002) and in Italy (Forlani et al., 1997) were much higher.

According to Kmecl et al. (2017) the nitrate content in vegetables depends on many factors, such as variety, fertilisers usage, soil properties, horticultural practices, weather conditions, light exposure, length of growth period … At the same time, the variation in nitrate content can be described by the variable intensity of metabolic action in the different parts of crops (Mahlangu et al., 2016).

Vitamin C content in the lettuce leaves tended to increase in lower nitrogen value of the nutrition solution. However, no statistically significant difference ($p > 0.05$) was found. The mean vitamin C concentration in cultivars ranged between 94.5 mg 100 g^{-1} DW ('Lollo Bianco') and 304.6 mg 100 g^{-1} DW DW ('Lollo Rosso'). The contents of vitamin C collected are higher than that announcement in previous studies carried out in the same cultivars (i.e. 'Lollo Rosso') (Llorach et al., 2008). According to Ignat et al. (2011), accumulation of vitamin C in vegetables depends on crop cultivars, cultivation method, climatic situation, as well as maturity at harvest.

The nutrient solution had an apparent influence on chlorophyll contents in lettuce leaves. The contents of total chlorophyll were highest in T4 (0.62 mg g^{-1} FW) solution followed by T3, T2 and T1. There may be a linear relationship between N in solution and total chlorophyll. Fraile et al. (2017) also mentioned that nitrogen is important to chlorophyll accumulation and that reducing N content in a nutrient solution reducing chlorophyll synthesis. Furthermore, the results of present study are in agreement with those obtained by Fallowo et al. (2009). In our study, the highest contents of chlorophyll were recorded in cultivar 'Vanity'. Cassetari et al. (2015) revealed that genetic variability impact chlorophyll in lettuce.

Carotene contents followed a similar trend as total chlorophyll. These findings are in agreement with those obtained by Phibunwatthanawong and Riddech (2019).

Table 2. Mean values on the biochemical parameters of lettuce under different hydroponic solutions

Treatment	Nitrates (mg kg^{-1})	Vitamin C (mg 100 g^{-1} DW)	Total Ch. (mg g^{-1} FW)	Carotene (mg g^{-1} FW)
T1	216.6 c	176.2 a	0.56 c	0.24 b
T2	385.5 b	185.3 a	0.74 b	0.32 ab
T3	410.6 b	195.4 a	0.82 b	0.36 ab
T4	504.2 a	208.3 a	1.15 a	0.41 a
Cultivar				
'Vanity'	384.8 a	186.8 b	0.76 b	0.31 b
'Kiloma'	410.2 a	207.3 b	0.72 b	0.28 b
'Lolo Rosso'	362.4 a	304.6 a	0.51 c	0.17 c
'Lollo Bianco'	376.2 a	94.5 c	1.02 a	0.42 a

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Conclusion

In summary, this study aimed to assess how different planting media, represented by nutrient solutions and lettuce cultivars, influence the growth characteristics of lettuce plants cultivated using a NFT hydroponic system. The results provide insights into the potential effects of these factors on lettuce production, which can be valuable for optimizing hydroponic cultivation techniques in the future. Our findings reveal that that the plant growth and yield are multiple characteristics that associated by plant genetic (cultivar) variation and nutritional variation. In future experiments, we will examine the impact of a hydroponic nutrient solution on the growth and nutritional quality of vegetable crops under various environmental conditions (i.e. air temperature and humidity, water stress, photoperiod and solar radiation).

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A NEW PROMISING LATE MATURING CLEMENTINE MANDARIN 'MARTIATIKI'

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Abstract

'Martiatiki' is a clementine selection by Vitsios nurseries made several years ago due to its late maturation (mid-February). The aim of the current trial was to assess the physiological, organoleptic, and phytochemical properties of the fruit and/or juice, as well as the amount of essential oil in the peel and its aroma, in comparison to the 'Clementine SRA63' and 'Nova' mandarin hybrid. Mature fruits of all three mandarins were harvested, and their physiological characteristics (fruit weight, diameter, and length; peel thickness and color; juice content), organoleptic properties (juice pH, total soluble content, total titratable acidity), and juice phytochemical content (total phenolic compounds, antioxidant capacity, soluble sugars, and carotenoids) were assessed. Significant differences were observed among the three mandarins. 'Martiatiki' exhibited a higher ratio of total soluble solids to total titratable acidity and β-carotene content compared to the other two cultivars, although it generally had lower values than 'Nova' but similar values to 'Clementine SRA63'. The principal component analysis clearly distinguished the three mandarins. The volume of essential oil extracted from the peel was higher in mature fruits of 'Clementine SRA63', followed by 'Martiatiki', and then 'Nova'. The essential oil produced had a fatty odor in 'Clementine SRA63', a tangerine aroma in 'Nova', and a citrus scent in 'Martiatiki'. Based on the results of the present study, 'Martiatiki' is an interesting clementine mandarin. However, further research is needed to fully evaluate its properties, both at the fruit level and at the plant level (abiotic and biotic tolerance or sensitivity, etc).

Keywords: *Antioxidant capacity, Carotenoids, Essential oils, Fruit phenolics.*

Introduction

Mandarins are a special species as they are among the progenitor species of citrus fruits, and they are classified into three types (Type 1, Type 2a, Type 2b, Type 3) based on the percentage of the genome derived from the Pommelo species (Ziogas, 2023). Cultivated mandarins primarily belong to Type 2b and Type 3, which encompass varieties such as Mediterranean mandarin, Ponkan, Dancy, King, Satsuma, Clementines, Nadorcott, and hybrids resulting from crosses between mandarins, oranges, or grapefruits (Ziogas, 2023). Mandarins are known for their resistance to cold and frost, making them well-suited for tropical as well as subtropical climates.

In Greece, mandarins are extensively cultivated, particularly in Peloponnese (3,567.6 ha) and Epirus (3,213.2 ha) counties, with diverse cultivars adapted to local climatic conditions and possessing desirable quality characteristics such as aroma, taste, and antioxidants. However, due to the low selling prices, citrus growers prefer cultivars with extended maturation periods, few or no seeds, and high-quality fruits or juice (Simón-Grao *et al*., 2014). To meet these preferences, citrus growers and nurseries are introducing new cultivars, primarily hybrid ones and tangor mandarins (Simón-Grao *et al*., 2014). It is crucial thus for growers to understand

the internal and external characteristics, organoleptic properties, and nutritional value of these new mandarin cultivars (Beltrán *et al*., 2008) to select the appropriate ones for specific regions or markets and identify potential opportunities for market expansion.
The primary objective of this trial was to assess the quality parameters of three different groups of mandarins: a) 'Clementine' ('Clementine SRA63'), b) hybrid ('Nova'), and c) a Clementine selection by Vitsios Nurseries ('Martiatiki'), cultivated in the Epirus region of Greece. Therefore, several physiological, organoleptic, and phytochemical properties of the fruit and/or juice, as well as the amount of essential oil in the peel and its aroma, were evaluated.

Material and Methods

The study was conducted in the city of Arta, located in the Epirus region of Western Greece during the years 2020-2021. Three groups of mandarins were used in this study, classified as follows: a) 'Clementine': 'Clementine SRA63'; b) hybrid: 'Nova'; and c) a clementine selection by 'Vitsios Nurseries' made several years ago due to its late maturation (mid-February): 'Martiatiki'. The experiment was arranged as a completely randomized design, with three replications of four trees per group. The trees were budded on sour orange rootstock, were healthy, and showed no signs of any mineral deficiencies. Fruits were harvested at the stage of commercial maturity. Subsequently, at least 30 fruits per replicate were randomly sampled from the harvested fruits, placed into labeled plastic bags, and immediately send to the laboratory for further phytochemical analyses.
In the laboratory, the weight of each individual fruit (at least 20 fruits per sample) was determined using an electronic balance, along with their diameter, length, peel thickness, and flesh thickness, which were measured using a digital caliper. The ratio of fresh-to-dry weight of both the peel and the flesh was also determined. Fruit peel color was measured at three different points around the equatorial region of each fruit, using a Minolta CR 300 reflectance Chroma Meter (Minolta, Osaka, Japan). Additionally, to determine the volume of juice per fruit and the number of seeds, the juice was individually extracted from each fruit using an electric household juicer. The volume of the extracted juice was measured as well as the number of seeds remaining in the juicer separator.
A 10 mL juice sample was centrifuged at 4000 g for 6 min, and the supernatant was analyzed for total soluble solids (TSS) expressed as °Brix, pH, and titratable acidity (TA) expressed as % (w/v) citric acid, following the method described by Roussos et al. (2019). Furthermore, a portion of the extracted juice was transferred to 50 mL Falcon tubes and stored at −25°C for the analysis of sugars, organic acids, and vitamin C, as described by Roussos et al. (2019). Another portion was filled into 15 mL Falcon tubes, flushed with nitrogen gas, and placed at −80°C for the analysis of carotenoids, based on the method of Roussos et al. (2019). Finally, to analyze phenolic compounds and antioxidant activity, the fresh squeezed juice was diluted 1:1 with methanol and extracted at 40 °C with periodic agitation for 1 hour, following the method by Roussos *et al*., (2019). The methanolic solution was then centrifuged at 4000 g for 6 min, and the supernatant was stored at −25 °C until analysis.
Finally, to determine the quantity of the oil produced, the fruit peel was removed, and 100 g of fresh weight were extracted using approximately 800 mL of distilled water with a Clevenger apparatus. Subsequently, the aromatic characteristics of the oil were evaluated.
The raw data were subjected to analysis of variance and significant differences were determined using Tukey's HSD test at α=0.05.

Results and Discussion

'Nova' has the highest fruit weight, surpassing 140 g, followed by 'Martiatiki' (102 g), while 'Clementine SRA63' is the lightest among the studied mandarins, with values below 100 g (Table 1). Moreover, 'Nova' exhibits the largest fruit diameter and length, as well as the highest peel and flesh weight. 'Nova' also yields the highest amount of juice, averaging 90 mL per fruit. This juice volume represents 64% of the total flesh weight of the fruit, which is significantly higher than the average of 50.7% observed in the other mandarins. 'Martiatiki' demonstrates the highest ratio of flesh fresh to dry weight, while 'Clementine SRA63' has the highest peel thickness and peel fresh to dry weight ratio. In terms of seeds, 'Clementine SRA63' presents the highest number of seeds, with an average of 3.35 seeds per fruit (Table 1). Generally, a high number of seeds is considered an undesirable quality characteristic and can negatively affect consumer demand for a mandarin cultivar.

Table 1. Mandarin fruit's physiological parameters.

	Martiatiki	Nova	Clementine SRA63
Fruit weight (g)	102.05 ab	140.57 a	98.12 b
Fruit diameter (mm)	61.19 b	65.41 a	60.28 b
Fruit length (mm)	50.35 b	54.19 a	51.93 ab
Peel weight (g)	20.36 b	31.11 a	22.22 b
Flesh weight (g)	80.39 b	111.27 a	72.89 b
Peel thickness (mm)	2.59 b	2.81 ab	3.47 a
Flesh fresh/dry weight	8.16 a	7.26 b	7.37 b
Peel fresh/dry weight	3.38 b	3.52 b	4.08 a
Seed number	1.33 b	2.66 ab	3.35 a
Juice volume per fruit (mL)	52.08 b	90.00 a	49.09 b
Juice volume per fruit (v/v %)	51.59 b	63.94 a	49.92 b

Means within the same row followed by the same letter, do not differ significantly according to Tukey HSD test at a = 0.05.

The juice pH of fruits belonging to the 'Martiatiki' was found to be less acidic (pH 3.91) compared to the other two groups of tested mandarins, which had an average pH of 3.58 (Table 2). The TA of the juice from all mandarins ranged from 0.5 to 1.02 %. Once again, 'Martiatiki' exhibited lower TA compared to the cultivars of the other two groups (Table 2). Notably, the 'Nova' mandarin exhibited a high TA (1.02%) when compared to the other mandarins. Furthermore, the cultivars had a significant impact on the TSS of the juices, which ranged from 11.16 to 13.1 °Brix. It is evident that 'Nova' fruits had the highest TSS concentration, surpassing both 'Martiatiki' and 'Clementine SRA63'. Consequently, 'Martiatiki' had the highest TSS to TA ratio, which is often used as an indicator of fruit maturity. Additionally, the 'Clementine SRA63' had the highest peel L^* value, while the hue and color intensity of the peel varied between 'Nova' and 'Clementine SRA63' (Table 2).

Table 2. Mandarin fruit's organoleptic characteristics (pH, TA, TSS) and peel color attributes (*L**, hue, Chroma).

	Martiatiki	Nova	Clementine SRA63
pH	3.91 a	3.49 b	3.67 b
TA (% w/v citric acid)	0.51 c	1.02 a	0.88 b
TSS (^{o}Brix)	11.16 b	13.1 a	11.66 b
TSS/TA	21.87 a	12.80 b	13.18 b
*L**	63.03 b	62.77 b	65.63 a
Hue	26.15 ab	28.23 a	23.52 b
Chroma	68.67 ab	69.74 a	67.77 b

Means within the same row followed by the same letter, do not differ significantly according to Tukey HSD test at a = 0.05.

The 'Nova' mandarin exhibited higher concentrations of all detected sugars (fructose, glucose, sucrose, and total sugars) compared to 'Martiatiki' and 'Clementine SRA63' (Table 3). Additionally, there were no significant differences in the concentrations of malic acid, fumaric acid, and ascorbic acid among the juices of the three mandarins. However, the juice of 'Nova' contained higher levels of citric acid and total organic acids (Table 3). The presence of ascorbic acid in citrus fruits is considered a valuable parameter as it is a strong antioxidant (Simón-Grao *et al*., 2014). In our experiment, all mandarins demonstrated ascorbic acid levels surpassing the minimum desirable level of 300 mg L^{-1} of vitamin C in mandarin juices, as established by the Expert Committee of European Juice Producers (AIJN, 2006). Therefore, these cultivars could be marketed to consumers by highlighting their high vitamin C content as an added value.

Table 3. Mandarin fruit's soluble sugars and organic acids concentration (both expressed as mg mL^{-1} juice).

	Martiatiki	Nova	Clementine SRA63
Fructose	15.26 b	29.12 a	8.93 c
Glucose	16.64 b	28.65 a	8.91 c
Sucrose	62.03 b	66.53 a	35.07 c
Total sugars	93.95 b	124.32 a	52.92 c
Malic acid	0.42 a	0.48 a	0.45 a
Ascorbic acid	0.53 a	0.54 a	0.49 a
Citric acid	1.15 b	2.19 a	1.34 b
Fumaric acid	0.25 a	0.24 a	0.25 a
Total organic acids	2.36 b	3.47 a	2.55 b

Means within the same row followed by the same letter, do not differ significantly according to Tukey HSD test at a = 0.05.

Significant differences were observed in the concentration of phenolic compounds among the different juices (Table 4). 'Nova' displayed higher levels of total phenolics, total *o*-diphenols, total flavonoids, and total flavanols compared to 'Martiatiki' and 'Clementine SRA63'. However, there was no significant difference in terms of antioxidant capacity between 'Nova' and 'Martiatiki' as determined by the FRAP and DPPH methods. Finally, 'Nova' fruits outperformed in terms of carotenoid concentration compared to the other two mandarins, as it exhibited significantly higher concentrations of lutein, zeaxanthin, and cryptoxanthin (Table 5). On the other hand, 'Martiatiki' presented the highest concentration of β-carotene compared to 'Nova' and 'Clementine SRA63'

Table 4. Mandarin fruit's total phenolic compounds and antioxidant capacity.

	Martiatiki	Nova	Clementine SRA63
Total phenols (mg GAE 100 mL^{-1})	0.72 b	0.99 a	0.77 b
Total *o*-diphenols (mg CAE 100 mL^{-1})	0.03 b	0.06 a	0.04 ab
Total flavanols (mg CAE 100 mL^{-1})	0.06 b	0.19 a	0.02 b
Total flavonoids (mg CtE 100 mL^{-1})	0.03 b	0.04 a	0.01 c
FRAP (μmol equiv. Trolox 100 mL^{-1})	4.51 ab	5.05 a	3.63 b
DPPH (μmol equiv. Trolox 100 mL^{-1})	2.93 a	3.30 a	2.58 a

Means within the same row followed by the same letter, do not differ significantly according to Tukey HSD test at a = 0.05. Abbreviations: GAE, gallic acid equivalents; CAE, caffeic acid equivalents; CtE, catechin equivalents.

Table 5. Mandarin fruit's carotenoids concentration (expressed as μg 100 mL^{-1} juice).

	Martiatiki	Nova	Clementine SRA63
Lutein	1.27 b	6.8 a	0.98 b
Zeaxanthin	2.04 a	2.69 a	0.59 b
Cryptoxanthin	644.89 b	1900.09 a	462.38 b
β-Carotene	28.42 a	13.87 b	4.03 b

Means within the same row followed by the same letter, do not differ significantly according to Tukey HSD test at a = 0.05.

Besides, the texture and color of fruits, taste plays a crucial role in the consumer's acceptance of edible products. The taste is influenced by organic acids, sugars, and aromas. A delightful and distinct aroma often assures the consumer's acceptance of the product. In the present study, the essential oil volume produced from the peel was higher in mature fruits of 'Clementine SRA63' followed by that of 'Martiatiki' and then from 'Nova' (Table 6). Furthermore, Table 6 presents the aroma of the essential oils extracted from the peels of the examined mandarin fruits, with the primary/main dominant aromas, according to the testers, indicated in bold. Therefore, the produced essential oil was characterized by a fatty odor in 'Clementine SRA63', by tangerine aroma in 'Nova' and by citrus scent in 'Martiatiki'.

Table 6. The produced volume of essential oils (μl 100 g^{-1} FW) from the peel of mandarins using the Clevenger apparatus and the aroma of the essential oils.

Mandarins	Fruit maturity stage	Essential oil volume	Essential oil aroma
Clementine SRA63	Mature fruits	760	**Fatty**, spicy, floral, geranium, grassy
Nova	Mature fruits	220	**Mandarin**, fatty, soapy, sweet, floral,
Martiatiki	Small, intensely green fruits	360	**Woody**, Sweet, lemony, herbal,
	Small, light green fruits	300	**Grassy**, lemony, geranium
	Mature fruits	320	**Citrus**, humidity, wood, orange, sweet, floral

From the principal component analysis, it became clear that the three examined mandarins in the present study are distinct and can be completely separated using the parameters presented above, as shown in the Figure 1. Similar differences among mandarin cultivars have been extensively described by Lado *et al.* (2016).

Conclusions

The new late maturing clementine 'Martiatiki' showcases unique characteristics that differentiate it from the globally cultivated 'Clementine SRA63' and the hybrid cultivar 'Nova', also known for its exceptionally favorable traits.

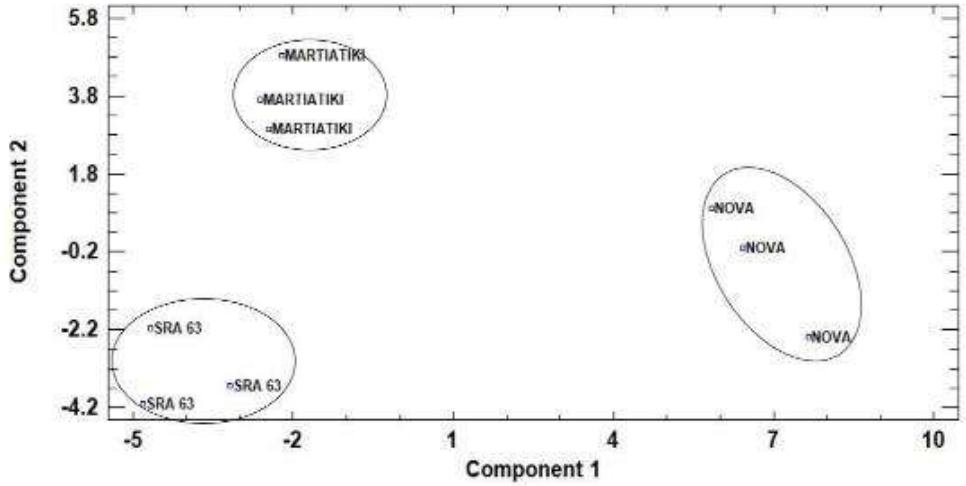

Figure 1. Distribution and relationship of mandarins according to principal component analysis, taking into account physiological, sensory, and functional characteristics of the fruits.

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EFFECT OF DIFFERENT FERTILIZERS ON SEED GERMINATION AND EARLY SEEDLING DEVELOPMENT OF TAGETES PATULA L.

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Abstract

French marigold or dwarf marigold (*Tagetes patula* L.) is well-known annual low-maintenance ornamental species. Besides its ornamental use, French marigold is an edible and medicinal plant, and it is also used in the food and cosmetics industries. The essential oil extracted from dwarf marigold is used in perfumery and this plant can also produce allelopathic compounds toxic to some species of plant-parasitic nematodes. French marigold is propagated by seed and application of fertilizers can promote early seedlings growth. However, many different commercial fertilizers are available on market and marked as suitable for application in early stages of plant propagation, without proper evidence of their effect on germination and early seedling growth. For this reason the aim of this study was to examine the effect of commercial fertilizers: EkoBooster 1®, Unistart and Bakterije on *T. patula* seed germination and seedling growth. The seeds were sown in mixture of peat, sand and perlite which was treated with fertilizers before seed sowing and on 2nd, 3rd and 9th day after seed sowing. Fertilizers affected germination, and the highest germination rate was obtained after the treatment with Unistart (84%) and Bakterije (81.3%) while the germination was lower after treatment with EkoBooster 1® and in the control (65.3%). However, the seedlings were smaller after the treatment with Unistart and Bakterije compared to control. Contrary to this, treatment with EkoBooster 1® promoted seedlings growth, and they formed longer roots and true leaves compared with seedlings treated with other fertilizers and control.

Keywords: *French marigold, dwarf marigold, fertilizers, germination, propagation.*

Introduction

French marigold or dwarf marigold (*Tagetes patula* L.) is native from Mexico to Argentina, but it is grown in Europe since 16th century. Today, it is well-known annual low-maintenance ornamental species, tolerant to a moderate drought which can grow on poor soils. In Serbian gardens it is usually self-seeding plant. *T. patula* cultivars are usually 20 – 30 cm tall, with single, semi-double or double fragrant flowers heads which are in different shades of yellow, orange, or dark red color, 3-4 cm in diameter. Leaves are pinnate, with toothed leaflets, dark green (Cicevan et al. 2014; Grbić et al. 2017; Armitage 2001). Besides its ornamental use, French marigold is an edible and medicinal plant, young leaves and petals can be eaten raw in salads, dried or cooked. As a medicinal plant, *T. patula* has antioxidant, anti-inflammatory, diuretic, antifungal and antibacterial properties, and it is used against rheumatism, stomach and intestinal problems, kidney and hepatic disorders, fever, and pneumonia. French marigold is also used in the food industry for food coloring and in cosmetics industries because the essential oil extracted from dwarf marigold is used in perfumery. French marigold can also produce allelopathic compounds toxic to some species of plant-parasitic nematodes and mosquito larvaes (Amrouche et al., 2022; Kashif et al., 2015; Araújo et al., 2015; Dharmagadda et al., 2005; Chitrakar et al., 2019; Chkhikvishvili et al., 2016). Besides, *T.*

patula can be used for a phytoremediation as a potential hyperaccumulator in Cd-polluted areas (Shi et al., 2022).
T. patula is propagated by seed and application of fertilizers can promote early seedlings growth (Gao et al. 2020), especially can promote plant growth through nitrogen fixation, phosphate or potassium solubilization, or if they contain plant growth regulators (Bashan and Bashan, 2005). Today, many different commercial fertilizers are available on Serbian market and they are marked as bio fertilizers suitable for application in early stages of plant propagation, without proper evidence of their effect on germination and early seedling growth for the specific plant species or a group of plant species. For this reason, the aim of this study was to examine the effect of commercial fertilizers: EkoBooster 1®, Unistart and Bakterije on *T. patula* seed germination and seedling growth. According to the product specifications, EkoBooster 1® contains 14% Nitrate (N), 2% Phosphorus and 5% Potassium (K). Unistart contains not-specified strains of proteolytic and cellulolytic bacteria that increase availability of carbon, nitrogen and phosphorus for plants. Bakterije contains *Bacillus subtilis*, *Bacillus megaterium* and *Saccharomyces* spp.

Material and Methods

The seeds of *T. patula* were purchased from Semenarna Ljubljana (product code 5438) and were sown in trays (dimensions 25 × 15 × 5 cm) in spring, 25 seeds per tray, into the mixture of peat, sand and perlite. Each fertilizer was applied according to the product specifications, they were diluted in distilled water, EkoBooster 1® was applied as a 33% solution, Unistart as a 1% solution and Baketrije as 0.5% solution. The substrate was irrigated with prepared solutions of fertilizers before seed sowing, and later, fertilizer was applied simultaneously with irrigation on the 2^{nd}, 3^{rd} and 9^{th} day after seed sowing. Each tray was irrigated with 33 mL of fertilizer solution. The seeds in the control treatment were irrigated with water without fertilizers. The seed was kept in the greenhouse of the Faculty of Forestry in Belgrade (Serbia), the humidity was controlled daily and temperature ranged between 16°C and 25°C.
Each treatment consisted of three replicates with 25 seeds each. Three weeks after setting the experiment, the germination percentage as well as the length of shoots, roots, and leaves were recorded. Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p<0.05$) and the least significant difference (LSD) test.

Results and Discussion

Germination percentage of *T. patula* was relatively low in the control treatment, without any fertilizers, only 65.3% (Table 1). Besides, treatment with fertilizer containing only NPK macroelements did not affect germination, because the germination percentage was the same as in the control (Table 1). However, fertilizers containing bacteria had a positive effect on germination and germination percentage was much higher, 81.3% (after treatment with Bakterije) and 84.0% (after treatment with Unistart). Recent researches showed that bio-fertilizers can have a positive effect on germination, but it can depend on bio-fertilizers composition (Delshadi et al. 2017; Bákonyi et al. 2013).

Table 1. The effect of fertilizers on germination of Tagetes patula seeds

Treatment	Germination rate (%)
control	65.3b
EkoBooster 1	65.3b
Bakterije	81.3a
Unistart	84.0a

Despite the expectations, the treatment with Unistart and Bakterije did not positively influence development of seedling (Table 2). The average length of the longest root was smaller after treatment with Unistart and Bakterije (18.7 mm) compared to the control (25.8 mm) or o the seedlings treated with EkoBooster 1 (41 mm). The fertilizers treatment did not influence the length of shoots, which ranged from 53.2 mm to 56.2 mm, because obtained differences were not statistically significant (Table 2). However, all fertilizers promote development of true leaves, and number of seedling which developed the true leaves in 3 weeks after germination was the lowest in the control treatment (63.6%), but the best results were achieved with EkoBooster 1 where 90.7% of seedlings developed true leaves during this period and these leaves were considerably longer (8.1 mm) compared to other treatments (2.4 – 2.5 mm) (Table 2). Similarly Prayogo et al. (2021) also achieved better results with application of NPK or combination of NPK and bio-fertilizers than when only bio-fertilizers were used. The other researches also proved positive influence of combination of organic fertilizer, bio-fertilizer and chemical fertilizer indicating that adding NPK macroelements is important for improving plant growth despite the positive effect of bio-fertilizers (Duraid et al. 2021).

Table 2. The effect of fertilizers on length of shoots, roots and true leaves of T. patula

Treatment	Shoot length (mm)	Longest root length (mm)	Presence of true leaves* (%)	Length of true leaves (mm)
Control	56.2a	25.8ab	63.6b	2.4b
EkoBooster 1	54.9a	41.0a	90.7a	8.1a
Bakterije	53.2a	18.7c	78.0ab	2.4b
Unistart	55.6a	18.7c	72.7ab	2.5b

*The percentage of seedling which developed a first pair of true leaves

Conclusions

French marigold is an important ornamental, edible and medicinal plant, also used in the food and cosmetics industries. The wide range of possibilities to use *T. patula* is the reason to improve its production. The fertilizers available on the Serbian market: EkoBooster 1®, Unistart and Bakterije can be used in early propagation of marigold, but their effect on seed germination and seedling growth was different. The bio-fertilizers Unistart and Bakterije enhanced seed germination, which was much higher compared to control and EkoBooster 1 treatment, but EkoBooster 1, as a fertilizer with NPK macroelements considerably promoted seedling growth. For this reason, the effect of combination of bio-fertilizers and chemical fertilizers on the *T. patula* seed germination and seedling growth should be examined.

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COMPARED AGRICULTURAL MACHINE SIZE MEASUREMENT WITH A CAD AND AN IMAGE ANALYSIS SOFTWARE

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Abstract

The ability to quantify things is fundamental to contemporary thought. It's how we explain the world to one another. A further impetus for the development of the theory and concepts of measurement has come from the advancement of computing, which can handle the numerical information. In this study, we explore the idea of a CAD and image analysis-based measurement. Measuring, in its broadest sense, is the act of assigning characteristics to things and events in the physical world in order to characterize them. In the study, a CAD and an image analysis software dimension measurement results were compared based on image analysis techniques for an agricultural machine dimension measurement. The aim of the study, obtaining data from the CAD software (Autodesk Inventor Pro) and the image analysis software (OpenText Brava Desktop) for the aim of comparing measurement results of the software and determining their efficiency for agricultural machine dimension measurement. The efficiency of measurements is important because, without an image analysis software, it is possible to measure the dimensions of agricultural machines with the help of a CAD software. And, during all design and production procedures generally a CAD software use. For this reason, this research gives us an idea to determine and select the appropriate measurement method for agricultural machine production.

Keywords: *Image analysis, Autodesk Inventor Pro, OpenText Brava Desktop, Dimension measurement, Agricultural machine design.*

Introduction

To accurately describe and comprehend the natural world, measurement is indispensable. Measurement, in the realist view, forces us to accept the reality of real numbers and the existence of qualities and relations that may be expressed in numerical form (Michell, 2005; Finkelstein, 2005). Measuring used to be strictly limited to the physical sciences and technologies, but now it is used in many other fields as well (Finkelstein, 2003).

The advancement of agricultural production in terms of quality and quantity is a primary responsibility of agricultural engineering. This responsibility includes the disclosure, application, economic analysis, and evaluation of the most cutting-edge techniques for this purpose. Agricultural machinery dimension measurements are one of the most important field to consider when implementing these methods. This requires an in-depth familiarity with the technical characteristics of agricultural machinery, or metals. Understanding the technical characteristics of farm equipment is crucial for several reasons, including better tool and machine design, greater business success, more thorough product processing, and higher-quality finished goods (Beyaz, 2008).

However, it's impossible to ignore the fact that computers have made tremendous strides in recent years. In addition to decreasing in price, computer technology is becoming increasingly user-friendly. Because of these benefits, computers have been widely adopted by the agricultural industry in recent years. Two such applications, the computer-aided design

(CAD) systems, and image processing and analysis systems, which see heavy use in the agricultural industry as well. These technological advancements have improved the efficacy and efficiency of agricultural applications, some of which were previously only a pipe dream. Many programs are organized and created for specific purposes, such as computer-aided design (CAD) or image analysis. Due to their respective industries' initiatives, Autodesk Inventor Pro and OpenText Brava Desktop software were used in the research. There is a wealth of literature on the topic.

For instance, Mitta et al. (1995) conducted several tests and emphasized the significance of AutoCAD interface design in terms of user productivity. In this view, the success of image area selection and measurement is directly tied to the quality of the user interface and, by extension, to the efficiency of the user.

Gadelmawla (2008) centered his research on a computer-aided system created for microscopic measurement, and he used measurements exported to AutoCAD to verify the system's efficacy.

AutoCAD was used for the control system developed by Licari et al. (2001) for CNC machines with three axes of automatic movement. They claimed that the AutoCAD program is useful for the sizing, diagnosis, and control of CNC machines and that this is because it has a higher success rate than numerically controlled (NC) machines.

Hao et al. (2002) stress that a maintainability analysis visualization and its development under the AutoCAD environment is an important product characteristic, and is also a design parameter.

Since these two types of software operate in such different ways, this research focuses on the potential of comparing them in the design and manufacture of agricultural machinery.

Material and Methods

To compare the results of CAD and image analysis software, a semi-automatic potato planter was chosen because of the dimensions and shape properties that set it apart as an agricultural machine, and 10 samples were individually read by each program. There was a total of 20 measurements taken. Image analysis software measurements were accepted as the primary measurement method after being compared to those of other methods and found to be superior. The images of the farm machine (a semi-automatic potato planter) were taken with a digital camera on a Samsung S9+ phone. The Samsung S9+ phones digital camera with a resolution of 12 Megapixels is used to get an image of an agricultural machine. The camera used to obtain the image is positioned 150 cm far from the machine. A digital photograph of an agricultural machine was taken without a calibration plate measuring because one part with a known dimension was used for calibration (Figure 1). This calibration part provides the image analysis software with descriptive information about the size of the objects on the image. Because of the use of a single component with a known dimension, a digital photograph of an agricultural machine could be taken without the use of a calibration plate (Figure 1). This calibration step gives the analysis program specifics on how big things are in the image.

Figure 1. Image of a semi-automatic potato planter

After the imaging process of the semi-automatic potato planter, some measurement points were selected for the comparison of the efficiency of the measurements (Figure 2).

Figure 2. Selected measurement points on the image of a semi-automatic potato planter

Autodesk Inventor Pro (a CAD program with a student license) and OpenText Brava Desktop (an image analysis program with a trial license) were used to extract dimensions from digital photographs for this research. It is common knowledge that various algorithms can be used in the development of software for evaluating images, or that existing software can be utilized. The primary instrumentation in this investigation was an image analysis software called OpenText Brava Desktop. The software has two stages: the image calibration process and the correct image selection process (Beyaz 2008). Various options are presented by the software for selection. The line selection method was used because it is the most suitable for this task (Figure 3).

Figure 3. Dimension measurement (inch) process from a semi-automatic potato planter

The accuracy rate and drawing precision of CAD softwares, which are similar to image measurement software, are rapidly improving. This increase in precise measurement has opened up new applications for CAD softwares. Image analysis is one special area, and CAD softwares has begun to take the lead in this field due to its potential to provide a graphic and high-precision visual area, simple dimension calculations, and the flexibility to rapidly alter and assess measurement results. CAD softwares must allow users to make visual dimensional measurements due to the extreme precision required for such measurements. Therefore, Autodesk Inventor Pro was used in the research.

Autodesk Inventor Pro allows you to take measurements in any direction, horizontally, vertically, or at an angle. Different measurement methods can be applied thanks to its structure's multiple toolbars, allowing for highly accurate and precise measurements. Also, these object dimensions can be recorded and retrieved later. To accurately measure the dimensions needed for agricultural machines, the captured images with a cell phone camera (Samsung S9+, 12 megapixels) loaded into Autodesk Inventor Pro and transferred the actual dimensions to the software's workspace using a calibration part measured in-app (Figure 4).

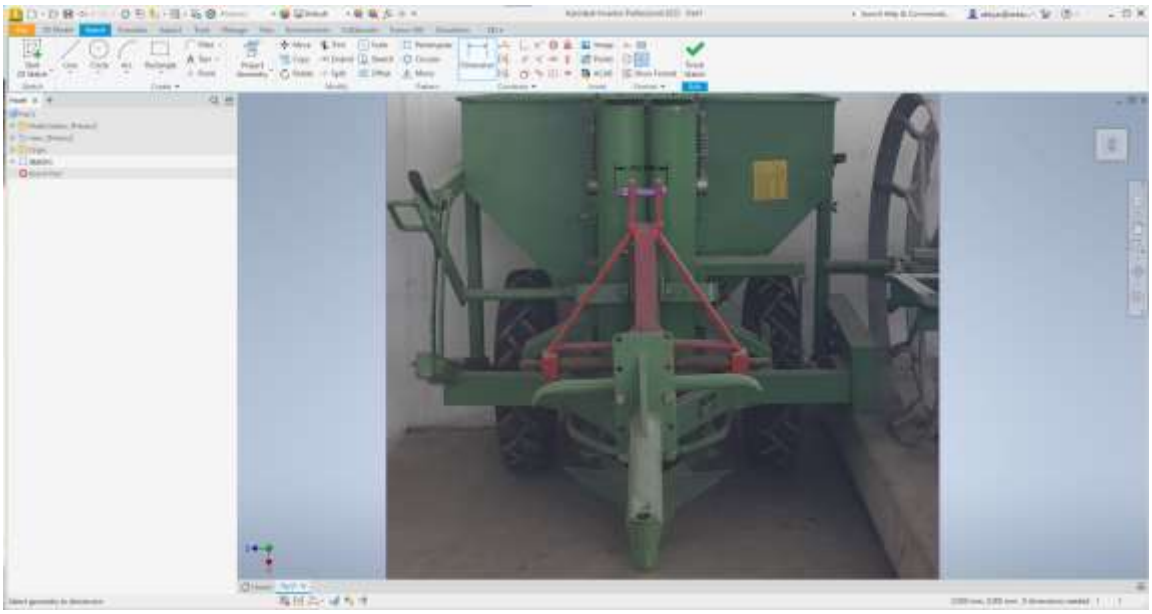

Figure 4. Dimension measurement (mm) of the agricultural machine with the Autodesk Inventor Pro software

Results and Discussion

The CAD and image analysis software measurements of the image sample are presented in Table 1. Additionally, the data plots, regression values, and regression forecast equations of these measurements are shown in Figure 5.

Table 1. The CAD, and image analysis software measurement values of the Semi-automatic potato planter

Sample ID	Image analysis software measurement (cm)	CAD measurement (cm)
1	8,8900	8,3255
2	9,4742	8,3255
3	9,9822	9,1918
4	18,3388	15,8405
5	18,7706	16,5168
6	21,4630	18,2668
7	28,7274	24,5492
8	29,1338	33,0336
9	29,2862	24,9181
10	74,5236	67,5443

It is found that there is a 98,2 % regression between the Image analysis measurements and CAD software measurements. Also, it is possible to get a 100 % regression coefficient after using an extra calibration coefficient between software. Between the two methods, this research only focused on the direct measurement comparison.

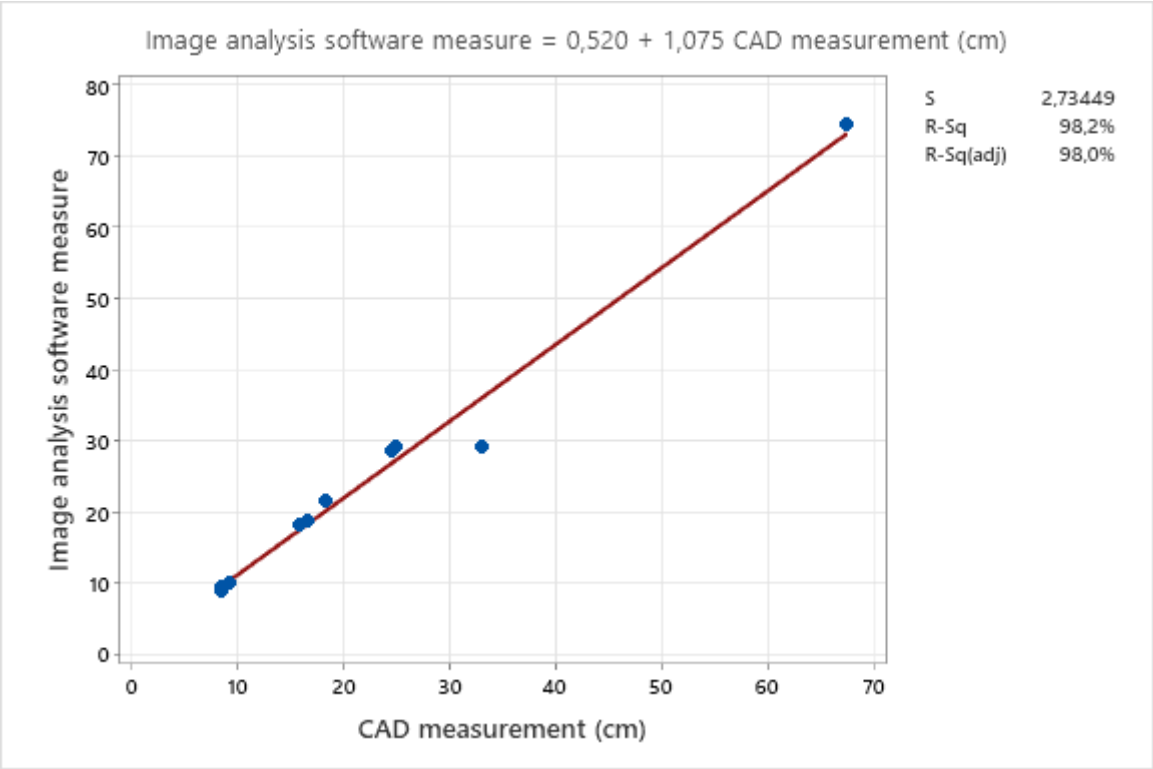

Figure 5. The graphs of the data with the regression value and regression forecast equations of the measurements.

Conclusions

This research aimed to uncover how useful computer-aided design (CAD) and image analysis software are for agricultural machinery. The literature review and the measurements show that there are benefits and drawbacks to using each software. For instance, due to the challenges of visual selection over photographs, the accuracy of CAD measurement values is essentially useless in some sides. Although product surface, color, and lighting conditions do not impact CAD measurement outcomes, they are important to consider when assessing the image analysis method. As a measurement problem dependent on the color light diffusion, this light can alter the detection efficiency of agricultural machine dimensions that need to be determined with the image analysis and reduce the success of perception.

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THE ROLE OF ADDITIVE MANUFACTURING COMPOSITE SHEETS FOR MECHANICAL PROPERTIES IN AGRICULTURAL MACHINERY PRODUCTION

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Abstract

Composites have been gaining attention in several industries, in recent years. However, they present a difficult research gap because of their high costs and distinctive features, which are the result of their heterogeneity. Therefore, the finite element method has been used to analyze composite materials in the most extreme conditions. Thus, the purpose of this work is to take a stab at modeling composite materials with an eye toward their properties, failure criteria, element types, and primary industries of use like agricultural machinery. Mechanical features, theories, and constitutive relationships necessary to model these materials are presented in this study. The analysis also covers the various failure criteria developed and used for the simulation of composites, as well as the element types most commonly used to do. Aeronautics, aerospace, automotive, naval, energy, civil, sports, electronics, and even agricultural machinery manufacturing are just some of the main industries that use composite material simulation, and the benefits they receive from it, are listed in this article. Composite materials are becoming increasingly prevalent in today's farm implements. Components made from composites are increasingly used in farm equipment due to their low weight, high durability, and low requisite for maintenance. Composites with a frame are frequently can be manufactured to serve the agricultural sector.

Keywords: *Agricultural machines production, Composite material, Plate element, Additive manufacturing, CAD Analysis.*

Introduction

There has been a rise in the desire for useful materials having qualities that can't be replicated by metal mixes as a result of technological developments (Rezende et al., 2011; Callister and Rethwisch, 2007). Fortunately, composite materials were created to meet this demand. The materials used to construct them have differing mechanical, physical, and chemical properties (Rezende et al., 2011; Kaw, 2006). Composites are multiphase-designed materials that consist of a matrix that binds the reinforcement together and transfers loads between the fibers, and reinforcement that provides structural rigidity and prevents fracture propagation properties (Rezende et al., 2011; Neto and Pardini, 2016; Teti, 2002). The matrix material (metal mixes) and the reinforcing type (plates or particles) are two ways to categorize composites (Rezende et al., 2011; Berthelot and Ling, 1999; Gay, 2022; Gay et al., 2003). Excellent mechanical qualities, strong thermal stability, and low density make matrices and continuous plates particularly important and relevant (Li et al., 2019).
When combined, composite materials exhibit superior performance over the sum of their parts (Berthelot and Ling, 1999; Chung, 2010). As a result of combining these properties, a lightweight design with high strength and stiffness is feasible, along with other desirable qualities including resistance to high temperatures, corrosion, and impacts. When taken as a

whole, these benefits make composites a more compelling, practical, and desirable option (Katsoulis et al., 2012; Jia et al., (2019). These qualities make composites useful in a broad variety of contexts, including the manufacturing sectors (Rezende et al., 2011; Chung, 2010; Katsoulis et al., 2012; Boumbimba et al., 2014; de Moura et al., 2005; Martinelli et al., 2014). Despite their widespread use across a wide range of industries, the complexity and high cost of characterizing these materials are largely attributable to the fact that their properties are determined by their heterogeneity and lamination arrangements. Increasing the abrasion resistance of components of farming gear and tools typically involves making the working surface of those components harder than the abrasive particle itself (Melibaev et al., 2022).

About this subject, Aulin et al. (2020) concentrated on the usage of polymer composites in the construction of agricultural equipment for tillage in the field of agricultural machinery. Polymer composite materials were offered as a means to update the cultivation equipment manufacturing process. Polymer composite CCPA-6-30 is highly recommended for use in cultivator construction due to its advantageous technical properties.

Alves et al. (2009) explored the potential of composite materials to integrate mechanical and ecological benefits in farm machinery. They emphasize that many businesses' primary focus is on enhancing their products' environmental performance. It has not been a common practice for businesses to incorporate environmental inputs into the development phase of the design process, even though doing so is essential to a successful outcome.

This essential to a successful outcome can be done with Additive Manufacturing, additive manufacturing, sometimes known as 3D printing, is a cutting-edge technique in the manufacturing sector. To put it simply, additive manufacturing technology allows for the direct translation of digital information into a material good. The steps in the Additive Manufacturing process chain, which involve sketching a 3D model in CAD software, may seem elementary at first glance. Next, the data is sent to a specific 3D printer, which produces the component immediately (Győri and Ficzere, 2017).

In today's manufacturing, composites are gradually replacing traditional materials (such as iron, steel, aluminum, and wood). Composites' key benefits include their lightweight, strength, flexibility, weather, and corrosion resistance, low maintenance, and long lifespan (Gay and Hoa, 2007; Petrů et al., 2015). Composites provide an advantageous combination of material qualities and low production costs. The manufacturing of agricultural equipment is a major user of composites. In addition to the benefits of composites in general, their application in the manufacture of agricultural gear allows for the creation of unique cargo compartment shapes. When made of composites, the agricultural machine can weigh significantly less, reducing the amount of stress placed on valuable cropland. Tractors, grain harvesters, and other types of agricultural cultivators all benefit greatly from the usage of composite frames. Specifically, composite frames are frequently utilized to strengthen the chassis and driver's cab of agricultural machinery. Machine cargo bays, operator compartment doors, and other covers can all benefit from composite frame reinforcement (Mlýnek et al., 2019).

Scientists use computer simulations to better understand the properties of structural materials and to aid in the optimization and improvement of building projects. When the composite is subjected to extreme conditions, such as static and dynamic loading, varying temperatures, and high pressures, among others, the matrix, the fiber, and their interface can sustain damage, and this damage can be understood through the application of the finite element method (FEM) (Cook, 1995; Tenek and Argyris, 1997). So, the text it is looked at the possibility of adopting new materials with strong tribological qualities and zero maintenance needs to improve the dependability of agricultural machines based on FEM. Because learning composite material behaviors for additive manufacturing affects the cost of production. So,

this information gives scientists and agricultural machinery producers to decrease their costs based on material compositions that they need at a large variety of prices.

Material and Methods

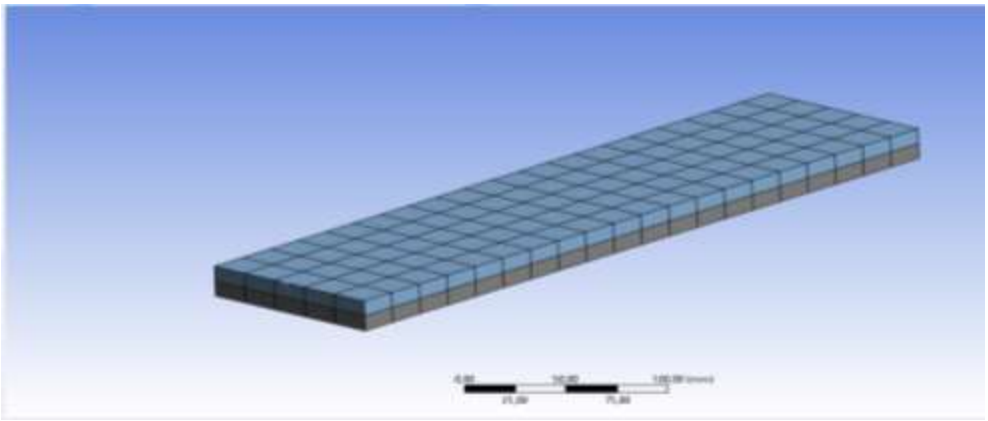
Material

17-4PH stainless steel, 316 stainless steel, gray cast iron, all of which have slightly different properties, were chosen for the sample’s construction so that their effects on the surface (form) deviation could be studied. The materials were combined as the list below to see the additive manufacturing effects on material combinations based on stress analysis;

1-17-4PH Stainless Steel - Gray Cast Iron,
2- 316 Stainless Steel - Gray Cast Iron,

Method and Modeling

Many factors affect additive manufacturing. These factors may vary by additive manufacturing category. Like the part design, energy fraction, thermal expansion factor, material, cost, and energy consumption. When the properties of composite materials are considered in terms of additive manufacturing; They can be supplied in large quantities depending on the model designed with the parameters of width, length, thickness, and the shape of the model. Generally, all experiments and tests depend on these factors. For this reason, a basic study was carried out on the sample material with specified dimensions. In this research and implementation, the plate model features are used as follows; length: 400 mm, width: 100 mm, and thickness: 10 mm as sample composite materials for additive production for scientists and agricultural machinery producers (Figure 1). As mentioned, in cases where materials are used together for our purpose, their behavior under 1000 N, 2000 N, and 3000 N loads has been examined to the sample composite material model for general maximum load needs and interpreted for two analysis aspects. There is equivalent elastic strain and total deformation. There used to be a finite element method for modeling and interpretation. All design and analysis procedures were done by using ANSYS 2023 software (Under Student License and "Images used courtesy of ANSYS, Inc.").



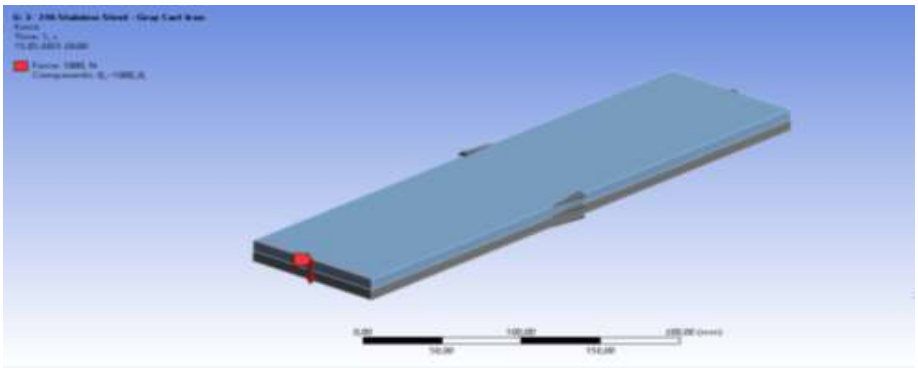


Figure 1. Composite material architecture and load direction

Results and Discussion

The quality of the finished composite product is determined mainly by the caliber of the materials used to make the frame and the precision of the production. To ensure a high-quality composite frame, it is crucial to maintain a consistent plane orientation. Manufacturing composite frames using conventional methods that rely on the physical abilities of experts is a time-consuming and laborious process (Mlýnek et al., 2019). Sample equivalent elastic strain and total deformation results of the material combination properties were presented in Figure 2 and 3 based on their behavior under 1000 N, 2000 N, and 3000 N loads, respectively.

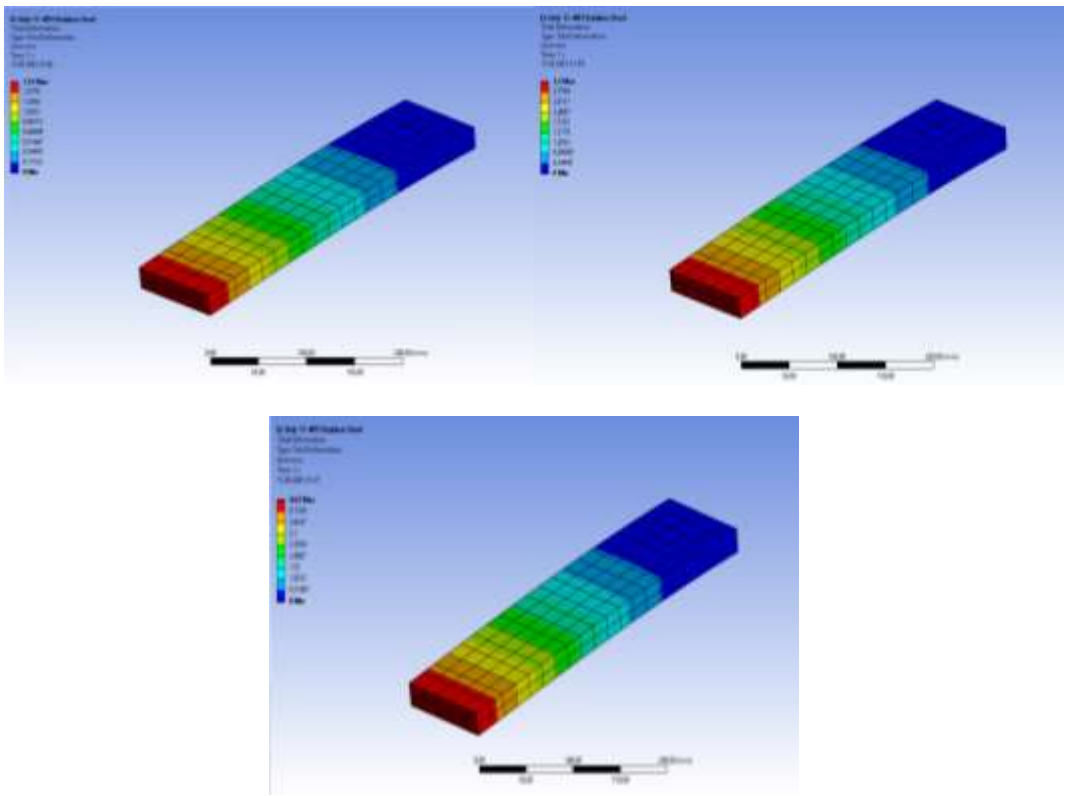


Figure 2. Total deformation of the 17-4PH stainless steel under 1000 N, 2000 N, and 3000 N loads

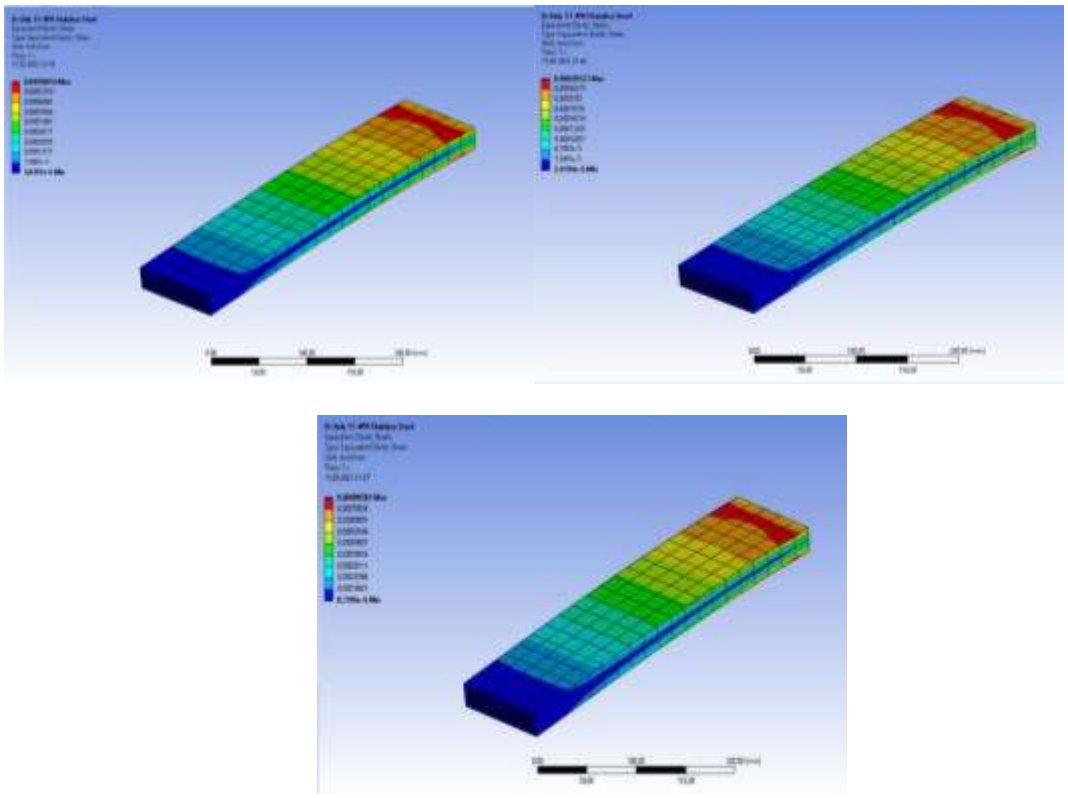


Figure 3. Equivalent elastic strain deformation of the 17-4PH stainless steel under 1000 N, 2000 N, and 3000 N loads

Between all equivalent elastic strain and total deformation results of the material combinations presented in Table 1 and 2 based on their behaviour under 1000 N, 2000 N, and 3000 N loads, the best results found from 0,00029527 mm/mm for 17-4PH stainless steel material under 2000 N load for equivalent elastic strain and 1,55 mm 17-4PH stainless steel material under 1000 N load for total deformation for the aim of agricultural machinery usage. Also, the worst results were obtained from 0,001597 mm/mm Gray Cast Iron material under 3000 N loads combination for equivalent elastic strain and 8,5875 mm Gray Cast Iron material under 3000 N load for total deformation. Table 1. shows the all-equivalent elastic strain with materials and loads.

Table 1. Equivalent elastic strain with materials and loads

Equivalent elastic strain (mm/mm)	**Materials and Loads (N)**
0,00029527	17-4PH stainless steel under 2000 N
0,00030796	316 Stainless Steel under 2000 N
0,00046543	17-4PH Stainless Steel - Gray Cast Iron material combination under 2000 N
0,00047126	316 Stainless Steel - Gray Cast Iron material combination under 3000 N
0,00053233	Gray Cast Iron under 2000 N
0,00059054	17-4PH stainless steel under 1000 N
0,00061592	316 Stainless Steel under 1000 N
0,00088581	17-4PH stainless steel under 3000 N
0,00092387	316 Stainless Steel under 3000 N
0,00093085	17-4PH Stainless Steel - Gray Cast Iron material

	combination under 3000 N
0,00094252	316 Stainless Steel - Gray Cast Iron material combination under 1000 N
0,00106470	Gray Cast Iron under 1000 N
0,00139630	17-4PH Stainless Steel - Gray Cast Iron material combination under 1000 N
0,00141380	316 Stainless Steel - Gray Cast Iron material combination under 2000 N
0,00159700	Gray Cast Iron under 3000 N

Also, Table 2. presents the total deformation with materials and loads. So, composite materials based on additive manufacturing seems to be useful to some core material for agricultural machine design and production but other material options under different loads can be seen in Table 1 and 2.

Table 2. Total deformation with materials and Loads

Total deformation (mm)	**Materials and Loads (N)**
1,5500	17-4PH stainless steel under 1000 N
1,6303	316 Stainless Steel under 1000 N
2,1630	17-4PH Stainless Steel - Gray Cast Iron material combination under 1000 N
2,2108	316 Stainless Steel - Gray Cast Iron material combination under 1000 N
2,8625	Gray Cast Iron under 1000 N
3,1000	17-4PH stainless steel under 2000 N
3,2607	316 Stainless Steel under 2000 N
4,3266	17-4PH Stainless Steel - Gray Cast Iron material combination under 2000 N
4,4216	316 Stainless Steel - Gray Cast Iron material combination under 2000 N
4,6500	17-4PH stainless steel under 3000 N
4,8910	316 Stainless Steel under 3000 N
5,7250	Gray Cast Iron under 2000 N
6,4899	17-4PH Stainless Steel - Gray Cast Iron material combination under 3000 N
6,6325	316 Stainless Steel - Gray Cast Iron material combination under 3000 N
8,5875	Gray Cast Iron under 3000 N

According to Aulin et al. (2020), using polymer composite materials in the hinged joints of the parallelogram mechanisms of soil tillage machines will increase the efficiency with which agrotechnical requirements are met, get rid of the need for costly downtime whenever the mechanisms need to be maintained and lower the overall cost of running and caring for modernized cultivators. The maximum load on the hinge is 2400 N, making it ideal for use in parallelogram systems.

Conclusions

It is a well-known reality that material properties are the main parameter for every production the industries. So, additive manufacturing and composite materials give us a chance to improve material properties for the production processes. This change is also important for agricultural machine design and production. But, the main problem is to know the behaviors of the material combinations under different loads. Computer-aided design and analysis software helps us to see these behaviors like ANSYS. So this application aims to give an idea about these properties for agricultural machinery producers to reduce their costs. As a reality, there should be more literature to give more information about the new material compositions because material science, also computer-based design and analysis software, and software material libraries are developing in short terms.

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THE EFFICACY OF GLUTAMINE PRIMING ON GERMINATION AND SEEDLING DEVELOPMENT OF ROCKET IN SALT STRESS

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Abstract

The effects of glutamine (Gln), which acts as a signal molecule in living organisms but whose function in plants has not been fully determined, on germination and early seedling development were investigated. Three different rocket seeds (Eda, Geniş Yaprak, Bengi) pre-treated with glutamine (1, 2, 3 mM) were subjected to germination under unstressed and salt stress (150 mM) conditions. As germination parameters; germination percentage (GP), mean germination time (MGT), coefficient of velocity of germination (CVG) were calculated. Seedling vigor index (SVI), shoot and root length (cm), leaf width and length (cm), shoot and root fresh weights (g) were measured in order to reveal their effects on early seedling growth. In unstressed conditions, 3 mM Gln increased the germination percentage in all cultivars. It was determined that 1 mM Gln in Eda cultivar and 3 mM Gln in other cultivars had a positive effect on germination parameters in general under salt stress conditions. In terms of seedling growth, the effect of Gln under stress-free conditions differed according to the cultivar, and it had a positive effect on the SVI value depending on the dose. It was determined that plantlets developed from seeds pre-treated with 1 mM Gln in Eda and Bengi and 3 mM Gln in Geniş Yaprak showed better growth under salt stress condition. As a result, it has been determined that Gln priming can be applied as an exogenous treatment under salt stress conditions in rocket, depending on the cultivar.

Keywords: *Germination, Glutamine, Priming, Rocket, Salt Stress*

Introduction

The germination time and performance of seeds in plant production are closely related to the health of the seed, which is the starting material. In addition, keeping the seeds in osmotic solutions before planting is an important practice to increase seed viability and vigor (Sivritepe and Şentürk, 2011). Various treatments to the seed before sowing are generally called "Priming" and can be done with water and many water-soluble substances (Heydecker and Gibbins, 1978; Sivritepe, 1999). These pre-applications increase seed germination, seed vigor and plant growth, as well as enable them to tolerate stress factors. It improves biochemical processes in plant metabolism, regulates proteins, initiates protein synthesis and DNA repair processes (Paparella *et al*., 2015; Diya *et al*., 2021). As a result of the application of organic priming materials to watermelon seeds (Özkaynak *et al*., 2015), it was determined that the seedlings developed earlier and in a shorter time. Similarly, organic priming applied to pepper seeds positively affected the viability and strength of seeds (Sivritepe *et al*., 2015). In mung beans (Rashid, 2004), the seeds emerged faster and at a higher rate, and a significant reduction in yellow mosaic virus disease symptoms occurred in plants.

Glutamine is a precursor amino acid for the synthesis of nitrogenous compounds, which can also function as a signaling molecule in all living organisms. In nitrogen assimilation,

inorganic nitrogens are converted to glutamine and glutamate in plants. In addition, plants can directly take in amino acids, including glutamine, to support their growth. However, unlike other living things, the functions of glutamine in plants have still not been fully determined (Kan *et al.*, 2015). It is known that amino acids have a biostimulant effect, and in a study on onions, the effects of 10 different amino acids on germination were determined (Abdelkader et al., 2023). Researchers have stated that glutamine gives the best results in terms of growth parameters, and that they also reach the highest germination rates of seeds applied glutamine, proline and tryptophan. In this study, the effect of glutamine applied as a pre-application under salt stress conditions on rocket varieties was revealed.

Materials and Methods

In the study, the seeds of three different rocket cultivars (Eda, Geniş Yaprak, Bengi) obtained from a commercial company were used. The research was carried out at the Laboratory of the Department of Plant and Animal Production, Vocational School of Technical Sciences at Akdeniz University.

The washed seeds were kept in sodium hypochlorite for 10 minutes for surface sterilization. After kept in 70% alcohol for 3 minute, they were washed with distilled water and kept in solutions containing Glutamine at different concentrations (1, 2, 3 mM) for 24 hours. The control group was kept in distilled water.

The study was carried out in petri plates with 25 seeds in each replicate, in 4 replicates, at 24±1ºC in a climate chamber with a photoperiod of 16/8 hours. Irrigation was done with distilled water containing 150 mM NaCl to provide salt stress conditions. The control group was irrigated with distilled water. In order to determine the effect of glutamine on germination, the number of germinated seeds was recorded daily and according to the data obtained; germination percentage (Gosh *et al.*, 2014), coefficient of velocity of germination (Kotowski, 1926) and mean germination time (Ellis and Roberts, 1981) were calculated. In order to determine the vegetative development at the end of the experiment; shoot length (mm), root length (mm), leaf length (mm), leaf width (mm), shoot and root fresh weights (g), plant fresh weight (g) and seedling vigor index (Baki and Anderson, 1973) were calculated.

Statistical analyzes of the data were made using Minitab 21 package program and Jamovi program. The differences between treatments were detected by the Tukey Test ($p< 0.01$, $p< 0.05$).

Results and Discussion

Descriptive statistics and analysis of variation results showed that cultivar, application, and cultivar-application interaction were statistically significant at 0.01 level on all germination parameters (Table 1). Varieties were examined separately in terms of these parameters and the results are presented in Figures 1, 2, 3. The highest germination percentage was obtained from 1 mM Gln application in Eda cultivar and 3 mM Gln application in Bengi and Geniş Yaprak under salt stress conditions. CVG values revealed that the fastest germination was obtained from 1 mM Gln application in all cultivars. Similarly, average germination times were positively affected by 1 mM Gln and it was determined that Gln applied to all cultivars increased seedling vigor under salt stress conditions. Similarly, the promoting effect of proline, glutamine and tryptophan on onion germination was detected (Abdelkader *et al*., 2023). In a study conducted in Arabidopsis, protein hydrolysates were used in priming and it was stated that they had positive effects on plant growth and stress tolerance (Sorrentino *et al*., 2021).

Table 1. Descriptive and Variance analysis result with respect to the germination parameters of rocket cultivars.

Variation source	df	GP (%)	CGV	MGT (day)	SVI

C	2	***	***	***	***
A	7	***	***	***	***
C*A	14	***	***	***	***
Mean		0.63	67.3	1.60	35.7
Minimum		0.07	33.3	1.02	0.00
Maximum		0.93	97.2	3.01	92.3
Standart deviation		0.226	16.9	0.483	19.5

C: Cultivar, A: Application, * p<0.05, **p<0.01, ns: not significant ***p<0.001

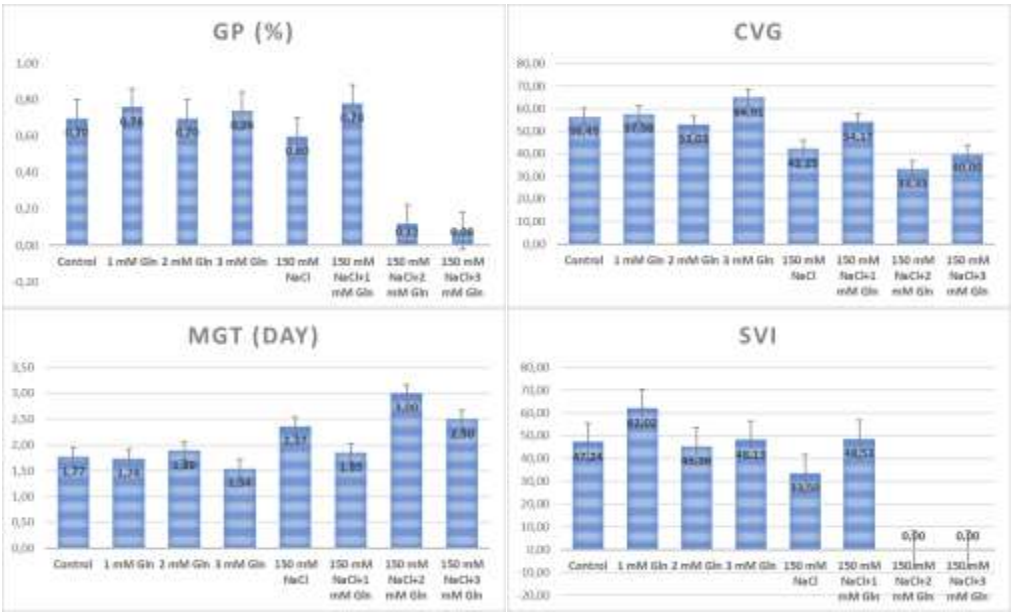


Figure 1. Changes in the germination parameters of Eda cultivar

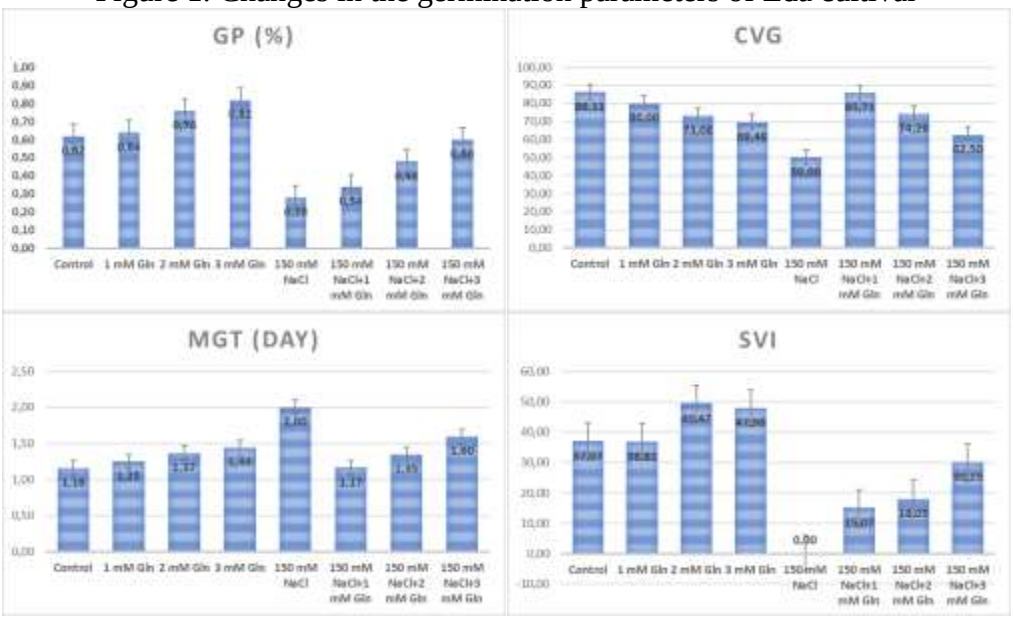
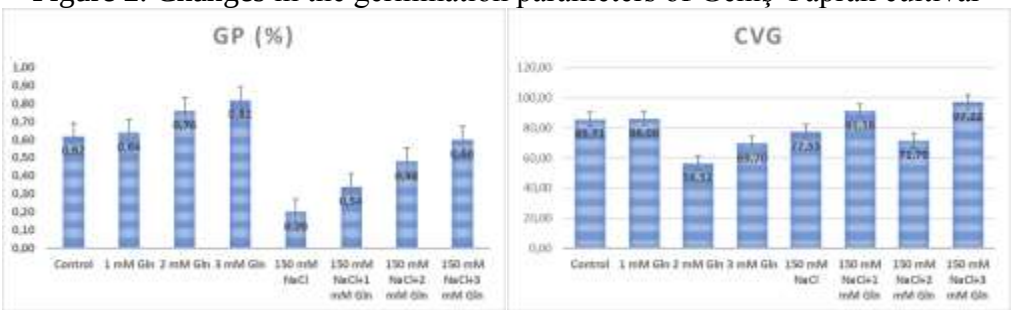


Figure 2. Changes in the germination parameters of Geniş Yaprak cultivar



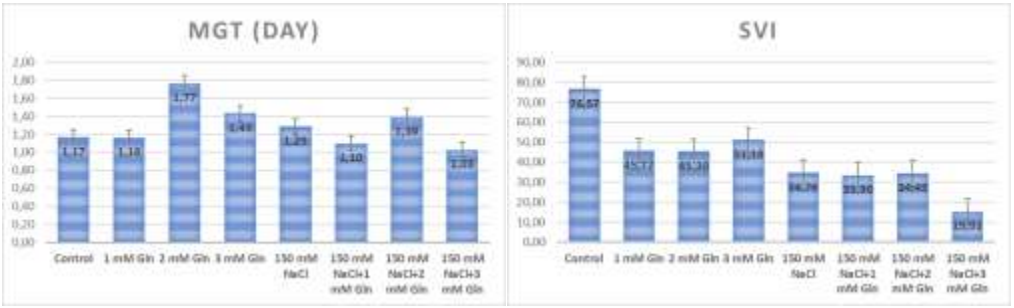


Figure 3. Changes in the germination parameters of Bengi cultivar

Statistical analyzes showed that, as in germination results, cultivar, application and cultivar-application interaction were statistically significant at the 0.001 level on vegetative parameters of all seedlings (Table 2). In Eda cultivar, it was not possible to obtain enough plants for statistical analysis from the application of 2 and 3 mM Gln under salt conditions. In other words, 2 and 3 mM Gln applications negatively affected seedling growth. On the other hand, sufficient plant was obtained from the seeds applied 1 mM Gln, but it was determined that this dose did not stimulate plant growth much (Table 3). In Geniş Yaprak, 3 mM gave the best results, although there was not much variation between applications (Table 4). In the Bengi, the prominent application was 1 mM Gln (Table 5). When the correlation between vegetative parameters was examined, it was determined that the highest correlation was between LL and LW (r= 0.804) (Figure 4).

Table 2. Descriptive and Variance analysis result with respect to the vegetative growth of rocket cultivars.

Variation source	df	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SFW (g)	RFW (g)
C	2	***	***	***	***	***	***	***
A	7	***	***	***	***	***	***	***
C*A	14	***	***	***	***	***	***	***
Mean		20.8	29.1	5.38	4.22	0.0186	0.0140	0.00502
Minimum		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum		42.1	97.8	8.36	8.24	0.0327	0.0273	0.0192
Standart deviation		9.96	15.2	2.01	1.72	0.00776	0.00640	0.00358

* p<0.05, **p<0.01, ns: not significant ***p<0.001

Table 3. Vegetative growth of Eda cultivar treated with glutamine

Application	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SFW (g)	RFW (g)
Control	26.96	36.62 ab	6.08	4.54 ab	0.0239 ab	0.0191 ab	0.00655
1 mM Gln	29.75	47.09 a	6.06	4.93 ab	0.0211 b	0.0160 b	0.00403
2 mM Gln	28.42	34.44 b	5.82	4.91 ab	0.0226 ab	0.0196 ab	0.00442
3 mM Gln	26.74	32.98 b	6.57	5.75 a	0.0277 a	0.0222 a	0.00593
150 mM NaCl	23.12	24.79 b	5.67	3.95 b	0.0203 b	0.0153 b	0.00405
150 mM NaCl+1 mM Gln	28.00	30.04 b	6.47	4.29 ab	0.0236 ab	0.0175 ab	0.00613
150 mM NaCl+2 mM Gln	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150 mM NaCl+3 mM Gln	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Vegetative growth of Geniş Yaprak cultivar treated with glutamine

Application	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SFW (g)	RFW (g)
Control	22.50 ab	31.62 ab	5.42	4.05	0.0194	0.0157	0.0033 ab
1 mM Gln	26.20 ab	27.00 bc	5.50	3.92	0.0186	0.0152	0.0046 ab
2 mM Gln	27.29 a	38.69 a	5.36	4.72	0.0211	0.0178	0.0038 ab
3 mM Gln	22.09abc	30.17 ab	5.59	4.01	0.0183	0.0164	0.0038 ab
150 mM NaCl	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150 mM NaCl+1 mM Gln	15.38 d	26.36 bc	5.11	4.19	0.0204	0.0143	0.0052 a
150 mM NaCl+2 mM Gln	15.85 cd	19.78 c	5.01	3.94	0.0214	0.0159	0.0064 a
150 mM NaCl+3 mM Gln	20.14bcd	25.75 bc	5.87	4.38	0.0237	0.0185	0.0066 a

Table 5. Vegetative growth of Bengi cultivar treated with glutamine

Application	SL (mm)	RL (mm)	LW (mm)	LL (mm)	PFW (g)	SFW (g)	RFW (g)
Control	20.20 a	70.21 a	7.08 ab	6.13 ab	0.0234 a	0.0113 ab	0.0038 de
1 mM Gln	23.65 a	26.50 bc	7.18 a	6.44 a	0.0232 a	0.0117 ab	0.0115 ab
2 mM Gln	21.34 a	31.49 b	6.55abc	5.57 ab	0.0228 a	0.0171 a	0.0092abc
3 mM Gln	20.91 a	28.28 bc	6.38abc	4.53 bc	0.0219 a	0.0089 bc	0.0138 a
150 mM NaCl	13.04 bc	30.07 bc	5.31 bcd	4.76 abc	0.0193 a	0.0110 ab	0.0060cde
150 mM NaCl+1 mM Gln	17.88 ab	37.17 b	5.78abc	5.39 ab	0.0232 a	0.0165 a	0.0102abc
150 mM NaCl+2 mM Gln	13.46 bc	23.74 bc	4.91 cd	3.49 c	0.0173 a	0.0118 ab	0.0071bcd
150 mM NaCl+3 mM Gln	6.85 c	14.10 c	3.82 d	3.17 c	0.0031 b	0.0026 c	0.0018 e

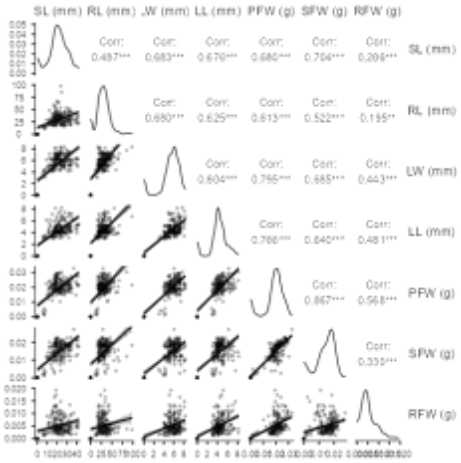


Figure 4. Correlation graph between vegetative parameters

Kan *et al*. (2015) stated that glutamine may increase the signaling of plants for the regulation of stress responses and plant growth and may interact with other signal transduction pathways. It has been determined that 24-hour hydropriming in rice (Nakao *et al*., 2018) and *Solanum villus* (Forti *et al*., 2021) positively affects germination and provides rapid plant growth in rice. Nakao et al (2018) reported that hydropriming promotes water absorption and activates metabolic pathways, while Forti *et al.* (2021) determined that there was an increase in tocopherol, which provides protection against oxidative damage with hydropriming application. Hydropriming applied to pumpkin rootstock seeds (Ermiş *et al.*, 2021) increased germination percentage and seedling growth under salt and drought stress conditions. Barcanu *et al.* (2021) determined that arginine applied as a priming study increased the seed emergence rate in pepper, but glutamine decreased it in some cultivars. On the other hand, Nejadalimoradi *et al*. (2014) reported that arginine reduces the negative effects of salt stress on sunflower plant growth.

Conclusion

The effect of Gln differed according to whether the environment was stressed or unstressed and according to the cultivar. In terms of plant growth, 1 mM Gln application was prominent in Eda and Bengi cultivars under both stressful and non-stressful conditions, while 3 mM Gln application under stressful conditions and 2 mM Gln application under non-stressful conditions was prominent in Geniş Yaprak. As a result, external applications to seeds should be evaluated according to species and cultivars, and the effects of amino acids, which are the building blocks of proteins, should be examined in detail.

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PEAR BREEDING FOR THE FUTURE

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Abstract

This study was carried out to determine the future expectations of primary and secondary school students on fresh pear fruit, to raise awareness on the social and environmental effects of resistance breeding studies, and to provide information about the process of obtaining new pear varieties. 360 students from elementary and secondary schools took part in the activities planned as part of the pear fruit breeding studies. Students created works in the story, picture, slogan, and logo design categories during the activities. 383 works participated in the competitions. It was attempted to determine the "new pear variety perception" in the students' minds using the content analysis method. Not only students, but also teachers and parents were reached through awareness trainings in primary and secondary schools. The results show that students in the 10-14 age group like green and yellow pears, as well as red, sweet, crunchy, soft, or crispy pears. The findings are expected to be significant for future forecasts on the characteristics of new pear cultivars, which could take up to 25-30 years to evolve.

Keywords: *New varieties, elementary school, consumer perception, hybridization*

Introduction

Fruits are important nutritional components because of their high biochemical content, which is required for health. Awareness of these foods, which play an important part in human nutrition, is especially important for young people, who eat a lot of unhealthy foods. In this context, social studies to enhance fruit consumption among young people are extremely important (Klepp et al., 2005; Hakim and Meissen 2023; Ingman et al., 2022). However, access to fruits is getting more and more challenging every day. Fruit production is endangered by changing climatic conditions and increased pressures caused by abiotic and biotic stress. Aside from the obstacles in production, the fact that the fruits produced must meet the tastes and expectations of consumers adds to the sector's stress. This necessitates the development of new fruit varieties that will satisfy consumers for a better future. At this point, the attitudes and behaviours of early age groups will determine the acceptance of new varieties and the sustainability of fruit production and consumption. Studies conducted under topics such as identification of fruit, taste tests, nutrition, creative snacks, computer games, social messages, fun fruit activities, continuous reinforcement, parent involvement (Sharps and Robinson, 2016; Klepp et al., 2005; Zeinstra et al., 2021), besides raising awareness for healthy nutrition, are also very important in determining the future expectations, tastes, and preferences of new cultivars. In this study, activities and outputs organized specifically for the pear fruit for primary school students are included to raise awareness about healthy nutrition and new fruit varieties in society.

Material and Methods

This study, carried out by the Fruit Research Institute (MAREM), was designed within the framework of the MAREM pear breeding program. 360 4th and 7th grade students in Eğirdir district of Isparta province, where fruit growing is intense, participated in the study (Table 1). 51% of 4th grade students are female and 49% are male. On the other hand, 54.9% of the 7th grade students are female and 45.1% are male.

Table 1. Number of students participating in the study and gender distribution.

	4th Grade	7th Grade	Total
Student number	207	153	360
Female	106	84	190
Male	101	69	170

The study was conducted in three stages. In the first of the two-stage awareness trainings, entertaining and interactive activities were organized on topics such as the importance of pear fruit in terms of health, the effects of pesticides used in the production process, why we need new pear varieties?, and the development processes of new pear varieties. At the end of the section, two new pear cultivars, "ArTroya" and MarSalda," developed by MAREM, which are tolerant to fire blight disease, were especially emphasized, and additional information about them was given to the participants. In the second of the awareness activities, the participants visually examined the real hybrid fruit samples and experienced their flavors. In both sections, the activities were carried out face-to-face. The third part, organized to receive feedback, consists of award-winning competitions in the fields of story, picture, slogan, and logo design related to the "ArTroya" and MarSalda varieties of the participants. 383 original works produced by 240 participating students were evaluated with the content analysis method, and the "new pear variety perception" in their imagination was tried to be determined.

Results and Discussion

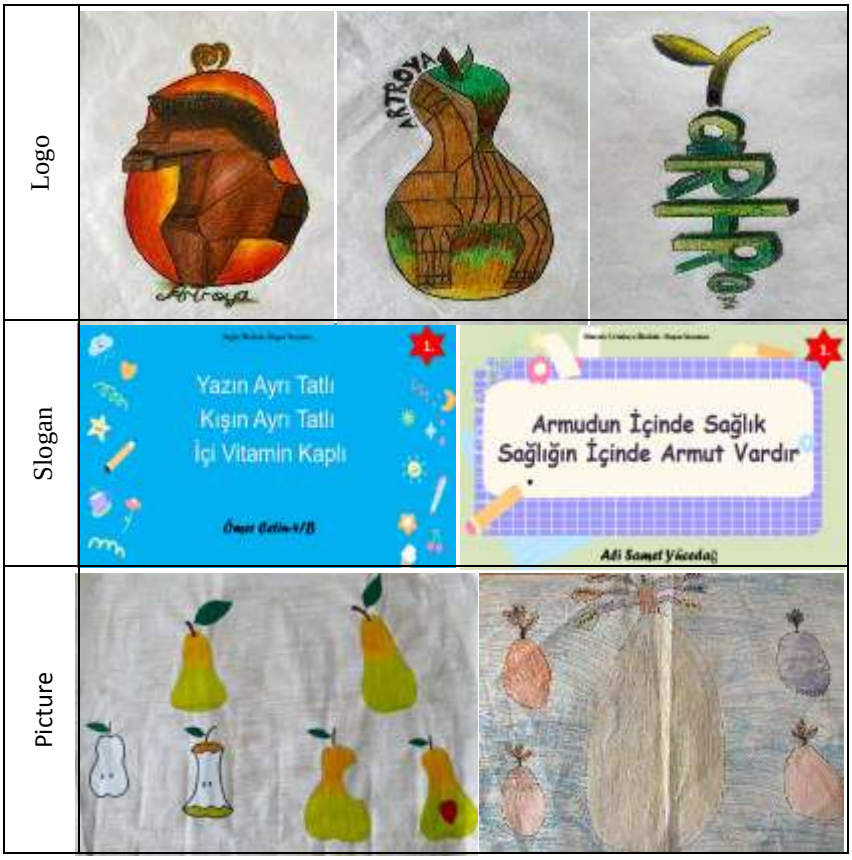
Awareness trainings

360 4th and 7th grade students attended the awareness training titled "My Pear" (Figure 1). In the content of the activity, the benefits of pear consumption and the problems experienced in the supply chain due to fire blight disease (tree losses and additional struggle costs for producers, increase in pear prices for the whole supply chain, negative effects of chemicals used in production on human health and the environment, etc.) were discussed. Afterwards, the participants examined the cultivar candidates developed within the scope of the MAREM pear breeding program visually and by tasting. All the students who participated in the activities stated that they participated in such a training for the first time and that "their perspectives have changed" about the pear fruit. Some of the students stated that they ate pears for the first time and that they would continue to consume pears from now on. A few of them stated that they do not consume pears due to their allergies. Before and during the trainings, the teachers and families of the participating students were actively interviewed, and it was observed that the activities were effective in the school and in the family. In this way, it is thought that there is a stronger possibility that the gains will be permanent.

Figure 1. “My Pear” awareness training.

Competition

The theme of the competitions was ArTroya and MarSalda varieties, where detailed information was given about their features in awareness activities. The students who participated in the trainings were asked to produce works in the fields of story, picture, slogan, and logo design for one of these two varieties that are tolerant to fire blight disease, registered in 2020. Some images of 383 works participating in the competitions are presented below (Figure 2).



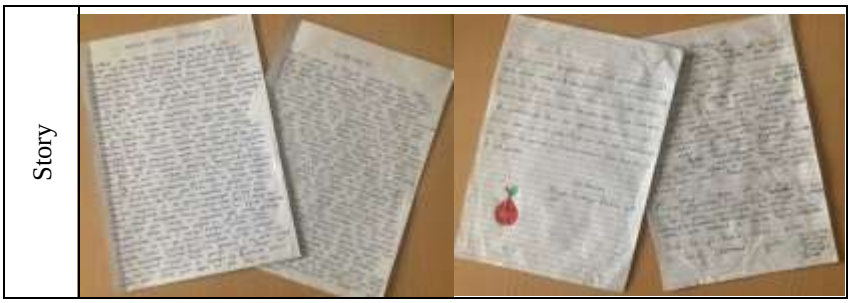


Figure 2. Some photos of the works in the competitions organized within the scope of "My Pear" awareness trainings.

The works were evaluated separately for each category at the school and grade level (4th and 7th grades) by the juries consisting of subject experts (Figure 3a), and their awards were presented at the ceremony held at the Fruit Research Institute (Figure 3a). Figure3b).

Figure 3. Evaluation of the works (a) and award ceremony (b).

The works prepared by the students within the scope of the competitions provided a rich content that could give an idea to the breeders about the future expectations about the pear. Some market information was also obtained from these contents (Figure 4).

(a) **(b)**

Figure 4. Word clouds a) student's expectations of appreciation regarding the internal and external quality of pear fruit, b) students' perceptions of pear fruit.

36,77% of the students defined new pear varieties as health, 35% as happiness, and 11.76% as the future. Other than these, 16.47% consisted of nature, Anadolu, and others (Figure 5).

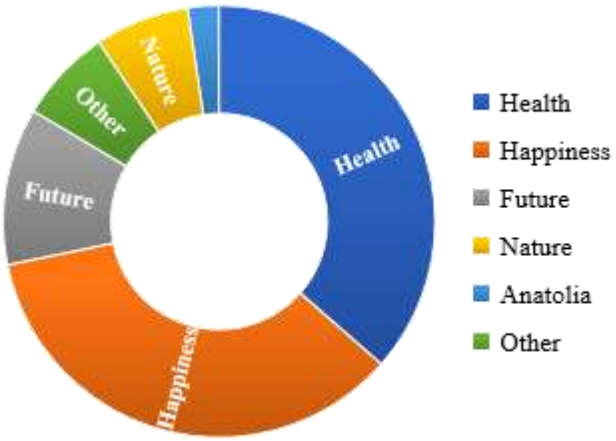


Figure 5. Distribution of future expectations

Figure 6 shows the students' expectations for the new varieties based on the sensory attributes of the pears. While 16% of the students stated that they wanted green pears, this was followed by red (16%) and yellow (12%) pears, respectively (Figure 6). Orange, pink, purple and blue colors other than these colors constituted 5% of the total color appreciation. Few of the students stated that they liked pears with oval and large fruit (Figure 6).

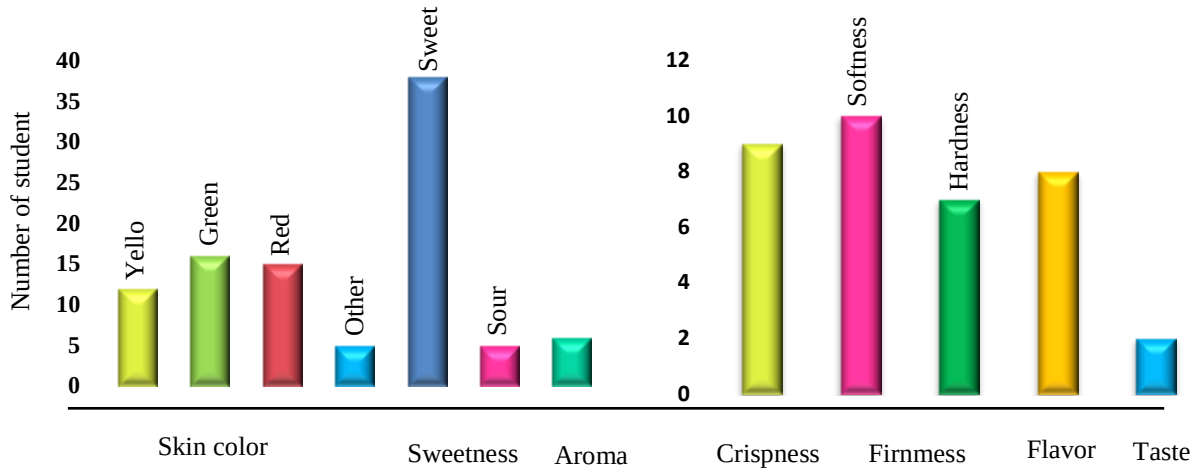


Figure 6. Students' internal and external quality appreciation levels.

Although the nutritional properties and health benefits of fruit are important to consumers, the eating quality of the fruit ultimately influences their decision to purchase it (Farruggia et al., 2016). It was determined that the students were more selective in terms of internal quality characteristics, and the majority of them (38%) preferred sweet pears. The level of appreciation for aroma and sourness was around 5%. While softness, which is one of the features affecting eating quality, had a share of 18% in taste, this feature was followed by crispness (16%), flavor (14%), and hardness (13%). Taste expectations were found to be low compared to other characteristics (Figure 6).

Conclusion

The consumption of pears, which is very beneficial for health, is relatively limited due to the low production amount, higher prices, and inconsistencies in fruit quality. This situation also leaves unanswered questions about what should be expected of pear fruit, especially in children. In the study, it was determined that children prefer traditional green- and yellow-colored sweet pears, and crispy or buttery pears can be consumed by this age group. In addition, they were found to be more innovative in terms of visual quality. These results can provide pear breeders with information on what the fruit quality should be. The works prepared in the competitions organized within the scope of the study have strikingly revealed the richness of children's imaginations. The mentioned works are quite original and can be used in marketing communication. It is thought that these and similar studies will contribute to an increase in the level of education and awareness in society, indirectly improving the quality of life and the sustainability of fruit production.

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CHARACTERIZATION OF HWM-GS AND EVALUATION OF QUALITY CHARACTERS IN ELITE SYNTHETIC WHEAT LINES

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Summary

In the study, 44 synthetic wheat lines and Selimiye, Flamura 85, Pehlivan, Aldane, Bereket and Gelibolu bread wheat varieties were used as material. When the genotypes were examined in terms of quality scoring for gluten band patterns, the highest values (10 points) were obtained in ZFSN 4, HRSN 2-14, ZFSN 12, HRSN 13-2 and HRSN 8-6, while HRSN 13-17, HRSN 9-7, HRSN 11-11, ZFSN 7, HRSN 2-16, HRSN 15-2, ZFSN 2, HRSN 4-10 and HRSN 14-2 had the lowest glutenin score values in terms of quality. According to the data obtained, in synthetic bread wheat genotypes, in terms of gluten score, 21 lines had a gluten score of 8 and above, while 23 lines had a gluten score of 7 and below. In particular, 6 synthetic lines with a gluten score of 10 and 1 line with a gluten score of 9 are superior lines in terms of quality.
The 7 synthetic lines with the highest gluten score showed variation in protein content, gluten content, and sedimentation rate. This result reveals that the quality characteristics of the growing region are significantly affected by the climate data and cultural practices. When the synthetic lines are examined in terms of quality characteristics, it is seen that there are genotypes with superior characteristics in terms of glutenin score and that these genotypes can be a good source material for breeding studies. The fact that the quality characteristics obtained change according to the glutenin scores reveals that quality studies should be evaluated according to the results to be obtained in different years and locations.

Key Words: *Wheat, synthetic line, glutenin score, quality*

Indroduction

The main purpose of breeding studies is to develop stable and high-quality varieties in terms of yield. The performance of the genotypes is evaluated by cultivating the lines that are seen as promising in breeding studies in different years and places. In recent years, significant losses in yield and quality have occurred in wheat cultivation areas with the effect of abiotic and biotic stress factors. Drought and high temperatures occurring during the head period in wheat agriculture cause significant decreases in yield and quality in wheat varieties. Therefore, it is necessary to expand the wheat genetic pool in the breeding studies to be carried out for the region. In recent years, there are opinions that synthetic wheat genotypes can be a good source material for resistance to abiotic and biotic stress factors. However, studies on synthetic wheats are still at a very low level.
Synthetic wheat is a useful genetic resource used to transfer agronomically important genes from a wide range of tetraploid or diploid species, including wild species, to improve the performance of bread wheat. A set of reference genomes has recently been made available for both bread wheat and its progenitors. Draft genome sequences of the A and D donor genomes were obtained while several hexaploid wheat genome versions were under development (Jia et al. 2013; Ling et al. 2013, Mayer et al. 2014). Studies in synthetic wheats are about analyzing gluten subunit composition, changes in grain hardness and germination

resistance before harvest (Gedye, et al. 2004; Nelson et al. 2006). According to Pena et al. (1995) analyzed the effects of synthetic hexaploid wheat material on quality properties and found that 5 + 12 and 1.5 + 10 subunits gave higher quality performance at the Glu-D1 location.

Gliadin and glutenin proteins are major determinants of breadmaking quality in hexaploid wheat. These proteins, which are found in almost equal proportions and make up 80% of endosperm proteins, are analysed by chromatographic and electrophoretic methods. Gliadin is composed of many polypeptides with low molecular weight and glutenin with high molecular weight (Artık, 1988). Gliadins show codominant inheritance according to the 3-set chromosome number in the endosperm (Metakovsky, 1984, Du Cros and Hare, 1985). Gliadin band patterns are determined directly by genotype and are not affected by environmental conditions. Wheat breeders determine the best genotypes by evaluating agronomic and quality traits with multiple trials in different years and locations. For this purpose, in addition to agronomic characters such as plant height, grain yield, disease resistance and number of days to ear, selection of varieties with superior protein ratio, sedimentation ratio, gluten ratio, flour extraction, dough resistance and cooking performance are important selection objectives. Wheat breeding programs are based on the goal of supplying end users, including breeders, millers, bakers and consumers.

Quality characteristics differ from agronomic characteristics, quality analyses require large samples, time and specialized personnel to carry out these analyses. For this purpose, MAS selection has been suggested in the development of superior cultivars, in a shorter and more effective development of cultivars (Raman et al. 2009; Simons, et al. 2012). For this purpose, wheat breeders have carried out numerous genetic studies in recent years to identify suitable markers associated with quality traits in wheat (Carter et al. 2012, Tsilo, et al. 2013;).

The band patterns of gliadin and glutenin in wheat are used to determine the biochemical similarities and differences of breeding lines, to study the origin and ancestors of wheat, and to develop superior genotypes using characters such as quality, resistance to abiotic and biotic stress factors. Studies have reported the relationships of genes regulating gluten proteins with molecular markers. The score values of gluten obtained in their study in wheat (Shuster et al. 1997) are given in Table 1.

Glutenins are responsible for dough strength and are formed by high molecular weight (HMW) subunits and low molecular weight (LMW) subunits. The main genes controlling gluten high molecular weight subunits (Glu-1, Glu-A1, Glu-B1, and Glu-D1) are located in the long arms of the homeologous group chromosome, while genes controlling low molecular weight glutens (Glu-A3, Glu-B3), and Glu-D3) are on the short arms of the same chromosome (Tang et al. 2010).

Table 1. Gluten scores in wheat

Glutenin scores	Genome			Glutenin scores	Genome		
	1A	1B	1D		1A	1B	1D
4	-	-	5+10	1	Null	7	4+12
3	1	17+18	-	1	-	6+8	-
3	2*	7+8		1	-	20	-
3	-	13+16	-	No information	-	21	2,2
2	-	7+9	2+12	No information	-	22	2+11
2	-	-	3+12	No information	-	13+19	2+10

Different researchers conducting research on glutenins in wheat have revealed that the quality characteristics of synthetic wheats vary (Li et al. 2014; Tang et al. 2016; Doneva et al. 2018). It has been determined that high molecular weight glutens have a quality-enhancing effect in

bread wheat, while low molecular weight glutens have a negative effect on quality. While 1 and 2* in Glu A, 7+9 and 17+18 in Glu B1, and 5+10 in Glu D1, one of the subunits of gluten, provide high quality in wheat. It was determined that 6+8 in Glu B1 and 2+12 in Glu D1 locus had a negative effect on quality in wheat (Horvat et al 2006). In this study, it was aimed to reveal the potential of 44 synthetic bread wheat genotypes in terms of gluten score values, protein ratio, gluten ratio and sedimentation quality characteristics.

Material and method

In the study, 44 synthetic wheat lines and 5 bread wheat varieties (Selimiye Flamura 85, Pehlivan, Aldane, Bereket and Gelibolu) were used. SDS-PAGE method was used to reveal the genotypic differences of the cultivars and lines used in the experiment (Mujeeb-Kazi et al. 1996). Six wheat genotypes (Norman, Courtot, Kadett, Opatas, Troll, and Moluing) with standard banding patterns were used for identification and comparison of the bands created (Table 2).

Table 2. Standard varieties glutenin band patterns and band numbers

Varieties	Genome		
	1A	1B	1D
Norman (N)	-	6+8	3+12
Courtot (C)	2*	7+8	2+12
Kadett (K)	1	7+9	5+10
Opata (O)	-	13+16	-
Troll (T)	-	14+15	-
Moulin (M)	-	17+18	-

Forty-four synthetic hexaploids were characterized separately for Glu-A1 and Glu-B1 subunits to confirm the respective subunits in wheats. One wheat grain was taken and after it was thoroughly crushed, the sample was placed in the ependof tube. Then, 500 µl of 70 ethanol was added to the sample in this tube and left in the tubes for 2 hours.
It was shaken in vortex for 1 minute every 10 minutes during holding. At the end of this period, the tubes were centrifuged at 13,000 rpm for 5 minutes. 100 µl was taken for each centrifuged sample and transferred to a separate tube. 100 µl of SDS solution, 25 µl of mercaptoethanol, 190 µl of 60% glycerin, 190 µl of 0.005% bromphenol blue solution were added to the tubes and kept in a water bath set at 90°C for 2.5 minutes. Finally, 10 µl of sample was taken from the tubes and the gel was loaded.

Results and discussion

In the study carried out with forty-four synthetic bread wheat genotypes and 5 standard cultivars, the band patterns of high and low molecular weight obtained for the bread wheat cultivar and line and their scores are given in Table 3.

Table 3. High and low molecular weight band patterns obtained from genotypes

Varieties	Glutenin scores				Varieties	Glutenin scores			
	Glu A1	Glu B1	Glu D1	GLU 1		Glu A1	Glu B1	Glu D1	GLU 1
HRSN 11-11	2*	7+8	5+10	10	HRSN 1-14	2*	5+10	-	7
HRSN 15-17	1	7+8	5+10	10	ZFSN 28	2*	7	2+12	6
HRSN 7-12	1	7+8	5+10	10	HRSN 12-14	2*	7+8	-	6
ZFSN 7	2*	7+8	5+10	10	ZFSN 12	null	7+8	2+12	6
Gelibolu	1	7+8	5+10	10	HRSN 7-6	null	7+8	2+12	6
HRSN 12-11	2*	7+8	5+10	10	HRSN 1-16	null	6+8	5+10	6
ZFSN 6	2*	7+8	5+10	10	HRSN 13-2	null	7	5+10	6
Aldane	2*	7+9	5+10	9	HRSN 6-2	1	7+8	-	6
HRSN 14-17	null	7+8	5+10	8	HRSN 15-11	1	7+8	-	6
HRSN 11-4	null	7+8	2-12	8	ZFSN 30	1	7+8	-	6
ZFSN 8	null	7+8	5+10	8	ZFSN 32	2*	7	2+12	6
HRSN 15-2	1	7	5+10	8	Bereket	null	7	5-10	6
HRSN 12-9	2*	7+8	2+12	8	ZFSN 5	null	7	5-10	6
HRSN 6-8	2*	7+8	2+12	8	HRSN 14-2	1	7+8	-	6
ZFSN 31	2*	7+8	2+12	8	ZFSN 33	2*	7	2+12	6
ZFSN 4	2*	7+8	2-12	8	HRSN 11-14	2*	7	2+12	6
HRSN 4-10	null	7+8	5-10	8	ZFSN 2	2*	7+8	-	6
ZFSN 3	null	7+8	5-10	8	HRSN 15-13	null	5+10	-	5
HRSN 14-18	1	7	5+10	8	ZFSN 18	null	5+10	-	5
HRSN 13-17	null	7+8	5+10	8	ZFSN 23	2*	7+9	-	5
ZFSN 13-9	null	7+8	5+10	8	ZFSN 24	null	5+10	-	5
Pehlivan	null	7+8	5+10	8	HRSN 2-14	2*	7	-	4
ZFSN 26	2*	7+8	2+12	8	HRSN 9-15	2*	7	-	4
ZFSN 16	1	7	5+10	8	HRSN 8-6	2*	7	-	4
Flamura 85	null	7+9	5+10	7					

It was observed that there were genotypical differences in terms of band patterns and band scoring of bread wheat genotypes. When the gluten obtained in the synthetic wheat genotypes included in the experiment was examined in terms of scoring, the highest values with 10 points were obtained in HRSN 11-11, HRSN 15-17, HRSN 7-12, HRSN 7, HRSN 12-11 and ZFSN 6. According to the data obtained, HRSN 11-11, HRSN 15-17, HRSN 7-12, HRSN 7, HRSN 12-11 and ZFSN 6 and HRSN 14-17 are superior genotypes in terms of quality. It can be said that these genotypes have an important potential in cultivar breeding studies. In addition, with 8 glutenin score values, HRSN 14-17, ZFSN 24, HRSN 15-11, HRSN 1-16, HRSN 11-14, HRSN 12-14, ZFSN 31, HRSN 12-11, HRSN 15-17, ZFSN 32, ZFSN 5, HRSN Genotypes 14-18, ZFSN 33, ZFSN 16, HRSN 14-10 and HRSN 6-8 also offer variation to breeders in terms of quality.

ZFSN 15, ZFSN 18, Flamura 85, HRSN 7-6, ZFSN 26, HRSN 15-13 and ZFSN 30 with 7 scale values in terms of quality, ZFSN 23, HRSN 11-4, HRSN 12-9, ZFSN 15 with 6 quality values , HRSN 9-15, HRSN 1-11, ZFSN 14, HRSN 1-6, ZFSN 3, HRSN 4-11, ZFSN 21 and HRSN 15-6 lines were in the middle group in terms of quality characteristics. Among the examined synthetic wheat genotypes, HRSN 13-17, HRSN 9-7, HRSN 11-11, ZFSN 7, HRSN 2-16, HRSN 15-2, ZFSN 2, HRSN 4-10 and HRSN 14-2 were the lowest in terms of quality. have values. Protein ratio, gluten ratio and sedimentation values of synthetic wheats and bread wheat genotypes examined in the study are given in Table 4.

In the synthetic and bread wheat genotypes examined in the study, the protein ratio varied between 17.00-11.93%, the gluten ratio between 38.67-25.20% and the sedimentation ratio between 83.0-38.00 ml. According to the two-year average values, the gluten value, which

was 30.53% on average in bread wheat varieties used as standard, was 29 synthetic lines and 7 synthetic lines higher than the Pehlivan variety (33.64%), which had the highest gluten content.

While the average of bread wheat cultivars in terms of protein content was 13.85%, 20 synthetic lines gave higher value than this value, and 11 synthetic lines gave higher value than Flamura 85 cultivar, which had the highest protein value with 14.30%. While the sedimentation value was 39.6 ml on average in bread wheat varieties, 29 synthetic lines exceeded this value, and 7 synthetic lines gave a higher value with 51.84 ml than the Aldane variety, which has the highest gluten content. The results obtained show that the synthetic lines contain suitable genotypes in terms of gluten content, protein content and sedimentation value. In the study, the quality criteria of the HRSN 11-11, HRSN 15-17, HRSN 7-12, ZFSN 7, Gelibolu, HRSN 12—11 and ZFSN 6 genotypes with the highest gluten scores changed in terms of protein content, gluten content and sedimentation value. The HRSN 11-11 genotype was at the bottom in terms of all three traits, while HRSN 15-17 was at the top in terms of gluten, protein and sedimentation.

Synthetic genotypes ZFSN 7 and HRSN 7-12 rank in the middle for the three traits examined. HRSN 12-11 synthetic line is in the middle ranks in terms of gluten and protein ratio, and in the lower ranks in terms of sedimentation rate. ZFSN 6 line, on the other hand, was in the middle in terms of gluten ratio and lower in terms of protein and sedimentation value.

The HRSN 14-17 line, on the other hand, was in the lower ranks in terms of all three features. In the study, HRSN 2-17, which had the lowest gluten score values, were at the top in terms of protein ratio, HRSN 9-15, and in the middle in terms of gluten and sedimentation ratio, while HRSN 8-6 synthetic line was in the last place in terms of all three characteristics.

Table 4. Protein ratio, gluten ratio and sedimentation values of wheat genotypes

Varieties	Gluten rate (%)			Protein rate (%)			Sedimentation (ml)		
	2015	2016	Average	2015	2016	Average	2015	2016	Average
HRSN 11-11	28.67	30.00	29.34 r-v	12.67	12.93	12.80 y	50.00	19.67	34.84 H
HRSN 15-17	36.07	28.67	32.37 e-k	13.20	16.20	14.70 b	60.00	56.57	58.29 b
HRSN 7-12	29.07	27.00	28.04 t-y	13.00	13.07	13.00 uv	50.00	45.67	47.84 l
ZFSN 7	33.27	28.00	30.64 k-s	12.67	15.07	13.87 hıj	49.00	36.67	42.84 u
Gelibolu	30.80	23.33	27.07 y	12.20	14.73	13.47 no	42.00	23.67	32.84 L
HRSN 12-11	32.93	30.33	31.63 h-o	12.67	15.00	13.84 ıjk	40.00	31.67	35.84 G
ZFSN 6	32.07	42.00	37.04 a	11.93	14.13	13.03 tuv	42.00	26.67	34.34 J
HRSN 14-17	29.73	30.00	29.87 o-u	12.93	13.27	13.10 stu	50.00	16.67	33.34 K
Aldane	32.07	28.00	30.04 n-t	12.93	14.67	13.80 ı-l	59.00	44.67	51.84 g
HRSN 11-4	31.00	28.00	29.50 r-v	13.13	14.27	13.70j-m	47.00	43.67	45.34 r
ZFSN 8	29.67	37.00	33.34 c-h	14.00	13.87	13.94 hı	75.00	27.00	51.00 ı
HRSN 15-2	32.20	33.00	32.60 e-j	14.67	14.67	14.67 bc	40.00	37.67	38.84 B
HRSN 12-9	31.07	32.00	31.54 h-o	13.13	14.67	13.90 hı	56.00	29.67	42.84 u
HRSN 6-8	32.73	30.00	31.37 ı-p	14.00	14.8	14.40 de	56.00	52.67	54.34 e
ZFSN 31	33.07	40.00	36.54 b	14.67	14.87	14.77 b	57.00	21.67	39.34 A
ZFSN 4	26.20	32.00	29.10 r-y	13.67	13.00	13.34 opr	52.00	44.67	48.34 k
HRSN 4-10	33.87	38.00	35.94 b	14.30	14.73	14.52 cd	58.00	29.67	43.84 s
ZFSN 3	27.93	27.00	27.47 vy	11.93	13.2	12.57 z	38.00	31.00	34.50 I
HRSN 14-18	30.07	30.33	30.20 m-t	12.93	14.27	13.60 mn	44.00	40.00	42.00 v
HRSN 13-17	34.13	32.00	33.07 bcd	13.67	14.93	14.30 ef	57.00	35.67	46.34 p
ZFSN 13-9	36.93	39.00	33.64 ef	14.87	17.2	16.04 a	56.00	26.67	41.34 z
Pehlivan	34.27	33.00	33.6 c-g	12.80	15.67	14.24 ef	43.00	31.67	37.34 E
ZFSN 26	29.87	25.00	27.44 vy	13.63	13.67	13.65 lm	72.00	22.67	47.34 m
ZFSN 16	34.00	30.33	32.17 e-l	13.20	14.87	14.04 gh	55.00	31.67	43.34 t
Flamura 85	38.67	25.00	31.84 g-n	11.93	16.67	14.30 ef	44.00	34.67	39.34 A
HRSN 1-14	31.93	33.00	32.47 e-j	12.87	14.2	13.54 mn	42.00	26.67	34.34 J
ZFSN 28	25.20	29.00	27.10 y	13.00	12.8	12.90 vy	55.00	54.67	54.84 d

HRSN 12-14	30.00	36.00	33.00 d-ı	14.00	13.67	13.84 ıjk	60.00	25.67	42.84 u
ZFSN 12	30.67	33.00	31.84 g-n	12.93	14.00	13.47 no	58.00	24.67	41.34 z
HRSN 7-6	31.80	28.00	29.90 o-t	13.20	14.20	13.70 j-m	49.00	24.67	36.84 F
HRSN 1-16	26.73	31.00	28.87 r-y	13.20	14.67	13.94 hı	47.00	28.67	37.84 D
HRSN 13-2	31.27	34.00	32.64 e-j	12.93	14.8	13.87 hıj	43.00	31.67	37.34 E
HRSN 6-2	30.87	28.33	29.60 p-v	13.80	13.27	13.54 mn	54.00	52.67	53.34 f
HRSN 15-11	30.67	32.00	31.34 ı-p	13.20	14.13	13.67klm	56.00	31.67	43.84 s
ZFSN 30	28.67	37.00	32.84 e-ı	14.67	14.13	14.40 de	57.00	53.67	55.34 c
ZFSN 32	31.27	29.00	30.14 m-t	13.67	14.13	13.90 hı	57.00	52.67	54.84 d
Bereket	32.13	28.00	30.07 m-t	12.67	14.2	13.44nop	39.00	34.67	36.84 F
ZFSN 5	28.80	35.00	31.90 f-m	13.26	13.27	13.27 prs	69.00	24.00	46.50 o
HRSN 14-2	31.13	32.67	31.90 f-m	13.67	14.67	14.17 fg	63.00	39.67	51.34 h
ZFSN 33	28.07	30.00	29.04 r-y	13.13	12.8	12.97uvy	59.00	34.67	46.84 n
HRSN 11-14	28.87	27.00	27.94 uvy	12.80	13.27	13.04 tuv	42.00	34.67	38.34 C
ZFSN 2	33.87	36.00	34.94 bc	17.00	15.07	16.04 a	82.00	79.33	80.67 a
HRSN 15-13	31.00	32.67	31.84 g-n	13.67	14.07	13.87 hıj	61.00	40.00	50.50 j
ZFSN 18	29.40	38.00	33.70 c-f	13.13	13.13	13.13 stu	64.00	22.67	43.34 t
ZFSN 23	33.80	28.17	30.99 j-r	14.13	15.13	14.63 bc	42.00	41.67	41.84 y
ZFSN 24	28.93	39.00	33.97 cde	15.33	14.07	14.70 b	52.00	9.67	30.84 M
HRSN 2-14	31.73	29.00	30.37 l-s	14.00	14.07	14.04 gh	50.00	46.67	48.34 k
HRSN 9-15	30.67	32.67	31.67 h-o	13.73	13.93	13.83 ıjk	53.00	29.67	41.34 z
HRSN 8-6	29.73	27.00	28.37 s-y	12.13	14.27	13.20 rst	41.00	37.67	39.34 A

HRSN 13-17(8), HRSN 4-10, ZFSN 31 and HRSN 4-10 lines, which are at the forefront in terms of protein ratio, gluten ratio and sedimentation value, received 8 gluten score values. When the correlations between the obtained gluten score, sedimentation rate, gluten ratio and protein value were examined, a statistically insignificant but positive correlation was found between the gluten score and gluten ratio of 0.0260. This shows that the gluten score is generally high in genotypes with high gluten content. While there was a statistically insignificant and negative relationship of -0.0570 between gluten score value and protein ratio, a statistically insignificant and negative relationship was found between gluten score and sedimentation value of -0.1386. This shows that especially the sedimentation rate is more affected by growing conditions and environmental conditions.The obtained data show that there is significant variation in glutenin scores and quality characteristics of the examined synthetic wheat lines. When the 7 synthetic lines with the highest gluten score are analysed in terms of protein ratio, gluten ratio and sedimentation ratio, it is seen that there is a significant change over the years. These results reveal that the examined quality characteristics are significantly affected by the genetic structure of the genotype as well as the ecological characteristics of the region, soil structure and cultural practices, and this reveals that quality studies should be evaluated according to the results to be obtained in different years and locations as much as possible.

Conclusion

The data obtained show that synthetic wheat genotypes have a gluten score of 8 and above in 21 lines in terms of glutenin score, while 23 lines have a gluten score of 7 and below. In particular, 6 synthetic lines with a gluten score of 10 are seen as the most superior lines in terms of quality. The 6 synthetic lines with the highest gluten score showed variation in protein content, gluten content, and sedimentation rate. This result reveals that the quality characteristics of the growing region are significantly affected by the climate data and cultural practices. When the synthetic lines are examined in terms of quality characteristics, it is seen that there are genotypes with superior characteristics in terms of glutenin score and that these genotypes can be a good source material for breeding studies. The fact that the quality

characteristics obtained change according to the glutenin scores reveals that quality studies should be evaluated according to the results to be obtained in different years and locations.

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THE EFFECT OF SOIL CONDITIONERS APPLIED TO SEED ON GRAIN YIELD AND YIELD TRAITS IN WHEAT

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Abstract

This study was conducted in the experimental field of Tekirdağ Faculty of Agriculture, Department of Field Crops during the 2016 and 2017 growing seasons. In the study, the effects of 4 different soil conditioners (1. control, 2. 13-5-8+glycine betaine, 3. 15% organic matter, 15% humic and fulvic acid+0.03% potassium, 4. 25% organic matter + 65% humic acid + 6% potassium) applied to the seed on the grain yield and yield components of Flamura-85, Selimiye,and Esperia bread wheat varieties were investigated. The experiment was arranged according to split-plot experimental design with 3 replications. The results showed that the effects of 4 different soil conditioners on grain yield and yield components of wheat varieties were statistically significant. In soil conditioner applications, plant height 90.15-91.83 cm, spike length 9.47-9.95 cm, number of spikelets 20.82-21.81 per spike, number of grains per spike 45.18-46.82, grain weight per spike 1.94-2.01 g, spike harvest index 68.47-78.28%, harvest index 42.59%-44.82% and grain yield varied between 737.1-767.9 kg da-^{1}.
According to the results of the research, T4 application with 25% organic matter + 65% humic acid + 6% potassium content gave the highest values in terms of spike length, number of spikelets per spike, number of grains per spike, grain weight and grain yield per decare. In terms of grain yield, T2 application and T4 application containing 13-5-8+glycine betaine was in the same statistical group. The highest values in the harvest index were given by T2 applications containing 13-5-8 + glycine betaine and T3 applications containing 15% organic matter, 15% humic and fulvic acid + 0.03% potassium.

Keywords: *soil, seed, yield, wheat*

Introduction

Wheat is the agricultural product that takes the first place as a basic nutrient and strategic product in our country and in the world. In the 2018-19 production season, the total wheat production in the world is 733 million tons. Wheat cultivation in Turkey in 2019 is 6.9 million hectares, production is 19 million tons (Anonymous, 2020). Environmental factors have a high impact on the yield and quality characteristics of wheat. Annual precipitation amount, distribution, temperature and relative humidity in the growing period, especially in the grain filling period, were determined to be the most important environmental factors (Smith and Gooding, 1999). In addition to climatic factors in cultivated areas, agricultural practices and the structure of the soil where cultivation is made are also features that directly affect grain yield and quality.
In studies with wheat grown in different ecologies and soils conditions, it was determined that there were significant changes in yield and quality characteristics (Korkut et al., 1993; Ayçiçek and Yıldırım, 2006; Kurt and Yağdı, 2013). In recent years, the effect of the increasing global climatic changes and the in appropriate agricultural processes in the soil have adversely affected many soil properties, especially the organic matter. For this reason, producers to achieve higher yields, apply intensive agricultural inputs day by day, which causes the soil structure to worsen. For this reason, the need for soil conditioner applications that regulate the soil structure in agricultural areas is increasing day by day. Soil conditioners

are products or components that act as soil conditioner. The effect of humic/fulvic acid application on plant height, number of spikes per square meter, grain yield and thousand grain weight in wheat was statistically significant at the 1% level. According to the average of two years, the highest grain yield was obtained with 392.2 kg da-1 from 300 ml humic/fulvic acid application per decare (Arduç et al. 2020.) In order to eliminate the germination delay problem of wheat in winter, wheat seeds were kept in different gibberellic acid concentrations, 250 ppm gibberellic acid application completely eliminated the germination delay (Pavlista et al. (2014).

Increasing soil fertility using various methods and estimating yield increases is one of the current and research priorities. The physical and chemical properties of soils have a significant impact on soil formation processes, productivity and plant growth. Rasaei et al. (2017); in their study on wheat, they found that the effects of IAA, GA3 and BAP hormones were not effective on the number of spikelets per spike, but on meristem elongation. According to the results obtained, hormone applications have a positive effect on shoot length. Koç (2021); while the highest grain yield was obtained in IAA and BAP applications (BAP: 775.31 kg/da, IAA: 767.28 kg/da) in hormone and hormone dose applications, hormone applications has a significant positive effect on the protein ratio and sedimentation. Determining the physical-chemical properties of soils and the relationship between these properties and agronomic characters of plants; it has been stated that it is very important in the creation of methods to increase productivity (Ekberli et al. 2015; Özyazıcı et al., 2016).

The study was carried out to determine the effects of 4 different soil conditioner applications on grain yield and yield components of bread wheat.

Materials and methods

The study was carried out as a split plot design with 3 replicates in the experimental area of Department of Filed Crops, Faculty of Agriculture, Tekirdağ Namık Kemal University. In the study, 4 different soil conditioners were applied to the seeds of wheat varieties (1. Control, 2. 13-5-8+glycine betaine, 3. 15% organic matter, 15% humic and fulvic acid+0.03% potassium, 4. 25% organic matter + %65 humic acid + 6% potassium) was applied. Seeds subjected to four different soil conditioner applications were sown with a seed drill in the Tekirdağ Agricultural Faculty experimental area. For 1 kg of seeds for each variety, 2 grams of the sample from the T4 application was dissolved in pure water and added to the water in which the seeds were soaked. In the T3 application, 5 ml was added to 1 kg of seeds for each variety in pure water and then applied to the seeds. In the T2 application, for 1 kg of seeds of the each variety, 3 ml of pure water was placed, and the seeds were applied. After applications, the seeds were dried and then sown in plots.

In the trials conducted in 2016 and 2017, sowings were made in 6 rows, 5 meters of rows, 17 cm between rows, and 500 seeds per square meter. 20 kg/da of 20.20.0 compound fertilizer during the sowing of the seeds, 17 kg/da of urea fertilizer during the tillering period and 20 kg/da of Ammonium nitrate (26%) fertilizer during the stem elongation period were applied In the study, weed spraying was done to prevent weed growth. pesticides were not used in the trial area against diseases and pests. Plant samples were taken for the necessary measurements and weighing of the plants that reached the harvest maturity and the plants were harvested with a parcel combine.

In the experiment, plant height, spike length, number of spikelets per spike, number of grains per spike, grain weight per spike, spike index, harvest index values and grain yield were determined. After removing 0.5 m from the beginning and end of the parcels consisting of 6 rows, the remaining parts were cut with a HEGE-160 parcel combine harvester and the obtained parcel yields were determined as kg per decare. Analysis of variance was performed

using the JUMP 5.0 statistical package program in the data obtained from the experiment, and the significance levels of the differences between the obtained means were determined by the Least Significant Difference (LSD) test.

Results and discussion

Four different soil conditioners were applied to the seeds of Flamura 85, Selimiye and Esperia bread wheat varieties, which are widely grown in the Thrace region. In this study, it was tried to determine the effect of soil conditioners on grain yield and yield components in wheat. In the study, in the different soil conditioners applied to 3 different bread wheat varieties for 2 years resulted; plant height (PH), spike length (SL), number of spikelets per spike (NSS), number of grains per spike (NGS), grain weight per spike (GWS), spike index (BFI), harvest index (HI) and grain yield (GY) values were determined. Variance analysis results of the obtained data are given in Table 1.

According to the results of the analysis of variance, years, varieties and soil conditioner applications were found to be statistically significant at the 0.01 level in all examined traits except plant height. When the interactions are examined, the year x variety interaction is statistically significant at the 0.01 level in all characters examined. While the year x soil conditioners interaction was statistically insignificant for plant height, significant at the 0.05 level for the number of spikelets per spike. It was found statistically significant at the 0.01 level for the spike fertility index (SFI) and harvest index (HI).

Table 1. The results of analysis of variance

Source of variation	**PH (cm)**	**SL (cm)**	**NSS (no)**	**NGS (no)**	**GWS (g)**	**BFI**	**HI (%)**	**GY (kg ha^{-1})**
Replication	1.470	0.036	0.032	1.150	0.059	0.136	0.584	1074.764
Year (Y)	233.460	38.749**	526.501**	110.509*	3.516**	3217.223**	1053.023**	1795512.500**
$Error_1$	18.946	0.003	0.195	4.858	0.036	1.365	0.957	2244.042
Variety (V)	1095.723**	2.555**	3.565**	411.202**	0.304**	503.672**	53.623**	38446.181**
Y x V	14.862**	1.725**	24.708**	157.032**	0.110*	94.685**	23.230**	28519.292**
$Error_2$	1.435	0.032	0.222	1.612	0.024	1.197	0.938	327.382
Treatment (T)	10.469*	0.694**	3.668**	9.029**	0.019	301.440**	16.608**	2915.111**
Y x T	2.561	0.097	0.417*	13.806**	0.020	128.885**	12.257**	855.315
V x T	27.201**	0.178*	0.578**	1.304	0.038	96.700**	8.109**	5515.292**
Y x V x T	22.899**	0.151*	0.156	6.901**	0.025	125.610**	3.245**	4944.662**
Error	2.945	0.055	0.097	1.325	0.018	3.463	0.807	397.278

In terms of year x variety x soil conditioner interaction, the difference between the number of spikelets per spike is statistically insignificant, the differences between spike length are statistically significant at the 0.05 level. The differences between plant height, number of grains per spike, grain weight per spike, spike fertility, harvest index and grain yield were found statistically significant at the 0.01 level. As a result of the variance analysis performed on the data obtained, the average values and statistical significance level groups for the characters examined are given in Table 2 and Table 3.

When varieties and applications were examined, plant height values varied between 83.51-96.77 cm. While the longest plant height was 96.77 cm in Flamura 85 variety, the lowest plant height value was in Esperia variety with 83.51 cm. When examined the soil conditioners applied to the seed, the longest plant height was 91.83 and 91.21 cm in T3 and T2 applications, while the shortest plants were 90.15 cm in T1, that is, control application, without soil conditioner.

In the study, the spike length values varied between 9.405-10.049 cm in varieties and between 9.467-9.946 in soil conditioner applications. While the longest spikes were obtained in Flamura 85 variety with 10.049 cm, the lowest spike length was obtained in Esperia variety with 9.405 cm. When the effect of soil conditioners on the spike length was examined, the longest spikes were 9.946 cm in T4 application, and the lowest spike length was obtained with 9.467 cm in plants without soil conditioners.

Table 2. Soil conditioner application and variety averages, importance levels regarding grain yield and some yield characteristics

Soil conditioners	**PH (cm)**	**SL (cm)**	**NSS (no)**	**NGS (no)**	**GWS (g)**	**SFI (%)**	**HI (%)**	**GY (kg ha^{-1})**
T1 (Control)	90.15 b	9.467 c	20.817 b	45.178 c	1.942	68.472 d	42.592 c	7371 c
T2	91.21 ab	9.706 b	20.978 b	45.650 bc	1.994	78.284 a	44.822 a	7568 ab
T3	91.83 a	9.670 b	20.967 b	46.211 ab	2.002	71.623 c	44.274 a	7538 b
T4	90.43 b	9.946 a	21.811 a	46.817 a	2.014	73.046 b	43.597 b	7679 a
$LSD_{0.05}$	1.160	0.159	0.210	0.778		1.258	0.607	13.474
Variety								
Flamura 85	96.77 a	10.049 a	20.729 b	47.967 a	2.117 a	76.376 a	43.723 b	7640 b
Selimiye	92.43 b	9.637 b	21.208 a	41.204 b	1.909 b	67.677 c	42.379 c	7098 c
Esperia	83.51 c	9.405 c	21.492 a	48.721 a	1.939 b	74.516 b	45.363 a	7879 a
$LSD_{0.05}$	1.160	0.119	0.314	0.845	0.103	0.728	0.645	12.045

The number of spikelets per spike in the bread wheat varieties varied between 20,729 and 21.492 unit, and between 20,817 and 21.811 unit in soil conditioner applications. While the highest number of spikelets per spike was found in Esperia variety with 21,492, the lowest value was in Flamura 85 variety with 20,729. While the number of spikelets per spike was 20,817 in the control, it increased with the application of soil conditioner and the highest value was obtained in the T4 application with 21,492. These values were followed by T2 and T3 applications with 20,978 and 20,967 unit.

The number of grains per spike, which is one of the important yield criteria, varied between 41.204-48.721 for bread wheat varieties and between 45.178-46.817 for soil conditioner applications. While the number of grains per spike was 45,178 unit in plants obtained from seeds that were not treated with soil conditioner. A significant increase in soil conditioner applications was observed. The highest number of grains per spike was obtained with 46,817 units and 46,211 units in T4 and T3 in the same statistical group.

In the study, the spike length values varied between 9.405-10.049 cm in varieties and between 9.467-9.946 in soil conditioner applications. While the longest spikes were obtained in Flamura 85 variety with 10.049 cm, the lowest spike length was obtained in Esperia variety with 9.405 cm. When the effect of soil conditioners on the spike length was examined, the longest ear was 9.946 cm in T4 application, and the lowest spike length was obtained with 9.467 cm in plants without soil conditioners.

The number of spikelets per spike in the bread wheat varieties included in the experiment varied between 20,729 and 21.492 unit, and between 20,817 and 21.811 in soil conditioner applications. While the highest number of spikelets per spike was found in Esperia variety with 21,492, the lowest value was in Flamura 85 variety with 20,729. The effects of different soil conditioner applications on the number of spikelets per spike were 20,729 unit in those who did not apply control, that is, soil conditioner, while it increased with soil conditioner applications and the highest number was obtained in T4 application with 21,492. This application was followed by T2 and T3 applications with 20,978 and 20,967 applications. The number of grains per spike, which is one of the important yield criteria, varied between

41.204-48.721 for bread wheat varieties and between 45.178-46,817 for soil conditioner applications.

While the number of grains per spike was 45,178 in plants obtained from seeds that were not treated with soil conditioner, an increase was observed in all soil conditioner applications. The highest number of grains per spike was obtained with 46,817 and 46.211 unit in T4 and T3 applications, which were in the same statistical group.

In the bread wheat varieties, the grain weight per spike varied between 1.909-2.117 g, and in soil conditioner applications, it was between 1.942-2.014 g. While the highest grain weight per spike was found in Flamura 85 variety with 2.117 g, the lowest value was in Selimiye variety with 1.909 g. In soil conditioner applications, the number of grains per spike increased in all applications compared to those without soil conditioners. While the grain weight per spike was 1.942 g in untreated plants, 0.72 g increase in grain weight per spike achieved in T4 soil conditioner application.

For high grain yield in the plant, it is important to have a high level of grain in the spike harvest index, that is, the total spike weight, as well as the spike length. Spike fertility index values varied between 67.677-76.376% in varieties and between 68.472-78.284% in soil conditioner applications. The highest spike harvest index among the varieties was Flamura 85 with 76.376%, while the lowest value was in Selimiye with 67.677%.

Table 3. Mean values and significance groups for variety x soil conditioner interaction

Variety	Soil conditioner	**PH (cm)**	**SL (cm)**	**NSS (no)**	**NGS (no)**	**GWS (g)**	**SFI (%)**	**HI (%)**	**GY (kg $^{-1}$)**
Flamura85	T1	95.09 bc	9.88 bc	20.22 g	47.15 c	2.13 ab	65.87 g	42.96 fg	7232 ef
	T2	96.78 ab	10.01 b	20.90 de	47.50 bc	2.07 abc	83.73 a	43.65 e	7573 cde
	T3	96.12 b	9.83 bc	20.47 fg	48.80 a	2.20 a	77.66 cd	45.70 b	8055 a
	T4	99.13 a	10.48 a	21.33 bc	48.42 abc	2.07 abc	78.25 bc	42.58 gh	7698 cd
Selimiye	T1	93.96 bcd	9.48 de	21.30 bcd	40.27 e	1.80 e	68.84 f	40.56 ı	7210 ef
	T2	92.00 de	9.63 cde	20.73 ef	40.68 e	1.92 cde	70.88 ef	43.54 e	6977 gh
	T3	94.18 bcd	9.76 bcd	21.10 cde	41.23 de	1.88 de	65.60 g	42.19 h	6795 h
	T4	89.5g7 e	9.67 cde	21.70 b	42.63 d	2.04 a-d	65.40 g	43.23 ef	7410 def
Esperia	T1	81.45 g	9.04 f	20.93 cde	48.12 abc	1.90 cde	70.71 ef	44.25 d	7672 cd
	T2	84. 84 f	9.48 de	21.30 bcd	48.77 ab	2.00 bcd	80.25 b	47.28 a	8153 a
	T3	85.18 f	9.42 e	21.33 bc	48.60 abc	1.93 cde	71.61 e	44.94 c	7763 bc
	T4	82.58 g	9.68 cde	22.40 a	49.40 a	1.93 cde	75.50 d	44.98 c	7928 ab
$LSD_{0.05}$		2.204	0.301	0.400	1.478	0,172	2.390	0.489	25.59

When soil conditioner applications were examined, while the spike fertility index was 68.47% in control parcels, it caused a significant increase, 14%, especially in T2 application. This was followed by T4 with an increase of 6.7% and T3 with an increase of 4.6%.

One of the main goals of plant breeding studies is to obtain plants with high harvest index. Harvest index values varied between 42.379-45.363% in varieties and between 42.592-44.822% in soil conditioner applications. While the highest harvest index among the varieties was obtained in Esperia variety with 45.36%, the lowest value was obtained in Selimiye variety with 42,379 %. While the harvest index was 42.592% in those who did not apply soil conditioner, an increase was observed in all those who applied soil conditioner. The highest harvest index was found in T2 and T3 soil conditioner application with 44.822% and 44.274%.

High yield in plant production is the main breeding target. The grain yield in the varieties varied between 7098-7879 kg ha^{-1}, and in soil conditioner applications it was between 7371-8055 kg ha^{-1}. While the highest value was 7879 kg ha^{-1} in Esperia variety, the lowest value

was in Selimiye variety with 7098 kg ha^{-1}. While the lowest grain yield (7371 kg ha^{-1}) was obtained from the control plants. T3, T2 and T4 applications had 308, 197 and 167 kg ha^{-1} yield increases, respectively.

According to the results of the analysis of variance, all the characters examined in the variety x soil conditioner applications were found to be statistically significant. In other words, the responses of varieties to soil conditioner applications were at different levels. The highest value in terms of plant height was 99.13 cm in Flamura 85 variety with T4 application, followed by T2 application of Flamura 85 variety with 96.78 cm. The lowest plant height was obtained with of 81.45 cm in Esperia variety that did not apply soil conditioner. While soil conditioners caused an increase in plant height in Flamura 85 variety, there was increase or decrease plant height in Selimiye and Esperia varieties, and plant height decreased significantly in both varieties, especially in T4 application.

When the effect of soil conditioners on the variety was examined in terms of spike length, while there was a difference in spike length in Flamura 85 variety according to the soil conditioner application, soil conditioners caused an increase in spike length in Selimiye and Esperia varieties. The longest spike was obtained with 10.48 cm in T4 application of Flamura 85 variety, followed by T2 application with 10.01 cm. The lowest spike length was observed in control applications of Selimiye and Esperia varieties.

The effect of soil conditioner applications on varieties was examined in terms of number of spikelets per spike, there was an increase with the soil conditioner application in Flamura 85 and Esperia varieties, while there was a change according to the application in Selimiye variety. The highest number of spikelets per spike was obtained in Esperia variety with 22.40 pieces in T4 application, followed by T3 application in Selimiye variety with 21.73 unit. The lowest number of spikelets per spike was 20.22 in the control application of Famura 85 variety.

In the all soil conditioner applications to three bread wheat varieties, the number of grain per spike varied between 49.40-40.27 unit. Soil conditioner applications caused an increase in the number of grains per spike in all three varieties compared to the control. The highest number of grains per spike was obtained with 49.40 and 48.80 pieces, in T4 of Esperia variety and T3 application in Flamura 85 variety. These applications were followed by T3 and T2 of Esperia variety and T4 of Flamura 85 variety with 48.77, 48.60, 48.42 and 48.12 values. The lowest number of grains per spike was 40.27 unit in the control application of Selimiye variety.

When the effect of soil conditioners in bread wheat varieties was examined in terms of grain weight per spike, there were fluctuating values in Flamura 85, while soil conditioner applications was increased in Esperia and Selimiye varieties. The highest grain weight per spike was 2.20 grams in T3 application of Flamura 85 variety, followed by control, T2 and T4 applications of the same variety. In the Selimiye variety, whose grain weight was 1.80 g in the control, 2.04 g in T4 application was achieved. The lowest grain weight per spike was generally in control applications.

When the spike fertility index values in the variety x soil conditioner application interaction were examined, the spike fertility index values increased in all three varieties, except for the T3 and T4 applications of Selimiye, compared to the control applications. The spike fertility index of Flamura 85 variety, which was 65.87% in the control application, became 83.73% in the T2 application and increased by 27%.

In the Selimiye variety 68.84% in the control application, the spike fertility index increased by 2.96% in the T2 application and in the Esperia variety, the spike fertility index was 70.71% in the control applications. It caused an increase of 13.49. The lowest values in all three varieties were obtained in the control, that is, the plants that did not apply soil conditioner. In Selimiye variety, the spike fertility index which was 68.84% in the control application increased by 2.96% in the T2 application, and in the Esperia variety it was 70.71%

in the control application and increased by 13.49%. The lowest values in all three varieties were obtained in the control, that is, the plants that did not apply soil conditioner.
The harvest index of different soil conditioners in bread wheat varieties caused an increase in all three varieties compared to control. In Flamura 85 variety, while the harvest index was 42.96% in the control application, there was an increase of 45.70% and 6.4% in the T3 application. The harvest index of Selimiye variety, which was 40.56% in the control application, was 43.54% in the T2 application and 43.23% in the T4 application. While harvest index in control application of Esperia variety was 44.25%, T2 soil application increased 47.28%. While the responses of the varieties to soil conditioners were different, T4 and T2 soil conditioners caused a higher increase in the harvest index.
The effects of different soil conditioners on grain yield of Flamura 85 and Esperia varieties were positive. T4 application caused an increase in Selimiye variety, while T2 and T3 applications caused a decrease, slightly, compared to the control.
The grain yield, which was determined as 7322 kg ha^{-1} in the control plants of the Flamura 85 variety, increased by approximately 10% in the T3 application and was determined as 8055 kg ha^{-1}.. Grain yield of Selimiye variety increased by 2.77% in T4 application compared to the control application (7210 kg ha^{-1}) and was determined as 7410 kg ha^{-1}. Grain yield of Esperia variety increased by 6.27% in T2 application, 1.19% in T3 application and 3.33% in T4 application compared to the control application.
The highest grain yield was obtained from T3 application of Flamura 85 variety, T4 application of Selimiye variety and T2 application of Esperia variety. This reveals that varieties respond differently to soil conditioners.

Conclusion

The data obtained with 4 different soil conditioners applied to wheat seeds showed that soil conditioner applications in wheat cause a significant increase in the investigated characters. Soil conditioner applications affected all the characters examined in bread wheat varieties. Increases in the number of grains per spike, grain weight per spike, spike index, harvest index and grain yield are more remarkable. The obtained data indicated that the response of bread wheat varieties to different soil conditioners is different. In general, T4 and T2 soil conditioner applications caused more increase in examined traits. While 7371 kg ha^{-1} yield was obtained in plants obtained from control plants, in T4, T3 and T2 soil regulatory applications, an increase of 308, 197 and 167 kg ha^{-1} was achieved, respectively. When the effects of different soil conditioners on grain yield in bread wheat varieties were examined, it was found that the effect on grain yield was positive in Flamura 85 and Esperia varieties. In Selimiye variety, there was a decrease in T2 and T3 applications compared to the control, while T4 application caused an increase. The highest grain yield was obtained in T3 application in Flamura 85 variety, T4 applications in Selimiye variety and T2 applications in Esperia variety. As a result, although the responses of bread wheat varieties to soil conditioners are at different levels, soil conditioner applications to seed cause a remarkable increase in yield and yield characteristics of wheat.

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GERMINATION AND EARLY SEEDLING DEVELOPMENT OF PURPLE CARROT TREATED WITH AMINO ACIDS

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Abstract

Amino acids, which are the building blocks of proteins, have very important functions for plant development and are applied externally in different plants. In this study, the effects of some amino acids (Aspartic acid, Glutamic acid, Glycine) applied as pre-treatment in terms of germination and early seedling development in purple carrots (*Daucus carota* L) with low germination rate were investigated. As germination parameters, germination percentage, mean germination time, coefficient of velocity of germination, and germination index were calculated. Seedling vigour index, shoot and root length (cm), and plant fresh weights (g) were measured in order to reveal their effects on early seedling growth. As a result, it was determined that amino acids positively affect both germination and plant growth in carrots. While 4 mM Aspartic acid and Glutamic acid, 1 mM Glycine were effective at the germination stage, 2 mM Aspartic acid, 1 mM Glutamic acid, 4 mM Glycine were prominent in terms of plant growth. When these three amino acids are evaluated together, 4 mM Aspartic acid application gave the best results in terms of germination and 2 mM Aspartic acid in plant growth. These results show that different doses of amino acids are effective in different periods of plant development and are important in terms of showing the positive effect of aspartic acid on germination and carrot growth.

Keywords: *Aspartic acid, Daucus carota, Germination, Glutamic acid, Glycine.*

Introduction

Carrot (*Daucus carota*), which is a protective vegetable against many diseases, including cancer, heart diseases and diabetes, due to the carotenoid, vitamin and antioxidant substances it contains, is a vegetable that is widely consumed all over the world. Today, mostly orange-colored carrots are consumed in kitchens, but the original carrot varieties are yellow and purple. Although mostly orange carrots are consumed in Europe and America, purple and yellow carrots are used in Turkey, India and China, and red carrots are used as an alternative to orange carrots in Japan. Different colored carrots obtained as a result of breeding process of these varieties have strong antioxidant properties due to the anthocyanin and carotenoid compounds they contain. Increasing awareness on this issue around the world has made carrot an increasingly important vegetable economically (Arscott and Tanumihardjo, 2010; Simon, 2010).

The seed, which is used as the most important production material in plant reproduction, rapidly loses its germination ability when exposed to adverse environmental conditions. Carrot seeds are highly affected by adverse environmental conditions and are faced with germination losses (Akbıyık and Aktaş, 2022). Some exogenous applications to be made before germination mitigate the effects of environmental conditions and increase germination yield and quality. Seed priming, which accelerates and synchronizes seed germination and has

a positive effect on seedling viability and growth, is an easily applied cost-effective method (Zulfiqar, 2021; Karim *et al*., 2020). Different organic and inorganic compounds such as salicylic acid (Nasircilar *et al*., 2019), melatonin (Tiwari *et al*., 2020) mannitol, H_2O_2 (Ellouzi *et al*., 2017) and silicon (Nasircilar *et al*., 2021) are used for seed priming.
Amino acids, which are the building blocks of proteins and function as precursors for the synthesis of nucleotides that make up the structure of DNA (Heinemann and Hildebrandt, 2021¸ Nasırcılar and Ulukapı, 2023), are accepted as biostimulant in organic agriculture because they support plant growth and development. Due to these properties, amino acids are among the organic compounds used in the priming process. Priming application with methionine, proline, tryptophan and glutamine increased the amount of germination and photosynthetic pigments in onions (Abdelkader *et al*., 2023). Considering their role in increasing plant growth and crop quality in agricultural practices, it becomes inevitable to use amino acids more effectively (Teixeira *et al*., 2017). Therefore, in this study, the effects of different amino acids on germination and vegetative growth parameters of purple carrot as a cultivar used in Turkey were evaluated.

Material and Methods

The purple carrot variety purchased from a commercial company was used in the study carried out in the Plant and Animal Production Laboratory of Vocational School of Technical Sciences in Akdeniz University, Antalya, Turkey.
Surface sterilized seeds were kept at room temperature for 24 hours at 4 different concentrations (1, 2, 3, 4 mM) of Aspartic acid, Glutamic acid and Glycine for priming. The seeds in the control group were kept in distilled water under the same conditions. After the priming process, the seeds were transferred to petri dishes with double-layer sterile blotter. Experiments were made in 4 repetitions and 25 seeds were placed in petri dishes for each trial. Petri dishes were kept in a climate chamber with a temperature of 24±1ºC and a photoperiod of 16/8 h, and the germinated seeds were counted daily during the experiment. During this period, the seeds were irrigated with an equal amount of water to prevent the seeds from drying out. The trial was terminated on the 14th day in accordance with ISTA rules and germination percentage (GP) (Gosh *et al.*, 2014), mean germination time (MGT) (Ellis and Roberts, 1981), coefficient of velocity of germination (CVG) (Kotowski, 1926), germination index (GI) (Benech *et al.*, 1991), seedling vigorous index (SVI) (Baki and Anderson, 1973) were calculated. As vegetative parameters, shoot length (mm), root length (mm), and plant fresh weight (g) were measured. The data were evaluated statistically with Minitab 21 and Jamovi programs and the differences between the applications were determined by the Tukey test (Minitab 2021).

Results and Discussion

Priming in carrot seeds is generally applied to provide tolerance to abiotic stress conditions. It was determined that silicon (Nasircilar *et al*., 2021), salicylic acid (Nasircilar *et al*., 2019) and acetylsalicylic acid (Akbiyik and Aktaş, 2022) applications in salt stress and α-tocopherol Hameed *et al*., 2021) in drought stress conditions had a positive effect on the germination parameters of different carrot varieties. In this study, priming applications with different amino acids under stress-free conditions similarly had an encouraging effect on germination and vegetative growth parameters. All concentrations of all three amino acid types increased the germination rate compared to the control, and the highest germination rates were obtained in the 4 mM application of glutamic acid and aspartic acid and the 1 mM application of glycine. CVG and GI parameters were also positively affected by amino acid priming (Figure

1). Descriptive statistics and analysis of variation results showed that all treatments were significant at the 0.05 or 0.01 level on germination and growth parameters except PFW (Table 1).

Table 1. Descriptives and Variance analysis result with respect to the vegetative growth of purple carrot.

Variation source	df	SL (mm)	RL (mm)	PW (g)	SVI	GP	MGT	CGV	GI
T	12 26 38	**	*	ns	**	*	**	*	*
Mean		81.8	26.2	0.0404	152	0.74	3.38	17.3	0.79
Minimum		46.2	13.2	0.0154	87	0.40	1.65	4.68	0.43
Maximum		112	48.2	0.189	274	1.00	3.91	30.7	1.07
Standart deviation		15.5	8.17	0.0272	44.1	0.13	0.417	6.78	0.15

T: treatment, SL: shoot length, RL: root length, PW: plant weight, SVI: seedling vigorous index, GP: germination persentage, MGT: mean germination time, CGV: coefficient of velocity of germination, GI: germination index, * $p<0.05$, ** $p<0.01$, ns: not significant

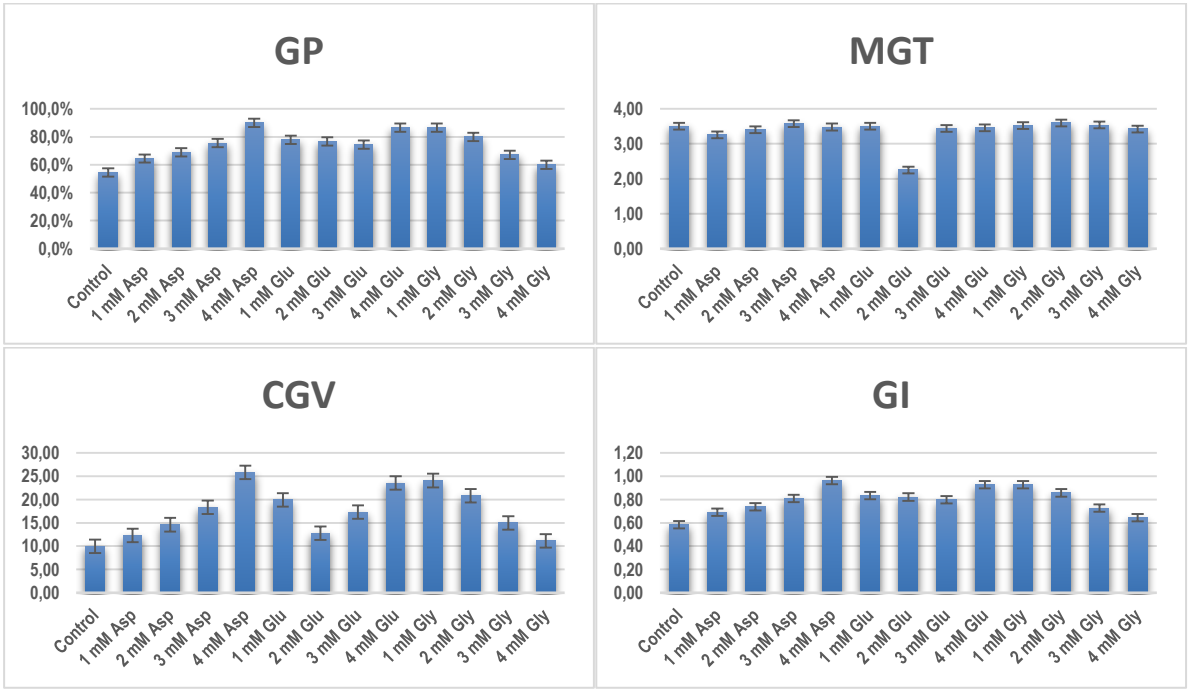

Figure 1. The effects of amino acid priming on germination parameters of purple carrot seeds. GP: germination persentage, MGT: mean germination time, CGV: coefficient of velocity of germination, GI: germination index, Asp: Aspartic acid, Glu: Glutamic acid, Gly: Glycine

The effect of different amino acid applications on vegetative parameters differed depending on the dose. All other parameters except SVI showed better improvement than control plants at all doses of aspartic acid. In particular, 2 mM of aspartic acid gave the best results in terms of all parameters, and the SL, RL, PFW and SVI values has risen from 78.3 to 102.63, 19.80 to 38.97, 0.0235 to 0.0548, and 186.733 to 211.88, respectively. Increasing doses of glutamic acid had a negative effect on vegetative parameters. The best results were obtained with the lowest dose of 1 mM application, and the root length reached 30.20 and PFW reached 0.0783 in this application. Glycine had the opposite effect of glutamic acid and the best results were obtained from the highest dose of 4 mM. At this dose, the shoot and root length of the plants increased by 32% and 46%, respectively. Similarly, plant fresh weight increased by 49% and SVI value increased by 10%, reaching the highest values (Table 2, Figure 2)

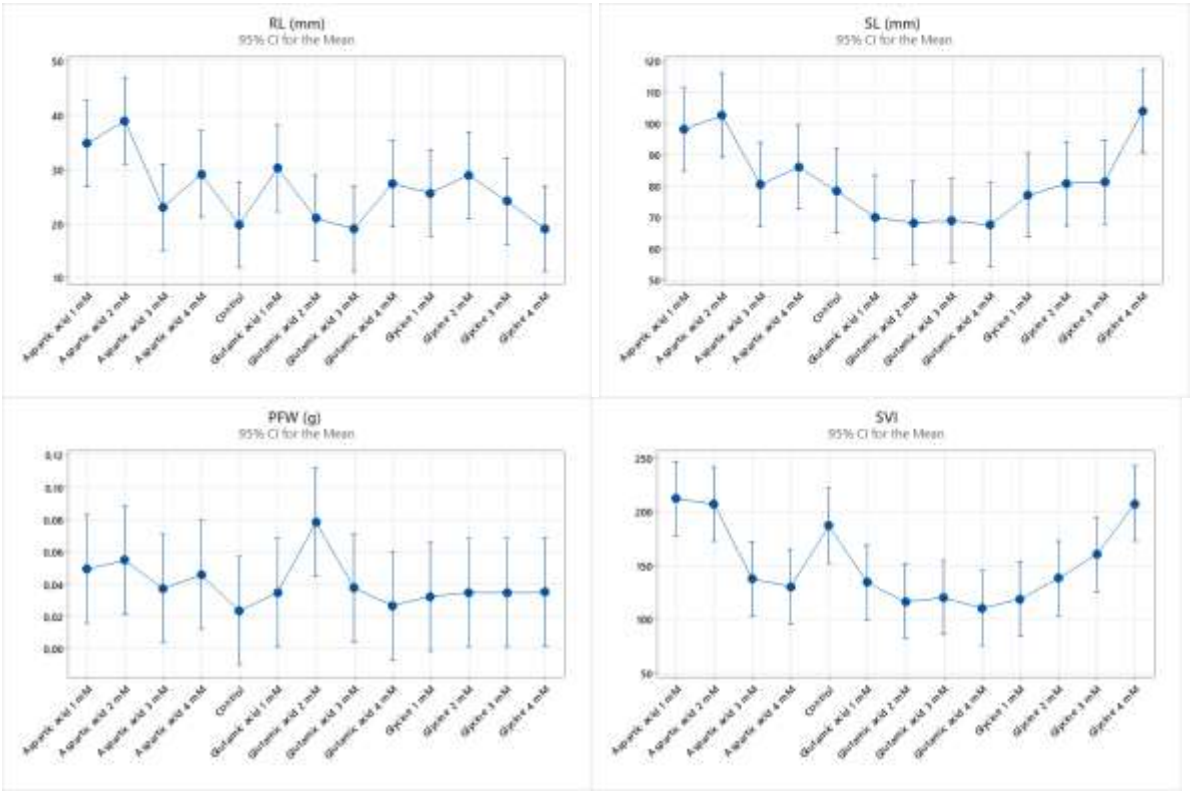

Figure 2. Changes in RL, SL, PFW and SVI values in different amino acid applications
(RL: Root Length, SL: Shoot Length, PFW: Plant Fresh Weight, SVI: Seedling Vigor İndex)

As a result of statistical analysis, it has been shown that there was a correlation between SVI and SL (r=0.689), SVI and GP (r=0.791), MGT and PFW (r=0.575), CGV and SVI (r=0.673)/ GP (r=0.945), GI and GP (r=1.00)/SVI (r=0.791)/ CGV(r=0.945) (Table 3).

Table 3. Correlation between germination and vegetative parameters of purple carrot

	SL (mm)	RL (mm)	PFW (g)	GP (%)	SVI	MGT	CGV	GI
RL	0.099							
	0.549							
PFW	0.056	0.046						
	0.735	0.783						
GP	0.230	0.107	0.055					
	0.160	0.517	0.737					
SVI	0.689	0.242	0.056	0.791				
	<0.001	0.138	0.734	<0.001				
MGT	0.236	0.185	0.575	0.083	0.125			
	0.148	0.258	<0.001	0.615	0.449			
CGV	0.143	0.156	0.204	0.945	0.673	0.375		
	0.384	0.343	0.212	<0.001	<0.001	0.019		
GI	0.230	0.107	0.055	1.000	0.791	0.083	0.945	
	0.160	0.517	0.737	<0.001	<0.001	0.615	<0.001	

SL: shoot length, RL: root length, PW: plant weight, SVI: seedling vigorous index, GP: germination persentage, MGT: mean germination time, CGV: coefficient of velocity of germination, GI: germination index

Exogenous amino acid applications in different vegetable species such as *Allium sativum* (Turfan and Turan, 2023), tomatoes (Eksi and Sönmez, 2022) and lettuce (Noroozlo *et al*., 2019) provided an increase in growth parameters and yield. Although all of these applications

were foliar spraying at the seedling stage, in the current study, amino acids were applied as priming to the seeds before planting and similarly supported plant growth.

Conclusion

Aspartic acid, Glutamic acid and Glycine had positive effects on both germination and plant growth in carrot. At the germination stage, 4 mM Aspartic acid and Glutamic acid, 1 mM Glycine applications had a positive effect on germination. On the other hand, 2 mM Aspartic acid, 1 mM Glutamic acid, 4 mM Glycine were effective on plant growth. As a result, it is thought that amino acids can be used reliably for sustainable agriculture under increasing environmental stresses due to their organic origin.

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EFFECTS OF DIFFERENT ORGANIC MATERIALS ON PLANT WEIGHT AND DEHYDROGENASE ACTIVITY IN THE RHIZOSPHERE OF STRAWBERRY PLANTS

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Abstract

In this study, we investigated the impact of various plant-based and animal-based organic materials on the growth of Albion strawberry plants and the dehydrogenase enzyme activity in the rhizosphere soil. The experiment was conducted using clayey soil, and eight different organic materials (cow manure, poultry manure, sheep manure, vermicompost, wheat straw, rice husk, tea waste, and hazelnut husk) were applied at a 5% rate. The experiment lasted for 61 days in a greenhouse with controlled environmental conditions. The results revealed that sheep manure, cow manure, and vermicompost significantly increased the fresh plant weight compared to the control group. In contrast, all other organic materials led to a reduction in fresh plant weight. Sheep manure emerged as the most effective organic material, promoting the highest increase in fresh plant weight. The differences in plant weight were attributed to the chemical properties of the organic materials, as the application rate and method were standardized. Furthermore, all organic material additions significantly increased the dehydrogenase activity in the rhizosphere soil compared to the control treatment. Animal-based organic materials, including sheep manure, exhibited a more pronounced effect on the dehydrogenase activity, surpassing the impact of plant-based organic materials. These findings highlight the importance of choosing suitable organic materials for soil amendments to optimize plant productivity and enhance soil microbial activity. Sheep manure, in particular, proved to be a highly effective organic material for promoting plant growth and stimulating dehydrogenase enzyme activity in the rhizosphere soil. Understanding the specific impacts of different organic materials on plant growth and soil enzymatic processes can aid in making informed decisions to support sustainable agricultural practices and improve overall soil health.

Keywords: S*trawberry, organic materials, dehydrogenase activity, rhizosphere soil, plant weight.*

Introduction

Soil microorganisms are integral to the ecological chain of soil, playing vital roles in nutrient cycling, organic matter formation and decomposition, soil-borne disease prevention, and crop development (Zak et al., 2003; Jacobsen and Hjelms, 2014). Maintaining high soil microbial activity and diversity is essential for effective soil quality management.

In Turkey, continuous protected strawberry cropping is a common agricultural practice, which can disrupt the soil microbial balance, resulting in reduced diversity and increased pathogenic bacteria, partly due to excessive chemical fertilizer application (Li et al., 2014; Fu et al., 2017). To address these concerns, the utilization of organic materials, such as livestock and poultry manure, and straw, in place of chemical fertilizers, has gained significance in facility agricultural production, offering benefits like improved soil structure, increased organic matter content, and reduced salt levels (Zhong et al., 2010; Hu et al., 2018). Long-term studies have shown that combined organic and inorganic fertilizer application promotes the growth of

beneficial bacteria, while solely relying on chemical fertilizers favors fungi growth (Ding et al., 2016). Moreover, organic manure and straw incorporation in fields can enhance soil health by encouraging the proliferation of growth-promoting bacteria and mycorrhizal fungi (Zhu et al., 2017; Wang et al., 2015). However, the impact of organic material application on dehydrogenase activity in continuous cropping soil remains uncertain. Dehydrogenase activity, widely used to evaluate soil microbial metabolic activity, serves as an indicator of overall microbial health, as it is closely linked to microbial cells (Trevors, 1984; Obbard, 2001).

In this study, we investigated the changes in dehydrogenase enzyme activity in the rhizosphere soil of Albion strawberry plants grown in clayey soil, with the addition of various plant-based and animal-based organic materials (cow manure, poultry manure, sheep manure, vermicompost, wheat straw, rice husk, tea waste, and hazelnut husk) at a 5% rate. Additionally, we examined the effects of these organic materials on strawberry yield through a 61-day greenhouse experiment.

Material and Methods

Soil and Analyses: The experimental soil used in this study was collected from an agricultural field at Ondokuz Mayıs University in Samsun, Türkiye (41° 21' 49.9" N, 36° 11' 19.7" E). The site experiences a mean annual maximum temperature of 27.7°C, a relative humidity of 73%, and a minimum temperature of 5°C, with an annual precipitation of 937.26 mm. Standard techniques were employed to measure the physical and chemical properties of the soil, including particle size distribution using the hydrometer method, $CaCO_3$ content using the volumetric method, pH, and electrical conductivity (EC) in a 1:1 soil-water suspension using a pH-meter and EC-meter, and Total N determined via the Kjeldahl method. Total soil organic matter was determined using the Walkley-Black wet oxidation method with $K_2Cr_2O_7$ (Rowell, 2014).

Strawberry: For the greenhouse experiment, Albion variety Strawberry (Fragaria x ananassa) plants were utilized.

Organic Wastes and Analyses: Various organic wastes were collected from Ondokuz Mayıs University Agricultural Faculty Farms in Samsun, Türkiye. The organic wastes consisted of animal wastes (Cow Manure, Poultry Manure, Sheep Manure, Vermicompost) and plant residues (Wheat Straw, Rice Husk, Tea Waste, Hazelnut Husk). The chemical analysis of the organic wastes was performed using standard methods, measuring pH (1:1, w/v, waste-water suspension, pH-meter) and electrical conductivity (1:1, w/v, waste-water suspension, EC-meter). Ash content was determined by dry ashing, while organic matter content was estimated by dry ashing and calculating the organic carbon using a conversion factor of 1.724. The total phosphorus in organic wastes was determined through the dry ashing method, and the Kjeldahl method was employed to measure the total nitrogen content (Jones, 2001).

Experimental Design: The soil was collected, air-dried, crushed, and sieved through a 4 mm sieve to achieve homogeneous soil conditions for each treatment. The experiment was conducted using 27 pots, each containing 3 kg of soil. Organic material was applied at a rate of 5% of the pot weight (150 grams per pot), followed by transplanting the Albion strawberry plants and watering with rainwater. Soil moisture content was regularly monitored and adjusted to maintain it close to field capacity. The experiment lasted for 61 days and followed a completely randomized design. Each treatment had three replicates. Pots without any organic material application were considered as the control group. It was conducted in a greenhouse with controlled environmental conditions, including temperature regulation. At the end of the experiment, the each strawberry plant taken from each pot were weighed.

Additionally, soil samples were collected from the rhizosphere of strawberry plants to determine dehydrogenase activity at the end of the experimental period.

Dehydrogenase Activity in Rhizosphere Soil: Dehydrogenase Activity was determined following the method described by Pepper et al. (1995). Six grams of soil, 30 mg glucose, 1 ml of 3% 2,3,5-triphenyltetrazolium chloride (TTC) solution, and 2.5 ml of pure water were mixed. The samples were incubated for 24 hours at 37°C. The formation of 1,3,5 triphenylformazan (TPF) was measured spectrophotometrically at 485 nm, and the results were expressed as μg TPF g^{-1} dry soil.

Statistical Analyses: The mean values were calculated, and the least significant difference (LSD) test was used for statistical analyses to identify significant differences among treatments.

Results and Discussion

The characteristics of the soil used in the experiment are provided in Table 1. Accordingly, the experimental soil is clayey in texture, slightly acidic in reaction, free from salts, and has low total nitrogen and lime content, along with a moderate level of organic matter. The C/N ratio of the experimental soil is 9.92.

Table 1. The physical and chemical properties of the experimental soil

Soil Properties	Results	Evaluation
Sand, %	29.91	
Silt, %	21.68	Clayey
Clay, %	48.41	
pH	6.89	Slightly asidic reaction
Electrical Conductivity (EC), dS m^{-1}	0.55	Non-saline (0.98 dS m^{-1})
Lime content ($CaCO_3$), %	1.79	Highly calcareous (15-50%)
Organic matter, %	2.18	Moderate (1.71-3.00%)
Total Nitrogen (N), %	0.13	Low (<0.15%)

The chemical analysis results of the organic materials used in the experiment are presented in Table 2. According to these results, tea waste exhibits a strong acidic reaction, while the organic material with the highest pH value is vermicompost. Among the organic materials, sheep manure has the lowest salt content, whereas vermicompost is determined to be the highest in salt content. The organic material with the highest organic matter content is identified as rice husk and the one with the lowest C/N ratio is poultry manure.

Table 2. Chemical properties of organic materials

Organic waste	pH	EC, μS cm^{-1}	OM, %	OC, %	Ash, %	N, %	C/N
Cow Manure	7.94	2141	57.62	33.42	42.38	1.56	21.42
Poultry Manure	7.53	5441	65.53	38.01	34.47	5.22	7.28
Sheep Manure	8.88	1244	60.03	34.82	39.97	2.03	17.15
Vermicompost	9.11	5662	59.25	34.37	40.75	2.17	15.84
Rice Husk	6.72	1093	82.04	47.58	17.96	0.40	118.96
Wheat Straw	6.80	1509	70.30	40.77	29.70	0.33	123.56
Tea Waste	4.78	3018	43.62	25.30	56.38	1.19	21.26
Hazelnut Husk	7.26	2024	80.51	46.70	19.49	0.78	59.87

The changes in strawberry plant fresh weight induced by different organic materials are presented in Figure 1. According to the obtained results, when compared to the control treatment, applications of sheep manure, cow manure, and vermicompost increased the fresh

plant weight, while all other organic materials decreased the fresh plant weight. The changes in plant fresh weight caused by the organic materials were found to be statistically significant. Among the organic materials, sheep manure had the most significant increase in plant fresh weight, while Poultry Manure and Hazelnut Husk were found to reduce the plant fresh weight compared to the control. The differences in the origin, application rate, and chemical properties of organic materials lead to varied responses in plants grown in the soil after the application. These variations are reflected in the yield and plant biomass, resulting in differences in their weights. Similarly, studies conducted by Kızılkaya et al. (2012), Durmuş and Kızılkaya (2022), Türkkan and Kibar (2022) and Ye et al. (2022) also obtained different results in plant weights based on the properties of the organic materials used. The differences in plant weight observed among the organic materials used in the experiment can be attributed to their chemical properties since the application rate (5%), application method, and particle sizes were the same. In general, it can be said that, except for poultry manure, animal-based organic materials increased the fresh plant weight compared to the control, while plant-based organic materials reduced the fresh plant weight. The main reason for Poultry Manure's reduction in plant weight might be its high salt content. Similarly, despite having high salt content, vermicompost did not decrease plant fresh weight. This can be explained by the fact that other organic plant growth regulators present in vermicompost might have a more dominant effect on plant growth compared to the effect of salt content in it. In the experiment, it was determined that plant-based organic materials, when compared to the control group, reduced the fresh plant weight. This is undoubtedly due to the slow decomposition of plant-based organic materials in the soil compared to animal-based organic materials and the high application rate (5%) of these materials in the soil, which can lead to deficiencies of certain nutrients such as nitrogen (N) in plants, resulting in a decrease in plant weight.

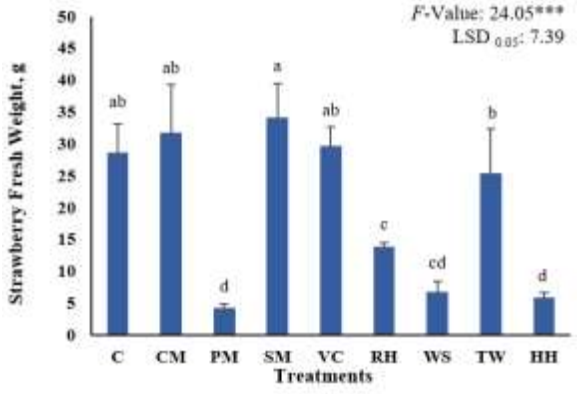

Figure 1. Effects of the different organic materials on strawberry fresh weight

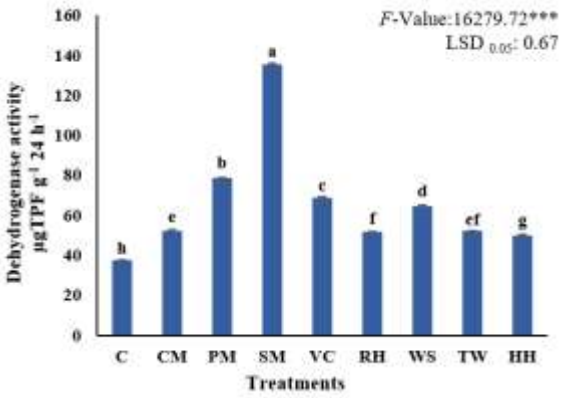

Figure 2. The effect of different organic materials on the dehydrogenase activity of the strawberry plant's rhizosphere soil

The changes in the dehydrogenase activity of the strawberry plant's rhizosphere soil caused by different organic materials are presented in Figure 2. According to the obtained results, all organic material additions to the soil significantly increased the dehydrogenase activity of the strawberry plant's rhizosphere soil compared to the control treatment. Additionally, it was found that animal-based organic materials significantly increased the dehydrogenase activity of the rhizosphere soil compared to plant-based organic materials. This is undoubtedly due to the faster decomposition and easier utilization of animal-based organic materials by the soil microflora compared to plant-based ones. Studies have shown that different organic materials applied to the soil can affect the microbiological properties and enzymatic processes of the soil to varying extents. However, in general, these materials tend to stimulate the microbiological properties and enzymatic processes of the soil, such as dehydrogenase

activity (Kızılkaya, 2005, 2008; Kızılkaya and Hepşen, 2007; Kızılkaya et al., 2012; Sürücü et al., 2014; Durmuş and Kızılkaya, 2022). The highest effect on the strawberry plant's fresh weight was observed in Sheep Manure, and similarly, the dehydrogenase activity in the rhizosphere of the strawberry plant was also found to be the highest in Sheep Manure among all organic materials used.

Conclusion

In this study, we investigated the changes in dehydrogenase enzyme activity in the rhizosphere soil of Albion strawberry plants grown in clayey soil, with the addition of various plant-based and animal-based organic materials (cow manure, poultry manure, sheep manure, vermicompost, wheat straw, rice husk, tea waste, and hazelnut husk) at a 5% rate. Additionally, we examined the effects of these organic materials on strawberry yield through a 61-day greenhouse experiment.

The results demonstrated that sheep manure, cow manure, and vermicompost applications significantly increased the fresh plant weight compared to the control group. On the other hand, all other organic materials led to a reduction in fresh plant weight. These findings indicate that sheep manure, among the tested organic materials, is the most effective in promoting plant growth and increasing fresh plant weight.

Regarding the dehydrogenase activity in the rhizosphere soil, all organic material additions significantly increased this enzymatic activity compared to the control treatment. Moreover, animal-based organic materials, including sheep manure, exhibited a more pronounced effect on the dehydrogenase activity of the rhizosphere soil compared to plant-based organic materials. This observation suggests that sheep manure is the more effective organic material in stimulating soil microbial activity, as indicated by the increase in dehydrogenase enzyme activity.

In conclusion, sheep manure emerged as the most effective organic material in terms of promoting both plant growth, as evidenced by increased fresh plant weight, and enhancing the dehydrogenase activity in the rhizosphere soil. The results highlight the importance of selecting appropriate organic materials for soil amendments to optimize plant productivity and soil microbial activity. By understanding the specific impacts of different organic materials on plant growth and soil enzymatic processes, farmers and researchers can make informed decisions to support sustainable agricultural practices and improve overall soil health. Further investigations focusing on the long-term effects of sheep manure and other organic materials on soil quality, nutrient cycling, and crop productivity will be valuable for advancing agricultural sustainability and environmental stewardship.

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SEED PRIMING AGAINST TO DROUGHT STRESS

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Abstract

The increasing world population, climate changes by global warming, and disappearance of natural sources cause quite a big risk factor against feeding the world population. In this context, seed priming is extremely important to have a good germination level from seeds and to have high tolerance plants under water-limited stress conditions which might cause yield loss. Seed priming basis on the seed's osmotic pressure for a while through seed germination phases. Regarding achieve of the priming treatment directly have relation with age and vigor of the seeds, storage period and conditions, ingredient type of priming solution, concentration of treatment, treatment period, genetic effects of seeds; variety and crops, drying method and period. These several techniques of primings as; halo-priming, hormonal-priming, bio-priming, and osmo-priming which serve to increase plant tolerance against to stress factors besides increasing seed germination allowing by holding seeds into the solutions with several ingredients. The purpose of this review is to draw attention to the importance of priming techniques in terms of drought stress management.

Keywords: *Drough stress, priming, seed, treatment.*

Introduction

Drought stress one of the abiotic stress which can be cause most of yield loss around world. Also plant tolerance against drought stress quite variable across different crops, varieties and genetics. Due to these facts, the increasing world population and climate changes by global warming, finding some way to avoid yield loss quite important.

On the other hand, plant nutrition, hormones, photosynthesis, respiration, morphogenesis, stress physiology, germination and phytochemistry are various fundamental processes which consist plant physiology. One of the first reactions of the plants is thought to be stomatal conductance. Due to the fact that would affect the photosynthesis rate, and it directly cause change energy level produced by the plant and biomass. And this is the major reason of the yield loss under drought conditions (Kocaoglu, 2019).

The most critical cycles of plants are seed germination and seedling establishment. Surive of seedlings and also, final yield and quality correlative through with germination process. Water, temperature, oxygen and light are the major factors which has direct affects on germination. Among these factors water is key needed for seed germination get start by metabolism activate and after then for growth of young plants. And another major factor is temperature which affects on germination and period of seed germination. Also, it effects on control of dormancy (Emiralioglu *et al.*, 2022).

Priming is a treatment before sowing on seeds which might allow an increase seed performance which would ensure uniformity and healthy plant establishment. Also, it would enhance the yield in diverse ecosystems by allowing greater tolerance to environmental stress and helps to overcome dormancy. In the process this treatment seeds are soaked dedicated solution with hight osmotic potential, and it allows a controlled metabolic progression necessary for germination until radicle protrusion. In addition to having a good level of

germination and a uniform seedling/plant establishtment, Priming seeds commonly has been used for having plants growth from primed seeds to increase plant productivity under abiotic stress conditions (Dhal, 2022).
Hydropriming, osmopriming, hormonal priming, biopriming and solid matrix priming are various method of priming. Optimization of these parameters of methods are dependent through genotype (Kocaoglu, 2019).
In this context, seed priming techniques have gaining importance increasingly, as these treatments can allow to increase seed germination which allow to reach aimed number of plant. By the way, plants from the priming treated seeds gaining a higher tolerances against to drought conditions. Both of benefits quite critical to avoid risk factors in the case of the yield losses causes in drought stress. Therefore, seed priming allows to us to be able to produce food sufficiently for increasing world population under the risky climate changes by having plants with higher tolerance against to drought conditions.
The purpose of this study is to draw attention to the importance of priming techniques against to drought stress management which occures increasingly as one of the results of climate change by reviewing on the local and international literature.

Materials and method

In the study, literature on seed priming and effects on germination, seedling emergence and under drought stress conditions was reviewed and the subject was examined in detail.

Results and Discussion

Effect of seed priming on germinatıon and seedling emergence

Germination of seeds were defined that the process begins with uptake of water by the quiescent dry seeds and terminate with elongation of the embryonic axis (Bewley, 1997). Physiological and biochemical changes followed by morphological changes during germination are strongly related to seedling survival rate and vegetative growth which affect yield and quality. Food reserve of starch and protein are mainly stored in the endosperm. In general, germination process can be distinguished into three phases: phase I, rapid water imbibition by seed; phase II, reactivation of metabolism; and phase III, radicle protrusion. The most critical phase II whereas, the essence physiological and biochemical processes such as hydrolysis, macromolecules biosynthesis, respiration, subcellular structure, and cell elongation are reactivated resulting in initiation of germination (Awatif *et al.*, 2017) (Fig.1).

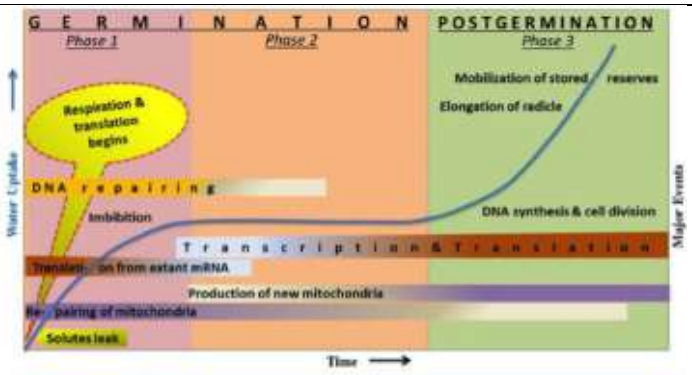

Fig 1: Major events associated with seed germination and post-germinative growth phases. Germination stages are represented by phase 1 and phase 2; postgermination events includes phase 3. The time (x-axis) for events varies from several hours to many weeks, depending on different plant species and germination conditions (Bewley, 1997; Shabari *et al.*, 2015).

Regarding water and temperature are major effects on germination of seeds, drought has a negative effect on seed germination (Emiralioglu *et al.*, 2022). Seed germination and plant growth can be affected with water limitation as drought in arid and semi-arid regions (Souhail

et al., 2019) due to the fact water and temperature are key factors for germination of seeds. Priming of seeds is one of the treatments which allows to minimise drought negative affect on seed germination. The priming basis on controlled water uptake by seeds without allowing radicle protrusion (Emiralioglu *et al.*, 2022). Seeds are been immersed in water (hidro-priming) or in a solution with a low osmotic potential (osmo-priming, halo-priming, hormo-priming) in determined temperature and period (Ceritoglu *et al.*, 2021). While these treatments allow to minimise risk in germination of seeds also might increase plant tolerance against to environmental stress conditions (Emiralioglu *et al.*, 2022). Regardless of the genotypes, increasing drought at germination stage, by lowering the water potential reduce the capacity for germination (Souhail *et al.*, 2019).

Effect of seed prımıng under drought stress

Drought stress is water deficit or unability of accession to water by plant as result of insufficient rainfall, water holding capacity of soil and water lost due to the evaporation is drought stress is the most common and restrictive factor of crop yield among abiotic stress factors. Some morphological, physiological and biochemical changes commonly seems when plants cannot get enough amount of water from the soil (Kocaoglu, 2019). Since, the germination of seeds begins with water uptake, drought stress can cause a reduction in germination significantly. However, pre-germinate the seed with priming treatments allows to seed germinate well and seedling establishment. Negative osmotic conditioning on seeds by priming treatments allows plants tend to have higher tolerance upcoming negative osmotic pressure conditions causes with drought stress.

And an experiment showed that vegetable seeds was significantly affected by drought stress which induced with PEG solutions. Germination capacity was drastically reduced by expose the seeds with highly negative osmotic potentials. Although responses were variable across different vegetable crops, the most common responses of plants to reduction of osmotic potential are a delay in initial germination and a reduction in the rate and total germination (Fig2) (Steiner *et al.*, 2019).

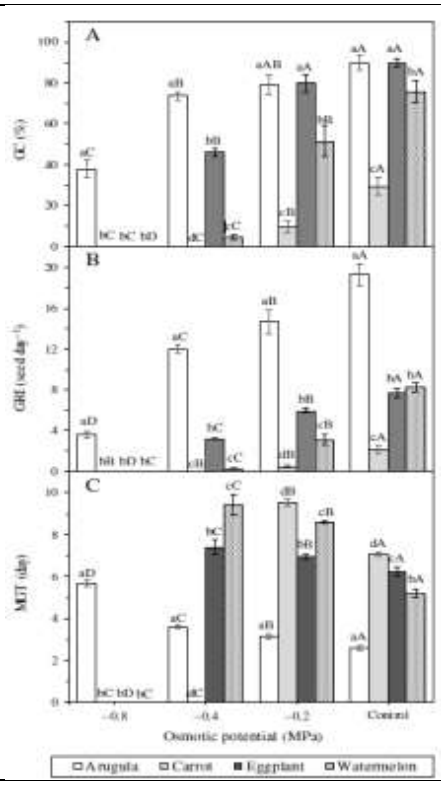

Fig 2: Effects of drought stress induced by polyethylene glycol on germination capacity (GC, in A), germination rate index (GRI, in B), and mean germination time (MGT, in C) of vegetable seeds. Bars followed by the same lower-case letters, between the vegetable species or same upper-case letters, for the osmotic potential levels within each species are not significantly different by Tukey test at the 0.05 level of confidence. Data refer to mean values (n = 4) ± standard error.

On soybean showed that seed priming significantly effect on plant height, shoot/root ratio, chlorophyll content and protein content. However, the results for RGR, number of stomata, 100 grains weight and grain yield were not significantly different. Absence of significant effect of seed priming on grain yield in the present study may be due to short period of priming, varietal response, priming conditions and osmoticum concentration (Table 1) (Syatrianty *et al.*, 2014).

Table 1. The effects of seed priming at different levels of irrigation on field performance of soybean

Treatment (Priming)	Irrigation (%F C)	Plant height Increment (cm)	RGR	Shoot/Root ratio	Chlorophyll content (mg g^{-1})	Number of stomata	100 grain weight (g)	grain yield (kg ha- 1)	Protein content (%)
dry seed	100	34,17	0,134	7,240 a	0,314 b	25,00	12,73 bcd	1321,50	31,19 de
	75	31,70	0,141	5,440 abc	0,338 b	29,67	13,99 abcd	1101,04	29,32 ef
	50	38,50	0,135	6,760 ab	0,320 b	24,33	10,46 e	1089,54	31,35 de
0 g PEG L- 1 water	100	20,67	0,162	5,833 ab	0,300 b	31,00	12,89 bcd	1036,21	32,68 cd
	75	25,67	0,178	5,430 abc	0,310 b	28,00	14,21 abc	1034,83	32,14 cd
	50	24,50	0,138	4,510 bc	0,291 b	20,00	11,25 de	903,92	28,67 f
100 g PEG L- 1 water	100	17,50	0,136	6,053 ab	0,332 b	21,33	11,13 de	1167,33	32,05 d
	75	37,33	0,169	4,580 bc	0,312 b	28,33	14,88 abc	1110,67	35,00 ab
	50	20,67	0,123	7,390 a	0,336 b	17,33	11,44 de	884,46	34,49 bc
200 g PEG L- 1 water	100	40,83	0,163	2,387 c	0,337 b	30,00	14,39 abc	1563,29	30,10 ef
	75	28,67	0,138	4,870 bc	0,323 b	25,00	12,33 cd	1116,67	30,39 e
	50	30,83	0,128	4,967 abc	0,344 b	19,00	15,27 ab	999,83	35,22 ab
300 g PEG L- 1 water	100	33,00	0,161	5,257 abc	0,309 b	31,00	13,00 bcd	1448,25	30,66 e
	75	29,57	0,161	3,057 c	0,326 b	38,33	11,02 de	1132,00	35,23 ab
	50	24,00	0,154	4,387 bc	0,963 a	29,00	15,45 a	1111,00	35,95 a

Different letters in each column indicate significant difference at p≤0,05

While short-term physiological adaptations of wheat to soil water deficit are well-known, the impact of seed ethephon priming on stress memory against drought during the tillering stage remains less explored. A study involving 42 wheat cultivars from 1991 to 2012 subjected to drought stress (45% Field water-holding capacity) and well-watered conditions (75% Field water-holding capacity) for three weeks aimed to assess wheat's genetic progress against drought. For a drought-sensitive cultivar (CM104), seed ethephon priming was applied to

investigate its effects at organic, cellular, and molecular levels. The research found that drought at the tillering stage led to yield losses, but genetic improvement over the years reduced these losses. Ethephon-primed seeds helped maintain leaf water content, enhance drought avoidance through root improvements, and increase drought tolerance via reduced oxidative stress and enhanced osmotic regulation. Transcriptome analysis revealed genes and pathways related to ethylene-induced drought tolerance. Ultimately, seed ethephon priming enhanced grain yield by improving both drought avoidance and tolerance mechanisms (Yang *et al.*, 2021).

One the other hand, priming of seeds led for epigenetic modification which accomplished the stress memory led for beneficial under recurrent drought exposure (Fig.3). Drought stress has adverse effects on plant growth and productivity. Various seed priming techniques are used to mitigate these effects and enhance drought tolerance, proving to be cost-effective and sustainable. Recovery from drought stress, often through rain or irrigation, is crucial for plant recuperation, with the severity of damage influencing recovery kinetics. Seed priming involves processes like DNA repair, osmotic adjustment, antioxidant activity, improved photosynthesis, and stress-responsive gene expression, aiding in alleviating drought stress and promoting recovery. While the benefits of seed priming for drought tolerance are well-documented, its role in recovery mechanisms remains underexplored, highlighting a research gap addressed in this paper (Aswathi *et al.*, 2022).

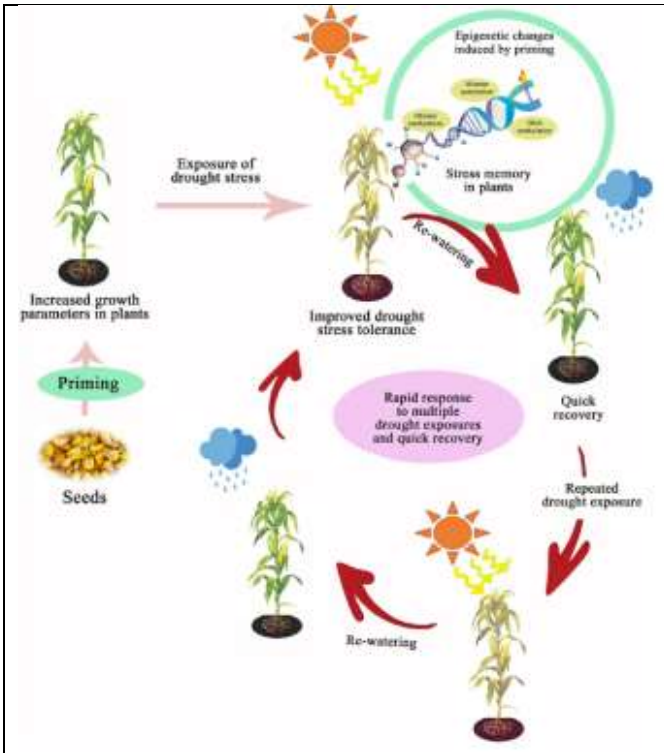

Fig 3: Epigenetic modifications induced by priming is helping in imprinting stress memory. It is beneficial under recurrent drought exposure and also favours rapid stress recovery (Aswathi *et al.*, 2021)

Conclusions

Drought stress is the major abiotic stress factor which cause reducing yield for all crops. And responses on seed priming across genetics are variable in regard to seed germination, seedling establishment and plant productivity. It can be inferred it can be that seed priming can improve plant growth and also impart tolerance to drought stress. Due to the fact seed priming techniques is a promising strategy to avoid effects of drought stress on plant growth and productivity, germplasm selection against drought stress could be improved among germination percentage, shoot lenght and root lenght.

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POSSIBILITY OF USING BANANA WASTE AS A SUBSTRATE FOR SUSTAINABLE AGRICULTURE UNDER SALT STRESS

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Abstract

In this study, the use of banana peels as a sustainable approach was investigated in order to overcome salt stress during the germination stage, which is the most important stage of plant development. Dried banana peels were ground and added to the peat perlite mixture at different concentrations (1:1:1, 1:1:2 peat: perlite: banana respectively) to determine the effects of purple carrot seeds, which have low germination rates, on germination and early seedling growth under both non-stress and salt stress conditions. As germination parameters; germination percentage (GP), mean germination time (MGT), coefficient of velocity of germination (CVG), germination index (GI) were calculated. Seedling viability index, shoot and root length (cm), leaf width and length (cm), shoot and root fresh weights and dry weights (g) were measured in order to reveal their effects on early seedling growth. It has been determined that it has a positive effect on germination under salt stress and on seedling growth under salt-free conditions. Banana waste application in salt stress increased the germination percentage to 93%. It also increased germination rate coefficient (57.40), germination index (0.27) and germination rate index (15.44) and decreased mean germination time (6.61). In salt-free conditions, banana application added to the medium at a ratio of 1:1.1 increased all parameters except shoot dry weight. The results revealed that banana peels are promising for use in sustainable agriculture and that biochemical changes should also be evaluated.

Keywords: *Banana, Carrot, Germination, Salt Stress, Sustainable Agriculture.*

Introduction

The fact that conventional agriculture is based on the intensive use of synthetic chemical fertilizers adversely affects living things and ecosystems. Contrary to the pressure exerted by conventional agriculture on the ecosystem, sustainable agriculture aims to protect the environment and soil fertility, support the sustainable management of natural resources and preserve the diversity of species (Anonymous, 2023; Singh *et al.,* 2021). Banana, which is a tropical fruit, can be grown in microclimate regions of subtropical regions as in Turkey (Pekmezci *et al.,* 1997). Depending on the climatic characteristics, it can be grown both under cover and in the open (Kafkas *et al.,* 2022), increasing its production. According to 2021 data, world banana production has reached approximately 125 million tons (FAO, 2023). A large amount of the peel of the banana, which is a product whose fruit is consumed, comes out as waste (El Barnossi *et al.,* 2021). The use of these waste peels in agricultural production will contribute to sustainable agriculture. The powder obtained from banana peels is alkaline and contributes to reduce the acidity of the soil. Jariwala and Syed (2016) reported the high

phosphorus and potassium content of banana peel powder and its use as fertilizer can be used to regulate soil pH and enrich it with micronutrients. Mercy et al. (2014) 1 g each of powdered banana, pomegranate, sweet lime and orange as fertilizer positively affected the growth of fenugreek. In this study, it was aimed to determine the effect on germination and early seedling growth by applying dried banana peels at different rates to purple carrot seeds, which have low germination rate and are sensitive to salt stress.

Material and Methods

Purple carrot seeds, a glycophytic vegetable species sensitive to salt stress, were used as plant material. First, the banana peels were dried in an oven at 39°C. Dried banana peels were brought into powder form in the homojenizator and germination media were prepared by mixing peat and perlite in different proportions (Table 1). Salt stress was applied to carrot seeds planted in these substrates. In order to create salt stress, 150 mM NaCl, which was determined as the threshold value in our previous study (Ulukapi and Nasircilar, 2021), was applied to purple carrots. Seeds were sown in viols in 4 repetitions with 25 seeds in each replication. At the end of the experiment, germination percentage (Gosh *et al.*, 2014), mean germination time (Ellis and Roberts, 1981), coefficient of velocity of germination (Kotowski, 1926), germination index (Benech *et al.,* 1991) were calculated. In addition, seedling viability index (Baki and Anderson, 1973), shoot and root length (cm), leaf width and length (cm), shoot and root fresh weights and dry weights (g) were measured and early seedling development was determined.

Table 1. Schematic representation of the trial plan

Treatments	Peat	Perlite	Banana peels	NaCl (150 mM)
T1 (control)	1	1	-	-
T2	1	1	1	-
T3	1	1	2	-
T4	1	1	-	+
T5	1	1	1	+
T6	1	1	2	+

Statistical analyzes of the data were made using Minitab 21 package program. The differences between treatments were detected by the Tukey Test ($p< 0.01$, $p< 0.05$).

Results and Discussion

Analysis of variance results showed that treatments had statistically significant effects on all germination parameters and plant growth values (Table 2, Table 3).

Table 2. Descriptives and Variance analysis result with respect to the vegetative growth of purple carrot.

Variation source	df	GP	MGT	GCV	GI
T	5 12 17	**	**	**	**
Mean		0.83	7.01	44.2	0.24
Minimum		0.69	6.51	30.0	0.19
Maximum		0.94	7.79	57.4	0.28
Standart deviation		0.0869	0.447	8.95	0.0272

T: treatment, * p<0.05, **p<0.01, ns: not significant

Table 3. Descriptives and Variance analysis result with respect to the vegetative growth of purple carrot.

Variation source	df	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	LL (mm)	LW (mm)	SVI
T	5 12 17	ns	*	*	*	**	*	*	*	*
Mean		60.3	94.8	0.197	0.0694	0.0330	0.0108	16.9	17.9	192
Minimum		38.9	50.5	0.0887	0.0134	0.0109	5.00e-4	8.90	11.3	128
Maximum		82.6	230	0.362	0.322	0.124	0.0591	27.6	27.2	440
Standart deviation		11.2	47.6	0.0791	0.0722	0.0319	0.0144	5.13	3.93	78.7

T: treatment, * p<0.05, **p<0.01, ns: not significant

Banana peels had no stimulating effect on germination parameters under stress-free conditions. On the other hand, it increased germination percentage (93%), germination index (0.27) and coefficient of velocity of germination (57.4) under salt stress conditions. The decrease in the mean germination time (6.61) shows that the seeds applied to the banana peel germinate faster. The increased dose of banana peel in nano-fertilizer extracted from banana peels rose germination of tomato (97%) and in fenugreek (93.14%) (Hussein *et al.*, 2019). Analysis results of banana peel revealed that it is rich in phenolic substances and has higher radical scavenging activity than other fruit peels (Morais *et al.*, 2015; Vu *et al.*, 2018). It is thought that the positive effect of seed germination under salt stress of banana peels is related to these properties.

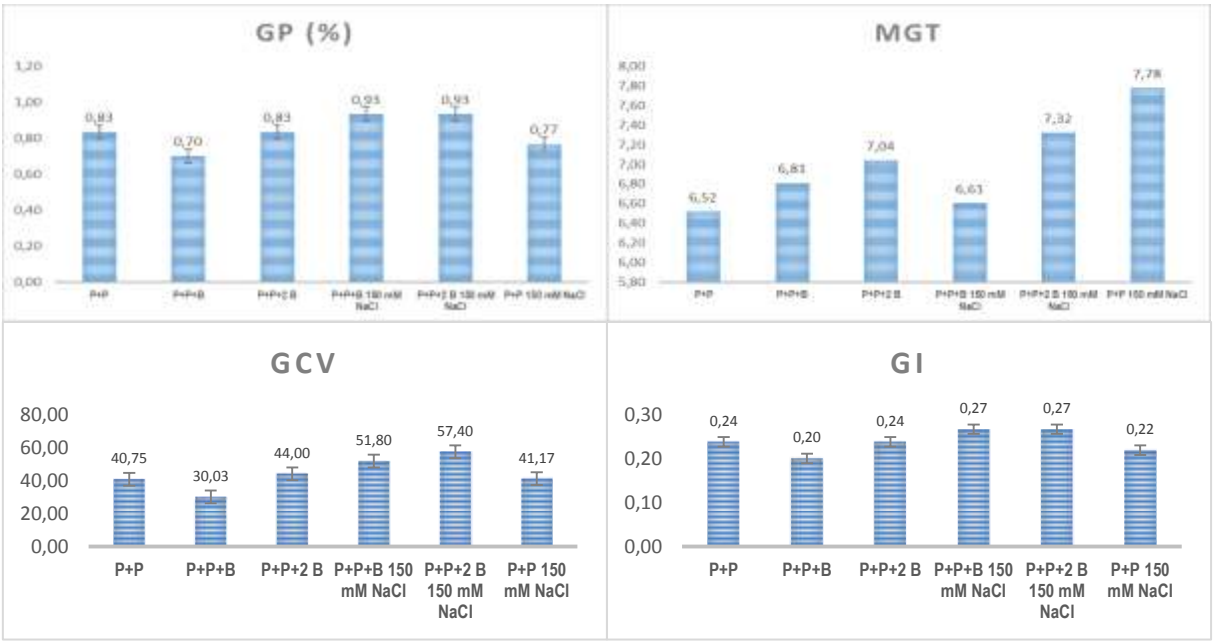

Figure 1. Change of germination parameters according to the banana peel dose. P: peat, perlite, B: banana peel

Banana peel applications had a stimulating effect on plant growth in non-stressed conditions, but did not have a positive effect except leaf growth under salt stress conditions (Table 4). Leaf length increased by 35.9% and leaf width by 12.8% under salt stress conditions. Kalemelawa *et al.* (2012) determined that the composts obtained by using banana peels and different animal manures in compost preparation have a high potential as a source of K and N fertilizers. The researchers' demonstration of the potential of banana peels as a fertilizer source and the fact that banana peels promote leaf growth, which is very important for photosynthesis, under stress conditions in the current study suggested that better results can be obtained with higher dose application or in the later stages of plant development. Also in another study, Jariwala and Syed (2016) determined the pH value of the powder obtained from banana peels to be 7.9. They showed that this material, which can be used to reduce soil acidity, also has high P and K content. El Barnossi *et al.* (2022) stated that pomegranate, banana and tangerine peels affect the germination and plant growth of pea seeds positively and that these peels can be used as environmentally friendly bioorganic fertilizers that can replace chemical fertilizers.

Table 4. Change of growth parameters according to the banana peel dose

Treatment	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	LL (mm)	LW (mm)	SVI
P+P	52.70	59.40 b	0.2557 ab	0.0376 b	0.0877 a	0.0045 b	16.67 ab	17.33 bc	134.83 b
P+P+B	65.97	152.53 a	0.2853 a	0.1727 a	0.0453 b	0.0280 a	22.40 a	22.53 a	311.25 a
P+P+2 B	57.90	122.70 ab	0.1394 c	0.0460 b	0.0149 b	0.0025 b	13.43 b	13.40 c	217.13 ab
P+P 150 mM NaCl	60.97	91.53 ab	0.2020 abc	0.0547 b	0.0199 b	0.0091 ab	15.53 ab	18.23 abc	200.69 b
P+P+B 150 mM NaCl	55.77	80.67 b	0.1575 bc	0.0812 ab	0.0187 b	0.0169 ab	12.30 b	15.53 bc	146.78 b
P+P+2 B 150 mM NaCl	68.50	61.93 b	0.1393 c	0.0240 b	0.0117 b	0.0040 b	21.10 a	20.56 ab	140.08 b

P: peat, perlite, B: banana peel

Conclusion

Instead of creating waste, banana peels, which cost nothing, can be used in agricultural production again to contribute to sustainable agriculture. The results obtained in this study show that banana peels are a promising application under both normal and salt stress conditions. In terms of plant growth, it was determined that P+P+B application had a stimulating effect under normal conditions. However, P+P+2B increased only leaf growth under salt stress conditions. In order to determine the stimulating dose under stress conditions, it is recommended that the subject should be put into practice and disseminated with studies to be carried out at different doses and in different species.

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INFLUENCE OF INTERCROPPING MAIZE WITH CLIMBING BEAN ON QUALITY AND YIELD OF FRESH FODDER

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Abstract

Cereals are highly important in feeding ruminant animals for their high dry matter production and low cost. Maize forage is poor in protein content which shows its low quality and nutritive value. Regarding to high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content and, thus, providing cost savings. Since legumes have low dry matter yield, acceptable forage yield and quality can obtained from intercropping cereals and legumes compared with their sole crops. In this study, maize (*Zea mays* L.) and climbing bean (*Phaseolus vulgaris* L.) intercropped in different sowing densities and pure maize crops were evaluated to the best intercropping system with respect to quality and yield of fresh fodder. Maize was sown alone (7.5 seeds m^{-2}) and intercropped with bean as follows: 7.5 seeds m^{-2} of maize and 3.7 seeds m^{-2} of bean, 7.5 seeds m^{-2} of maize and 5.0 seeds m^{-2} of bean and 7.5 seeds m^{-2} of maize and 7.5 seeds m^{-2} of bean. The highest dry matter yield was produced by 7.5 plants m^{-2} of maize and 7.5 plants m^{-2} of bean (19.4 t ha^{-1}), and the lowest by 7.5 plants m^{-2} of maize and 3.7 plants m^{-2} of bean (17.5 t ha^{-1}). Intercropping of maize with climbing bean reduced neutral detergent fiber content, which in turn, resulting in increased forage digestibility. Based on forage yield and quality, this study showed that among all intercropped forages by 7.5 plants m^{-2} of maize and 7.5 plants m^{-2} of climbing bean treatment was better performing than other intercrops.

Keywords: *Intercropping, Maize, Climbing Bean, Quality, Yield.*

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used in feeding cows and fattening cattle. Despite its high energy content, the protein content is low (88 g kg^{-1}) compared with legumes silage (Anil *et al.*, 2000) and needs to be supplemented with proteins for better feed quality (Stoltz *et al.*, 2013). Intercropping maize with legumes for silage is a feasible strategy to improving the level of crude protein (Contreras-Govea *et al.*, 2009; Zhu *et al.*, 2011). Although maize provides high yield in terms of dry matter, it produces low protein content in fodder. The bean (*Phaseolus vulgaris* L.) is a common legume cultivated for its edible seeds all over the world. It is slightly hairy with a well-developed root system and the stems are many branched. The bean is a fast growing, warm season legume, and, it can grow in a diverse range of environmental conditions worldwide because of its adaptability. There are many varieties of beans grown in all the regions. However, selecting high yielding (seed and herbage), disease resistant variety is most important factor for successful cultivation. In addition, the bean serves as an adequate source of protein. Furthermore, it can be planted alone or intercropped with other crops such as corn and sorghums. Javanmard *et al* (2009), worked on intercropping of maize with different legumes, and showed that dry matter yield and crude protein yield of forage were increased by all intercropping compositions compared with the maize monocrop. Physiological and morphological differences between intercrop constituents influence their ability to use resources; especially cereals with legumes, have

several advantages such as higher overall yields, better soil utilization (Dhima *et al.*, 2007), yield stability of the cropping system (Lithourgidis *et al.*, 2006), better use of light, water and nutrients (Javanmard *et al.*, 2009), improved soil conservation (Anil *et al.*, 1998), soil fertility through biological nitrogen fixation, increases soil conservation through greater soil coverage as compared to sole cropping, and ensures better soilsusceptible crop in monoculture (Lithourgidis *et al.*, 2006), and better control of pests and weeds (Banik *et al.*, 2006; Vasilakoglou *et al.*, 2008). Atmospheric nitrogen fixation using legumes plants can reduce nitrogen competition in the reciprocal intercropping system of legumes and cereals enabling the cereals to use more nitrogen in the soil (Eskandari *et al.*, 2009). This can affect the quality of the fodder intercrop components because the protein content is directly related to the content of nitrogen in the forage plants (Putnam *et al.*, 1985). This study was designed to determine the influence of different patterns of maize and climbing bean intercropping on the yield and quality of forage.

Material and Methods

A field experiment was carried out during the 2017 and 2018 growing season at experimental fields in Oborovo (45°40'54"N, 16°15'12.5"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Mean monthly air temperatures and rainfall during the 2017 i 2018 growing season

Year	Meteorological data	Month					
		April	May	June	July	August	September
2017	Mean monthly air temperature (°C)	11.9	17.3	22.4	23.3	22.7	14.8
	Rainfall (mm)	41.6	49.2	57.8	91.8	32.0	186.3
2018	Mean monthly air temperature (°C)	15.7	19.2	20.9	22.4	23.0	17.2
	Rainfall (mm)	51.6	50.5	144.4	94.4	66.1	36.4

The experiment was set up as a randomized complete block system with three replicates. Maize hybrid seed (KWS Kolumbaris) was obtained from Seed Company "KWS". Seed of the climbing bean cultivar "Anellino verde" was obtained from "Green Garden" company. The individual plot size was 50 m × 2,8 m for each treatment. The maize population 7.5 plants m^{-2} (SM) were spaced at 70 cm × 19 cm and climbing bean population 3.7 (MB_1), 5.0 (MB_2) and 7.5 plants m^{-2} (MB_3) were spaced at 70 cm × 38.1 cm, 70 cm x 28.6 cm and 70 x 19 cm, respectively, in rows alternating with maize. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 200 kg of K_2O ha^{-1}. Maize and climbing bean were sown to a depth of approximately 5 cm by maize drill on May 6, 2017 and on May 8, 2018. Herbicide Wing P (active substances 212.5 g/l dimethenamide-p and 250 g/L pendimethalin) was applied pre emergence in intercropping maize with climbing bean at a dose of 4 L ha^{-1}. The soil of the research area has a pH 4.2 (M-KCl), 3.2% humus (organic matter), and is poorly supplied with physiologically active phosphorous (4.6 mg P_2O_5/100 g^{-1} soil), medium supplied with physiologically active potassium (20.0 mg K_2O/100 g^{-1} soil), while the total nitrogen content averaged 0.17% in topsoil. The crops were hand harvested when the maize reached at soft dough stage and climbing bean was at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at

a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldahl (AOAC, 2000) and neutral and acid detergent fiber according to Van Soest *et al.* (1991). Calcium were analysed by atomic absorption spectrophotometry by analyzer Spectrophotometer 2010 Model M530 Infrared Spectrophotometer (USA). Statistical analyses: Analyses of variance were made for dry matter yield and forage quality parameters ($P<0.05$), and the Tukey test was used for comparing means ($P<0.05$). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and Discussion

The differences in the yield of dry matter (Table 2) were statistically significantly ($P<0.05$). Dry matter yields ranged from 18.5 t ha^{-1} (MB_3) to 16.9 t ha^{-1} (MB_1) in 2017 and 20.2 t ha^{-1} (MB_3) to 18.1 t ha^{-1} (MB_1) in 2018. Consequently, dry matter yields were higher in 2018 than in 2017. This could be due to the impact of more favorable environmental factors (effect of the year) such as solar radiation, water and temperature during plant vegetation. According to obtained results, when climbing bean seed number increased in intercrop, dry matter yields on parcels increased. The intercropped maize with cowpea (*Vigna unguiculata* (L.) Walp.) and bean (*Phaseolus vulgaris* L.) produced higher dry matter yield than monocrop maize (Horvatić *et al.,* 2018., Uher *et al.,* 2019). Since crude proteins are very important in cattle fodder, silage containing more crude proteins is desirable. In this study it was found that the yield of crude proteins of intercropped fodder MB_2 and MB_3 was significantly ($P<0.05$) higher than SM (sole crop maize) during a two years research (Table 2). Results in the present study were in agreement with other studies where legumes also increased crude protein concentration when in a mixture with maize (Htet *et al.,* 2016; Dawo *et al.,* 2007). The results suggested that the contributions provided by legume components in the mixtures increased crude protein yields of fodder. This could be due to the greater availability of nitrogen in intercropping arrangement compared to the pure maize crop (Eskandari *et al.,* 2009).

Table 2. Yield of dry matter and yield of crude proteins of maize and maize-climbing bean intercropped

Treatments	Yield of dry matter in t ha^{-1}			Yield of crude proteins in t ha^{-1}		
	2017	2018	Mean	2017	2018	Mean
SB	18.3a	19.4ab	18.9a	1.35c	1.51c	1.43c
MB_1	16.9c	18.1b	17.5b	1.50c	1.52c	1.51c
MB_2	17.6b	18.9ab	18.3a	1.74b	1.80b	1.77b
MB_3	18.5a	20.2a	19.4a	1.98a	2.08a	2.03a
Mean	**17.8b**	**19.2a**		**1.64a**	**1.73a**	

Different letters in the column indicate significant difference ($P<0.05$)

In this study it was found that the value of crude proteins of intercropped fodder MB_1, MB_2 and MB_3 was significantly ($P<0.05$) higher than SM (sole crop maize) during a two year research (Table 3). According obtained results, when climbing bean seeds number increased in intercrops, the content of crude protein in the mixture increased. Armstrong *et al.* (2008) found that climbing bean intercropped with corn had the greatest potential among the climbing beans to increase crude protein concentration compared with monoculture corn. The intercropping of maize with climbing bean may serve as a way to increase crude protein and improve the overall nutritive value of silage (Grobelnik *et al.,* 2005). For this reason, the best option in maize-climbing bean intercropping is the use of climbing bean genotypes that provide forage with the greatest amount of pods at harvest. In addition, the level of neutral

detergent fibers is associated with the stage of maturity of the fodder due to the level of the cell wall components, mainly cellulose, hemicellulose and lignin (Mugweni *et al.*, 2000). The value of a neutral detergent fiber refers to the total cell wall and consists of an acid detecting fiber fraction plus hemicellulose. In this study it was found that the values of neutral detergent fibers of intercropped MB_1, MB_2 and MB_3 were statistically significantly ($P<0.05$) lower than SM (Table 3). According to the results, when climbing bean seed number increased in intercrop, the values of neutral detergent fibers in the mixture decrase.

Table 3. Content of crude protein and content of neutral detergent fiber of maize and maize-climbing bean intercropped

Treatments	Crude protein in g kg^{-1} dry matter			Neutral detergent fiber in g kg^{-1} dry matter		
	2017	2018	Mean	2017	2018	Mean
SB	74d	78d	76d	385a	373a	379a
MB_1	90c	84c	87c	359b	353b	356b
MB_2	99b	95b	97b	350b	342b	346b
MB_3	107a	103a	105a	335c	327c	331c
Mean	**92.5a**	**90.0a**		**357a**	**349a**	

Different letters in the column indicate significant difference ($P<0.05$)

The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis *et al.*, 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh *et al.*, 2009). Many researchers stated that the nutritional value of cell wall components decreased with plant age was related to increased lignin content (Atis *et al.* 2012; Zhao *et al.*, 2012). Since smaller amounts of fiber components are used for better digestion, the climbing bean intercropped plots to be superior to monocrop maize in terms of neutral detergent fiber.

Table 4. Content of ash and calcium of maize and maize-climbing bean intercropped

Treatments	Ash in g kg^{-1} dry matter			Calcium in g kg^{-1} dry matter		
	2017	2018	Mean	2017	2018	Mean
SB	29d	33d	31d	2.2d	2.4d	2.3d
MB_1	34c	40c	37c	2.4c	2.6c	2.5c
MB_2	41b	45b	43b	2.7b	2.9b	2.8b
MB_3	45a	51a	48a	3.1a	3.4a	3.3a
Mean	**37.3b**	**42.3a**		**2.60b**	**2.83a**	

Different letters in the column indicate significant difference ($P<0.05$)

Contreras-Govea et al. (2011) ensiled corn and forage sorghum with different proportions of lablab bean and reported that legume must make up at least 50% of the mixture to affect fermentation and nutritive value. In this paper, the value of ash and calcium of intercropped forage MB_1, MB_2 and MB_3 was statistically significantly ($P<0.05$) higher than SM (Table 4). According to the results, when the climbing bean seed number increased in intercrop, the values of ash and calcium in the mixture increased. Contribution of legumes with sweet sorghum in mixtures was significant increased potassium, phosphorus, calcium and magnesium in fresh fodder (Terzić *et al.*, 2004; Basaran *et al.*, 2017).

Conclusion

The conclusion of this study is that intercropping of maize with climbing bean at various different sowing densities was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced nutrient quality of fresh fodder. Intercropping of maize with climbing bean has increased the values of crude protein, ash and calcium and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the MB_3 treatment (7.5 plants m^{-2} of maize and 7.5 plants m^{-2} of climbing bean) was most effective regarding the nutrient composition in fresh forage.

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PhytoTester: PORTABLE INSTRUMENT FOR ASSESSMENT OF VITAL ACTIVITY OF THE VEGETABLE ORGANISMS

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Abstract

PhytoTester is intended for measurement of bioelectric potentials of the vegetated plant and its organs in rest and action conditions (bioelectric response to electric pulse disturbance). By means of the original PhytoTester, we have experimentally determined correlation between vital activities of the vine planting materials (as well as grafting components and stratified grafts) and bioelectric potentials. Researches established the following aims: (a) To assess vital activity and suitability for use of the grafted nurslings of the plants in the rest condition and their suitability for use;. It is reasonable to use bioelectric potentials of the entire nursling roots system and above-root part in rest condition; (b) To determine suitability of the grafted nurslings base material for grafting, use of the rest bioelectric potential of the material, as well as material bioelectric response to electric pulse disturbance regarding provision of good results; (c) For use of the budding material diagnostics, it would be reasonable to use rest bioelectric potential of the bud; (d) For assessment of stratified grafted material callus suitability, it would be reasonable to use bioelectric response of the upper part of the callus (grafting material) to the disturbance of the lower part of callus (base) with electric pulses. Obtained results and our studies on citruses and other cultivated plants clearly show the prospects of use and introduction of pocket PhytoTester use as the tool for express-diagnostics of the vegetable organisms in plant-growing researches and practice.

Keywords: *active potential, electric signal, bio object, input resistance.*

Introduction

Irrespective of the technologies' rapid development, in the crops husbandry, suitability of the planting and grafting materials is assessed only visually, based on the materials' morphological signs and mechanical properties, e.g. degree of vine shoot maturity and suitability for use is established by:

- Uniformity of the sprout color characteristic for the variety – in the nodes intense dark and dark green color without spots;
- Bunch stalk elasticity – it does not break when bent, skin bursting indicates that the bark is well developed, its cells split in case of bending;
- Sprouts timber color and diaphragm density – well matured sprouts have dense diaphragm of timber color, immature ones – porous, light green loose diaphragm;
- When bending the material, color of its cross section as a result of treating with different chemical compounds, e.g. content of carbohydrates (mostly the starch) in the bunch stalk is measured by dying of its perpendicular cross-section with iodine solution;
- Proportion between the stalk timber and core diameters and proportion between water soluble and insoluble substances in the sprouts;
- Assessment of the vital activity of the stratified grafts, grafted nurslings and buds on the branches for grafting is provided on the basis of visual signs as well.

Subjectivity and poor accuracy of assessment of the object's physiological condition by means of the above methods was clearly demonstrated in practice. For example, frosty winter damages more than half of the grafting buds, making them unsuitable for grafting. While the graft produced with such shoot develops callus at a time of stratification, it dries after transfer to the nursery. This, together with the other factors, comprises the main cause of low productivity of the nurseries frequently no more than 33-40%).Non-transportable laboratory methods are relatively accurate but they require involvement of the specialists, expensive reagents, destruction of biological integrity of the materials and long process of testing. These factors make laboratory tests too expensive and limit their use in the production conditions. There are also the physical methods for information collection and control (chemo-luminescence, paramagnetic resonance etc.) that, similar to the above described methods, require stationary equipment, participation of the highly qualified specialists and are almost unsuitable for gaining of information from plants, in the field/production conditions. Regarding that it is desirable to determine the physiological condition of the grafting components and nurslings without destruction of the object integrity, to deal with such issues, preference should be given to the express diagnostics methods relying on measurement of bioelectric potentials of integral energetic characteristics of the plants' vital activity [1,2,..,8]. The work presents the results of testing of our portable phyto tester for the purpose of express diagnostics of physiological conditions of the vine nurslings and grafting components.

Materials and Methods

Research object is original / pocket PhytoTester and biophysical methods of express diagnostics of the vital activity of the vine nurslings and grafting components.

a) Technical characteristics of the PhytoTester: range of measurement of bioelectric potential: 0-200 mV; input resistance 100 MΩ;

Original non-polarizable electrodes; measurement error $\leq$1%;

Time per test: 5-15 S; power supply voltage: 9-12 V; device dimensions: 117x68x20 mm; device weight with the electrodes: 50 g;

b) Methods of express diagnostics of the vegetable organisms' vital activity:

- Method for evaluation of base material vital activity and suitability for grafting;
- Method for evaluation of suitability of one-year grafted nursling with the roots;
- Method for testing of budding material suitability for grafting.

Results and Discussion

a) Measurement of the rest bio-potential of the vegetated plant.

Structural diagram of the measurement is provided on the figure below.

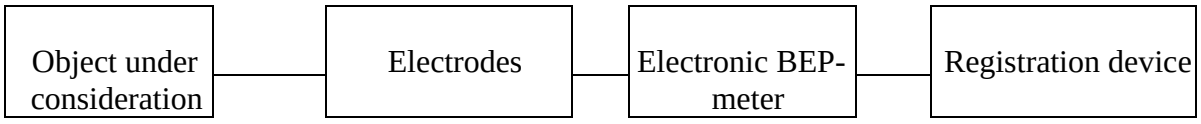

Fig. 1. Structural diagram of bio-potential measurement

As the diagram shows, from the live organisms, the signal, via electrodes, is delivered to the measurement unit and is registered on the digital display. The device must ensure stability and reliability of the parameters, saving power supply, low weight and small dimensions, simile operation. As the equipment is intended for measurement of permanent and infra-low frequency weak signals, in the conditions of external noise, it must also have high metrological characteristics, with respect of measurement accuracy, signal selection, sensitivity and electric parameters. Methodology of measurement of the plants' bioelectric

signals must not cause damage integrity of the biological object and must not impact the processes ongoing in the plant.

Equivalent electric schemes of definition of bioelectric activity of over-surface part and root system of the plants are shown in Fig.2 and Fig.3. As it is shown in the figures it is not allowed to earth metering electrodes or any clamps during the measurement of biopotentials because it will cause short circuit of BEP on the some part of plant, changes of physiological processes in it and high inaccuracy of measurement. In contrary, during the measurement of BEP of root system one electrode should be earthed (Fig.3) and another one should be connected to bole above the root neck.

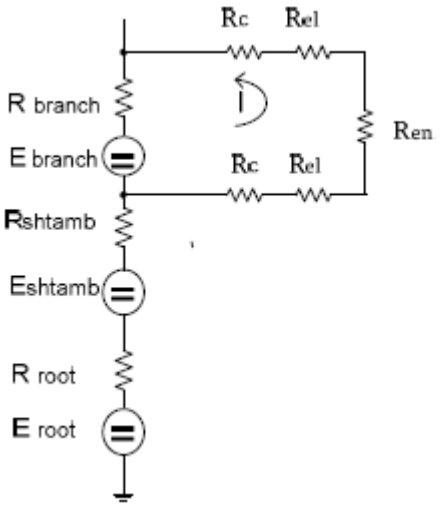

Fig.2. Equivalent electric scheme of measuring of BEP of over-surface part of the plant

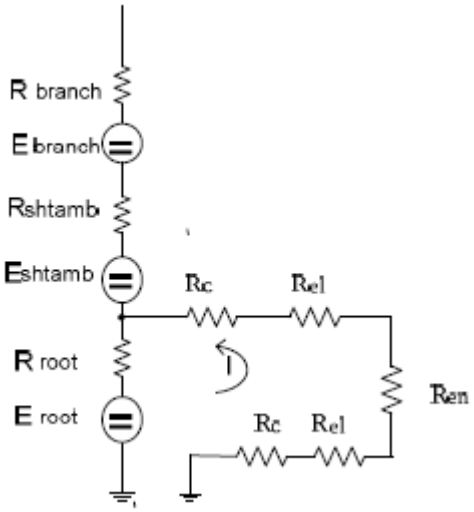

Fig.3. Equivalent electric scheme of measuring of BEP of root system of the plant

Where R_c is the resistance of contacts; R_{eL} - resistance of electrode; R_{en} - resistance on the entry of the equipment. R_{branc}- E_{branc}, R_{stamb} - E_{stamb}; R_{root}- E_{root} – resistances and electromotive forces of the branches, stambs and roots system.

b) Study of dependence of the grafting material action bioelectric potentials on maturity

According to technological regulation of vine nursling production, before commencement of grafting, the base and grafting materials are stored in the special conditions. While, in the storage period, the material is in the physiological rest condition, metabolic and other vital processes are still present, as reflected in the amplitude of their electric signals. Measurements showed that in the vegetation process, on the developed shoots of the vine, the bioelectric potential values vary within 1 ÷ 20 mV range, Studies showed that the amplitude of vine sprout bioelectric response to the electric current impact is more real indicator of its physiological condition, while rest potentials are less effective, due to small amplitudes. There was also established that measurement of bioelectric response is more effective. It was also established that it is more effective to measure bioelectric response to the pulse disturbance that, in a form of excitation wave, is a launching mechanism of the whole number of physiological and geochemical processes.

To find out, whether the vine stalk is suitable for grafting, in studying of the amplitude and time characteristics of the material response to electric pulse disturbance, asymmetric structure of the vine stalk was taken into consideration.

The experiments were conducted as follows:

On each side surface of the vine stalk, there were placed four disturbing electrodes (points 1, 2, 3, 4 and 1^1, 2^1, 3^1, 4^1) Fig. 3. Distance between these electrodes was up to 2 cm. First four

electrodes, by means of the switchboard C1, was connected to the negative pole of impact unit and second four electrodes by means of the switchboard C2 was connected to the positive pole of the impact unit and the measuring electrodes were located on the sides of vine stalk, in 2 cm, above the upper disturbing electrodes.

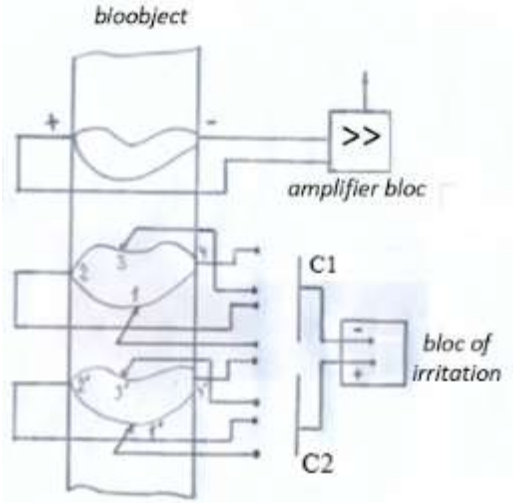

Fig. 3. Diagram of experimental test of bioelectric response of the base material to the electric pulse disturbance

Measurements were conducted in the following sequence: switchboard C1 was placed in point 1 and switchboard C2 connected in series the positive pole of excitation unit with the points 1^{I}, 2^{I}, 3^{I}, 4^{I}. Further, switchboard C1 is connected to point 2 and switchboard C2, similar to the previous case, connects in series, the positive pole of the excitation unit. Further, the characteristics of the response were taken similarly. Diagram describing changes of the responses of the vine shoot characteristic sides is provided on Fig.4.

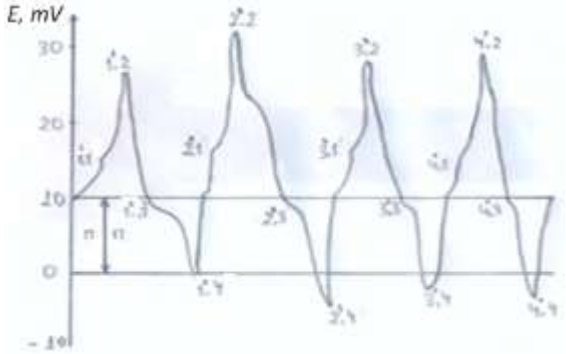

Fig. 4 Vine sprout bioelectric response to disturbance by electric pulse, depending on dislocation of the electrodes

Studying of the different variants of the measuring electrodes attachment points and change of their polarity, as well as change of exciting electrodes polarity causes on the diagram only change of the relevant response signals.

As a result of diagram analysis, characteristics of bioelectric response to pulse excitation of the vine shoot, regarding one-year vine sprout asymmetric structure, the conclusion can be made that the amplitude of the bioelectric response drastically changes with the exciting areas. Maximal amplitude was indicated, when the measuring and exciting electrodes are in a single plane, with the middle points of the bud sides, apparently showing that the sides are more developed than the groove and back sides. Supposedly, this should be caused by greater content of starch in these sides.

It could be noted that based on the bioelectric response amplitudes, on the side surface, groove and flat sides, we can assess the degree the vine one-year branch maturity.

To prove this phenomenon, using the same methodology, we have conducted experiment on the young sprouts; at this time, the response amplitudes in all points, i.e. o all sides are equal.
We have also experimentally observed the values of bioelectric potential values of the bud, on the plant's grafting shoot. The experiments showed that to evaluate the bud vital activity, the rest bioelectric potentials can be used.
Study of bioelectric potentials of the grafted nurslings showed that for diagnosing of vital activity of the one-year nursling, rest bioelectric potentials provide good results. Measurement is provided between the root end and over-root part, amplitude of bioelectric potentials, in this case, vary between 50 and 150 millivolts. For the purpose of improvement of the testing process productivity, the roots of the nurslings bundle are placed into the polyethylene vessel, on the bottom of which, at 0.5 cm depth, there is poured water. Tip of one electrode is placed into the water together with the roots and by manipulating of the other electrode on the over-root parts of different nurslings, the amplitudes of the relevant nurslings are measured and their suitability is evaluated.

Conclusions

Pocket PhytoTester is a compact, light device, simple for use. Non-polarizable electrodes of bioelectric potentials are simple in use and ensure proper contact with the bio organisms.
By means of PhytoTester, physiological condition of the plant nurslings and grafting components can be evaluated in 5-10 seconds, without any damage to the object, based on the bioelectric signals obtained directly from the object. Low quality nurslings, as well as grafting materials can be identified very simply, saving time and finance, reducing expected losses and additional expenses in the future, to deal with the scarceness of plantations
PhytoTester can be regarded as the instrument for demonstration and verification of the planting and grafting materials produced by the farmers, as well as image of the producer. It can be also used to measure bioelectric response to the treatment and other impacts on the plants and setting of the effective doses of such impact.
Operation manual of the device provides detailed information about device operation for each specific case. We shall launch serial production of the device in 2024. At current stage, we accept individual orders for portable / pocket PhytoTesters.

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INFLUENCE OF MOWING HEIGHT AND PHENOLOGICAL STAGE OF MATURITY ON NUTRITIONAL VALUE OF ALFALFA HAY

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Abstract

The cutting height increased linearly with the increase in movement speed. This increase during Phase I (budding phase) was most pronounced in mower B (regression slope coefficient was b = 0.4653), and least in mower C (b = 0.2156). During Phase II (full flowering phase), we also note the greatest increase in mower B (b = 0.4084), and the smallest in mower C (b = 0.2313). The phenological stage of maturity significantly affects the crude protein content. During Phase I, the maximum crude protein content of 19.62% was recorded by mower C, and the minimum by mower B (18.43%). The lowest sensitivity during Phase I is observed in mower A (b = 0.6019) and mower B (b = 0.8657), while in mower C we observe a very strong sensitivity (b = 1.3986). In Phase II, mower C recorded the maximum crude protein content of 16.11%, and mower B the minimum (15.34%). In Phase II, mower A recorded the lowest sensitivity (b = 0.2785). For mowers B and C, the increase is almost identical (0.5832 and 0.5868). The content of crude fiber in alfalfa hay decreases with increasing cutting height, and increases significantly with increasing maturity. The maximum values of crude fiber content were achieved by mower A (Phase I = 31.62%, Phase II = 35.53%). Mower C shows a very strong sensitivity (b = -4.7486) in Phase I and a significant sensitivity in Phase II (b = -1.5396). The phenological stages of maturity did not significantly affect the crude ash content of alfalfa hay.

Keywords: *Mowing, Cutting height, Budding phase, Flowering phase, Crude proteins*

Introduction

Alfalfa and alfalfa hay is one of the highest quality nutrients used in animal nutrition. It is characterized by a high crude protein content of 18 - 25% (Kebede *et al.*, 2017) and a lower crude fiber content (23 - 25%) at the beginning of budding. By the stage of seed formation, the crude protein content decreases to 14%, and the crude fiber content increases to 36%. The intensive development of a sophisticated grassland system based on leguminous cultivation will have a great importance in ruminant nutrition in the future (Lüscher *et al.*, 2014). The production of alfalfa hay with high nutritional value is unthinkable without the use of appropriate mowers. The mowing operation is very important, it must be done in the optimal period (Karayilanli and Ayhan, 2016) and significantly affects the yield and nutritional value of alfalfa (Jin *et al.*, 2021).

Alfalfa mowing time affects its nutritional value because the protein content and nutritional value of hay decrease with the age of the plant. According to (Chang *et al.*, 2020) mowing time had a significant effect on the composition of nutrients of mowed alfalfa. The time of mowing at a certain phenological stage of maturity has a great influence on the quality of forage (Atis *et al.*, 2014; Basbag *et al.*, 2021). Studies by Sayar *et al.*, (2022) and Yari *et al.*, (2012) proved that with the progress of the maturity stage, the crude protein content decreases

and the crude fiber content increases. Crude protein content depends on the number of mowing, variety, phenological stage of development (Kebede *et al.,* 2017), and plant maturity affects protein and fiber fractions in alfalfa hay (Palmonari *et al.,* 2014). Alfalfa is characterized by high nutritional value if mowing is done at an early stage (budding period) (Abdushaeva, 2020). Investigations by Stavarache *et al.,* (2015) showed that with the progression of phenological stages, from early bud to full flowering, the yield and content of crude fiber increases, while the content of crude protein decreases from 21.2-24.0% to 13.3-16.5%. The result is a lower nutritional value of alfalfa hay. Information on the quality of alfalfa is important for making decision on the profitability of a livestock production system based on feeding animals with alfalfa fodder (Arnold *et al.,* 2019). There are controversies about the optimal cutting height and its interaction with mowing time in different geographical locations and climatic conditions (Jungers *et al.,* 2020). It is considered that the optimal mowing height is from 6 to 10 cm. Mowing height had no effect on the total crude protein content, but an increase in yield and a slight decrease in nutritional value were observed when alfalfa mowing was done at a lower height (Jin *et al.,* 2021; Savoie *et al.,* 2011). As the cutting height increases, the ash content decreases (Digman *et al.,* 2013). The aim of research is to determine whether mowing height and phenological stages of maturity have an effect on the nutritional value of alfalfa hay under dry land conditions.

Material and methods

Under the dry land conditions in central Serbia in the municipality of Kruševac (430 33' 33"N; 210 12' 53"E) comparative studies of the impact of an oscillatory mower with one cutting bar IMT 627.667 (Type A), a rotary mower with drums PÖTTINGER CAT 185 (Type B) and a rotary mower with discs JF-STOLL SB 200 (Type C) on the nutritional value of alfalfa hay with an accent on the content of crude proteins, crude fiber and crude ash were performed. The mowers were tested while mowing the alfalfa variety NS Mediana ZMS V in two phases of phenological maturity: the budding phase (Phase I) and the full flowering phase (Phase II), on the same plot. In conditions of extensive production, the average yield of green mass in Phase I was 4.5 t ha^{-1}, and in Phase II 4.7 t ha^{-1}. The average yield was determined in three trials with three repetitions each by measuring the mass of alfalfa collected from one square meter across the diagonal of the plot in the swath width for each type of tested mower, calculated per area of one hectare (Al-Gaadi, 2018). All mass samples were measured using the analytical electronic scale Mettler Toledo JS1603C. Mowing of the green mass was done with a set cutting height of 6 cm, which is compatible with the recommendations of the authors (Digman *et al.,* 2013; Jin *et al.,* 2021). The working speed was determined using the chronometric method - measuring the time interval of machine travelling along the 500 m long trace. The length of trace was measured using a laser distance meter PCE LDM 50, while the chronometry was based on a digital chronometer TFA Dostmann Triple Time XL-Digitaler 3-fach Timer (Al-Gaadi, 2018). The working speed and stem cutting height was determined in three tests (three repetitions within the test). Based on the obtained parameters, the average for each trial was determined.

Using appropriate laboratory methods (AOAC, 1990), the content of crude protein, crude fiber and crude ash was determined by taking samples in three trials. For each trial, three samples were taken, from which the average sample for the given trial was finally obtained. The content of crude proteins was determined on the Kjeltec Auto 1030 apparatus, manufactured by "Tecator." The method is based on the decomposition of organic matter with concentrated sulfuric acid in the presence of a catalyst (Cu) and the titration of the resulting ammonia. The obtained values were multiplied by the coefficient 6.25. The crude fiber content was determined using the Fiber Bag-Gerhardt apparatus. The samples were treated

with a solution of sulfuric acid and sodium hydroxide. The rest is separated by filtration and weighed after drying. The method for determining crude ash is based on the decomposition of samples by annealing at 550 °C and measuring the residues. The obtained experimental data were processed using the free software package R-Statistics. Tukey's multiple range test and coefficient of variation were used to test effects of the treatments. A linear regression model was used to analyze the influence of mower working speed on alfalfa cutting height and the effect of the cutting height on the content of nutritional elements.

Results and Disscusion

The working speed of the tested mowers affect the cutting height of the alfalfa stem (Table 1). The highest cutting height according to the phenological phases was achieved by the rotary mower with discs (C) in the third trial (6.82 cm at a working speed of 11.84 km h-1 in Phase I and 6.93 cm at a working speed of 12.03 km h-1 in Phase II). The minimum cutting height was achieved by the drum mower (B) in the first trial (5.42 cm at a working speed of 8.58 km h-1 in Phase I and 5.54 cm at a working speed of 8.61 km h-1 in Phase II). No statistically significant differences was found in the cutting height by phenological stages. The obtained results are consistent with the results (Digman *et al.*, 2013; Jin *et al.*, 2021;).

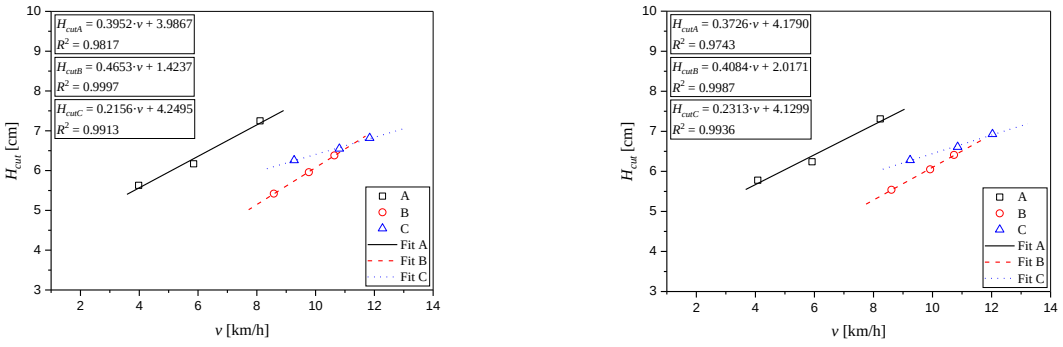
Table 1- Results of experimental measurements

Param.	Mower	Phaze I				Phaze II			
		Repetition			$\overline{X}$	Repetition			$\overline{X}$
		1	2	3	CV %	1	2	3	CV %
Yield ($t\ ha^{-1}$)		4.48	4.47	4.55		4,72	4.67	4,71	
v ($km\ h^{-1}$)	A	3.98	5.85	8.11	5.98 ± 2.07^{b}	4.08	5.92	8.23	6.08 ± 2.08^{b}
	B	8.58	9.77	10.64	9.66 ± 1.03^{a}	8.61	9.92	10.73	9.75 ± 1.07^{a}
	C	9.27	10.8	11.84	10.64 ± 1.29^{a}	9.24	10.85	12.03	10.71 ± 1.40^{a}
	CV %	-	-	-	28.05	-	-	-	27.62
Hcut (*cm*)	A	5.63	6.17	7.25	6.35 ± 0.82^{ns}	5.78	6.24	7.31	6.44 ± 0.78^{ns}
	B	5.42	5.96	6.38	5.92 ± 0.48^{ns}	5.54	6.05	6.41	6.00 ± 0.44^{ns}
	C	6.26	6.55	6.82	6.54 ± 0.28^{ns}	6.28	6.61	6.93	6.61 ± 0.32^{ns}
	CV %	-	-	-	5.07	-	-	-	4.96
CP %	A	18.51	18.76	19.47	18.91 ± 0.50^{b}	15.58	15.81	16.03	15.81 ± 0.22^{a}
	B	18.43	18.68	19.28	18.80 ± 0.44^{b}	15.34	15.37	15.88	15.53 ± 0.30^{b}
	C	18.84	19.38	19.62	19.28 ± 0.40^{a}	15.73	16.06	16.11	15.97 ± 0.21^{a}
	CV %	-	-	-	1.32	-	-	-	1.41
CF %	A	31.62	30.57	28.86	30.35 ± 1.39^{a}	35.53	34.95	34.68	35.39 ± 1.00^{ns}
	B	31.37	30.42	28.65	30.15 ± 1.38^{b}	35.41	34.83	34.43	34.89 ± 0.49^{ns}
	C	31.21	30.28	28.54	30.01 ± 1.35^{c}	35.32	34.74	34.32	34.79 ± 0.50^{ns}
	CV %	-	-	-	0.57	-	-	-	0.92
CAsh %	A	9.24	9.18	9.03	9.15 ± 0.11^{b}	8.56	8.51	8.44	8.50 ± 0.06^{b}
	B	9.28	9.22	9.07	9.19 ± 0.11^{a}	8.61	8.54	8.50	8.55 ± 0.06^{a}
	C	9.18	9.13	8.96	9.09 ± 0.12^{c}	8.53	8.48	8.42	8.48 ± 0.05^{c}
	CV %	-	-	-	0.55	-	-	-	0.42

v - working speed; Hcut - cutting heigh; CP – crude protein; CF – crude fiber; CAsh – crude ash; A - oscillatory mower with cutter bar; B – rotary mower with drums; C rotary mower eith discs; The Tukey test was applied for the influence of the type of mower (treatment average), significance level $p \leq 0.05$

The cutting height increased linearly with an increase in the working speed (Fig. 1). This increase during Phase I (Fig. 1A) was most exposed for the mower B (regression slope coefficient was b = 0.4653), and least for the mower C (b = 0.2156). The R^2 factor values show a strong dependence for all three tested mowers A = 0.9817, B = 0.9913 and C = 0.9997 respectively. During Phase II (Fig. 1B), the largest increase of the cutting height was recorded

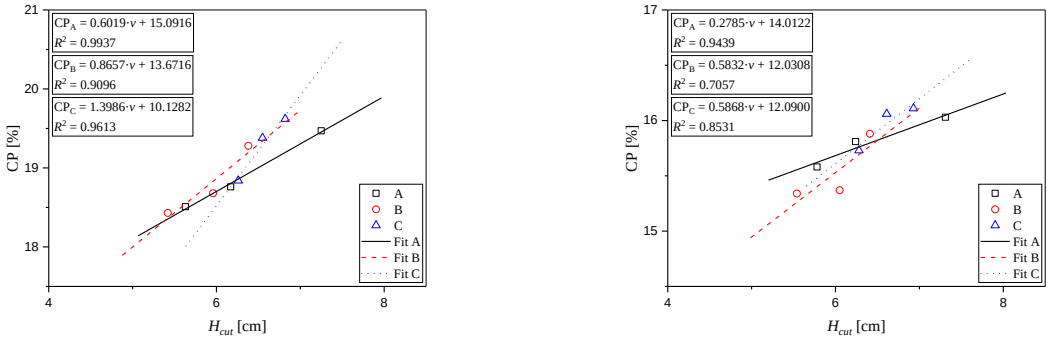
also during the operation of the mower B (b = 0.4084), and the smallest with the mower C (b = 0.2313). The value of the R^2 factor in this phase also shows a strong dependence (A = 0.9743, C = 0.9936 and B = 0.9987).



A B

Figure 1 - Effect of working speed (v) on cutting height (Hcut): (A) Phase I, (B) Phase II

The phenological stage of maturity significantly affects the crude protein content (Fig. 2). The maximum crude protein content by stages of maturity was achieved by the mower C in the third test (19.62% at a cutting height of 6.82 cm in Phase I and 16.11% at a cutting height of 6.93 cm in Phase II). The minimum crude protein was achieved by the mower B in the first trial (18.43% at a cutting height of 5.42 cm in Phase I and 15.34% at a cutting height of 5.54 cm in Phase II). Depending on the type of mower, low variability of crude proteins was determined for Phase I (CV% = 1.32) and Phase II (CV% = 1.41). However, between the mower B (18.80%) and the mower C (19.28%) for Phase I, a significant difference ($p \leq 0.05$) was found, as well as for Phase II (B - 15.53%, C - 15.97). The crude protein content increased linearly with an increase in cutting height. The lowest sensitivity during Phase I (Fig. 2A) is observed in the case of the mower A (b = 0.6019) and the mower B (b = 0.8657), and very strong in the mower C (b = 1.3986). Very strong dependence was found in all three cases A (R^2 = 0.9937), B (R^2 = 0.9096) i C (R^2 = 0.9613).

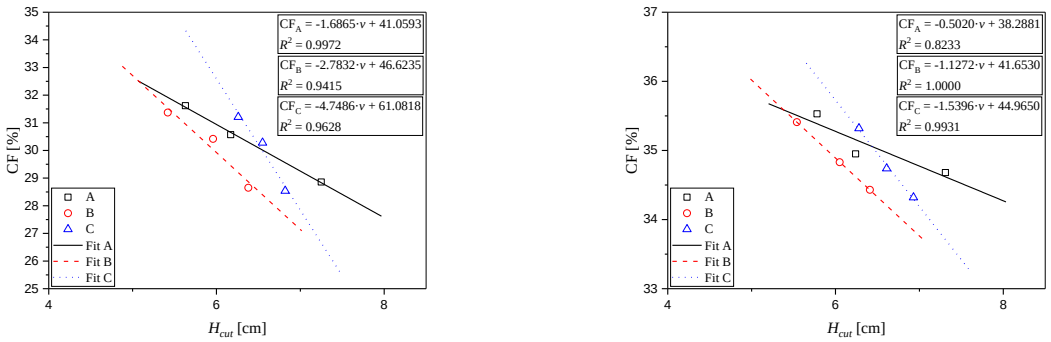


A B

Figure 2 - Effect of cutting height (Hcut) on crude protein content (CP): (A) Phase I, (B) Phase II

In Phase II (Fig. 2B), mower A (b = 0.2785) has the lowest sensitivity. For the mowers B and C, the increase is almost identical (0.5832 and 0.5868). Value of the R^2 factor shows a strong dependence for all types of mowers, B = 0.7057, C = 0.8531 and A = 0.9439. Crude protein content values in Phase I were significantly higher compared to Phase II in all mowers. The obtained results are consistent with the results of Abdushaeva (2020), Palmonari *et al.*, (2014), Sayar *et al.*, (2022), Yari *et al.*, (2012) who state who state that in the budding phase the crude protein content was 18.15 - 23.70%, and in the full flowering phase 16.2 - 19.15%.

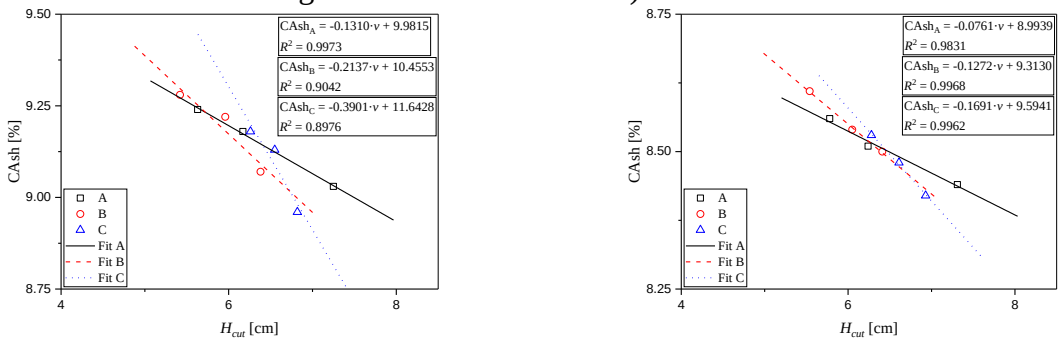
As alfalfa maturity increases (Phase II), the crude fiber content increases significantly. The maximum crude fiber content was achieved by the mower A in the first test (31.62% at a cutting height of 5.63 cm in Phase I and 35.53% at a cutting height of 5.78 cm in Phase II). The minimum crude fiber was achieved by the mower C in the third test (28.54% at a cutting height of 6.82 cm in Phase I and 34.32% at a cutting height of 6.93 cm in Phase II). The crude fiber content decreases linearly with an increase in cutting height. During Phase I, the mower A was the least sensitive to cutting height (b = -1.6865), (Fig. 3A). The mower B shows a very strong sensitivity (b = -2.7832), and the mower C an extremely strong sensitivity (b = -4.7486). This dependence is very strong for all three types of mowers, B (R^2 = 0.9415), C (R^2 = 0.9628) and A (R^2 = 0.9972). In Phase II (Fig. 3B), the smallest decline was recorded by the mower A (b = -0.5020), and significant decline by the mowers B (b = -1.1272) and C (b = -1.5396). This relationship was medium strong for the mower A (R^2 = 0.8233), and strong for the mowers C (R^2 = 0.9931) and B (R^2 = 1.0000). The achieved values match the results of Dubljević and Mitrović (2013).



A B

Figure 3 - Effect of cutting height (Hcut) on crude fiber content (CF): (A) Phase I, (B) Phase II

A high ash content indicates a low energy value of the food and soil contamination. The phenological stages of maturity did not significantly affect the crude ash content (Fig. 4). The maximum concentration was achieved by the work of the mower B in the first trial (9.28% at a cutting height of 5.42 cm in Phase I and 8.61% at a cutting height of 5.54 cm in Phase II), and the minimum by the work of the mower C in the third trial (8.96% at a height of 6.82 cm in Phase I and 8.42% at a height of 6.93 cm in Phase II).



A B

Figure 4 - Effect of cutting height (Hcut) on crude ash content (CAsh): (A) Phase I, (B) Phase II

The crude ash content decreases linearly in all tested mowers. The lowest sensitivity during Phase I (Fig. 4A) is observed during operation of the mower A (b = -0.1310), and the highest with the mower C (b = -0.3901). The numerical value of the R^2 factor shows a strong dependence in the mower A (R^2 = 0.9973) and moderately strong in the mowers C (R^2 =

0.8976) and B (R^2 = 0.9042). In Phase II (Fig. 4B), the minimum sensitivity is observed in the mower A (b = -0.0761), and higher in the mowers B (b = -0.1272) and C (b = -0.1691). Value of the R^2 factor shows a strong dependence in all types of mowers A (R^2 = 0.9831), C (R^2 = 0.9962) i B (R^2 = 0.9968). The values that we present are in line with the results of Dubljević and Mitrović (2013) and Yari *et al.*, (2012).

Conclusions

The operating speeds of the tested mowers affect the cutting height of alfalfa. The rotary mower with discs C achieved the maximum cutting height (6.82 cm at an operating speed of 11.84 km h-1 in Phase I and 6.93 cm at 12.03 km h-1 in Phase II). The minimum cutting height was achieved by the rotary mower with drums B (5.42 cm at a working speed of 8.58 km h-1 in Phase I and 5.54 cm at a speed of 8.61 km h-1 in Phase II).

The crude protein content increased linearly in stages with an increase in cutting height. It has been proven that the phenological stage of maturity significantly affects the content of crude proteins and crude fiber. The maximum crude protein content was achieved by the operation of the mower C (19.62% in Phase I and 16.11% in Phase II) with a very strong sensitivity to the increase in cutting height (b = 1.3986) in Phase I. The minimum content was achieved by the operation of the mower B (18.43% in Phase I, and 15.34% in Phase II) which shows less sensitivity (b = 0.8657).

The crude fiber content decreases linearly with an increase in cutting height, but increases significantly with increasing of alfalfa maturity. The minimum value of crude fiber was achieved by the mower C (28.54% in Phase I and 34.32% in Phase II) with an extremely strong sensitivity (b = -4.7486) in Phase I. The maximum values are recorded in the mower A (31.62% in Phase I and 35.53 % in Phase II). The phenological stages of maturity did not significantly affect the crude ash content of alfalfa hay.

The results of our study favor the mower C over the mowers A and B and recommend its use in dryland conditions for alfalfa hay preparation.

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IMPACT OF POLYETHILENE MULCH AND LOW TUNNEL ON YIELD AND WATERMELON FRUIT CHARACTERISTICS

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Abstract

The use of low tunnels and mulching both gives significant results in the early vegetable production, but mostly in warm-season vegetables which are sensitive to low temperatures. The aim of this paper was to analyze the effect of black polyethylene (PE) mulch and low tunnels on the yield and fruit characteristics. The trial was carried out during 2019 in Despotovo, Republic of Serbia on a chernozemic soil type. The experiment was set in a randomized block system with three replications. Three treatments bare soil as control, mulching, and mulching in low tunnels have been tested on three watermelon hybrids 'New Fantasy F1', 'Celine F1', and 'Top Gun F1'. The application of black PE mulch resulted in significantly higher yield per plant (average increase of 35.8%) compared to the control treatment. The highest average rind thickness was observed in the combination mulching and low tunnel (13.4 mm) which was significantly higher than control (12.2 mm). The highest percentage of flesh content was found in hybrid Top Gun F1 with mulching (66.3%), and the lowest in hybrid Celine F1 with mulching in a low tunnel and control (57.0%).

Key words: *Mulching, yield, fruit characteristics, watermelon, low tunnel.*

Introduction

Watermelon is one of the most economically important vegetable crops in the Republic of Serbia. According to FAOSTAT (2023), watermelon and melon are grown on an area of around 6233,4 ha in Serbia, with the average yields of 28.6 t/ha and total production of about 179 348 tons in last five years. Watermelon is sensitive to temperature and requires hot, dry climate with mean air daily temperatures of 22-30^{o}C (Balliau and Sallaku, 2017), The use of plastic mulch and low tunnels for watermelon production is a popular cultural practice in the Republic of Serbia (Ilin et al., 2016).

Black plastic mulch can give a harvest earlier by some 7-14 days (Dadheech et al., 2018), better quality fruit and higher yields (Spizewski et al., 2010). Earlier harvest is caused due to the higher soil temperature and the enhanced growth and development of the crop (Ban et al., 2009). Effects of mulching with black PE mulch depends on the climate and weather conditions during growing period, and should be used in early vegetable production during cool conditions of early spring (Adamović et al., 2023).

Low tunnels are a special type of row covers who modify microclimate around crop increasing air and soil temperature and provides benefits: frost protection, earliness in planting and harvesting, and season extension (Ranjan et al., 2019). They are usually used for low temperature sensitive vegetable crops and enhance plant growth and development (Soltani et al., 1995). Effects of combination mulching and low tunnel are better microclimate for the crop resulting earlier and higher yields (Soltani et al., 1995).

The aim of this paper was to analyze the effect of black polyethylene mulch and low tunnels on the yield and fruit characteristics.

Material and Methods

The trial was carried out during 2019 in Despotovo ($45^0$27' N, $19^0$31' E), Vojvodina Autonomous Province (Serbia) on a chernozemic soil type. The experiment was set in a randomized block system with three replications. Three treatments bare soil as control, mulching, and mulching in low tunnels have been tested on three watermelon hybrids New Fantasy F1, Celine F1, and Top Gun F1. The black PE film that was used for mulching in this experiment was 14 μm thick and 1.1 m wide. In parallel with the mulching a drip irrigation system was installed with a 30 cm distance between the drippers. Transparent PE foil with 14 μm thickness and 1.4 m wide was used as a covering material for the low tunnels. The supporting construction was made by wooden arches placed on 1 m distance. The planting distance was 2.0 meters between rows and among plants, with 5 plants for each treatment and each replication. A single experimental plot was 60 m^2. The transplant production was carried out in a walk-in tunnel wit supplementary heating. For this trial, the transplants were grafted on 'Emphasis F1' rootstock. The plants were transplanted in the combined treatment black PE mulch+ low tunnel on April 4th after which the low tunnel was covered, the plants under black PE mulch and the control ones were transplanted on April 30th. The cover foil was removed from the low tunnel on May 5th. The fruit harvest was carried out in physiological maturity. It earliest started in the treatment Black PE+ low tunnel, beginning from July 7th to August 2nd. Watermelons grown on black PE in open field reached full maturity 26 days later, i.e. on August 6th. During the harvesting period the following observations were made: fruit yield per plant and percentage of flesh content given by correlation between flesh mass and fruit mass. The thickness of fruit rind (mm) was determined at the equatorial axis with a caliper after cutting fruit with a knife and removing the edible part (flesh).

The average monthly temperature during the vegetation period was above the multiannual average for all the months, except for May when temperatures were lower for 2.7^{0}C than the average was recorded (Table 1). The air relative humidity was higher compared to the multiannual average, except for August when it was equal to the multiannual average. Regarding the rainfall, the sum of the monthly rainfall was 116 mm lower than the multiannual sum for that period. However, this fact did not have any influence on the examined watermelons since they were not rain-fed.

Table 1. Average air temperature, relative humidity and amount of rainfall during the vegetation period

	Year	April	May	Jun	July	August	Average
Temperature (^{0}C)	2019	13.2	14.6	23.2	22.1	23.6	19.3
	Multiannual avarage	11.8	17.3	20.1	21.9	21.6	18.5
Relative humidity (%)	2019	67	81	76	74	70	73.6
	Multiannual avarage	67	66	69	68	68	67.6
							Sum
Rainfall (mm)	2019	10.2	49.0	85.6	63.8	0.8	209.4
	Multiannual avarage	49.2	63.0	91.4	64.3	57.5	325.4

The multiannual average for the period 1981-2010

The results of the experiment were analyzed using two-factor analysis of variance (ANOVA) in the statistical software Statistica 14.

Results and Discussion

The yield per plant

Yield per plant representing total fruit mass of one plant. Average amount of yield per plant in this study is 36.6 kg (Table 2). The largest yield per plant (40.3 kg) was obtained in the mulching treatment, whereas the lowest one was (29.9 kg) recorded in the control. The application of black PE mulch resulted in significantly higher yields (average increase of 35,8%) in all three hybrids compared to the ones grown on control plot. Higher yields on mulching treatments are recorded by Soltani et al. (1995) and Dadheech et al. (2018). Higher yield per plant is a result of increased soil temperature on the root zone and the enhanced growth and development watermelon (Ban et al., 2009; Dadheech et al., 2018).

Table 2. Yield per plant (kg)

Treatments (A)	Hybrids (B)			Mean (A)
	New Fantasy F1	Celine F1	Top Gun F1	
Control	27.1 f	31.6 e	30.9 e	29.9 B
Mulching	40.3 b	42.2 a	38.3 c	40.3 A
Mulching+low tunnel	35.4 d	40.2 bc	42.8 a	39.5 A
Mean (B)	34.3 B	38.0 A	37.3 A	36.6

Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

The yield per plant was significantly higher on mulching and mulching+low tunnel treatments compared to the control. These two treatments had very similar values for yield per plant (40.3 kg and 39.5 kg), but 26 days earlier harvest were recorded on mulching+low tunnel treatment compared to only mulched plots. A little bit lower yield per plant can be result of highest temperatures below low tunnel which had a negative influence on the watermelon plants (Ibbara-Jimenez et al., 2006). Removal of the cover PE after a certain period to reduce the negative effect from the accumulated heat in the low tunnels is advice by Soltani et al. (1995).

The largest mean of average yields per plant (38.0 kg) was recorded for the 'Celine F1' hybrid and the lowest one (34.3 kg) for the New Fantasy F1 hybrid. There was no significant difference in the average yield per plant between Celine F1 and Top Gun F1, but these two hybrids had significantly higher yield per plant compared to the New Fantasy F1. New Fantasy F1 is very early hybrid and that has effect on this results which is confirmed with three-year trial by Rašković (2010).

Rind thickness

The rind thickness is an important varietal characteristic, because transportability and time storage depends on rind thickness (Milošević and Kobiljski, 2011). Average amount of rind thickness in this study is 12.7 mm. The largest average rind thickness (13.4 mm) was observed in the treatment mulching+low tunnel (Table 3) and the lowest one (12.2 mm) in the control. This is opposite with Alan et al. (2007) who reported lower rind thickness in low tunnel compared to open field. There was no significant difference between mulch treatment and bare soil. The plants grown in a low tunnel with mulching had a 9,1% higher average rind thickness compared to the ones grown on bare soil. This difference was statistically significant. This is in accordance with Fernandes et al. (2023) who reported that rind thickness depends on growing conditions and applied agrotechnology during plants vegetation.

Table 3. Rind thickness (mm)

Treatments (A)	Hybrids (B)			Mean (A)
	New Fantasy F1	Celine F1	Top Gun F1	
Control	12.8 b	12.7 bc	11.2 e	12.2 B
Mulching	13.0 ab	13.0 ab	11.8 de	12.6 B
Mulching+low tunnel	13.7 b	14.5 a	12.0 cd	13.4 A
Mean (B)	13.2 A	13.4 A	11.7 B	12.7

Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

The highest average rind thickness was measured in the hybrid Celine F1 (13.4 mm), and the thinnest rind in Top Gun F1 hybrid (11.7 mm). Recorded difference was 8,7%. There was no significant difference in rind thickness between Celine F1 and New Fantasy F1, while both had a significant higher rind thickness compared to the Top Gun F1 hybrid. Similar to this study, Tegen et al. (2021) found significant differences in rind thickness among varieties. Based on the rind thickness, Gusmini et al. (2004) divided watermelons into three groups. In the first group are hybrids with the rind thickness >19 mm, the second from 10 to 19 mm and the third <10 mm. According to this division, hybrids in this research were distributed in the second group.

Watermelon flesh content

Average amount of flesh content in this study is 60.9%. The highest average flesh content (62.9%) was measured in the watermelon fruits grown in mulching treatment (Table 4), with a statistically high difference compared to the treatments mulching+low tunnels and control. The lowest average flesh content (58.7%) was recorded in the watermelon fruits grown in low tunnels with mulching. This is in agreement with Rico et al. (2020) who reported watermelon flesh accounts for approximately 60% of the total watermelon mass. Mulching resulted in an increase of 1.7% flesh content compared to the control, and 4.2% higher flesh content compared to the mulching with low tunnel.

Table 4. Flesh content (%)

Treatments (A)	Hybrids (B)			Mean (A)
	New Fantasy F1	Celine F1	Top Gun F1	
Control	61.0 b	57.0 c	65.7 a	61.2 B
Mulching	60.7 b	61.7 b	66.3 a	62.9 A
Mulching+low tunnel	61.3 b	57.0 c	57.7 c	58.7 C
Mean (B)	61.0 B	58.6 C	63.2 A	60.9

Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

In regards to the tested hybrids, the largest flesh content (63.2%) was recorded in Top Gun F1 hybrid and the lowest one (58.6%) in Celine F1 hybrid. Top Gun F1 hybrid had significantly higher flesh content compared to other two tested hybrids. Flesh content was in range from 57.0% in hybrid Celine F1 on the control and low tunnel with mulching up to 66.3% in hybrid Top Gun F1 on mulched plot. This is in agreement with Gvozdanović-Varga et al. (2004) who reported that flesh content depends on variety and amounts 50-65% of total watermelon fruit mass.

Conclusions

Application of mulching significantly increased the fruit yield per plant and flesh content compared to the control, whereas it did not have a significant impact on the rind thickness. Mulching within low tunnel resulted in significantly higher yield per plant and rind thickness,

but the flesh content was significantly lower compared to the mulching treatment and control. A combination of mulching and low tunnel enabled earlier harvesting.

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INDOOR SMART GARDEN: A NEW ERA IN PLANT PRODUCTION

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Abstract

Indoor smart gardens are becoming increasingly popular as a way to grow plants indoors without the need for natural sunlight and soil. Consequently, this paper is aimed at providing a comprehensive overview of indoor smart gardening, its benefits, and a comparison with traditional agriculture. The paper's methodology involves desk research, where existing literature and research on indoor smart gardening and traditional agriculture will be analyzed and synthesized to provide a holistic understanding of the topic. The paper will also conduct a comparative study of indoor smart gardening and traditional agriculture, examining their respective advantages and disadvantages in terms of resource utilization, yield, and environmental impact. The research findings will provide insights into the potential of indoor smart gardening as a sustainable and efficient alternative to traditional agriculture. Our study found that the use of an indoor smart garden can result in increased plant growth and yield, as well as improved plant health and resistance to pests and diseases. The smart garden system allows for precise control of environmental factors such as light, temperature, humidity, and nutrients. The potential benefits of indoor smart gardens for sustainable agriculture and food security include reduced dependence on traditional farming methods, increased production of fresh produce, and the ability to grow crops in areas where traditional agriculture is not possible. Overall, this paper aims to educate and inform readers about the benefits of indoor smart gardening and its potential to transform the way we grow food in the future.

Keywords: *plant productions, indoor smart garden, smart agriculture, hydroponic systems.*

Introduction

Indoor smart gardens are a recent technological innovation that could revolutionize the way we grow plants. By providing an optimal growing environment within the comfort of our own homes, indoor smart gardens offer a sustainable and convenient alternative to traditional outdoor gardening. The technology development is paving the way for the automation to be made to the existing machines leading to the new technology called Internet of Things (Kuppusamy, 2016). With advancements in technology and increasing concerns about the environmental impact of food production, indoor smart gardens have become a popular solution for individuals who want to grow their own fresh produce without relying on traditional farming methods. Smart garden is an electronic control and garden monitoring system for the process of watering plants so that it can help people care for plants (Astutiningtyas et al., 2021). Namely, indoor smart gardens typically consist of a small self-contained system that provides the necessary conditions for plants to thrive. These systems often include LED growth lights, automated watering systems, and sensors that monitor temperature, humidity, and nutrient levels. Some indoor smart gardens even come equipped with features such as voice control, mobile app integration, and automatic nutrient dosing.
The benefits of indoor smart gardens extend beyond just convenience and sustainability. They also offer a way for individuals to connect with nature and experience the therapeutic benefits of gardening, even if they live in an urban environment with limited outdoor space.

Additionally, indoor smart gardens can be used to grow a wide variety of plants, from herbs and leafy greens to fruits and vegetables.
Overall, the emergence of indoor smart gardens represents a significant shift in the way we think about plant production and food sustainability. As more individuals seek out innovative solutions to reduce their carbon footprint and live a more eco-friendly lifestyle, indoor smart gardens have the potential to play a crucial role in shaping the future of food production.

Materials and methods

To conduct this study on the indoor smart garden and its potential to revolutionize plant production, a detailed methodology has been developed. The methodology includes extensive desk research, where existing literature and research on indoor smart gardening and traditional agriculture will be thoroughly analyzed and synthesized to provide a comprehensive understanding of the topic. This desk research will include academic journals, books, conference proceedings, and online resources that cover the topic of indoor smart gardening and traditional agriculture. Additionally, to fully understand the potential of indoor smart gardening, this paper will conduct a comparative study of indoor smart gardening and traditional agriculture. The comparative study will involve an analysis of their respective advantages and disadvantages in terms of resource utilization, yield, and environmental impact. This analysis will be carried out by reviewing various case studies, empirical research, and industry reports.
Moreover, the paper will also examine the current state of the indoor smart gardening industry, including the latest technologies and innovations in this field. By conducting this analysis, the paper aims to identify the key factors driving the growth of indoor smart gardening and explore the potential implications for the future of plant production.
Overall, this study seeks to provide valuable insights into the potential benefits of indoor smart gardening and how it can transform traditional agriculture practices. By comparing and contrasting these two methods, this paper aims to provide a comprehensive understanding of indoor smart gardening and its potential to revolutionize plant production.

Results and discussion

The Earth's water resources have been strained due to population growth, accelerated urbanization, and climate change. However, the implementation of wireless networks of sensors that measure soil moisture can significantly enhance the efficient utilization of water resources (Abbas et al., 2014). Indoor smart gardening and traditional agriculture are two different approaches to plant production. Traditional agriculture involves cultivating crops in outdoor fields or greenhouses, using soil and natural sunlight. In contrast, indoor smart gardening uses technology such as LED lights, hydroponic systems, and climate control to create an optimal growing environment for plants indoors. In accordance with the changing times, a garden system has emerged that makes it easy to supply the ideal temperature, humidity, sunlight, and moisture conditions to grow plants (Woo and Suh, 2021). The amount of moisture present in the soil is a critical factor that impacts the growth of plants. The level of soil moisture is determined by the quantity of water it holds, and if the soil is dry, it can impede the process of photosynthesis, ultimately leading to subpar crop yields. The Internet of things (IoT) helped creating smart garden systems to solve this problem (Ayu et al., 2022). Namely, the advent of the Internet of Things (IoT) has shown a new direction of innovative research in the agricultural domain (Ray, 2017). The smart garden system mainly composes of several elements, such as sensors, microcontrollers, LCD display, plant water sprinklers, and small water pumps (Kwok, 2015). The Internet of Things (IoT) is a network of devices

that connect to the internet and communicate with each other, allowing them to gather and share data with consumers. For those who are interested in cultivating plants but tend to forget to water them, IoT technology can be particularly useful (Sadli, 2019).
Here are some potential advantages and disadvantages of indoor smart gardening and traditional agriculture:

- Resource utilization: *1) Indoor smart gardening.* Controlled environments allow for precise resource management, reducing waste and optimizing resource use. For example, hydroponic systems use up to 90% less water than traditional soil-based agriculture. Additionally, indoor smart gardens can be located in urban areas, reducing transportation costs and emissions associated with long-distance food transportation. *2) Traditional agriculture.* Relies on natural resources such as soil, water, and sunlight, which can be unpredictable and subject to variability. However, traditional agriculture may have lower upfront costs for resources such as land and equipment. Also, one of the main difficulties faced by people is that they do not know when to water the plants and also the amount of water to be poured, resulting in the death of the plant (Srithar et al., 2021).
- Yield: *1) Indoor smart gardening.* Automatic garden monitoring and controlling is a way where all the parameters are automatically monitored without any human support (Ramya et al., 2021). Precise control over environmental conditions and plant nutrients can lead to higher yields and faster growth rates. Indoor smart gardens can also grow crops year-round, allowing for consistent production. *2) Traditional agriculture.* Yields may vary depending on weather patterns, soil quality, and other factors outside of farmers' control. However, traditional agriculture can benefit from long-established knowledge and techniques that have been developed over centuries of farming.
- Environmental impact: *1) Indoor smart gardening.* Can be more sustainable than traditional agriculture in terms of reducing water usage, fertilizer runoff, and pesticide use. Indoor smart gardens can also use renewable energy sources to power their systems. *2) Traditional agriculture.* Can have negative impacts on the environment such as soil erosion, water pollution from fertilizer and pesticide runoff, and greenhouse gas emissions from transportation and equipment use. It's worth noting that the specific advantages and disadvantages may vary depending on the specific implementation and location of both indoor smart gardening and traditional agriculture.

In the modern mechanized era, we can hardly think of any activity that doesn't require technology (Muhtasim et al., 2018). Consequently, researchers are working to integrate modern technologies in agriculture to develop new practices for the enhancement of healthy agriculture and production (Rasel et al., 2022). Namely, indoor smart gardens offer the convenience of year-round cultivation of fresh flowers, herbs, and produce. With the aid of smart technology, it is possible to create an optimal environment for any plant variety irrespective of the season. Setting up and maintaining these gardens is as simple as clicking a button from the comfort of your home or office. It is recommended to begin with a single small plant on your desk and gradually expand as you become familiar with the benefits of indoor gardening. Eventually, you may find yourself transforming your entire living space into a sprawling indoor hydroponic garden. The benefits of gardening have been emphasized to improve the quality of life (Min, Park, 2018). Consequently, what are the benefits of Indoor Smart Gardens (https://www.healthline.com)?

- It's easy: You don't need a degree in horticulture to grow an indoor garden. Smart gardens leverage advanced technology to automatically water your plants, herbs, flowers, and food on your behalf. These machines are also programmed to provide the

perfect amount of artificial sunlight. Once your indoor smart garden is setup and plugged in, you can simply relax and watch it grow.

- It's space-saving: You don't need a massive yard to grow your own food and flowers from home. Indoor smart gardens take up minimal space, and can be conveniently stationed on your desk, in your bedroom, or anywhere you'll see them and remember to monitor them. Indoor hydroponic gardens let you grow your own organic food from home, with minimal space or effort required.
- It's convenient: There's little as frustrating as toiling in the garden for weeks to nurture a plant, only to see it die out of the blue one morning. With an indoor smart garden, this is never a concern. The best indoor smart gardens leverage something called "smart soil", bringing NASA-inspired technology down to Earth. Smart soil looks like an ordinary pot of dirt, but it's been engineered to release nutrients in line with the plant's natural life cycle. Smart soil is also infused with small oxygen pockets to ensure breathing room, even if the soil it wet. This keeps the soil's pH balanced at all times, while regulating the plant's root system.
- It's natural: An indoor smart garden might look like something out of the space-age, but the fundamentals of the technology are completely natural. The food and herbs you grow with a smart garden are free from harmful substances, plus, you can relax knowing your plants are receiving nutrients automatically.
- It's spiritual: Some plants – like sage, basil, thyme, and peppermint – are considered to be more spiritual than others. But you don't need to sow the seeds of spiritual plants in particular to enjoy the infinite benefits of an indoor hydroponic garden. There's something inexplicably fulfilling about creating something out of nothing. Gardening gives you the chance to sow seeds and watch them throw into thriving plants. Not only is this a fun and relaxing activity after a long, hard day of work, but the journey also satisfies our emotional need to have an impact on our surroundings.
- It's great for your body: There are countless surprising health benefits of indoor gardening. When we feel good, we release little "happy" hormones known as endorphins. A better mood is directly linked to a faster metabolism, a healthier heart, and better brain health. There's no need to jog around the block ten times to get your heart racing and your endorphins flowing.
- It fuels mental health: Countless studies show that being in the presence of greenery is directly linked to lower instances of anxiety and depression. Gardening can also make you feel more at peace and content with life, while boosting your self-esteem and lowering your stress levels. That's what's so great about having an indoor garden. You don't need to leave your home or office to improve your memory and attention span by at least 20%! The benefits of indoor plants are backed by science, and science never lies.

Smart indoor gardens are the low maintenance gardens of the future. They offer even city apartment owners the opportunity to grow their own fresh produce, flowers, and plants in the comfort of your own home. So, whether you're looking to add a little nature to your living room or start a home-grown healthy diet, here are 5 of the best plants for smart indoor gardens (https://www.realsimple.com):

1) Grow fruits at home. Imagine a world where you wake up in the morning and pack lunch for work that contains fruit of your own labor. Well, if you have a smart indoor garden, you can grow almost any fruit your heart and taste buds desire. Become a citrus super-grower and install potted oranges, grapefruits, lemons, and limes. Or start a small strawberry or banana farm in the four corners of your very own home. When you grow fruits at home, you'll finally unlock the freshness and goodness grocery stores can't compete with.

2) Grow vegetables at home. Greens, beans, and aubergines – these are just some of the many vegetables your smart indoor garden can produce. When you decide to grow vegetables at home, you can be absolutely certain that only the best of the best will be entering your body. Growing vegetables in your smart indoor garden gives you the guarantee that no pesticides, insecticides, additives, or preservatives have contaminated your otherwise healthy produce. And best of all, having your own vegetables to cook with will fill every meal with taste-bud-bending flavor you've never experienced before.

3) Grow herbs at home. If there's one thing you should know about cooking, it's that salt and pepper are not suitable seasoning. They're standard ingredients in almost every dish. So if you're ready to ramp up the flavor on your home-cooked meals, you need a handful of herbs to help you turn up the heat. A smart indoor garden empowers you to grow herbs at home. You can find fresh lime basil to bring out other flavors, Italian herbs to spice up your pasta dishes, and even steak seasoning herbs to beef up your Sunday barbecue.

4) Grow tea leaves at home. Some of us can't live without coffee. And for some of us, a day without tea is too terrible to even think about. If you're a part of the latter, then a smart indoor garden is exactly what you need to grow tea leaves at home. As a tea lover, you know the difference between tea bags and loose-leaf tea. Equipped with an indoor garden, you'll never have to settle for a bad cup of tea again. You can grow peppermint, lemon balm, holy basil, and more. Simply wait until your leaves are fully grown and prune as much of them as needed for your next pot of tea. Best of all, your tea leaves will grow back just as beautiful, so making use of them is something you should do as often as you can.

5) Grow flowers at home. The final fresh item on the list is none other than beautiful and fragrant flowers. You see, smart indoor gardens offer more than a place to grow herbs and produce. They are the perfect indoor gardening system that enables you to add a bit of color to your living space. If you want to grow flowers at home, a smart indoor garden can help you grow pansies, petunias, gazanias, lavender, painted needles, snapdragons, and alyssum – to name a few. So, not only will your home be vibrant in color and natural texture, but you can enjoy the sweet-smelling benefit of your flowers' aromas filling the space too.

What are the limitations of an indoor smart garden?

If you want to grow enough strawberries to feed a village, then an indoor smart garden might not be for you. Hydroponic gardens offer a relatively small output of plants, making them ideal for small homes and apartments – but the benefits of indoor gardening far outweigh any limitations you might experience. Another reason why people avoid buying indoor smart gardens is that they believe refill seed pods are unaffordable. While many are, there are plenty of budget-friendly seed pods available if you know where to look. The benefits of indoor gardening are truly infinite, giving you the chance to nurture your green thumb and take control of your anxiety for good! Ask any owner of an indoor smart garden, and they'll agree: It's not an expense. It's an investment into your physical, mental, spiritual, and emotional wellbeing. The creation of a smart and robust garden through the use of simple IoT devices can encourage the development of a sustainable system that can be constructed and utilized with ease. Making this system open-source would enable everyday users to incorporate a smart garden into their households, thereby promoting sustainability and collaboration in community gardens (Penzenstadler et al., 2018). Additionally, it would encourage open-source development to improve and enhance this technology in the future.

Conclusion

Indoor smart gardens offer a promising new approach to plant production, providing a controlled environment for optimal growth and yield while reducing resource use and promoting sustainable agriculture. As technology continues to advance, it is likely that indoor smart gardens will become increasingly popular and widely used in both residential and commercial settings. Moreover, indoor smart gardens can be adapted to any location, making it possible to grow food and other crops in urban areas, further reducing the carbon footprint of transportation. However, indoor smart gardening also presents some challenges, such as high initial investment costs and the need for technical expertise to operate the system effectively. The use of artificial lighting and other energy-consuming equipment also raises concerns regarding the sustainability of the system. Overall, indoor smart gardening has the potential to revolutionize plant production and contribute to a more sustainable future. As technology continues to evolve, it is likely that we will see further advancements in indoor smart gardening, making it an even more attractive option for farmers and individuals looking to produce their own food.

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YIELDS OF NEW ONION CULTIVARS UNDER TWO SOWING METHODS

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Abstract

Onions belonging to the species *Allium cepa*, are essential biennial plants cultivated in the Autonomous Province of Vojvodina in Serbia, serving diverse roles in fresh consumption, spice production, and pickling. The comprehensive study, conducted in 2022 in Gospođinci (Province of Vojvodina), aimed to investigate the impact of various cultivars and sowing methods on onion yields. The field experiment was organized using a split-plot method with randomized treatments and replicated three times. Different onion cultivars included Elenka (standard as control), Magika, KNS 314, Lisa, and Zelda. The sowing methods involved strips of six double rows (M1) and strips of four double rows per strip (M2). Findings revealed an average yield of 69.2 t/ha for first-class bulbs, with Lisa displaying the highest mean yield of second-class bulbs (1.1 t/ha) among the cultivars. The overall onion production averaged 70.7 t/ha. These findings emphasize the significant influence of cultivar selection on onion yields, with Magika and Zelda cultivars demonstrating particularly promising results. Notably, the M2 method showcase higher first-class yields (82.7 t/ha) compared to M1 (55.7 t/ha), with a significant difference of 27.0 t/ha in favor of M2, substantiating its potential for optimizing bulb size through sowing methods. When comparing the averages of total yields, it becomes evident that M2 exhibited a remarkable superiority over M1, showcasing a substantial yield difference of 44.4%. The implications of this research extend to enhanced yields and modern onion cultivation practices, enriching agro-technical approaches in regions like Vojvodina Province.

Key words: *Sowing, cultivars, onion, yield, bulb.*

Introduction

Onion is a biennial monocotyledonous plant belonging to the *Alliaceae* family and *Allium* genus, with *Allium cepa* being the most cultivated species in the agroecological conditions of Vojvodina. Onions are essential vegetable species, and their bulbs are used fresh, processed as a spice, or pickled (Barzegar et al., 2008; Mahanthesh et al., 2008). Depending on the production goals, onions can be grown through direct seed sowing, seedlings, or sets. However, regardless of the production method, proper selection of cultivars can significantly impact the yield and quality of bulbs (Kumar et al., 2007). In modern vegetable farming practices, hybrid cultivars are commonly chosen for direct seed sowing, which have a genetic potential for yields over 100 t/ha. According of official statistics, the average yield of onions in the Republic of Serbia is 8.5 t/ha (Republic Statistical Office, 2023), while the global average is 18.4 t/ha (FAOSTAT, 2023). The low yield in our country is often a result of the mismatch between the cultivar and the level of applied agrotechonology. Therefore, it is necessary to evaluate newly developed onion cultivars and find new solution to improve their yield and quality. In a study by Kahsay et al. (2013) investigating cultivars with purple-colored dry outer leaves, they found that the Adama Red cultivar had a yield of 29.86 t/ha, while the Bombay Red cultivar yielded 34.68 t/ha. In a similar study, Abou-El-Hassan et al. (2018) reported that the Giza 20 cultivar achieved a bulb yield of 52.63 t/ha, Giza Red yielded 50.25 t/ha, and the least productive was Giza-6 with 49.23 t/ha.

This research aims to investigate the effects of two sowing methods and different cultivars on onion yields, with the goal of selecting suitable techniques and cultivars that meet modern vegetable production standards.

Material and Methods

The experiment was conducted during 2022 in the village of Gospođinci, located in the Vojvodina Province, Serbia. It was organized using a split-plot method with randomized treatments and replicated three times.

Main plot (**Factor A**) consisted of the sowing method with with the following treatments:

M1 - Onion was sown in strips with six rows (three paired rows), spaced 50 cm apart. Within each strip, there was a spacing of 35 cm between two double rows, and a spacing of 10 cm between two individual rows within the double row. The plants within a row were spaced at intervals of 4-6 cm.

M2 - Onion was sown in octuple strips, with four double rows per strip, using the same distances between strips and rows as in treatment M1.

Sub-plot (**Factor B**) included different cultivars of onion, and their details are presented in Table 1.

The experimental sub-plots, for each cultivar and replication measured 1.5 m x 6.67 m, approximately 10 m^2, and were used to determine the yields of onion.

Table 1. The tested cultivars onion in this study

Cultivars	Basic characteristics of the cultivar
Elenka (standard as control)	Dark bronze dry outer leaves. It is a medium-late hybrid, and its bulbs have a long storage capacity.
Magika	Cultivar with bronze-colored dry outer leaves. It is a medium-early hybrid, and its bulbs also have a long storage capacity.
KNS 314	Orange-bronze-colored dry outer leaves. It is an early hybrid, and it is suitable for fresh consumption or processing.
Lisa	Cultivar with purple-colored dry ourter leaves. It is a medium-early hybris, and its bulbs have a long storage capacity.
Zelda	Light purple dry outer leaves, medium-early hybrid, belongs to the groups of sweet onions with reduced allicin content (burger onion).

The applied agrotechnical practices in this experiment were carried out in accordance with the material and methods explained in Vojnović et al. (2023).

During the vegetative period, onion cultivation experienced above-average temperatures, ranging from 0.9 ^{0}C in the second decade of April to 37.8 °C in the third decade of July. The total precipitation during sowing to bulb harvesting was 462.8 mm. March had 37.4 mm less rainfall than the multi-year average, while July and August received above-average rainfall of 105.3 mm and 49.7 mm, respectively.

The data on onion yields were analyzed using two-factor analysis of variance (ANOVA) in the statistical software Statistica 14.

Results and Discussion

The yield of first-class bulbs (t/ha)

The contribution of first-class bulbs consists of healthy and undamaged bulbs with a diameter exceeding 40 mm (Off. Gaz. SFRY 29/79, 53/87). The average yield of first-class bulbs in this study amounts to 69.2 t/ha (Table 2). By observing the average A, it is noted that the M2 sowing method resulted in a yield of 82.7 t/ha, while with M1, the yield was 55.7 t/ha, with a significant difference of 48.37% in favor of M2.

Table 2. The yield of first-class bulbs (t/ha)

Cultivar (B)	Sowing Method (A)		Average (B)
	M1	M2	
Elenka (control)	58.3 c	84.6 b	71.4 B
Magika	57.8 c	97.3 a	77.5 AB
KNS 314	39.1 d	42.6 d	40.8 C
Lisa	58.1 c	93.2 ab	75.6 AB
Zelda	65.4 c	95.9 ab	80.7 A
Average (A)	55.7 B	82.7 A	69.2

M1- sowing method involved sowing in three paired rows; M2- sowing method involved sowing in four paired rows.
*Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

Depending on the interaction between factors A and B, it is observed that under M1 sowing method, the highest yield of first-class bulbs was recorded with the Zelda cultivar (65.4 t/ha), while the lowest yield was obtained with KNS 314 (39.1 t/ha), with a significant difference of 67.26% (Table 2). Abou-El-Hassan et al. (2018) reported that the Giza 20 cultivar achieved a bulb yield of 52.63 t/ha, Giza Red yielded 50.25 t/ha, and the least productive was Giza 6 with 49.23 t/ha. On the M2 treatment, the highest yield of first-class bulbs was observed with the Magika cultivar (97.3 t/ha), while the lowest yield was obtained with KNS 314 (42.6 t/ha), with a significant difference of 128.4%, Comparing these results with those of other researchers, it can be noted that the yield of first-class bulbs for the KNS 314 cultivar corresponds to the findings of Das Ratan et al. (2017), who reported an average bulb yield of 34.67 t/ha for the PBR-15-26 cultivar under agroecological conditions in India.

The yield of second-class bulbs (t/ha)

Second-class bulbs are defined as healthy and undamaged bulbs with a diameter smaller than 40 mm (Off. Gaz. SFRY 29/79, 53/87). In this study, the average yield of second-class bulbs was 1.5 t/ha (Table 3). When considering factor A, the average yield of second-class bulbs under the M2 treatment was 0.94 t/ha, while under M1 treatment, it was 2.1 t/ha, resulting in a significant difference of 123.4% in favor of the M1 treatment.

Table 3. The yield of second-class bulbs (t/ha)

Cultivar (B)	Sowing Method (A)		Average (B)
	M1	M2	
Elenka (control)	2.5 ab	0.82 c	1.6 AB
Magika	0.86 c	1.7 bc	1.3 AB
KNS 314	2.4 ab	0.33 c	1.3 AB
Lisa	1.6 bc	0.61 c	1.1 B
Zelda	3.3 a	1.2 bc	2.2 A
Average (A)	2.1 A	0.94 B	1.5

M1- sowing method involved sowing in three paired rows; M2- sowing method involved sowing in four paired rows. *Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

Comparing the averages of factor B, the Zelda cultivar exhibited the highest yield of second-class bulbs (2.2 t/ha), while Lisa cultivar had the lowest average yield (1.1 t/ha), with a significant difference of 100%. Observing the averages of the other cultivars, their yields were statistically similar, but all were significantly higher than KNS 314 in terms of the yield of second-class bulbs.

In the M1 treatment, the Zelda cultivar exhibited the highest yield of second-class bulbs (3.3 t/ha), whereas the Magika cultivar had the lowest yield (0.86 t/ha), with a significant difference of 283.72%. Similary, under the M2 sowing method, the Magika cultivar demonstrated the highest yield (1.7 t/ha), while the KNS 314 cultivar had the lowest yield (0.33 t/ha), and their difference was statistically significant according to Table 3. Second-class bulbs are primarily processed using hot or cold processing methods because their market price is significantly lower compared to first-class bulbs when sold fresh.

The total yield of bulbs (t/ha)

On average, considering factors A and B, the total yield of onions in this research amounted to 70.7 t/ha (Table 4). Comparing the averages of factor A, the M2 treatment significantly outperformed M1, with a yield difference of 44.38%. In terms of cultivars, regardless of the sowing methods, the highest yield was obtained with the Zelda cultivar (82.9 t/ha), while the lowest yield was recorded with KNS 314 (42.2 t/ha), with a statistically significant difference of 96.44% (Table 4). The lower yield of the KNS 314 cultivar can be attributed to its shorter vegetation period compared to the other cultivars. It is intended for consumption after the previous year's stored onions have been depleted, while the medium-late and late maturity cultivars are still not ready for harvest.

Table 4. The total yield of bulbs (t/ha)

Cultivar (B)	Sowing Method (A)		Average (B)
	M1	M2	
Elenka (control)	60.8 c	85.4 b	73.1 B
Magika	58.6 c	99.0 a	78.8 AB
KNS 314	41.5 d	42.9 d	42.2 C
Lisa	59.7 c	93.8 ab	76.8 AB
Zelda	68.7 c	97.7 ab	82.9 A
Average (A)	57.9 B	83.6 A	70.7

M1- sowing method involved sowing in three paired rows; M2- sowing method involved sowing in four paired rows. *Values in the column marked with the same letter do not significantly differ at a significance level of α=5%

In the interaction factors A and B, the M1 treatment exhibited the highest yield with the Zelda cultivar (68.7 t/ha), while the KNS 314 cultivar had the lowest yield (41.5 t/ha), with a statistically significant difference of 65.54% (Table 4). According to Kahsay et al. (2013), studyng cultivars with purple dry out leaves, Adama Red yielded 29.86 t/ha, whereas Bombay Red yielded 34.68 t/ha. Under the M2 treatment, the Magika cultivar showed the highest yield (99.0 t/ha), while the KNS 314 cultivar had the lowest yield (42.9 t/ha), with a statistically significant difference.

Conclusions

In the study on onions, it was found that the average yield of first-class bulbs was 69.2 t/ha, while the Lisa cultivar showed the highest average yield of second-class bulbs (1.1 t/ha) among all cultivars. The overall average total yield of onions was 70.7 t/ha. These results emphasize the significant influence of sowing method and cultivars on the yields of onions. Notably, the Magika and Zelda cultivars demonstrated exceptional yields. When comparing the averages of total yields, it becomes evident that M2 exhibited a remarkable superiority over M1, showcasing a substantial yield difference of 44.4%. The implications of this research extend to enhanced yields and modern onion cultivation practices, enriching agro-technical approaches in regions like Vojvodina Province.

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WEEDNESS AND FORAGE YIELD OF FIELD PEAS AND OATS MIXTURE CROP

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Abstract

The paper shows the influence of the mixture cultivation of field peas and oats on the degree of crop weediness and forage and hay yield. Domestic varieties, NS Junior field peas and Lovćen oats were used in the experiment. The trial variants were: field peas in monoculture, oats in monoculture, field peas and oats in the ratio 75% : 25% and field peas and oats in the ratio 50% : 50%. The determination of weed species and the determination of their abundance were carried out at a field pea height of 15-20 cm. After harvesting the crops in the optimal phase, the forage yield, hay yield, the share of hay, as well as the percentage mass share of field peas, oats and weeds in the total yield of hay were determined. A significantly higher yield of hay was recorded on all other variants compared to the pure field pea crop. In the variants with mixture crops, compared to pure crops, a lower number of weed plants per unit area was recorded in the stem growth phase. However, no significant impact of competition on the mass share of weeds at the moment of mowing was recorded, in spite of the noted presence of a tendency to decrease their.

Keywords: *mixture crop, oats, field peas, yield, weeds.*

Introduction

The mixture crops in crop production offers great opportunities both in terms of human nutrition and domestic animal nutrition. In bulk forage production systems, mixtures of annual legumes and grains have a number of advantages over pure crops. One of the most important advantages is providing the soil with nitrogen, originating from nitrogen fixation, and its utilization by non-leguminous crops. Annual legumes basically have a relatively low potential for forage yield, especially in regions with low rainfall, and their harvest is difficult due to their tendency to lie down. The complementarity of legumes and cereals in terms of utilization of agroecosystem resources is often manifested in higher yield and better forage quality of combined crops compared to pure crops (Balabanli et al., 2010). In addition, better control of weed flora, as well as biotic and abiotic stresses is achieved (Anil et al., 1998). The mechanism of action of combined sowing on weeds is such that, most often, one of the crops in the mixture enables the reduction of weeds with its competitive ability (Vandermeer et al., 1998).

The fight against weeds normally involves a series of measures aimed at reducing or completely destroying weed vegetation in cultivated crops. Considering the numerous negative effects that weed plants can have on cultivated plants, the world is increasingly searching for new methods of weed control. In order to obtain quality and health-safe food, alternatives to chemical control are sought. Most often, these are spring and cover crops, combined crops, etc. (Knežević and Ulloa, 2007). During selection, today more and more attention is paid to the development of systems in which the crops themselves would be more capable of competing with weeds for water, food and light (Mohler, 2001).

Based on previous decades of research, related to the ways of combining mixtures of cereals and grain legumes, it was determined that the yield and stability of the yield in the mixture are significantly more reliable than the same crops grown in monoculture (Raseduzzaman and Jensen, 2017). In order to reduce the competition between crops in combined sowing, a number of authors recommend that the usual sowing rate of legumes and cereal-grains should be reduced, which requires adjusting the sowing density, i.e. the number of sub-crop and over-crop plants per unit area (Smith et al., 1975; Lanini et al., 1991; Tan et al., 2004).
Independently grown field peas are weakly competitive against weeds compared to spring oats, and therefore their combination is suitable in order to obtain high-yielding crops for the production of quality forage in sustainable agricultural production systems.
The aim of the work was to analyze the impact of joint sowing of spring field peas (*Pisum sativum* L.) and spring oats (*Avena sativa* L.) on the degree of weediness and crop biomass yield, in different variants of combinations.

Material and methods

The experiment was carried out on the Ješić family farm, in the village of Gorobilje near Požega in Serbia, during the 2022 growing season in conditions without irrigation. Plowing and pre-sowing soil preparation were carried out in the spring immediately before sowing.
The experiment was set up on arable land using the randomized block system method, in three replications. The size of the elementary plot was 5 m² (5×1 m). The trial variants were: field peas in a monoculture, oats in a monoculture, field peas and oats in a ratio of 75% : 25% (G75O25) and field peas and oats in a ratio of 50% : 50% (G50O50). Domestic cultivars the NS Junior field pea and the Lovćen oat, were used for the experiment. The experiment was carried out on previously unused land. Sowing was done on April 6, 2022, by hand at an inter-row distance of 20 cm. The amount of seeds in pure sowing was 160 kg of field pea and 120 kg of oats. The amounts of seeds in the mixtures were recalculated based on the specified share. Field pea and oat were sown in the same rows.
In the year 2022, the mean annual temperature for Gorobilje was 10.82 °C, and the annual amount of precipitation was 528 mm. During the cultivation of crops, a dry period was recorded from the beginning of the third decade of April to the middle of May. In contrast, the second half of May and the first half of June were characterized by higher amounts of precipitation.
Crop emergence was registered on April 18, and the appearance of the first flowers was recorded on May 29. Crop weediness was determined when the pea was between 15 and 20 cm tall on an area of 1 m^2.
The mowing was done by hand on June 29, at the time of the milky-waxy maturity of the oats and the growth of the field pea pods. After mowing, the total yield of green biomass was measured. Green forage yield was recalculated and expressed in t ha^{-1}. Hand-mown samples of 1 kg of forage were randomly taken from each plot and weeds, peas and oats were separated from it.. The samples were dried naturally, and then the proportion of hay (%), yield of hay (t ha^{-1}) and the proportion (%) of field peas, oats and weeds in the dry sample were determined.
The results were processed by the method of one-factor analysis of variance (ANOVA) using SPSS software (1995). The significance of the differences between treatment means was tested by the LSD test.

Results and discussion

The number of weeds per unit area in the phase when the field pea was 15-20 cm high was lower on the variants with combined sowing compared to pure crops (Table 1). The greatest

divergence and abundance of weed species were recorded in the pure field pea crop. The greatest impact of the competition was recorded on the number of weed species such as lamb's quarters, bindweed, wild carrot, canadian thistle and chickweed. Similar results are indicated by other authors. Independently grown field pea is weakly competitive against weeds compared to cereals (Kadziuliene et al., 2009). Šaūranitė (2013) states that independently grown field peas compete weakly with weeds, while combined crops have the same effect as independently grown cereals. Combining, i.e. growing crops in a mixture, is a good measure for reducing the number of weeds per unit area (Dolijanović and Simić, 2015; Simić et al., 2018). Combined sowing prevented the growth and development of some weed species in the research of Davies and Welsh (2001), as well as Koocheki et al., (2009). Dolijanović et al., (2009) confirm that the weediness of combined crops is lower than that of individually grown crops.

Table 1. Overview of weed species and their abundance in a field peas and oats mixture crop

Weed species		Number of plants m^{-2}			
Species	Family	Pure pea	Pure oat	G75O25	G50O50
1. Wild radish *Raphanus raphanistrum*	Brassicaceae	4	4	4	2
2. Lamb's quarters *Chenopodium album*	Chenopodiaceae	8	5	6	4
3. Quackgrass *Agropyron repens*	Poaceae	20	4	10	8
4. Bindweed *Convolvulus arvensis*	Convolvulaceae	27	20	16	18
5. Wild carrot *Daucus carota*	Apiaceae	2	3	1	2
6. Common dandelion *Taraxacum officinale*	Asteraceae	1	1	/	/
7. Common knotgrass *Polygonum aviculare*	Polygonaceae	1	/	/	/
8. Birdeye Speedwell *Veronica persica*	Scrophulariaceae	1	/	5	/
9. Creeping Cinquefoil *Potentilla reptans*	Rosaceae	1	/	3	/
10. Curlytop knotweed *Polygonum lapathifolium*	Polygonaceae	7	3	9	2
11. Canadian thistle *Cirsium arvense*	Asteraceae	2	3	1	/
12. Chickweed *Stellaria media*	Caryophylaceae	3	13	2	6
13. Yellow Wood Sorrel *Oxalis Fontana*	Oxalidaceae	9	/	9	12
14. White clover *Trifolium repens*	Fabaceae	3	1	/	1
15. Purple Archangel *Lamium purpureum*	Lamiaceae	12	15	10	19

There were no significant differences in forage yield between the variants (Table 2). Forage yield varied from 27.0 t ha^{-1} on the variant with a pure oat crop, to 31.7 t ha^{-1} on the G75O25

variant. Contrary to these results, Krga et al., (2016) in a two-year experiment achieved a higher forage yield by growing spring field peas and oats in a mixture compared to individually grown crops, and there were no significant differences in yield between the variants with different proportions of oats. The reason for the discrepancy in these results may be due to different growing conditions. In this experiment, in April and May, which are crucial for the formation of forage yield, there was a significantly lower amount of precipitation, and the temperatures were higher compared to multi-year average, which indicates that the weather conditions were not favorable for the cultivated plants. In addition, the basic tillage of the soil was not carried out in the fall, so there was no accumulation of moisture and all the other advantages provided by autumn plowing, and the pre-sowing preparation was carried out immediately before sowing. According to Raseduzzaman and Jensen (2017), yield and yield stability in the mixture represent a more reliable way of growing than individually grown crops.

A significantly higher yield of hay was recorded in all other variants compared to the variant with pure field peas. In addition, a significantly higher yield of hay was recorded on the variant with pure oats compared to the G75O25 variant. Discrepancies in forage and hay yields are the result of different forage water content between variants. This is also confirmed by the results on the share of hay in relation to the total forage yield. The proportion of hay on the variants with mixtures of field peas and oats, as well as on the variant with a pure oat crop, was significantly higher compared to a pure field pea crop. The highest proportion of dry matter was in the pure oat crop. This is expected considering that legumes have a significantly higher proportion of water in feed compared to plants from the grass family. The results point to the fact that under the mentioned conditions, oats proved to be a more productive crop compared to field peas and more tolerant to poor growing conditions.

Table 2. Forage yield, yield hay and hay share in relation to the total forage yield of field pea and oat mixture

Treatment	Forage yield (t ha^{-1})	Hay yield (t ha^{-1})	Hay share (%)
Field pea (G) 100%	29.0 a	5.77 c	19.93 b
Oat (O) 100 %	31.7 a	9.05 a	28.61 a
G75O25	27.0 a	7.54 b	28.26 a
G50O50	31.2 a	7.92 ab	25.63 a

Values marked with different lowercase letters per column are significantly different at the P≤0,05 level according to the LSD-test

Variants did not differ significantly among themselves in terms of the mass fraction of weeds. It ranged from 9.4% in the pure oat crop to 18.7% in the G75O25 variant (Table 3). Small grains are highly competitive crops (Ćupina et al., 2010) with significantly higher co-current ability compared to annual forage legumes (Kadziuliene et al., 2009). However, their competitive ability was not expressed in the reduction of the mass share of weeds due to specific weather conditions with a small amount of precipitation at the beginning of the vegetation and a large amount of precipitation and early laying of field peas in the second part of the vegetation. In such conditions, in addition to the fact that the competition affected the reduction of the number of weed individuals per unit area, due to the later laying of crops, the remaining weeds were given space for development, and this affected their mass to be greater, and it did not significantly differ from control.

Table 3. Mass share of the components in hay (%)

Treatment	Share (%)		
	Pea	Oat	Weeds
Field pea (G) 100%	87.8 a	/	12.2 a
Oat (O) 100 %	/	90.6 a	9.4 a
G (75%) + O (25%)	52.5 b	28.8 b	18.7a
G (50%) + O (50%)	40.5 b	48.9 b	10.5 a

Values marked with different lowercase letters per column are significantly different at the P≤0,05 level according to the LSD-test

Conclusion

On the variants with combined cultivation of field peas and oats, a significantly higher yield of hay was recorded compared to the variant with a pure crop of field peas, and at the same time, there were no significant differences compared to a pure crop of oats. This phenomenon is primarily a consequence of the higher content of dry matter in the yield of oats compared to field peas.

By growing field peas and spring oat together, it is possible to achieve weed control, primarily in terms of the diversity and number of weed individuals per unit area. However, in the variants with combined crops, compared to pure crops, a significant influence of competition on the mass share of weeds was not recorded, even if such tendencies existed.

Acknowledgement

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RESEARCH OF PHOSPHORUS (P) CONTENT IN AGRICULTURAL LAND

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Abstract

Phosphorus belongs to essential and deficient elements. It participates in energy processes in plants, especially in the youngest growing parts. The lack of phosphorus in plants slows down growth, the color of the leaves changes from dark green to red-purple, and necrosis may also occur. An excess of phosphorus rarely occurs since phosphorus ions are quickly bound in the soil. Agriculture Extension Service "Sombor" researched the amount of phosphorus in agricultural land in the area of the city of Sombor. The analysis was done in a chemical laboratory using the extraction method with the help of Al solution and calorimetry. According to the obtained results of the agrochemical analysis in the area of the city of Sombor, 35.26% of the investigated lands belong to the group of lands poor in phosphorus, while 33.33% belong to the lands well supplied with phosphorus. Lands that contain from 25 to 50 mg/100 gr of land are classified as very well supplied with phosphorus, and 21.15% of them are in the area of the city of Sombor. The toxic content of phosphorus per 100 g of soil was determined at 10.26% of the examined samples, at a depth of 0 to 30 cm, while the lowest content was 1.80 mg. Also, in point 64, 322 mg of phosphorus was detected per 100 g of land. This is a toxic value that represents a potential danger for cultivated plants, both because of the high phosphorus content and because of its very negative impact on the availability of microelements for plants. The phosphorus content in the soil varies greatly and is subject to large changes.

Keywords: *phosphorus, soil, content, concentration, toxic dose, investigation.*

Introduction

Phosphorus belongs to the necessary, constitutive and deficit elements. It is important for the functioning of all living organisms because it is part of numerous organic compounds. Nucleic acids and nucleotides are certainly the most important. Phosphorus participates in energy processes in plants, especially in the youngest growing parts. Most of the phosphorus in plants is in organic forms, but a smaller part is found in inorganic forms. This phosphorus serves as a reserve for plants. The total phosphorus content does not exceed 1%, and most often it is between 0.2 and 0.3% (Kabata, 2011). Most phosphorus is found in the reproductive organs. In humans and animals, phosphorus is also a necessary element for nervous activity, normal heart muscle work, participates in the metabolism of carbohydrates, fats and proteins, has antihistamine properties, etc. Phosphorus deficiency (also causes Mg deficiency) causes a number of disorders in organisms. An excess of phosphorus is also undesirable. It leads to kidney diseases, catabolism increases, stress occurs, etc. Phosphorus absorption is supported by vitamins A, D and F, followed by Ca, Fe, Mg and proteins, while Al, excess Fe and Mg as well as the use of white sugar degrease (Kabata, 2020).

The lack of phosphorus in plants slows down growth, the color of the leaves changes from dark green to red-purple, and necrosis may also occur. The life of the leaves is shortened, the root system is poorly developed, and the whole plant becomes resistant to abiotic and biotic stresses. In the case of grain, budding is reduced, the leaves are smaller, fertilization is

weaker, grain quality is reduced, as is the yield. Phosphorus deficit limits nitrogen uptake (Sabadoš, 2019).

An excess of phosphorus rarely occurs since phosphorus ions are quickly bound in the soil. However, with an excess of phosphorus, there is a lack of Fe, Zn, Mn, Cu and B, due to which the plant also reduces the total growth, the size of individual leaves, and increases the speed of their aging, all of which affects the yield reduction (Petrović, 2010).

According to the total content in the soil, phosphorus ranks thirteenth on average. Its amounts vary from 0.03 to 0.3%, which depends on the parent substrate. It is most abundant in the arable layer, and its content decreases with depth. For the assessment of soil availability with phosphorus, its accessible forms are important. It is found in organic and mineral forms. Mineral phosphorus, which can be found in the soil solution, is more important for plant nutrition. Phosphorus is subject to circulation in nature, changing its forms and accessibility. Plants adopt it in form of the primary or secondary ion of orthophosphoric acid. The primary ion (H2PO4-) predominates in acidic conditions, and the secondary ion (HPO42-) at a pH higher than 7. When determining phosphorus, extraction is performed, i.e. converting the easily soluble part of phosphorus into a solution, and then phosphorus is determined colorimetrically. A diluted Al solution is used for extraction (Sabadoš, 2019).

Land classification based on the content of readily available phosphorus (in mg P2O5/100 g of soil): 0 to 10 mg/100 g of soil – indicates poor soil, 10 to 20 mg/100 g of land – medium-secured land, more than 20 mg/100 g of land - well-secured land (Sabadoš, 2019).

Optimum land security is considered if the content of P2O5 and K2O is between 16 and 25 mg/100 g of land. If there is more than 50 mg of phosphorus, it is necessary to pay attention to the content of microelements due to antagonism (Kabata, 2011).

More than 100 mg of phosphorus represents the toxic content of this element. According to available analyzes in Vojvodina, more than 22% of land is poor in phosphorus. The same percentage of land, however, has an excess of phosphorus.

In both cases, there are problems with mineral nutrition of plants and achieving good yields of appropriate quality (Sabadoš, 2019).

Material and Methods

In this survey of agricultural land in the area of the city of Sombor in Serbia, the content of the basic parameters of soil fertility was examined at the depths of 0 to 30 and 30 to 60 cm, while the P content was examined at the depth of 0 to 30 cm. Laboratory analyzes of the basic parameters of soil fertility and content of P, were performed in the laboratory of the Agricultural Extension Service Sombor in Sombor. Land sampling was carried out at fixed points with the determination of GPS coordinates for each point individually, cartographic basis for the area of the city of Sombor and triangulation network with a list of GPS coordinates is presented permanent points on agricultural land in the area of the city of Sombor. Determination of phosphorus was done in the agrochemical laboratory according to the Al method according to Egner-Riehm. When determining phosphorus, extraction is performed, i.e. converting the easily soluble part of phosphorus into a solution, and then phosphorus is determined calorimetrically. A diluted Al solution is used for extraction. Reagents required for analysis are: concentrated Al solution, diluted Al solution, standard solutions, 2.5% ascorbic acid, ammonium molybdate solution.

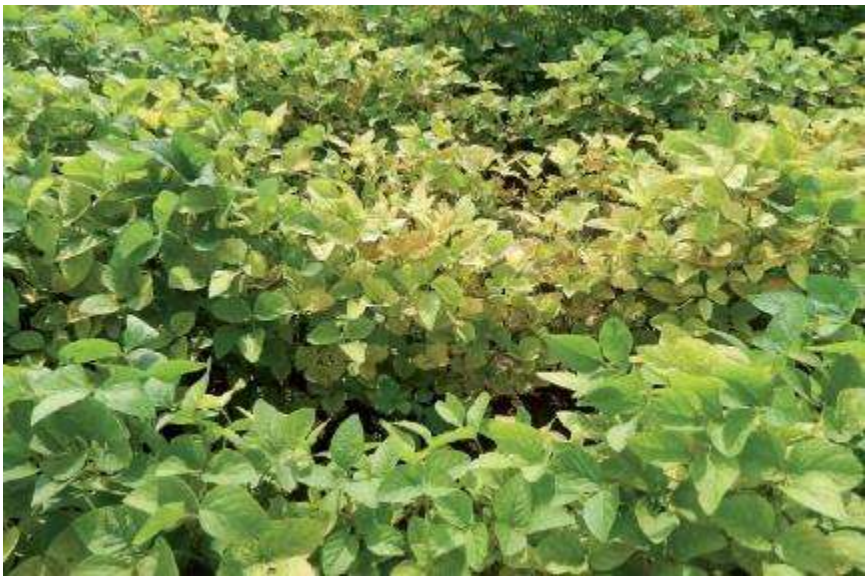
Procedure: Weigh 5 g of air-dry soil on an analytical balance, transfer to plastic bottles and add 100 ml of diluted Al solution, close tightly, put on a shaker and shake for 2 hours. After that, turn on the water bath at 90°C. Place the stand for the funnel, plastic funnels, filter paper and glasses. Measure out 2.5 g of ascorbic acid and add distilled water to the line in a normal 100 ml beaker. After 2 hours of shaking, filter the solution and since the first portions are

usually cloudy, throw them away and collect only the clear solution. Phosphorus are determined from the filtrate thus obtained.

Results and Discussion

According to the obtained results of the agrochemical analysis of agricultural land in the area of the city of Sombor, 35.26% of the examined lands belong to the group of lands poor in phosphorus, while the lands with good provided with phosphorus belongs to 33.33%. Soils containing from 25 to 50 mg/100 gr of soil are classified as very well supplied with phosphorus, and 21.15% of such soils are in the area of the city of Sombor.

The toxic content of phosphorus above 50 mg per 100 g of soil was determined in 10.26% of the examined samples, at a depth of 0 to 30 cm (graph no. 1). The lowest content of readily available phosphorus (P2O5 mg/100 gr) was determined in the territory of the cadastral municipality of Kolut, at point number 46; it was 1.80 mg per 100 g of land. The content of phosphorus (P2O5) in the soil varies greatly and is subject to large changes. It varies from plot to plot and depends a lot on the type of land and on the production technology, that is, on the applied fertilizers on a certain plot. Proof of this is the value of the content in point 64, which is located in close proximity, and which has the highest phosphorus content in the soil - 322 mg per 100 g of soil. This is a toxic value that represents a potential danger for cultivated plants, both because of the high phosphorus content and because of its very negative impact on the availability of microelements for plants. Often, the symptoms of excess phosphorus in the soil are manifested as a lack of microelements in the development of plants during the growing season.



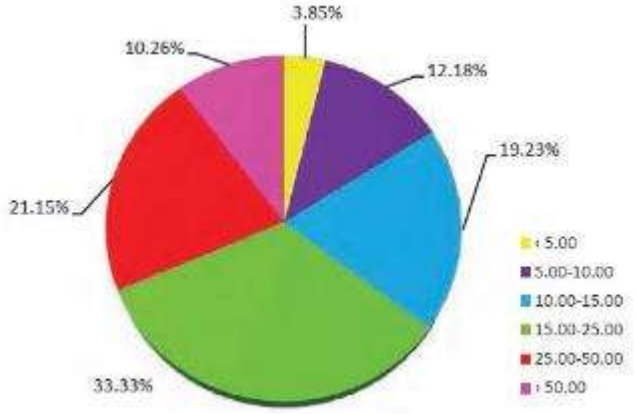
Picture number 1: Symptom of excess phosphorus above 200 mg/100gr of soil on the soybean crop (V.Sabadoš)

The average value of the phosphorus content of all tested samples in the layer from 0 to 30 cm is 27.63 mg per 100 g of soil. In the layer from 30 to 60 cm, the lowest phosphorus content was determined in the sample from point no. 46 in the area of the cadastral municipality of Kolut and amounted to 0.50 mg per 100 g of land. The highest phosphorus content is in the layer from 30 to 60 cm in the sample from point 64, which is located in the area of the settlement Bezdan. The average content of phosphorus in the layer from 30 to 60 cm is 14.76 mg per 100 g of land, which is lower than the upper layer from 0 to 30 cm by 46.57%.

These data confirm the very significant influence of man, that is, agricultural production, on the content of easily accessible phosphorus in the soil, as well as the need to omit fertilization

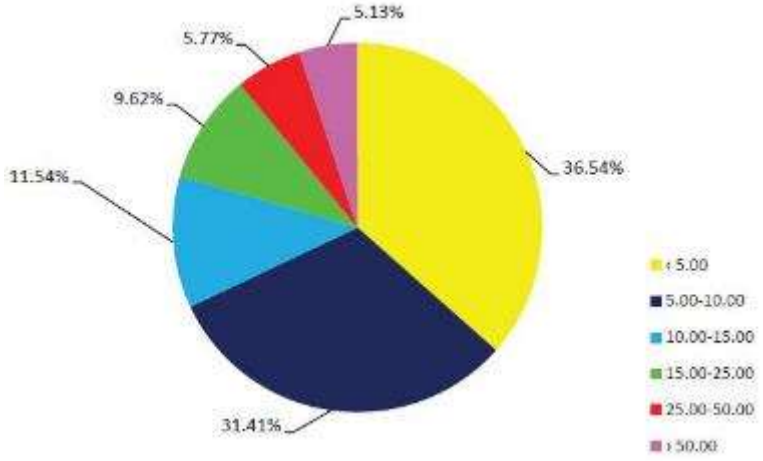
on soils where the phosphorus content is above 30 mg per 100 g of soil, in order to preserve agricultural land.
It is recommended that this be done on the basis of specific results of agrochemical soil analysis for each plot and for each crop that will be grown on a specific plot. By applying the obtained results of phosphorus content at high values, it is possible not only to reduce production costs, but also to increase the yield of cultivated plants.
A significant part of the plots in the area of the city of Sombor (35.33%) is characterized by a reduced phosphorus content in the soil. On such plots, it is necessary to fertilize with phosphorus as a regular agrotechnical measure. On plots with a content of less than 10 mg/100 gr of land, ameliorative fertilizing with phosphorus fertilizers is necessary, but this should be done only on the basis of agrochemical analysis of the agricultural land. The average phosphorus content by type of agricultural land in the city of Sombor is given in graph number 1, as well as in table number 1.
The highest average content of phosphorus in the layer from 0 to 30 cm has soils that belong to the type of chernozem cultivated (41.54 mg per 100 g of land), as well as carbonate chernozem on the loess terrace (38.00 mg per 100 g of land). The lowest average phosphorus content is in the soil type chernozem on sand (14.93 mg per 100 g of land) and in alluvial soils (17.28 mg per 100 g of land).

Graph number 1: Percentage representation of tested samples of agricultural land in the layer from 0 to 30 cm in the area of the city of Sombor, according to categories of phosphorus content (mg/100 gr of land)



In the layer of 30 to 60 cm of agricultural land in the area of the city of Sombor, the highest average content phosphorus was found in the chernozem ogajnjaceni type, namely 27.35 mg per 100 g of soil, while the lowest average content determined for agricultural land type chernozem on sand, namely 4.36 mg per 100 g of land.

Graph number 2: Percentage representation of tested samples of agricultural land in the layer from 30 to 60 cm in the area of the city of Sombor, according to categories of phosphorus content (mg/100 gr of land)



The smallest variation of the average phosphorus content in relation to both tested layers is in the soil type Rit black.

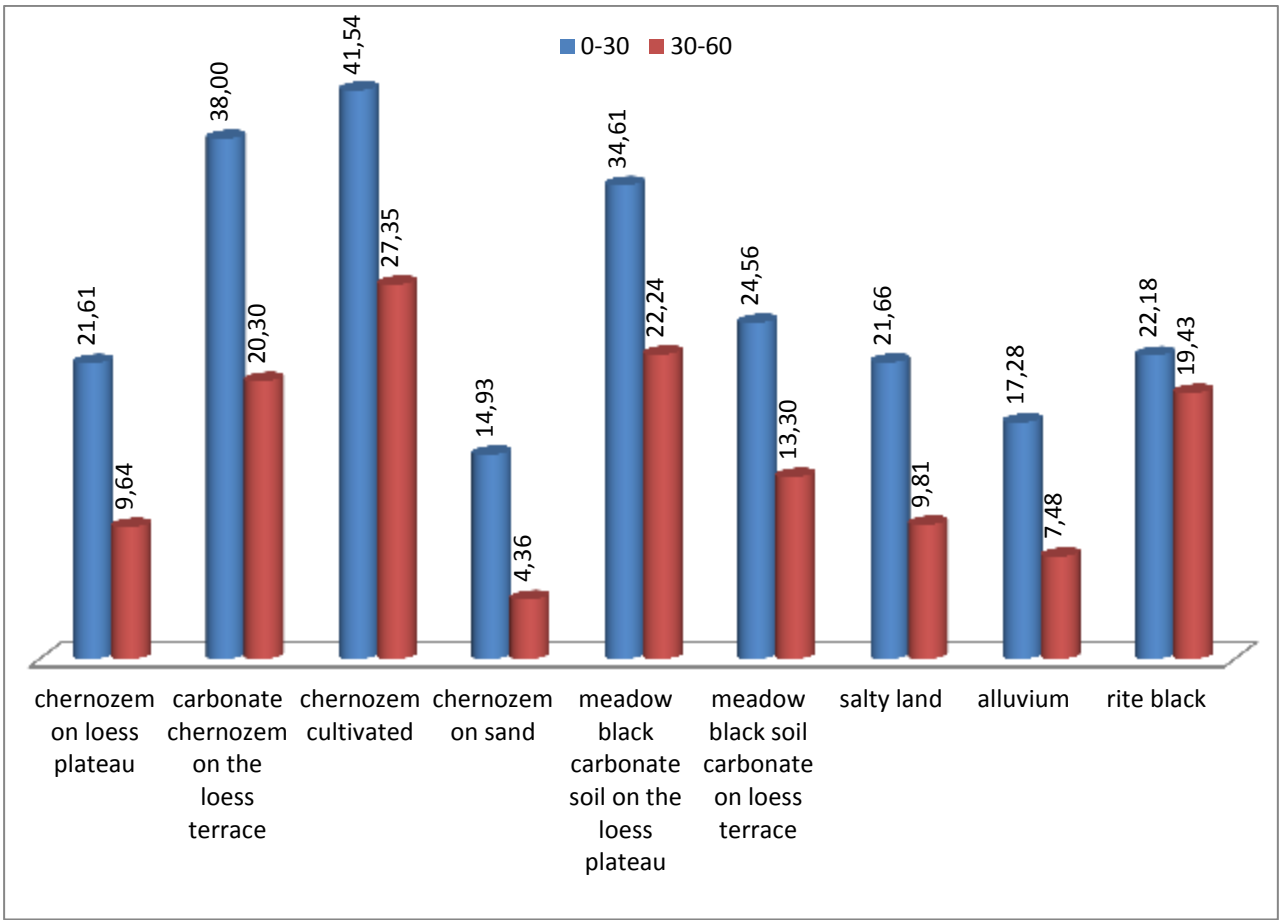
According to the method of agricultural land use, the highest average phosphorus content was found in the areas lands that are used as gardens and in both tested soil layers, from 0 to 30 cm and from 30 to 60 cm. 92.42 and 68.44 mg per 100 g of land were found, which indicates that these areas were uncontrollably harvested with high quantities of phosphorus fertilizers of mineral or organic origin.

Due to the excess of readily available phosphorus, as well as the consequences and symptoms of the impossibility of adopting microelements from the soil, on many crops bad consequences were caused. Such lands, on which vegetables are most often produced, regardless of the area, need to be controlled more often, that is, perform an agrochemical analysis of the land so as not to jeopardize the production and quality of the product.

The lowest average phosphorus content was found in forest lands at a depth of 0 to 30 cm and is 12.49 mg at 100 grams of land; the lowest phosphorus content in the layer from 30 to 60 cm was recorded in pastures.

Based on the way agricultural land is used, owned land has the highest phosphorus content of male faces, namely 34.89 mg per 100 g of land. This fact shows that the owners of the land themselves lead account of the phosphorus content on their plots. Most often they use organic fertilizers as well as mineral fertilizers and on the basis of agrochemical analyzes of the land, which were mainly carried out by the owners of agricultural land. Among tenants, the most common technology is without the use of mineral phosphorus and potassium fertilizers, a organic fertilizers are almost never used on leased plots. This was especially noticed in the last one time, for a one-year lease of both state and private land. As one of the ways of reduced of production technology on leased land, is the extension of the lease of state land by local governments and the Ministry of Agriculture, Water Management and Forestry of the Republic of Serbia. By this measure it would lease to farmers by local entities were also extended due to a more stable land market and land lease. The lowest phosphorus content in the layer from 0 to 30 cm and from 30 to 60 cm is on state land, which is rarely or never issues and it averages 12.04 mg of phosphorus per 100 g of land, while in the second layer there is 6.06 mg of phosphorus on 100 gr of land.

Graph number 3: Average values of phosphorus content in agricultural land in the area of the city of Sombor in the layer from 0 to 30 cm and from 30 to 60 cm

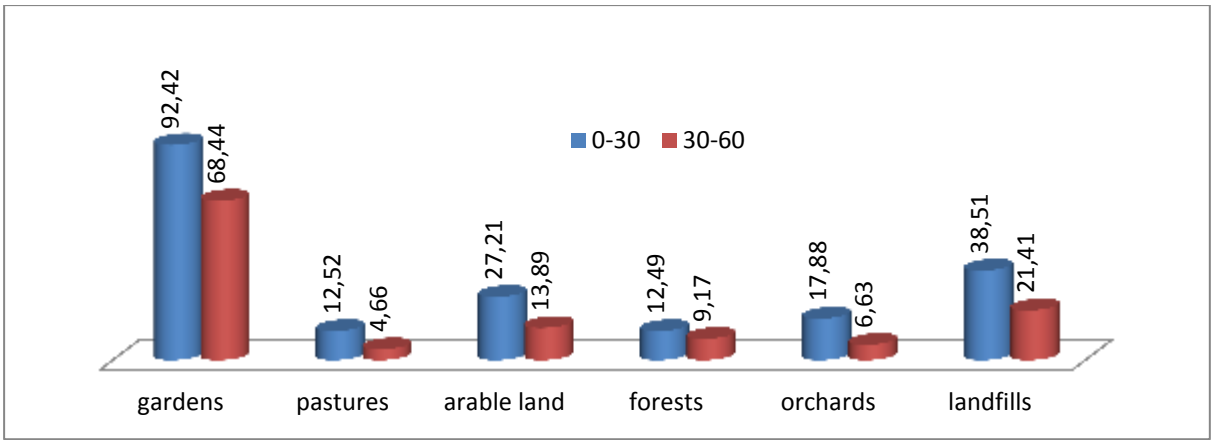


According to these data, we can see that we have the highest content of phosphorus on cultivated chernozem, 41.54 mg at a depth of 0 to 30 cm, and also at another depth of 30 to 60 cm, we have 27.35 mg, which is the highest amount on that depth.

Table number 1: Phosphorus content in agricultural land in the city of Sombor (by land type)

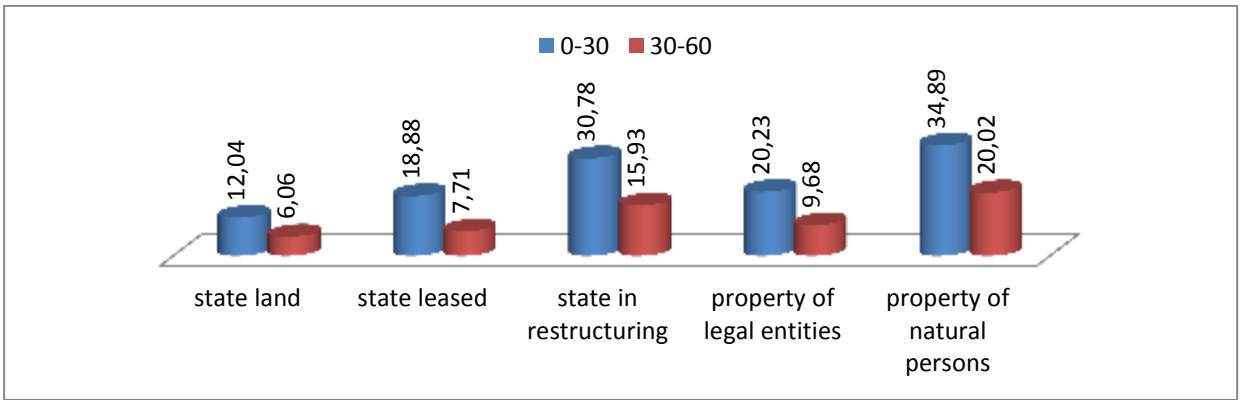
depth	min max aver	chernozem on loess plateau on loess plateau	carbonate chernozem on the loess terrace	chernozem cultivated	chernozem on sand	meadow black carbonate soil on the loess plateau	meadow black soil carbonate on loess terrace	salty land	alluvium	rite black
0-30	min	6,90	5,10	1,80	1,50	4,90	7,90	4,40	3,60	3,10
0-30	max	52,3	309	322	25,3	75,9	60,3	69,7	38,5	53,5
0-30	medium	21,61	38	41,54	9,64	28,43	24,56	21,66	17,28	22,18
0-60	min	1,50	1,10	0,50	4,60	15,5	2,20	0,90	2,00	0,60
0-60	max	46,8	213	226	25,3	75,9	69,1	42,8	21,4	43,10
0-60	medium	9,64	20,30	27,35	14,93	34,61	13,34	9,81	7,48	19,43

Graph number 4: Average values of phosphorus content by land use in the city area Sombor



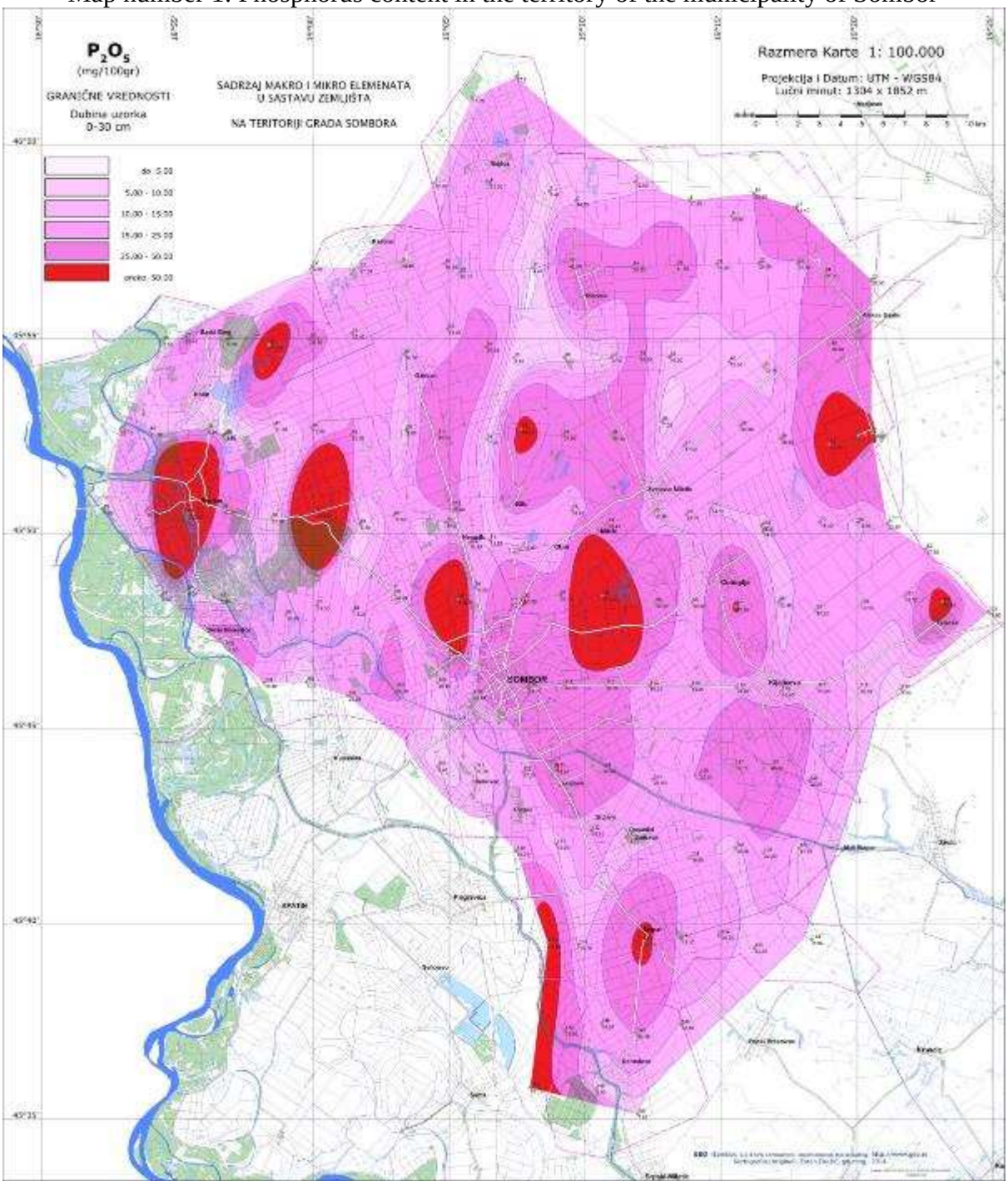
If we look at this graphic, we can see that in the garden we have 92.42 gm at the depth from 0 to 30, while at the depth from 30 to 60 we also have the highest phosphorus content of 68.44 mg.

Graph number 5: Average values of phosphorus content by land ownership



On graph number 6, we can see that the largest amount is owned by natural persons, 34.89 mg at a depth of 0-30 cm.
On the cartographic display, we can see exactly located places with the lowest and highest phosphorus content in the territory of the municipality of Sombor.

Map number 1: Phosphorus content in the territory of the municipality of Sombor



Conclusions

Phosphorus is an element that is both necessary and deficient. According to research, it is most abundant in the arable layer. For the assessment of soil availability with phosphorus, its accessible forms are important. The results of the research show that in the area of the city of Sombor, almost 36% of the examined lands belong to the group of poor phosphorus supply, while almost 34% of the land is well supplied. Also, almost 22% of the land is very well supplied with phosphorus, while 10% of the tested samples had toxic content. Based on the results, we can see that we have 56% of the tested samples with a good phosphorus content. The phosphorus content in the soil varies greatly and is subject to large changes. It changes

from plot to plot and depends a lot on the type of land and on the production technology, i.e. on the applied fertilizers. This data also speaks about the results of the analysis with a high or toxic phosphorus content of 322 mg/100 grams of soil. It is recommended to do an agrochemical analysis before agricultural production.

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ANALYSIS OF PHYLOGENETIC VARIATION THE DOMINANT MICROORGANISMS GENUS OF *STENOTROPHOMONAS MALTOPHILIA*REVEALED FROM MUSHROOM

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Summary

The aim of work was to determine the taxonomic position of dominant microorganisms in the fruitiest body of mushroom of the representatives of the genera *Stenotrophomonas* based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene. To identify the bacteria they used the analysis of the nucleotide sequence of the 16S rRNA gene. Bacterial DNA was extracted from the suspension of bacterial cells using GeneJet Genomic DNA Purification Kit (Thermo Scientific) according to the protocols of the manufacturer. Amplification of the 16S rRNA gene was performed with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3'), 1492R (5'-GGTTACCTTGTTACGACTT-3'). The taxonomic position is determined by dominant microorganisms in the fruitiest body of mushroom (strains of *Stenotrophomonas Maltophilia* 11 from the working collection of the Department of Ecobiotechnology and Biodiversity, NULES of Ukraine) based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene. The nucleotide sequence of a fragment of the gene 16S rRNA of the mentioned above strains was registered in the international database GenBank (NCBI) with number TI-2019 (1) respectively. Obtained amplicon in size of ~1500 BP was cut out from the gel and purified using GeneJet PCR Purification Kit (Thermo Scientific). The DNAconcentration was determined on a spectrophotometer DS11FX+ (DeNovix, USA). The purified PCK-product was sequenced in two directions on the device 3130 «Genetic Analyzer» (Applied Biosystems, USA) using a set of reagents «BigDye Terminator v 3.1 Cycle Sequencing Kit». Conclusions. Analysis of the isolated strain of *Stenotrophomonas Maltophilia* 11 for the similarity of the nucleotide sequences of the 16S rRNA gene revealed 99% similarity with sequences of typical representatives of the species concerned. Promising strains of *Stenotrophomonas Maltophilia* 11 can be successfully introduced in the metagenome of aboriginal groups of the substations as biological agents of microbial preparations. They can provide metabolic functions of biological systems of the *Agaricus bisporus*, and be practically valuable agents of bioprotector action, induction of systemic resistance of plants against bacterial pathogens.

Key words: *sequencing, 16S rRNA, Stenotrophomonas Maltophilia, phylogenetic identification.*

Introduction

The genus *Pseudomonas* is a data and complex heterogeneous group of organisms belonging to the family *Pseudomonanaceae.* They contain approximately 211 described species and more than 56 of which are reclassified to another genus. These genera have permanently undergone continuous taxonomic regrouping by improvements in methodologies identification of species. Organisms previously classified within the genus *Pseudomonas* (rRNA homology groups I-V) are now divided among the genera *Pseudomonas, Burkholderia, Ralstonia, Comamonas, Acidovorax, Delftia, Hyrodenophaga, Brevundimonas,*

Stenotrophomonas, and Xanthomonas (Dodds, P.N., Rathjen, J.P., 2010; Ivanova, 2015, 2018, 2019, 2020, 2021). Many identified stamps have no designated species. Commercial identification test systems do not provide definitive speciation of many of the clinically significant. In agriculture and mushroom farm situations where precise identification is important for determining optimal measures, prognosis, and appropriate infection control intervention (DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006; Hodkinson, B.P., Grice, E.A., 2015; Msimbira, L.A., Smith, D.L., 2020).

Respectively increasing environmental problems are leading to a decrease in the resistance of mushroom grown in industrial conditions to abiotic and biotic factors. Therefore, recently the development of the mushroom industry has focused on studying the ways of transmission of fungal, bacterial, and viral diseases and creating an methodology which capable of responding quickly to stress and further regulation (Msimbira, L.A., Smith, D.L., 2020).

According to the estimates of scientists emphasize that one of the reasons for the rapid spread of diseases is the low-quality processing of mushroom growing chambers, which are often infected with pathogens (Patyka, V.P., Pasichnyk L.A., Dankevych, L.A. 2014;Radchenko, O.S., 2012).

It is noteworthy that the diagnosis of viral rather than bacterial infections by molecular biological methods is more common in Ukraine. The development of highly accurate and effective test systems for the identification of mixed bacterial infections of champignons will make it possible to provide express diagnostics and successful cultivation of mushrooms (Tulivetrova, K., Ivanova, T., Patyka, M., 2020; Xhalhun, L., Kipgen, Bora, L.C., 2017)

Material and methods

Identification of *Pseudomonas* isolates by the PCR method described by H. I. Lee is carried out using a set of primers (Pt-1A, Pt-1D1). This is a specific aspect of the detection of these bacteria. The reference strain *P. tolaasii* CFBP 2068T is used as a positive control. The resulting sequences were assembled using Pregap4 from the Staden software package (Patyka, V.P., Pasichnyk L.A., Dankevych, L.A., 2014; DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L.,2006; Radchenko, O.S.,2012).

In 2000, Korean scientists developed a method of multiplex PCR analysis for the detection of *Pseudomonas tolaasii* and *Pseudomonas agarici* using PTOF/PTOR and PAGF/R23-1R primer sets.

It is worth noting that the named method is successfully used in world practice. Iranian scientists identified strains of *P. tolaasii* and *P. reactans* in different centers of mushroom cultivation using the above-mentioned method. Similar tests were also conducted in Serbia. Pathogenic samples of *P. tolaasii* (forty six samples) were isolated by REP, ERIC-PCR analysis, and Southern blotting. Scientists also determined their genotypic diversity. This helped to identify among the studied isolates those that cause the brown color of spotted mushroom disease and differ both phenotypically and genotypically **Error! Reference source not found.**.

We have selected to analyze the fruiting bodies with disease symptoms. The fruiting bodies that didn`t have symptoms according to a visual assessment and electron microscopic analysis were as control (Melnychuk, M.D., Valverde, R.A., 2001).

Isolation nucleic acids

These methodologies were carried out the detection of total and double-stranded RNAs in *Basidiomycetes* (Melnychuk, M.D., Valverde, R.A., 2001; Valverde, R.A., Guttierez, D.L., 2005; Ivanova, T., 2021).

Molecular biological method of extraction total DNA, cDNA, reverse transcription of PCR, amplification, sequencing of viral cDNA, and dsRNA were performed according to the

method described previously. Biotechnological (obtaining and subcultivation of samples of mycelium in vitro using electrophoresis in agarose and polyacrylamide gels (PAGE), determining the hydrogen index (pH) of the nutrient medium mycological (a measurement of growth).

Bacterial DNA was isolated from a suspension of bacterial cells using the GeneJet Genomic DNA Purification Kit (ThermoScientific), according to the manufacturer's protocol. Amplification of the 16S rRNA gene was performed with primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-CGGTTACCTTGTTACGACTT-3') at the following temperature: 95°C, 2 min.; 30 cycles – 95°C, 30 sec.; 55°C, 45 sec.; 72°C, 90 sec.; final elongation 72°C, 7 min. The 25 μL PCR mixture contained 12.5 μL of 2x DreamTaq PCR Master Mix (ThermoScientific), 30 μmol of each primer and 50 ng of DNA. PCR was performed on an amplifier Mastercycler Personal 5332 (Eppendorf, Germany). PCR products were separated in a 1.7% agarose gel containing 0.01% ethidium bromide. The results were visualized in UV light. The resulting amplicon measuring ~ 1500 bp cut from the gel and purified using the GeneJet PCR Purification Kit (ThermoScientific). The DNA concentration was determined on a DS-11FX + spectrophotometer (DeNovix, USA). The purified PCR product was sequenced in two directions on a Genetic Analyzer 3130 (Applied Biosystems, USA) using the BigDye Terminator v 3.1 Cycle Sequencing Kit.

The resulting nucleotide sequence was compared with GenBank database data using the NCBI Blast program (http://www.ncbi.nlm.nih.gov/blast). Phylogenetic analysis, alignment of nucleotide sequences of 16S rDNA of representatives of different species of the genera Bacillus and Phyllobacterium was performed using the program MEGA 10 [3, 6, 10]. The dendrogram of phylogenetic relationships was constructed using the Neighbor Joining method using a two-parameter Kimura model based on 1000 replicates of bootstrap analysis. The 16S rRNA gene sequences of the reference cultures of bacteria of the genera Pseudomonas were used from the GenBank database.

Statistical analysis of data

Statistical analysis of data has been carried out with the help of statistical and other computer software.

Results and discussion

The essence a bioinformative analysis which we performed to create primers specific to the nucleotide components of Pseudomonas on the mushroom is as follows. The first stage was the screening of conservative diseases of the genes that encode the disease protein of the corresponding pathogen. We screened it using genetic data (GenBank). Based on general data of known nucleotide components of microbial genomes, strictly specific conservative nucleotide components were identified. They can be used as matrices for oligonucleotide primers in the process of synthesis of specific fragments of nucleic acids.

Our monitoring of bacterial diseases on mushroom farms and fresh produce markets in Ukraine revealed the presence of brown spot symptoms. We noted different degrees of the brown coloring of A. bisporus fruiting bodies (Figure 1).

In particular, after extraction, more than one hundred bacterial isolates were obtained from symptomatic samples. Gram-negative isolates in quantity thirty-seven samples from different sources and locations were selected for pathogenicity tests. Bacterial isolates showed different degrees of color change and tissue degradation on the fruiting bodies of mushrooms, varying from light to dark brown (Fig. 1).

Fig. 1. The presence of brown spots on the fruiting bodies of the mushroom

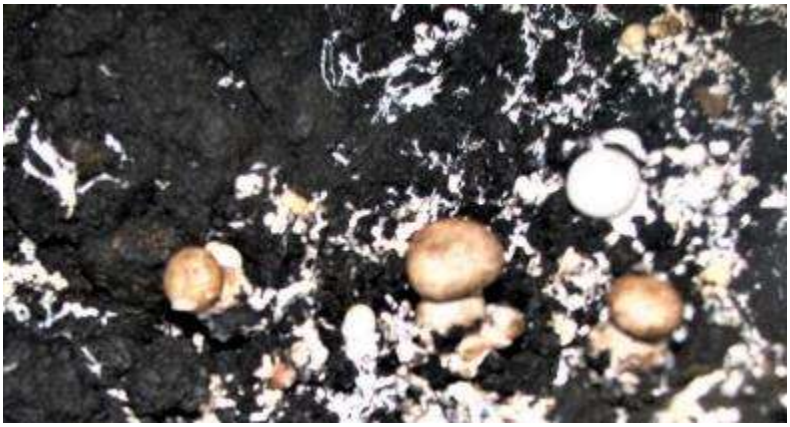

Fig.2. Changes in color and degradation of tissues on mushrooms

Bacterial isolates caused sunken brown lesions on tissue blocks of *A. bisporus* carp after 72 h. This indicates that they are caused by the reference strain *Pseudomonas fluorescens*. The rest of the isolates showed a light brown surface color on both tissue blocks and sporophores, similar to the reference strain *P. agarici*.

In figure 2 shows superficial brown lesions on *A. bisporus* caps caused by *Stenotrophomonas Maltophilia*.

According to the studied biochemical properties, all studied bacterial isolates be divided into two groups. Those exhibiting LOPAT characteristics (series of defining tests: L, levan production; O, oxidase production; P, pectinolytic activity; A, arginine dihydrolase production; and T, tobacco hypersensitivity). They also had the following biochemical properties: catalase-positive, esculin hydrolysis negative, Tween 80 hydrolysis positive, casein hydrolysis positive; negative hydrolysis of gelatin and reduction of nitrates. They used mannitol, erythritol, sorbitol, inositol, and trialose as carbon sources, but did not use sucrose, arabinose, or D(−) tartrate.

The obtained results of the analysis demonstrate the characteristics of Pseudomonas fluorescens. Identification was confirmed in PCR tests. Another group of isolates, as well as the reference strain *Stenotrophomonas Maltophilia*, showed characteristics of the LOPAT group III and did not use sorbitol, erythritol, L-arabinose, L-rhamnose, L-arabitol, -ketogluconate or D-tartrate.

The identity of these seven species was confirmed by analysis of 16S rDNA components as *P. agarici* (deposited in NCBI GenBank under accession numbers: TI-2019(1).

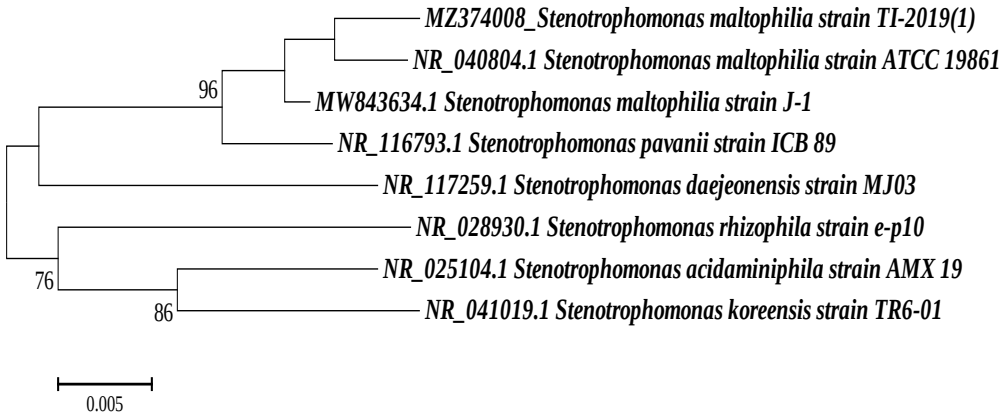

Fig. 3. Results of phylogenetic analysis of PHLF regulatory protein bacteria TI-2019 (1) in the form of dendrogram of the studied isolates of pathogenic bacteria

Moreover, the pathogenicity tests in our study also showed a difference in the change in the color of the inoculated fungus of the mushroom hat, indicating that some other fluorescent pseudomonads participated in the expression of symptoms of the disease.

On the other hand, *Stenotrophomonas Maltophilia* was discovered and identified in seven different places between 2015-2021. Further monitoring of fungal products in the sale of markets did not show repeated release of this bacterium. It is also worth noting that the typical symptoms of bacterial spotting (rust brown) were not observed in samples infected with *Stenotrophomonas Maltophilia.* The same observation was made by L.O. Cantore and Icobellis (2004) and Svetlana Milijašević- Marčić* et al. (2016) assuming the ability of the pathogen to cause different symptoms under different environmental conditions. As for the economic impact of Pseudomonas Fluorescens as a predominant bacterial pathogen in the fungal farms of Ukraine, this depends mostly on the phytosanitary measures taken in the cultivation chambers. Although small manufacturers are still prevalent, they are ready to introduce and use strict sanitary measures and new products in mushroom cultivation techniques. The next step was to carry out and apply our data from the strains received to the Genbank (Fig. 5).

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   1 tgcaggcgct aggcctacca tgcaagtcga acggcagcac aggagagctt gctctctggg
  61 tggcgagtgg cggacgggtg aggaatacat cggaatctac tctgtcgtgg gggataacgt
 121 agggaaactt acgctaatac cgcatacgac ctacgggtga aagcagggga tcttcggacc
 181 ttgcgcgatt gaatgagccg atgtcggatt agctagttgg cggggtaaag gcccaccaag
 241 gcgacgatcc gtagctggtc tgagaggatg atcagccaca ctggaactga gacacggtcc
 301 agactcctac gggaggcagc agtggggaat attggacaat gggcgcaagc ctgatccagc
 361 cataccgcgt gggtgaagaa ggccttcggg ttgtaaagcc cttttgttgg gaaagaaatc
 421 cagctggtta atacccggtt gggatgacgg tacccaaaga ataagcaccg gctaacttcg
 481 tgccagcagc cgcggtaata cgaagggtgc aagcgttact cggaattact gggcgtaaag
 541 cgtgcgtagg tggttattta agtccgttgt gaaagccctg ggctcaacct gggaactg
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Fig. 5. Fragments of nucleotide sequences of *Stenotrophomonas Maltophilia* TI-2019 (1) gene 16S Ribosomal RNA (598 NP Linear DNA)

The analysis was performed through the Multiple Sequence Alignment software. Nucleotide sequences from the genetic bank were chosen by accident, but so as to cover the as a possible area of distribution of pathogens of mushrooms in each case. The selection of primers is

designed in such a way that the annealing temperature of all oligonucleotides was within +50-55 ° C.

The taxonomic position is determined by dominant microorganisms in the fruitiest body of mushroom (strain *Stenotrophomonas Maltophilia* 11 from the working collection of the Department of ecobiotechnology and biodiversity, NULES of Ukraine) based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene. The nucleotide sequence of a fragment of the gene 16S rRNA of the mentioned above strains was registered in the international database GenBank (NCBI) with number TI-2019 (1). Obtained amplicon in size of ~1500 BP was cut out from the gel and purified using GeneJet PCR Purification Kit (Thermo Scientific). The DNAconcentration was determined on a spectrophotometer DS11FX+ (DeNovix, USA). The purified PCK-product was sequenced in two directions on the device 3130 «Genetic Analyzer» (Applied Biosystems, USA) using a set of reagents «BigDye Terminator v 3.1 Cycle Sequencing Kit».

Analysis of the isolated strains of *Stenotrophomonas Maltophilia*11 for the similarity of the nucleotide sequences of the 16S rRNA gene revealed 99% similarity with sequences of typical representatives of the species concerned. Promising strains of *Stenotrophomonas Maltophilia*11 can be successfully introduced in the metagenome of aboriginal groups of the substations as biological agents of microbial preparations. They can provide metabolic functions of biological systems of the *Agaricus bisporus*, and be practically valuable agents of bioprotector action, induction of systemic resistance of plants against bacterial pathogens.

Conclusions

The experiment was the detection and identification of *Stenotrophomonas Maltophilia* in mushroom farms in Ukraine. Most markets have been infected with Pseudomonas Fluorescens, which indicates that this pathogen was the main cause of bacterial diseases in the fungal farms of Ukraine over the last 10 years.

The results of this experiment showed that Pseudomonas fluorescens was a predominant bacterium associated with the symptoms of brown color change on A. Bisporus.

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PLOIDY STUDY OF FESTUCA TAXA IN CARPATHIAN BASIN

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Abstract

There are more than 200 *Festuca* species in the world. It is hard to identify the species of the genus of *Festuca* and determine their distributions in the Carpathian basin. Researchers generally use morphological and genetical materials for their definition. Nevertheless, most of the *Festuca* in Hungary that examined by genetical researches were cultivated individuals, and not wild ones. In this study our objective was to detect and identify the species of the *Festuca* taxon which occurring along the Danube and compare their data. Also, our aim was to clarify the taxonomic poisition of *Festuca rupicola*, *Festuca wagneri* and *Festuca tomanii*. In addition, our intension was to find out whether we could confirm the presence of *Festuca javorkae* in Hungary. To research, we collected alive specimens from five sample areas. The tests were carried out at the Institute of Genetics and Biotechnology of the Hungarian University of Agricultural and Life Sciences. The stems were placed in a row according to where we collected them and both rows and stems were numbered. A minimum of six plants per taxon were in a row. The ploidy test was conducted with a CytoFLEX flow cytometer. A total of 101 individuals were observed. Based on the present investigation, the specimens from Győrszentiván which were previously thought to be *Festuca javorkae* are hexaploids which means that they may belong to the *Festuca rupicola* species. So, the occurrence of *Festuca javorkae* in Hungary requires further investigations. The taxonomic position of the *Festuca* individuals with fibrous outer sheaths, strongly silvery and with rough leaves, collected from the Homoktövis Conservation Area was also questionable. They could have belonged to the taxon *Festuca tracyhyphylla*, but they are tetraploid and not hexaploid. Although *Festuca wagneri* and *Festuca tomanii* are morphologically different, their ploidy levels are the same, so separation based on ploidy cannot be used on them.

Keywords: *ploidy analysis, festuca, Hungary, Danube*

Introduction

More than 200 species belonging to the Festuca taxon have been described worldwide (Šmarda 2008, Šmarda and Kočí 2003, Šmarda et al. 2008). In habitats where the conditions are already too extreme for other plant species, the species of the *Festuca* taxon are the defining elements of the vegetation (Ruemmele et al., 2003, Borhidi et al., 2012), henceforth they are the key species of the Pannonian vegetation (Borhidi, 2003, Borhidi et al., 2012). From the point of view of habitat protection, *Festuca* species can represent a significant nature conservation value since they provide habitats for several protected species of insects, small mammals, and reptiles (Vinczeffy, 2004, Tasi et al., 2013, Halász et al., 2017, 2018). From the point of view of grassland management, *Festuca* species appear in most cases on grasslands with low productivity, to which category 65% of the dry grasslands in our country belong (Tasi, 2020). Their role in plant management and food production is significant, since a significant part of domestic lawn management activities takes place in the above-mentioned

plant management and vegetation zone (Tasi, 2011). Considering all these it's not easy to determine their species. Their determination is made difficult by the high degree of variability between the taxa. Initially, morphological characteristics were used to define *Festuca* species (Sent Yves, 1928, Šmarda et al., 2008). More recently, however, genetic and histological studies have shown significant, sometimes surprising results (Simon, 1992, 2000, Loureiro et al., 2007, Penksza, 2009).

It turned out that similarities or differences between species are best shown by their genetic distances and genomic differences. So far, more detailed molecular and genetic studies have been carried out in our country, mostly on economically important and cultivated Festuca species. Although there are results for natural flora taxa including domestic species (Galli et al., 2006, Bauer et al., 2003, Qiu, 2019), the information we can obtain in this area is still incomplete and further research is needed. From now on, the taxonomic delimitation of *Festuca* species is made more difficult by the environmental dependence of the defining features, the foreign fertilization nature of the majority of species, and the varied ploid level (Verseczki and Wichmann 2003, Penksza et al. 2019, 2020). Unfortunately, our domestic dry grassland associations are constantly decreasing and transforming, which can have significant effects both from an economic and nature conservation point of view. As a result, clarifying the situation of our native species and determining their distribution will become more and more important and urgent. Our research thus provides important information for knowledge and future research in this field.

Material and Methods

The individuals that form the basis of our investigations were collected from 5 locations following the line of the Danube. The living roots collected from the sampling sites in 2018 and in the case of foreign collections in 2019, The roots were planted in the Genetic Experiment Field of the Szent István University. The sampling areas and species were as follows*:* *Festuca vaginata* - Homoktövis nature reserve, *Festuca pseudovaginata* – Homoktövis nature reserve, *Festuca tomani* – Shomoktövis nature reserve, *Festuca brevipila* - Barcs, *Festuca rupicola* - Győrszentiván, *Festuca javorkae* - Csenke (Čenkov), *Festuca wagneri* – Kunbaracs. We planted at least 6 stems from each individual, where the rows and the planted individuals were numbered. These stems were also used during the morphological and genetic studies. The stems from each growing area were placed in a row, with both rows and stems numbered. For the ploid level test, we cut 100 mg of young, green, viable leaves per sample from each taxon intended for testing. We worked with a total of 101 individuals. The flow cytometric measurements were carried out at the Szeged Grain Research Institute, (Lantos et al., 2012) using the CytoFLEX Flow Cytometer (Beckman Coulter Inc., Brea, California, USA). The samples were partially prepared based on the 1989s protocol of Doležel et. al. The samples were minced into Galbraith buffer with a homogenizer and then filtered through a 20 micron filter. The samples were treated with RNase for 60 minutes and then stained with 40 microliters of 1 mg/ml Propidium iodide solution for 30 minutes at room temperature. Propidium iodide binds statiometrically to the nucleotides of the tested individual and behaves as a fluorescent. The amount of fluorescents is the same as the amount of DNA in the cell. Once inside the analyser, the given sample is placed in a tube containing a sheath fluid. The sheath fluid surrounds the sample liquid in concentric circles, causing the cells to arrive one by one in the capillaries. A laser illuminates the cells in the capillaries and built-in sensors detect scattered and reflected light. The scattered light detects the size of the cell and indicates its passage. And the light reflected at the wavelength given by fluorescence is determined by the DNA in the cell. After the computer evaluates the data, it is displayed on a graph where the x-axis measures the relative DNA content, while the y-axis measures the

number of cells that have passed through the given time. As a reference, we used the ploid analysis graph of proven diploid *Festuca vaginata* individuals collected at the Homoktövis nature reserve.

Results and Discussion

As a result of preliminary morphological and histological studies, the taxa were potentially determined and the ploidy level was also given based on the literature data. After the examination, we corrected the definitions based on the knowledge of the ploidy level and gave the names of the species as well. The instrument we used could measurably detect the G0/G1 phase of the cell cycle. This is the growth phase of the cell cycle.
In figure 1.: The ploid level of *Festuca pseudovaginata* from the Hokomtövis Nature Reserve is shown, showing a tetraploid species. The tetraploid nature of the species can be seen from the fact that the elevation (cell proliferation) measured in the G1 growth phase on the x-axis is twice as far as the diploid reference *Festuca vaginata*.

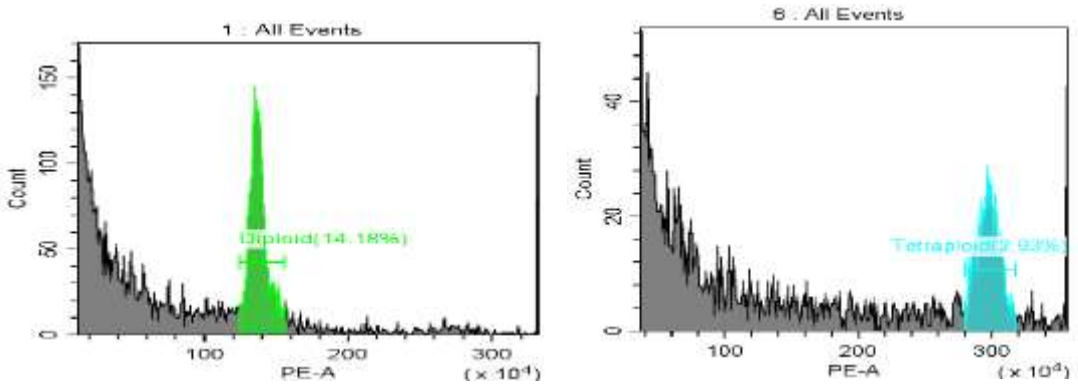

Figure 1. Comparison of the ploidy of diploid *Festuca vaginata* (Sandbuck TT) and tetraploid *Festuca pseudovina* (Homoktövis nature reserve).

In Figure 2.: *Festuca brevipila* samples from Barcs were compared with *Fesstuca vaginata* samples from diploid Homoktövis TT used as reference material. It is clear from the result that the prominence of *Festuca brevipila* is located three times as far on the graph as that of *Festuca vaginata*.

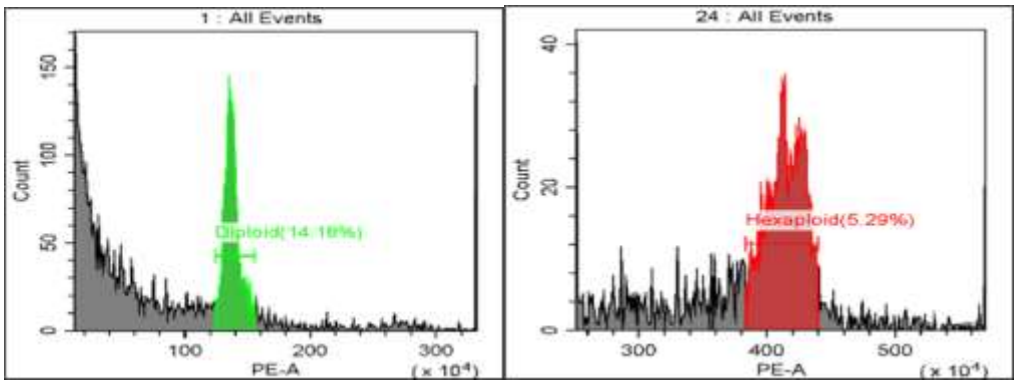

Figure 2. Comparsion of the ploidity level of the diploid *Festuca vaginata* (Homoktövis nature reserve) and *Festuca brevipila* (Barcs).

Figure 3.: shows a comparison of the ploidy level of the samples taken from the assumed *Festuca javorkae* population in Győrszentiván, The species determination was based on surveys which were based on previous morphological marks, with individuals from the locus classicus area in Csenke, Slovakia, belonging to the certified *Festuca javorkae* species, and with the reference result of *Festuca vaginata* from the diploid Homoktövis Nature reserve. The figure clearly shows that the samples from Győrszentiván are hexaploid, because the emergence stage is three times as far as the diploid *Festuca vaginata* used in the reference material. Henceforth, they differ from the tetraploid *Festuca javorkae* samples from Csenke, which stand out twice as far from the diploid reference values. Based on this, it is conceivable that the Győrszentiván species belongs to the known hexaploid *Festuca rupicola*.

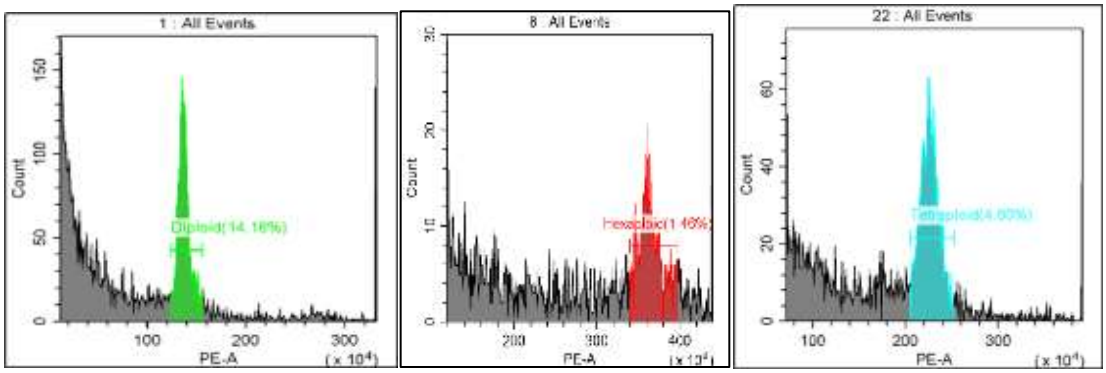

Figure 3. Comparsion of the ploidity level of the diploid *Festuca vaginata* (Homoktövis nature reserve), the hexaploid *Festuca rupicola* (Győrszentiván) and the tetraploid *Festuca javorkae* (Csenke).

The graphs shown in Figure 4 are slightly different from the others. In these figures, purple shows the location on the graph of the *Festuca vaginata* ploid test data used as reference material, while green shows the location of the *Festuca wagneri* samples collected at Kunbarac and the *Festuca tomanii* samples collected at the Homoktövis nature reserve. The figures clearly show that *Festuca wagneri* and *Festuca tomanii* are located at almost the same distance on the graph and that both are diploid, so their ploidy level is the same.

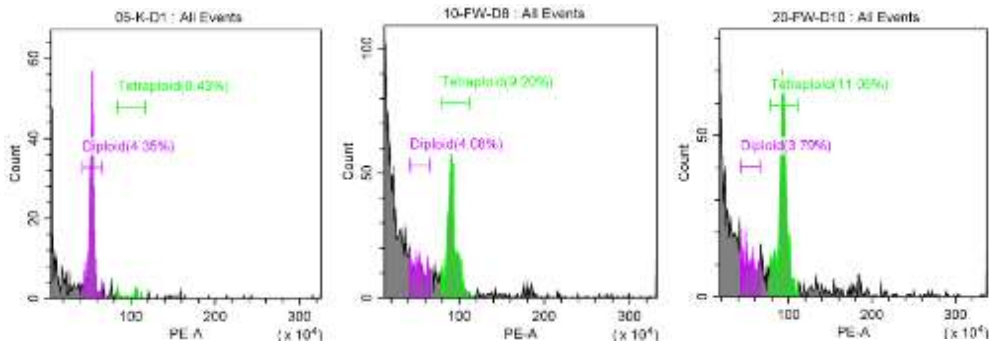

Figure 4. Compariom of the ploidity level of the diploid *Festuca vaginata* (Homoktövis nature reserve) the tetraploid *Festuca wagneri* (Kunbaracs) and the tetraploid *Festuca tomanii* (Homoktövis nature reserve).

Conclusions

The ploidy analysis of one hundred and one samples collected at four national and one foreign location yielded several new, interesting results. In the course of the present study, the national and potentially *Festuca rupicola* specimens collected near Győrszentiván were hexaploid, which confirms the mentioned assumption. On the contrary, the research rejects the possibility that the species belongs to the previously assumed *Festuca javorkae* species (Soó 1964). The national status and the occurrence of *Festuca javorkae* in Hungary requires further investigations. The affiliation of the *Festuca* individuals with fibrous outer sheaths, strongly silvery, and with rough leaves, collected from the Homoktövis Nature Reserve was also questionable. They could have belonged to the hexaploid *Festuca tracyhyphyll*a taxon, but they are tetraploids, so the mentioned assumption can be ruled out. Although *Festuca wagneri* and *tomanii* are morphologically different, their ploidy levels are the same, both species are diploid, so separation based on ploidy cannot be used for this species. In the future, therefore, a more detailed genetic determination will be necessary: for example, using and examining separation based on DNA synthesis.

Acknowledgments

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CONTROLLED-ENVIRONMENT VERTICAL FARMING IN ORNAMENTAL PLANT PRODUCTION AND APPLICATION OF A DECISION SUPPORT SYSTEM: THE EXAMPLE OF HYBRID AFRICAN VIOLET

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Abstract

Considering the sustainable use of natural resources, the decreasing agricultural lands, and the expected increase in the world population, this situation has become a problem for governments and institutions today. To overcome this emerging problem, it is necessary to prevent the waste of energy and resources. Achieving this depends on the correct and efficient use of all production parameters. A controlled-environment produces high-quality products at high production rates consistently, economically, efficiently, and sustainably. African Violet is one of the most produced and marketed plants in the global ornamental plant trade. This plant can be easily propagated and grown in closed and controlled environments. Furthermore, it is also a very suitable plant for researchers because it is a fast-growing plant that responds quickly to changes in production parameters. This study was conducted to determine the most accurate production process and cultivation parameters of hybrid African Violet in a controlled environment. For this purpose, an experimental setup was designed and created based on a controlled-environment system. A database was created by recording environmental parameters, hourly images of plant growth, and symptoms occurring during the plant's growing process. Finally, a decision support system was developed with the help of artificial intelligence, image processing, and machine learning techniques, with the support of this database with expert opinion.

Keywords: *Controlled-environment agriculture (CEA), vertical farming, IOT, computer vision,*

Introduction

In recent years, ornamental plant cultivation has increasingly become important. Ornamental plants are grown commercially in more than 50 countries, according to the World Ornamental Plants Report 2020 data, creating an economic income of more than 43 billion dollars. African violet is one of the most preferred ornamental plants due to its ease of production, easy adaptation to indoor areas, and blooming throughout the year. Hybrid African violet is obtained by crossing the different species of African Violet, leaf and flower forms have been enriched and have reached approximately 30,000 named species. Some species are registered and cataloged by independent organizations such as the AVSA (African Violet Society of America) and AVSC (African Violet Society of Canada). Many hybrid African violet species are grown by collectors in homes, offices, or greenhouses. Optimara Holtkamp Greenhouses Inc., and Lyndon Lyon Greenhouses produce hybrid African violets with a worldwide market share. For example, Optimara exceeds 10 million units and 100 varieties per year (Russell, 2007).

The hybrid African Violet is more valuable (2 or 3 times) than the standard African Violet and is also rich in visual diversity. However, the growing conditions and production costs are

the same as for the African Violet, the hybrid African Violet is more sensitive to external factors and pests. In addition, it is a plant that is very sensitive to growing parameters such as light, irrigation, nutrients, humidity, and temperature and requires special care. For this reason, it is a very suitable plant for intelligent agriculture and vertical farming applications where these parameters can be controlled continuously.

In the literature, studies on innovative farming systems have generally focused on the Internet of Things and Expert Systems. Studies on expert systems are mostly based on offline and static information that does not fully reflect the actual situation (Kang & Wang, 2017). Most of them consist of studies that collect, analyze and statistically present the available data on agricultural products and environments (Navulur et al., 2017) (Ray, 2017) (Kapoor et al., 2016) (Arnal Barbedo, 2013). Although these studies contribute to the literature in technical terms, they have some deficiencies in "Contribution to Management." Especially today, intelligent solutions such as artificial intelligence and decision support systems are demanded to enable managers to make quick decisions (Ray, 2017). The outputs obtained with smart agriculture applications using wireless sensor networks and data analytics provide summary information about the current situation to the producer (Elijah et al., 2018). However, in recent years, studies have shown that small-scale enterprises benefit from Decision Support Systems in sustainable agricultural activities to increase their productivity and improve their expertise due to financial constraints (Aiello et al., 2018). Current smart agriculture applications require advanced techniques such as artificial intelligence and image processing (Hadidi et al., 2021) (Ray, 2017) stated that the use of Decision Support Systems (DDS) in smart agriculture will significantly contribute to previous studies. (Despomer, 2010) state that any crop can be grown by a vertical farming method. However, ornamental plant applications in the global vertical farming market are limited. Due to their high profitability, most vertical agriculture production consists of vegetables that can be harvested quickly, micro vegetables, green leafy vegetables, fruits, and a small part of aromatic plants. This study proposes an intelligent farming model that will enable the hybrid African Violet to be produced in a controlled environment due to its high commercial value.

Material and Methods

This section describes the proposed controlled environment vertical farming framework for growing hybrid African violets. The framework consists of four parts for estimating the shape and leaf size of African Violet plants during the growing process.

IT (Information Technology) Infrastructure

The diagram of the controlled-environment plant production system is given in Figure 1. One computer is virtualized to be used as a server. The server has AMD Ryzen 7 5800X 8-Core Processor CPU and 64GB DDR4-RAM. A Nvidia GeForce RTX 3090 graphics card was selected for image processing. The captured plant images were stored on a 2 TB Nvme SSD disk. Different programs are needed for the controlled-environment system to achieve different purposes. For these programs to run on different machines, virtual machines have been created on the server with virtualization technology. A virtual machine for Home Assistant (HA) software; a virtual machine with the operating system Windows 10 for temporary storage of photos and remote camera control and a virtual machine with the operating system Linux Ubuntu 22.04 was created for image processing and artificial intelligence operations. The virtualization process was performed using Proxmox software as type 1. A Cuda-supported graphics card is installed on the server so that the image and artificial intelligence models can work.

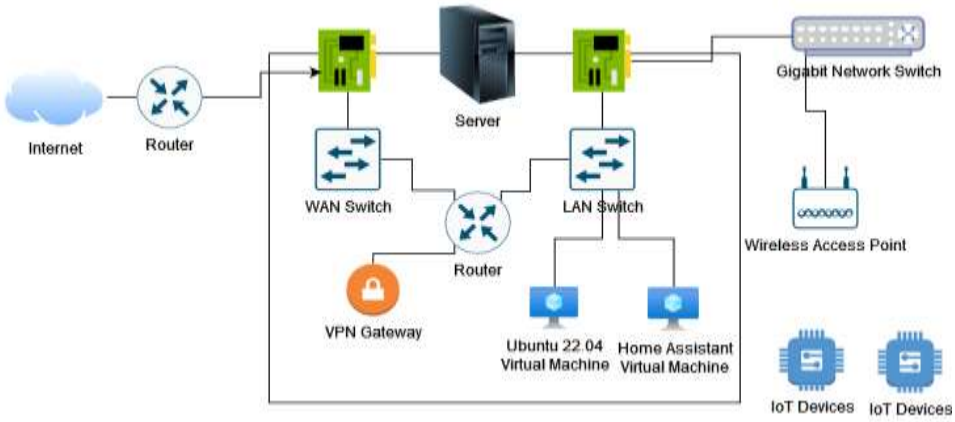

Figure 1. IT (Information Technology) Architecture

The system has two networks, WAN and LAN. Internet access is provided via WAN. A local network is created with LAN. The IP address 192.168.31.0/24 was used in the local network, and in this way the devices on the network could communicate with each other. NAT operations are performed with a router in the virtual computer to exit from the local network to the WAN network. Virtual LAN switches connect virtual machines and physical gigabit network switches to the local network. Thus, the data collected from the IoT devices on the system are transferred to virtual machines in the local network via Wireless AP

IoT Infrastructure

The IoT devices in the system are controlled by firmware software produced by the open-source software platform ESPHome. With this platform, IO controls of the devices are performed using a YAML-based configuration file. The configuration file is converted into a C++ program by ESPHome, and the prepared firmware file is sent to the device remotely via Over The Air (OTA). Changes made to the software of IoT devices are made automatically only by updating the configuration file. The ESPHome platform can work with the Home Assistant, and all API functions supported by the HA server can be used on IoT devices. Since IoT devices are recognized as devices on the HA server, it is quite easy to receive or control data from all devices with HA.

Plant Image Acquisition System

A schematic view of the system established to take pictures of the plants is given in Figure 2. Image acquisition was performed for two shelves separately. The devices used in both racks are the same. These devices consist ESP32, TB6600, a stepper motor, and two end-stop sensors. This image acquisition system uses two Canon 250D DSLR cameras and a Canon EF-S 10-18 mm f/4.5-5.6 IS STM lens. A wide-angle lens and low focusing distance are required for efficient use of shelf height. At 50 cm mounting distance, thanks to the lenses used, vertically 45 cm; horizontally 70 cm, an area can be focused. Each experimental design group consists of 9 or 12 plants. This group is displayed in a single frame with a resolution of 4000x6000px, and each image is saved in the library in JPG image format. These images are taken pictured at 1-hour intervals with a camera moving on a rail thoroughout the shelves as shown in Figure 3. An IoT-controlled stepper motor realizes the movement of the camera and an IoT device that enables the camera to take images automatically and controls the shutter. The plant pictures are stored in JPG image format in the storage unit on the virtualized server with local network integration. BME680 sensor was used to measure environmental pressure, moisture and temperature values. Also SEN0193 soil sensor was used to measure of soil moisture for each group of plants.

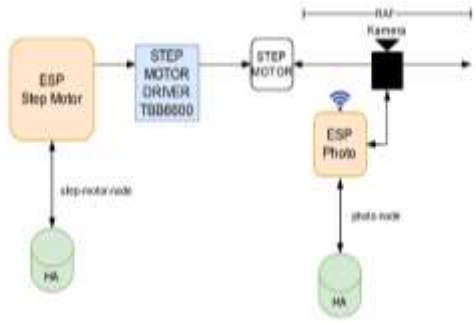

Figure 2. Schematic view of the image acquisition system

Figure 3. Real application of the system

Adaptation Cabinets

The study used plants produced by tissue culture at Sakarya University of Applied Sciences, Faculty of Agriculture, Tissue Culture Laboratory to ensure that hybrid African violet plants were of the same sizes. Afterwards, these plants were kept for one month in adaptation cabinets (Figure 3) developed for rooting and adaptation to the soil.

Figure 3. Adaptation Cabinets

Cabins operate in 16/8 hour (dark/light) periods. The fans operate for 1 min per hour in the light period and 1 min per 30 min in the dark period. The fans operate at 700 rpm and evacuate the air in the cabin and ensure the humidity in the cabin remains at the appropriate values for the development of the plant, and rooting occurs in a short time.

Plant Growing System

A 2:1 ratio of the peat-perlite mixture was prepared for the mixing process. With the prepared mixture, the plants were planted and placed in the adaptation cabinet. The adaptation cabinet was adjusted to keep the humidity range 50-70%, the exposure time 8/16 hours (light/dark), and the temperature at 24±2°C. At the end of 1 month, the plants that developed homogeneously were moved to 8 cm diameter pots with one plant in each pot. The plants ready for experiment were placed on shelves which have an area of 50x150cm annd the height between the shelves was determined to be 55cm. Two PHILIPS- LED tubes (T8 1500 mm 20W/865 G13 LED Fluorescent Bulbs) were used on the shelves. Air-conditioning has been made so that the ambient temperature of the production shelves is 24±2°C. The automation system was adjusted according to the 8/16 (light/dark) hour lighting requirement, and approximately 2500-3000 lux of light was given to the plants. The plants used for the experiment were established in 3 groups (control, 300 ppm, 600 ppm nutrient solution) in a 3x3 arrangement according to the randomized plots experimental design. Micronutrients of 20% Nitrogen, 10% Phosphorus, 20% Potassium, 0.05% Mg, 0.0068% B, 0.0036% Cu, 0.05% Fe, 0.025% Mn, 0.00009% Mo and 0.0025% Zn have in the primary stock nutrient solution prepared during the experiment. The prepared leading stock solution was adjusted to

be 300 ppm and 600 ppm according to the time of use and distilled water, and the pH values of soil were provided to be 6.5-7 pH.

Results and Discussion

This section explains the processes of analyzing the plant data collected by the image acquisition system with image processing techniques. Firstly, the plants were labeled and identified by the created model. Secondly, plant leaves were labeled, and the leaves of the plants were identified. In the third stage, the number of leaves of each plant was counted to follow the plants' development.

Plant Labeling, Model Training and Plant Detection

Data labeling is the process of correctly classifying samples in a data set, and generally, this process is performed by Domain experts. Labelers add the specified tag or category information to the dataset. This process improves the training and performance of machine learning and artificial intelligence algorithms. In this study, the collected plant image data was labeled with an open-source software tool called Labelme. Then a class called “plant” was created with the tagged plants. Screenshots of the labeled plants are given in Figure 5. The tagged data is used as input for model training. Model training was implemented with the YOLOv8. This algorithm can make fast object detection using convolutional neural networks. The YOLO algorithm borders the objects it detects on the images with a bounding box. After validating the model, the screen output of the results obtained by entering unlabeled plants into the trained model is given in Figure 6.

Figure 5. Tagged plant images

Figure 6. Trained model output

Leaf Labeling, Detecting and Counting

The leaves of African violet plants are labeled with Labelme, and a class called “leaf” has been created for the labeled leaves. A screenshot of the labeled plant leaf is given in Figure 7. After the validation of the trained model, the screen output of the results obtained by entering the unlabeled leaf data into the model is shown in Figure 8. Figure 9 shows the leaf number and positions of the plants identified by image processing. So we can find the number of leaves and size of each plant over time. In this way, changes in the number of nutrients given to the plant can be monitored.

Figure 7. Labeled leaf image

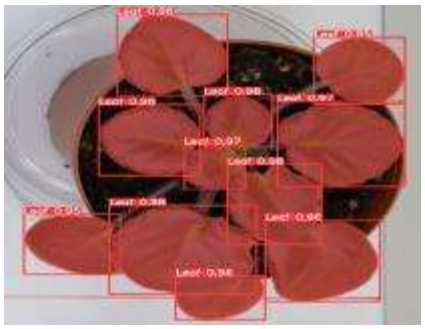

Figure 8. Leaf output obtained with the trained model.

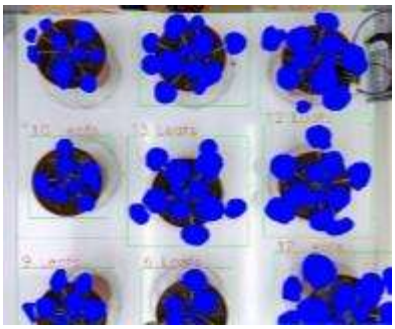

Figure 9. Counting of Plant Leaf Numbers

Conclusions

Vertical farming practices are widely used especially in the cultivation of products such as lettuce and parsley. In outdoor ornamental plant cultivation, production is usually carried out in large soil areas. African violet is a plant used as an ornamental plant. This plant has growing difficulties compared to other outdoor plants. In this study, an environment-controlled imaging system was designed to develop this plant in a vertical farming environment and to monitor its growth. The environmental control of the system is managed by IoT devices. Growth control is provided by taking hourly images of the plants with two cameras. As a future study, we are going to analysis the leaves for diseases detection of the plants. In addition, the training data set will be enlarged with the plant images taken by the system. We will use this data to improve the performance of the system.

Acknowledgement

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PLANT PROTECTION AND FOOD SAFETY

SCLEROTINIA MINOR AS A NEW PATHOGEN OF LETTUCE IN SERBIA

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Abstract

Lettuce (*Lactuca sativa* L.) is the world's most popular and widely grown leafy vegetable. *Fungal pathogens* are a *major threat for lettuce producers and can cause significant* losses under favorable conditions. During February 2021 and 2022, poor growth, plant stunning, and blanching of the leaves of lettuce, cultivated in unheated plastic tunnels located in three different regions of Serbia, were observed. From diseased plants, originating from all three locations, 31 morphologically identical isolates were derived. Morphological features of three representative isolates were investigated after 7-day incubation at 20ºC on potato dextrose agar (PDA), water agar, Czapek agar, V8 juice agar, and malt extract agar media, while the effect of different temperatures (4, 10, 15, 20, 22, 25, 30, and 32°C) on fungal growth, was studied on PDA. The isolates had fast-growing, white, cottony aerial mycelium, producing profuse number of small, black, irregularly shaped sclerotia, less than 2 mm in diameter. Based on morphological features, the isolates were preliminary identified as *Sclerotinia minor*, the causal agent of a lettuce drop disease. To confirm the species identity, a species specific pair of primers, SMLcc2F/SMLcc2R, was used for molecular detection. Pathogenicity tests were conducted by inoculation of 55-day-old lettuce plants. The isolates caused typical lettuce drop symptoms on inoculated plants after 7–10 days of incubation, whereas the control plants remained symptomless. Since *S. minor* is recently detected in Serbia, more research is needed to better understand this pathogen, establish control strategies and minimize the spread of the pathogen to other lettuce-producing areas.

Keywords: *lettuce drop, Sclerotinia disease, soilborne pathogens, identification*

Introduction

Lettuce (*Lactuca sativa* L.) is the most popular and widely grown leafy vegetable. Fungal pathogens are major threat for producers and can cause significant losses under favourable conditions. Sclerotinia lettuce drop, caused by two closely related soilborne species of the genus Sclerotinia, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* Jagger, is one of the most economically damaging diseases of this crop (Mamo et al., 2021). These pathogens can infect all lettuce types, particularly the leaf, romaine, and head lettuce types (Patterson and Grogan, 1985). The most typical symptoms of disease are watery soft rot accompanied by appearance of white, fluffy mycelial growth. As the disease develops, the host suffers extensive tissue maceration, girdling of the stem near the ground, wilting of leaves, and collapse within a few days after infection (Subbarao, 1998). Afterward, the pathogens may survive as active mycelia within– infected or dead host plants and on adjacent soil surfaces (Burgess and Hepworth, 1996). Over time, the fungus produces hard, black survival structures called sclerotia, which can persist in the soil for several years (Boland and Hall, 1988). The most common Sclerotinia disease in Serbia is Sclerotinia wilt, caused by *S. sclerotiorum*. It occurs on sunflower and many other field crops and in some years the incidence may exceed even 50% (Marić et al., 1988). Information about the damage and distribution of diseases caused by Sclerotinia species, especially on leafy vegetables, is limited (Živković et al., 2016). *S. minor* was recently detected in Serbia (Mihajlović et al.,

2022) and, to our knowledge, there is no other literature data about lettuce drop caused by this pathogen in Serbia. More research is needed to better understand biology of pathogen, establish control strategies, and minimize the spread of the pathogen to the other lettuce-producing areas. Therefore, the objective of this study was to characterize the causal agent of this new Sclerotinia disease in lettuce using morphological and molecular methods.

Material and methods

Over the winter of two successive years (2021 and 2022), poor growth, plant stunning, and blanching of the leaves of lettuce plants cultivated under unheated plastic tunnels in three different regions of Serbia were observed. The crowns of the infected plants were softened by spreading decaying lesions covered with white mycelium, particularly on the leaves near the soil surface. In order to identify the causal agent, symptomatic tissues from diseased plants were cut into small pieces, surface sterilized with 70% ethanol for 1 min, rinsed three times in sterile distilled water, and placed on potato dextrose agar (PDA) for four days at 20°C. From the developed colonies mono-hyphal-tip isolates were derived and used for pathogenicity tests.

To confirm the pathogenicity of obtained isolates, artificial inoculation of 55-day-old lettuce plants cv. 'Majska kraljica', grown on commercial growth substrate in 1 L pots, was conducted. Mycelial plugs, 5 mm in diameter, were cut from the margin of 7-day old colony grown on PDA and placed mycelium-side down on undamaged ground-level leaves of lettuce plants. Two plugs per isolate were placed onto five plants each, for a total of ten replicates per isolate. Negative control plants were inoculated similarly with sterile PDA plugs. Inoculated plants were covered with transparent plastic bags, sprayed with water (under the plastic) twice a day for 3 to 5 days to maintain high humidity, and kept in a growth chamber at 20°C (13 h light). Inoculated plants were inspected daily for the occurrence of symptoms. After the appearance of disease symptoms in the inoculated plants, the pathogen was reisolated on PDA. Then, the morphological features of the isolated fungi were compared and matched with the original ones used for inoculation.

Morphological characteristics of the selected isolates were investigated 3 days after incubation on PDA medium at 20ºC in darkness, as described by Morral et al. (1972). The following parameters were observed: colony colour, colony growth and texture. The presence of sclerotia, size, shape, number, and pattern of sclerotia formation was observed after the incubation of 15 days.

Mycelial growth rate of the isolates was determined on PDA medium at 20°C and expressed as mm/day. Further, mycelial growth was tested on five different media (PDA, water agar (WA), Czapek agar (ChA), V8 juice agar (V8), and malt extract agar mediim (MA)) at 20°C in darkness, and at various incubation temperatures (4, 10, 15, 20, 22, 25, 30, and 32°C) on PDA medium. Mycelial plugs (5 mm in diameter) were cut from the margin of 4-day-old colonies, placed on medium, and incubated. The growth was measured after 72 h of incubation and expressed as mm per 72 h. The mean value of three replicates was calculated for each isolate.

To confirm the species identity of representative isolates, a species-specific pair of primers based on laccase 2 gene, SMLcc2F/SMLcc2R (Table 1), was used for molecular detection. Genomic DNA was isolated from mycelium scraped from the surface of a seven-day-old PDA culture with the DNeasy Plant Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The PCR amplification process consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 90 s, and extension at 72°C for 90 s. The final extension was at 72°C for 7 min (Abd-Elmagid et al., 2013).

Table 1. Primer codes, targets and sequences for *Sclerotinia minor* detection (Abd-Elmagid et al., 2013)

Target gene	Primer code	Primer Sequence (5'- 3')	Product size (bp)
Laccase 2	SMLcc2 F SMLcc2 R	CCCTCCTATCTCTCTTCCAAACA TGACCAATACCAATGAGGAGAG	264

Results and discussion

From all collected symptomatic lettuce plants only *Sclerotinia* -like colonies were recovered, all with uniform colony appearance. A total of 31 mono-hyphal-tip isolates were obtained, three of which from different districts were selected for further characterization.

Table 2. Isolates of *Sclerotinia minor* chosen for further characterization

Isolate	Location	Region	Year of isolation
11-9	Trbušani (Čačak)	Moravica	2022
15-1	Potočanje (Užice)	Zlatibor	2021
18-1	Blace (Prokuplje)	Toplica	2022

The pathogenicity of selected isolates was confirmed by artificial inoculation of lettuce plants. After 7 to 10 days, all pathogen-inoculated plants developed lettuce drop symptoms, whereas the control plants remained symptomless. The pathogen was reisolated from symptomatic leaves, and Koch's postulates were completed by confirming the identity of the isolates.

All selected isolates, grown on PDA at 20°C in darkness, formed a well-developed whitish mycelium, initially white, homogeneous and airy, and later exhibiting radially distributed cottony clusters of hyphae, indicating the initiation of sclerotia formation. All tested isolates produced whitish sclerotia that became slightly darker after 5 days. Completely developed sclerotia were small (1.5–1.7 x 1.2-2 mm in diameter), dark-grey to black in colour, hard in consistency and irregular in shape. The number and pattern of sclerotia formation varied among isolates. The highest production of sclerotia was recorded for isolate *Sm* 15-1 (360 sclerotia per plate). Two patterns of sclerotia formation were observed: scattered all around the plaque (15-1 and 11-9) and near the rim of the plaque (18-1) (Table 3).

The highest growth rate at 20ºC was recorded for the isolate 18-1 (50.3 mm/day), followed by15-1 (36.5 mm/day) and 11-9 (22.4 mm/day). Ten days after incubation, the isolate *Sm* 11–9 produced silver pigment thus becoming darker in colour, while the other two isolates remain whitish without pigmentation. Based on morphological characteristics, all isolates obtained from the diseased lettuce were identified as *S. minor* (Kohn, 1979; Willetts and Wong, 1980).

Table 3. Cultural features of *Sclerotinia minor* isolates on PDA at 20°C

Cultural appereance			Sclerotia formation		
Isolate	**Colony colour**	**Type of growth**	**Initiation day**	**Pattern of sclerotia production**	**Number of sclerotia per plate**
11-9	Whitish	Sparse regular	5	Scattered all around	88
15-1	Whitish	Fluffy regular	5	Scattered all around	360
18-1	Whitish	Fluffy regular	6	Near the rim	200

Cultivation of the isolates at different temperatures showed that the isolates *Sm* 18-1 and *Sm*15-1 had the highest growth rate at 20ºC (79.75 mm/72 h and 65.5 mm/72 h, respectively) , while the most favourable temperature for isolate *Sm* 11-9 was 22ºC (66.5 mm/72 h) (Figure 1). The formation of sclerotia was observed at temperatures between 10 and 25ºC. The results of the present study revealed that the optimal temperatures for sclerotia production overlapped with the optimal temperatures for mycelial growth of the tested isolates. Thus, for isolates *Sm* 18-1 and *Sm* 15-1 the highest number of sclerotia was recorded at 20ºC (200 and 350 sclerotia per plate, respectively), while for the isolate 11-9 the higest number was recorded at 22ºC (380 sclerotia per plate), exactly the same optimal temperature for mycelial growth. Similarly, Domingues et al. (2016) showed that 22°C was the most favourable for the growth of *S. minor* isolate from lettuce plants. Our results are also in agreement with the findings reported by Kim and Cho (2002) that the optimal temperature for *S. minor* was 20-22°C. However, neither of these authors studied the influence of temerature on sclerotia production.

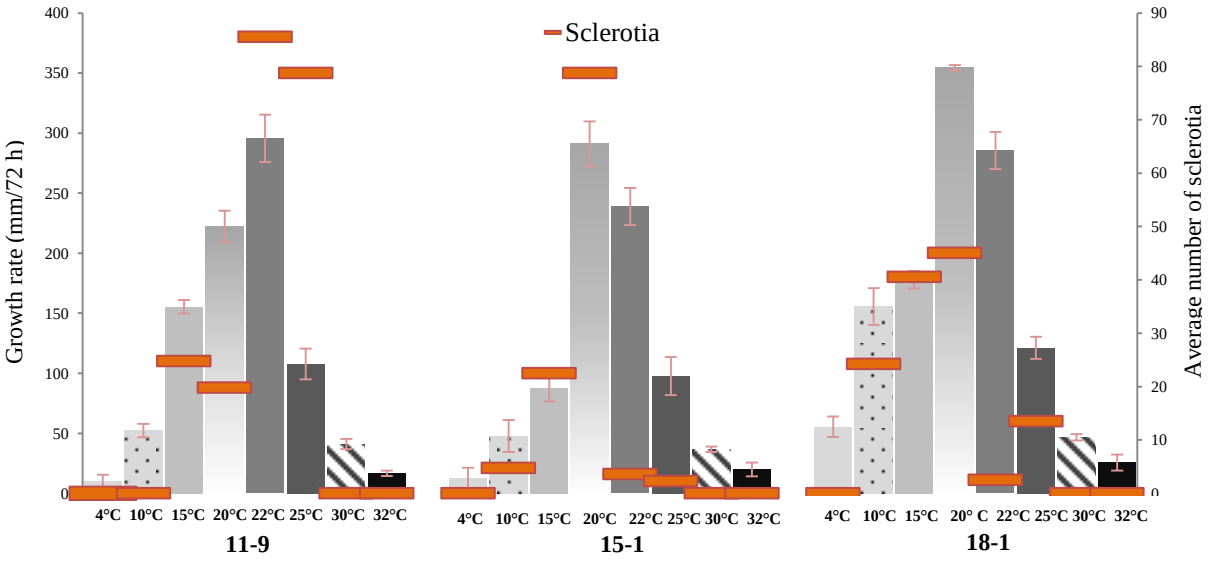

Figure 1. Effects of different temperatures on the growth rate of *Sclerotinia minor* isolates 72 h after inoculation on PDA medium

Since cultural features of *S. minor* have not been widely described in the literature, one of the main goals of this research was to provide an in-depth characterization of this economically important pathogen. All selected isolates were able to grow on all tested media, but generally the highest growth rate was recorded on MA and PDA media (Figure 2). Isolates cultivation on different media showed that PDA medium was the best medium for the growth. Conversely, the lowest growth rate was recorded on the WA medium.

Besides differences in growth rate on different media, it was noticed that all tested isolates had compact mycelium on PDA, MA, and V8A media, while transparent and sparse mycelium was developed on WA and ChA media. The optimal media for sclerotia production was MA for the isolates 11-9 (490 sclerotia/plate) and 15-1 (425 sclerotia/plate), while for the isolate 18-1 the highest number of sclerotia was recorded on ChA media (200 sclerotia/plate) despite sparse appearance of the mycelium. Similarly, Prajapati et al. (2022) showed that PDA and MA media were the most favourable for the growth of *Sclerotinia* spp. and sclerotia production.

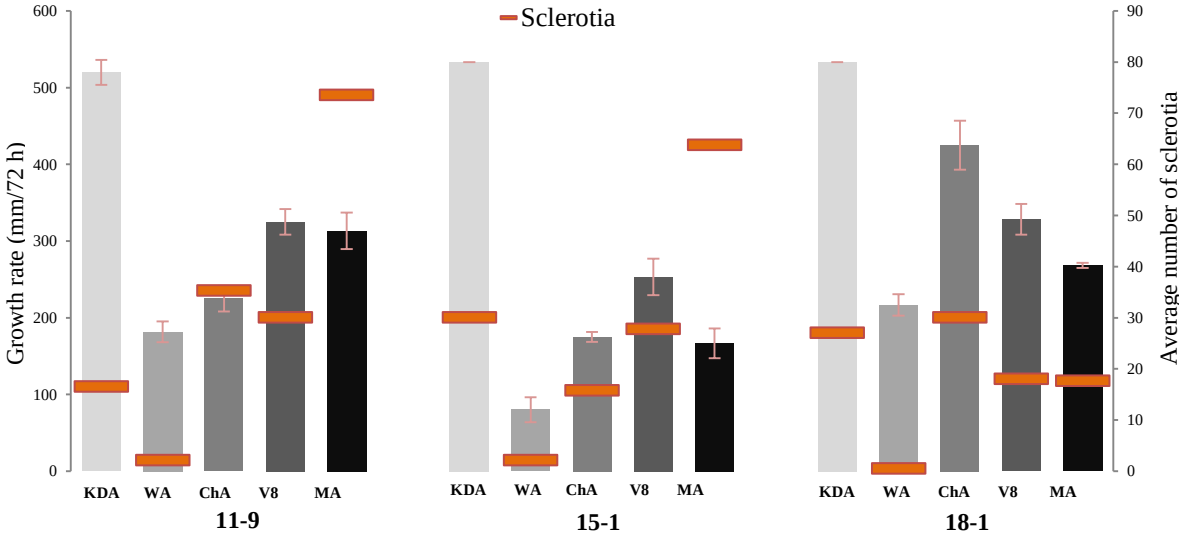

Figure 2. Effects of cultivation media on growth rates of *Sclerotinia minor* isolates 72 h after inoculation

Using the species-specific primers SMLcc2F/SMLcc2R, a product of predicted size of 264 bp for all three tested isolates were amplified, providing conformation of identification based on morphological and cultural characteristics. Erper et al. (2020) used the same specific primers to confirm the presence of white mold caused by *S. minor* on sunflower in Turkey.

Conclusion

Based on pathogenicity test, morphological and cultural characteristics, and molecular analysis, representative isolates from this study were identified as *S. minor*. To our knowledge, after the recent detection of *S. minor* in Serbia, these are the first study data about this newly discovered pathogen. Knowledge about populations of *S. minor* on lettuce plants in Serbia is of great importance because of its ability to reduce the quality of production. In addition, obtained results provide the basis for the development of effective disease management strategies.

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INSECTICIDES FLONICAMID AND PYRIPROXYFEN IN THE CONTROL OF GREENHOUSE WHITEFLY IN TOMATOES

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Abstract

The aim of this study was to evaluate the efficacy of insecticides flonicamid and pyriproxyfen in the control of *Trialeurodes vaporariorum* (Westwood) in tomatoes grown in the greenhouse, and their behavior and dissipation dynamics. Plant protection product (PPP) based on these two insecticides (160 g/l flonicamid + 120 g/l pyriproxyfen, SE) was applied in the amount of 0.6 l/ha when the tomato was in BBCH 76. The trial was set up as a randomized block design in a greenhouse (t=27.7-31.7 ˚C, H=36-49%), according to EPPO methods. Two weeks after the treatment, the efficacy of the applied PPP was 84.4-91%, depending on the time of application and the stage of the insect. For the insecticide residue analysis, sampling was performed after drying the deposit and daily, for five days. Extraction was carried out using the QuEChERS-based method, followed by chromatographic analysis. One day after the application, flonicamid in tomato fruits was below the MRL (0.5 mg/kg), while the amount of pyriproxyfen below its MRL of 1 mg/kg was achieved after two days. The results indicated that under greenhouse conditions, flonicamid and pyriproxyfen degraded rapidly in tomato fruits and exhibited first-order kinetics dissipation, with a half-life of 2.03 and 3.41 days, respectively, and calculated pre-harvest interval of two days. This research showed that PPP based on flonicamid and pyriproxyfen are efficient and safe for the control of *T. vaporariorum* in tomatoes in greenhouse conditions.

Keywords: *Flonicamid, pyriproxyfen, tomato, Trialeurodes vaporariorum (Westwood), dissipation dynamics*

Introduction

One of the most important pests of tomato in greenhouse is *Trialeurodes vaporariorum* (fam. *Aleyrodidae*). Conditions in greenhouse favor the development of this whitefly, and it constantly reproduces and develops, giving a large number of generations. These features, as well as the intensive application of insecticides, have led to the development of resistance to a large number of active ingredients and thus made control even more difficult.

Combination of flonicamid and pyriproxyfen has a good perspective to control *T. vaporariorum*, due to different modes of action and therefore targeted developmental stages of insects.

Flonicamid is pyridinecarboxamide compound (Figure 1) used as insecticide in control sucking pests in various crops, such as apple, tomato, cucumber, cotton and pepper. Pre-harvest interval (PHI) of PPPs based on flonicamid in tomato, in Republic of Serbia is one day (Plant Doctor, 2021). This substance has translaminar and systemic movement in plants, and it has contact and digestive effect on insects (EFSA, 2010). Flonicamid blocks the potassium chanel of insects nervous system, causing disorientation and rapid cessation of feeding and honeydew production (Heim *et al.*, 2006). It is toxic to larvae and adult insects. The activity of flonicamid was not reduced in the neonicotionoid-resistant populations of *T. vaporariorum*, indicating that there is no cross-resistance between those substances (Heim *et al*., 2006). It has a good toxicological and environmental profiles; degrades rapidly in soil by

microorganisms. Flonicamid has been tested on a number of beneficial arthropods in the laboratory and field conditions, and it is proved to be non-toxic to all species that have been tested so far, such as *Apis mellifera*, *Chrysoperla carnea*, *Orius insidiosus*, *Encarsia formosa*, *Trichogramma spp.* (Heim *et al*., 2006).

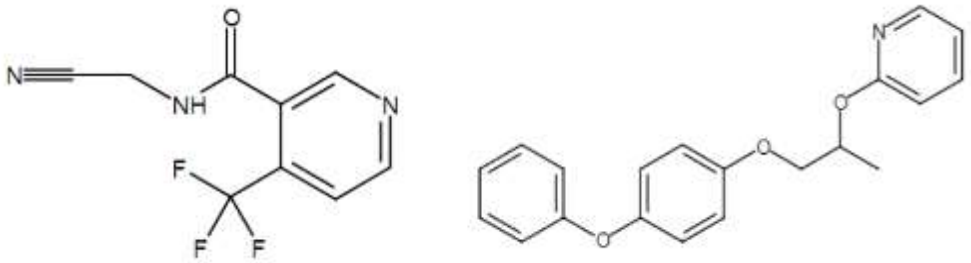

Figure 1. Chemical structure of flonicamid (left) and pyriproxyfen (right)

Pyriproxyfen is an aromatic, non-terpenoidal insecticide, from the class called Insects Growth Regulators, IGRs (Figure 1) (Dhadialla *et al*., 1998). Due to IGRs specific mode of action, they have very low mammalian toxicity (Ishaaya *et al*., 1994). It is toxic to insects during their embryonic development, last larval stage, or reproductive phase, and effective against insects that are developing resistance to conventional insecticides, such as whiteflies, scale insects, thrips and mealworms (Sullivan & Goh, 2008).

Pyriproxyfen mimics the action of juvenile hormones which affects the hormonal balance in insects (Dhadialla *et al*., 1998). That results in a suppression of embryogenesis, metamorphosis, and adult formation (Ishaaya *et al*., 1994). The action of IGRs is usually slow to moderately slow (*https://irac-online.org*). Pyriproxyfen is applied foliar and it has translaminar activity in plants, which affects hidden developmental stages of the pests. It has contact and digestive effect on insects (Ishaaya *et al*., 1994).

This compound is used for the control of greenhouse whitefly (*T. vaporariorum*), sweetpotato whitefly (*Bemisia tabaci*), green peach aphid (*Myzus persicae*) and pear psylla (*Psylla pyricola*). It is also used to control a diaspidid and coccid scales (Dhadialla *et al*., 1998). In Republic of Serbia, it is registered to control *T. vaporariorum*. PHI of PPPs based on pyriproxyfen in tomato, in Republic of Serbia is three days (Plant Doctor, 2021).

Due to the excessive use of pesticides in greenhouse, soil and vegetables are more contaminated than those grown in the open field (Ma et al., 2020). In order to protect human health, it is necessary to monitor the amount of pesticide residues in food. The guarantor of food quality, in addition to various organoleptic properties, is the amount of pesticide residues, which must be below the legally regulated value. Tomato belongs to the vegetables that are treated with a wide range and a large amount of plant protection products against diseases and pests, and accordingly, numerous residues of PPP are regularly detected (Hepsağ *et al.*, 2021).

The dissipation rate of a pesticide after application is a useful tool for evaluating the behavior of its residues. Furthermore, residue dissipation curves can be used to estimate the time required to decrease residue level below maximum residue level (MRL).

In this work, the effectiveness of the insecticide based on flonicamid+pyriproxyfen in the control of the whitefly (*T. vaporariurum*) and the dissipation dynamics in tomato under greenhouse conditions will be evaluated. Based on the results of the research, we will gain insight into the effectiveness and persistence of the insecticide while the half-life will be calculated by monitoring the content of these compounds from the drying of the deposit and the next five days.

Material and Methods

Field trial

The field trial was set up in July 2022, at the locality Kovilj (Republic of Serbia) in order to control greenhouse whitefly, *T. vaporariorum*, in the tomato crop under greenhouse conditions. The experiment was carried out according to the standard EPPO methods (Anonimus, 2009; Anonimus, 2012; Anonimus, 2012a). Plant protection product based on flonicamid+pyriproxyfen (160 g/l+120 g/l, SE) was foliar applied by backpack sprayer in rates of 0.24 l/ha (minimal effective dose), 0.4 l/ha and 0.6 l/ha, respectively, with water consumption of 400 l/ha. Tomato was in phase BBCH 76 (fruit development, the sixth fruit on the main stem has reached typical size and shape). The experiment was set up as a randomized block system, in four replications.

In order to evaluate the flonicamid+pyriproxyfen efficacy against *T. vaporariorum*, three assessments were performed on the number of living adults and larvae per one leaf, out of examined 10 plants per repetition. The first assessment was performed 2 days after treatment (July 7, 2022), the second 7 days after treatment (July 12, 2022) and the third 14 days after the treatment (July 19, 2022). The results are presented through average number of adults and larvae. Also, the standard deviation (Sd) was determined. The efficacy of the insecticide was carried out according to Henderson and Tilton (Wentzel, 1963).

In order to assess the flonicamid and pyriproxyfen residues in the tomato fruits, PPP was applied in rate of 0.6 l/ha. Approximately 1.0 kg of representative vegetable samples from field experiments were randomly picked, delivered to the laboratory and stored at −20 °C before analysis, according to the FAO/WHO recommendations (FAO, 1986). Samples were taken after drying the deposit and daily for five days. The untreated samples were also collected and used as a control.

Analitical procedure

Flonicamid and pyriproxyfen were simultaneously extracted from tomato fruit samples using the QuEChERS method described by Anastassiades *et al.* (2003). Residues were determined by HPLC using an Agilent 1100 HPLC system (USA), with photodiode array detector, and C_{18} Zorbax XDB (50 mm × 4.6 mm, 1.8 μm film thickness) column. The column was kept at room temperature (25ºC), flow rate of mobile phase (acetonitrile/water, 70/30 v/v) was 0.650 ml/min and injection volume was 10 μl. Flonicamid and pyriproxyfen were detected at wavelength 230 nm. The retention time of flonicamid was 1.137 min, while the retention time of pyriproxyfen was 2.830 min.

The method was validated through linearity, precision, limit of detection and quantification (LOD, LOQ), matrix effect and accuracy. A six-point standard solution and matrix-matched standard solutions were prepared at the concentrations of 0.027–3.0 μg/ml. The recovery assays were performed to estimate the accuracy and precision of the method. The recovery experiments were conducted by three replicates at three spiked levels (0.1, 1.2 i 3.0 μg/ml). The accuracy and the precision under these conditions for repeatability were expressed as the average recovery and the relative standard deviation (RSD) (Table 1). All parameters fulfilled SANTE criteria (SANTE 11312/2021).

Table 1. Validation parameters

	Linearity, 0,027–3,0 µg/ml	Precision, RSD (%)	Recovery 0.1, 1.2 and 3.0 µg/ml (%)	LOD (mg/kg)	LOQ (mg/kg)	ME (%)
Flonicamid	R^2= 0.9629	0.47	93.11–95.23	0.02	0.06	101.2
Pyriproxyfen	R^2= 0.9676	0.55	92.96–95.19	0.05	0.15	102.9

Results and discussion

In order to examine effectiveness of pesticide based on flonicamid+pyriproxyfen, it was applied in rates of 0.24 l/ha (minimal effective dose), 0.4 l/ha and 0.6 l/ha. The efficacy 2, 7 and 14 days after the treatment are given in Tables 2–3. After the treatment, average number of *T. vaporariorum* adults was at a significantly lower level compared to the control. Efficacy of the tested preparation was in the range of 72–90.8%, depending on applied dose and the moment of evaluating the effects (Table 2).

Table 2. Average number of adults and efficacy of PPP based on flonicamid+ pyriproxyfen

Applied rate (l/ha)	2nd day		7th day		14th day	
	$\bar{x}$ ±Sd	Ek %	$\bar{x}$ ±Sd	Ek %	$\bar{x}$ ±Sd	Ek %
F+P*, 0.24	14.2±2.1	72.0	16.75±5.8	72.9	14.75±2.2	79.7
F+P*, 0.4	10.5±2.4	78.0	11.5±2.9	80.2	11.0±2.2	83.8
F+P*, 0.6	9.2±0.9	84.5	9.5±3.1	86.9	7.75±1.7	90.8

F+P* – flonicamid and pyriproxyfen; $\bar{x}$ – average number; Sd± – standard deviation; Ek – effectiveness versus control

Table 3. Average number of larvae and efficacy of PPP based on flonicamid+pyriproxyfen

Applied rate (l/ha)	2nd day		7th day		14th day	
	$\bar{x}$ ±Sd	Ek %	$\bar{x}$ ±Sd	Ek %	$\bar{x}$ ±Sd	Ek %
F+P*, 0.24	15.0±3.7	71.9	13.2±2.2	81.1	19.7±4.6	69.5
F+P*, 0.4	11.2±1.9	84.5	11.0±2.2	88.3	13.0±2.2	85.0
F+P*, 0.6	9.5±1.9	85.9	8.5±2.9	91.0	12.7±1.2	84.4

F+P* – flonicamid and pyriproxyfen; $\bar{x}$ – average number; Sd+ – standard deviation; Ek – effectiveness versus control

Average number of *T. vaporariorum* larvae 2, 7 and 14 days after the treatment, was at a notably lower level compared to the control. Efficacy was in the range of 71.9–91.0%, depending on the rate and the time of assessment (Table 3).

Dissipation dynamics were evaluated after application of PPP based on flonicamid and pyriproxyfen in the rate of 0.6 l/ha. One day after the treatment, the average flonicamid content was below MRL (0.5 mg/kg), while second day after the treatment, the average pyriproxyfen content was below MRL (1 mg/kg). The residues of pyriproxyfen were dissipated to undetectable limits four days after the treatment (Table 4).

Table 4. Dissipation of flonicamid and pyriproxyfen in tomato fruits

Day after the treatment	Average residues of flonicamid (mg/kg)	Remains of flonicamid (%)	Loss (%)	Average residues of pyriproxyfen (mg/kg)	Remains of pyriproxyfen (%)	Loss (%)
0 day	0,78	100	0,00	2,53	100	0,00
1st day	0,46	69	30,77	1,43	40,54	59,49
2nd day	0,40	51	48,95	0,79	22,46	77,57
3rd day	0,30	39	61,48	0,35	10,00	90,03
4th day	0,22	29	71,51	<LOD	<LOD	<LOD
5th day	0,12	16	84,05	<LOD	<LOD	<LOD
MRL, mg/kg		0,5			1	

Residue dissipation of flonicamid and pyriproxyfen in tomato fruits was studied by subjecting the data to the first-order kinetic equation $C_t=C_0e^{-kt}$, where C_0 represents the initial deposit of the compound, k is the constant of dissipation rate in days and C_t is the amount of pesticide residues over time. The results show a gradual decrease of flonicamid and pyriproxyfen in tomato fruits, with a correlation coefficient of 0.984 and 0.915, respectively (Table 5). The half-life of degradation (DT_{50}) was calculated according to the formula $DT_{50}=\ln(2)/k$, and it is shown in Table 5. Based on the obtained results, half-lives of flonicamid and pyriproxyfen, were 2.03 and 3.41 days, respectively.

While the residues of pyriproxyfen are studied in tea (Li et al., 2022) and bee products (Wu et al., 2021), the dissipation behaviors and the terminal residues of flonicamid remain unclear, particularly in some crops. According to Wang et al. (2018), the half-lives of flonicamid as total residues (the sum of flonicamid and its metabolites) were 1.49-4.59 and 1.97-4.99 days in cabbage, and 2.12-7.97 and 2.04-7.62 days in soil. Due to available data, this study provided the first results of the residues and behavior of flonicamid and pyriproxyfen in tomato fruits.

Table 5. Half life of flonicamid and pyriproxyfen in tomato fruits

Insecticide	Regression equation	Constant	R^2	DT_{50} (day)
Flonicamid	$y = 0.78e^{-0.341x}$	0.341	0.984	2.03
Pyriproxyfen	$y=2.25e^{-0.203x}$	0.203	0.915	3.41

Conclusion

Based on the results obtained in this study, flonicamid+pyriproxyfen has great potential for the control of *T. vaporariorum* in tomatoes, with the efficacy of 71.9–90%. Furthermore, their residues have shown a gradual decrease of the content in tomato fruits, with a correlation coefficient of 0.984 and 0.915, and half-lives of 2.03 days and 3.41 days, for flonicamid and pyriproxyfe, respectively. Based on the results obtained in this study, flonicamid+pyriproxyfen has great potential for the control of *T. vaporariorum* in tomatoes, with an efficacy of 71.9–90%. Furthermore, their residues have shown a gradual decrease of the content in tomato fruits, with a correlation coefficient of 0.984 and 0.915, and half-lives of 2.03 days and 3.41 days, for flonicamid and pyriproxyfen, respectively. Thus, the results confirmed the safe use of flonicamid+pyriproxyfein the tomato protection.

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INFLUENCE OF INSECTICIDES APPLICATION ON CHANNEL LENGTH IN STEM OF MAIZE PLANTS CAUSED BY FEEDING OF LARVAE OF EUROPEAN CORN BORER (*Ostrinia nubilalis* Hbn.)

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Abstract

In maize plants the attack of European corn borer can cause significant damages, which can lessen by insecticide application in crop protection. The aim of this study was to determine the effect of insecticides on the total length of channels in maize plant stem formed by the feeding of larvae *Ostrinia nubilalis*. The research was carried out in Maize Research Institute "Zemun Polje" on the experimental field in 2018. Study used three maize genotypes of different FAO ripening groups (ZP 434, ZP 600 and ZP 666) and three insecticides chlorantraniliprole (200 g l^{-1}), bifenthrin (100 g l^{-1}) and lufenuron (50 g l^{-1})+(cypermethrin (50 g l^{-1}) + chlorpyrifos (500 g l^{-1})), which were applied only after the maximum flight of the first generation to protect plants from attack of European corn borer. The result showed significantly different total length of channels in the stem of plants, caused by feeding of the larvae of European corn borer, which varied from the lowest on treatment with insecticide chlorantraniliprole in maize genotype ZP 600 (170.00 cm) to the highest in maize genotype ZP 666 (278.33 cm) on the control variant (without insecticide application). For all treatments, the average value of total length of channels in stem, was the lowest 192.92 cm in ZP 434, slightly higher 195.42 cm in ZP 600, while the highest average value of total length of channels was 233.34 cm in maize genotype ZP 606. The established differences for total length of channels depended on genotype and type of insecticide applied.

Key words: *maize, genotype, pest, insecticide, length of channels.*

Introduction

One of the most important crops in the Republic of Serbia, along with wheat, is maize. Looking at the structure of harvested field crops, maize dominates with 53% of the crop area, followed by wheat with 38% (Grčak et al., 2018). With such great importance, the protection of maize from diseases and pests plays a big role (Gošić et al., 2020). In the agroecological conditions of the Republic of Serbia, *Ostrinia nubilalis* Hbn. is one of the most dangerous pests on maize that can cause very significant yield losses (Szőke et al., 2002; Grčak et al., 2022). The insect species itself attacks over 200 species of wild and cultivated herbaceous plants, but prefers maize (Bourguet et al., 2000). It originates from Europe, but occurs on almost all continents where maize is grown.

Ostrinia nubilalis attack and damage varies from year to year depending on climatic conditions (Frolov and Grushevaya, 2020). In their experiment, the dynamics of the European

corn borer (ECB) population was significantly correlated with environmental conditions. The number of hatched eggs and surviving caterpillars depended on air temperature and air humidity in the first decade of May. Yield reductions caused by ECB attacks have been reported to be 10-20% in Hungary (Szőke et al., 2002), 20-30% in Poland (Bereś and Konefaá, 2010), around 23% in the USA (Tiwari et al., 2009), 17-80% in Belarusia (Trepashko et al., 2013), and from 37% to 90% in Croatia (Raspudić et al., 2009).
European corn borer (ECB) larvae feed on almost all parts of the maize plant, except the root system. Therefore, all damages caused by this insect can be very large. ECB has two to three generations per year (Gaspers, 2009), in the agroecological conditions of the Republic of Serbia, it has two generations per year (Almaši et al., 2002). The development of the first generation is coordinated with the period of panicle formation, while the development of the second generation coincides with the formation of the cob, and they can feed on unripe grain under the cob or on the stem of the cob, which in this case leads to cob drop (Bereś, 2012). The larvae of both generations bore into the corn stems. There, they feed on the parenchymal tissue and, in addition to its damage, they also lead to damage to the conducting tissues. In this way, they directly influence the difficulty and reduction of absorption and transfer of nutrients and water. This type of damage contributes to and enables the attack of fungi and bacteria, and helps virus vectors to infect the plant more easily (Franeta, 2018).
The aim of this work was to investigate the effect of insecticides on the length of channels in corn stems that were formed by the feeding of *Ostrinia nubilalis* larvae.

Materials and methods

In this research was included three maize hybrids of different maturation groups (ZP 434, ZP 600 and ZP 666) with four variants of treatment: control variant (C) - without chemical protection and three insecticides: (T1) chlorantraniliprole (200 g l^{-1}), (T2) bifenthrin (100 g l^{-1}) and T3 [lufenuron (50 g l^{-1})+(cypermethrin (50 g l^{-1}) + chlorpyrifos (500 g l^{-1}))], which were applied only after the maximum flight of the first generation to protect plants from attack of European corn borer.
On the field of the Maize research institute „Zemun Polje" in Belgrade, the experiment was carried out in 2018. The experiment was designed in three repetitions on a plot of 10.5 m². The sowing of maize hybrids was performed by machines in three rows of 5 m length with a distance between rows of 0.7 m and a distance between plants in a row of 0.25 m.
After the maize harvest was completed, the plants were cut to the ground and tied into bundles according to the treatments. Later, these plants were dissected, and among other morphometric measurements, the total length of all channels per experimental group was measured.
The obtained results were statistically processed, using various statistical methods of the program package "SPSS Statistics 20" (trial version). The significant differences among the average values were estimated according to the LSD test.

Results and discussion

In the studied maize hybrids in the 2018 growing season, a variation in the total length of all channels formed by the feeding of the larvae of the European corn borer in the maize plants, and measured during dissection, was found. The average length of all the channels created by the feeding of *O. nubilalis* larvae varied from a minimum of 170.00 cm in hybrid ZP 600 treated with the insecticide chlorantraniliprole to a maximum of 278.33 cm in hybrid ZP 666 in the control variant (without application of insecticide). The total length of all channels in the stems formed by feeding on *O. nubilalis* was the smallest in hybrid ZP 434 (192.92 cm)

and the largest in hybrid ZP 666 (233.33 cm) - table 1. The average length of all channels formed by feeding ECB was highly different ($p<0.01$) between ZP 666 and the other two hybrids, while the channel length between them (ZP 434 and ZP 600) was not significantly different (table 2).

Table 1. Variation of the total length of the channels in the stems formed by feeding of *O. nubilalis* larvae in maize plants under insecticide application only during the flight of the first generation

Treatments*	Maize hybrids							
	ZP 434		ZP 600		ZP 666		Average	
	$\bar{X}$ (cm)	CV (%)	$\bar{X}$ (cm)	CV (%)	$\bar{X}$ (cm)	CV (%)	$\bar{X}$ (cm)	CV (%)
C	$238,33^{b}$	4,37	$233,33^{b}$	3,27	$278,33^{a}$	1,04	250,00	8,94
T1	$173,33^{fg}$	1,67	$170,00^{g}$	7,78	$186,67^{de}$	1,55	176,67	5,83
T2	$181,67^{def}$	4,20	$191,67^{d}$	3,98	$216,67^{c}$	7,05	196,67	9,25
T3	$178,33^{efg}$	1,62	$186,67^{de}$	3,09	$211,67^{c}$	1,36	192,22	8,03
Average	192,92	14,59	195,42	13,07	233,33	16,07	203,89	15,93

*C = control variant; T1 = treated with chlorantraniliprole in the first generation of insect; T2 = treated with bifenthrin in the first generation of insect; T3 = treated with combination of lufenuron + (cypermethrin + chlorpyrifos) in the first generation of insect

In Table 1, lowercase letters above the length of stems channels produced by pest feeding indicate whether there was a statistically significant difference between treatments ($p<0.05$) - the same letters indicate no statistically significant difference, including and comparing all treatments and all three hybrids. Among the studied maize hybrids, the total length of all channels in the stems formed by the feeding of *O. nubilalis* larvae was highly significantly greater ($p<0.01$) in the control varieties than in the treatments with insecticides.

In hybrid ZP 434, there were no significant statistical differences in the total length of channels in the stems in all three treatments. The treatment with the insecticide chlorantraniliprole had the slightly smallest length of all channels (173.33 cm), followed by the combination of active substances lufenuron + (cypermethrin + chlorpyrifos) with a length of 178.33 cm and the treatment with the insecticide bifenthrin which had the slightly highest channel length (181.67 cm) created by feeding ECB.

Analyzing the ZP 600 hybrid, we observe that the treatment with chlorantraniliprole had significantly the smallest channel length (170.00 cm) in the stems compared to the other two insecticide treatments. There were no significant statistical differences between the other two treatments (T2 and T3) in hybrid ZP 600.

In the ZP 666 hybrid treated with the insecticide chlorantraniliprole, the total length of all channels formed by the feeding of *O. nubilalis* larvae was significantly smaller ($p<0.01$) than in the treatments with other insecticides, between which there were no significant statistical differences.

Observing each treatment with insecticides separately, we recognize that in all three treatments (T1, T2 and T3) but also in the control (C) there are no significant statistical differences for the length of the channel in the stems produced by ECB feeding between the hybrids ZP 434 and ZP 600, while both hybrids had significantly shorter channel length compared to the ZP 666 hybrid.

The length of channel in the stems produced by ECB feeding on treatments with the insecticide chlorantraniliprole was the lowest in hybrid ZP 600 (170.00 cm), which was statistically slightly less than the length of channel in hybrid ZP 434 (173.33 cm), while the channels length of both hybrids was statistically significantly lower ($p<0.01$) than in the hybrid ZP 666 (186.67 cm) on the same treatment.

When treated with insecticides with the active substance bifenthrin, the channels length in the stems formed by ECB feeding was the lowest in hybrid ZP 434 (181.67 cm), which was statistically slightly less than the channel length in hybrid ZP 600 (191.67 cm), while in both hybrids the channel length was statistically significantly lower ($p<0.01$) than in the hybrid ZP 666 (216.67 cm).
In the treatments with the combination of lufenuron + (cypermethrin + chlorpyrifos), the length of the channel in the stems formed by feeding *O. nubilalis* was the lowest in the hybrid ZP 434 (178.33 cm), which was statistically slightly less than the length of the channel in the hybrid ZP 600 (186, 67 cm), while the channel length of both hybrids was statistically significantly lower ($p<0.01$) than that of hybrid ZP 666 (211.67 cm).

Table 2. Analysis of variance for the total length of channel in the stems, caused by the feeding of *O. nubilalis* larvae on maize plants with the application of insecticides only during the flight of the first generation (Zemun Polje, 2018)

Source	df	Sum of Squares (SS)	Mean Square (MS)	F test	LSD 0,05	LSD 0,01
Genotype	2	6843,056	3421,528	53,554	9,525	12,907
Treatment	3	27500,000	9166,667	143,478	10,998	14,904
Gen x Tre	6	1029,167	171,528	2,685	19,049	25,815
Error	24	1533,333	63,889			
Total	35	36905,556				

The analysis of variance shows that in the growing season of the study, highly significant and significant differences ($p<0.01$, $p<0.05$) were manifested for the total length of all channels in the stems, caused by the feeding of the larvae of the ECB between the hybrids, between the insecticide treatments, as and in the hybrid/treatment interaction (Table 2).
The result showed that the longest channels lengths were recorded in plants that were not treated with insecticides. *Ostrinia nubilalis* overwinter in the channels they feed on and can cause significant damage to the corn yield. Yield losses when the plant had at least one larva in stem ranged from 4.1% in the late growing season, 6.8% during silking and 1.8% at the beginning of grain filling (Jordan, 2008). And in his research, Bağdatlı (2019) observed that the genotypes with the smallest channel length in the stem that were created with the ECB had the highest yield. Sandoya et al. (2008) demonstrated in their research that the genotype and the genotype/external environment interaction have a significant influence on the length of the channel in the stem and on the yield of maize. In our research, too, the genotype played a significant role in the length of the channels that were formed in the stems due to the feeding of *O. nubilalis* larvae. The studied hybrids differed from each other in the size of the channela in the stems, where the hybrid ZP 666 had statistically significantly the worst results of the other two hybrids. Hybrids ZP 434 and ZP 600 did not significantly differ from each other. We observe these differences between hybrids both on the control (untreated plants) and on all three insecticide treatments. In his three-year study, Bereś (2012) confirmed that channel length in ECB maize plants differed significantly in relation to genotype. Varying plant damage caused by ECB attack was also found in research with 18 different maize hybrids during 2013 and 2015 (Georgescu et al., 2015).
The differences between C - control variant (without insecticide protection) and other three variants T1, T2 and T3 with application of different insecticides were significant. In this study, we found that with the application of insecticides with the active substance chlorantraniliprole in all three hybrids, the length of the channels from ECB in the stems was the lowest. In hybrids ZP 600 and ZP 666, the channel length on maize plants treated with this insecticide was statistically significantly smaller than the channel length of plants treated with

the other two insecticides (table 1). While in the ZP 434 hybrid, the chlorantraniliprole insecticide treatment had a slightly smaller channel length than ECB compared to the plants treated with the other two treatments (T2 and T3). The average channel length in all three hybrids in the treatment with the insecticide chlorantraniliprole (T1) was 176.67 cm, in the treatment with the combination of active substances lufenuron + (cypermethrin + chlorpyrifos) - (T3) was 192.22 cm , while treatment with bifenthrin (T2) had the worst results with 196.67 cm.

In the research by Georgescu et al., (2019) where a large number of active substances were tested in order to control the ECB population as effectively as possible, the results showed that the least damage was on plants that were treated with the active substance chlorantraniliprole, which is in agreement with our research. Also, in a two-year study on small plots for the control of *Ostrinia nubilalis* where the insecticide cyantraniliprole (also a diamide, with a similar composition to chlorantraniliprole) and bifenthrin (a pyrethroid) were applied, the results showed that the application of the insecticide cyantraniliprole in the same terms had a stronger effect on the pest (Huseth et al., 2015).

Conclusion

Based on the analyzed results, it can be concluded that the insecticide with the active substance chlorantraniliprole had the best effect on the length of the channels in the stems that resulted from feeding ECB in maize hybrids, which confirms the results of other research. The treatment with this insecticide (T1) proved to be the most effective in hybrid ZP 600 with a length of 170.00 cm of channels formed by feeding ECB. The best results on plants treated with the insecticide bifenthrin (T2) are found in hybrid ZP 434 with a channel length of 181.67 cm, while the combination of treatment lufenuron + (cypermethrin + chlorpyrifos) (T3) had the best results on the same hybrid (ZP 434) with 178.33 cm.

All three hybrids in the experiment had a similar response to the length of the channels in the stems, but the least resistant hybrid was ZP 666, which had the worst results both on the control variant and on each treatment. The hybrids ZP 434 and ZP 600 did not significantly differ from each other in the length of the channels made in the stems by the pest. Overall, hybrid ZP 434 found a slightly smaller channel length in stems (192.92 cm) compared to hybrid ZP 600 (195.42 cm), while looking only at untreated plants, hybrid ZP 600 (233.33 cm) had better results than hybrid ZP 434 (238.33 cm).

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EFFECTS OF PLANTS ESSENTIAL OIL AND PLANT POWDER ON THE MORTALITY OF SITOPHILUS GRANARIUS L.

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Abstract

Post-harvest losses of cereals and pulses are a major problem during storage. The responses to combat insects, have been essentially chemical. However, given the nuisances associated with the use of pesticides, the search for alternative methods is necessary. The current study was conducted in Lebaa station laboratory at the Lebanese Agricultural Research Institute (LARI) to evaluate the effect of essential oil (EO) and leaf powder (LP) obtained from four plant species (Origanum, Cannabis, Rosemary, and Lavender) on the mortality of a major stored product insect, *Sitophilous granarius* (Family: Curculionidae).The obtained results showed that all plant essential oils (EO) exhibited higher mortality rate (82.2 to 96.7%) than plant powders (26.7 to 46.7%) against *S. granarius.* The majority of died insects by EO was recorded at the first (24h) and second (48h) exposure times accounting for about 90% of the total mortality. The powder of the tested plants did cause the major mortality at third exposure time (120h). In both essential oil and powder toxicity tests, the concentrations used did not cause a significant variation in mortality rate. The results obtained in this study suggest that essential oils of four plant species possess a high insecticidal activity that can be leveraged for pest control purposes.

Keywords: Stored seeds, *Cannabis sativa, Rosemary officinalis, Origanum vulgare L., Lavendula angustifolia,* insecticidal activity, repellency, Contact toxicity.

Introduction

The world's growing population has increased demand for food, with cereals being essential for most people (FAO, 2023). Pests infesting stored cereals are the primary cause of food loss in developing countries (Fornal et al., 2007, Demeter et al., 2021) due to inadequate infrastructure (Kumar and Kalita 2017, Greeley 1986, Fox 2013) during storage. There are both direct and indirect forms of losses in agriculture, which can affect factors such as weight, quality, nutritional value, seed viability, and commercial value (Sugri et al., 2021). The five major pests causing insect damage to grains during storage and transportation (Upadhyay and Ahmad 2011) are the granary weevil (Geuttal et al., 2021), rice weevil (Batta 2004), maize weevil (Fekrimeriam et al., 2009), lessor grain borer, and Angoumois grain moth (Schwardt 1933). Botanical pesticides, such as plant essential oils, powders, and leaf extracts, are environmentally-friendly and effective alternatives to synthetic chemical insecticides (Chandrasekaran 2015, Trivedi et al., 2018), which have negative effects on people and the environment (Aktar et al., 2009). These treatments have become the primary components of Integrated Pest Management to combat pest infestation (Aulakh 2013, Alam et al., 2019).
Plant essential oils are biodegradable organic molecules that are secondary metabolites of plants and their derivatives, making them an efficient and less expensive alternative to synthetic pesticides (Gupta and Dikshit, 2010). Synthetic pesticides have negative effects on human health, persist in the environment, and can lead to the development of insect resistance, as well as harming beneficial insects (Nicolopoulou-Stamati et al., 2016).Plant

essential oils are a blend of 20-60 components that give them their distinctive flavor and aroma, and many have a harmful effect on stored-food insects and pests (Regnault-Roger et al., 2012). The major components of rosemary oil are 1, 8-cineol, camphor, α-pinene, limonene, camphene, and linalool, while lavender oil contains linalool, linalyl acetate, 1, 8-cineole, β-ocimene, terpinen-4-ol, and camphor. Cannabis oil contains four major compounds, including d-9-THC, CBD, d-8-THC, and cannabinol, and the main components of oregano oil are αp-cymene, γ-terpinene, thymol, carvacrol, and β-caryophyllene (Pamplona and Takahashi, 2012).

This study was conducted to assess the insecticidal efficiency (contact toxicity) plant essential oils and plant powders made from four plants: Cannabis sativa, Rosemarinus officinallis, Origanum vulgare L, and Lavendulla angustifolia against an insect species called "Sitophilus granarius" by contact or by ingestion.

Material and methods

To rear a homogenous population of S. granaruis, chickpeas were purchased from a local market and placed in glass jars with 25 unsexed insects each, maintaining a temperature of 25 ± 2 ºC and 70±5% RH. The insects were supplied with healthy chickpea grains for feeding (Kfar-Rouman,Lebanon).

Four plant extracts (cannabis, rosemary, lavender, and thyme) were evaluated, and the aerial parts of the plants were collected from the field of the Lebaa station at the Lebanese Agricultural Research Institute during May-June. Only the leaves were used for essential oil extraction and powder preparation. The leaves were partially or fully dried at a temperature of 18-25°C and then subjected to hydro-distillation in a Clevenger-type apparatus to extract the oil. The oil was conserved in amber glass vials in the refrigerator. For powder preparation, the leaves were dried at room temperature and manually blended before being stored in paper bags in a cool place (Samadi et al., 2017).In the toxicity test, concentrations of 1%, 2%, and 3% were utilized for each essential oil, with the dissolving agent being 96% ethanol. Powder concentrations for Cannabis: 15mg, 25mg, and 30 mg; Rosemary: 15mg, 20mg, and 50 mg; Lavender: 15mg, 25mg, and 50 mg; Origanum syriacum: 15mg, 20mg, and 30 mg.

Contact toxicity bioassays using essential oils and powders: the experiment was conducted in sterile plastic boxes, each containing 5 grams of healthy chickpea seeds treated with designated concentration of essential oils or powder. Ten Sitophyllus granarius insects were introduced into each box, with the experimental design being randomized and repeated three times. The boxes were placed in a dark environment with 27°C temperature and 75% relative humidity, and mortality was recorded at 24, 48, and 120-hour intervals by probing the insects.

Statistical Analysis: the results are presented as means ± standard deviation (±SD). Data were analyzed using the statistical SAS system for windows 9.0 and were evaluated by analysis of Variance (ANOVA). Mauchly's test was used to assess the assumption of equal variances of the differences between all related exposure times. Tukey's Studentized Range (HSD) Test was used for comparisons among different treatments. Statistical differences were considered significant at the $p < 0.05$ level 3. The mortality rate data were corrected for control mortality using Abbott's formula.

Results and Discussion

Toxicity test of the essential oils: A multivariate analysis (MANOVA) was then conducted, indicating that time had a significant effect on the mortality rate (Wilks' Lambda, $F(2, 29) = 20.38$, $p<.0001$), with significant interactions between time and treatments (Wilks' Lambda, $F(8,58) = 2.49$, $p=0.0214$), as well as between time, treatments, and dose (Wilks' Lambda,

F(16,58) = 2.07, p=0.0226). However, there was no significant interaction between time and dose (Wilks' Lambda, F (4, 58) = 1.64, p=0.1768).
The mortality rate of Sitophilus granarius was significantly affected by treatments (F (4, 30) = 30.06, p<.0001), but not affected by dose (F (2, 30) = 0.91, p=0.4143), and there was no significant interaction between treatment and dose (F (8, 30) = 0.89, p=0.5398). Essential oils caused high mortality rates (82.2-96.7%) compared to the control (6.1%) and were particularly effective up to 48 hours of exposure. Mortality rates were significant at each exposure time, and the tested essential oils were equally effective at the first exposure time. Cannabis and Rosemary oils showed even distribution of mortality rates between the first and second exposure times (24h, 48h). Origanum essential oil showed statistically higher mortality rates (22%) at the third exposure time compared to other essential oils and the control Table 1).

Table 1. Mortality Rate (%) of Sitophilus granaries exposed to four essential oil treatments and concentrations recorded at three exposure times.

	Exposure time			
Treatments	24h	48h	120h	Total
Cannabis	38.9^{a} (29.3)	40.0^{a} (28.3)	3.3^{b} (5.0)	82.2^{a} (27.7)
Lavender	53.3^{a} (37.8)	23.3^{ab} (24.5)	12.2^{ab} (17.3)	88.9^{a} (16.2)
Origanum	41.1^{a} (35.9)	33.3^{a} (28.0)	22.2a (25.6)	96.7^{a} (7.1)
Rosemary	41.1^{a} (36.3)	41.1^{a} (35.2)	2.2^{b} (4.4)	83.3^{a} (39.7)
Control	1.1^{b} (3.3)	3.3^{b} (5.0)	4.4^{b} (7.3)	6.1^{b} (8.5)
P	***0.0008***	***0.0177***	***0.0165***	***<.0001***
Concentration				
1%	26.7 (29.6)	39.3 (32.4)	8.7 (9.0)	68.1 (41.7)
2%	36.0 (38.3)	26.0 (27.70	13.3 (22.9)	68.2 (39.9)
3%	42.7 (35.9)	19.3 (24.3)	4.7 (8.3)	66.7 (40.5)
P	***0.1921***	***0.0983***	***0.2211***	***0.984***
Interaction Trt*dose				
P	***0.0013***	***0.4266***	***0.1119***	***0.998***

Mean values within the same column followed by different letters are significantly different at P < 0.05 based on Tukey's Studentized Range (HSD) Test. Figures within parentheses are standard deviation.

The significant interaction between treatment and concentration at 24h exposure time indicates that the effect of concentration differs between the treatments at that time point. The lavender essential oil was more effective (76.7% mortality) at a concentration of 1% compared to other essential oils (Rosemary 23.3%, Cannabis 10%, Origanum 23.3%) and control (0%) in the first exposure time. With the concentration of 2%, rosemary showed the highest mortality level (76.7%) compared to Origanum (13.3%) and control 0%). Cannabis and lavender oils at this concentration showed an intermediate mortality level of 50% and 40% respectively. With the concentration of 3%, Origanum oils expressed 86.7% mortality, while the three other essential oils showed an intermediate mortality level (Lavender 43.3%, Rosemary 23.3%, and Cannabis 56.7%).
Toxicity test of the plant Powder: the multivariate analysis (manova) revealed a significant effect of time on the mortality rate (Wilks' Lambda, F (2,29) = 20.38, p<.0001). The interact treatments by time (Wilks' Lambda, F (8, 58) = 1.37, p=0.2307), dose by time (Wilks' Lambda, F(4,58) = 0.38, p=0.8149), and treatment*dose*time (Wilks' Lambda, F(16,58) = 0.89, p=0.5827) were all not significant respectively. Univariate analysis revealed a significant effect of treatments (F(4,30)= 2.75, p=0.0462), and non-significant effect of dose

(F(2,30)= 0.04, p=0.9628), and treatment*dose interaction (F(8,30)= 0.42, p=0.9012) on mortality rate.

Table 3 displays significant effects of treatments on insect mortality (P = 0.046), with Lavender showing the highest overall mean mortality rate (46.7%) followed by Rosemary (44.4%). However, the standard deviations indicate some variability in mortality rates within each treatment. No insect mortality was observed at the first exposure time (24 hours) for either the control group or the treatment groups exposed to plant powder. Plant powders caused the majority of insect deaths at the third exposure time (120 hours), and while mortality rates caused by treatments (20-35.6%) were relatively higher than the control (6.6%), the differences were not statistically significant. Concentrations used in the study exhibited comparable effects on insect mortality at each exposure time, with no significant interaction between treatments and concentrations indicating that the effectiveness of treatments is not strongly dependent on concentration or the specific combination of treatment and concentration.

Table 2. Mortality Rate (%) of Sitophilus granaries exposed to four leaf powder treatments and concentrations recorded at three exposure times.

	Exposure time			
Treatments	24h	48h	120h	Total
Cannabis	0.0	6.7 (10.0)	20.0 (22.4)	26.7^{ab} (24.5)
Lavender	0.0	11.1 (17.6)	35.6 (21.9)	46.7^{a} (28.3)
Origanum	0.0	13.3 (14.1)	24.4 (26.0)	37.8^{a} (33.8)
Rosemary	0.0	11.1 (14.5)	33.3 (20.0)	44.4^{a} (13.3)
Control	0.0	4.4 (8.8)	6.6 (10.0)	11.1^{b} (14.5)
P	**---**	***0.591***	***0.073***	***0.046***
Concentration				
1	0.0	9.3 (32.4)	22.7 (9.0)	32.0 (29.1)
2	0.0	12.0 (27.70	22.7 (22.9)	34.7 (27.7)
3	0.0	6.7 (24.3)	26.7 (8.3)	33.3 (24.7)
P	**---**	***0.539***	***0.855***	***0.962***
Interaction Trt*dose				
P	**---**	***0.228***	***0.104***	***0.901***

Mean values within the same column followed by different letters are significantly different at P < 0.05 based on Tukey's Studentized Range (HSD) Test. Figures within parentheses are standard deviation.

Discussion

The study tested the insecticidal effects of essential oils and powders from four plants (cannabis, lavender, oregano, and rosemary) on grain weevils (Sitophilus granarius). The EOs showed maximum mortality (96%) compared to powders (<50% mortality). This aligns with Lougraimezi et al. (2018), which found differences in essential oil vs. powder mortality for Mentha pulegium on rice weevils. EOs contain higher concentrations of insecticidal compounds. Their volatility, penetration, and multiple modes of action likely explain their higher mortality rates compared to powders. However, powders can still provide some insect control at lower effectiveness (Bitner et al 2010, Tozlu et al 2011). Most EO mortality occurred within 24-48 hours while most powder mortality occurred at 120 hours, showing EOs have a more potent and immediate effect. EOs contain effective insecticidal compounds that contribute to their toxicity (Regnault-Roger et al., 2012; de Oliveira et al., 2014). The concentrations independently had no significant effect, but did interact with treatment type.

While 1% lavender was most toxic, 3% origanum and 2% rosemary were most potent (Pavela, 2015).

Conclusion

This study found that essential oils from oregano, lavender, rosemary, and cannabis are effective insecticides against the granary weevil, with higher mortality rates compared to leaf powders. Essential oils also acted more quickly, with most mortality occurring within the first 48 hours. Concentrations showed interactions with treatment, suggesting that optimal doses may vary depending on the essential oil's chemical makeup. These plant-based compounds could be used as natural insecticides for stored product pest control, but further research is needed to determine ideal doses and formulations under realistic storage conditions and their compatibility with integrated pest management systems.

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GLYPHOSATE RESIDUES IN GROUNDWATER IN SLOVAKIA

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Abstract

Glyphosate is a non-selective herbicide which is strongly sorbed to soil particles. It is widely used in agricultural, urban as well as forestry areas in Slovakia. Although the sorption of this active substance is very strong and mobility towards groundwater is limited there is a high occurrence of glyphosate in groundwater in Slovakia. The exceedance of the limit for groundwater was observed in 29 out of 293 samples by 2021 with a maximum of 8.42 μg/l in one sample. The main reason for high concentrations of glyphosate in groundwater is most probably huge consumption of plant protection products containing this active substance. There are 38 plant protection products registered in Slovakia containing active substance glyphosate with total consumption of more than 300 000 kg per year. The authorisation of new products containing glyphosate is continuing because of good results obtained from the modelling of predicted environmental concentrations in groundwater, which is made for individual products. The regulation of the consumption is needed because when used extensively, monitoring shows high concentrations, often exceeding the limit 0.1 μg/l. This is raising concerns about the potential risks associated with glyphosate exposure through drinking water because of potential health effects, including its possible carcinogenicity and effects on endocrine and immune system.

Keywords: *Glyphosate, Groundwater, Predicted contamination, Monitoring*

Introduction

Glyphosate targets a wide range of weeds and is used to protect corn, soybean and various kind of fruits and vegetables. In addition, glyphosate can be used for no-till and low-till farming to reduce soil erosion, and to facilitate integrated pest management as well as in non-agricultural settings such as forests, parks or public spaces. It is approved for use in railway lines and even for use in water reservoirs, rivers and irrigation channels. Glyphosate is also a key herbicide for production of genetically modified, herbicide resistant crops, what can lead to even higher consumption of this substance (Benbrook, 2012; Clémentine et al., 2020; EPA, 2022).

The huge consumption of the pesticides containing glyphosate raises concerns about the safety of this active substance. Glyphosate has been already worldwide detected in soils, crop products, crop-fed animals, freshwater and organisms living in freshwater (Battaglin et al., 2014; Pérez et al., 2011) but also in groundwater, bottled drinking water or human urine (Rendón-von Osten et Dzul-Caamal, 2017). Numerous studies have indicated a potential link between glyphosate exposure and increased risks of possible carcinogenic and neurotoxic effect (Cattani et al., 2014; Krüger et al., 2014; Portier, 2020). Glyphosate is also being associated with endocrine disruption, which can cause reproductive and developmental issues as well as kidney and liver damage, respiratory and immune system problems and potential birth defects (Benbrook, 2016; Majewski et al., 2014). However, during re-evaluation process of glyphosate, ECHA´s Committee for Risk Assessment (RAC) have discussed the hazard classification of glyphosate and agreed to keep glyphosate´s current classification as causing

serious eye damage and being toxic to aquatic life. Based on new evidence, the committee concluded that classifying glyphosate as a carcinogen or mutagen is not justified (RAC´s opinion, 2022).

It cannot be excluded that health effects observed in organisms exposed to glyphosate containing products are caused by co-formulants in the product. For instance the co-formulant POE-tallowamine was used in glyphosate-based herbicides until 2016. The significant toxicity has been observed in POE-tallowamine (RAC´s opinion, 2022). Concerns were highlighted for its genotoxic potential regarding DNA damage at concentrations not causing cytotoxicity and for potential reproductive and developmental toxicity. POE-tallowamine is more toxic than glyphosate when tested in glyphosate-based formulations (EFSA Journal, 2015b; Gandhi et al., 2021).

Glyphosate is assumed to be rapidly and strongly adsorbed to soil and exhibits low to very high persistence forming one major metabolite AMPA. Glyphosate shows low mobility or even immobility in soil and is unlike to leach into groundwaters (Pérez et al., 2011, EFSA Journal, 2015a). Laboratory and field studies have shown the immobility of glyphosate, but other field studies showed detectable concentrations of glyphosate in flume and streams after applications (Pérez et al., 2011). In natural waters, glyphosate dissipate rapidly due to adsorption, sedimentation and biodegradation. However, slower dissipation was observed in hard waters (Pérez et al., 2007). Based on new evidence, glyphosate is considered as not rapidly degradable and not readily biodegradable but having low potential for bioaccumulation (RAC´s opinion, 2022). Despite its relatively low mobility in soil, groundwater contamination can occur due to several factors. Glyphosate applied to fields can be carried away by spray drift, rainfall or irrigation water, leading to runoff into nearby surface water bodies, from where it can be infiltrated and to get into groundwater (Battaglin et al., 2014). Significant pathway for glyphosate to reach groundwater is through runoff and erosion. Runoff is more likely to occur in areas with high rainfall or intense irrigation practices. Leaching occurs when glyphosate moves downward through the soil profile and reaches the saturated zone, where groundwater is present. Factors that influence glyphosate leaching include soil properties, such as texture and organic matter content as well as climatic conditions and application practices (Barber et al., 2014). Some authors have reported that glyphosate can be easily desorbed from soil and has the potential to become extensively mobile in the soil environment (Pérez et al., 2011). Adsorption to soil particles is determined by chemical and physical characteristic of the soil. The release from soil particles could be potentially caused by addition of inorganic phosphorus through competition for sorption sites (Pérez et al., 2011). In some cases, plant protection products containing glyphosate are directly applied in aquatic ecosystems what can lead to infiltration and percolation to groundwater (Battaglin et al., 2014).

The persistence of glyphosate in the environment combined with its frequent and extensive use can contribute to long-term contamination of groundwater. Its persistence allows it to remain in the environment for longer periods of time, when repeated applications and the accumulation of glyphosate in soil can increase the likelihood of leaching to groundwater (Ruark et al., 2016).

Material and Methods

Glyphosate is the herbicide with the biggest consumption in Slovakia. The consumption of substances is reported in accordance with the Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No. 491/2011 Coll. to The Central Control and Testing Institute in Agriculture (ÚKSÚP). In 2021, 346 157 kg of the active substance glyphosate was used in Slovakia, what is nearly 20 % of total amount of all applied pesticides. The consumption has growing tendency, which was in 2021 more than doubled compared to year 2011 (Figure 1). In Slovakia, glyphosate is authorized for use in the most cultivated crops such us cereals, oilseed rape and corn, in various types of fruits and vegetables, in orchards, grasslands, woodlands, railways, in lands not intended to wear vegetation as well as in water reservoirs, waterways and irrigation canals. *In total, 38 glyphosate based plant protection products are authorized in Slovakia (Vestník MPaRZV SR, 2022).*

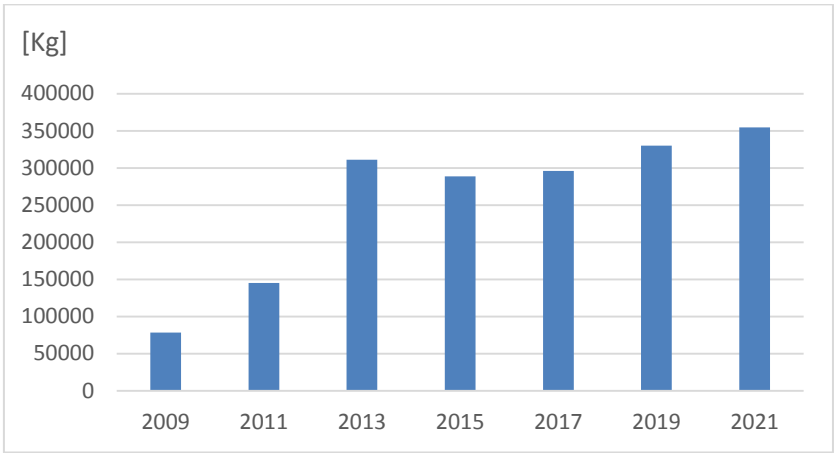

Figure 1: Total consumption of glyphosate in Slovakia per year [kg]

The authorization of plant protection product is conditioned by low concentrations resulting from modelling of predicted environmental concentrations in groundwater *(PECgw).* Among main parameters entering to groundwater modelling are half live, soil adsorption coefficient, Freundlich sorption exponent and plant uptake factor coefficient. In the case of glyphosate, which has low geometric mean of half live and very high adsorption coefficient, resulting concentration are very low, even if high dose of the active substance has been applied. *Values PECgw for glyphosate* <0.001 μg/L (EFSA Journal, 2015a) are well below the limit 0.1 μg/L, what is parametric value for the individual pesticide allowed in water intended for human consumption (Directive (EU) 2020/2184). There is the tiered approach in the groundwater risk assessment. The modelling of the predicted environmental concentrations is the first tier and since this tier shows the results which comply with the requirements and meet the limit values, there is no need for performing higher tiers of risk assessment. The first tier represents the worst case scenario. It is more conservative compared to higher tiers such us leaching studies, lysimeter studies or monitoring, which stand for more realistic assessment of the leaching behaviour of the active substance (SANCO/13144/2010). Because of these reasons, the risk evaluation process of pesticides containing glyphosate is readily done and authorization can be granted.

The targeted monitoring of glyphosate in groundwater was conducted in Slovakia in 2020 and 2021, with 80 monitoring objects in 2020 and 102 objects in 2021. The monitoring was realized by Water Research Institute due to high risk associated with glyphosate in surface waters, since many underground sources of drinking water are in areas where high interaction between surface and groundwater occurs. The limit concentration 0.1 μg/L was exceeded in

9.9 % of measured samples. The maximum of 8.5 μg/L was reached in one measured sample, while other exceedances of the limit ranged between 0.1 – 0.33 μg/L. In 21.5 % of samples the national limit 0.05 μg/L (limit for exclusion of use from the protection zone of water resources) was exceeded (Monitoring WRI 2009-2021). It must be mentioned that the detection limit for glyphosate was 0.075 μg/L, so number of samples with concentrations of glyphosate over 0.05 μg/L may be higher.

Results and discussion

According to EFSA conclusion on glyphosate, further information is needed to assess the contamination route through run off, especially in situations where application to hard surfaces may occur as well as subsequent surface water contamination and bank infiltration to groundwater. In addition, it is necessary to investigate the degradation of the major soil metabolite AMPA in acidic soils. Glyphosate and its metabolite AMPA have specific ionic characteristics due to which, leaching mechanisms and routes simulated by FOCUS GW models may not be the most relevant to assess the potential of leaching to groundwater (EFSA Journal, 2015a). *EFSA conclusion on active substance glyphosate states that the active substance glyphosate as well as its metabolite AMPA have been detected in groundwater in Europe above the parametric limit 0.1* μg/L during monitoring in Italy, Germany, Sweden, France, Spain and The Netherlands. It was assumed that the high concentrations may not be directly related to representative uses and other authorised good agricultural practices, but can be caused by misuses; however the origin of individual results was not specified (EFSA Journal, 2015a). Since re-evaluation process for glyphosate is ongoing, new EFSA conclusion on active substance glyphosate is expected in 2023 which may clarify some processes and fulfil the data gaps. Although according to RAC´s opinion glyphosate is not the carcinogenic substance, the fact is, that drinking water sources in Slovakia are contaminated with this substance exceeding parameter limit. The biggest problem is wide and enormous consumption of this herbicide and not fully reliable prediction of its concentrations in groundwater. Prediction of the glyphosate leaching to groundwater for individual products does not reflect the reality, when big amount of the products containing this substance is applied. The contamination caused by one product may not be significant or can be even negligible, but sum of all uses may become problematic.

Conclusions

Contamination of groundwater by glyphosate is a growing concern due to its widespread use, its ubiquity as well as potential health effects. Monitoring in Slovakia has shown the presence of glyphosate in various samples of groundwater. Although glyphosate may not exhibit high mobility in soil, the combination of runoff, leaching, accumulation and contaminant transport can lead to groundwater contamination. Understanding these pathways is important for effective management strategies and the protection of groundwater resources. It is very important for regulatory agencies to continue monitoring and regulating glyphosate use to minimize contamination and protect human´s health. Glyphosate and its metabolite AMPA were selected among monitored indicators in groundwater in the Slovak State Hydrological Network for the following years, according to Framework program for water monitoring in Slovakia for the period of 2022-2027. There is also need for further research to better understand the risks associated with huge consumption of glyphosate-based products and its occurrence in groundwater as well as to develop effective mitigation strategies.

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SUSTAINABILITY IN OLIVE OIL PACKAGING

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Abstract

Packaging of olive oil is highly crucial to prevent quality deterioration. decreasing oxygen and eliminating light are important aspects in decreasing lipid oxidation and flavor development, and so preserving olive oil quality. The absorption of aromatic components in olive oil by polymeric resin materials during storage can considerably impair oil quality by causing a partial loss of oil scent. Large stainless steel containers (capacity 10-20 tons) (bulk packed) with the ability to discharge nitrogen gas into the container's headspace are the ideal storage materials. Glass, metals, polyethylene terephthalate, composites, and active and new novel packaging materials are the most common commercial packaging materials used to preserve the quality of olive oil. Foods packaged in innovative ways leave less of an environmental effect. Although virgin olive oil is frequently packaged and distributed in dark glass, the growing interest in sustainability necessitates specific consideration in the selection of packaging materials. Package has a little environmental impact. According to this theory, flexible packaging materials with unique oxygen barrier capabilities can be employed to preserve the extra virgin olive oil's finest quality while posing less environmental risks. Future developments are undoubtedly predicted given the amount of attention this topic has received, including from the scientific community. It's likely that some materials will be improved and new container types will be suggested for a market for olive oil that is more environmentally friendly.

Keywords: *Extra virgin olive oil, packaging, active and sustainable packaging*.

Introduction

Extra virgin olive oil is produced by harvesting, washing, crushing, and malaxing olives. The oil phase is then separated and centrifuged. Olive oil is a primary source of lipids in the Mediterranean diet and contains a number of biologically active components. It is a functional food that is high in monounsaturated fatty acids (MUFAs) and contains a number of trace elements with biological qualities. Among the beneficial lipids with antioxidant and protecting effects was extra virgin olive oil (Kiritsakis and Keceli, 2023). In the 2021/22 crop year, global olive oil output totaled 3 098 500 tons (IOC, 2023). The Mediterranean countries generate 93.9% of this, including Spain, Italy, Greece, Tunisia, Turkey, Morocco, Algeria, Syria, and Portugal. Given its health-promoting characteristics, it is projected that demand for olive oil, particularly EVOO, would rise in the near future (Accorsi *et al*., 2015). Extra virgin olive oil contains saponifiable fraction (98-99%) made up of triacylglycerols and such as oleic, linoleic, palmitic, and stearic acids, as well as minor unsaponifiable fraction (1-2%) contains vitamins, such as tocopherols, polyphenols (primarily tyrosol, hydroxytyrosol, oleuropein), sterols, phospholipids, waxes, squalene, other hydrocarbons. One of the key health benefits of olive oil is oleic acid. Phenolic chemicals, oleic acid, polyunsaturated fatty acids, phytosterols, squalene, and tocopherols are beneficial to consumer health (Kritsakis *et al*., 2020; Kiritsakis and Keceli, 2023; Kiritsakis *et al*., 2023).
However, due of its sensitivity to numerous elements such as oxygen, light, and elevated temperatures, it must be properly processed, packaged, and stored under prescribed settings in

order to keep its long-term therapeutic characteristics. Such settings ensure that the food does not deteriorate or change in terms of nutritional content or sensory qualities (Kiritsakis *et al*., 2002; Kanavouras *et al*., 2006). The preservation of olive oil's beneficial qualities is critical for the olive oil industry during the transition from production to purchasing and consumption. Proper olive oil packing and bottling is regarded as crucial for minimizing quality deterioration prior to consumption. After pressing, the oils are moved to tanks and poured into storage containers, where they acquire their sensory characteristics. Containers vary in size and substance; they are generally composed of AISI 304 inox steel, which is also used in containers for olive oil purification. The packaging in the selling container is the final unit operation: if done correctly, it is critical for product stability, maintaining high quality levels, and adding value. Reduced oxygen in the packaging headspace and light exposure are important variables in minimizing lipid oxidation and off-flavor development, and so maintaining olive oil quality. (Piscopo and Poiana, 2012).

Olive oil packaging method

The restriction of consumer sales of on-tap olive oils was sanctioned by Regulation 1019/2002/CE. This effect encourages consumer protection in terms of product quality as well as the valorization of agricultural identity. In this sense, olive farms might offer packaging or request it from outside companies. In the case of other foods, the oil storage and packing mill area must be kept clean, aired, and lit, and all unit operations must adhere to the hygienic-health requirements outlined in Regulation 852/2004/CE. As previously said, olive oil should be stored at a consistent temperature of 14-15 °C, away from light and air.

When storing oils in large containers (inox steel is the best material), the headspace should be kept as full as possible or filled with nitrogen. For lesser quantities, depression bottling and pre-bottling with inert gas are now diffused by utilizing bottlers device that saturate the headspace with nitrogen during the bottle filling operation. Bottles are connected to a special machine that inverts them and blows inside liquid nitrogen, which turns into gas when it comes into touch with the environment and dilutes and pushes out the oxygen contained in the free volume of the bottle. The bottle is then straightened and moved on to the filling operation, during which nitrogen remains inside because it is heavier than air. As the bottle fills and the oil level rises, the nitrogen is transferred outside, preventing oxygen penetration. The pace of consecutive capping ensures that only nitrogen is present between the cap and the oil. Using inert gases such as argon and nitrogen can address various problems and enable excellent product preservation across multiple manufacturing procedures such as pressing, kneading, and, most importantly, storing and bottling. A study found that extra virgin olive oils obtained from the previous harvesting season and held under a nitrogen atmosphere could be packaged in glass bottles with no discernible quality changes compared to seasonal extra virgin olive oil packaged in comparable bottles (Guil-Guerrero and Urda-Romacho, 2009). Nitrogen is the most commonly employed gas to protect the oil from air contact because it is capable of removing oxygen from container headspace and pre-formed volatile pollutants. Bottling lines are quite versatile in terms of packaging typologies and are very easy to clean. A previous study on Italian plant typologies indicated that the semi-automated packaging line is the most common (50%), followed by the automatic (27%) and manual (23%). The first and third exhibit a low level of process specialization and a limited bottling capacity. Bottle capping can be done with cork or, more commonly, with metal caps. A good quality cork must not be porous or contain fungal pollutants; yet, due to its high oxygen permeability, it is not recommended. Metal caps with a screw and a plastic soft lining allow for a flawless airtight acting as a dripper. A thermally retractable capsule can also be featured on the cap to seal the packing and improve the product's aesthetics. Only these last are the most popular

(72%) compared to those with a basic screw (15%) and cork (11%) (Piscopo and Pionna, 2012).

Effect of packaging materials on olive oil quality

The effectiveness of several active packaging options in protecting the quality of EVOO during storage, as well as their influence on shelf life, were evaluated using an examination of the primary olive oil quality indicators. According to Regulation 1019/2002/CE (European Union, 2002), olive oil may only be sold in standardized, packaged form. Olive oil must be stored at a constant temperature of 14-15 °C, away from light and oxygen, until it is ready for sale packing. Large tanks (capacity 10-20 tons) composed of stainless steel (bulk packaging) with the ability to flush their headspace with nitrogen are the ideal packaging material for such applications. Several studies have been conducted over the last decade to determine the viability of various retail packaging materials for preserving the quality of olive oil. Glass, metals (tinplate and stainless steel), polymers (mostly PET), and composites (Tetra Brik/Tetra Prisma, bag-in-box, and single-use multilayered sachets) are the most common commercial packaging materials used for this purpose. In addition to these integrated tasks, new and creative packaging materials should be designed to decrease their persistence in the environment, addressing the long-term critical environmental issue of plastics disposal. Simultaneously, modern packaging is an important aspect in addressing rising difficulties of sustainable food consumption, which includes reducing the environmental impact of packaged food. Plastics are mistakenly perceived as having the greatest environmental footprint because they are almost exclusively viewed from an end-of-life (EOL) perspective, with no consideration of material recyclability or the impacts associated with the production and transportation of the packaging materials (Farris *et al*, 2021). Oil packaging into PET bottles has increased in recent years. Nonetheless, PET monomers, oligomers, colorants, and breakdown products are all susceptible to migration. Acetaldehyde migration from PET bottles is a big issue since its presence might impair the sensory characteristics of oil. Several studies have demonstrated that plastic packing materials can absorb significant amounts of fragrance molecules, resulting in a loss of aroma intensity or an imbalanced flavor of the food. Several strategies have been proposed to minimize the diffusion of O2 into bottled olive oil. The most common is the use of oxygen scavengers (OSs), which remove dissolved oxygen in the oil as well as that present in the bottle headspace. An OS also adsorbs oxygen penetrating through the bottle wall into the oil during storage in the case of plastic packaging. OSs can readily be included into packaging materials without affecting their other qualities. Sensory examination revealed an earlier production of off-flavors (rancid) in oils stored in bottles without OS and UV-Vis absorber. The packaging with the UV-Vis light radiation barrier effect outperformed all others in terms of restricting oil quality decline and oxidative degradation.

Conclusions

Storage and packaging of olive oil are the last steps in the production process and are just as significant as the others. Deterioration agents can reduce the quality of this crucial food even during these unit operations, thus proper control and monitoring of some indicators can help extend the shelf life of olive oil. The type of packaging also affects the stored olive oil qualities, with various results depending on the material. Various containers were used and reviewed throughout time, with clear input for qualitative aspects (some materials were forbidden for health reasons). Furthermore, traditional usage can give rise to differing preferences (for example, in Italy, glass is particularly difficult to substitute aside from its established value as a packaging medium for olive oil). A variety of modern materials are suitable for olive oil packaging. The ideal option for olive oil packaging is dark-tinted glass,

preferably placed inside a paperboard or corrugated fiberboard container. Alternatively, olive oil can be stored in tinplate containers, which, while less inert than glass, provide adequate protection from both oxygen and light. Finally, plastics (PET) and composites (bag-in-box and Tetra Brik/Tetra Prisma type containers) are suitable packaging materials for olive oil, but the latter requires additional research. Despite the widespread use of glass as the primary material for packaging and distribution of extra-virgin olive oil, growing sustainability consciousness necessitates careful consideration of packaging materials with a lower overall environmental impact. According to this viewpoint, considering new materials other than glass bottles appears to be a trend that will continue in the next years, particularly in emerging economies. In this study, we showed that a flexible packaging material with excellent oxygen barrier performance may be used efficiently for extra-virgin olive oil, outperforming traditional glass on the most important quality metrics.

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MICROBIOLOGICAL PURITY IN PRODUCTION, PROCESSING AND FOOD DISTRIBUTION

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Abstract

Samples of swabs of equipment, devices, utensils, work surfaces, work clothes and hands of workers in production, processing and traffic in facilities and means of transport that come into contact with food, where there is a risk of the appearance and spread of an infectious disease, were used as test material. The samples come from facilities for the production, processing and distribution of food, restaurants and other catering establishments where food is served, facilities in the field of upbringing, education and social protection and means of transport that come into contact with food. The examination was carried out during 2021 and included 4,078 samples. The aim of the research was to assess the state of microbiological purity in the food chain. For microbiological testing used methods BAS EN ISO 4833-1, BAS EN ISO 21528-2, BAS EN ISO 11290-1 and BAS EN ISO 6579-1. Of the total number of swab samples examined, 97.52% were satisfactory and 2.48% were unsatisfactory. Observed in relation to the total number of tested samples, 85.59% of samples were unsatisfactory due to an increase in colony count and 14.41% due to increased number of *Enterobacteriaceae*. Pathogens *Salmonella* and *Listeria monocytogenes* were not isolated in any swab sample. The general level of hygiene in food production and distribution is at a high level, but there is a risk of contamination with *Enterobacteriaceae*, which are indicators of the hygiene of the production process. The risk of *Salmonella* in the business of meat, meat products and semi-products and *Listeria monocytogenes* in the business of ready-to-eat food is minimal.

Keywords: *Microbiological purity, Swab, Food chain.*

Introduction

The current regulation defines the criteria for microbiological purity and the frequency of control of equipment, devices, accessories, work surfaces, work clothes and hands of food handlers in production and traffic in facilities and means of transport that come into contact with food, in which there is a risk of the appearance and spread of an infectious disease (FSA, 2013; Official Gazette, 2018; Official Gazette, 2019). It is common for hygiene checks in facilities to be directed to surfaces that are most often in contact with food (Watnick and Kolter, 2000).

One of the main risks of food contamination comes from the work process of food handling personnel and microorganisms, the cause of disease, that are present in or on the personnel, which are then transferred from the personnel to the food during the handling process. (Gordon-Davis, 1998). Food handlers must maintain a high level of personal hygiene and wear appropriate protective clothing, and be trained in food hygiene requirements (Gill and Jones, 1999). Training of food handling personnel in relation to the basic concept and requirements of personal hygiene is an integral part of the measures to obtain a safe product for the consumer (Adams and Moss, 1997). Food handling areas, conveyances, items, utensils and equipment that come into contact with food must be regularly cleaned and disinfected to avoid any risk of contamination, and food and staff must be regularly monitored (Official

Gazette, 2004). Improper cleaning and disinfection are directly related to the occurrence of various foodborne diseases (Johns, 1991). The absence of *Listeria monocytogenes* indicates an effective cleaning and disinfection program (Samelis and Metaxopoulos, 1999). The assessment of surface hygiene is mainly based on the determination of the colony count and *Enterobacteriacea* per cm^2 **(**Aarnisalo *et al*., 2006**)**. Sometimes testing is done for the presence of other microorganisms, because it has been proven that *Salmonella spp., Shigella spp., Escherichia coli, Staphylococcus aureus, Bacillus cereus* and faecal streptococci originate from infected food handlers (Lawrie, 1998). Due to the risk to public health, mandatory tests for *Listeria monocytogenes* in ready-to-eat foods and for *Salmonella* in meat, semi-finished products and meat products are carried out in production, processing and trade (Official Gazette, 2012; Official Gazette, 2019).
The aim of the research was to assess the state of microbiological purity in the food chain.

Material and methods

Samples of swabs of equipment, devices, utensils, work surfaces, work clothes and hands of workers in production, processing and traffic in facilities and means of transport that come into contact with food, where there is a risk of the appearance and spread of an infectious disease, were used as test material. The samples come from facilities for the production, processing and distribution of food, restaurants and other catering establishments where food is served, facilities in the field of upbringing, education and social protection and means of transport that come into contact with food. The examination was carried out during 2021 and included 4,078 samples.
Sampling and transport of samples was carried out according to BAS ISO 18593 (ISBIH, 2008).
Evaluation of the conformity of samples in relation to the colony count and *Enterobacteriaceae* (Official Gazette, 2012; FSA, 2013; Official Gazette 2018), *Salmonella* (Official Gazette, 2019) i *Listeria monocytogenes* (Official Gazette, 2012; Official Gazette 2019), was carried out on the basis of the categories of the tested samples and the microbiological criteria shown in Table 1.:

Table 1. Sample categories and microbiological purity criteria

Sample categories	Colony count	*Enterobacteriaceae*	*Listeria monocytogenes*	*Salmonella*
Porcelain, glass, smooth metal surfaces cfu/cm^2	≤10	0-1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Other surfaces (wooden, plastic, stone) cfu/cm^2	≤30	0-1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Plates, bowls, cutlery and small dishes; dishes and utensils that come in contact with food cfu/ml (cm^2)	≤100	0-1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Hands of food handlers cfu/ml (cm^2)	≤200	0-1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Bottles or containers for liquid products cfu/ml	0-1	0-1	Absence in swab	Absence in swab

cfu: colony forming units

For microbiological testing used methods BAS EN ISO 4833-1 for colony count (ISBIH, 2006), BAS EN ISO 21528-2 for *Enterobacteriaceae (*ISBIH, 2018c), BAS EN ISO 11290-1 for *Listeria monocytogenes* (ISBIH, 2018b) and BAS EN ISO 6579-1 for *Salmonella* (ISBIH, 2018a).
In our research and analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables and figures.

Results and discussion

Microbiological purity criteria are an important link in the microbiology of the food chain. The occurrence of food-borne diseases in 81% of cases is the result of food contamination during the production of which the principles of good production practice were not observed (Rašeta *et al*., 2012). The structure of the tested swab samples by category is as follows (Figure 1.).

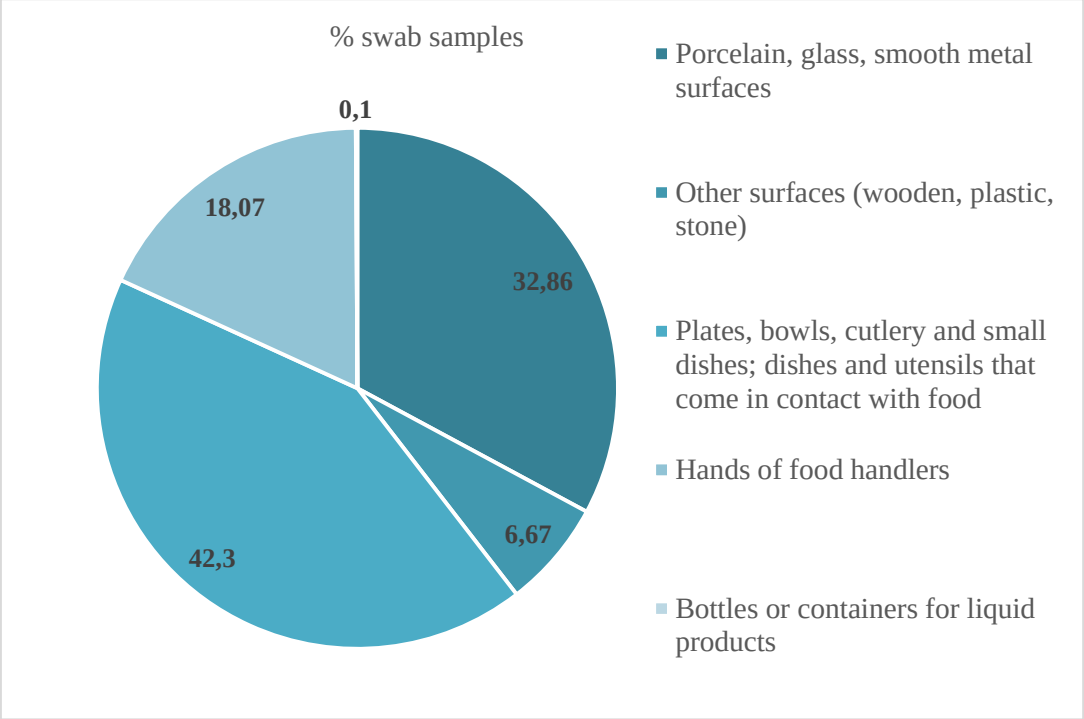

Figure 1. Samples of swabs by category

The structure of the tested samples in relation to the category was similar to the study by Golić *et al*. (2019). A negligibly small number of swab samples referred to the category "bottles or containers for liquid products" for the reason that a very small number of subjects in the food business, which were included in this research, use this type of samples. Therefore, the test results of these samples are presented, but not taken into account for the discussion, because we considered that the number of samples is not representative in relation to the total number of samples included in the research.
The structure of the tested swab samples in relation to the test parameter is shown on Figure 2.

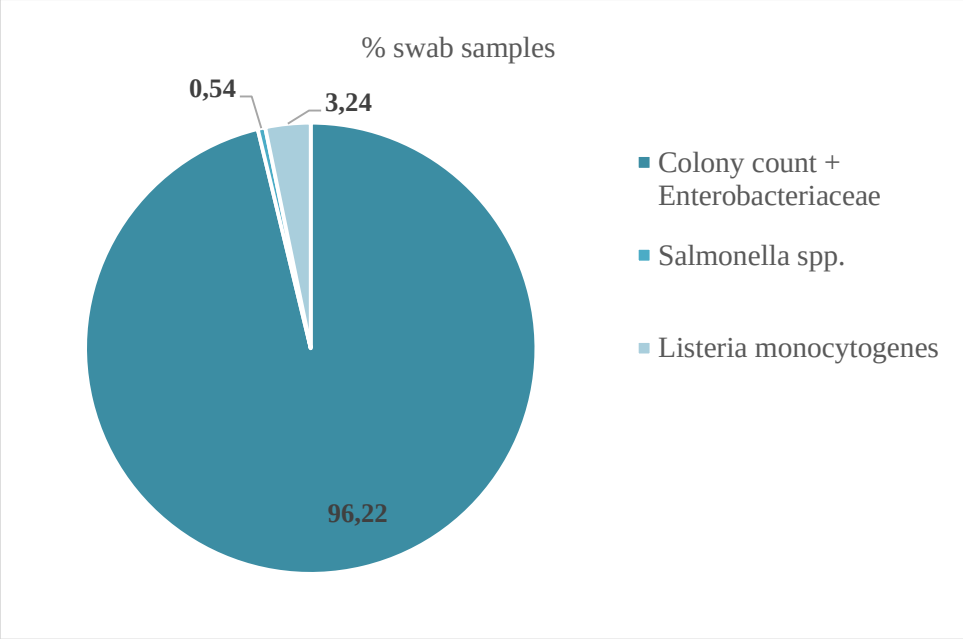

Figure 2. Swab samples by test parameter

The structure of the tested samples in relation to the test parameter was similar to the study by Golić *et al*. (2019) for colony count, *Enterobacteriaceae* and *Salmonella spp*.
Comparing the obtained test results with the microbiological purity criteria, the acceptability of the microbiological purity of the sample is evaluated. A sample is of acceptable microbiological purity if it meets the microbiological criteria. If it contains up to 50% more microorganisms, the sample can be of acceptable microbiological purity, provided that it does not contain pathogenic bacteria (Official Gazette, 2018; Official Gazette, 2019).
Of the total number of swab samples examined, 97.52% were satisfactory and 2.48% were unsatisfactory. Of the unsatisfactory samples, 77.19% were due to an increased colony count, and 22.81% were due to an increased number of *Enterobacteriaceae*.
In some cases, it is necessary to determine the presence of *Listeria monocytogenes*, as the cause of listeriosis, a serious infectious disease of humans and animals, on surfaces that come into contact with food (Aguado *et al*., 2001; Lundén *et al*., 2002; Lundén *et al*., 2003; Suihko *et al*., 2002; Fonnesbech-Vogel *et al*., 2001). In the process of food production, the environment is a more significant source of *Listeria monocytogenes* than live animals and carcasses, so special attention should be paid to the cleaning and disinfection process (Samelis and Metaxopoulos, 1999). In our study, the pathogens *Salmonella* and *Listeria monocytogenes* were not isolated in any swab sample. These results are in accordance with the results obtained by Kalaba *et al*. (2017) and Golić *et al*. (2019).
Figure 3. shows the test results of swab samples by category.

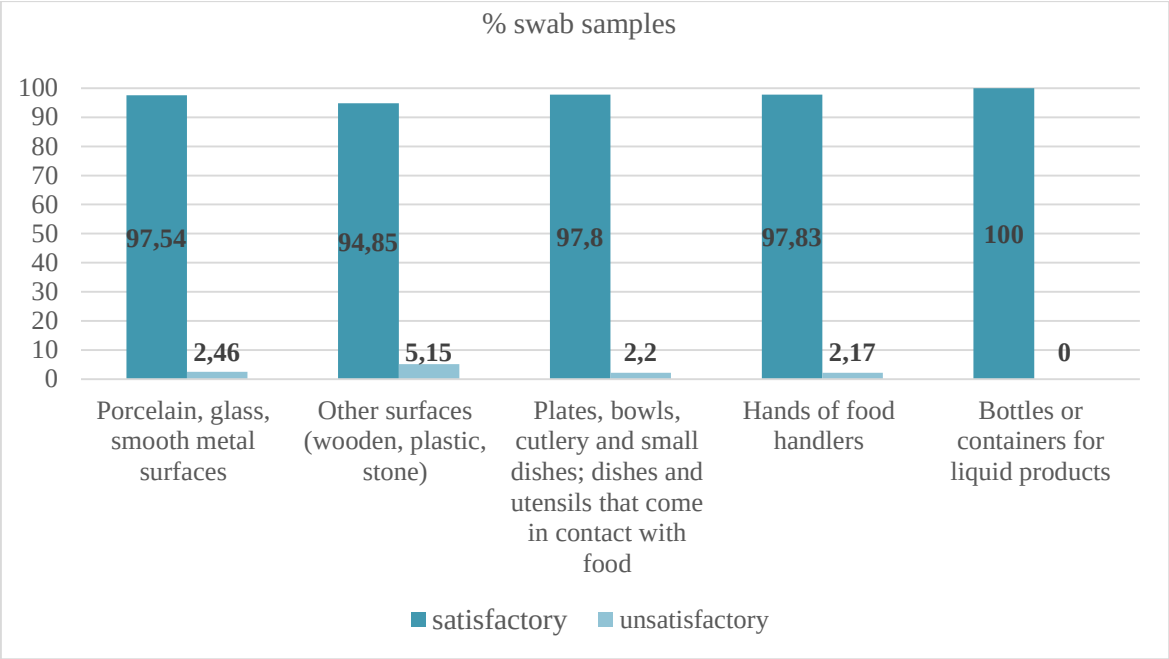

Figure 3. Test results of swab samples by category

The test results are in accordance with the results obtained by Ivanović *et al*. (2013) and Golić *et al*. (2019), in contrast to the results of Kalaba *et al*. (2017).
The representation of unsatisfactory swab samples by category is shown in Figure 4.

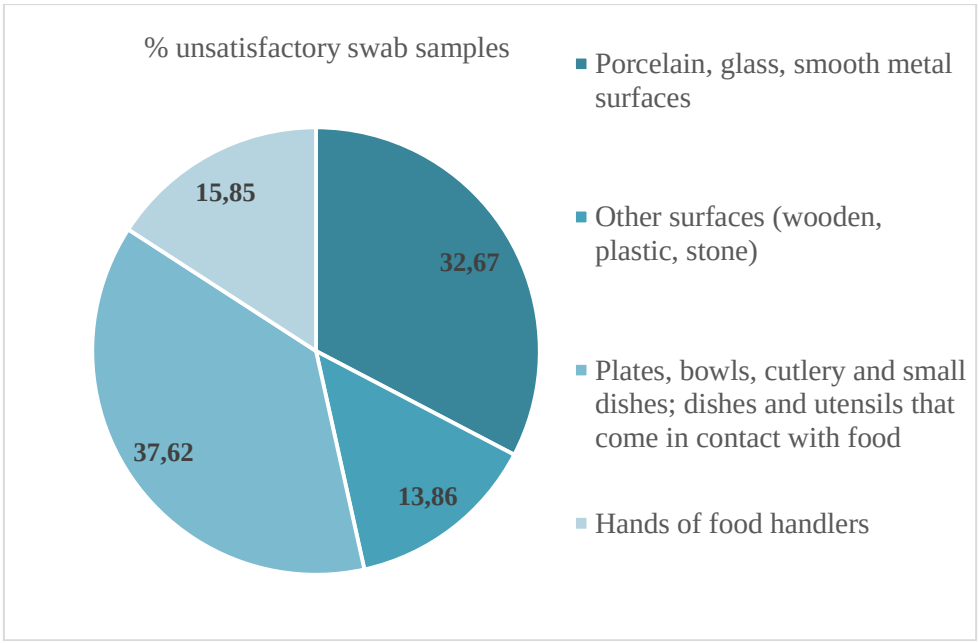

Figure 4. Representation of unsatisfactory swab samples by category

The most unsatisfactory samples were from the categories "Plates, bowls, cutlery and small dishes; dishes and utensils that come in contact with food", which is are in accordance with the conclusions of Watnick and Kolter (2000).
Figure 5. shows the results of unsatisfactory swab samples by category in relation to the tested parameter.

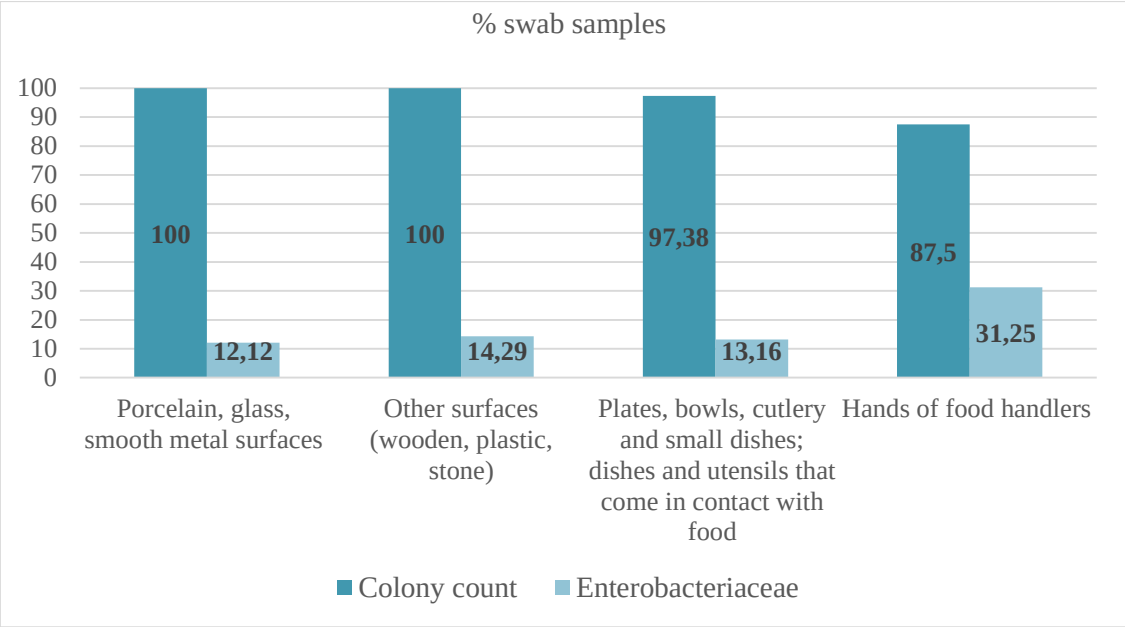

Figure 5. Test results of unsatisfactory swab samples by category in relation to the test parameter

When looking at the results of unsatisfactory swab samples by category in relation to the investigated parameter, it is observed that most of the samples were unsatisfactory due to an increased colony count, while significantly fewer unsatisfactory samples were due to an increased number of *Enterobacteriaceae*, which is in accordance with the results of Golić *et al*. (2019). Also, in accordance with the mentioned research, we determined that the category "hands of persons in contact with food" had the least unsatisfactory samples due to an increased colony count, but also the most unsatisfactory samples due to an increased number of Enterobacteriaceae (almost one third of unsatisfactory samples from this category) which is of great concern given the high prevalence of *Enterobacteriaceae* in the hands of food handlers.

Conclusion

The general level of hygiene in food production and distribution is at a high level, but there is a risk of contamination with *Enterobacteriaceae*, especially in food handlers, which are indicators of the hygiene of the production process. The risk of *Salmonella* in the business of meat, meat products and semi-products and *Listeria monocytogenes* in the business of ready-to-eat food is minimal.

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VARIABILITY OF TWO-YEAR-OLD SEEDLINGS OF *GINKGO BILOBA* L. GROWN FROM SEED COLLECTED IN THE CITY AREA OF BELGRADE (SERBIA)

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Abstract

Ginkgo biloba L. (*Ginkgoaceae*), known as ginkgo, is a species of gymnosperm tree native to China, but also a popular ornamental tree in Europe and North America. It is a very suitable species for cultivation in shelterbelts, multiple-purpose plantations, and urban conditions. On the territory of the Republic of Serbia, ginkgo occurs in green areas mainly as individual trees, or in smaller groups, rarely in tree rows. In the wider zone of the city of Belgrade, 94 trees are found. The analysis of several morphological traits and phenotypic characteristics of two-year-old ginkgo seedlings in the test with seven half-sib lines sampled from green spaces of Belgrade was conducted in order to provide the basis for planting stock selection and the establishment of plantations adapted to urban conditions. The obtained numerical data was processed employing descriptive statistics and analysis of variance (ANOVA) with post hoc Fisher's least significant difference (LSD) test. Compared to the other sampled trees, the progeny of a tree sampled in Zemunski Quay have superior phenotypic characteristics [number of leaves /arithmetic mean ± standard deviation/ 12.60 ± 1.55; seedling height (cm) 18.97 ± 2.57; root collar diameter (mm) 5.91 ± 0.93; mass of above-ground part (g) 1.40 ± 0.38; mass of under-ground part (g) 2.62 ± 0.80], representing a promising source of reproductive material for plant production. ANOVA and LSD show that, in most cases, there are statistically significant differences between the mean values of the analyzed characters, suggesting there is a significant variability among the analyzed genotypes.

Keywords: *Ginkgo, morphometric variability, plant stock selection, urban greenery, Belgrade*

Introduction

Maidenhair tree or ginkgo (*Ginkgo biloba* L.) is a dioecious species of gymnosperm, a stenoendemic of the eastern part of China and a secondary wild species in other parts of the country, Japan and Korea. It is the last living species in the order Ginkgoales and the family *Ginkgoaceae*, which first appeared over 250 million years ago. It is a large tree, reaching a height of 40 m and a trunk diameter of up to 2.5 m. Owing to its decorative fan-like two-part leaves which have a characteristic venation and turn deep saffron yellow in autumn, ginkgo is often used as an ornamental tree worldwide, especially in Europe and North America (Vilotić, 2004). Ginkgo is a very suitable tree species for cultivation in shelterbelts, multiple-purpose plantations, and urban conditions (Šijačić-Nikolić et al., 2009). It is classified into the group of most resistant ornamental tree species, tolerating the conditions in urban environments and showing high resistance to low temperatures and pathogens (Vilotić, 2004; Ocokoljić and Grbić, 2009; Ocokoljić et al., 2011). Ginkgo is also efficient in the storage of pollutant

concentrations, which justifies its utilization in urban green areas (Ocokoljić et al., 2011). In addition, its wood is used in the wood industry, leaves for pharmaceutical purposes, and seeds as food or drug (Vilotić, 2004; Šijačić-Nikolić et al., 2009).

On the territory of the Republic of Serbia, ginkgo occurs in green areas mainly as individual trees or in smaller groups, very rarely in tree rows. The origin of the planting stock is not known, but it is assumed that the seeds and seedlings were not brought from Asia and that they are of European origin. There are 55 trees of ginkgo above the age of 100 years that are protected by the law. In the wider area of the city of Belgrade, 94 trees are known to exist (Vilotić, 2004).

As a result of its multiple benefit value, this species has been increasingly studied in Serbia in the past 20 years (e.g., Vilotić, 2004; Knežević and Tucović, 2005; Šijačić-Nikolić et al., 2006a, 2006b, 2009; Ocokoljić and Grbić, 2009; Jovanović, 2010; Ocokoljić et al., 2011). The study of individual variability of trees has considerable significance for genetic studies, whose results could be applied to the production of nursery stock (Stilinović and Tucović, 1975). Ecological adaptability can also be confirmed by morphologically variable characteristics (Lavadinović and Marković, 2012) and the variability of seedling characters can be the indicator of further seedling development, which emphasizes the significance of such research for the advancement of the technology of plant production (Šijačić-Nikolić et al., 2009). Bearing in mind the aforementioned information, this paper analyzes several morphological traits and growth elements of two-year-old ginkgo seedlings in the test with seven half-sib lines sampled from green spaces of Belgrade city, in order to provide the basis for planting stock selection and the establishment of plantations adapted to urban conditions.

Materials and Methods

Depending on the yield quantity, seven mother trees appearing individually on five green areas of Belgrade city were selected (Table 1; Fig. 1) and used as seed sources for the establishment of the experiment and the production of half-sib progeny. After preparation, the seeds were sown in plastic tubs ("Plantagrah") in a mixture of peat, sand and forest humus (2:2:1). During winter and early spring, the tubs were stored in the greenhouse of the Nursery of the Faculty of Forestry, University of Belgrade, and transferred to the open in May. Applying intensive tending measures, the seedlings were grown in containers placed on a flat surface in seclusion, at an altitude of 127 m (44°46'59"N, 20°25'31"E).

Table 1. Analyzed ginkgo mother trees on green spaces in Belgrade city.

Tree mark	Site	Coordinates
T1	Zemun Quay	44°50'35"N, 20°25'01"E
T2	Zemun Quay	44°50'35"N, 20°25'01"E
T3	Zemun Quay	44°50'35"N, 20°25'01"E
T4	Tasmajdan Park	44°48'35"N, 20°28'14"E
T5	Kalemegdan Park	44°49'12"N, 20°27'06"E
T6	Jevremovac Botanical Garden	44°48'53"N, 20°28'26"E
T7	Park Milutin Milankovic	44°47'55"N, 20°27'51"E

Seedling analysis was performed at the end of the second vegetation period on the sample of 15 individuals per half-sib line, covering the variability of the following growth elements and morphometric characters: root collar diameter (mm), seedling height (cm), number of leaves, mass of above-ground part (g), and mass of below-ground part (g). The obtained numerical data was processed employing descriptive and univariate statistical methods. The descriptive statistics included determining the following statistical parameters: minimum value (MIN), maximum value (MAX), mean value ($\bar{X}$), standard deviation (±SD), and coefficient of

variation (CV, %). The statistical significance of differences between the mean values of the analyzed seedling characters was determined by analysis of variance (ANOVA) with post hoc Fisher's least significant difference (LSD) test. All statistical analyses were performed using Statgraphics Centurion ver. XVI.I (2009; Statpoint Technologies, Inc., Warrenton, VA).

Fig. 1. Some of the analyzed ginkgo mother trees on green spaces of Belgrade city and the analyzed seedling characters: (a) Zemun Quay (trees T2, T3, and T1) (photo: Šijačić-Nikolić M., 2005); (b) Park Milutin Milankovic (tree T7) (photo: Jovanović F., 2005); (c) seedling morphometric characters (height, root collar diameter, mass of above-ground part, mass of under-ground part, and number of leaves).

Results and Discussion

The results of the statistical analysis of morphometric characters and growth elements of two-year-old ginkgo seedlings, grown from seed collected in green spaces of Belgrade city, are presented in Table 2 and Fig. 2. Seven half-sib lines were analyzed in this study, but given that the seed germination percentage of tree T2 was insufficient for seedling sampling, the statistical analysis covered the remaining six half-sib lines. It was found that all analyzed seedling characters (except for the mass of the under-ground part) of tree T1 have the highest mean values, and all analyzed seedling characters of tree T3 have the lowest mean values (Table 2; Fig. 2). ANOVA and LSD show that, in most cases, there are statistically significant differences between the mean values of the analyzed characters (excluding root collar diameter) (Table 2), suggesting there is a significant variability among the analyzed genotypes based on the seedling characters at the end of the second vegetation period. In other words, for the most analyzed characters, the progeny of T1 was superior in comparison to the progeny of the other sampled trees. According to literature (Šijačić-Nikolić et al., 2009; Jovanović, 2010), the same mother three, along with T7, had the highest germination percentage and the highest mean values of all seedling characters in the juvenile phase of development, which makes this tree a promising source of seed material for plant production. On the other hand, the poor germination percentage of T2 may be the consequence of poor pollination due to the distance and the lower number of male individuals compared to female individuals on the sampling site in Zemun Quay (Šijačić-Nikolić et al., 2006a). The best

indicators of seedling quality are seedling height, root collar diameter, root length, and the ratio of seedling under-ground part to above-ground part (Stilinović, 1987, 1991), which are conditioned by the genetic constitution of mother trees, the presence of sufficient amount of pollen, the compatibility of parent genotypes, and the environmental conditions in the year of seed formation (Stilinović and Tucović, 1975). Taking into account the presented statistical results and the fact the seedlings were grown in the same environment conditions, the factor of environmental impact can be eliminated and the resulting morphometric variability can be considered as the result of the differences in genotypes.

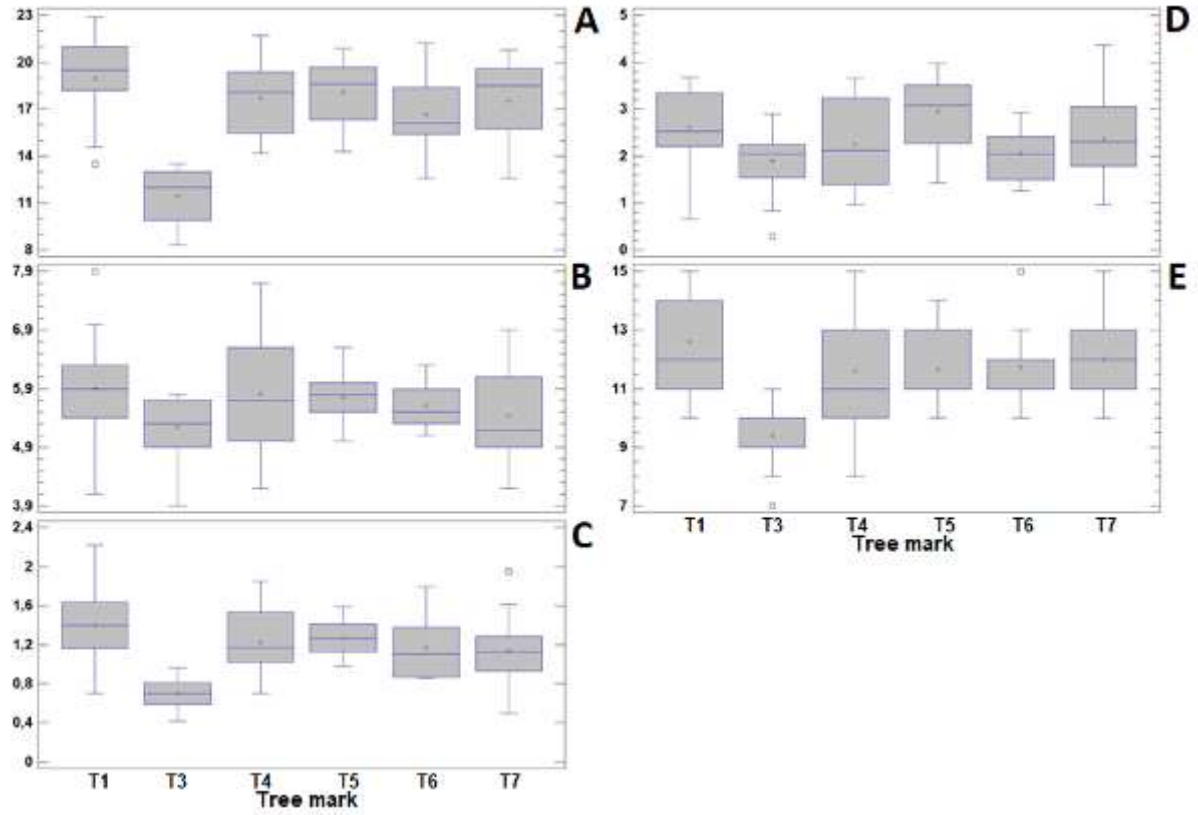

Fig. 2. Box and whisker plots of basic parameters of seedling morphometric characters: (A) seedling height (cm); (B) root collar diameter (mm); (C) mass of above-ground part (g); (D) mass of under-ground part (g); (E) number of leaves. Legend: middle sign = mean, middle line = median, box = mean and standard deviation, whisker = variation range.

The genetic potential of ginkgo mother trees was assessed by the analysis of morphometric traits of their progeny in the two-year stage of development, aiming at the improvement of reproductive material by the detection of superior genotypes. The obtained results conform to the observations made on the progeny of the same trees in the juvenile phase of development (Šijačić-Nikolić et al., 2009; Jovanović, 2010), confirming that there is a significant genetic variability among the analyzed genotypes. The analyses included the quantitative characters controlled by polymer genes, i.e., numerous multiple genes whose effects are added. As father trees are unknown, because the reproduction material originated from free pollination, it can be argued that mother tree T1 has a good genetic constitution given that the results of the analyzed characters were repeated in its half-sib progeny during two successive vegetation periods. Since comparative analysis of seedlings from half-sib lines enables the identification of the planting stock which is significant for further propagation (Ocokoljić et al., 2011), the results of this study can serve as the base for the selection of mother trees which could be the

source of reproductive material for the production of seedlings with targeted characteristics and the establishment of plantations adapted to urban conditions.

Table 2. Statistical analysis of seedling morphometric characters of six ginkgo mother trees.

Tree mark		Seedling height (cm)	Root collar diameter (mm)	Mass of above-ground part (g)	Mass of under-ground part (g)	Number of leaves
T1	$\bar{X}$	18.97 **a**	5.91 **a**	1.40 **a**	2.62 a**b**	12.60 **a**
	MIN	13.50	4.10	0.70	0.65	10
	MAX	22.90	7.90	2.22	3.68	15
	SD	2.57	0.93	0.38	0.80	1.55
	CV%	13.54	15.81	27.03	30.74	12.30
T3	$\bar{X}$	11.45 **c**	5.24 **b**	0.70 **c**	1.90 **c**	9.40 **b**
	MIN	8.30	3.90	0.42	0.30	7
	MAX	13.50	5.80	0.96	2.89	11
	SD	1.67	0.58	0.16	0.67	1.06
	CV%	14.55	11.12	23.11	35.08	11.23
T4	$\bar{X}$	17.71 **ab**	5.81 **a**	1.23 **ab**	2.25 **bc**	11.60 **a**
	MIN	14.20	4.20	0.70	0.96	8
	MAX	21.70	7.70	1.85	3.67	15
	SD	2.39	0.92	0.33	0.94	2.03
	CV%	13.48	15.94	27.06	41.85	17.49
T5	$\bar{X}$	18.09 **ab**	5.75 **ab**	1.26 **ab**	2.95 **a**	11.67 **a**
	MIN	14.30	5.00	0.98	1.43	10
	MAX	20.90	6.00	1.59	3.97	14
	SD	2.01	0.46	0.16	0.77	1.29
	CV%	11.09	8.02	12.92	26.05	11.07
T6	$\bar{X}$	16.63 **b**	5.61 **ab**	1.17 **b**	2.06 **bc**	11.73 **a**
	MIN	12.60	5.10	0.86	1.26	10
	MAX	21.20	6.30	1.79	2.94	15
	SD	2.31	0.38	0.31	0.53	1.28
	CV%	13.91	6.69	26.21	25.47	10.91
T7	$\bar{X}$	17.49 **ab**	5.44 **ab**	1.14 **b**	2.36 **bc**	12.00 **a**
	MIN	12.60	4.20	0.50	0.96	10
	MAX	20.80	6.90	1.95	4.36	15
	SD	2.48	0.76	0.34	0.92	1.36
	CV%	14.17	13.96	29.55	39.03	11.36
ANOVA	F	21.38	1.86	9.85	3.54	8.39
	p	**0.0000**	0.1102	**0.0000**	**0.0059**	**0.0000**

*Note: Boldfaced values denote variables with $p < 0.05$ and any two means within a column not followed by the same letter(s) are significantly different at the corresponding confidence level.

Conclusion

Based on the results of the morphometric analysis of two-year-old ginkgo seedlings grown from the seed material collected in the urban green areas of Belgrade city, it can be concluded that the differences between the mean values are statistically significant for almost all analyzed characters, except for root collar diameter.

Considering the fact that the experimental conditions were homogenous, it can be concluded that the established differences between the progeny of the analyzed ginkgo mother trees are of a genetic nature. This research of progeny at the two-year stage of development confirms the presence of major genetic variability. Most of the analyzed characters have a quantitative character and it is governed by polymeric genes whose effect is additive. The highest mean values of seedling morphometric characters and growth elements were assigned to the

progeny of tree T1, originating from Zemun Quay. Possessing a high genetic potential, this mother tree represents a promising source of seed material for seedling production.
The results of the study should be considered significant information in selecting mother trees for sourcing reproductive material for plant production and the establishment of plantations adapted to urban conditions.

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OVERVIEW OF THE FACTORS THAT INFLUENCE FEED SAFETY AND QUALITY

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Abstract

Feed mills are responsible for producing high-quality animal feed that meets the nutritional needs of various livestock and poultry species. However, animal feed production is a complex process involving multiple stages, including the selection and processing of raw materials, formulation, mixing, and packaging. In addition, the feed industry must comply with several regulations and standards related to quality control and feed safety. The relevance of feed safety systems is essential. Feed contaminated with harmful substances such as mycotoxins and pathogens can pose serious risks to animal and human health. There are many challenges that the feed industry faces in ensuring the safety of its products. To address these challenges feed mills must implement a range of quality control and feed safety measures. The four pillars of quality control and feed safety systems (ingredient quality, process control, finished feed quality, laboratory analysis) are interdependent and interconnected. Each one of these pillars can be impacted by issues related to: poor plant design and construction, deficient employee training, poor employee hygiene, difficult-to-clean equipment, no preventive maintenance, contamination of raw materials, contamination during processing, post-process contamination at the plant, poor plant and equipment sanitation, and incorrect labeling or packaging. This paper will contribute to a better understanding of the usefulness of feed safety systems in feed mills and the ways in which they can ensure the safety and quality of their products.

Keywords: *animal feed, feed safety, mycotoxin, pathogen.*

Introduction

Feed mills are responsible for producing high-quality animal feed that meets the nutritional needs of various livestock and poultry species. However, the production of animal feed is a complex process that involves multiple stages, including the selection and processing of raw materials, formulation, mixing, and packaging. In addition, this sector must comply with a range of regulations and standards related to quality control and feed safety (EC, 2005; EC, 2001).

The value of quality control and feed safety systems in feed mills cannot be overstated. Poor quality feed can lead to reduced animal performance, increased susceptibility to disease, and even death. Moreover, feed contaminated with harmful substances such as toxins and pathogens, can pose serious risks to both animal and human health (Godde, 2021). Therefore, it is crucial for feed producers to implement robust quality control and feed safety systems to attest that their products are safe and of high quality.

Despite the influence of quality control and feed safety systems in feed mills, there are still many challenges they face in ensuring the safety and quality of their products. For example, poor plant design and construction can create opportunities for cross-contamination and other hazards. Deficient employee training and poor employee hygiene can also lead to

contamination of feed during processing. Difficult-to-clean equipment can harbor harmful bacteria and other microorganisms, while a lack of preventive maintenance can increase the risk of equipment failure and contamination. Contamination of raw materials can occur at various stages of the supply chain, and post-process contamination at the plant can also be a concern. In addition, poor plant and equipment sanitation and incorrect labeling or packaging can further compromise the safety and quality of feed products (Motarjemi, 2016).
Effective quality control and feed safety systems can not only reduce the risk of animal and human health hazards, but also improve the overall efficiency and profitability (Aung, 2014).
The purpose of this research paper is to provide a review of the literature on quality control and feed safety systems in feed mills. Specifically, this paper will examine the different challenges that feed operators face in ensuring the safety and quality of their products, as well as the various measures that can be taken to address these challenges.
Overall, this review will contribute to a better understanding of the importance of quality control and feed safety systems in feed mills. Additionally, this paper can serve as a valuable resource for feed mill managers, regulators, and other stakeholders in the animal feed industry.

Materials and methods

The methodology section of this review paper details the process used to collect and consolidate literature concerning feed safety and quality control within feed mills. The objective was to thoroughly explore existing research and literature to offer an insight into the factors that impact feed safety and quality. The review paper seeks to provide an extensive overview of these factors. The methodology encompassed systematic literature search, source selection, analysis of significant findings, and synthesis of information.

Literature Search
A comprehensive literature search was carried out to locate peer-reviewed articles, academic papers, industry reports, and publications related to feed safety, quality control, and associated challenges within feed mills. Databases like PubMed, Scopus, Web of Science, and Google Scholar were extensively used to ensure an inclusive coverage of relevant literature. The search terms employed included "feed safety," "feed quality control," "feed mills," "contamination in animal feed," and similar keywords. The search encompassed a two decade time frame to ensure an all-encompassing representation of available literature.

Source Selection Criteria
Sources were meticulously chosen in accordance with their alignment to the review's focus. Particular attention was directed towards sources published over the last two decades, aiming to encompass contemporary advancements and insights in feed safety and quality control. These selected sources encompassed original research articles, review papers, and authoritative reports, originating from reputable organizations within the realm of animal nutrition and feed production. Inclusion criteria entailed the pertinence of articles to the subject of feed safety and quality control within feed mills. This incorporated studies, reviews, and articles delving into the discussion of challenges, practices, and solutions tied to feed safety and quality. Articles not available in English, those exclusively centered on unrelated topics, or those displaying insufficient relevance to the review's scope were excluded.

Data Extraction and Analysis

Essential insights were extracted and structured from the chosen sources, encompassing challenges pertinent to feed safety and quality control, as well as proposed solutions and optimal strategies. This data extraction procedure involved the identification of recurrent themes, emerging trends, and patterns within the literature. A particular focus was directed towards unveiling the correlations between the challenges and solutions highlighted in the literature. The review process comprised two phases, initiated by identifying potentially important sources. The first phase involved a scrutiny of titles and abstracts to ascertain whether the articles adhered to the inclusion criteria. In the subsequent phase, full-text articles underwent evaluation to evaluate their importnace and quality. The chosen articles were then meticulously analyzed to distill pivotal discoveries, methodologies, and recommendations tied to the challenges and solutions explored in this review.

Synthesis of Information

The synthesized information forms a structured overview of the diverse challenges encountered by feed mills, accompanied by corresponding actions to tackle these issues. The interrelatedness between challenges and the four pillars of quality control and feed safety systems was underscored to emphasize their interconnected nature. Insights derived from the chosen articles were classified into specific themes and subjects, encompassing aspects like poor plant design, staff training, hygiene, equipment sanitation, and other factors impacting feed safety and quality. These categories provided the foundation for organizing the ensuing discussion section of the paper.

The methodology employed in this review paper relies on the analysis and synthesis of existing literature, and consequently, the drawn conclusions hinge on the accuracy and dependability of the sources integrated. While the paper's scope may not encompass every conceivable challenge or solution, its goal is to offer a comprehensive comprehension of the pivotal factors influencing feed safety and quality control within feed mills. In essence, the methodology chosen ensures a methodical and extensive exploration of literature concerning feed safety and quality control in feed mills, affording valuable insights and recommendations for stakeholders and feed producers in the industry.

Results and Discussion

Poor plant design and construction

The design and construction of a feed mill can have a significant impact on the safety and quality of its products. Poor plant design and construction can create opportunities for cross-contamination and other hazards. For example, inadequate segregation of different production areas can increase the risk of cross-contamination between different feed types. Similarly, poor air flow and ventilation systems can lead to the spread of dust and other airborne contaminants throughout the plant (Clark, 2008). To address these subjects, feed mills must ensure that their facilities are designed and constructed to minimize the risk of contamination and other hazards. This may involve the installation of appropriate barriers and separation systems, the use of positive air pressure to prevent the spread of contaminants, and the execution of effective cleaning and sanitation protocols (Wang, 2020).

Deficient employee training

Another common challenge faced by feed mills is deficient employee training. Employees who are not properly trained in safety and hygiene practices can unwittingly contribute to the contamination of feed during processing. For example, employees who do not understand the effect of proper hand washing and sanitation procedures may inadvertently introduce harmful bacteria into the production process (Orriss, 2000). When focusing on this issue, feed mill employees must undergo comprehensive training in safety and hygiene practices. This may involve the implementation of regular training programs and the provision of clear and concise instructions for all employees. In addition, feed producers' employees have to be equipped with the necessary tools and equipment to maintain proper hygiene, such as gloves, aprons, and hand sanitizer (Maloni, 2006).

Poor employee hygiene

Related to deficient employee training is poor employee hygiene. Even with proper training, employees may still neglect to maintain good hygiene practices, such as hand washing and the use of protective clothing. This can result in the introduction of harmful bacteria and other contaminants into the production process (Pellegrino, 2015). Feed producers need to implement strict hygiene protocols for all employees when engaging on this matter. This may involve regular monitoring and supervision of employee hygiene practices, as well as the provision of training and support to help employees maintain good hygiene. In addition, feed mills should ensure that all employees are aware of the significance of hygiene and its role in ensuring the safety and quality of feed products (Fung, 2018).

Difficult-to-clean equipment

Another challenge faced by feed mills is difficult-to-clean equipment. Equipment that is not designed for easy cleaning and sanitation can harbor harmful bacteria and other microorganisms, increasing the risk of contamination during the production process (Park, 2019). To address this problem, feed mills have to prioritize the use of equipment that is designed for easy cleaning and sanitation. This may involve the installation of equipment with smooth surfaces and nooks and crannies that are easy to access and clean. In addition, feed mills ought to make sure that all equipment is regularly cleaned and sanitized according to established protocols (Wang, 2020).

No preventive maintenance

A lack of preventive maintenance is another common challenge faced by feed mills. Equipment that is not properly maintained can break down or malfunction, increasing the risk of contamination and other hazards (Setiawan, 2021). On this topic, feed mills should carry out regular preventive maintenance programs for all equipment. This may involve the development of maintenance schedules and checklists, as well as the provision of training and support to the employees so they will be able to maintain and repair equipment as needed (Nguyen, 2004).

Contamination of raw materials

The quality of raw materials used in the production of animal feed can also have a significant impact on feed safety and quality. Raw materials that are contaminated with harmful substances such as toxins and pathogens can pose serious risks to animal and human health (Streit, 2013). When aiming to answer this question, feed mills can implement rigorous monitoring and testing programs for all raw materials. This may involve the use of various tests and analyses to detect the presence of contaminants, as well as the development of protocols for rejecting contaminated raw materials (Shurson, 2022).

Contamination during processing
Even with rigorous monitoring and testing programs, contamination can still occur during the processing of feed. This can happen due to a variety of factors, including improper handling and storage of raw materials, equipment malfunction, and human error (Gebhardt, 2018). On this point, feed mills must introduce effective control measures throughout the production process. This may involve the use of physical barriers and separation systems to prevent cross-contamination, as well as carrying out rigorous cleaning and sanitation protocols. In addition, feed mill employees can be trained to identify and address potential sources of contamination (Kamboj, 2020).

Post-process contamination at the plant
Once feed has been processed and packaged, there is still a risk of contamination at the plant. This can happen due to improper storage or handling of finished products, or due to cross-contamination from other sources within the plant (Santillana Farakos, 2014). Feed mills should enable effective storage and handling protocols for finished products. This may involve the use of dedicated storage areas and the implementation of rigorous monitoring and inspection programs to detect potential sources of contamination. In addition, feed mills must ensure that all employees are trained to identify and address potential sources of post-process contamination (Manning, 2006).

Poor plant and equipment sanitation
In addition to addressing specific sources of contamination, feed mills must also maintain high standards of plant and equipment sanitation. Poor sanitation can allow harmful bacteria and other microorganisms to proliferate, increasing the risk of contamination (Betta, 2011). To address this topic, feed mills must use effective cleaning and sanitation protocols for all plant and equipment. This may involve the use of specialized cleaning products and equipment, as well as the development of rigorous cleaning schedules and checklists. In addition, feed mills must prove that all employees are trained to maintain proper sanitation practices and that all equipment is regularly inspected for signs of wear and tear that could compromise sanitation (Burnett, 2014).

Incorrect labeling or packaging
Finally, incorrect labeling or packaging can also pose a risk to feed safety and quality. Incorrect labeling can lead to the misidentification of feed types, which can result in animals receiving the wrong type of feed. Similarly, incorrect packaging can compromise the integrity of feed products, increasing the risk of contamination and spoilage (Soon, 2021). To attend this problem, effective labeling and packaging protocols are needed. This may involve the use of clear and concise labeling systems that clearly identify the type and quality of feed products. In addition, feed mill packaging area has to be designed in a way to protect feed products from contamination and spoilage, and all employees are trained to properly handle and store packaged products (Kleter, 2009).

Conclusion

The production of safe and high-quality feed is critical for ensuring the health and well-being of animals, as well as for protecting public health. The quality control and feed safety systems implemented in feed mills play a critical role in achieving this goal. As we have discussed in this paper, there are several factors that can impact the safety and quality of feed products.
It is essential for feed mills to stay up to date with the latest developments and best practices in feed safety and quality control. This can implicate investing in new technologies and tools for monitoring and analyzing feed products, as well as engaging in ongoing research and development efforts to improve the safety and quality of feed products.
It is also significant for the feed mills to work closely with regulatory agencies and other stakeholders to certify that their products meet all relevant safety and quality standards. This may involve working collaboratively with other industry stakeholders to share best practices and develop new standards for feed safety and quality. Overall, ensuring the safety and quality of feed products is a complex and multifaceted task that requires a comprehensive and proactive approach.

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ANTIFUNGAL ACTIVITY OF RHIZOBIA AGAINST RED CLOVER PATHOGENS FROM GENUS FUSARIUM

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Abstract

Red clover (*Trifolium pratense* L.) is an important forage legume that can be found in nature or as cultivated crop in monoculture or in grass-legume mixtures. The great importance of red clover is the fact that it lives in symbiosis with *Rhizobium leguminosarum bv. trifoli*. In addition to promoting plant growth, productivity and biomass, the bacteria also have an important role in disease control. Apart from useful microorganisms, many soil pathogenic microorganisms inhabit red clover roots and under favorable conditions they cause infections and diseases of the cultivated plants. On the infected red clover plants *Fusarium* spp. can cause several symptoms: vascular wilt on older plants, dumping of seedlings, root rot, rot of root base and crown rot. In the present study, the antagonistic potential was evaluated for two bacteria isolates preliminarily determined as *R. leguminosarum bv. trifoli* (CD1 and CD6) against three isolates *Fusarium* spp. (G1, G2 and G3) who were obtained from the roots of symptomatic red clover plants. The research was performed in *in vitro* agar plate (PDA medium) by methods of dual culture ("bacterial ring"). The comparison of growth assessment of fungi after they were grown together with rhizobia in a single plate and growth of fungi in individual plates was performed. Both rhizobia isolates showed significant antifungal activity against all the tested phytopathogenic fungi. Growth inhibition of G1, G2 and G3 was evaluated (%) using both rhizobia isolates, CD1 varied from 30 to 43 % and CD6 from 14 to 32 %.

Key words: *Rhizobia, Fusarium, Red clover*

Introduction

Red clover (*Trifolium pratense* L.) is very important perennial forage legume that is grown in different agroecological conditions (Lugić *et al*., 2006). As a forage crop, red clover is characterized by high potential for production of biomass with high content of crude protein) and rapid regrowth post cutting (Tomić *et al*., 2007). Compared to alfalfa, red clover better tolerates lower pH, less fertility and poorer drainage of the soil (Vasiljević *et al*., 2005). Red clover could be used as a potential source of bioactive compounds for the production of pharmaceutical supplements (Vlaisavljević *et al*., 2014). It is very important in crop rotation (Lugić *et al*., 2010), especially in organic agriculture (Vasiljević *et al*., 2023).
Red clover lives in symbiosis with *Rhizobium leguminosarum bv. trifoli* (Ištvánek *et al*., 2017). As a result, nitrogen from the atmosphere becomes available to plants and contributes to reduction of need of synthetic nitrogen fertilizers (Tomić *et al.*, 2012). Rhizobia is a soil-inhabiting bacterium that has the capacity to be an effective nitrogen fixing microsymbiont (Reeve et al., 2010).

Formation of the symbiosis requires a highly specific and complex signal exchange between the plant and the bacteria (Dénarié and Cullimore, 1993). The plants exude flavonoids into the rhizosphere and, in response, compatible rhizobia produce lipochitin oligosaccharide (Ferguson *et al*., 2020). The bacteria contributes to plant nutrition by fixing nitrogen, also it can synthesize various phytohormones, such as IAA, riboflavin, rhizobitoxine, gibberellins, ethylene, cytokinins etc. (Jaiswal *et al.*, 2021). These bacteria can contribute to biological control of soil pathogens by competing with them for space and nutrients or by producing secondary metabolites such as antibiotics and hydrocyanic acid (Deshwal *et al*., 2003). *Fusarium* spp. are most known as plant pathogens that can parasitize almost every plant species in the world, besides that, these fungi can cause various infections of animals and can be harmful to humans in more than one way. These fungi can maintain their vitality for a long period of time colonizing very different substratum materials, like soil, plant leftovers, dead trees, water, bread, grain, meat and meat products, dairy products and various canned food. Although *Fusarium* spp. have a very wide distribution area, it's noticed that these pathogens have a preference to warmer climates of the planet (Gilardi *et al*., 2017). *Fusarium* spp. can produce secondary metabolites, mycotoxins, of which the most significant are trichothecenes, zearalenones, fumonisin B1, fumonisin B2 and fusaric acid (Ohberg, 2008). On the infected red clover plants (*Trifolium pratense* L.) *Fusarium* spp. can cause several symptoms: vascular wilt on older plants, dumping of seedlings, root rot, rot of root base and crown rot. These primal symptoms lead to secondary ones, chlorosis and necrosis of leaves and shoots, which can be seen on the overhead part of the plant. *Fusarium* spp. exists in the soil and with that contaminated soil it is distributed and spread in new uninfected areas of the world. The fungus survives unfavorable part of the year in the soil and in red clover plant debris on the fields. Pathogen infects red clover plants through wounds on the roots and after that it slowly progresses in the plant (Krnjaja *et al*., 2004).
The aim this study was assessment antifungal potential of rhizobia against red clover pathogens from genus *Fusarium* under *in vitro* conditions.

Material and Methods

Infected red clover plants used in this study were sampled during the year 2021. on the several fields of red clover monoculture that are situated in Globoder, Central Serbia. The sampled plants showed obvious stunting and growth retardation compared to nearby plants, as well as mildly chlorosis and necrosis on the leaves. Roots were a little more murky than usual with some parts that were darker than the rest. These darker parts showed rot symptoms and isolation was performed from them. Several isolates were obtained, and three isolates were chosen, they were designated G1, G2 and G3 and further characterized on potato dextrose agar (PDA) based on morphological and pathogenic characteristics.
All three isolates formed fast growing mycelium, forming colonies with diameter 66.17 mm (G1); 81.67 mm (G2); 75.67 mm (G3) after 7 days of incubation at 25°C. G1 had very abundant aerial mycelium with uneven edges and pale coloration, with shades of brown or beige. G2 formed extremely fast growing and plentiful colonies that filled both the medium and all the left space in the petri dish with its aerial mycelium. G2 mycelium was pale in the edges but closer to the center it had pink, red and yellow pigmentation. Aerial mycelium of G3 was a little less abundant than the other two isolates, but still pretty ample and fast growing; overall colony color was pale with shades of light brown to dark red.

The roots of plants red clover were completely rinsed and nodules of typical appearance were isolated and sterilised. Each nodule was crushed and the content of the nodule was transferred onto a Petri dish with YEM agar (Vincent, 1970). Petri dishes were incubated at 28°C until typical colonies of rhizobia appeared. Colonies of rhizobia were examined microscopically, and purified by a series of successive dilutions. Two bacteria isolates CD1 and CD6, obtained from the nodules of the plants that were growing on different locations preliminarily determined as *R. leguminosarum bv. trifoli*.

The research was performed in *in vitro* agar plate (PDA medium) by methods of dual culture ("bacterial ring"). In determining the growth rate, antagonists and target pathogens were grown in a 90 mm Petri dish containing 18 mL PDA. The target organism is enclosed in a complete ring of the test bacterium, by which the inhibitory activity is easy to quantify. Pure cultures of rhizobia isolates (CD1 and CD6) were formed by seeding the entire surface YEM agar.

The rim of a sterile glass tube of desired diameter was gently placed on the bacterial colony and then centrally placed on the surface of a fresh culture medium, leaving a complete ring of the bacterial inoculum on the agar surface. Using a small agar culture disk place *Fusarium* sp. in the center of the ring and incubate for seven days. At the same time, the tested isolates were sown in monoculture (control). After that period, the radius of the grown mycelia was measured.

At incubation end, the growth diameter of the fungal colony was measured in millimeters and compared to the bacteria-free control. The tests were performed in 3 repetitions, and the antagonistic activity of the bacterial isolates was determined based on the following equation (Dikhoba et al., 2019):

(PI) (%) = (FR- AR)/FR*100

PI- the percentage of inhibition;

FR - the growth radius (mm) of the fungus in control dish;

AR- the radius of the fungal growth in direction of the growth of rhizobia.

Results and Discussion

Fusarium species pose a serious threat to animal and human health because of their mycotoxin production, with that in mind we should do everything in our power to reduse infections by these pathogens. Use of resistant red clover cultivars would always be the best choise in controlling the disease in the field, but this control measure is not always practical and applicable. On the carnation leaf agar medium (CLA) tested isolates formed three types of asexual spores, which are typical for *Fusarium* sp. (Burgess *et al*., 1994). Koch's postulates were examined via different types of plant pathogenicity tests using detached plant parts and whole plants of red clover in controlled environments. Morphological features of three isolates were in accordance with Burgess *et al*. (1994) for suspected preliminary species, *Fusarium* sp.

The comparison of growth assessment of fungi was performed after they were grown together with rhizobia in a single plate and via growth of fungi in individual plates. Both rhizobia isolates showed significant antifungal activity against all the tested phytopathogenic fungi.

Highest percentige of inhibition was notised by fungal isolate G2 via application of bacterial isolate CD1 (Figure 1.). Inhibition of G1 isolate was a bit lesser than G2, and G3 remained in third spot by the diameter of inhibition zones that were formed. Growth inhibition of G1, G2 and G3 was evaluated (%) using both rhizobia isolates, CD1 varied from 30 to 43 % and CD6 from 14 to 32 % (Figure 2). The *in vitro* dual culture test shows mutual effects of the paired organisms and provides a preliminary screening of the interaction between antagonist and pathogen (D'Ambrosio *et al*., 2022).

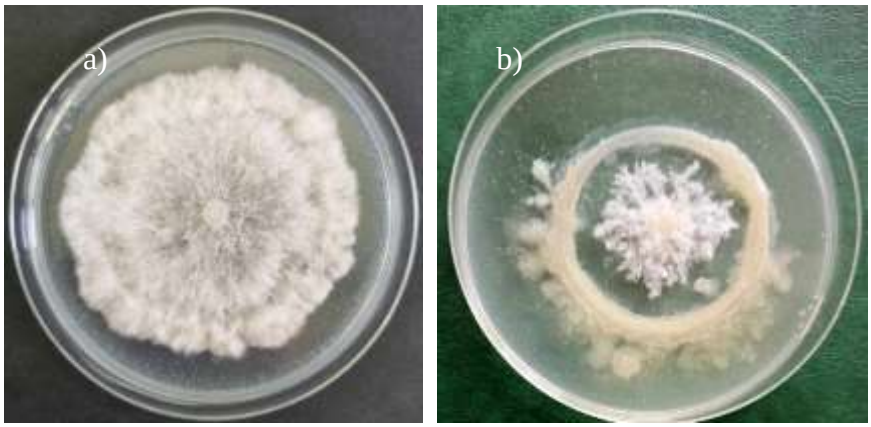

Figure 1. *In vitro* growth inhibition of *Fusarium* spp. by rhizobial isolates in dual-culture plate assay: a) The fungus in the control dish; b) The fungal growth in presence of rhizobia

Rhizobia provide plants with nitrogen from the soil and also synthesize polysaccharides, vitamins B_{12}, B_1, B_2 (Denison and Kiers 2004). Research results obtained by Chao (1990) showed antagonistic activity of six different *Rhizobium* strains against 10 isolates of fungi (all the tested strains inhibited the growth of fungi). Höflich (2000) reported that *R. leguminosarum bv. trifolii R39* strain showed *in vitro* antagonistic activity against *Fusarium* spp. and other soil plant root pathogens. Antibiotics produced by rhizobia have been presumed to play an important role in disease control (Deshwal *et al*., 2003). Breil *et al*. (1996) noted the secretion of secondary metabolite, peptide antibiotic trifolitoxin (TFX) *by R. leguminosarum bv. trifolii* T24. According to Nautiyal (1997) *Rhizobium* sp. inhibited growth of *F. oxysporum f.sp. ciceri*, *Rhizoctonia bataticola* and *Pythium* sp. under *in vitro* conditions.

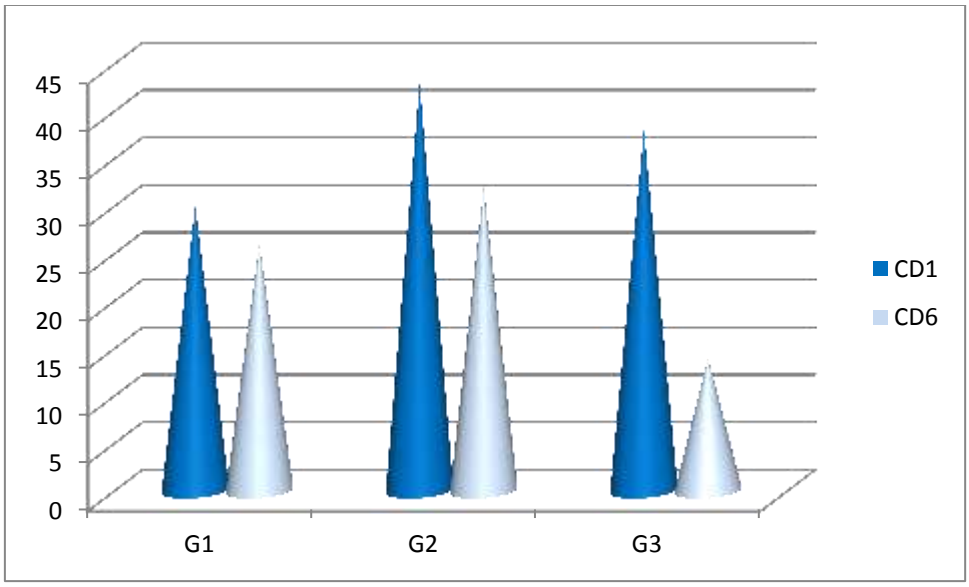

Figure 2. The percentage of growth inhibition of *Fusarium* spp. by rhizobial isolates

Conclusions

By using means of biological control we can control the pathogens in the soil by adding bacteria that are beneficial to plant via its nitrogen production. Results from this study show potential for further research on this matter and suggest use of rhizobia as effective biocontrol

agent. The use of this microorganisms interaction should always be considered when in search for viable plant growth promoters for sustainable agriculture.

Acknowledgement

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ENTOMOFAUNA ON WHEAT AND BARLEY IN BIJELJINA AREA (BOSNIA AND HERZEGOVINA)

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Abstract

During the vegetation, wheat and barley are exposed to many harmful insects that feed on them and cause direct and indirect damage, thus significantly reducing quality and yield. Entomofauna on wheat and barley in Bijeljina area (entity of Republic of Srpska, Bosnia and Herzegovina) was examined in 2022, in the location Srednji Dragaljevac.
Using entomological methods such as visual examination of plants, the method of „mowing" using an entomological catcher and collecting insects using an exhauster, their presence, abundance and symptoms of damage were determined. Total number of determined insect species is 20, from three orders and 13 families. The largest number of species from the order Hemiptera have been determined, 11 species from 7 families, while from the order Diptera determined only one species from the fam. Syrphidae. Fourteen species are phytophagous and six species are zoophagous. From phytophagous species, six species are related to small grains in their development. Eight species aren´t related to small grains and their presence is most likely accidental, because there were plants in the immediate vicinity that serve as food for them. The largest number of insect species was found at the beginning of the vegetation when the most numerous was *Sitobion* (=*Macrosiphum*) *avenae* Fabricius) which inhabited the leaf, stem and spike wheat and barley.

Keywords: *insects, wheat, barley, Bijeljina.*

Introduction

In the Republika of Srpska, in the structure of sown areas, wheat is present by 66.4 %, barley by 13.4 %, oats by 7.9 %, triticale by 6.1 %, rye by 0.6 %, while 2.8 % of the total area was sown under other crops (Statistical Institute of the Republic of Srpska, 2022). The largest granary in the Republic of Srpska is Semberija, a region in the northeastern part of Bosnia and Herzegovina, and the center of the region is the town of Bijeljina.
During the vegetation, the wheat and barley are exposed to many harmful insects which cause direct and indirect damage through their feeding and reproduction, and significantly reduce the yield and quality of the product. The important harmful insect species are: aphids (Aphididae), wheat thrips (*Haplothrips tritici* Kurd.), wheat bugs (*Eurigaster* spp. - *E. maura* L., *E. austriaca* Schrank, *E. integriceps* Puton), (*Aelia* spp. - *A. acuminata* (L.), *A. rostrata* (Boheman)), wheat grain beetle (*Anisoplia austriaca* (Herbst)), barley leaf beetle (*Oulema melanopus* (L.)), corn ground beetle (*Zabrus tenebrioides* (Goeze)) and the others species.
From aphids, on wheat and barley more species are present. The english grain aphid (*Sitobion* (=*Macrosiphum*) *avenae* Fabricius) and the bird-cherry oat aphid (*Rhopalosiphum padi* (L.)) are more present and numerous aphids than the others. *S. avenae* damages spike, leaf and stem. If this aphid is less present, it´s concentrates on the top of the leaf, and in the case of a larger attack, the entire surface of the leaf is covered with colonies. It´s mostly inhabits the spikes of small grains (Petrović-Obradović et al., 2017). *R. padi* numerous colonies usually form a spike. The less present aphid than the previous are the rose-grain aphid

(*Metopolophium dirhodum* Walker), the spring grain aphid (*Schizaphis graminum* (Rondani)) and the Russian aphid (*Diuraphis noxia* (Mord.)). The aphids cause direct and indirect damage. Direct damage is caused by sucking the sap from plant organs, which leads to slowed plant growth. If aphids are present in large numbers on the spikes in the milk stage and milk-dough stage, the grain remains shrunk, which directly reduces the quality and quantity of the yield. However, the indirect damage is far greater, because aphids make large amounts of honeydew which disturbs the normal physiological processes. Also, aphids are known as vectors of viruses. The most significant virus is Barley Yellow Dwarf Luteovirus (BUDV). The most important vector of this virus is *R. padi.* The damage begins to manifest late in autumn, which is the most common case, and during the spring of the following year. Infected plants are visibly smaller than healthy, with yellow color. Diseased plants don´t to come into ear and they dry up and completely decay. The disease occurs on wheat and barley, but it causes far greater damage to barley, especially in conditions of warm autumn and mild winter when aphids successfully overwintering and continue to transmit the virus (Jasnić and Jevtić, 1997; Stamenković and Jasnić, 2002; Trkulja and Stojčić, 2002; Stamenković and Petrović-Obradović, 2005).

Wheat and barley are damaged by wheat thrips also (*Haplothrips tritici*). Damage is caused by larvae and adults. After the spikes are formed, thrips suck the sap from the chaff and grains, so the chaff turns white color. The quality of the product is reduced and its commercial value decreases (Özsisli, 2011).

The most important bugs that feed on small grains, primarily wheat, are species from the genus *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). The dominant species in our area are *Eurygaster austriaca* Schrank and *E. maura* L. (Kereši, 1999; Konjević, 2008). Both nymphs and adults of the *Eurygaster* and *Aelia* wheat beetles insert their mouthparts which penetrate the wheat grains and extract substances from them. To facilitate the absorption of the nutritional elements of the endosperm, the grains are digested externally by injecting saliva rich in proteolytic enzymes (Sivri et al., 1998; Özkan and Babaroğlu, 2015) and amylases (Kazzazi et al., 2005). Pierced kernels in the field usually continue to ripen. When ripe, beetle-damaged kernels have a whitish spot and sometimes a small black spot where the kernel has been punctured (Hariri et al., 2000).

One of the most important harmful insects species of small grains in general in Europe, is the cereal leaf beetle (*Oulema melanopus*) (Dedruver, 1990). This species can reduce yields by 5-20 % every year (Deutsch et al., 2018). Larvae and adults cause damage. Adults gnaw the leaves between the leaf veins, leaving longitudinal stripes (Stamenković, 2004). The larvae gnaw the upper epidermis and parenchyma of the leaf. White lines remain on the leaves, resulting in reduced photosynthetic activities and yield reduction (Buntin et al., 2004). The decrease in yield on barley can be up to 75 %, on winter wheat 23 % and 23-49 % on spring wheat (Dimitrijević et al., 2001).

The significant damage is also caused by the corn ground beetle (*Zabrus tenebrioides* (Goeze.)). The adults feed on the grains, while the larvae damage the leaf tips of the lower plants by splitting them and entering a vertical hallway in the soil.

In addition to harmful insect species, wheat and barley are also inhabited by beneficial species (predators and parasitoids) from the orders Neuroptera, Hemiptera, Coleoptera, Diptera and Hymenoptera. These species have a great importance in reducing the population of harmful species. The most important predators are ladybirds (Coccinelidae: Coleoptera), which are predators in the larval and adult stadium. They feed on aphids and larvae from orders Hymenoptera and Coleoptera. Among them, particularly important are *Coccinella septempunctata* L. (adult eats about 500 aphids, larva about 190 aphids), and *Adalia bipunctata* L. (adult eats about 400 aphids, larva, about 70) (Rana, 2005; Markova and Tomchev, 2013). Also, predatory species from the family Carabidae (*Carabus, Calosoma,*

Nebria, *Poecilus*) are very significant. Predators are adults and larvae, and they feed on larvae, cockchafer and the other soft-bodied insects. In wheat, these species reduce the population of harmful species by up to 40 % (Mikić, 2012). The most important predator of aphids and the other harmful insects are species from the family Nabidae, such as *Nabis ferus* (L.). This species reduce population aphids on wheat, feeding on 10-15 individuals per day.
Very widespread and significant predators of aphids on small grain are species from fam. Syrphidae. Syrphids perform very important ecological roles like plant pollination and predation of plant pests (Rotheray and Gilbert, 1999; Dunn et al., 2020). Their larvae are very diverse. Some species are saprophagous, mycophagous or phytophagous, but predation is one of the most common ways of feeding (Rojo et al., 2003). Important parasitoids are parasitoid wasps from the family Trichogrammatidae. These wasps parasitize the eggs of harmful species on small grains. The most important species are *Trichogramma evanescens* Westv. and *Trichogramma embryophagum* Htg (Pehlivan, 2021).

Materials and Methods

The survey was completed in 2022, in the field, in Bijeljina area (location Srednji Dragaljevac) and in the laboratory of the Faculty of Agriculture in East Sarajevo.
The area under wheat was 5000 m^2, and the barley was 6000 m^2. The basic crop of the soil was realized in the fall to a depth of 30 cm. Both crops were sown on October 15, 2022. Barley was harvested on June 19, 2022, while wheat was harvested on June 20, 2022.
Using entomological methods such as visual examination of plants, the method of "mowing" using an entomological catcher and the collection of insects using an exhauster, the presence, abundance and simptoms of damage of insects was determined.
In wheat and barley crops, on 10-20 test areas of size 50 x 50 cm, at intervals of 7-10 days, a visual examination of all aerial parts of plants was realized for the presence of different developmental stadium of insects and symptoms of damage.
Using the entomological catcher and the "mowing" method were used to pass over the plants, so that all the insects that were on the surface of the plant organs fell into the catcher. Diagonally across the crop, 20 swings were made in 5 places, which is a total of 100 swings with the catcher in one collection period. This method of collection was realized in the period from April 22 to June 14, at intervals of ten days.
Small insects were collected directly from the plants using an exhauster. All sampled infested plant matherial was observed in laboratory. All collected larvae and pupa of phytophagous and entomophagous insects, were reared in the lab to adult stadium. In this purpose was used plastic containers and Petri dishes. In the bottom placed a layer of cellulose wadding occasionally misted in order to maintain the freshness of the leaf. An analysis of symptoms of damage, the total number of species and individuals of different stadium and determination to the species level was carried out. The collected insects were fixed in 70 % alcohol; some of them were prepared, making microscopic preparations or the entomological collection.

Results and Discussions

One year research of entomofauna on wheat and barley in Bijeljina area, resulted in 20 species, from 3 orders and 13 families. Fourteen species are phytophagous, and six species are zoophagous.
From the order Hemiptera, the largest number of species have been determined, a total of 11 from 7 families. Four species are phytophagous and are attached to small grains: *Eurigaster maura* (L.) (fam. Scutalleridae), *Aelia acuminata* (L.) and *Dolycoris baccarum* (L.) (fam. Pentatomidae) and *Sitobion avenae* (Fabricius) (fam. Aphididae). Six species are also

phytophagous, but associated with the other plants. These are *Eurydema oleracea* (L.) and *Graphosoma lineatum* (L.) (fam. Pentatomidae), *Cercopis vulnerata* Rosii (fam. Cercopidae) and two species from the family Miridae, *Lygus pratensis* (L.) and *Adelphocoris lineolatus* (Goeze). From the fam. Cicadellidae, *Cicadela viridis* (L.) was determined. One species is predatory, *Nabis ferrus* (L.), from the fam. Nabidae.

From the order Coleoptera, 8 species from 7 families have been determined. From the phytophagous species, two species are related to small grains in their life cycle, *Oulema melanopus* (L.) (fam. Chrysomelidae) and *Omophulus lepturoides* (Fabricius) (fam. Tenebrionidae). Two species are phytophagous also, but not associated with small grains. One of them is polyphagous, *Epicometis* (=*Tropinota*) *hirta* Poda (fam. Scarabaeidae) and *Tanymecus dilaticollis* Gyll. (fam. Curculionidae). Four species are zoophagous, *Cocinella septempunctata* L. and *Adalia bipunctata* L. (fam. Coccinellidae), *Malachius bipustulatus* (L.) (fam. Melyridae) and *Cantharis fusca* (L.) (fam. Cantharidae).

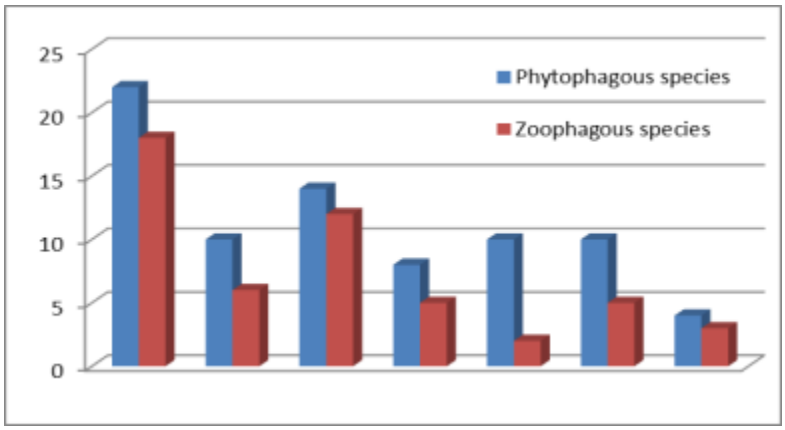
From the order Diptera, six larvae and three pupae of syrphids (fam. Syrphidae) were found, which were reared in the lab to adult stadium. The determined species is *Episyrphus balteatus* (De Geer).

Table 1. The presence of insects on wheat and barley in the Bijeljina area in 2022 year

Species/date of collection		April 22	May 2	May 12	May 22	June 2	June 11	June 19
1.	*Eurigaster maura*	2-A	2-A		1-A			
2.	*Eurydema oleracea*						1-A	
3.	*Aelia acuminata*	2-A				2-A		
4.	*Dolycoris baccarum*				1-A			
5.	*Graphosoma lineata*							1-A
6.	*Oulema melanopus*	1-A		3-LA	4-LA			
7.	*Tropinota hirta*	2-A				1-A		
8.	*Cercopis vulnerata*		1-A					
9.	*Adelphocoris lineolatus*						1-A	
10.	*Tanymecus dilaticollis*			1-A	1-A			
11.	*Lygus pratensis*				1-A		2-A	
12.	*Malachius bipustulatus*						1-A	
13.	*Omophlus lepturoides*	3 – A						
14.	*Sitobion avenae*	10-LA 2-A	7 -LA	8-A 2-LA		5- LA 2-A	3-LA 2-A	
15.	*Cocinella septempunctata*	13-LA	1- A	2-A				1-A
16.	*Adalia bipunctata*	3-LA		2-A		2-A		3-A
17.	*Cicadela viridis*						2-A	
18.	*Nabis ferus*		1-A	1-A				
19.	*Cantharis fusca*		3-A	7- A	5-A			
20.	*Episyrphus balteatus*	2-LA	1-PU				2-LA 1-PU	3-LA

Phytophagous species Zoophagous species *A – adult; LA – larva; PU – pupa

The largest number of species (phytophagous and zoophagous) was determined at the beginning of the vegetation, according to the literature data (Balaško and Gašparić, 2022). In that period, the most numerous was *S. avenae*. The smallest number of species was determined on June 19 (Tab. 1, Graph. 1).



Graph. 1. The presence of phytophagous and zoophagous species

The identified phytophagous species which are related to small grains in their development are: Eurigaster maura, Aelia acuminata, Dolycoris baccarum, Oulema melanopus, Sitobion avenae and Omophulus lepturoides.

E. maura was determined on April 22, May 2 and May 22 (Fig. 1). This species was identified at the beginning of the vegetation, during earing and dough stage, according to the literature data about bionomy of this species (Kereši, 1999). Adults and nymphs of E. maura cause damage by feeding on the leaves, stems and grain kernels of the wheat plants (Özkan and Babaroğlu, 2015), and can lead to economic losses as high as 100 % in the absence of control measures (Özbek and Hayat, 2003). The species O. melanopus was determined at the end of April 22, when adults and characteristic damage in the form of longitudinal stripes on the leaves were found. The larvae were found during May (May 12, May 22) and they were damaging only the upper epidermis of the leaves (Fig. 2), according to the literature data (Dimitrijević et al., 2000; Gueorguieva and Mateeva, 2001; Malschi, 2009; Kher et al., 2011; Tanasković et al., 2012; Kher et al., 2016).

Figure 1. *E. maura*

Figure 2. Larva *O. melanopus*

From the aphids, only *S. avenae* was identified. This species represents the most economically significant species of all species of aphids on small grains. It´s primarily inhabits the spike and upper leaves of small grains (Stamenković and Jasnić, 2002; Kereši and Konjević, 2022). Colonies of this aphid were found at the end of April, during May and June, on the leaf, stem and spike, according with literature data about the bionomy of this species (Dedryver et al.,1998, Petrović-Obradović et al., 2017) (Fig. 3).

The identified phytophagous species which aren´t related to small grains in their development are *Eurydema oleracea* that occurs on cabbages, *Graphosoma lineata*, mainly present on plants from the family Apiaceae, polyphagous species *Epicometis hirta*, *Cercopis vulnerata* Rosii (Fig. 4) which mainly sucks the plant sap from grasses and the other plants, *Tanymecus dilaticollis* which mainly attacks corn, *Lygus pratensis*, *Adelphocoris lineolatus* and *Cicadela*

viridis, which occurs on alfalfa and the other legumes. The presence of these species on wheat and barley is most likely accidental, because there were plants in the immediate vicinity that serve as food for them, so they were present there at the time of collection.

Figure 3. Colony *S. avenae* on the leaf

Figure 4. *C. vulnerata*

The determined zoophagous species are: Cocinella septempunctata, Adalia bipunctata, Cicadela viridis, Nabis ferrus, Cantharis fusca and Episyrphus balteatus. The largest number of larvae Cocinella septempunctata was found in April (April 22), at the time when the S. avenae colonies were the most present, accordance with the literature data about the predatory role of this ladybug species (Fig. 5) (Rana, J.S., 2005; Markova and Tomchev, 2013). Later, during the vegetation, the adult of this species, as well as the imago of A. bipunctata, were determined. In addition to the mentioned species, adults of Cantharis fusca were found during May (15 adults in 3 sampling dates) (Fig. 6). The adult of this species feeds on various parts of the flower, but can also feed on various small insects (Traugot, 2016). From the predatory species, Nabis ferrus (fam. Nabidae) during May was found. In the larval and adult stadium, this species feeds by sucking body fluids from aphids or from some the other harmful insect (Franin and Barić, 2012).

Figure 5. Larva *C. septempunctata*

Figure 6. *C. fusca*

Figure. 7. *E. balteatus*

From the order Diptera, during May and June, in colonies of *S. avenae* larvae and pupae of syrphids were found. The determined species is *Erispiphus balteatus* (Fig.7), according to literature data about predatory role this species in relation to *S. avenae* (Winder et al., 1994).

Conclusion

Harmful insects on wheat and barley reduce the yield and quality of the final product by their diet. Therefore, it is important to know the harmful species present in order to implement adequate protection measures. Except harmful species, these small grains inhabit very important beneficial insects which reduces the population of harmful species, thus reducing the need to apply chemical measures, thus contributing to the preservation of biodiversity and the natural environment. Total number of determined insect species is 20, from three orders and 13 families. Fourteen species are phytophagous and six species are zoophagus. The

largest number of species from the order Hemiptera was determined which indicates the importance of monitoring them in the following growing seasons, in order to take plant protection measures in time. The largest number of insect species was found at the beginning of the vegetation.

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A SAMPLE PREPARATION METHODOLOGY USING DIFFERENT d-SPE SORBENT FOR AMITRAZ ANALYSIS IN HONEY

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Abstract

An analytical method was validated using gas chromatography with tandem mass spectrometry (GC/MS) for the precise analysis of amitraz and its metabolites 2, 4-dimethylaniline (2, 4 - DMA) in honey. The QuEChERS technique, which comes from the words Quick, Easy, Cheap, Effective, Rugged, and Safe extraction of the pesticides was used. Amitraz is both an acaricide and insecticide and is often used in beekeeping to protect honeybee colonies against *Varroa destructor*. The procedure was validated according to SANTE/11325/2021 and the values of recovery, precision, and linearity, limit of detection and limit of quantification were established. The average recoveries obtained for amitraz were ranged from 79.4 to 100.8% and for 2, 4 dimethylaniline from 78.1% to 103.6 % at the two spiked levels 0.01 and 0.02 mg/kg. Several clean-up approaches were tested: d-SPE with Enhanced Matrix Removal-Lipid (EMR-Lipid), combination of anhydrous magnesium sulfate, primary secondary amine (PSA) and C18 sorbents, and a third method using Z-Sep. The most precise recovery, i.e. the highest accuracy, was achieved using purification with EMR lipid. The linearity of the analytical response across the studied range of concentrations (0.010 - 0.10 mg/kg) was excellent, obtaining correlation coefficients higher than 0.99. Limit of quantification was 0.004 mg/kg for amitraz and 0.005 mg/kg for 2, 4-DMA. The method was used for the determination of amitraz and 2, 4-DMA in real samples of acacia and flower honey.

Key words: *honey, QuEChERS method, GC/MS, amitraz.*

Introduction

Amitraz is a synthetic pesticide that acts as an insecticide and acaricide. It is used primarily for the control of animal ectoparasites, i.e. an acaricide used against the parasite *Varroa destructor* in bees. It can be applied in the form of tablets, but it is much more often used as an organic solution of amitraz in a concentration of 20%, which is applied by smoking using self-burning smoke sheets. The effect of amitraz is reflected in its ability to kill the nervous system of the *Varroa destructor* parasite. It is most often sold in the form of a 20% solution under different trade names. Amitraz is readily hydrolyzed (under acidic conditions) to 2, 4-dimethylphenylformamide, which can be rapidly hydrolyzed under alkaline conditions to 2, 4-dimethylaniline. After use, amitraz molecules have the ability to hydrolyse through the intermediate metabolites N-2,4-dimethylphenyl-N-methylformamidine (DPMF), and N-2,4-dimethylphenylformamide (DMF) and 2,4-dimethylaniline (2,4- DMA). It is generally accepted that 2, 4-dimethylaniline is the stable endpoint of amitraz degradation (Caldow *et al*., 2007). The compound 2, 4-dimethylaniline is environmentally stable and toxic jedinjenje (Pohorecka et al., 2018). For this reason, the legal regulation defines amitraz which, in addition to the amount of amitraz, includes the metabolites containing the 2, 4-dimethylaniline moiety expressed as amitraz (EU, Annexes II Reg. 396/2005). The maximum allowed value of amitraz is regulated to a value of 0.2 mg/kg based on Legislation Reg. (EU) 2017/623 applicable from 26/04/2017.

This paper describes the validation of the QuEChERS method for determination of amitraz in honey samples. The QuEChERS method is an extraction method which has been developed for analysis pesticides from fruit, vegetables and cereals (Ananstassiades *et al.*, 2003). Therefore, various modifications of this method are used for the determination of pesticides in honey and other bee products (Česnik *et al*., 2019; Juan-Borras *et al*., 2016). One of the methods of preparation and extraction of 2,4-DMA involves acid extraction and heating in a water bath at - 80°C, and then extraction in a basic medium (Caldow et al., 2007).

Control of the quality and safety of honey is important, given that, in addition to its nutritional value, it also has therapeutic potential. Honey is the sweet product that has been used in medicine since ancient times due to its antioxidant, anti-inflammatory and antimicrobial activity. The use of honey in the diet is a significant combination of the carbohydrates, pollen, aromatic compounds, minerals, enzymes, vitamins, pigments, and various acids (Mititelu *et al.*, 2022). The chemical composition of honey is very variable and depends on the place where it is produced, due to the nature of the soil and the quality of water and air, which affect the quality of the raw materials used by the bees. The results of this study show that residues of pesticides, especially neonicotinoids, may occur in different regions and in greater frequency and concentration in areas where more technology is applied and more pesticides are used in agricultural production systems, such as southern Jalisco, Mexico (Ponce-Vejar *et al.*, 2022). In bee feeding, the use of products with added medicines or products purchased on the basis of honey and non-sterilized pollen is prohibited, as they contaminate bee families with spores and mycelia that cause bee-specific diseases. Artificial feeding is stopped 15 days before the beginning of the harvest. On the other hand, the quality of the environment has a significant impact on the degree of its pollution with various toxic pollutants (Mititelu *et al.*, 2022). In modern beekeeping, contamination of honey can be direct (i.e., in colonies treated for veterinary purposes) or indirect, as honeybees travel long distances for foraging and come into contact with contaminated pollen, nectar, and water (Panseri *et al.*, 2020).

The main objective of this study was to evaluate and optimize a modified method for the determination of amitraz and 2, 4 - DMA in honey samples using gas chromatography with mass spectrometry (GC-MS). Another specific objective was to develop and optimize a sample preparation procedure with a minimal solvent volume and high selectivity without the need for additional purification procedures. To achieve maximum efficiency for the target residues, a final extract dilution was applied to simplify the sample preparation procedure and reduce analyte loss during sample preparation. However, it should be emphasized that this approach could also be used for the determination of other pesticides. The proposed method was evaluated in terms of limit of detection (LOD) and limit of quantification (LOQ), linearity, specificity, precision and trueness.

Material and methods

Amitraz and 2, 4 -DMA were purchased from Dr Ehrenstorfer (LGC). The sample preparation was carried out using three methods of purification until the establishment of purification with satisfactory recovery. The first stage of preparation involved the homogenization of honey samples and the establishment of the method through the preparation and validation process. Then, a validated method with satisfactory performance was applied for the determination of amitraz and its metabolite in real samples. The analysis was carried out against the presence of pesticides in real samples of acacia and flower honey. For analysis, there were 17 samples of acacia honey and 28 samples of flower honey from the territory of the Republic of Serbia, i.e. the vicinity of Belgrade, a place called Batajnica. Honey samples were sampled in the amount of 500 g in a sterile plastic jar, closed and thus delivered to the laboratory. The samples were sampled and delivered to the laboratory in the period from the beginning of September to the middle of October 2022 and were from harvest 2022. All the samples were

from the territory of Batajnica, which is known for beekeepers and raising bees for many years. Batajnica is an urban settlement in the northwest of Belgrade, it is a lowland village that donated significant amounts of honey to hospitals in Serbia during the COVID-19 pandemic.

The methods for the determination of the pesticides residue and also amitraz and 2,4-DMA, was the already described for honey samples (Kubiak *et al.*, 2020). Before the analysis, the honey samples were homogenized and 5 g was weighed into a 50 ml plastic tube. Water (5 ml) and acetonitrile (10 ml) were added and then the samples were vortexed. Then, an internal standard was added (ISTD, triphenyl phosphate), and for the samples for validation and verification of the recovery rate and for the determination of the recovery rate, the standards amitraz and 2,4-DMA were added at 0.01 and 0.02 mg/kg, respectively, and amounts of 0.1 and 0.2 μg/kg were added for precision when the maximum residue level was exceeded. Prior to analysis of the recovery samples, the honey was tested to ensure that it did not contain amounts of the specified analytes.

In the first phase of preparation the samples are shaken and a salt and buffer mixture is added and the samples are shaken again. After centrifugation for 5 min (4000 rpm) the supernatants are put in -20 degree freezer. In the second stage for purification, three methods of checking recovery and purification were used: d-SPE with Enhanced Matrix Removal-Lipid (EMR-Lipid), combination of anhydrous magnesium sulfate, primary secondary amine (PSA) and C18 sorbents, and a third method using Z-Sep. A stock solution of amitraz and 2,4-dimethylaniline were prepared in acetonitrile and stored at –20 °C. Intermediate dilutions were prepared in hexane before analysis. The amitraz and 2, 4 -DMA compound are separated on a Elite-CLP column (PerkinElmer, 30 m; 0,25 mm ID; 0,25 umdf) and determinated by GC/MS operating in the multiple reaction monitoring mode (MRM). Quantification analysis was performed using an Clarus 680 GC gas chromatograph connected to a SQ8T mass detector (PerkinElmer, USA). The system is equipped with Electrin Impact (EI) ionisation and 255L/sec turbomolecular pump. The operation of the system is controlled and also data analyzed through the PerkinElmer TurboMassTM GC/M software system. The mass analyzer in the mass detector is a quadropole with pre-filter mass range: 1.0 – 1200 u (amu). The injection volume of calibration standards, recovery samples and analyzed samples was 2 μl. The temperature of splitless was set at 250°C and the detector temperature was set at 280°C. The quantifer ions monitored for 2,4- dimethylaniline were m/z 106, 120, 121,77 and for amitraz m/z 121, 132, 162, 293. Validations and performance validation parameters were carried out according to the SANTE 11312/2021 document (EC, 2022), which includes the criteria for pretreatment which is should be carried out during method validation in order for the results of the analysis to be considered satisfactory. The final extract is prepared for GC/MS analysis. Before sample analysis, samples were calibrated in a solvent (hexane:toluene, 1:1, v:v) and then calibrated in a honey matrix to reduce matrix interferences during quantification and matrix effect during calibration.

Results and discussion

The applied extraction procedure is shown in Figure 1. The chromatographic separation was carefully optimized. Five-point calibration curves were plotted using standard mixtures of the analytes at various concentrations added to the honey sample (Table 1). The limits of detection achieved were determined to be 0.004 mg/kg for amitraz and 0.005 mg/kg for 2, 4 – DMA. Linearity was checked by using matrix-matched standards (Table 1). Linearity and range were determined by linear regression using calibration with an internal standard, Method repeatability was calculated as the relative standard deviation (RSD) of five

replicates, whereas instrument repeatability was determined as the RSD of five consecutive injections of the same sample concentrations.

Table 1.GC-MS validation data for amitraz and metabolite 2, 4 -DMA in honey

Compound	Retention time, min	Calibration curve	Correlation coefficient, r^2	LOQ, mg/kg	Expanded Uncertainty (%)
2,4- DMA	5.07	y=1.05368 – 3.69617	0.999048	0.005	25
Amitraz	29.79	y=1.23725 – 35.7889	0.999052	0.004	28

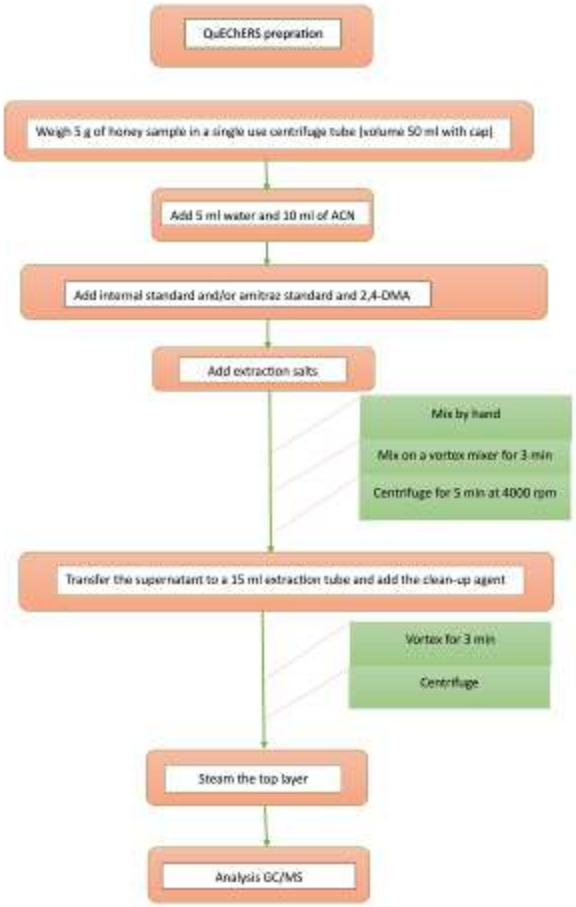


Figure 1. Extraction preparation scheme

The applied extraction scheme is shown in stages in Figure 1. The results of the RSD and recovery for spiked honey samples at four concentration levels are shown in Figure 2. Three different types of sorbents were used for pretreatment. Common sample preparation procedures include the post-extraction cleaning step, which is time-consuming and requires the use of solvents that are not environmentally friendly. The comparison of various SPE phases included Z-sep (ZrO_2-coated silica phase), $MgSO_4$/PSA/C18 and EMR-lipid. When a 15 ml EMR-Lipid tube was used after separating the supernatant after the extraction and before the purification phase the EMR-Lipid salts were preactivated with 3 ml of water. All tested samples were in compliance and with a value lower than the MDK prescribed by the European and the regulations of the Republic of Serbia (EC, 2005; Rulebook 91/2022).

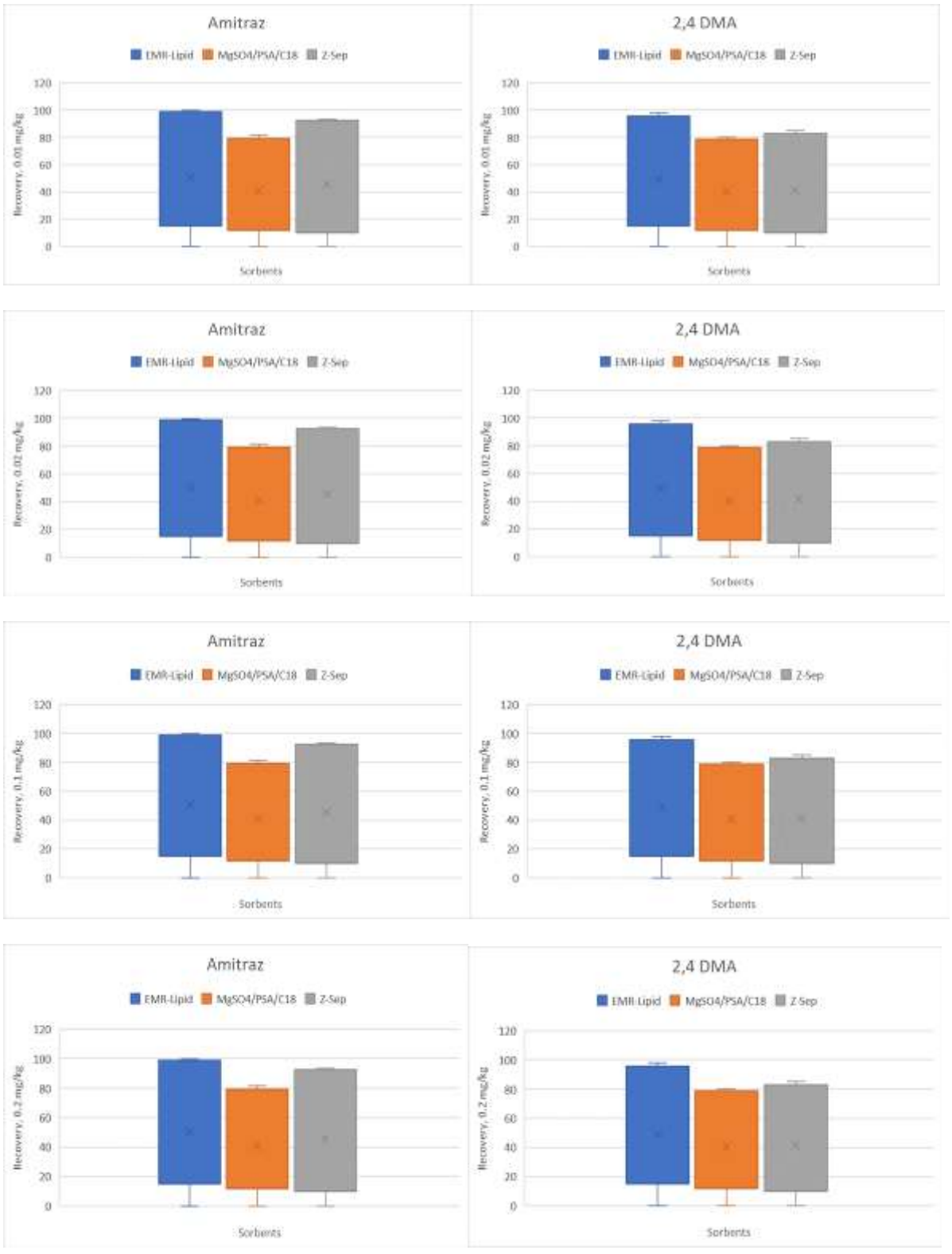
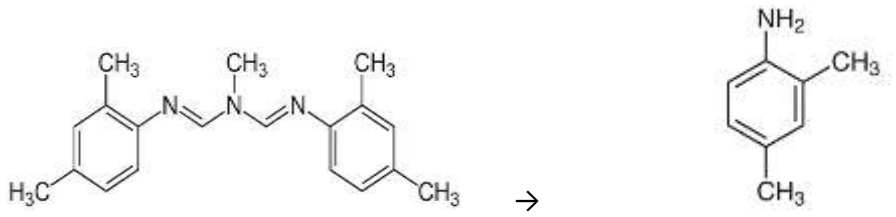


Figure 2. Recovery results using three type od sorbents at the four concentration levels

Table 2. Results obtained by testing real honey samples

Compound	Type of honey	Tested samples	Attendance percentage	Min value, mg/kg	Max value, mg/kg	Mean value, mg/kg
2, 4- DMA	acacia honey	17	11.8	0.015	0.052	0.034
Amitraz	acacia honey	17		n.d.	n.d.	
2, 4- DMA	flower honey	28	17.4	0.024	0.086	0.055
Amitraz	flower honey	28	17.4	0.028	0.036	0.032



Amitraz → 2, 4 –DMA

Figure 3. Structures of amitraz and its degradation product

Furthermore, results also showed that in the case of $MgSO_4$/PSA/C18 clean-up, the low recoveries with a pre-spiked standard solution originated mainly from the incomplete recovery in relation to other sorbents. As can be seen in Figure 2, the amount of sorbent and the type of sorbent have a small effect on the recovery of amitraz and metabolites. Similar recoveries (from 79.4% to 89%) were obtained for amitraz and 2,4-DMA when the sorbent amount was PSA, C18, and Z-sep. Better recoveries were obtained when EMR lipid purification was used (100.8% for amitraz and 103.6% for 2,4-DMA), and this sorbent was chosen for conducting further experiments with honey samples.

Investigations by other authors have confirmed that in most honey samples only the final products of amitraz decomposition such as 2,4-dimethylformanilide (DMF) and 2,4-DMA can be detected while amitraz was not present (Kubiak *et al.,* 2020). The structure of amitraz and the degradation product are shown in Figure 3. Their molar masses are important for the calculation of the conversion factor and calculation of the amitraze sum. Tests of Australian honey samples did not confirm the presence of acaricides and neonicotinoids, but the presence of polycyclic aromatic hydrocarbons was confirmed in some honey samples. From a total of 212 honey samples tested, PAHs were detected in 23 honeys, where only 4 of 33 PAHs were found, while the presence of tested pesticides (Cyhalothrin isomers) was confirmed (Hungerford *et al.*, 2021). The examination of samples from the territory of Batajnica is in agreement with other examinations, because the obtained amounts were below the MRL, and in a larger percentage of the examined samples below the LOQ (Table 2).

In the scientific literature, there are test results of honey samples treated with agents containing amitraz (eg Apiwarol). Colony treatment by Apiwarol single treatment or a four times every four days during a mount after the harvest, the presence of amitraz was not determined in the examined honey. While of the examined metabolites, the most frequently determined compound was 2, 4 DMA (Pohorecka *et al.,* 2018). After two long-lasting treatments with Apiwarol (strips with 500 mg amitraz each, contact action, 42 days), no residue of the parent compound was detected in honey. Tests of honey samples from conventional production in Slovenia, only the active substances amitraz, coumaphos and

thymol were found ≥LOQ, the amount of amitraz in the samples ranged from 0.01 to 0.12 mg/kg (Česnik *et al*., 2019).

Conclusion

In this study, the presence of amitraz and 2, 4-DMA was investigated by different analytical methods in honey samples from production areas in the Batajnica region. A rapid, simple and efficient GC-MS method for the determination of amitraz and 2, 4-DMA in honey samples was achieved using water and acetonitrile with an EMR lipid purification step. The QuEChERS extraction was modified and applied to the samples. A dilution approach was applied to the final extract, which was found to be sufficiently reliable for the selected matrices. In this way, the process is simplified and the possible loss of analytes during sample preparation is minimized. Chromatographic conditions, optimized sample preparation and minimization of matrix effects were optimized. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection, and limit of quantitation.
Satisfactory validation parameters were obtained for the determined residues according to the SANTE/11312/2021 requirements. The applicability of the method was tested on a real sample and proved to be suitable for routine analysis of the pesticides investigated in this study.

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CONTROL OF *CYDIA POMONELLA* L. IN APPLE ORCHARDS USING A MIXTURE OF INSECTICIDES

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Abstract

The codling moth (*Cydia pomonella* L.) is the most important apple pest causing fruit damage, significantly decreasing their market value, and complicating the storage process. To establish the level of apple protection against the codling moth, the trials were carried out during 2022, according to the EPPO methods using three plant protection products (PPPs) based on chlorantraniliprole + abamectin (45 g a.i./L+18 g a.i./L, SC), acetamiprid + pyriproxyfen (200 g a.i./L+100 g a.i./L, OD), emamectin benzoate + spinosad (40 g a.i./L+160 g a.i./L, SC). In apple orchards (Idared, Modi, Golden Delicious, and Granny Smith), at localities in Vojvodina, Serbia (Kać, Čelarevo, Mala Remeta, and Budisava), PPPs were foliarly applied by backpack sprayer at concentration/amount of 0.075-0.11% 0.25-0.5 L/ha and 0.3-0.5 L/ha, respectively. The experiment was set up in four replications in randomized block design. By visual examination of plants in orchards, the presence of *C. pomonella* eggs was registered, and the hatching of larvae of the first generation was underway. The efficacy of the products was performed according to Abbott, and the significance of differences (ANOVA) for the confidence interval of 95%. Fifteen days after the application the efficacy ranged from 56.3-100%, while after 24 days efficacy ranged from 74.3-96.9%, depending on the applied insecticides, the amount, as well as the locality. In both assessments, the number of damaged apples in the variants with insecticide application was significantly lower regarding the control. The applied insecticides showed satisfactory efficacy for the protection of apple fruits from *C. pomonella* in agricultural production in the localities of Serbia.

Keywords: *Apple, C. pomonella, Insecticide, Efficacy.*

Introduction

Plant protection is one of the main prerequisites for achieving successful, intensive, and economical agricultural production. When it comes to contemporary fruit production, efficient protection must ensure a high yield and fruit quality with necessary expenses, and obligation that no pesticide residues, which could be harmful to consumers, can be found in the fruits. The apple is considered one of the fruit species where the use of pesticides is the highest, both per treated surface and the number of treatments during the growing season because it is exposed to the attack of numerous harmful agents.

The codling moth (*Cydia pomonella* L.) is one of the most important pests of apples and pears. It is a cosmopolitan insect widely distributed in the Republic of Serbia. *C. pomonella* occurs every year and causes great damage to apples, which significantly reduces the market value of the fruits and prevents their storage. This pest has two to three generations per year and overwinters as a mature larva in a white cocoon under the bark of a tree (trunk or branch). First-stage larvae bore into the fruits, most often in places where two fruits or a leaf and a fruit touch, making tunnels, and leaving excrement behind, after which they burrow into the core

where they feed on apple seeds. Several caterpillars can be burrowed into one fruit (Kereši *et al*., 2019).
Control of the codling moth should be adapted to each orchard, and the strategy primarily depends on the number of insects that needs to be determined and monitored. The first measure that should be implemented in orchards is the use of pheromone traps for monitoring the occurrence and number of moths, as well as determining the optimal period for treatment. In the Republic of Serbia, a large number of insecticides (14 active substances and 46 plant protection products (PPPs)) with different modes of action (10) have been registered for the control of *C. pomonella* (Group of authors, 2022). As a consequence of the frequent application of insecticides, in many apple orchards, we often encounter reduced efficacy of certain active substances, i. e. resistance. Therefore, the alternating application of insecticides with different modes of action, use of mixtures, as well as other integral measures, play a key role in preventing resistance of this pest to the most commonly used insecticides.
This research aimed to determine the efficacy of PPPs based on chlorantraniliprole + abamectin, acetamiprid + pyriproxyfen, and emamectin benzoate + spinosad in the control of *C. pomonella* within intensive apple orchards at four locations on the territory of Vojvodina. Another goal was to determine whether there are significant differences in efficacy between the listed insecticides.

Material and Methods

The trial was conducted in apple orchards (varieties Idared, Modi, Red Delicious, and Granny Smith varieties), at the localities Kać, Čelarevo, Mala Remeta, and Budisava (Autonomous Province of Vojvodina, Serbia). The experiment was set up according to standard OEPP methods for experimental design and data analysis (Anonymous, 2012) and for the efficacy of insecticides for *C. pomonella* (Anonymous, 2004). The experiment was set up according to a random block design in four replications. The size of a basic plot consists of four trees, and PPPs used in the experiment are given in Table 1.

Table 1. PPPs used for control of the *C. pomonella* in field trials

Active substance	Formulation	Amount	Applied amount/concentration
chlorantraniliprole + abamectin	SC	45 g/L+18 g/L	0.075 and 0.11%
acetamiprid + pyriproxyfen	OD	200 g/L+100 g/L	0.25 and 0.5 L/ha
emamectin benzoate + spinosad	SC	40 g/L+160 g/L	0.3 and 0.5 L/ha

The treatment was performed on Jun 2022 when the apples were in phase BBCH 73-74 (fruit size up to 40 mm). Two evaluations of the applied insecticide effects were performed. The evaluation was based on the number of damaged apple fruits, out of 300 examined apples per repetition. The first evaluation was performed 15 days after treatment, and the second 24 days after treatment. The results of the experiment are presented through mean values for the number of damaged apple fruits. Also, the standard deviation (Sd+) was determined, as well as the significance of the differences (LSD 5%) (ANOVA). Efficacy (E%) was calculated according to Abbott (Wentzel, 1963).

Results and Discussion

Results of the efficacy of insecticides in the control of the codling moth (*C. pomonella*) in apple orchards are shown in Tables 2-3. In all examined localities, the number of damaged fruits, 15 days after the application of PPPs based on chlorantraniliprole + abamectin, acetamiprid + pyriproxyfen, and emamectin benzoate + spinosad was significantly lower compared to the control.

Table 2. The number of damaged apple fruits and the efficacy of insecticides in the control of *C. pomonella* 15 days after treatment

Insecticide (%, L/ha)	**Kać**		**Čelarevo**		**Mala Remeta**		**Budisava**	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
chlorantraniliprole + abamectin(0.075)	**10.2**±2.9 b	**76.7**	**2.7**±0,9 b	**87.2**	**2.0**±0.8 b	**70.4**	**0.5**±0.6 c	**96.4**
chlorantraniliprole + abamectin (0.11)	**8.5**±2.6 bc	**80.7**	**1.7**±1,7 b	**91.8**	**1.2**±1.2 b	**81.5**	**0.0**±0.0 c	**100**
acetamiprid + pyriproxyfen (0.25)	**5.2**±0.9 c	**88.1**	**2.5**±0,6 b	**88.4**	**1.5**±1.3 b	**77.8**	**3.2**±1.5 b	**76.3**
acetamiprid + pyriproxyfen (0.5)	**5.0**±1.4 c	**88.7**	**1.2**±0,9 bc	**94.2**	**1.2**±0.9 b	**81.5**	**2.0**±1.4 b	**85.4**
emamectin benzoate + spinosad (0.3)	**10.5**±3.1 b	**76.1**	**2.2**±0,5 b	**89.5**	**0.7**±0.9 bc	**88.9**	**1.7**±0.5 bc	**87.2**
emamectin benzoate + spinosad (0.5)	**6.7**±0.9 c	**84.6**	**1.5**±1,7 b	**93.0**	**0.2**±0.5 c	**96.3**	**1.0**±1.4 c	**92.7**
Control	**44.0**±4.97a	/	**21.5**±2.6a	/	**6.7**±1.3 a	/	**13.7**±2.1 a	/
F	99.9		98.0		13.6		36.1	
p	<0.01		<0.01		<0.01		<0.01	
LSD 5%	3.26		1.73		1.38		1.78	

$\bar{x}$ – average value; Sd± - standard deviation; E%-efficacy.

Table 3. The number of damaged apple fruits and the efficacy of insecticides in the control of *C. pomonella* 24 days after treatment

Insecticide (%, L/ha)	**Kać**		**Čelarevo**		**Mala Remeta**		**Budisava**	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
chlorantraniliprole + abamectin(0.075)	**18.2**±2.9 b	**83.0**	**4.0**±0.8 c	**88.9**	**3.75**±0.9 c	**84.7**	**0.75**±0.9 d	**95.5**
chlorantraniliprole + abamectin (0.11)	**16.7**±2.2bc	**84.4**	**2.5**±1.3 cd	**93.1**	**2.25**±0.9 c	**90.8**	**0.5**±0.6 d	**96.8**
acetamiprid + pyriproxyfen (0.25)	**17.2**±1.5 b	**83.9**	**7.2**±0.6 b	**79.8**	**2.00**±1.3 c	**91.8**	**4.2**±0.9 b	**74.3**
acetamiprid + pyriproxyfen (0.50)	**10.5**±1.3 b	**90.2**	**5.2**±0.8 c	**85.4**	**0.7**±0.5 c	**96.9**	**2.5**±0.6 c	**84.8**
emamectin benzoate + spinosad (0.30)	**17.0**±2.6 b	**84.1**	**3.7**±0.9 c	**89.6**	**10.0**±2.9 b	**59.2**	**2.5**±0.6 c	**84.8**
emamectin benzoate + spinosad (0.50)	**12.5**±1.9 b	**88.3**	**3.5**±1.0 c	**90.3**	**10.7**±0.9 b	**56.3**	**1.2**±1.5 cd	**92.4**

Control	**107**±28.7a	/	**36.0**±3.6 a	/	**24.5**±5.5 a	/	**16.5**±1.3 a	/
F	39.5		222.1		40.2		103.9	
p	<0.01		<0.01		<0.01		<0.01	
LSD 5%	13.14		1.87		3.09		1.24	

$\bar{x}$ – average value; Sd± - standard deviation; E%-efficacy.

The efficacy of PPPs based on chlorantraniliprole + abamectin ranged from 70.4 to 100%, acetamiprid + pyriproxyfen 76.3 - 94.2%, while the efficacy of PPP based on emamectin benzoate + spinosad was 76.1-96.3%, depending on the applied amount and locality (Table 2). Also, the number of damaged fruits, immediately before harvest, was significantly lower in the variants where the examined insecticides were applied compared to the control. The efficacy of the PPPs based on chlorantraniliprole + abamectin, applied in the recommended concentrations, was 83.0-98.5%, acetamiprid + pyriproxyfen 74.3-96.9%, while the efficacy of the insecticides emamectin benzoate + spinosad was 56.3-92.4% (Table 3), depending on the applied amount and locality.

Chlorantraniliprole showed an efficacy of over 90% in the protection of apples against *C. pomonella* (Bassi *et al*., 2009). Thanks to its high efficacy, selectivity, and favorable toxicological and eco-toxicological properties, chlorantraniliprole can be pertinent in insecticide resistance management (IRM) as well as in integrated pest management (IPM) (Milanese *et al*., 2014). Based on previous research (Bosh *et al*., 2018) it was also concluded that chlorantraniliprole manifested high efficacy, ranging from 85 - 97%, in the control of *C. pomonella*. Moreover, it was determined that there is no resistance to chlorantraniliprole, which is in accordance with our research. In 2013, the efficacy of insecticides belonging to the groups of avermectins and diamides for the control of codling moth was tested. The results of these experiments indicated a good efficacy (92.0% and 95.6%) of insecticides from the mentioned chemical groups (Tamaš *et al*., 2014). In the same study, acetamiprid showed very low efficacy in the control of this pest (54.6%-55.1%) in two localities.

In our trials, the PPP based on acetamiprid + pyriproxyfen showed acceptable efficacy. Some data (Cichon *et al*., 2013), indicate that certain populations of codling moth showed a lower percentage of mortality when PPPs based on acetamiprid were used, and higher ethoxycoumarine-O-deethylation (ECOD) activity, which may indicate the occurence of resistance. It was observed that the phenomenon of resistance was expressed in larvae and adults, both in the field and laboratory conditions.

PPP based on spinosad in control of *C. pomonella* in apple orchards in Spain showed a high efficacy ranging from 94 to 100% (Bosch *et al*., 2018), and some research (Thompson *et al*., 2006) found reduced sensitivity of *C. pomonella* larvae to spinosad and thiacloprid. The resistance of *C. pomonella* to spinosad was correlated with the resistance to diflubenzuron. Due to the absence of prior use of neonicotinoids and spinosad, resistance to these compounds in the control of codling moth is likely the result of non-specific metabolic detoxification (Thompson *et al*., 2006). In the previous research (Iraqui and Hmimin, 2016), the PPP based on spinosad showed high efficacy (up to 100%) in the control of *C. pomonella*. In our field experiments, the PPP based on emamectin benzoate + spinosad showed acceptable efficacy (56.3-96.3%), depending on the locality and the time of efficacy evaluation.

Conclusions

In all localities, the number of apple fruits damaged by *C. pomonella*, two weeks after the treatment and immediately before harvesting, was at a significantly lower level compared to the control variant. The obtained results indicate the sensitivity of *C. pomonella* populations

in the mentioned localities of Vojvodina, to insecticides chlorantraniliprole + abamectin, acetamiprid + pyriproxyfen, and emamectin benzoate + spinosad.

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SURVEY OF THE POSSIBLE PRESENCE OF *Xylella fastidiosa*, THE CAUSAL AGENT OF PIERCE'S DISEASE OF GRAPEVINE AND OTHER PLANT DISEASES IN REPUBLIC OF SRPSKA

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Abstract

Xylella fastidiosa survey in Republic of Srpska (RS) in Bosnia and Herzegovina started in 2015, not long after outbreak in southern Italy. Considering the quarantine bacterium *X. fastidiosa* is listed on quarantine I A1 list in Bosnia and Herzegovina, and that it has a very wide range of host plants, survey in RS was approved and financed by the Ministry of Agriculture, Forestry and Water Management of RS. Visual inspections and sampling of different host plants were carried out in registered nurseries and seedling production places, farms and gardens, as well as in public areas in RS. Laboratory analysis was carried out in accordance with the EPPO diagnostic protocols: PM 7/24 (1): Bulletin 34, 187-192; PM 7/24 (2): Bulletin 46 (3), 463–500; PM 7/24(3): Bulletin 48 (2), 175–218 and PM 7/24 (4): Bulletin 49 (2), 175–227. Protocols include two tests based on a different principle: ELISA for the serological and a conventional PCR test for the molecular detection. CTAB method was used for DNA extraction and a set of primers RST31/RST33 was used for the detection of *X. fastidiosa*. The PCR products were separated on a 1.5% agarose gel in 1 x TAE buffer. During 2015 and 2016, 29, 131 samples were analyzed, respectively. In 2017 and 2018 40 samples were analyzed along with 83 visual inspections. Number of analyzed samples in 2019 was 65, in 2020 - 65, in 2021 - 74 and in 2022 - 70. All tested samples were *Xylella fastidiosa* negative, but considering consequences if bacteria occur, surveillance program is continued in 2023.

Keywords: *Xylella fastidiosa, different host plants, survey, Republic of Srpska.*

Introduction

Xylella fastidiosa Wells *et al.* is a gram-negative, slow-growing, fastidious bacterium that colonizes the xylem vessels of its host plants and is transmitted by insect vectors that feed by sucking xylem sap (Uceda-Campos *et al.*, 2022). In 2013, the occurrence of *Xylella fastidiosa* was reported in southern Italy (near Lecce, in the Salentopeninsula, Apulia region), associated with quick decline symptoms on olive trees (*Olea europea*), oleander (*Nerium oleander*) and almond (*Prunus dulcis*) (Saponari *et al*., 2013). Not long after its first detection, *X. fastidiosa* was detected in 2015 in France and Switzerland, followed by Germany and Spain in 2016, as well as Portugal in 2019 (Trkulja *et al.*, 2022; EPPO, 2023).

Bacterium *X. fastidiosa* is a polyphagous pathogen that attacks a wide range of host plants, from weeds, ornamental plants and shrubs to economically very important fruit-growing, woody and forest plants (Girelli *et al.*, 2022; Godefroid *et al.*, 2022). Currently, the list of host plants maintained by EFSA includes 694 plant species from 299 genera and 88 botanical families that have been confirmed to be infected with *X. fastidiosa* regardless of the detection method used, with 412 plant species from 190 genera and 68 botanical families of infections confirmed by at least two different detection tests. Moreover, 174 host plant species from 91

genera and 44 botanical families are currently identified in Europe (European Food Safety Authority et al., 2022a, 2022b).
Records in other European countries showed a major change regarding *X. fastidiosa* distribution, ranging from tropical and subtropical areas, as well as in temperate or even continental climate zones. Even in European Union *X. fastidiosa* is listed in A2 Quarantine pest list (Annex II B), in Bosnia and Herzegovina (B&H) this bacterium has quarantine status and it is listed on quarantine I A1 list (Trkulja *et al.*, 2012). *X. fastidiosa* causes a number of diseases of economic importance, namely Pierce's disease (PD) in grapevine, olive quick decline syndrome (OQDS), citrus variegated chlorosis (CVC) or citrus X disease, pony peach disease and plum leaves scald, as well as diseases such as margin necrosis and leaf scorch affecting oleander, coffee, almonds, pecans, as well as other types of cultivated and ornamental plants and forest trees (Trkulja, 2014; Trkulja *et al.*, 2017, 2022; Uceda-Campos et al., 2022). The phytopathogenic bacterium *X. fastidiosa* is currently one of the greatest phytosanitary threats to agricultural production in Europe (EFSA Panel on Plant Health, 2018; Trkulja *et al.*, 2019, 2022). Among host plants, severe damage can be caused in major crops such as grapevine, citrus, stone fruits, as well as forest, landscape and ornamental trees widely present in B&H. Thus, considering the above mentioned risk analysis, the survey in Republic of Srpska (RS) was approved and financed by Ministry of Agriculture, Forestry and Water Management of Republic of Srpska from 2015.

Materials and methods

Visual inspections and sampling of host plants were carried out in a number of registered nurseries and seedling production places, farms and gardens, as well as public areas in Republic of Srpska and from host plants on border crossing during their import. From Table 1, it can be seen the number of samples and places where host plant samples were taken for laboratory analysis of the presence of quarantine bacteria *X. fastidiosa* in the territory of the Republic of Srpska in the period 2015-2022.

Table 1. Sample number, locality and host plant samples were taken for laboratory analysis of the presence of bacteria *X. fastidiosa* in the period 2015-2022

Locations where host plant samples were taken for laboratory analysis	**Sample number for laboratory analysis per year**							
	2015	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Registered nurseries and producers of seedlings of agricultural plants	0	38	11	11	10	10	10	12
Production orchards, farms and gardens	17	73	20	20	37	37	44	43
Public areas	12	14	6	6	15	15	15	12
Host plants from import	0	6	3	3	3	3	5	3
Total:	29	131	40	40	65	65	74	70

According to the "Special surveillance program for the presence of quarantine pest *X. fastidiosa* (Wells *et al.*) – Pierce's disease in Republic of Srpska" (Official Gazette of the Republic of Srpska 62/17, 47/18, 53/19, 36/20, 43/21 and 31/22) every visual inspection and sampling were mapped, recorded and digitized using FITO GIS software (Photo 1-6).

Laboratory analyses were carried out in accordance with the EPPO diagnostic protocols: PM 7/24 (1): 2004 OEPP/EPPO Bulletin 34, 187-192; PM 7/24 (2): 2016 OEPP/EPPO Bulletin 46 (3); 463–500; PM 7/24(3): 2018 OEPP/EPPO Bulletin 48 (2), 175–218 and PM 7/24 (4): 2019 OEPP/EPPO Bulletin 49 (2), 175–227. Protocols include two tests based on a different principle: ELISA for the serological and a conventional PCR test for the molecular detection. CTAB method was used for DNA extraction and set of primers RST31/RST33 was used for the detection of *X. fastidiosa*. The PCR products were separated on a 1.5% agarose gel in 1 x TAE buffer.

Results and discussion

In 2015 visual inspections and sampling of host plants in Republic of Srpska was carried out in registered nurseries and seedling production places, farms and gardens, as well as public areas in regions of Banja Luka, Bijeljina and Trebinje. During 2015, 29 samples were analyzed in Laboratory for plant protection, seed and biotechnology PI Agricultural Institute of Republic of Srspka, Banja Luka: 12 grapevine samples from the Banja Luka and Trebinje region, 5 peach samples from the Banja Luka, Trebinje and Bijeljina regions, 2 samples of oak from the Banja Luka region, as well as 5 olives, 2 citrus, 1 almond and 2 oleander samples from the Trebinje region. All tested samples were negative for the presence of the quarantine bacterium *X. fastidiosa.*

During 2016, Ministry of Agriculture, Forestry and Water Management of Republic of Srpska published "Special surveillance program for the presence of quarantine pest *Xylella fastidiosa* (Wells *et al*.) – Pierce's disease in Republic of Srpska" (Official Gazette of RS 101/16). A total of 131 samples of host plants were analyzed (Table 1), of which 38 samples were taken from registered nurseries and from producers of seedlings of agricultural plants (of which 7 grapevine samples, 6 peach samples, 5 cherry samples, 4 rosemary samples, 5 lavender samples , 3 samples of vinca and 8 samples of other host plants), while 73 samples were taken from production orchards, farms and gardens (of which 19 samples are vines, 17 samples of plants from the genus Prunus (peach and cherry), 4 samples of olive, 4 samples of citrus, 7 samples of rosemary, 10 samples of lavender, 5 samples of vinca and 7 samples of other host plants), 14 samples taken from public areas (3 samples of oleander and 11 samples of other host plants), as well as 6 samples taken from host plants on border crossing during their import. The presence of the quarantine bacterium *X. fastidiosa* was not detected in any of the tested samples.

In 2017 and 2018, "Special surveillance program for the presence of quarantine pest *Xylella fastidiosa* (Wells *et al*.), the causal agent of Pierce's disease in Republic of Srpska" is published in Official Gazette of the RS 62/17 and 47/18. The host plants and samples number, as well distribution published in the program remained the same during both years. A total of 40 samples were analyzed in each year. In Table 1 number of samples taken from registered nurseries and producers of seedlings of agricultural plants, from production orchards, farms and gardens, from public areas, and from imported host plants in 2017 and 2018 is presented. Additionally, during 2017 and 2018, 83 visual inspections of host plants were carried out, of which 17 visual inspections were carried out in registered nurseries and producers of seedlings of agricultural plants, 45 visual inspections of host plants from production orchards, farms and gardens, as well as 22 visual inspections of host plants from public areas. All laboratory tested and visually inspected samples were negative for the presence of bacteria *X. fastidiosa*. The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2017 and 2018 are shown in Photo 1 and 2.

Photo 1. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska, 2017*

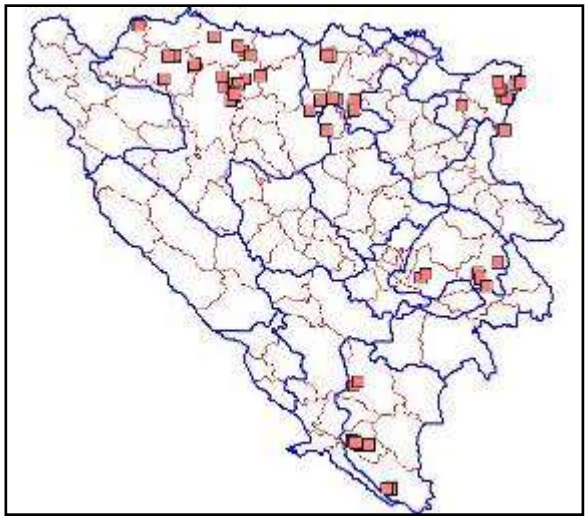

Photo 2. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska,2018*

During 2019, according to the "Special surveillance program for the presence of quarantine pest *Xylella fastidiosa* (Wells et al.) – Pierce's disease in Republic of Srpska" which was published in the Official Gazette of the RS 53/19, a total of 65 samples of host plants were analyzed, of which 10 samples were taken from registered nurseries and from producers of seedlings of agricultural plants, while 37 samples were taken from production orchards, farms and gardens, 15 samples were taken from public areas, and 3 samples taken from imported host plants (Table 1). The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2019 are shown in Photo 3. All tested samples were negative for the presence of the quarantine bacterium *X. fastidiosa.*

In 2020 according to the "Special surveillance program for the presence of quarantine pest *X. fastidiosa* – Pierce's disease in RS" which was published in the Official Gazette of the RS 36/20, a total of 65 samples of host plants were analyzed, of which 10 samples were taken from registered nurseries and from producers of seedlings of agricultural plants, while 37 samples were taken from production orchards, farms and gardens, 15 samples were taken from public areas, as and 3 samples taken from imported host plants (Table 1). The geographic distribution of the host plant samples taken in the territory of the RS during 2020 are shown in Photo 4. The presence of the quarantine bacterium *X. fastidiosa* was not detected in any of the tested samples.

Photo 3. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska,2019*

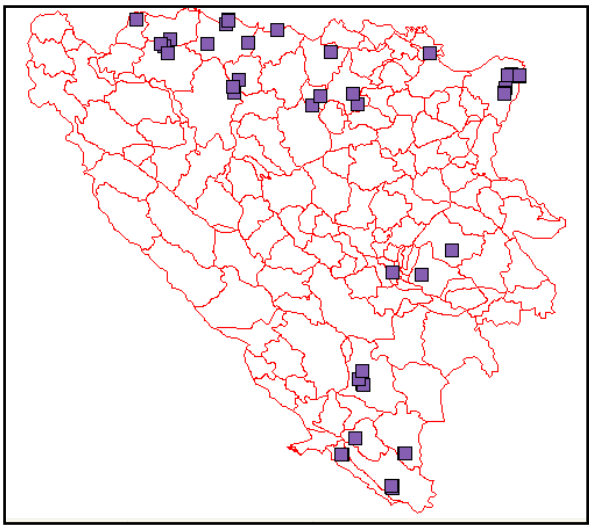

Photo 4. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska,2020*

During 2021, according to the "Special surveillance program for the presence of quarantine pest *X. fastidiosa* – Pierce's disease in RS" which was published in the Official Gazette of the RS 43/21, a total of 74 samples of host plants were analyzed, of which 10 samples were taken from registered nurseries and from producers of seedlings of agricultural plants, while 37 samples were taken from production orchards, farms and gardens, 15 samples were taken from public areas, as and 3 samples taken from imported host plants (Table 1). The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2021 are shown in Photo 5. All laboratory tested samples were negative for the presence of bacteria *X. fastidiosa*.

In 2022 , according to the "Special surveillance program for the presence of quarantine pest *X. fastidiosa* – Pierce's disease in RS" which was published in the Official Gazette of the Republic of Srpska 31/22, a total of 70 samples of host plants were analyzed, of which 12 samples were taken from registered nurseries and from producers of seedlings of agricultural plants, while 43 samples were taken from production orchards, farms and gardens, 12 samples were taken from public areas, as and 3 samples taken from imported host plants (Table 1). The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2022 are shown in Photo 6. All tested samples were negative for the presence of the quarantine bacterium *X. fastidiosa.*

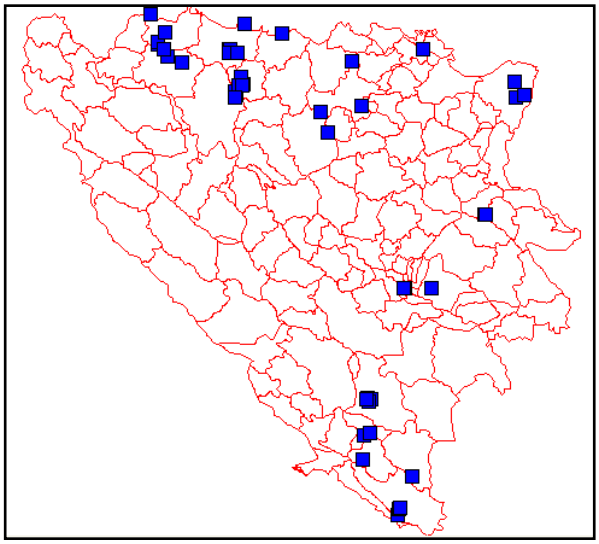

Photo 5. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska, 2021*

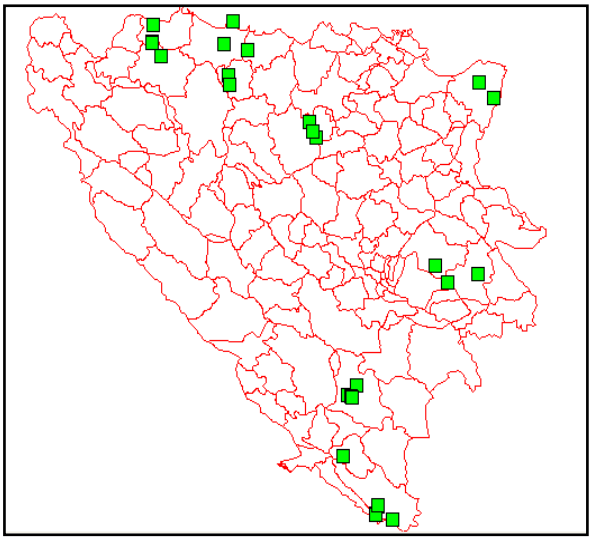

Photo 6. *Fito GIS mapping – X. fastidiosa, visual inspection and sampling in Republic of Srpska, 2022*

Conclusion

Based on the conducted laboratory analysis during 2015-2022, all tested samples were negative for the presence of quarantine pathogenic bacteria *X. fastidiosa*, whose special surveillance was carried out under the Program approved and financed by Ministry of Agriculture, Forestry and Water Management of Republic of Srpska. With regard to the consequences of the occurrence of these bacteria, as well as the expansion and huge economic importance of this pathogen, surveillance will be continued in 2023 in order to ascertain and monitor the presence status of this dangerous quarantine pathogen in the Republic of Srpska, with the goal that in the case of its occurrence emergency measures of eradication can be implemented and further spreading could be prevented.

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DETERMINATION OF CONTENT AND COMPOSITION OF ESSENTIAL OIL OF *ORIGANUM VULGARE* L. FROM THE REGION OF HERZEGOVINA

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Abstract

The composition of essential oils derived from wild and cultivated *Origanum vulgare* L., obtained through the process of hydrodistillation, was examined. The oil was subjected to gas chromatographic (GC) analysis and a combination of gas chromatography and mass spectrometry (GC-MS). Out of the 31 detected components, 25 were successfully identified, accounting for 98.4% of the total number of registered components. The predominant compound in the essential oil is carvacrol, constituting 59.27% in wild oregano and 67.11% in cultivated oregano. Other significant constituents include thymol (6.65% in wild, 6.173% in cultivated) and α-pinene (1.11% in cultivated, 0.99% in wild). A higher content of monoterpene hydrocarbons, such as ortho-cymene and γ-terpinene, was observed in the essential oil of cultivated oregano compared to the wild variety (14.928% - trace amounts; 5.357% - 4.412%). Additionally, the cultivated oregano essential oil contains sesquiterpenes, specifically γ-β-bisabolol and β-caryophyllene, which were not found in the wild sample. By comparing these findings with existing literature data, significant differences in the composition and content of essential oils between wild and cultivated *Origanum vulgare* L. have been observed. These variations can be attributed to annual climatic factors, habitat conditions, and the developmental stages of the plant species. Considering the diverse range of chemical constituents present in *Origanum vulgare* L. and their exceptional biological activities, further research in the fields of pharmacy, medicine, and agriculture is crucial.

Keywords: *Origanum vulgar* L, *wild and cultivated, essential oil, chemical composition.*

Introduction

Origanum vulgare L., commonly known as oregano, is a perennial herbaceous aromatic plant from the *Lamiaceae* family, native to the Mediterranean region. It is cultivated in regions with favorable climatic conditions. There are more than 61 species of oregano, each possessing a characteristic aroma and taste. Among them, the most renowned is *Origanum vulgare* L. (common oregano), which has been used for culinary purposes and in traditional medicine for thousands of years. It grows as a shrub, reaching a height of up to 50 cm, with small, green leaves. The flowers are also small, usually white, light pink, or pink in color. Oregano is easy to cultivate, and the leaves are harvested as needed between May and September.

The region of eastern Herzegovina, characterized by high temperatures, rocky soil, and abundant sunshine, offers ideal conditions for oregano cultivation. Large quantities of commercial spice for the global market are obtained from wild populations, while several species of the *Origanum genus* are cultivated and grown for their aromatic, medicinal, and ornamental properties (Stepanović and Radanović, 2011).

Origanum vulgare L. is widely known for its therapeutic properties, including diaphoretic, carminative, antispasmodic, antiseptic, and tonic effects, which have been utilized in

traditional medicine systems across various countries (Sahin et al., 2004). It is extensively employed in agriculture, pharmaceuticals, and the cosmetic industry as a culinary herb and a source of aromatic substances (Dorman and Deans, 2000). On average, oregano contains 0.3% to 4.0% essential oil, which is primarily found in its flowers and leaves. The oil content is generally higher in the flowers compared to the leaves. The chemical composition of oregano essential oil, like many other aromatic plants, is greatly influenced by the biological source used for herbal medicine production, the phenological stage of development (harvesting before, during, or after the flowering period), and the range of ecological factors specific to habitats (De Mastro et al., 2017; Morshedloo et al., 2018). The quantity of active compounds in oregano essential oil exhibits daily variations depending on the plant's developmental stage (Wogiatzi et al., 2009). Various chemotypes of oregano exist based on the content and ratio of carvacrol and thymol, including carvacrol chemotype, thymol chemotype, carvacrol/thymol chemotype, and thymol/carvacrol chemotype. Russo et al. (1998) reported four chemotypes for this species grown in Calabria (Southern Italy) based on their phenolic content: thymol/carvacrol and carvacrol/thymol chemotypes, with the majority of samples belonging to the thymol chemotype. Oregano essential oil is recognized as an interesting source of antimicrobial components (Bharti and Vasudeva, 2013). Numerous studies have focused on the antibacterial and antifungal effects of oregano tea and oil (Chaudhary et al., 2007). The anticarcinogenic and antihepatotoxic effects of carvacrol, the most significant active ingredient in the oil, are particularly noteworthy (Baser, 2008; Singletary, 2010). Dried aerial parts of the plant (Origani herba) are also utilized for treating cough, bronchitis, urinary tract infections, digestive disorders, and more (Džamić et al., 2008). The global market is witnessing an increasing number of food supplements based on oregano herb-derived essential oil (Prathyusha et al., 2009).

The objective of this study is to conduct qualitative and quantitative analysis of the essential oil from *Origanum vulgare* L. herb collected from natural habitats near Bileća and cultivated in Ljubinje.

Materials and Methods

Plant Material: The plant material was collected from the Herzegovina region as follows: Wild *Origanum vulgare* L. was collected on August 15, 2022, from Sitnica Mountain near the village of Dola, located in the Bileća municipality. Cultivated *Origanum vulgare* L. was collected on October 27, 2022, from a plantation in Ljubinje, in the same municipality. The herbaceous aerial parts of the plants in the flowering stage were collected. The material was cleaned and dried in a well-ventilated and shaded area for 10 days. The dried material was then packed in sacks and stored in a cool and dry place until further use for subsequent investigations.

Steam Distillation of the Material: The essential oil was isolated by steam distillation using a Klevendžer apparatus. Procedure: The dried material was ground, and the masses of the wild and cultivated *Origanum vulgare* L. herbs were measured (94.09 g and 84.72 g, respectively). The material was covered with 500 ml of water in the distillation flask. The distillation process lasted for 120 minutes, and the volume of the essential oil was recorded. Yellow-colored essential oils were obtained (0.15 mL; 0.1 mL, respectively).

Analysis of the Essential Oil: Gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) were used for the analysis of the essential oils. The analysis was performed using an Agilent 6890N GC system with a flame ionization detector (FID) coupled to an Agilent 5975C mass detector (MSD). The separation of components was carried out on an HP-5MS capillary column (30 cm × 0.25 mm × 0.25 μm). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The column temperature was linearly programmed from

60 to 280˚C with a temperature ramp of 3˚C/min. A 1 µl solution of the essential oil in hexane (2% v/v) was injected in split mode (1:10).
The identification of individual components was based on the comparison of experimentally determined retention indices (Kovats indices - KI), retention times, and mass spectra with corresponding data from databases (NIST/NBS; Wiley) and the literature. Linear KI values were determined relative to a homologous series of n-alkanes (C9-C40) chromatographed under the same experimental conditions. The relative abundance of each component in the essential oils was determined by normalizing the integrated peak areas on the chromatograms obtained from the flame ionization detector (Adams, 2002).

Results and Discussion

The yield of essential oil obtained by hydrodistillation of wild *Origanum vulgare* L. herb was 0.42%, while the plantation-grown oregano had a slightly higher yield of 0.49%, which is consistent with the literature data (Ph. Eur, 2002). Chromatograms of the obtained essential oils from wild and plantation-grown *Origanum vulgare* L. herb are presented in Figures 1 and 2, respectively.

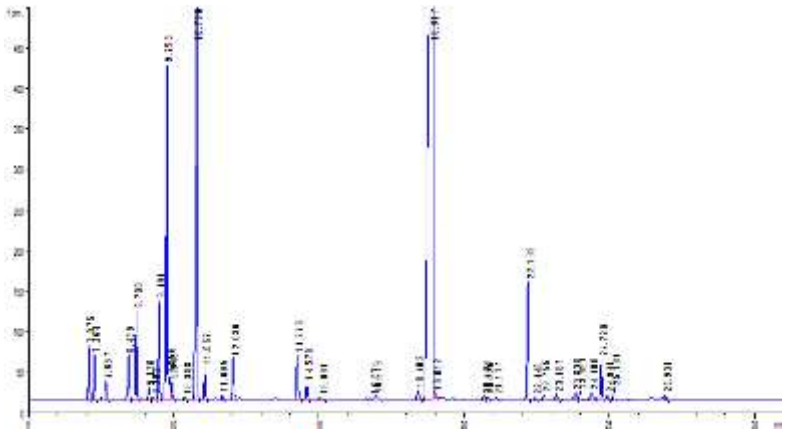

Figure 1: GC chromatogram of the essential oil from wild *Origanum vulgare* L. herb.

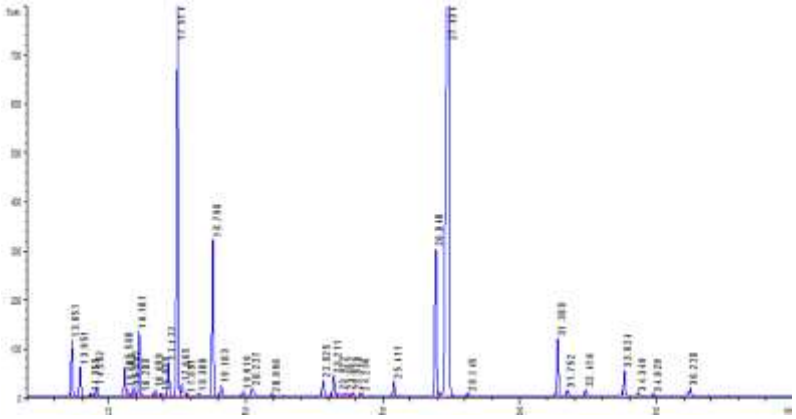

Figure 2: GC chromatogram of the essential oil from plantation-grown *Origanum vulgare* L. herb.

The analysis of Origanum vulgare L. essential oils obtained by hydrodistillation revealed a diverse range of chemical constituents. Gas chromatography analysis identified 25 out of 31 components, accounting for 99% of the essential oil. The results are expressed as the relative percentage (%) of each component in the essential oil and presented in Table 1.

Table 1: Chemical composition of the essential oil from *Origanum vulgare* L. herb

		KIE	KIL	RT	Wild	Cultivated
	Content (ml/100g)				0,42	0,49
	Compound (%)					
1.	α-thujene	915	924	6,745	1,422	1,865
2.	α-pinene	922	939	6,733	0,993	1,067
3.	Camphene	936	954	7,171	-	0,349
4.	Sabinene	960	975	7,881	0,325	-
5.	octen-3-ol	963	979	7,974	1,207	
6.	octanol-3	965	979	8,005	0,285	0,330
7.	Myrcene	977	991	8,368	1,834	2,233
8.	α-phellandrene	994	1017	8,883	0,830	0,242
9.	α -terpinene	1006	1024	9,317	1,003	1,241
10.	*orto*- cimene	1014	1026	9,570	14,928	tr
11.	p-cimene	1017	1029	9,705	0,673	14,269
12.	1.8-cineole	1021	1031	9,825	тр	0,702
13.	cis-β-ocimene	1025	1032	9,983	tr	0,052
14.	trans-β-ocimene	1035	1044	10,369	tr	0,089
15.	γ-terpinene	1047	1054	10,928	4,412	5,357
16.	Linalool	1089	1097	12,364	0,480	1,986
17.	Borneol	1156	1169	15,146	0,676	0,686
18.	terpinene-4-ol	1167	1177	15,595	0,946	tr
19.	Carvacrol methyl ether	1181	1189	16,159	0,578	tr
20.	thymol	1282	1289	20,494	6,649	6,173
21.	carvacrol	1302	1298	21,320	59,27	67,112
22.	β-caryophyllene	1408	1419	25,799	1,990	2,016
23.	α-humulene	1442	1455	27,170	0,240	0,244
24.	β-Bisabolene	1497	1505	29,342	0,938	tr
25.	Caryophyllene oxide	1573	1583	32,281	0,549	0,56835
	Identified				100,0	99,11

KIE- Kovats retention index (experimental) (AMDIS); KIL – Kovats retention index – literature data; RT- retention time; tr- in traces

The essential oil of the investigated *Origanum vulgare* L. samples had a high content of monoterpenes (60.5%) and a lower content of sesquiterpenes (37.9%). The dominant components in the essential oil were carvacrol (59.27% in wild oregano, 67.11% in plantation-grown oregano), thymol (6.65% in wild oregano, 6.173% in plantation-grown oregano), and α-pinene (1.11% in the plantation-grown sample, 0.99% in the wild sample). Ortho-cymene was identified in the wild sample (14.928%), while it was present in traces in the plantation-grown sample. Both samples contained γ-terpinene, with 4.412% in wild oregano and 5.357% in plantation-grown oregano. Additionally, plantation-grown oregano essential oil contained (Z)-β-ocimene (0.052%), which was not detected in the wild sample. Sesquiterpene β-caryophyllene was present in both samples, with 2.016% in the plantation-grown oregano and 1.99% in the wild oregano. This chemical composition of oregano essential oil is consistent with previous findings reported by other authors (Kosakowska et al., 2019), where carvacrol was the dominant compound. Kovačević et al. (2021) investigated the chemical composition of essential oil from wild *Origanum vulgare* L. in the Babušnica region, Serbia, and demonstrated significantly higher percentages of carvacrol (80.35%), p-cymene (4.82%), and thymol (4.21%). According to Baydar et al. (2004), the most abundant

components in oregano essential oil quantitatively are thymol (up to 68.0%), carvacrol (up to 85.4%), and γ-terpinene (up to 33.14%), along with significant amounts of cymene, caryophyllene, pinene, bisabolene, linalool, borneol, geranyl acetate, and linalyl acetate. Timol is an isomer of carvacrol, but its presence in oregano oil is lower than that of carvacrol (Bakkali et al., 2008). However, the dominant active component can also depend on the eco-geographical location. The variations in the abundance of carvacrol, p-cymene, and other constituents are likely due to different climatic conditions, which is in line with the study by Prathyusha et al. (2009), stating that variations in the quantitative content of components are influenced by external factors. The results of our research, indicating differences in qualitative and quantitative composition, are in agreement with findings by other authors. According to De Mastro et al. (2017), carvacrol was also the dominant compound (55.01%), while p-cymene and terpinenes were detected in significant amounts (14.71% and 11.09%, respectively). Kokini et al. (1997) investigated essential oils from *Origanum vulgare ssp. hirtum* plants collected in late autumn from six locations in three different geographical regions of Greece. They reported that oils from plants in the northern part of Greece were rich in thymol, while those from the southern part were rich in carvacrol. Several samples rich in carvacrol were also found in Bulgaria (Konakchiev, 2004). De Martino et al. (2009) examined the chemical composition of essential oil from wild oregano growing in three different locations in the Campania region, Italy. They identified three chemotypes: the first with a prevalence of carvacrol/thymol, constituting 15.90% and 12.50%, respectively; the second characterized by the prevalence of carvacrol, thymol, and their derivatives (23.34% and 22.16%, respectively); and the third with a prevalence of γ-terpinene and p-cymene (2.38% and 2.81%, respectively). De Falco et al. (2013) investigated the chemical composition and biological activity of essential oils from *Origanum vulgare* L. under different spatial plant arrangements (single and double rows). The percentage of essential oil was higher in double-row treatments at full bloom. Oils from plants grown in single rows were rich in sabinene, while those grown in double rows were richer in ocimenes.

Conclusions

The composition of essential oils from wild and cultivated *Origanum vulgare* L. herbs collected from the area of Eastern Herzegovina and obtained by hydrodistillation was investigated. The results indicate a diversity of chemical constituents in these oils. The essential oil of the investigated oregano samples has a high content of monoterpenes (60.5%), while the content of sesquiterpenes is significantly lower (37.9%). The dominant components are carvacrol, thymol, ortho-cymene, and γ-terpinene. In addition to these, the essential oil of cultivated oregano also contains sesquiterpene components such as γ-β-bisabolol and β-caryophyllene. They were not identified in the wild sample. As expected, a significant difference in composition and content of the essential oils between wild and cultivated *Origanum vulgare* L. has been observed. By comparing the obtained results with the literature data, it was noted that there is a significant influence, probably of annual climatic and habitat factors, on the interrelationship of dominant components in the investigated essential oils. For a more comprehensive determination of the composition of the essential oil, further studies are necessary, taking into account the locality and developmental stage of the plant species *Origanum vulgare* L.

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ANTIFUNGAL ACTIVITY OF THREE CHEMOTYPES OF *SATUREJA MONTANA* ESSENTIAL OIL AGAINST *ALTERNARIA ALTERNATA*

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Abstract

Winter savory (*Satureja montana* L.) is a perennial plant species native to warm temperate areas between Europe, the Mediterranean and Africa. The plant grows to a height of approximately 50 cm, and it has a semi-evergreen subshrub with lanceolate leaves and pink to white blooms that draw bees. Stems and leaves are used as spices and as tonics, carminatives, astringents, and expectorants in traditional medicine. It is used to treat a variety of diseases as an herbal tea. It's essential oil is combined with other essential oils or used alone in the food and perfume industry. The aim of the study was to assay the antifungal activity of three chemotypes of essential oil of *S. montana* against *Alternaria alternata* isolated from leaf of *Paeonia peregrina* Mill. (KB-BG/1). The essential oil was obtained by hydrodistillation of dried plant material. The chemical composition was determined by Gas Chromatography–Mass Spectrophotometric (GC-MS) analysis. The main components of winter savory essential oil are carvacrol and thymol, and the chemotypes differ according to the percentage of these components (66.0%, 73.0%, and mixed 43.5%-32.8%). The concentrations of essential oil 1.7, 2.5, and 3.33 µl/ml were used in the treatments, while the fungicide tebuconazole (Mystic) of concentration 1.7 µl/ml was used as a positive control. The chemotype 3 showed the strongest antifungal activity against *A. alternata*, while the chemotype 2 and the chemotype 1 slightly less. The isolate was completely inhibited (100%) by the lowest concentration of chemotypes 2 and 3 of oil (1.7 µl/ml).

Keywords: *Satureja montana* L., three chemotypes, essential oil, antifungal activity, *Paeonia peregrina*, *Alternaria alternata.*

Introduction

Satureja montana L., known as winter or mountain savory, is a perennial, aromatic plant species belonging to the Lamiaceae family. The plant can be found in nature, and it is cultivated as a culinary herb with strong and spicy taste (Silva et al., 2009). Savory is one of the best honey plants, and its honey is well-known as a traditional treatment for bronchitis (Mastelić and Jerković, 2003). Also, the plant is used as an antiseptic, expectorant, in tradicional medicine in the form of tea, in industrial processing to obtain tea, and in cooking as a spice and aromatic addition to food (Stepanović and Radanović, 2011). Essential oil (EO) is obtained from the air-dried plant of *S. montana*. Fresh plant in flower has 0.1-0.3% of the EO. Data from the literature indicates that the EO yield might vary from 0.5-2.9% depending on the climate. The EO yield in Serbia used to be around 1.5% with carvacrol, thymol, β-caryophyllene, γ-terpinene, ρ-cymene, and linalool as major components (Stepanović and Radanović, 2011). These components have strong antioxidative, antimicrobial, antiviral, and antidiarrheal activity (Wesolowska et al., 2014). The EO yield and its composition differ depending on the climatic conditions in which the plant may grow and the stage of plant development (Damjanović-Vratnica et al., 2011). Winter savory oil is applied as natural conservation agent in the cosmetic industry, and as active ingredient in medicinal preparations

(Chorianopoulos et al., 2004). Also, the oil is used in the food industry to flavor condiments, canned meats, sausages, soups, in the making of liqueurs, and in perfumery. It can be used in the pharmacological industry (Damjanović-Vratnica et al., 2011; Wesolowska et al., 2014). The genus *Alternaria* includes widespread saprobic, endophytic and phytopathogenic species, and their spores are the most common and potent airborne allergens (Woudenberg et al., 2013). Also, the genus is well known as post-harvest pathogens (Thomma, 2003). It grows in humid areas with a temperature range of 20-30°C and has wide range of plant hosts. Phytopathogenic species can cause economic damage in the field and during storage, and losses resulting from infection with species of *Alternaria*, especially section *Alternaria*, are also reflected in contamination with mycotoxins. *A. alternata* is one of the most important toxigenic species of *Alternaria* and produces a large number of mycotoxins, among which the most important are alternariol 1 (AOH), alternariol monomethylether (AME), altenuene (ALT) and tenuazoic acid (TeA) (Pinto and Patriarca, 2017). *Alternaria* species survive on infected crop debris, seeds and weeds, and are easily spread by wind, water and insects. The seeds are important for maintaining and spreading phytopathogenic species (Simmons 2007; Gilardi et al., 2013).

The aim of this study was to assay the antifungal activity of three chemotypes of essential oil of *Satureja montana* against *Alternaria alternata* isolated from the leaf of peony (*Paeonia peregrina* Mill.) (KB-BG/1).

Material and methods

Extraction essential oil

The plant material of *S. montana* was vegetatively propagated by cuttings in Pančevo, South Banat, Republic of Serbia (44° 52′ 20.0″ N; 20° 42′ 04.7″ E). The air-dried plant material was used for extraction of EO by hydrodistillation for 2h using a Clevenger-type apparatus according to Procedure 1 of the Ph. Jug. IV (1984). The oil was separated, dried over anhydrous sodium sulfate, and kept in closed dark vials at 4°C until further investigation.

Chemical analysis of essential oil components

Identification of EO components was performed by Gas Chromatography–Mass Spectrometer (GC/MS, Varian CP-3800/Saturn 2200) equipped with split/splitless injector and DB-5MS column (30 m x 0.25 mm, film of 0.25 mm thickness), using Wiley 7.0 library of mass spectra and by comparison of the obtained retention indices (RI) with the literature data (Adams, 2007). The hexane was used as solution of the EO (1 ml, 1% solution).

Antifungal activity of essential oil

The test fungus *A. alternata* was isolated from leaf of peony (*Paeonia peregrina* Mill.), and was characterized based on morphological characteristics and molecular identification. Isolation of pathogen was done on potato dextrose agar (PDA) medium and incubated for 7 days at 25°C. The method of mixing EO with PDA was used for the assay of the antifungal activity of EO against *A. alternata*. PDA was autoclaved (90 min at 1.03 kg/cm at 121°C) and then was cooled to about 45°C. Each EO was diluted 1:9 using 900 ml of dimethyl sulfoxide (DMSO) and 100 ml of the EO. From this stock solution was taken 100, 150, and 200 µl and added in 20 ml sterile PDA to obtain final concentrations: 1.7, 2.5, and 3.33 µl/ml. Tween 20 0.1% was used as a surfactant to disperse the oil. Twenty milliliters of media were poured into each Petri dishes. After 7 days, mycelial fragments of 5 mm diameter were cut from 7-day-old culture and placed on the centre of Petri dishes with treated and control PDA. Treated and control Petri dishes were incubated for 7 days at 25±1°C, after that the mycelial growth diameter was measured and compared with the control. The fungicide tebuconazole (Mystic)

of concentration 1.7 µl/ml was used as a control. Three replicates were done for each chemotype of oil. The antifungal activity of the oil was done by the macrodilution method. The inhibition percentage of mycelial growth was calculated following the formula: Mycelial growth inhibition (%) = DC-DT/DC x 100 where DC and DT are average diameters of fungal mycelia of control and treatments, respectively.

Results and discussion

Based on the chemical characterization of the oil and the morphological characterization of the plant, it was established that there are three chemotypes. In chemotypes 1 and 2 the plants were dark green, whereas in chemotype 3 they were light green (Picture 1). The chemical composition of chemotypes of the EO of *S. montana* was determined by GC-MS analysis. The components carvacrol and thymol were dominant in chemotype 1 of EO (43.5-32.8%), carvacrol was dominant in chemotype 2 of EO (66.0%), and thymol was dominant in chemotype 3 of EO (73.0%).

Picture 1. The plants of *Satureja montana* L. in the chemotypes 1, 2, and 3

The chemotype 3 showed the strongest antifungal activity against *A. alternata*, followed by the chemotype 2 and chemotype 1 slightly less. KB-BG/1 was completely inhibited (100%) by the lowest tested concentration of chemotypes 2 and 3 of EO (1.7 µl/ml) (Picture 2), whereas chemotype 1 inhibited only up to 50% (Picture 3). In the control treatment, the lowest concentration of fungicide was inhibited the mycelial growth of the isolate (100%) (Picture 4). Stević (2013) examined the antifungal activity EO of *Satureja hortensis* against *A. alternata*. Carvacrol and thymol were main components (50% and 9.65%). Minimal inhibitory concentration (MIC) values of the oil were 0.31 and 1.00 mg/ml. In the EOs of *Thymus vulgaris* and *Thymus tosevii*, the percentage of thymol was significantly lower (48.9% and 10.4%). Concentration of 0.25 µL/mL of oil inhibited *A. alternata* (Soković et al., 2009). Compared to our chemotypes of *S. montana* oil, the percentage of thymol and carvacrol in the EO of *Thymus striatus* was much lower (59.5% and 4.9%) (Couladis et al., 2004). Abdolahi et al. (2010) examined the antifungal activity of the EO of ajowan against the growth of *A. alternata*. Thymol was main component in the EO of ajowan (63%). The concentration >200 µL/L of oil showed good inhibitory effects (100%). The antifungal activity of EOs of *Aniba rosaeodora, Laurus nobilis, Sassafras albidum*, and *Cinnamomum zeylanicum* against *A. alternata*, using macrodilution method, were 0.5, 10.0, 10.0 and 1.0 µl/ml (Simić et al., 2004). The EOs of *T. vulgaris* and *Thymus zygis* had antifungal activity against *Candida* spp. strains.

The percentage of carvacrol in the EO of *T. vulgaris* was higher compared to the percentage in our oil (70.3%), whereas the percentage of this component in the EO of *T. zygis* was significantly lower (2.4%). MIC values of *T. vulgaris* were 0.08, 0.16, and 0.32 μL/mL, and MIC values of *T. zygis* were 0.16 and 0.32 μL/mL depending on the strains (Pina-Vaz et al., 2004).

Pictures 2., 3. and 4. Antifungal activity of chemotypes essential oil of *Satureja montana* against isolate *Alternaria alternata* (in the treated and control Petri dishes)

Conclusions

Three chemotypes of *Satureja montana* L. essential oil showed antifungal activity against *Alternaria alternata*, a pathogen of the plant *Paeonia peregrina* Mill. The chemotype 3 had the strongest antifungal activity (100%), followed by the chemotype 2 and chemotype 1 slightly less. The chemotypes 2 and 3 of the EO of *S. montana* (main components carvacrol and thymol) of concentration 1.7 μl/ml can be recommended in further investigations of the antifungal activity against *Alternaria alternata*.

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THE IMPACT OF *BOTRYTIS CINEREA* ON INDIVIDUAL PHENOLIC COMPOUNDS IN CABERNET SAUVIGNON WINE

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Abstract

Grey mold caused by *Botrytis cinerea* is the most serious disease of grapes (*Vitis vinifera*) exposed to cool and humid conditions during ripening. From an oenological point of view, *Botrytis cinerea* affects grapes and wine quality mainly through changes in chemical composition, which are also reflected in phenolic content. The phenolic compounds present in grapes show different resistance to the enzyme laccase produced by *Botrytis cinerea*. The main objective was to investigate the influence of *Botrytis cinerea* on the phenolic profile of wine. The focus was on six phenolic compounds: catechin, gallic acid, caffeic acid, ellagic acid, rutin and kaempherol. This experiment included two different vinifications. In the first, healthy grapes were fermented, while in the second, fungus-infected grapes were fermented. Healthy and fungus-infected grapes were separately crushed, destemmed and spiked with 10 g of $K_2S_2O_5$ per 100 kg. Both fermentations were inoculated with BDX yeast (Lallemand, Canada), and maceration lasted 14 days with racking twice daily - punchdowns. After stabilisation of the wine in the bottle, the wines were subjected to LC-MS / MS analysis. Before LC-MS / MS analysis, the wines were prepared by SPE (solid phase extraction). Our results showed that the content of these phenolic compounds was lower in wines from fungus-infected grapes compared to wines from healthy grapes. Phenolic compounds such as rutin and kaempherol were not detected in wines from fungus-infected grapes, and the content of other analysed compounds was reduced by up to 72.60% for caffeic acid. In grapes infected with Botrytis cinerea, the enzyme laccase causes rapid oxidation of phenolic compounds.

Keywords: *Botrytis cinerea, grapes, wine, phenolic compounds*.

Introduction

In many vineyards around the world, *Botrytis cinerea* is responsible for one of the most serious diseases of grapevines. This pathogen usually occurs in vineyards exposed to cool and humid conditions during the ripening period with morning fog, humid nights and sunny afternoons (Carbajal Ida et al., 2016). Berries infected with grey mold undergo biochemical changes that reduce wine quality, particularly reflected in aroma and phenolic content (Steel et al., 2013; Quijada Morin et al., 2018). On the other hand, when cold, humid nights alternate with relatively warm, dry days, the fungus slowly dries out the berry, concentrates the sugars, and forms glycerol, causing a occurrence known as noble rot. This type of rot is desirable when grapes are grown for the production of desert wines, and it enables the production of famous sweet white wines such as Tokaj in Hungary, Auslese in Germany and Austria, Sauternes, Côteaux du Layon in France (Steel et al., 2013; Carbajal Ida et al., 2016). However, this infection is undesirable in our vineyards, and studies on how noble rot changes the phenolic composition of botrytized grapes and wine are rare. It usually damages the colour of red wine as a result of a decrease in the concentration of anthocyanidins and other phenolic compounds that can be rapidly oxidised by laccase enzymes (Ky et al., 2012; Quijada Morin et al., 2018). This enzyme catalyzes with low specificity the conversion of *o*- and *p*-diphenols

to the corresponding quinones, thus oxidizing a large number of phenols. The decrease in phenol concentration is mainly due to the development of chemical oxidation processes (Zinnai et al., 2013). The release of laccase in must from grapes infected with *Botrytis cinerea* can lead to a significant reduction in the content of phenolic compounds (Claus, 2017). It has been reported that the quality of red wine is affected when produced from grapes with an infection rate as low as 5% (Ky et al., 2012). Because of the dramatic impact on wine quality, there is a great need to study the effects on various phenolic compounds. Some of the grape phenolic compounds may be synthesized by berries in response to *Botrytis cinerea* infection, while others are involved in oxidative reactions that contribute to their degradation via enzymatic pathways. The focus of our research was on six phenolic compounds (catechin, gallic acid, caffeic acid, ellagic acid, rutin and kaempherol) present in wine. The main purpose of this study is therefore to identify changes in phenolic content in wine under grape infection by *Botrytis cinerea*.

Materials and methods

Healthy grapes (cv. Cabernet Sauvignon) infected with *Botrytis cinerea* at full ripeness, harvested in 2020, orginate from vineyards of the "Trišić" winery in the village of Vranić near Belgrade (Serbia). The percentage of infected berries was determined visually for all grapes. Healthy grapes and those infected with *Botrytis cinerea* were separately crushed, destemmed, and spiked with 10 g $K_2S_2O_5$ per 100 kg. For both experiments, the yeast strain *Saccharomyces cerevisiae* (BDX, Lallemand, Canada) was added at 20 g/hl. The maceration lasted 14 days at a temperature of 25±2ºC. During this time, the grape must was mechanically punched down twice a day. After that, the liquid part was separated and transferred into glass jars to finish the fermentation. The next step was the bottling of the wine and aging until further analysis.

After stabilization of the wine, samples were prepared by solid-phase extraction (SPE), which was performed on a vacuum SPE Vacuum Manifold Baker SPE12G (Fisher Scientific, Göteborg, Sweden) using Oasis HLB bcc/200 μm cartridges (Waters, Milford, MA, USA). Determination of individual phenolic compounds was performed using an Agilent 1290 Infinity II LC liquid chromatograph system (Agilent Technologies, Santa Clara, CA, USA) with a binary pump in combination with an Agilent LCTQ 6495C Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA).

Results and discussion

During grape crushing, the release of laccase from grapes infected with *Botrytis cinerea* can lead to a significant reduction in phenolic compound content during grape pressing, which is reflected in the wine (Zinnai et al., 2013). In our wine produced from botritized grapes, the concentration of phenolic compounds analyzed was lower than in wine produced from healthy grapes, and some of them were even not detected (rutin and kaempherol). This could be related to the destructive or oxidative activity of the enzyme laccase, which is promoted by gray mold. The phenols found in grape skin are the ones exposed to direct contact with gray mold and cause its decline (Ky et al., 2012). The same study showed that the decrease in total phenolic content was mainly due to a decrease in phenolic compounds in the skins and then to a decrease in phenolic compounds in the seeds. This finding is consistent with our results showing an overall decrease in rutin and kamepherol content (Table 1). These reports are consistent with the study by Carbajal Ida et al. (2016), which found a sharp decrease in kamepherol from healthy to botrytized Chenin berries. This is also consistent with the results of similar experiments conducted on Chardonnay berries (Hong et al., 2012). Therefore, such

a report may describe an overall reduction of kamepherol in our Cabernet Sauvignon wine. In our wine samples, the content of catechin was reduced by 28.51%. The previous study highlighted that the content of catechins decreased by up to 52% (Ky et al., 2012). In addition, oxidation of catechin by laccase can lead to the formation of several catechin dimers, which cause brown-yellow coloration of grape skins (Jandric et al., 2023). Decrease of catechin results in an organoleptic change in wine which could affect its quality. However, it has been reported that the amount of catechin increases with the degree of noble rot (Carbajal Ida et al., 2016). According to Quijada-Morin et al. (2018), phenols containing at least two hydroxyl groups in the para and meta positions in the phenolic ring structure, such as gallic acid and caffeic acid are good substrates for laccase enzymatic activity. The range of substrates that laccases can oxidise is wide, but they are generally characterised by a diphenolic structure, although it has been found that some fungal laccase enzymes can affect monophenolic compounds (Steel et al., 2013). In a previous study by Claus (2017), the activity of fungal laccases with phenolic wine compounds was determined by oxygen consumption, which was highest for gallic acid (89%) and lowest for caffeic acid (69%). This could be an explanation for the low concentrations of these phenols in our wine from unhealthy grapes. A significant decrease in caffeic acid was also observed in Chardonnay berries affected by grey mold compared to healthy berries (Hong et al., 2012). Ellagic acid, content slightly decreased in wine from grapes affected by grey mold. Therefore, the content of ellagic acid and gallic acid in wine from unhealthy grapes was almost maintained.

Table 1. Content of some phenolic compounds in wines from grapes affected by grey mold and healthy grapes

Phenolic compound (mg/L)	**Wine from healthy grapes**	**Wine from unhealthy grapes**	**% of decrease**
Gallic acid	2,80±0,10	2,00±0,05	28,60
Catechin	23,71±0,50	16,95±0,33	28.51
Caffeic acid	4,34±0,15	1,19±0,03	72.60
Ellagic acid	1,96±0,06	1,61±0,03	17.86
Kaempherol	5,12±0,20	0,00	100
Rutin	0,15±0,01	0,00	100

Conclusions

This study showed the influence of the presence of the grey mold on phenolic profiles of the wine. For all analysed compounds (located in different parts of grape berry) were found lower content in wine from grapes affected by *Botrytis cinerea*. Skin phenols such as kaempherol and rutin are the most susceptible to oxidation under the action of the laccase enzyme while the caffeic acid is the most resistance among these phenolic compounds.

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THE SITE AND TEMPERATURE EFFECT ON *IN VITRO* POLLEN PERFORMANCE OF SOME CHERRY CULTIVARS

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Abstract

The study was carried out to determine the effect of the site and temperature regime on the *in vitro* pollen performance of sweet cherries 'Lapins', and 'Burlat', and sour cherry 'Šumadinka' in order to evaluate their ability as pollen donors for some Serbian cherry cultivars. For each cultivar pollen was collected from the two sites (Beograd and Čačak). In vitro pollen performance was conducted at 17°C and 20°C. Pollen germination was determined in a medium containing 0.7% agar-agar and 15% sucrose. For sweet cherries, increasing the temperature from 17°C to 20°C resulted in a significant decrease in pollen germination and pollen tube growth, while for 'Šumadinka' opposite result, but without a significant difference was observed. A significant influence of the site on pollen germination was established. Higher values were recorded for both sweet cherries in Čačak, while 'Šumadinka' showed a better response in Beograd. The shortest pollen tubes were observed in Burlat (370.6μm) while 'Šumadinka' had the longest ones (830.5μm). Site and temperatures affected pollen tube growth in vitro. Different germination percentages and pollen tube growth were found among both, different cultivars located in the same place and the same cultivars from two different locations. This leads to the different environmental conditions that influenced pollen formation in each location. More evaluation, including pollen performance *in vivo* and fruit settings in cross-pollinated combination with Serbian cherry cultivars is needed for the proper choice of pollen donors.

Keywords: *Prunus avium, Prunus cerasus, pollen, germination in vitro, pollen tube length.*

Introduction

The Republic of Serbia has favorable climatic conditions for sweet and sour cherries growing. In the structure of fruit growing, cherries have an important place, especially sour cherry production which, together with raspberry, represents the country's most important exporting fruit (Lukić et al., 2015).

In cherries, the gametophytic self-incompatibility system that prevents self-fertilization and facilitates fertilization with pollen of other genotypes, is controlled by the multiallelic two linked genes of the *S*-locus, expressed in the style and the pollen (Bošković and Tobutt, 2001). To optimize cultivation technology and to obtain yield of sweet and sour cherries, it is necessary to identify suitable pollenisers for self-incompatible (sweet and sour cherry) and partially self-compatible (sour cherry) genotypes, and to propose the correct varietal design of the orchard. In addition to compatibility with the main cultivar and overlapping blooming times, pollinating cultivars must also have good pollen germination (Nikolić et al., 2012).

Knowledge of the viable pollen percentage and germination capacity is very important in choosing and selecting the appropriate pollen donors (Brown et al., 1996; Nyéki et al., 2008). Pollen germination varies between individual cultivars within the same fruit species (Stösser et al., 1996). Previous research indicates great variation in the degree of pollen germination *in vitro*, ranging from 6 to 75% in sweet cherry (Pirlak, 2002; Tosun and Koyuncu, 2007; Radičević et al., 2008; Beyhan and Karakas, 2009; Milatović and Nikolić, 2017, Radičević et al., 2013), and from 19 to 86% in sour cherry (Davarynejad et al., 2008; Szpadzik et al., 2008; Fotirić-Akšić, 2013; Milatović and Nikolić, 2014; Radičević et al., 2021).

A study conducted by Popovska et al. (2005) showed the impact of temperature and drought on sour cherry microsporogenesis, which took place about 30 days before flowering. Temperature fluctuation and drought during this time were related to irregularity in pollen mother cell division and therefore to disturbance of sour cherry pollen development. Pollen and the pollen tube, the male gametophyte of seed plants, are known to be highly sensitive to temperature increases, more so than the female gametophyte (Hedhly, 2011). The occurrence of extreme high temperatures is more frequent due to global warming in recent years, which has seriously affected pollen germination during the flowering period, as well as fruit set. Global temperatures may rise by 1.4–5.8°C by 2100, which will directly cause serious economic losses, therefore, more and more attention should be paid to the effect of pollen germination under temperature stress. Rootstock, soil fertility and lack of nitrogen, soil drought or water stress, and agro-technical management are also, according to Kozma et al. (2003), important factors that influence the percentage of viability and germination capacity of sweet cherry pollen.

According to Thompson (2004), the first requirement for economical fruit production is the availability of an adequate source of viable and compatible pollen. The use of selected pollen with a high degree of viability will ensure a better fruit set and, consequently, an acceptable yield (Bots and Mariani, 2005). Pollen viability and germination are among the most important properties in cherry tree fertilization, together with the selection of a suitable polleniser while the orchard is being established (Brown et al., 1996; Nyéki et al., 2008). Germinability tests *in vitro* have the potential to provide the best basis for fast predicting pollen performance (Heslop-Harrison and Heslop-Harrison, 1970).

The aim of this study was to determine the site and temperature effect on *in vitro* pollen performance of sweet cherries 'Lapins' and 'Burlat', as potential pollenisers for 'Đuti', and sour cherry 'Šumadinka' as potential polleniser for 'Feketićka' and 'Lenka'.

Material and Methods

Site and plant material

The study was conducted in 2022 and included sweet cherries 'Lapins' and 'Burlat', and sour cherry 'Šumadinka'. The samples were collected from two sites, near Belgrade and Čačak in Serbia. All three cultivars were sampled from a collection orchard of cherries on 'Radmilovac', Experimental Station of the Faculty of Agriculture of the University of Belgrade, located near Belgrade (44° 45' N; 20° 35' E; altitude 130 m). The climate is temperate continental with an average annual temperature of 11.7ºC, and an average temperature over the growing season (April–October) of 17.5ºC with a total annual rainfall rate of 693.9 mm. Second site was the experimental facility of Fruit Research Institute, Čačak, representing with two locations: 'Šumadinka' was sampled from an experimental orchard at 'Čačak' facility (Čačak 1; 43°53' N; 20°20' E; altitude 239 m), while 'Lapins' and 'Burlat' were sampled from an orchard at 'Preljina' facility (Čačak 2; 43°53' N; 20°21' E; altitude 350 m). The climate is temperate continental, with an average annual temperature of 10.8°C and an average temperature over the growing period (April–October) of 16.8°C, while the total annual rainfall rate is 748.4 mm.

Air temperature monitoring

The air temperature in the orchards was monitored from the BBCH 55 (single flower buds visible) until the BBCH 61 (beginning of flowering) (Meier, 2018), and average mean, maximum and minimum daily temperatures were calculated. The air temperature was measured at a height of 1.5 m by an automatic MeteosCompact weather stations (Pessl InstrumentGmbH, Austria), placed in the immediate vicinity of the orchards.

Pollen performance *in vitro* at different temperatures

For examination of pollen germination, branches with flowers were collected one day before anthesis, in the 'balloon' phase and carried to the laboratory. In order to collect pollen from the flower buds, anthers were isolated in Petri dishes. They were stored at room temperature (20±2ºC) for 24–48 h to dry and release the pollen grains. Then, the pollen was sown with fine brushes in Petri dishes on the nutrient medium consisting of 15% sucrose and 0.7% agar-agar. After incubation of 24 h at 17°C and 20°C, the Petri dishes with the sown pollen were observed under a Leica DM LS light microscope (Leica Microsystems, Wetzlar, Germany) for counting of germinated pollen grains. The experiment was done in three repetitions, each contain 150–200 pollen grains. Pollen grains with tubes exceeding their radius were considered to be germinating (Galleta, 1983). The germination percentage was determined as the average of three replications. Pollen tube length was measured in pictures taken under the microscope 'Leica DM LS' using the 'Leica IM 100' programme. For each treatment (site and temperature regime), 60 pollen tubes were measured.

Statistical analysis

The data were statistically analyzed using a two-factor analysis of variance (ANOVA). For the results expressed in percentages, the arcsin square-root data transformation was performed. The significance of differences between the mean values was determined using Tukey's test for significance level $p \leq 0.05$. Data analysis was performed using the statistical software package Statistica, Version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA)

Results and discussion

Air temperature monitoring. The average daily temperatures before and during the flowering phenophase were 8.3°C (Čačak 2), 10.5°C (Čačak 1) and 12.7 (Radmilovac). The coldest was Čačak 2 with minimum temperatures below 5°C recorded in 16 days, among which eight days were below 0°C. Temperatures below 5°C were noticed in 15 days including three frosty days, and in 8 days without the occurrence of frost, for Čačak 1 and Radmilovac, respectively. During the observed period, the maximum temperature occasionally exceeded 20°C (five days for Čačak 1 and Radmilovac; six days for Čačak 2). Great temperature fluctuation can be noticed for Čačak 2, which reached maximum on April 14th and 15th, varied from -2°C to 24°C, and from -1°C to 28°C, respectively. The highest variation for Čačak 1 was observed on April 24th and varied from 0°C to 24°C, and for Radmilovac on April 9th when it varied from 5°C to 22°C.

Pollen germinaton in vitro

Pollen germination *in vitro* in our study was affected by both different temperatures and different sites (Figures 1 and 2). For sweet cherries, increasing the temperature from 17°C to 20°C resulted in a significant decrease in pollen germination (40.1% to 32.1% for 'Burlat', 30.4% to 19.5% for 'Lapins'). Contrary to sweet cherries, average pollen germination in 'Šumadinka' was higher at 20°C (29.5%), but no significant difference was observed in comparison with lower temperature (27.8%). Higher values at 17°C were observed in Čačak for all cultivars, and were statistically different for 'Burlat' and 'Lapins' (Figure 1). The same

relation was observed also for Belgrade, with the exception of 'Šumadinka' that showed statistically higher values at 20°C.

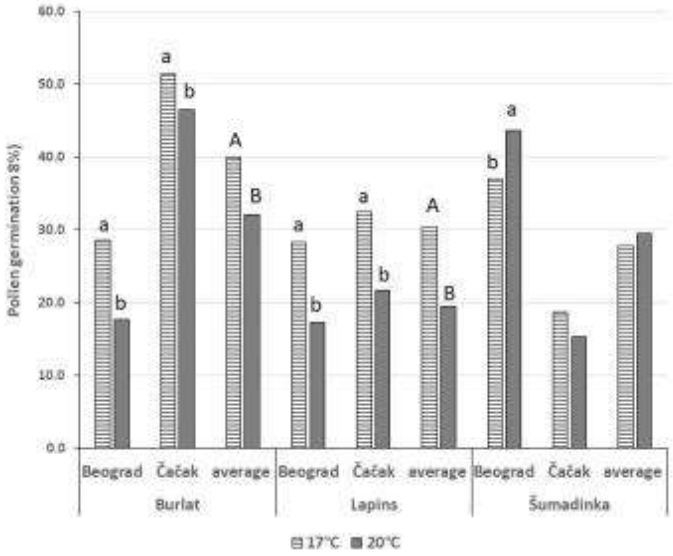

Figure 1. Temperature effect on pollen germination *in vitro* of 'Lapins', 'Burlat' and 'Šumadinka'. Different letters indicate statistically significant differences ($p \leq 0.05$).

Nenadović-Mratinić (1996) examined the effect of incubation temperatures (15, 25 and 35°C) on pollen germination *in vitro* of four sour and one sweet cherry cultivars, and found the highest germination at 25°C. Pirlak (2002) showed that sweet cherry pollen germination was optimal at 15 and 20°C. In studies of the influence of three different temperatures (5, 15 and 25°C) on pollen germination *in vitro* in sweet and sour cherry varieties, Milatović and Nikolić (2014, 2017) obtained high pollen germination at temperatures of 15°C and 25°C (66–75% for sweet and 50–70% for sour cherries).

A significant influence of site on pollen germination was also established (Figure 2), which is mainly related to air temperatures, but also affected by other environmental factors, differences in physical condition. Air temperature is considered as the main environmental factor affecting pollination and fertilization progress and was evident in sweet cherry (Zhao et al., 2008). Due to recorded frosts and in line with Waraich et al. (2012), who reported that low temperature stress during reproductive development induces pollen sterility and pollen tube distortion, we expected a significant decrease in pollen germination both for sweet and sour cherries from Čačak, but they respond differently. Regardless of different temperatures of incubation, 'Burlat' and 'Lapins' showed statistically higher pollen germination when collected from Čačak (49% and 27.1%) than from Belgrade (23.1% and 22.8%). Opposite, for 'Šumadinka' higher values were observed for Belgrade in comparison with Čačak (40.3% and 17.0%, respectively). That can be related to the fact that air temperatures during 'Šumadinka' microsporogenesis in Čačak occasionally exceeded 20°C which lowers pollen viability. The developmental phase from meiosis to the pre-vacuolate microspore was found to be the most temperature sensitive phase of pollen development, though sensitivity continued through the end of vacuolation and began in the late microsporocyte phase (Irenaeus and Mitra, 2014).

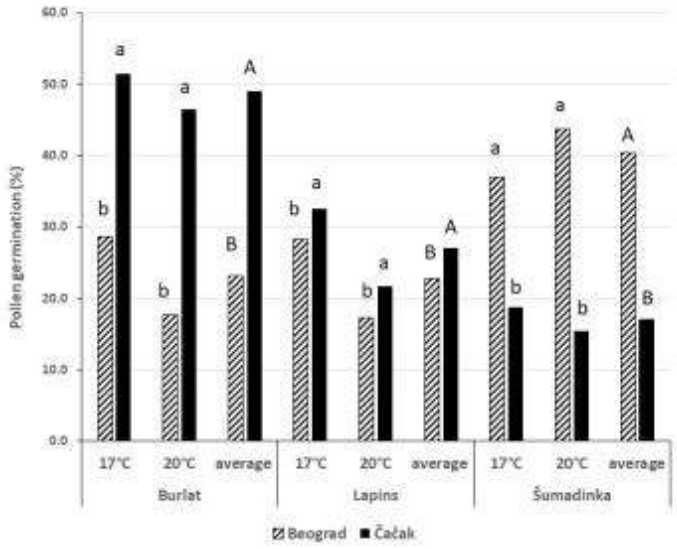

Figure 2. Site effect on pollen germination *in vitro* of 'Lapins', 'Burlat' and 'Šumadinka'. Different letters indicate statistically significant differences ($p \leq 0.05$).

Balas et al. (2017) analysed the pollen viability of 16 cherry cultivars from two different collections and found significant differences between pollen germination values for both, among sites and among cultivars. Great variation in the degree of pollen germination *in vitro* in sweet cherry was reported: Pirlak (2002) 24–75%, Tosun and Koyuncu (2007) 35–59%, Radičević et al. (2008) 25–50%, Beyhan and Karakas (2009) 6–63%. The results obtained in this study are within the specified values. Since our study included only one sour cherry cultivar, the obtained results were not enough to confirm results of Radičević et al. (2021) who reported that sweet cherry cultivars had generally better pollen germination *in vitro* than sour cherries.

In vitro pollen tube length

Pollen tube length *in vitro* in our study varied among cultivars, and in 'Šumadinka' was more than two fold greater than in 'Burlat' (830,5μm and 370.6μm, respectively), while in 'Lapins' was between the mentioned values (526.2μm). According to studies of Milatović and Nikolić (2014, 2017) in sour and sweet cherry the influence of temperature had more effect on the growth of pollen tubes than on pollen germination. The length of pollen tubes was three to six (sour cherry) and three to five (sweet cherry) times higher at 15 and 25°C in comparison with 5°C. In peach, Hedhly et al. (2004) reported that temperature, while affecting only slightly the final germination percentage, had a clear effect on the rate of germination. Within the range of temperatures studied (10, 20 and 30°C), at 20°C in the laboratory, the results show an accelerating effect of increasing temperature on pollen germination and pollen tube growth kinetics. Although an increase in temperature resulted in a significant increase in the length of pollen tubes was also reported (Cerović and Ružić, 1992; Pirlak, 2002). Our study does not support all these findings. As well as for pollen germination, increasing the temperature from 17°C to 20°C in sweet cherries decreased, while in 'Šumadinka' increased pollen tube length but no significant differences were found (Figure 3).

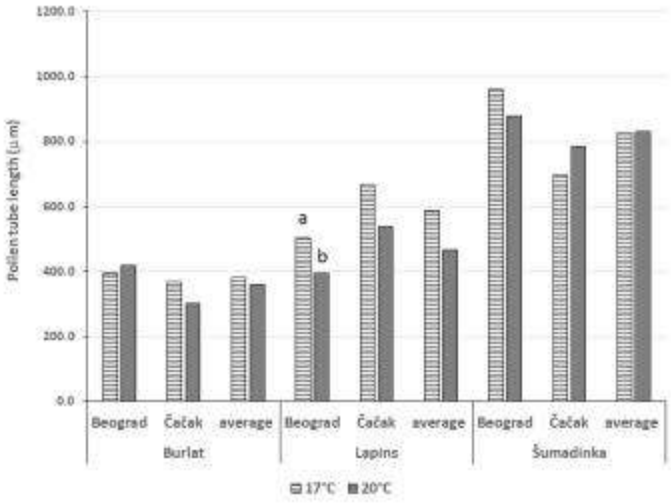

Figure 3. Temperature regime effect on pollen tube length *in vitro* of 'Lapins', 'Burlat' and 'Šumadinka'. Different letters indicate statistically significant differences ($p \leq 0.05$).

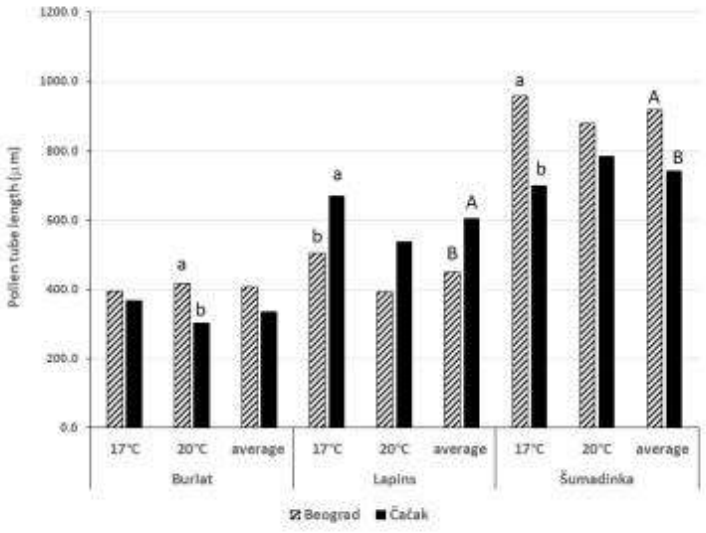

Figure 4. Site effect on pollen tube length *in vitro* of 'Lapins', 'Burlat' and 'Šumadinka'. Different letters indicate statistically significant differences ($p \leq 0.05$).

There is no unique influence of site on pollen tube length (Figure 4). For 'Lapins' and 'Šumadinka', it was in line with pollen germination and had statistically higher values on Čačak and Belgrade, respectively. For 'Burlat' on the same site, higher pollen germination leads to shorter pollen tube length, but values between sites were not statistically different. That indicates that genotype has a prevalent influence on pollen tube growth. Under the changing climate, early spring weather conditions are becoming more unpredictable. Cherry flowering phenological rhythm and floral organ viability are affected by more frequent events of erratic and extreme temperatures (Xu et al., 2023), which puts more importance on site selection.

Conclusions

The obtained results show that different germination percentages and pollen tube lengths were found among both, different cultivars located in the same place and the same cultivars from two different locations. This leads to the different environmental conditions (temperature, radiation, humidity, soil, chemicals) that influenced pollen formation at each location. Site and temperature had more influence on pollen germination than on pollen tube length, indicating that the influence of genotype is crucial. Further evaluation, including pollen performance *in vivo*, fruit setting in cross-pollinated combinations will give a more precise assessment of the male reproductive behavior of the investigated genotypes.

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MELISSOPALYNOLOGYCAL ANALYSIS OF HONEY SAMPLES FROM BOSNIA AND HERZEGOVINA

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Abstract

Melissopalynologycal research of honey samples from different localities in Bosnia and Herzegovina identified 32 types of pollen grains. Ten honey samples can be divided into six groups: rapeseed honey, acacia honey, chestnut honey, linden honey, wild cherry honey and bramble honey. Six families dominate, while the rest are present in a smaller percentage. The dominant pollen belongs to the families *Brassicaceae* with the highest percentage, *Fagaceae* with a smaller percentage and the families *Fabaceae, Rhamnaceae, Tiliaceae, Rosaceae*. In sample number 1, rapeseed (*Brassica napus* L.) pollen predominates and amounts to 74% in the sample. Samples number 2 and 3 have the highest percentage of acacia (*Robinia pseudoacacia* L.) pollen grains with 44% and 49%. According to the pollen analysis, samples 4 and 5 show the dominance of chestnut (*Castanea sativa* Mill) pollen grains in the values of 89% for sample number 4 and 91% for sample number 5. Samples number 6, 7 and 8 have the highest percentage of linden pollen grains (*Tilia cordata* L.). Samples number 9 and 10 show the highest abundance of wild cherry (*Prunus* sp.) and bramble (*Paliurus spina-christi*) pollen grains.
The plant species, oilseed rape, acacia and chestnut proved to be important plant species despite a very short flowering period of only one month.

Keywords: *Honey, Melissopalynological analysis, Bosnia and Herzegovina*

Introduction

The branch of botany that deals with the analysis of pollen and spores of plants is called palynology. The part of palynology that studies pollen and other microscopic elements of honey is called melissopalynology. This method gives us data on the botanical and geographical origin of honey by counting the amount of pollen grains in the honey sediment (Louveaux et al., 1978, Valencia et al., 2000). Geographical and botanical properties are important for honey quality, and the taste, smell and color of honey change according to the nectar from the flower (Kaya et al. 2005; Sabo et al. 2011; Brekalo et al. 2022). Data on the geographical origin of pollen refer to the composition of pollen grains of plants that are characteristic of a specific geographical area, while data on the botanical origin of honey are more focused on the pollen spectrum of plant species that are the source of nectar. Given that the morphological features of pollen are specific for each plant species, pollen analysis can be considered a reliable parameter for identification of plant species. (Hrga and Stjepanović, 2013). If we take into account the chemical composition of honey, it can be said that it is variable since the range of plant sources from which nectar is obtained is very wide (Ball, 2007). International standards specify that honey can be labeled according to the floral or plant species only if it comes entirely or mostly from the specified source. In this sense, it is also important that its organoleptic, physico-chemical properties correspond to the origin (Council of European Union, 2002).

Material and methods

Honey samples, ten of them, were collected from different localities in Bosnia and Herzegovina (Table 1). All analyses were performed according to national and international legislation in the Laboratory for Honey, Medicinal and Aromatic Plants of the Faculty of Agriculture and Food Technology, University of Mostar.

Table 1. List of localities from which honey samples were collected

Serial number of the sample	Location
1.	Brčko
2.	Derventa
3.	Odžak
4.	Konjic
5.	Bihać
6.	Jablanica
7.	Mostar
8.	Mostar
9.	Kreševo
10.	Stolac

Preparations for pollen analysis were made using the method according to Louveaux et al. (1978). The percentage (%) of pollen grains of a certain plant species in honey was obtained by counting and identifying at least 500 pollen grains. Microscopy was performed with a light microscope (Olympus Bx41) at 400x magnification. The dimensions of the pollen grains were measured using the Olympus DP-Soft program. Pollen grains were determined according to the relevant literature (Von der Ohe and Von der Ohe, 2003; Von der Ohe et al., 2004) and based on the dimensions of the pollen grains. Pollen grains were counted on two preparations for each honey sample and each type of pollen was expressed as a percentage in relation to the total number of pollen grains. Based on the number and frequency of pollen grains in honey samples, they were ranked by groups according to Louveaux et al. (1978), into dominant (>45%), and secondary groups (16-45%), then into the group where pollen grains are represented in small amounts (3-15%) and pollen grains found in traces or rare group (<3%).

Results and discussion

Melisopalynological research of honey samples from nine localities in Bosnia and Herzegovina identified 32 types of pollen grains, classified into 18 families. Five families dominate the honey samples, while the others are present in a smaller percentage.

Pollen of oilseed rape (*Brassica napus* L.), acacia (*Robinia pseudoacacia* L.), chestnut (*Castanea sativa* Mill.), wild cherry (*Prunus avium* L.) and linden (*Tilia cordata* L.) dominates in terms of abundance. Sabo et al. (2011) for Varaždin County (Republic of Croatia) state the dominance of pollen grains from the families *Brassicaceae, Fagaceae* and *Fabaceae*. Also, they state that rapeseed (*Brassica napus* L.) and chestnut (*Castanea sativa* Mill.) pollen dominates in terms of quantity.

The results of the botanical origin of the researched honey samples are shown in Table 2. Melisopalynological analysis revealed that three honeys belong to monofloral linden honey, two honeys belong to monofloral acacia honey, two to chestnut honey, and one is rapeseed, wild cherry and bramble honey (Table 2)

Table 2. Pollen analysis in the researched honey samples

Serial number of the sample	A type of honey
1.	Oliseed rape
2.	Acacia
3.	Acacia
4.	Chestnut
5.	Chestnut
6.	Linden
7.	Linden
8.	Linden
9.	Wild cherry
10.	Bramle

Monofloral rapeseed (*Brassica napus* L.) honey was found in sample number 1. Six types of pollen grains were found in sample number 1. Of this, 74% is dominated by rapeseed pollen (*Brassica napus* L.) and, to a lesser extent, sunflower (*Helianthus anus* L.) at 10%. Pollen grains of other plants were present in traces (Table 3).

Table 3. Pollen analysis of sample number 1

A plant species	Pollen (%)
Brassica napus L.	74
Helianthus anus L.	10
Prunus sp.	7
Trifolium pratense L.	5
Taraxacum officinale Weber	3
Aster spp.	1
Total	100

Pollen analysis of sample number 2 revealed a higher percentage of acacia (*Robinia pseudoacacia* L.) pollen grains in the amount of 44% (Table 4). Amorpha (*Amorpha fruticosa* L.) pollen grains with 26%, *Prunus* spp. with 11%, linden (*Tillia cordata* L.) with 7%, and *Aster* spp. and red clover (*Trifolium pratense* L.) with 3 %.

Table 4. Pollen analyzis of sample number 2 and 3

A plant species	Sample no. 2	Sample no. 3
Robinia pseudoacacia L.	44	49
Amorpha fructicosa L.	26	20
Prunus sp.	11	14
Tilia cordata L.	7	/
Aster spp.	3	4
Trifolium pratense L.	3	3
Taraxacum officinale Weber	/	7
Fraxinus excelsior L.	/	3
Total	100	100

In sample number 3, acacia pollen (*Robinia pseudoacacia* L.) is the most abundant, with 49%. In addition to acacia, secondary pollen is from acacia (*Amorpha fructicosa* L.) with 20%. Minor pollen includes fruit trees from the genus *Prunus* spp., dandelion (*Taraxacum*

officinale Weber), species from the genus *Aster* spp., and pollen grains from clover (*Trifolium pratesnse* L.) and white maple (*Fraxinus excelsior* L.) are sporadically represented (Table 4).
Sample number 4 has the presence of chestnut (*Castanea sativa* Mill.) pollen grains with a percentage of 89%. Pollen grains of other plants were present in traces in the sample (Table 5).

Table 5. Pollen analyzis of sample number 4 and 5

A plant species	Sample no. 4	Sample no. 5
Castanea sativa Mill.	89	91
Robinia pseudoacacia L.	3	3
Poaceae spp.	3	/
Sambucus nigra L.	2	2
*Taraxacum officinale*Weber	1	/
Juglans regia	1	1
Tilia cordata	1	/
Salix spp.	/	3
Total	100 %	100 %

Table 7 shows the results of pollen analysis for sample number 5, in which the dominant pollen is chestnut (*Castanea sativa* Mill.) with a share of 91%. Pollen grains of acacia (*Robinia pseudoacacia* L.) and species from the genus *Salix* are represented in a smaller presence with 3% each. Pollen grains of elder (*Sambucus nigra* L.) and walnut (*Juglans regia* L.) are found in traces (Table 5).
In sample number 6, linden pollen (*Tilia cordata* L.) is the most represented, with 42%. In addition to acacia, secondary pollen is from elder (*Sambucus nigra* L.) with 20%. The minor group includes pollen from the genus *Salix* spp., mint (*Menta piperita* L.), species from the genus *Plantago spp*. and Rubus *spp.* are sporadically represented (Table 6).

Table 6. Pollen analyzis of samples numbers 6, 7 and 8

A plant species	Sample no. 6	Sample no. 7	Sample no. 8
Tilia cordata L.	42	46	48
Sambucus nigra L.	20	18	7
Menta piperita L.	14	10	7
Salix spp.	7	/	/
Poaceae spp.	7	16	9
Plantago spp.	6	6	6
Robinia pseudoacacia L.	/	/	9
Rubus spp.	4	2	4
Lavandula L.	/	/	4
Sinapis arvensis L.	/	2	3
Olea europea L,	/	/	3
Total	100	100	100

In sample number 7, 7 types of pollen grains were found, 46% dominated by linden (*Tilia cordata* L.) pollen and, to a lesser extent, elder (*Sambucus nigra* L.) pollen with 18% and grass (*Poaceae* spp.) pollen with 16%. Pollen grains of other plants were present in traces. Sample number 8 has the presence of linden (*Tilia cordata* L.) pollen grains with a percentage of 48%.
Wild cherry (*Prunus avium* L.) monofloral honey was found in sample number 9. In addition to wild cherry, the secondary pollen is birch with 13% and elderberry with 11%.

Table 7. Pollen analyzis of sample number 9

A plant species	Pollen (%)
Prunus avium L.	54
Betula pendula Roth.	13
Sambucus nigra L.	11
Acer spp.	8
Taraxacum officinale Weber	7
Lamium spp.	7
Total	100

In sample number 10, bramble pollen (*Paliurus spina-christi* L.) is most represented with 44%.

Table 8. Pollen analyzis of sample number 10

A plant species	Pollen (%)
Paliurus spina-christi L.	44
Calluna vulgaris L.	13
Poaceae spp.	13
Mentha piperita	10
Hypericum perforatum L.	8
Plantago spp.	6
Rubus spp.	6
Total	100

Conclusion

Ten samples of honey from Bosnia and Herzegovina were melisopalynologically investigated, three honey samples are from linden, two are honey from chestnut and acacia and one honey each from rapeseed, wild cherry and bramble. The types of oilseed rape (*Brassica napus* L.), acacia (*Robinia pseudoacacia* L.) and chestnut (*Castanea sativa* Mill.), which proved to be the most dominant in the research, bloom for only one month, which shows the importance of the flowering of the mentioned plant species for bee grazing. Spring and summer is a very important period for beekeepers because around 60% of honey plants bloom then. Of the dominant pollen grains in the first sample, oilseed rape prevails with 74%, in the second and third samples acacia pollen prevails with 44% and 49%, and samples number 4 and 5 have a high proportion of chestnut pollen grains in the shares of 89% and 91%. Monofloral linden honey was found in samples six, seven and eight, with percentage values of 42%, 46% and 48%. In sample number nine, wild cherry pollen predominates with 54%, and in sample number ten, the presence of bramble is 44%. Pollen grains from the families *Asteraceae, Adoxaceae, Ericaceae, Hypericaceae, Juglandaceae, Lamiaceae, Oleaceae, Polygonaceae, Salicaceae, Poaceae* have a much smaller percentage share in the examined samples. Based on pollen analysis, the number and frequency of pollen grains and according to the Ordinance on honey and other beekeeping products ("Official Gazette of BiH" No. 37/09), the investigated honey samples can be determined as monofloral honeys.

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DIVERSITY OF SOIL-DWELLING INSECTS THAT ATTACK MAIZE AND SUNFLOWER CROPS FROM SOUTH AND SOUTHEASTERN ROMANIA

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Abstract

Maize and sunflower are two major crops that annually cover 35 to 40% of arable land in Romania. Biotic stresses exhibited by pest insects represent a key factor that influences the quantity and quality of harvest. The present study aimed to evaluate the community structure of the soil-dwelling insect pests associated with corn and sunflower crops in the area of south and south-east Romania, based on the specimens collected with pitfall traps, during 2 years (2017-2018), in 10 plots from 5 agricultural farms. Numerical abundance and relative frequency of each taxon, species richness, and dominance and Shannon-Weiner diversity index were calculated using pooled data sets of the soil pest insects' community for each field plot using Microsoft Excel and statistical software Past 4.03. In total, 2049 specimens of pest Coleoptera were identified from both crops, which are assigned to 24 species, 16 genus and six families, as follows: Elateridae (4 genus and 10 species), Curculionidae (5 genus and 6 species), Chrysomelidae (2 genus and 3 species), Tenebrionidae and Scarabaeidae (each with two genus and species), and Carabidae (represented by one species). Curculionidae family was the most abundant, constituting 45.5% of the total insects collected from all the ten sites during two years of study, closely followed by Elateridae family, with 38.3 % of the total catches. The paper further discusses the differences and similarities between the fauna structure and also the technological and other factors that influence the results at site or regional level.

Keywords: *soil-dwelling insect pests, soil biodiversity, maize insect pests, sunflower insect pests.*

Introduction

Maize and sunflower are major crops in Romania, annually cultivated on 3.5 to 4 million hectares (Romanian Statistical Yearbook 2021 cited by MADR data, 2022). In term of harvested surfaces Romania is often on the first place in the EU but not always as the producer of the largest amount of maize or sunflower (EUROSTAT, 2021). Apart from agricultural technologies and climate changes pressure, biotic stresses exhibited by pest insects are the main factors that influence the maize and sunflower crop production. The literature show that the major pests of both crops in the area of south and south-east of Romania are the maize leaf weevil (*Tanymecus dilaticollis* Gyll) and wireworms that in favorable conditions might induce significant yield losses or even compromise the crops on large surfaces, up to one million hectares annually (Georgescu et al., 2020; Manole et al., 1999; Popov et al., 2001, 2007, Fătu et al., 2023). This is the reason why the recent researches have been directed towards the control of these dangerous pests of the two crops, despite the fact that the list of their specific soil-dwelling pests is much wider (Iamandei and Rujescu, 2023). The present study aimed to evaluate the community structure of the soil-dwelling insect pests associated with corn and sunflower crops in the area of south and south-east Romania. This is as part of an extensive fauna collection effort, carried out over 5 years (2015-2019), in various agricultural farms. Our analysis concerns the specimens collected in

2017 and 2018, which were stored and identified in 2023, the study being partially conducted under a project financed by the Romanian Ministry of Agriculture and Rural Development.

Materials and methods

The study area cover 10 plots (5 each year) from 5 agricultural farms, cultivated with corn or sunflower, within 5 localities in 3 counties (Călăraşi, Ialomița, Constanța), the general conditions of agricultural plots are detailed in Table 1.

Table1. Details about maize or sunflower plots where soil-dwelling pest were sampled

Year	Locality (County)	Plot ID	Crop	Previous crop	Cultivar	Soil name
2017	Belciugatele(Calarasi)	PL1	Maize	Wheat	Alexxandra	Cambic chernozem
	Sapunari (Calarasi)	PL2	Maize	Maize	Alexxandra	Cambic chernozem
	Borduselu(Ialomita)	PL3	Sunflower	Maize	P64LE99	Cambic chernozem
	Agigea(Constanta)	PL4	Maize	Wheat	PO 412	Vermi-calcic chernozem
	Mihail Kogalniceanu(Constanta)	PL5	Sunflower	Maize	P64LE25	Vermi-calcic chernozem
2018	Belciugatele(Calarasi)	PL6	Maize	Maize	P 9903	Cambic chernozem
	Sapunari (Calarasi)	PL7	Maize	Oilseed rape	DKC4590	Cambic chernozem
	Borduselu(Ialomita)	PL8	Maize	Maize	DCK4670	Cambic chernozem
	Agigea(Constanta)	PL9	Sunflower	Maize	P64LE99	Vermi-calcic chernozem
	Mihail Kogalniceanu(Constanta)	PL10	Sunflower	Wheat	P64LE25	Vermi-calcic chernozem

The sampling of the soil-dwelling insects' fauna was performed monthly, between April and July, each year (2017 and 2018). At every plot, established with seeds not treated with insecticides, 4 pitfall traps, containing 4% formaldehyde solution, were installed and the content collected after 7 days. Afterwards, the samples were transferred to Research-Development Institute for Plant protection Entomology laboratory, sorted at order level and preserved in 70% alcohol. In 2023 the stored sample were analyzed under a stereomicroscope and the beetles were identified to species according to specific literature. For the present study, only the specimens belonging to community structure of the soil-dwelling insect pests were used. Numerical abundance and relative frequency of each taxon, species richness and Shannon-Weiner diversity indices were calculated using pooled data sets of the soil pest insects' community for each field plot using Microsoft Excel and statistical software Past 4.03.

Results and discussion

In total, 2049 specimens of pest Coleoptera were identified from both crops (Table 1), which are assigned to 24 species, 16 genera and six families, as follows: Elateridae (4 genus and 10 species), Curculionidae (5 genus and 6 species), Chrysomelidae (2 genus and 3 species), Tenebrionidae and Scarabaeidae (each with two genus and species), and Carabidae (represented by one species).

Table 2. The numerical abundance of soil-dwelling pest species in the maize and sunflower plots from southern Romania

Sp. No	Coleoptera Family/Species	Numerical abundance (total no. of specimens) per plot /year and relative abundance											
		2017					2018					2017-2018	
		PL 1	PL 2	PL 3	PL 4	PL 5	PL 6	PL 7	PL 8	PL 9	PL 10	Total No.	A %
1	*Zabrus tenebrioides*	12	19	2	6	1	1	12	5	0	0	58	2.83
2	*Aphthona lutescens*	0	0	1	0	0	0	0	0	0	0	1	0.05
3	*Aphtona* spp.	0	2	3	0	0	1	1	1	0	0	9	0.44
4	*Phyllotreta vittula*	9	3	2	1	0	7	4	1	0	0	27	1.31
5	*Bothynoderes punctiventris*	0	7	9	6	2	0	1	3	0	0	28	1.37
6	*Cleonus sp.*	1	5	0	0	0	0	1	0	0	0	7	0.34
7	*Otiorhynchus sulcatus*	9	0	1	1	1	0	0	0	2	5	19	0.93
8	*Sitona* spp.	12	8	33	14	18	6	2	13	5	7	118	5.76
9	*Tanymecus dilaticollis*	46	68	44	59	36	58	97	79	33	41	561	27.38
10	*Tanymecus palliatus*	6	7	32	4	53	8	6	8	36	39	199	9.71
11	*Blaps mortisaga*	1	5	1	0	0	1	3	1	0	0	12	0.59
12	*Opatrum sabulosum*	19	3	76	17	34	5	6	7	19	21	207	10.10
13	*Agriotes gurgistanus*	0	1	3	0	5	0	0	1	1	1	12	0.59
14	*Agriotes lineatus*	7	22	10	0	0	12	27	9	3	1	91	4.44
15	*Agriotes obscurus*	17	21	25	11	3	8	17	21	7	4	134	6.54
16	*Agriotes pilosellus*	2	4	0	7	0	1	5	0	3	0	22	1.07
17	*Agriotes sputator*	26	8	9	0	0	22	3	3	0	0	71	3.46
18	*Agriotes ustulatus*	18	16	46	11	7	7	14	29	12	16	176	8.59
19	*A. ustulatus var. flavicornis*	5	9	6	0	0	4	3	7	0	0	34	1.66
20	*Athous niger*	0	4	2	0	0	0	4	0	0	0	10	0.49
21	*Drasterius bimaculatus*	5	3	6	34	57	6	1	1	24	28	165	8.05
22	*Selatosomus latus*	0	0	0	14	26	0	0	0	12	18	70	3.42
23	*Anoxia villosa*	0	0	0	3	2	0	0	0	1	1	7	0.34
24	*Melolontha melolontha*	4	4	0	0	0	1	1	1	0	0	11	0.54
	Total	**199**	**219**	**311**	**188**	**245**	**148**	**208**	**190**	**158**	**182**	**2049**	**100.00**

Curculionidae family was the most abundant, constituting 45.5% of the total insects collected from all the ten sites during two years of study, closely followed by Elateridae family, with 38.3% of the total catches (figure 1).

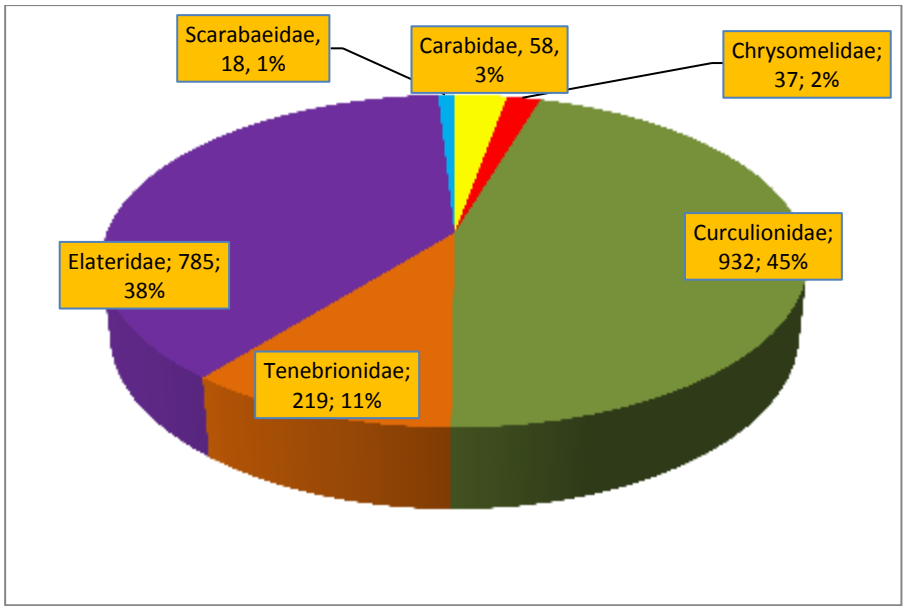

Figure 1. Numerical and relative abundance of Coleoptera families' individuals thorough the studyperiod

Curculionidae were represented by 6 species (Table 2). The most abundant were the species of the *Tanymecus* genus, respectively *T. dilaticollis* (27.38%) and *T. palliatus* (9.71%). While *T. dilaticollis* is generally recognized as the main pest of maize and sunflower crops, our study clearly shows that, actually, both species were present together in all 10 plots (table 2, figure 2). Their prevalence differs; *T. dilaticollis* was generally more abundant in maize crops while *T. palliatus* was more abundant in sunflower crop. Generally, mono-cropping and rotation like maize after sunflower or sunflower after maize favor the development of a higher reserve of *Tanymecus* complex in field. At regional scale, the most abundant species of Elateridae were found: *Agriotes ustulatus* (including *flavicornis var.*, accounting together 10.25% of total fauna catches), *Drasterius bimaculatus* (8.05%) and *Agriotes obscurus* (6.54%). The list of dominant click-beetle species was similar; but their prevalence differs depending on the location, so that in the localities from Calarasi and Ialomita counties species of *Agriotes* predominate, while *Drasterius bimaculatus* was dominant in Constanța county localities. These findings are in line with recently published data (Iamandei and Rujescu, 2023). The Tenebrionidae species *Opatrum sabulosum* was found the second species in term of overall relative abundance, and their populations were higher in all sunflower plots (table 2, figure 2). This is a polyphagous species; the attack of the adult is particularly dangerous in the first phases of the crop's vegetation, and up to 60% damages were mentioned in literature for sunflower crops, especially in years with dry springs (Ghizdavu et. al, 1997). Another dangerous pest of grain crops that may cause great damage to maize, the ground beetle *Zabrus tenebrioides*, had low abundance in samples over two years of study (2,84 %). Scarab beetle *Melolontha melolontha* and *Anoxia villosa* were also present but in very low abundance.

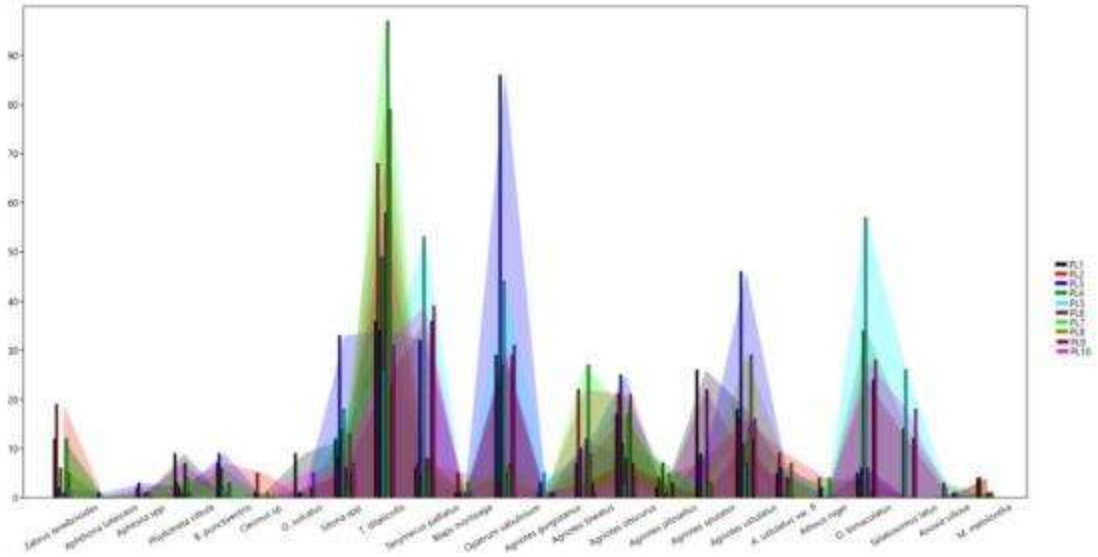

Figure 2. Presence and variation of numerical abundance of the 24 pest coleopteran species at plot level (The localities name corresponding to each of the plot ID are listed in Table 1)

In terms of sampling plots, the highest number of individuals was recorded in PL3 (311), while the lowest number of individuals was recorded from PL6 (148) (Tabel 2 and 3). At farm level, the species composition was relatively homogenous, and the species richness was almost similar within years (table 3). The maximum species richness (20 species) was recorded in 2017 at maize plot PL2 in Sapunari, and the minimum(12 species) was recorded at PL10, in 2018, at sunflower crop from Mihail Kogalniceanu. In 2017 Shannon–Weiner diversity indices ranged between 2.05 at PL5 and 2.489 at PL 1 and a slow decrease was noticed in 2018, with value from 1.974 (PL7) to 2.124 (PL 9). Overall conditions were less favorable for soil pests in 2018.

Table 3. Abundance and diversity indices of analyzed samples depending on plot

	Plot ID (Crop)									
	PL1 (Ma)	PL2 (Ma)	PL3 (Sf)	PL4 (Ma)	PL5 (Sf)	PL6 (Ma)	PL7 (Ma)	PL18 (Ma)	PL9 (Sf)	PL10 (Sf)
S	16	20	19	14	13	16	19	17	13	12
A	199	219	311	188	245	148	208	190	158	182
H	2.489	2.451	2.265	2.203	2.005	2.099	1.974	1.992	2.124	2.057

S- species richness; A- Total no. of specimens; H- Shannon–Weiner diversity index.
The localities name corresponding to each of the plot ID are listed in Table 1. Ma- maize, Sf- sunflower

Conclusion

The paper brings actual data on the community structure of the soil-dwelling insect pests associated with maize and sunflower crops in the main agricultural basin from south and south-eastern Romania. At regional scale, the study revealed the presence of 24 species, 16 genera and six families, as follows: Elateridae (10 species), Curculionidae (6 species), Chrysomelidae (3 species), Tenebrionidae and Scarabaeidae (each with two species), and Carabidae (represented by one species). Overall, the complex formed by the species *Tanymecus dilaticollis* and *Tanymecus palliatus* was dominant in all study locations in both crops. Other eudominant species, present in all study locations were: *Opatrum sabulosum*, *Agriotes ustulatus*, *Drasterius bimaculatus* and *Agriotes obscurus.* The structure of dominant

species is quite similar but variations of their abundance are usual and depend on the year, soil and technological conditions. The list of species of interest in the community structure of the soil-dwelling insect pests associated with corn and sunflower crops in the area of south and south-east Romania was completed by some secondary pest species: the click-beetles *Agriotes lineatus, A. sputator A. pilosellus, Selatosomus latus*, the ground beetle *Zabrus tenebrioides*, the weevils *Sitona spp, and Otiorhynchus sulcatus*, and scarab beetels *Melolontha melolontha* and *Anoxia villosa.* All these soil-dwelling pests, main or secondary, must be taken into account when developing integrated management programs. Future research efforts will have to be directed to areas with different soil and technological conditions and also to the others functional component of biodiversity in agroecosystems, like predators of pests or consumers of weed seeds, in order to fill the gaps in our knowledge and further integration of local, regional and national soil biodiversity data.

Acknowledgement

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SUSTAINABILITY IN OLIVE LEAVES FOR FUNCTIONAL FOOD INGREDIENTS PRODUCTION

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Abstract

Olive tree processing and agriculture generate significant amounts of food by-products, especially olive leaves, much of which is underexploited, posing a significant threat to the environment. Olive leaves are endowed with endogenous bioactive compounds. Olive leaves are a very rich source of bioactive compounds such as secoiridoids, flavonoids, and triterpenes. They can potentially have a higher added value if their fate is reconsidered. Valorization of the residual biomass derived from the agricultural and food sector is nowadays regarded as central to the emerging bio economy. Their health-promoting and beneficial potential, as well as environmental protection and the circular economy, deserve to be harnessed to recover and reuse natural ingredients that are potential alternatives and safer than synthetic ones. Olive leaves have great potential for wide industrial applications in food/diet systems. It is necessary to establish feasible strategies to optimize the extraction of valuable biomolecules from olive leaves and enable their conversion into high value-added products, while minimizing the generation of biomolecules agro-industrial waste streams. The major bioactive components naturally present in olive leaves must be obtained through proper analysis, extraction, application, and stability issues. Olive leaf enrichment allows for reduced operating costs, simple and flexible handling protocols, and easily accessible system components. Implementing a sustainable bioremediation strategy helps to achieve the goal of zero or green treatment of olive leaf by-products while also ensuring acceptable quality of functional or natural products to meet the needs of consumers and industrialists.

Keywords: *Olive leaves, functional compounds, sustainability.*

Introduction

The olive tree (Olea europaea L.) is one of the most important fruit trees in the Mediterranean countries, covering 8 million hectares and accounting for almost 98% of the world harvest. This demonstrates the enormous economic and social importance of this crop and the potential benefits of utilizing its by-products. Olive leaves are an important byproduct of pruning and fruit picking. They are also one of the by-products of olive milling, and their quantity may vary depending on the number of olive leaves accompanying the olive fruit. Olive mill leaves account for a relatively large proportion, accounting for about 4–10% of the total weight of processed olives. They are the result of pruning olive trees and account for almost 5 percent of total olive oil production. Horticulture also produces a large proportion of leaf residue. When pruning, leaf by-products amount to about 25% of the total weight of pruning residue, depending on geographic location, gardening routine, and tree age. Olive leaf is considered an inexpensive raw material that can be used as a useful source of high value-added products. (Keceli and Harp, 2014; Kiritsakis *et al*., 2017, Markhali *et al*., 2020, Contreas *et al*., 2020).

The increase of these residues is a major concern due to their adverse effects. Ecological sustainability, since a large proportion of residues are not fully utilized and/or not adequately disposed by burning. It is increasingly recognized that this underutilized biomass, if used

wisely, can be viewed as a valuable, health-promoting resource with enormous market potential in food and nutrition systems. Applications of olive leaves in various industrial fields such as food, modern medicine and pharmaceuticals. Despite great efforts to expand the use of leaves from traditional applications to modern and industrial applications, the sustainable extraction of natural biomolecules presents several challenges and better performance is required to ensure optimal extraction that is both time-efficient and economical. , as well as the stability of biomass and leaf extracts, the safety and non-toxicity of output and input, the bioavailability of products, and the scalability of processing systems (Markhali *et al*., 2020).

Bioactive compounds of olive leaves

The complex composition of OLE (olive leaf extract) includes flavonoids and their glycosylated derivatives, secoiridoids and their derivatives, simple phenols, phenolic acids and derivatives, terpenes, fatty acids, minerals, volatile compounds, vitamins, and phytosterols. However, it is challenging to get a broad picture of the composition of OLE due to the heterogeneity of these compounds, the use of various analytical techniques, and the development of various analytical methods. OLE is a by-product that can be used to create a variety of commercial products, including enriched foods, cosmetics, and nutraceuticals, due to its bioactive composition (Kiritsakis *et al*., 2017; Abi-Khattar *et al.*, 2019). Olive leaves are rich in a variety of phenolic compounds, which have gained a lot of attention in recent years due primarily to their antioxidant benefits to human health. Olive leaves contain 6 to 15% oleuropein, which is the main ingredient. The amounts of the individual flavonoids quercetin, luteolin, apigenin, and myricetin in olive leaves were found to be 62, 278, 28, and 14 mg/kg, respectively (Kiritsakis *et al*.,2017).

Sustainable Extraction of olive Leaves

Careful methodological selection is necessary for the extraction process, which is the separation of desired (endogenous) ingredients. In order to successfully satisfy processing, economics, and environmental sustainability, the extraction system should be optimally designed. According to Markhali *et al*. (2020), there are several key characteristics associated with the environmentally friendly extraction of useful ingredients from olive leaves. In light of this and the makeup of OO and its byproducts, it is crucial to choose the best extraction method and identify the precise uses for these bioactive compounds; consequently, various strategies have been used. Traditional methods like maceration have been used for this, as well as less traditional methods like microwave (MAE), infrared pressurized liquid, ultrasound-assisted (UAE), and supercritical fluid extraction. Since there is less compound degradation, fewer solvents are required, and better extraction yields are obtained, these extraction methodologies have some advantages over conventional ones. Maximizing polyphenol recovery while maintaining their chemical integrity and, consequently, their functional activities, is obviously how any given extraction technique is optimized (Abi-Khattr *et al*., 2019; Caballero *et al*., 2020; Otero *et al*., 2021). Increasing the bioaccessibility of phenolics can be accomplished using both MAE and UAE. Compared to conventional methods (maceration), UAE can be used to speed up adsorption uptake and kinetics. In order to break through the extraction matrix's wall and release more compounds, the cavitation bubbles produced by the ultrasound. By heating the target components from the inside out with microwave energy, MAE is known for accelerating the extraction process in contrast to maceration and other advanced extraction techniques. This minimizes the degradation of the target components and boosts extraction yield (Rosa *et al*., 2021).

Oleuropein may be deglycosylated and/or hydrolyzed to create hydroxytyrosol, which is highly valued for its biological activity and functionality thanks to the processing design. However, if oleuropein is the intended end product, the extraction system should be carefully planned to guarantee the recovery of the desired pure or intact molecules with the least amount of chemical reaction or degradation. In this regard, the precision of the processing system control ("from farm to final product") is of utmost importance given the high susceptibility of the compounds to be extracted, especially when aiming at more demanding applications, including pharmaceutical and medicinal. Olive leaves and OLEs are increasingly in demand because they are used as food additives and supplements. Under oxidative circumstances, OLEs also appear to be efficient stabilizers in vegetable oils. In order to extend the shelf life of olive oil, extract of polyphenols from olive leaves has been studied. As previously mentioned, among these are oleuropein, the most prevalent biophenol, followed by verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside, which act as biologically active natural antioxidants. The geographic region, plant nutrition, and cultivar all have an impact on the leaf extract's chemical makeup, just like they do with many other natural products (Kiritsakis *et al*., 2017)

Potential Industrial Applications of Olive Leaf Extracts

Olive leaves could be used to develop or functionalize a variety of food products due to their high potential for medical, cosmetic, and pharmaceutical uses. Olive leaf extract is thought to be useful in extending the shelf life of oils by promoting oxidation stability and nutrient retention (Markhali *et al*., 2020). Olive leaves have a variety of biological properties that have been linked to them, including antioxidant, anti-inflammatory, and anticancer effects. The various, previously mentioned bioactive compounds, particularly phenolic compounds (such as hydroxytyrosol, oleuropein, polyphenols, etc.), have generally been associated with these properties (Otero *et al*., 2021). Some applications, such as the addition of oil during storage or heating of refined olive oil, sunflower oil, and soybean oil, concentrate the antioxidant properties of leaf extracts. It takes only 1 kg of olive leaf extract to strengthen 50–320 l of refined olive oil to give it the same stability as virgin olive oil. The addition of phenolic compounds to processed foods has two advantages for oxidation resistance and human health. Olive leaf extracts (OLEs), which are excellent antioxidants and may replace synthetic antioxidants, have a great deal of potential for use as food industry antioxidants, according to earlier studies. The commercialization of olive leaf in various forms must be seriously considered by countries that produce olive oil. The composition of olive leaf extract can vary depending on factors like geographic location, plant nutrition, and cultivar, as is the case with many natural products (Keceli and Harp, 2014). In comparison to the expensive purified biomolecules such as oleuropein, including phenolic-rich olive leaf extract in the food system as an alternative functional source has the advantage of being a low-cost processing method because it eliminates the need for the purification step while being significantly effective. (Markhali *et al*., 2021).

Conclusions

It is obvious that a desired strategy is enhancing extraction effectiveness (taking into account both yield and functionality) while reducing costs overall. Although many studies on the analysis and use of olive leaves have been published to date, more information is required to close the current knowledge gap regarding the exploration of workable strategies based on environmental and economic sustainability. Although many studies on the analysis and use of olive leaves have been published to date, more information is required to close the current

knowledge gap regarding the exploration of workable strategies based on environmental and economic sustainability. Although many studies on the analysis and use of olive leaves have been published to date, more information is required to close the current knowledge gap regarding the exploration of workable strategies based on environmental and economic sustainability. It has been established that there is a direct link between the consumption of olive by products and the health claims. Due to their antioxidant properties, various food industries have utilized the phenolic fraction of olive by-products, which primarily contains hydroxtyrosol and oleuropein, as food additives and preservatives.

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FOOD SAFETY FOR OLIVE OIL

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Abstract

In order to ensure product quality in the olive oil and olive oil business, organizations have taken steps to ensure European standards and regulatory compliance in the manufacturing of high-quality products. Quality assurance and product safety certification show that a company has met the requirements of various standards (ISO and HACCP) and has implemented a traceability system. HACCP (Hazard Analysis and Critical Control Points) defines hygiene, occupational safety, environmental protection, hazard identification, and critical control point (CCP) evaluation criteria for olive oil mills and packaging departments to follow. A quality management system, for example. A HACCP program classifies and regulates hazards that affect food safety (physical, chemical, and microbiological). Olive oil can be hazardous both physically and chemically. The only potential microbiological dangers for olive fruits are fungal infestations during storage and before processing, particularly when stored in plastic bags. Because fungi are fully removed along with the wastewater during olive fruit processing, microbiological dangers are minimized in olive oil mills. The effective installation and execution of ISO 22000 HACCP and traceability criteria ensure the quality assurance and safety of extra virgin olive oil while satisfying the growing consumer demand for greater quality and functionality. From a management standpoint, there is a significant relationship between food safety, quality, and traceability systems.

Keywords: *Extra virgin olive oil, HACCP, IS0 22000, traceability, Food Safety*

Introduction

Consumer demand for high-quality products, as well as the need to align business with standards such as European standards and ensure compliance with legislation in the production of quality products, have led organizations involved in the olive oil and olives industry to take measures to ensure the quality of their products in recent years. The certification of quality assurance and product safety refers to a company's adherence to certain standards (ISO and HACCP) and the installation of a traceability system. HACCP (Hazard Analysis and Critical Control Points) is a quality control system that specifies the rules that olive oil mills and packaging units must follow in terms of hygiene, occupational safety, environmental protection, hazard identification, and critical control point (CCP) evaluation, among other things. The effective execution and application of these principles aim to achieve overall quality assurance and product safety, hence satisfying consumers' growing demand for superior quality and functionality in virgin olive oil. Traceability is linked to both quality and safety, while safety is more frequently implicated by traceability. They are two critical components of people's food perceptions and associated decision making (i.e., food selection). Traceability is primarily regarded as a tool for establishing food safety by giving a mechanism for recall as well as confirmation of food authenticity, but it is also associated with food quality (Aung and Chang, 2014). Authenticity and traceability means that quality certification is of major interest to the food industry. A variety of government legislation and regulations, as well as international agency guidelines, have been produced in this context. To be classified as EVOO in Europe, the oil must meet a set of requirements outlined in EU

Regulation No. 2568/91 and subsequently changed in EU Regulation 1348/2013, which are evaluated using chemical and sensory testing utilizing IOC methodologies and standards. Free acidity, the peroxide index, ultraviolet (UV) absorption (coefficients K232, K270, and K), fatty acid ethyl esters, and sensory qualities are the quality parameters examined in olive oil (Kiritsakis *et al*., 2020). These basic quality indicators, along with fatty acid and sterol content, as well as profiles in phospholipids, tocopherols, phenolic molecules, volatile compounds, and so on, can provide highly important information for determining the validity of EVOO. Among the EU rules put in place to standardize the production and commercialization of EVOO in Europe (the world's largest producer, exporter, and consumer of olive oil), certified labeling is a method of giving consumers with guarantee of food authenticity (Lozano-Castellon *et al*., 2021). Because they come from well-defined geographical areas, some olive cultivars are known for generating superior-quality oil. These oils attract higher prices and, in most cases, are legally protected. Indeed, the purpose of PDOs, PGIs, and TSGs is to add value to unique high-quality products from a specific origin. Instrumental advancements have resulted in better traceable success. The development of precise analytical fingerprinting methods for the identification of olive oils and the certification of geographical origin is a current problem and a significant challenge. However, the intensive study is still needed to incorporate new methodologies for better characterization and evaluation of olive oil functional components. Because both quality and safety have been linked to consumer confidence, traceability may improve consumer confidence through quality and safety inspections. Firms utilize the Total Quality Management (TQM) approach to promote continual improvement in the quality of their goods and operations. ISO 9000 can be viewed as a step toward applying TQM. From a management standpoint, there is a significant relationship between food safety, quality, and traceability systems. To qualify for a PDO, the olive oil must comply with specific requirements regarding the geographic origin, cultivar, organoleptic characteristics, production methods, and agronomic practice. Certification and denomination require EVOO traceability to be established, focusing on the region of the olive tree (geographic traceability) and the cultivar (botanical traceability). Authenticity covers many aspects, including adulteration, mislabeling, mischaracterization, and misleading origin. Therefore, the detection of edible oil adulteration is crucial in food quality, safety control, and the vegetable oil product trade (Keceli, 2018; Keceli, 2019; Keceli *et al*., 2023). The rapid and reliable detection of adulteration is a very challenging issue in the field of virgin olive oil authentication (violino et al., 2020; Keceli et al., 2023). Adulteration detection, is not easily possible and requires precise, time consuming and expensive methods. The analytical procedures (including sample preparation, analysis, data acquisition and processing) have been developed and proposed to control the adulteration of virgin olive oil (Heise *et al*., 2005; Lema-Garcia *et al*., 2010; Bajoub *et al*., 2018; Keceli *et al*., 2023). The aim of this paper is to idrntify HACCP, traceability and authenticity along the entire EVOO production chain to verify the economic sustainability and to Show relationship between food safety, quality, and traceability systems.

HACCP

The use of the HACCP program in an olive oil factory benefits numerous elements (Kiritsakis, 2007). Identify and trace all potential dangers (physical, chemical, and microbiological) in all processing processes. Analyze and assess the amount and severity of each risk, as well as the likelihood of this risk occurring. Control and resolve the issues brought on by each risk. Use systems and procedures to mitigate these hazards. Furthermore, the use of a HACCP system in an olive oil facility validates the following: The parameters of the olive oil produced are in accordance with current regulations. The health of consumers is safeguarded. The plant's benefits are protected against any legal issues. The promotion of

olive oil is preferred. International market competition is encouraged. The export of olive oil has increased, while the risk of order cancellation has been avoided. The price of olive oil has risen. Only until the oil refinery has implemented the required level of hygiene can the program be successfully implemented. Thus, in order to properly implement a HACCP system, the owner of the olive oil plant must assure the following: Appropriate staff training and uniforms. Sanitary-ware efficacy. Appropriate amenities (sanitary areas, drainage web, clean toilets, separate storage, rubbish area, and so on). After implementing the program, the company might be certified by a certification service. The International Management Standard for Food Safety ISO 22000 was published in 2005. ISO 22000 was developed in order to build a unified and homogeneous demand platform that would be accepted by all countries globally (El-Sayed *et al.*, 2015; Fernández-Segovia *et al*., 2014).

In general, certain criteria must be met to implement the HACCP program in olive oil plants: Good Manufacturing Practice (GMP) and Good Hygiene Practice (GHP) regulations will be followed. Staff will be adequately taught in hygiene and safety issues, as well as given updates on advancements. The following requirements must be met before implementing a HACCP program in an olive oil plant: To ensure active involvement in the program, a person in charge of the HACCP program should be designated and the personnel should be informed. Recording potential risks (physical, chemical, and microbiological) associated with the olive fruit process, recommending preventive actions, and detecting CCPs at each process step. Defining critical limits and control procedures for each CCP (a "critical limit" is the limit of a CCP beyond which there may be consequences for olive oil quality and consumer health). Specifying corrective actions for each CCP. Validation and verification. Validation of the HACCP system by maintaining and updating the necessary data.

HACCP program in the olive oil mill

The HACCP program assigns and regulates risks (physical, chemical, and microbiological) that compromise food safety. Physical and chemical risks may exist in olive oil. Only when the olive fruit is contaminated by fungi during storage and before processing, especially if it is stored in plastic bags, can microbiological problems develop. However, microbiological dangers are minimized in the olive oil mill since fungi are completely removed with the wastewater during the olive fruit processing. Physical hazards include small branches and pieces of wood attached to olive fruit during harvest, as well as metals, stones, plastics, and leaves, whereas chemical hazards include pesticide residues, packaging material, cleaning residues, polycyclic hydrocarbons, halogenated solvents, and hazardous metals (Pardo *et al*., 2002). There are CCPs, Hazardsi Critical limitations, monitoring action, and remedial action during the centrifugal system's olive oil extraction process. There are three critical phases before olive fruit reaches the olive oil mill. These include fruit picking, transporting fruits to the olive oil mill, and preserving the fruit before processing. Furthermore, there is a temporary process of olive oil storage following the final extraction of olive oil and before it reaches the packaging unit. Each phase of the olive oil plant requires an examination of the key dangers and the detection of CCPs.

Conclusions

Consumers are now very well informed about the health benefits that their food habits can provide. As a result, it is critical for food firms, particularly olive oil enterprises, to meet the requirements of international quality standards for quality assurance and product safety. The effective use of HACCP and traceability criteria, as stated in ISO 22000, ensures the quality and safety of virgin olive oil while also preserving the product's functional constituents. The world wide proliferation of EVOO quality and authenticity standards regulations, driven predominantly by the trade globalization of this product and the emergence of new producing

and consuming countries outside the Mediterranean region, has stimulated new discussion and debate that trade regulations should take into account the natural variation of olive oil composition due to environmental conditions and agro-technological practices, in order to assess the (E)VOO quality, authenticity, and origin (geographical and/or varietal) traceability of this product.

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COMBINING OF TWO PREDATOR, *ORIUS LAEVIGATUS* (FIEBER) (HEMIPTERA: ANTHOCORIDAE) AND *SERANGIUM PARCESETOSUM* SICARD (COLEOPTERA: COCCINELLIDAE) IN PROTECTED EGGPLANT GROWING

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Abstract

Biological control of *Frankliniella. occidentalis* Pergande has been carried out very successfully in many countries, including Turkey, by using the generalist predator *Orius laevigatus* (Fieber) (Hemiptera; Anthocoridae). There are also some promising results in the control of *Bemisia tabaci* by *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae). The present study investigated the possibility of simultaneous use of two predators under greenhouse conditions in a single-crop eggplant growing period between 2009-2010 and 2010-2011 in Antalya Province, Turkey. Each plot was 20 m^2 in size and had 30 plants. Pest populations were established by initial releases to maintain different pest densities. The predators were released on different plots with different pest populations on the eggplant plants. Four adults of *O. laevigatus* and *S. parcesetosum* per plant were released simultaneously in October. *O. laevigatus* suppressed *F. occidentalis* in all plots at varying pest densities approximately 4 weeks after the release date in both growing seasons. Thrips populations increased at a relatively high rate until mid-February. Due to the increase in thrips populations, an additional release of *O. laevigatus* was conducted in March. The specific whitefly predator, *S. parcesetosum*, was released only once and began suppressing *B. tabaci* populations about 4 weeks after the release date, especially in plots with high pest populations. In plots with low whitefly infestations, *S. parcesetosum* larvae were detected later than in the other plots. Both predators reduced their prey population when released together. In conclusion, a biological control strategy using a combination of *O. laevigatus* and *S. parcesetosum* against whitefly and thrips can be used in the future.

Keywords: *Orius laevigatus, Serangium parcesetosum, Bemisia tabaci, Frankliniella occidentalis, Biological control.*

Introduction

Cotton whitefly (*Bemisia tabaci* Genn.) and western flower thrips (*Frankliniella occidentalis* Pergande) are the most important pests in the western Mediterranean region of Turkey, where greenhouse vegetables are intensively produced (Tunç and Göçmen, 1994; Bulut and Göçmen, 2000; Keçeci *et al.*, 2007). Chemical control is generally preferred against these pests. The negative effects of this control method on human and environmental health are well known. In addition, the treatments also kill the natural enemies of these pests.

In many countries, especially in Europe, biological control methods are carried out against pests of greenhouse vegetables. Studies on biological control of these pests, especially *F. occidentalis*, are successfully conducted in the Netherlands, Italy, and Spain using *Orius laevigatus* (Fieber) (Dissevelt *et al.*, 1995; Sanchez *et al.*, 2000; Tommasini and Maini, 2001; Tavella *et al.*, 2003). In a study conducted in our country, it was found that *O. laevigatus* can be successfully applied especially on bell pepper and eggplant plants (Keçeci and Gürkan,

2013; 2017). It has been reported that the parasitoids *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich are successfully used against whiteflies in studies conducted in Europe (van Lenteren, 2000). In addition, *E. mundus* Mercet and the predator *Macrolophus melanotoma* (Costa) have also been reported to be used against whiteflies (Gabarra *et al.*, 2006, Karut *et al*., 2018). However, in a study conducted specifically in the eastern Mediterranean, *E. formosa* and *Verticillium lecani* used against whitefly in cotton were found to be ineffective (Yücel *et al*., 1995). In studies conducted in our country, *Serangium parcesetosum* Sicard, *Chrysoperla carnea* Stephan, *Conventzia hageni* Banks, *Clitostethus arcuatus* Rossi, and *Chilocorus bipustulatus* L. were among the most important natural enemies of whitefly (Anonymous, 1997). Promising results have also been obtained in the control of *B. tabaci* by *S. parcesetosum*. It has been reported that *S. parcesetosum* builds its population under greenhouse conditions and causes a strong reduction of *B. tabaci* population (Ellis *et al*., 2001; Al-Zyoud *et al*., 2007; Kutuk *et al*., 2008).
Biological control studies generally discuss the relationships between the pest and its natural enemy. However, in dynamic environments such as greenhouses, where 2-3 pests may occur simultaneously, it is very difficult to achieve success and apply the results to practice without determining the three- and four-way relationships between other pests and their possible natural enemies. The relationship between *O. laevigatus* and other natural enemies is important for successful biological treatment. Therefore, the present study investigated the combined release of *O. laevigatus* and S. *parcesetosum* for the control of whiteflies and thrips under greenhouse conditions.

Material and Methods

Protected eggplant cultivation is carried out as a single plant cultivation and lasts from September to June. The study of the first year of the field trials was conducted in the greenhouses in Aksu district during the 2009-2010 growing period. Before the plants were planted for the trial, each plot was separated with an insect net. The plants were planted in the greenhouse on September 17, 2009. Each plot was 20 m^2 and contained 30 plants.
Pest populations in these plots were established by initial releases. Adult stages of both pests were collected from the stock culture and transferred to the greenhouse, where they were released in the following ratios (Table 1).
The predators were released into the plots at 4 adults/plants on October 20, 2009. Adults collected from the stock culture in the laboratory were transferred to the greenhouse in glass jars. *S. parcesetosum* was released first, then *O. laevigatus* was released immediately. As thrips density increased, a second release of predatory insects in the same quantity was made on March 10, 2010, during the spring growing season. Beneficial insects were not released in the control plots. The experiment was set up in a randomized block design with 3 replicates for each different pest density.

Table 1. The number of pest and natural enemies released to the plots.

Treatments	No. of pests released		No. of benecials released	
Group A (Control)	*Bemisia tabaci*	10 adults/plant		
	Frankliniella occidentalis	10 adults/plant		
Group B	*Bemisia tabaci*	10 adults/plant	*Serangium parcesetosum*	4 adults/plant
	Frankliniella occidentalis	10 adults/plant	*Orius laevigatus*	4 adults/plant
Group C	*Bemisia tabaci*	5 adults/plant	*Serangium parcesetosum*	4 adults/plant
	Frankliniella occidentalis	15 adults/plant	*Orius laevigatus*	4 adults/plant
Group D	*Bemisia tabaci*	15 adults/plant	*Serangium parcesetosum*	4 adults/plant
	Frankliniella occidentalis	15 adults/plant	*Orius laevigatus*	4 adults/plant

The second year of greenhouse trials was conducted in the same greenhouse during the single crop growing period of 2010-2011. Plants were transplanted in the greenhouse on September 27, 2010. The size of the plots and the number of plants per plot were the same as in the first year study. Pest populations were established with initial releases on October 11, 2010. Predator releases were performed on October 26, 2010 with a dose of 4 adults/plant. A second release of *O. laevigatus* made on March 9, 2011, in the spring of the growing season, with the same dose.

Results and Discussion

The generalist predator, *O. laevigatus* suppressed *F. occidentalis* in almost all plots with varying pest densities, about 4 weeks after the release date. At this time, the *Orius* population reached its highest level. Beginning in January, a rapid decline in the predator population was observed as temperatures dropped. The thrips population, on the other hand, began to increase again in mid-February (Figure 1). The specific whitefly predator, *S. parcesetosum*, began to suppress the pest population about 4 weeks after the release date, and by the end of November the pest was reduced to below 10 larvae+pupae/10 cm^2/leaf. In the plots with low whitefly infestations, where 5 adults were released, *S. parcesetosum* larvae emerged later than in the other plots. An increase in the number of adult whiteflies was observed towards the end of February (Figure 2).

During the spring vegetation period, both thrips and whitefly populations began to increase. During this time, it was observed that the different pest densities that had initially developed in the cages also disappeared. With the additional release of *Orius*, especially after March 26, the thrips population was suppressed in all plots where the predator was released (Figure 1). Early in the spring, additional release of *S. parcesetosum* was not necessary because larvae were produced by overwintering adult *S. parcesetosum*. Since late March, the whitefly population has been suppressed. Towards the end of the season, the number of whitefly larvae+pupae on leaves decreased significantly and fell below the value of 10 larvae+pupae/10 cm^2/leaf (Figure 2).

Data from the greenhouse trial in the second year are shown in Figures 3 and 4.

In the control plot without *Orius* release, the thrips population increased to 15 nymphs+adults in leaf and 5 nymphs+adults in flower in the second half of December. On the other hand, the thrips population in all plots where different pest densities were produced was suppressed about 4 weeks after the release date in the plots where *O. laevigatus* was released. At this time, the *Orius* population reached its highest level. Beginning in January, a rapid population decline was observed as temperatures dropped (Figure 3). Although *Serangium* larvae were seen after two weeks primarily in plots where 10 and 15 whiteflies/plant were infested, they were not seen at the same time in plots where few whiteflies were released. This situation is thought to be due to the predator not laying eggs due to insufficient prey density. As whitefly density increased, the larvae of the predator became visible on these plots. Suppression of the whitefly population occurred in early December. By December 13, 2010, the pest dropped below the level of 10 larvae+pupae/10 cm^2/leaf in all plots (Figure 4).

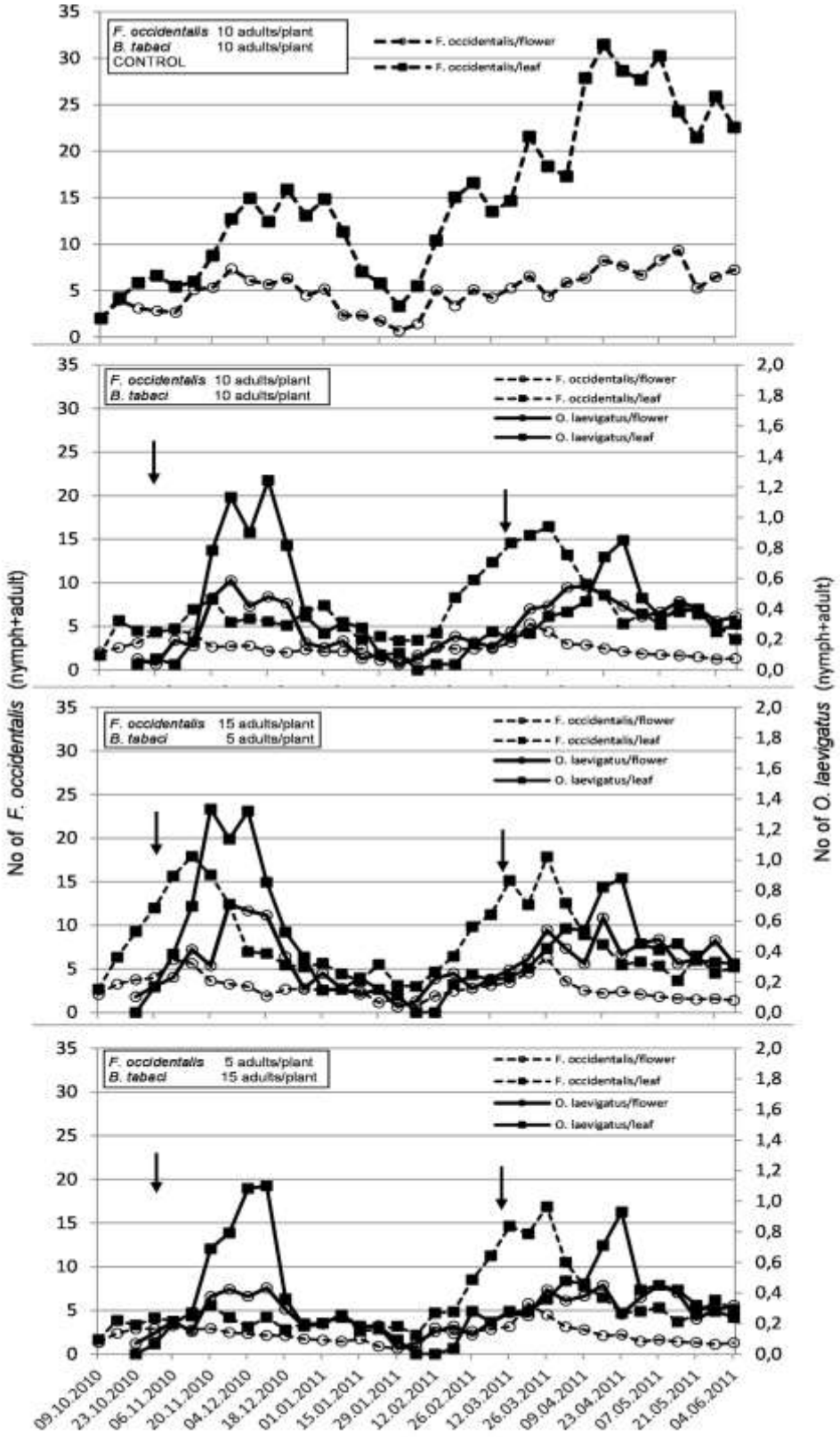

Figure 1. Population development of *Frankliniella occidentalis* and *Orius laevigatus* in plots with different pest densities in first-year study (↓: *Orius laevigatus* release date).

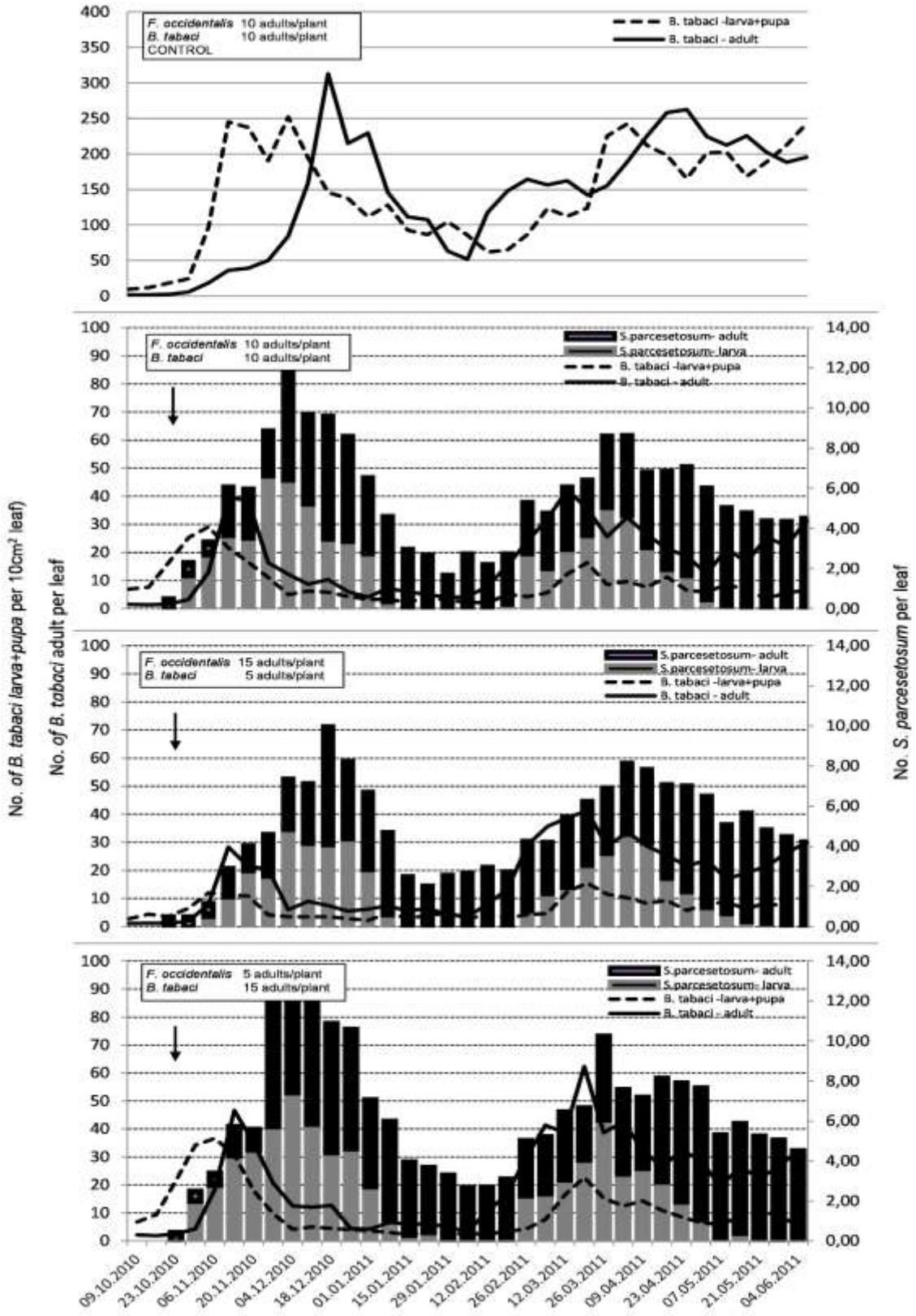

Figure 2. Population development of *Bemisia tabaci* and *Serangium parcesetosum* in plots with different pest densities in first-year study (↓: *Serangium parcesetosum* release date).

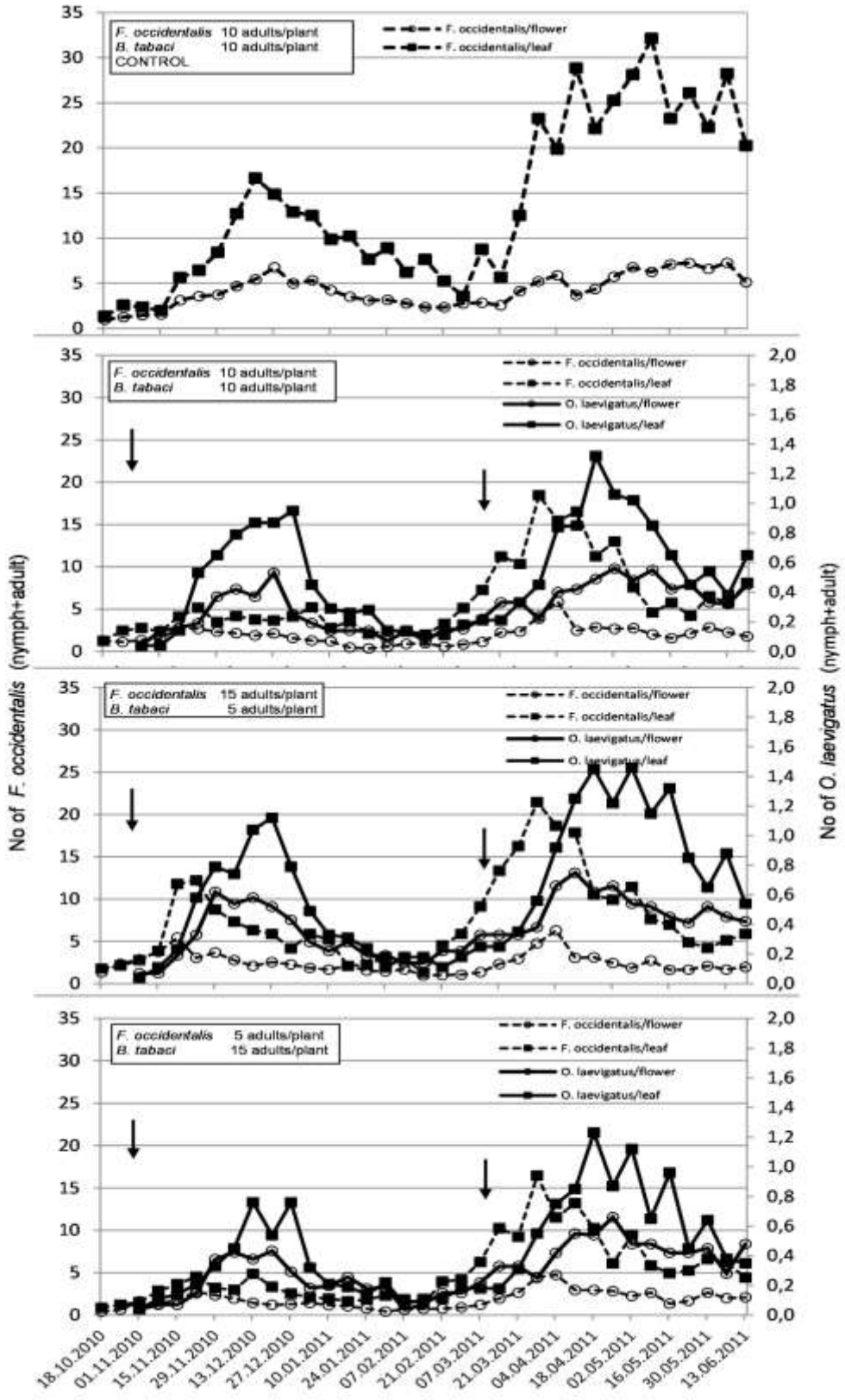

Figure 3. Population development of *Frankliniella occidentalis* and *Orius laevigatus* in plots with different pest densities in second-year study (↓: *Orius laevigatus* release date).

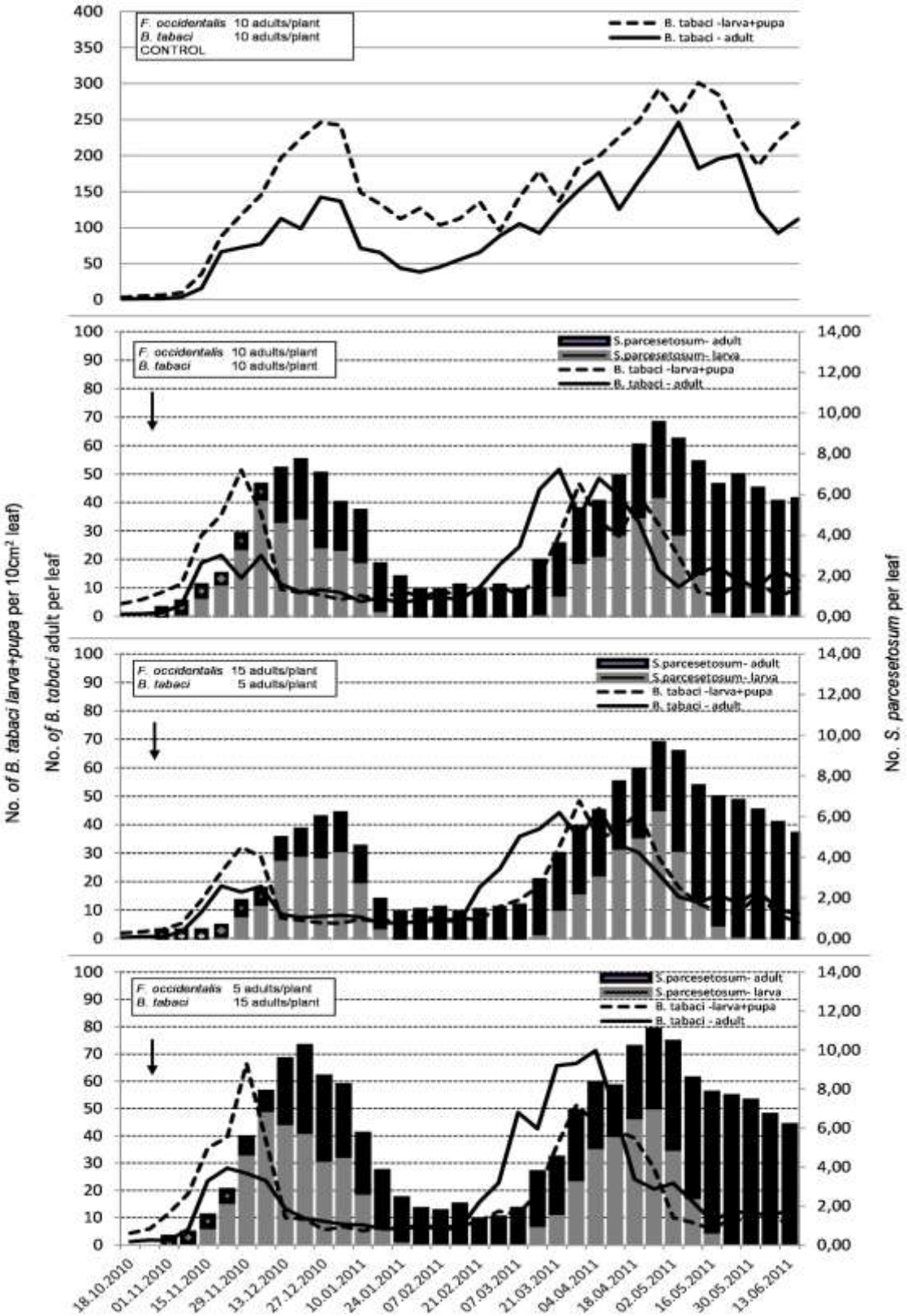

Figure 4. Population development of *Bemisia tabaci* and *Serangium parcesetosum* in plots with different pest densities in second-year study (↓: *Serangium parcesetosum* release date).

Greenhouse trial data for both years showed that the two beneficial insects did not adversely affect each other at different pest densities initiated at the beginning of vegetation. In this study, it was found that *S. parcesetosum* did not start to produce immediately, especially at low whitefly populations, but allowed the pest population to develop for a time. Therefore, it is believed that the predator may be more effective when whitefly populations are high. In addition, adult *Serangium* were observed in groups that were inactive, especially in January and February, and were found to have no feeding behavior. Although results from laboratory studies are sometimes disappointing, it is possible that different results may be obtained under field conditions. The feeding preference of *Cryptolaemus montrouzieri* Mulsant between healthy mealybug individuals, *Planococcus citri* Risso and *P. ficus* Signoret and parasitized by *Anagyrus pseudococci* Girault, was studied. It was found that the adult female and the fourth instar larva (L4) of the predator preferred the two- and four-day-old parasitized individuals of *P. citri* and *P. ficus* to the healthy ones for feeding (Muştu and Kılınçer, 2007). The result under these laboratory conditions may be different under field conditions. In particular, *A. pseudococci* prefers mealybugs located in hidden places such as under the sepals of the fruit to parasitize. For this reason, it is believed that the probability of encountering both beneficial insects decreases. It is thought that a similar situation may apply to the current study. It is thought that especially the smaller *Serangium* larvae are not attacked by the adult *O. laevigatus* because most of the *O. laevigatus* population is found in the flowers and young leaves of the plant, while the *Serangium* larvae are mostly found in the middle and lower leaves where the late stages of the whitefly larvae and the pupae are found. In view of these results, it can be assumed that both agents can be used together against thrips and whitefly in greenhouse cultivation of eggplant.

Conclusions

In recent years, the importance of alternative pest control methods has increased as the negative effects of chemical control on human health and the environment have become known. Biological pest control is one of the alternative methods in terms of its applicability and sustainability. In this study, the combined use of two predators, *O. laevigatus* and *S. parcesetosum*, against thrips and whitefly was investigated. In the greenhouse experiments, differential densities of *F. occidentalis* populations triggered by pest releases were suppressed by the release of *O. laevigatus*, which performed twice at a release rate of 4 adults/plant in October and March. Similarly, *B. tabaci* populations that had created at different densities were suppressed by the release of *S. parcesetosum* at a dose of 4 adults/plant in October. No negative effects of *O. laevigatus* on *S. parcesetosum* were observed in the greenhouse trials, even in plots with low thrips densities. It was concluded that both beneficial insects can be used together against thrips and whitefly in greenhouse eggplant cultivation.

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INVESTIGATION OF THE COMBINED HARMFUL EFFECTS OF CHLORPYRIPHOS AND CYPRODINIL ON PHEASANT EMBRYOS

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Abstract

The aim of this study was to determine the individual and combined toxic effects of Pyrinex 48 EC insecticide (480 g/l chlorpyriphos) and Chorus 50 WG fungicide (500 g/kg cyprodinil) on early and late development phase of pheasant embryos. In the European, Union chlorpyrifos is banned from use since 31 January 2020, but organophosphate pesticides are used in many countries except the European Union. Emulsion or suspension of the test materials was injected in 0.1 ml volume into the air chamber of the pheasant eggs before starting the incubation. Subsequently, on the third day of incubation permanent preparations were made from part of the embryos in order to study the early developmental stage. Based on the results of the experiment, embryonic mortality and the frequency of occurrence of pheasant embryos showing developmental abnormalities increased in the groups treated individually and together with the insecticide and the fungicide product compared to the control, but the differences could not be verified statistically. As a result of the treatments, developmental delay appeared in the form of poorly developed body and vasculature. The pheasant embryos were examined on day 21 by the followings: body weight, rate of embryonic mortality, rate and type of developmental anomalies by macroscopic examination. Except for the fungicide-treated group, the average body weight of the pheasant embryos in the treated groups was significantly lower than that of the control group. The single and simultaneous administration of the Pyrinex 48 EC significantly increased the mortality and the developmental anomalies of the pheasant embryos as compared to the control. There was presumably additive type toxic interaction between Pyrinex 48 EC and Chorus 50 WG. The test materials were embryotoxic in pheasant.

Keywords: *chlorpyriphos, cyprodinil, pheasant embryo, interaction, ecotoxicology.*

Introduction

Humanity's food necessity must be produced in an ever smaller area, as the cultivated area per capita is constantly decreasing, and the sizes suitable for crop cultivation are only available to a limited extent. For this reason, one of the biggest challenges of today and the future is how to fully meet the food needs of the growing humanity (Lal, 2016).

Among the harmful environmental effects of agricultural production, one of the most important is the impact of reducing biodiversity and endangering it. Pesticides used in agriculture are undoubtedly the most effective and dangerous means of plant protection. Their use can negatively influence and threaten the biodiversity (Ángyán *et al.*, 2003).

Due to the agricultural production and the development of various industries, humans and their living environment are exposed to significant chemical loads every day, among which

the harmful effects caused by pesticides cannot be neglected. The fauna of the areas used for agricultural cultivation is most exposed to the chemical load (Pintér *et al.,* 2020).
Hungary's small game population had its heyday in the first half of the 1900s. The first signs of its decline began to appear when agriculture became intensive. Many factors, such as the increasing use of pesticides, the rearrangement of field sizes and inadequate stock utilization, also contributed to their decline. There is a negative correlation between the plant protection products used during large-scale arable crop cultivation and the dynamics of our small game population. Many people misjudge or do not even recognize the effects of pesticide use on living beings because, looking only at their direct toxicity, many do not threaten wildlife. However, they forgot about the danger that affected the ecological systems of the field as a whole, and they indirectly affected the small game population (Faragó, 1997).
Plant production products used in agricultural production do not only exert their biological effects on the target organisms. With the expansion of ecotoxicology, we have more and more knowledge that describes the chemical effects on the living world that surrounds humans (Sinkovitsné and Benkő, 1993).
The harmful effects of various chemical compounds in the environment (adverse effects, after-effects) must be monitored (Pintér *et al.*, 2020) because the chemical load usually occurs in a complex way so that we can see a combined toxic effect as well (Juhász *et al.,* 2005).
The breeding period of the pheasant coincides with the time of chemical plant protection works in spring, which is why the sprayed agents pose a danger not only to the adult birds but also to embryos developing in the egg. During the hatching period, active substances can enter the egg from the spray that gets on the birds' eggs, disturbing embryonic development (Fejes *et al*., 2002; Juhász *et al.*, 2006).

Materials and methods

In our experiment carried out in 2020, the 1% emulsion of Pyrinex 48 EC (ADAMA Hungary Ltd., Hungary) insecticide with 480 g/l chlorpyrifos active ingredient and the 0.125% fungicide Chorus 50 WG (Syngenta Ltd., Hungary) with 500 g/kg cyprodinil active ingredient of suspension was used, which corresponded to practical spray concentration. Their single and simultaneous fetotoxic effect was investigated in the early and late stages of the development of the pheasant test organism.
Two hundred fertile pheasant eggs were used in our experiment came from the main colony of the Hubertus Hunting Society (Abádszalók, Hungary). The eggs were incubated after transportation and rested for 24 hours. The eggs were incubated for 23–24 days in a Ragus-type incubator (Vienna, Austria). During the incubation, the appropriate temperature (37.5–37.8°C), air humidity (48–65%) and the daily rotation of eggs were provided (Bogenfürst, 2004).
The treatment of eggs (n=50/group) was performed on the first day of incubation. In the individual treatments, suspensions and emulsions made from test chemicals in 0.1–0.1 ml end volume were used, while in case of combined treatment, 0.2 ml of the test materials were injected into the air chambers of eggs (Clegg, 1964; Lutz, 1974).
A hole was punched in the calcic eggshell and the shell membrane above the air space, and then the quantity of the suspensions and emulsion of the test materials was injected into the air chamber (Clegg, 1964; Várnagy *et al.,* 1996). After the injection, the hole was sealed with paraffin, and the eggs were placed into the incubator. In the control group, avian physiological saline solution (0.75 w/v%) was injected into the air space of the eggs in the manner described above.
The incubation was started immediately after the treatments. To study the early development phase, permanent preparations were made from 10 embryos per group on the third day of

incubation. Above the air chamber, the calcic eggshell and the shell membrane were removed. The germinal disk was cut around and, with a filter paper placed on it, was put into avian physiological saline (0.75 w/v %) at 38^{o}C temperature. After blotting up the saline solution, the embryo placed on a slide was stained with 0.1% osmium tetroxide solution and fixated, then mounted with DPX histological adhesive and covered with a coverslip. The permanent preparations were then examined by light microscopy (Sinkovitsné and Benkő, 1993). The number of embryonic death and developmental abnormalities of embryos were recorded.
Fourty eggs were prepared on day 21 of incubation in each group. During the necropsy we recorded the body weight of the embryos, evaluated the frequency and type of morphological changes, and recorded the number of embryos that died in the earlier development period.
In the case of the body weight data of live embryos, statistical comparisons among the groups were made with a one-way analysis of variances (ANOVA). In the case of the biometric processing of embryonic mortality and malformations, an exact test, according to Fisher, was used.

Results and discussion

On the third day after treatment, no dead embryo was found in the control group.
As a result of injection exposure to Pyrinex 48 EC, embryonic mortality increased by 10.0% compared to the control group; the difference was insignificant.
As a result of treatment with Chorus 50 WG, the rate of embryonic mortality increased to 10.0%. The change was not statistically significant.
As a result of the combined application of pesticides, the rate of embryonic mortality reached 30%. The difference was not significant compared to the control (Table 1).
During the light-microscopic evaluation of permanent preparations stained with osmium tetroxide, one of the embryos in the control group showed a developmental anomaly (10.0%).
Four embryos (44.44%) showed abnormal development due to treatment with insecticide alone. This change was not statistically significant as compared to the control group.
In the group treated with fungicide, the ratio of embryos showing developmental anomalies increased to 44.44% compared to the control group. The change was not statistically significant.
As a result of combined treatment with the insecticide and fungicide, the malformation rate of embryos reached 42.86%. The change was statistically insignificant compared to the control and the group treated with fungicide or insecticide alone. The developmental anomalies included the appearance of the retarded development of the vascular system and the body (Table 1).

Table 1. Embryonic mortality and developmental anomalies on pheasant embryos treated with Pyrinex 48 EC and Chorus 50 WG on day 3 of incubation

Treatment	No of dead embryos/ No of fertile eggs	No of embryos showing developmental anomalies/ No of alive embryos	Rate of embryonic mortality (%)	Rate of developmental abnormalities (%)
Control	0/10	1/10	0.00	10.00
Pyrinex 48 EC	1/10	4/9	10.00	44.44
Chorus 50 WG	1/10	4/9	10.00	44.44
Pyrinex 48 EC + Chorus 50 WG	3/10	3/7	30.00	42.86

Based on the embryonic mortality data observed during the necropsy on the 21st day, we found that embryonic death significantly increased compared to the control (6/40) in the

group treated with Pyrinex 48 EC insecticide alone (14/40; $p<0.05$), as well as the insecticide in combination with the agent and the fungicide product (21/40; $p<0.001$).
The combined treatment also resulted in a significant ($p<0.01$) increase in embryonic death compared to the group treated with the fungicide alone (7/40). Five embryos with abnormal development were observed in the group treated with Chorus 50 WG fungicide alone. Eight of the live pheasant embryos showed macroscopic developmental abnormalities in the group treated individually with the insecticide, which was already a significant ($p<0.05$) increase compared to the control group. As a result of the combination treatment, morphological changes were detected in eight live pheasant embryos, representing a significant increase ($p<0.01$; $p<0.05$) compared to the control group and the group treated individually with the fungicide. The malformations appeared primarily in limb deformity (bent foot) and growth retardation (Table 2).
As a result of the individual injection treatment with Pyrinex 48 EC the body weight values of the live embryos (9.70±2.09 g) were significantly ($p<0.001$) lower than the control values (11.83±2.03 g) compared. As a result of the combined treatment with Chorus 50 WG and Pyrinex 48 EC, the body weights of the live pheasant embryos decreased significantly ($p<0.001$; $p<0.05$) (8.98±1.84 g) compared to the control and to the data of the group treated with a fungicide alone. A decreasing trend was observed in the body weight of embryos in the group treated with fungicide alone (10.61±1.61 g). However, the difference was insignificant compared to the control group (Table 3).

Table 2. Embryonic mortality and developmental anomalies on pheasant embryos treated with Pyrinex 48 EC and Chorus 50 WG on day 21 of incubation

Treatment	No of dead embryos/ No of fertile eggs	No of embryos showing developmental anomalies/ No of alive embryos	Rate of embryonic mortality (%)	Rate of developmental abnormalities (%)
Control	6/40	0/34	15.00	0.00
Pyrinex 48 EC	14/40[a1]	8/26[a1]	35.00	30.77
Chorus 50 WG	7/40	5/33	17.50	15.15
Pyrinex 48 EC + Chorus 50 WG	21/40[a3, b]	8/19[a2, b]	52.50	42.11

[a]Significant difference compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$; [a3]$p<0.001$).
[b]Significant difference compared to the group treated with Chorus 50 WG alone ([b]$p<0.05$).

Table 3. Embryonic body weights (g) from teratogenicity test of Pyrinex 48 EC and Chorus 50 WG in pheasant embryos after single and combined administration on day 21 of incubation

Number of embryos (n)	34	26	33	19
Average (g)	11.83 ±2.03	9.70 ±2.09[a]	10.61 ±1.61[a1]	8.98 ±1.84[a, b]

[a]Significant difference compared to the control group ([a]$p<0.001$).
[b]Significant difference compared to the group treated with Chorus 50 WG alone ([b]$p<0.05$).

Based on the experiment's results, in the initial stage of embryo development, it can be established that the embryonic mortality found in the groups treated with the insecticide or the fungicide alone was not significantly different from that seen in the control group.

At the same time, it can be stated that combined treatment with the pesticides in enhanced embryotoxicity since the rate of embryonic mortality found in the group receiving the combined treatment was higher - but not significantly way - than that obtained in the control group or the group treated with the insecticide alone or with the fungicide alone.
As a result of the treatments, developmental delay appeared in the form of the retarded development of the vascular system and the body. Pyrinex 48 EC and Chorus 50 WG were applied alone and together proved embryotoxic for the pheasant embryo developing in the egg. Based on the incidence of malformations, a teratogenic effect cannot be confirmed because the detected developmental disorders can be compensated for later (Juhász et al., 2005).
These results are in harmony with the results of previous studies in which eggs were treated with various pesticides at different times of the incubation period and signs of embryotoxicity were detected at necropsy, but teratogenicity was not clearly proven (Budai et al., 2002; Varga et al., 1999).
In our study, individual and combined injection treatments with Pyrinex 48 EC and Chorus 50 WG in the late embryonic development stage proved embryotoxic on pheasant embryos. The combined toxic effect of the simultaneously applied plant protection agents prevailed. A small degree of teratogenic effect was confirmed. Szabó et al. (2021) observed a significant increase in embryonic mortality and a substantial decrease in body weight in the chicken embryo in their avian teratology study with Pyrinex 48 EC 1% emulsion using injection treatment. Pyrinex 48 EC is individually and combination caused a significant decrease in body weight during treatment. They experienced something similar copper sulfate (0.01%) and pendimethalin or herbicides containing metolachlor, and during the joint administration of different herbicides on avian embryos (Várnagy et al., 1996; Juhász et al., 2006). Farag et al. (2003) found in their mammalian teratology study on Fischer 344 rats that chlorpyrifos is fetotoxic and teratogenic at a maternally toxic 25 mg/kg dose. Tang et al. (2020) studied the developmental toxicity of cyprodinil in zebrafish embryos. During the 72-hour exposure, cyprodinil was used in concentrations of 0.1, 1.0, 10 and 100 µg/L. Finally, cyprodinil was established as a potential agonist of the aryl hydrocarbon receptor (AhR). As a result, it can cause abnormalities in cardiac development and heart function in zebrafish embryos. The results of the teratological studies performed on avian embrios prove that the bird embryo can be used in reproductive biology and teratology studies in addition to the mammalian and fish model, as it indicates the harmful effects of various chemical agents with adequate sensitivity (Várnagy et al., 2003, Keserű et al., 2004; Szabó et al., 2020; Lehel et al., 2021).

Conclusion

Based on our experimental data an additive effect was realised at the embryonic mortality and developmental anomalies due to the simultaneous injection treatment of Pyrinex 48 EC and Chorus 50 WG. The body weight of the pheasant embryos was reduced due to the experimental exposure without any combined relation.
Besides the injection treatment method applied during the studies, it would be advisable to perform complete examinations with immersing treatments that can be served as a model of expositional circumstances during the plant protection practice and compare.

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TROPHIC INTERACTIONS AND DYNAMICS OF *APHIS SPIRAECOLA* PATCH ON CITRUS TREES IN PROXIMATE PLOTS, MOSTAGANEM, ALGERIA

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Abstract

Aphis spiraecola Patch, the predominant aphid species infesting Algerian citrus trees, was the focus of this study. The research investigated the population dynamics of *A. spiraecola* and its trophic interactions with predators, primary parasitoids, and hyperparasitoids on Clementina and Thomson Navel citrus trees in Mostaganem, northwestern Algeria, during the initial leaf flush of 2021. Weekly sampling enabled the assessment of population fluctuations. A total of 200 young leaves (100 leaves per citrus species from 10 trees each) were collected. Laboratory analysis and species identification utilized a binocular microscope. Results unveiled a diverse array of species, encompassing aphid pests and their natural enemies. Identified predator species included *Coccinella algerica* Kovar, *Scymnus subvillosus* Goteze (Coleoptera: Coccinellidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), *Episyrphus balteatus* De Geer (Diptera: Syrphidae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and *Campyloneura virgula* Herrich-Schaeffer (Hemiptera: Miridae). Three primary parasitoid species emerged from mummies: *Lysiphlebus testaceipes* Cresson, *Binodoxys angelicae* Haliday, and *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae). Four hyperparasitoid species were also identified: *Phaenoglyphis* sp., *Pachyneuron aphidis*, *Dendrocerus* sp., and *Alloxysta* sp. Temperature played a crucial role in infestation onset and subsequent population fluctuations. Earlier infestations were observed in Clementina trees, with higher density recorded (8 aphids/cm²) compared to orange trees (3 aphids/cm²). Notably, the Clementina and orange blocks were in close proximity, facilitating accurate comparisons. Citrus species, unfavorable leaf physiological conditions, and temperature were identified as key factors influencing aphid population dynamics.

Keywords: *Aphis spiraecola, population dynamics, citrus, natural enemies, Algeria.*

Introduction

Aphis spiraecola Patch, commonly known as the spiraea aphid, poses a major threat to citrus trees, leading to significant economic losses in citrus-producing regions worldwide, including Algeria (Gómez-Marco, 2015 ; Barbagallo *et al.*, 2017 ; Labdaoui, 2019). The Algerian citrus industry plays an important role in the country's agricultural economy, but the infestation of citrus trees by *A. spiraecola* can result in reduced fruit quality, stunted growth, and yield losses (Labdaoui, 2019). Moreover, these aphids can transmit phytopathogenic viruses such as CTV (Citrus Tristeza Virus), further impacting citrus orchards (Ali Arous et al., 2017).
Understanding the trophic interactions and population dynamics of *A. spiraecola*, especially its interactions with natural enemies, is crucial for developing sustainable pest management strategies in citrus orchards. *A. spiraecola* is a polyphagous aphid species with a wide range of host plants, including citrus trees, and its rapid reproduction and ability to establish dense

colonies exacerbate its impact on citrus orchards (Blackman *et al.*, 1984 ; Hermoso De Mendoza *et al.*, 2006).

Trophic interactions involving predators, parasitoids, and hyperparasitoids within ecosystems play a pivotal role in shaping population dynamics and determining the impact of pests on their host plants. Various natural enemies have been identified in other citrus-growing Mediterranean regions that interact with *A. spiraecola* populations, such as predatory insects and parasitoid wasps, which can regulate aphid populations effectively (Gomez-Marco, 2015, Kavallieratos et al., 2004; Ben Halima-Kamel et al. 1994). However, the specific trophic interactions and population dynamics of *A. spiraecola* on citrus trees in Algeria especially in Mostaganem, (northwestern), remain largely unexplored (Labdaoui et Guenaoui, 2015). While research conducted in other regions has provided valuable insights, understanding the dynamics in this specific area is essential. Therefore, this study aims to investigate the diversity of natural enemies associated with *A. spiraecola* infestations on Clementina and Orange trees during the initial leaf flush of 2021. Key predator species, primary parasitoids, and hyperparasitoids will be monitored to understand their role in regulating *A. spiraecola* populations in the region.

Moreover, in order to enable a direct comparison of aphid population dynamic between two citrus species Clementina and orange, the same orchard containing both citrus species was chosen for this study. In this work, preliminary results obtained on the trophic interactions and dynamics of *A. spiraecola* infestations on two kinds of citrus trees in northwestern Algeria will be presented and discussed.

Material and methods

The study was conducted in a private citrus orchard located in Sayada, Mostaganem, Algeria (35°55'45.8"N and 0°08'01.8"E). The orchard covered an area of 4 hectares and consisted of Thomson orange trees (over 30 years old) and Clementina trees (approximately 10 years old) on sour orange rootstock. The trees were spaced 5 meters apart in rows, and the understory vegetation was mostly left undisturbed as weed control was performed mechanically.

Management practices in both orchards followed local farmer knowledge and customary practices common among citrus growers in the region. During the observation period, only one application of Acetamiprid insecticide was used after the flowering period on April 25th.

For data collection, a total of 100 young leaves (10 leaves per tree) were collected from each citrus tree. Leaves with *A. spiraecola* colonies containing at least one mummy were selected.

Leaf samples were collected, labeled, and examined in the laboratory on the same day. The different stages of *A. spiraecola* and their natural enemies (predators and parasitoids) on each leaf, along with mummies, were counted under a binocular microscope using a counter. Aphid density was calculated by determining the leaf area following the method of Onillon et al. (1973). To monitor the formation of new mummies, the samples were re-inspected after 72 h.

Mummies were individually placed in labeled Eppendorf tubes and kept under controlled conditions (25 ± 5 °C; 60-80% H.R.; L:D 18:6) until emergence. The emerged parasitoids were classified into primary and secondary parasitoids (hyperparasitoids) and preserved with their respective mummies in 96% ethanol for later identification based on morphological characteristics using multiple identification keys.

Predators were collected from *A. spiraecola* colonies based on their life stage (larvae or adults) using a soft brush. Larvae were observed until adult emergence. The collected adult predators were preserved in 96% ethanol and later identified using multiple identification keys.

Statistical analysis of the collected data was performed using Excel 2021 and IBM SPSS Statistics v.23. ANOVA was applied to assess significant differences among groups.

Result and discussion

Sampling took place from March 2nd to May 4th, 2021, in two plots: Clementina and Orange trees (Thomson Navel variety), coinciding with the first spring leaf-flushing in mid-February 2021, and *A. spiraecola* colonies appeared later that month.

In the Clementina plot, *A. spiraecola* infestation was evident during the first week of sampling (March 2nd) with an average density of 25 aphids per leaf (equivalent to 6.0 ± 2.7 aphids per cm²). The following week (March 9th), densities decreased to 6 aphids per leaf (2.0 ± 1.7 aphids per cm²), possibly due to climatic variation (39 mm precipitation and a temperature drop ranging from 10°C to 18°C). By March 30th, the density peaked at 34 aphids per leaf (8.0 ± 1.7 aphids per cm²) with temperatures ranging from 20°C to 26°C. In the first two weeks of April, infestation declined, with a slight increase during the week of April 20th. A chemical treatment with Acetamiprid on April 25th resulted in nearly complete aphid mortality, leading to a reduction in *A. spiraecola* populations in the following week.

In the Orange plot, during the first week of sampling (March 2nd), a low infestation of *A. spiraecola* was observed, with an average density of 0.1 ± 0.1 aphids per cm² (1 aphid per leaf). The infestation rate reached a peak of 0.6 ± 0.5 aphids per cm², equivalent to 7 aphids per leaf, on March 16th. The following week, a decrease in densities was observed, with 0.2 ± 0.2 aphids per cm² (2.0 aphids per leaf), which was possibly caused by a climatic variation (temperature drop from 18°C to 10°C). A second increase in infestation occurred, reaching a peak of 3.0 ± 1.0 aphids per cm², equivalent to 31 aphids per leaf, on April 6th, coinciding with temperatures ranging from 20°C to 25°C. These peaks are consistent with those found on oranges by Labdaoui (2019), who recorded 10 aphids per cm² (144 aphids per leaf) in 2016 and 6 aphids per cm² (52 aphids per leaf) in 2017. From this date until April 20th, the infestation experienced a decrease due to the rigidity of the leaves and the decrease in nutrients. Young shoots are rich in amino acids, forming the basic food for aphids. However, older leaves are generally rich in sugars, leading to a decrease in nutrients for the aphids (Kindlmann et al., 2010; Lebbal et al., 2015). The insecticide treatment conducted on April 25th with Acetamiprid resulted in a significant reduction in populations during the following sampling (-90%). The ANOVA test showed a highly significant demographic difference between the two samplings (before and after treatment) ($p < 0.05$).

The infestation curves (Figure 1) show a one-week lag between *A. spiraecola* infestations on Clementina and Orange trees. The ANOVA test reveals significant differences between the infestation rates of *A. spiraecola* on Clementina and Orange trees ($P \approx 0$). Clementina trees had higher infestation levels, with a peak of 8 aphids per cm² (11,770 living aphids) on March 30th, 2021, while Orange trees had lower levels, peaking at 3 aphids per cm² (7,177 aphids) on April 6th, 2021. *A. spiraecola* was the most dominant aphid species in both plots, which is consistent with findings in all citrus orchards in the Mediterranean Basin (Kavallieratos et al., 2002; Albanese et al., 2010; Tena et al., 2011; Labdaoui, 2019). Our results are in line with those found by Labbal et al. (2015), confirming the dominance of *A. spiraecola* in early spring in citrus orchards in eastern Algeria, and with Labdaoui and Guenaoui (2017) in western Algeria. This infestation is generally favored by a significant increase in average temperatures during winter and spring, leading to early growth of new shoots and aphid infestations on citrus (Kindlmann et al., 2010; Qureshi, 2010). Temperature has a major effect on the biology and life cycle of aphids (Satar et al., 2005).

The low infestation level of *A. spiraecola* at the beginning of spring on Orange trees, followed by a significant increase favored by temperature and shoot development, has been reported by several authors (Lebbal et al., 2015; Bouvet et al., 2019). Dartigues (1991) revealed that in spring, the importance of aphid infestations on Orange trees varies depending on physical factors, orchard care, nature of the branches, tree age, and especially the presence

of *Tapinoma simrothi* ants, which have a significant positive influence on aphid growth and survival.

Lebbal (2017) found that Orange trees are more attacked by *A. spiraecola*, in contrast to Marroquín (2004), who observed that lemon trees are the preferred host for citrus aphids compared to sweet Orange trees, grapefruit, and mandarin trees in Spain. Tsai et al. (2001) indicated that the fertility of *A. spiraecola* is higher on grapefruit compared to Orange trees. The host plant determines aphid mortality, development, and fertility (Satar et al., 2008). This difference may be attributed to various morphological or chemical factors. For instance, Da Costa et al. (2011) observed that volatile compounds emitted by plants, before and after infestation, are involved in their defense against aphids. In citrus, monoterpenes are likely to play a role in lemon tree defense against insects (Yamasaki et al., 2007). Monoterpene aldehydes and limonene, which are usually found in high quantities in young Orange leaves compared to Clementina leaves, act as repellents against phytophagous (Flamini et al., 2007; Lebbal, 2017). This could explain the lower infestation recorded on Orange trees compared to Clementina trees in the study orchard.

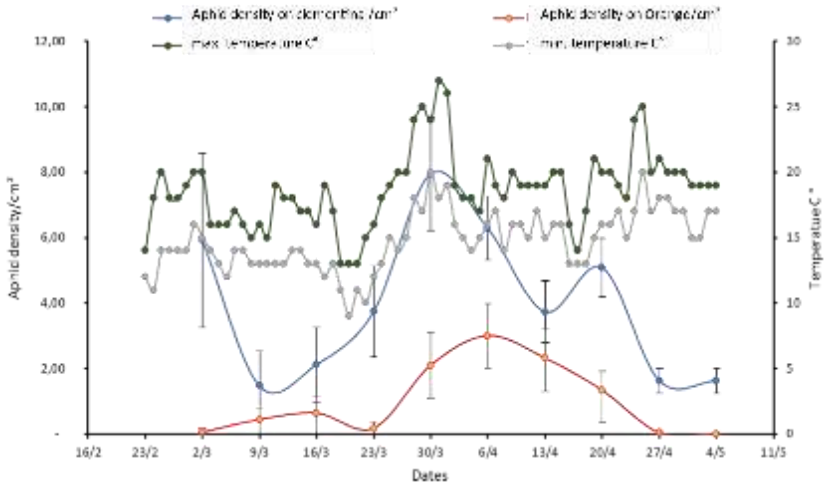

Fig.1: Demographic evolution of *A.spiraecola* in the two orange / Clementina plots

During the sampling period, six predators, three primary parasitoids, and four hyperparasitoid species were collected (Table 1). In the Coccinellidae family, the two most common citrus species in Algeria are *C. algerica* and *S. subvillosus* (Labdaoui and Guenaoui, 2015). *A. aphidimyza* was actively preying on *A. spiraecola* colonies in its larval form. While it is considered the most important predator in citrus orchards, its effectiveness remains limited (Kavallieratos et al., 2004; Bouvet et al., 2021). *E. balteatus* was found in both larval and adult forms within the aphid colonies, with only the larva being predatory. Previous reports have mentioned its presence in citrus orchards in the Mediterranean Basin on citrus aphids (Kavallieratos et al., 2004; Gómez-Marco, 2015; Labdaoui, 2019). *C. carnea* was less frequently found compared to other predators. Moradi et al. (2019) stated that this predator is not considered an effective biological control agent against *A. spiraecola* (Moradi et al., 2019). *C. virgula* was exclusively found on orange trees. As a polyphagous and cosmopolitan predator, it is commonly observed in poorly maintained orchards (Collyer, 1953). Its presence in citrus orchards has been documented in Algeria (Labdaoui, 2019).

The primary parasitoid *L. testaceipes*, the most frequent parasitoid attacking *A. spiraecola*, was found in both plots. A study in Tunisia reported *L. testaceipes* as dominant among primary parasitoids emerging from aphid mummies on citrus (Sellami et al., 2013). *B. angelicae* was found only in the orange plot in low quantities. This species is known as the only primary parasitoid completing its development on *A. spiraecola* in Spain (Gómez-

Marco, 2015), and it has been reported as one of the most frequent primary parasitoids attacking *A. spiraecola* on citrus in Algeria (Labdaoui, 2019). *A. colemani* was identified only in the Clementina plot in very small quantities on *A. spiraecola* (1 specimen). Several *A. spiraecola* mummies remained without emerging of parasitoids, and dissection of these mummies revealed the presence of parasitoid larvae with incomplete development. It is well known that *L. testaceipes* is unable to complete its development on *A. spiraecola* (Tremblay et al., 1983).

The hyperparasitoid *P. aphidis* emerged from *A. spiraecola* mummies in both plots and was the most frequent species. It is widespread on *A. spiraecola* in citrus orchards across several Mediterranean countries (Satar et al., 2014; Gómez-Marco, 2015; Labdaoui, 2019). In the orange plot, several individuals of the genus *Dendrocerus* were found on *A. spiraecola* mummies, which have been reported on citrus aphids in Algeria, Spain, and Turkey (Labdaoui, 2019; Gómez-Marco, 2015; Satar et al., 2014). Additionally, one unidentified species (1 specimen) of the genus *Phaenoglyphis* was found on *A. spiraecola* mummies in the Clementina plot. The genus *Phaenoglyphis*, is the most important genus in the subfamily Charipinae, with only two species reported in Algeria on citrus: *P. villosa* (Kieffer, 1909), and *P. heterocera* (Ferrer-Suay et al., 2017). Species of the genus *Alloxysta* were also found on *A. spiraecola* mummies in the Orange plot, with several species previously reported on citrus aphids in the Mediterranean Basin. In Algeria, new species of this genus have been reported (Labdaoui, 2019 ; Labdaoui et al., 2018).The presence of multiple hyperparasitoid species exploiting the two dominant primary parasitoids significantly reduces their effectiveness as regulating agents, likely contributing to the limited effectiveness of primary parasitoids in citrus orchards (Bañol et al., 2012).

Table 1. Auxiliary fauna associated with the *A. spiraecola* aphid collected in the two orange and Clementina plots

Antagonist	Order	Family	Species	Plot	
				C	O
Predators	Coleoptera	Coccinellidae	*Coccinella algerica* Kovar	x	x
			Scymnus subvillosus Goeze	x	x
	Neuroptera	Chrysopidae	*Chrysoperla carnea* Stephens	x	x
	Diptera	Cecidomyiidae	*Aphidoletes aphidimyza* Rondani	x	x
		Syrphidae	*Episyrphus balteatus* De Geer	x	x
	Hemiptera	Miridae	*Campyloneura virgula* H.-Schaeffer		x
Parasitoids	Hymenoptera	Braconidae	*Lysiphlebus testaceipes* Cresson	x	x
			Binodoxys angelicae Haliday		x
			Aphidius colemani Viereck	x	
Hyper-parasitoids	Hymenoptera	Pteromalidae	*Pachyneuron aphidis* Bouché	x	x
		Megaspilidae	*Dendrocerus* spp		x
		Figitidae	*Phaenoglyphis* spp	x	
			Alloxysta spp		x

C. : Clementina tree / O.: Orange tree

Conclusions

In conclusion, this study investigated the population dynamics of *A. spiraecola* on Clementina and Orange trees in northwestern Algeria. The infestation patterns differed between the two citrus species, with Clementina trees experiencing higher aphid densities compared to Orange trees. While six predator species were observed, their impact on regulating aphid populations was not clearly evident. Similarly, the effectiveness of three primary parasitoid species in controlling *A. spiraecola* infestations was not prominently demonstrated. Moreover, the

presence of at least four species of hyperparasitoids raises concerns about their potential to limit the effectiveness of biological control against *A. spiraecola*. Complementary experiments to understand the complex trophic interactions between aphids, predators, primary parasitoids, and hyperparasitoids in order to design more effective and sustainable pest management strategies are in progress and will be the subject of a subsequent publication.

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FIRST FINDING IN EUROPE OF *COLLETOTRICHUM SCOVILLEI*, A NEW AGENT OF PEPPER ANTHRACNOSE AND ASSESSSMENT OF POTENTIAL BACTERIAL BIOCONTROL AGENTS

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Abstract

Pepper (*Capsicum annuum* L.) is one of the most important vegetables grown in Kosovo. In September 2022, 40% of pepper fruits, in two commercial fields located in the municipalities of Peja and Rahovec (Kosovo), showed typical symptoms of anthracnose. Dark lesions developed on fruits, which later appeared sunken, necrotic and surrounded by brown haloes. After isolation onto PDA, colonies and single spores morphology of both isolates revealed to be consistent with the description of *Colletotrichum* sp. Amplification and phylotyping of *ITS*, *GAPDH* and *TUB2* gene sequences of both isolates were performed and the fungus was identified as *C. scovillei*. To confirm Koch's postulates, a conidial suspension from both isolates was inoculated into healthy pepper fruits and incubated in a humid chamber. Ten days after pathogen inoculation, typical anthracnose symptoms developed, from which the fungus was successfully reisolated. No symptom was ever observed on fruits inoculated with sterile water. To our best knowledge, this is the first report of anthracnose caused by *C. scovillei* in Europe. Given the economic importance of the crop and the significant quality and yield losses observed, measures are urgently needed aiming at pathogen eradication or, alternatively, to ensure an efficient disease control to avoid severe damage both in the field and in postharvest. Four bacterial isolates from the UNIMORE collection were tested *in vitro* against *C. scovillei*: preliminary data showed their effectiveness in reducing mycelium growth. Studies are ongoing to test such antagonists *in planta* to search for sustainable alternatives to the extensive use of fungicides.

Keywords: *Capsicum annuum, Anthracnose, Colletotrichum scovillei, First report, Kosovo.*

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop belonging to the genus *Capsicum*. Its consumption and its global surface area are estimated to be about 40 million tons and 3.8 million hectares, respectively (FAOSTAT 2018). Pepper is also one of the major agricultural crops cultivated in Balkan countries, especially in Kosovo (Aliu *et al.*, 2017), whose production has the largest area after potatoes, with more than 3,108 hectares (MAFRD, 2020).

Pepper is affected by common and emerging or newly introduced pathogens (Mijatovic *et al.*, 2005), including fungi (*e.g., Colletotrichum* sp., *Fusarium* sp., *Phytophthora capsici, Rhizoctonia solani, Verticillium dahliae*), viruses (*e.g.,* pepper mottle mosaic virus, potato virus Y, tobacco mosaic virus), bacteria (*e.g., Clavibacter michiganensis* subsp. *capsici, Xanthomonas* sp.) (Rodeva *et al.,* 2011) and insects (*e.g.,* aphids, *Lepidoptera* and thrips) (EPPO, 2022; Musa *et al.,* 2017; Yankova *et al.,* 2021), which represent a limiting factor for cultivation. Among fungal diseases, anthracnose fruit rot caused by various *Colletotrichum* sp. is a serious threat for pepper, resulting in extensive pre- and post – harvest decay of fruits.

Colletotrichum pathogens affecting *Capsicum* plants come from diverse species complexes with at least 28 different species implicated (De Silva *et al.*, 2021).
In September 2022, monitoring surveys in two commercial pepper fields located in western Kosovo, revealed the presence of symptomatic pepper fruits (*Capsicum annuum* cv. Somborka) with sunken and necrotic lesions. In both affected fields, disease incidence was approximately 40% and yield losses were estimated to be above 30%.
In this study the morphological and molecular identification of the causal agent causing softening and rotting of pepper fruits were performed, and Koch's postulates were confirmed. Moreover, to search for sustainable alternatives to the extensive use of fungicides as possible disease control strategies, four *Streptomyces* sp. isolates from the UNIMORE collection were tested *in vitro* to evaluate their effectiveness in reducing mycelial growth.

Material and Methods

During field surveys in two commercial fields located in Peja and Rahovec (western Kosovo), diseased pepper fruits were detected. The lesions developed on fruits appeared dark, later sunken, necrotic lesions and surrounded by brown haloes. Brown to gray conidial masses were also observed in association with lesions. Symptomatic fruits were collected, stored in a portable cooler and transported to the Laboratory of Plant Protection, Kosovo Institute of Agriculture (Pejë, Kosovo) for analysis. Pieces of diseased tissue cut from the margins of lesions were surface disinfected with 1% NaOCl for two minutes, rinsed in sterile distilled water, transferred onto potato dextrose agar (PDA) and incubated for five days at 27±1°C in the dark. For morphological identification, colony and single spore's observation was carried out under the microscope Olympus CX 43 Microscope (Tokyo, Japan) with 40-fold magnification . DNA was extracted from the two isolates obtained, DLS 1918-A and DLS1918-R, using the CTAB method, as described by Doyle and Doyle (1987), with modifications. For molecular identification of the fungus, three housekeeping genes were used, the internal transcribed spacer (*ITS*) region, the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the beta-tubulin (*TUB2*) genes. For both isolates, the amplification was carried out as described in White et al. (1990) with the primer pair P-ITS1/P-ITS4 and Dubrulle et al. (2020) for the primer pairs GDF1/GDR1 and BT2Fd/BT4R. The PCR products were then sequenced by Biofab Research s.r.l. (Rome, Italy) and deposited in GenBank. The pathogenicity of both isolates was tested on pepper fruits cv. Somborka. Four healthy fruits for each isolate were surface disinfected with 70% ethanol, rinsed twice with sterile-distilled water and dried. Each healthy fruit was then inoculated with 10 µl of a conidial suspension calibrated at the concentration 1×10^6 conidia/ml. Pepper fruits inoculated with sterile distilled water were used as a control. After inoculation, the fruits were kept in a humid chamber for ten days at 27±1°C and 90% relative humidity in the dark. The causal agent was re-isolated from inoculated symptomatic fruits and identified using PCR and sequencing of amplicons for the ITS gene. The assay was repeated twice.
The antimicrobial activity of 4 bacterial endophytes belonging to the UNIMORE collection was tested *in vitro* against the phytopathogenic fungus *C. scovillei*, both in dual culture and double plate assays. The selected endophytic strains DLS 1, DLS 2, DLS 3 and DLS 4 were isolated from tomato stems and identified as *Streptomyces* spp. (Ferrari, 2017). These antagonistic bacteria were grown on ISP-2 medium (yeast extract, 4 g; malt extract, 10 g; glucose, 4 g; agar, 20 g; H2O, 1000 ml, pH 7.2) at 27°C for 7 days. For the dual culture assay, a 20 µl droplet for each *Streptomyces* sp. (1×10^6 CFU/ml) was spotted at two opposite sides (approximately 1 cm from the edge) of the Petri dishes (diameter 90 mm) and then incubated at 27°C. The 5 mm fungal pathogen plug taken from the edge of an actively growing culture was placed 24 hours after the antagonist spot at the center of the Petri dishes. For negative

control, only a 5 mm plug of pathogen mycelium was plated onto PDA. Petri dishes were incubated at 27 ± 2°C for 7 days.

The effect of volatile organic compounds (VOCs) emitted by the four *Streptomyces* spp. was evaluated through double plate assay. Sterilized ISP2 and PDA media were plated into one half of each double plate, respectively. Bacterial strains used to inoculate Petri dishes in experiments were grown on ISP2 medium at 27 °C for 7 days. The day after, 40 µl of bacteria suspension (1×10^6 CFU/ml) were spread onto the Petri dish containing ISP2 medium, with a sterile plastic loop. As a negative control, double dishes containing non-inoculated ISP2 medium on the *Streptomyces* plate were used. Thereafter, a 5 mm pathogen plug taken from the edge of a young growing culture, was placed on the center of the Petri dish containing PDA medium. Subsequently, Petri dishes with *Streptomyces* sp. and *C. scovillei* were sealed with Parafilm (Bemis, Neenah, United States) to avoid VOCs escape and incubated for 10 days at 27°C. The test was replicated three times. For both the antagonistic assays, the mycelial growth inhibition (MGI) was determined according to the formula of Pandey et al. (1982), with modifications, using mycelium radius:

Inhibition of growth (%) = (RC-RT)/RC*100, where RC is the average radius in control fungal colony and RT the average radius of fungal colony in treatment.

The results were subject to ANOVA and Kruskal-Wallis tests at $P \leq 0.05$ using SPSS 15.0 for Windows® (SPSS Inc., Chicago, IL).

Results and Discussion

The morphology of both colonies and their spores revealed similarity with the characterization of *Colletotrichum scovillei* described by Damm et al. (2012). Moreover, the sequences for both isolates were identical to each other and to *C. scovillei* strain CBS 126529 (Damm *et al.,* 2012). Sequences were deposited in the GenBank under Accession Nos. OQ818873 and OQ819176 (ITS), OQ859040 and OQ874731 (GAPDH), and OQ859039 and OQ874732 (TUB2) for DLS 1918-A and DLS1918-R isolates, respectively. Regarding Koch's postulates, ten days after inoculation, pepper fruits showed typical anthracnose symptoms, similar to those observed in the two fields. No symptoms developed on pepper fruits treated with sterile distilled water. The amplification and sequencing of the re-isolated fungi from inoculated symptomatic fruits confirmed 100% identity to accessions OQ818873 and OQ819176 mentioned above.

C. scovillei has been described as the causal agent of anthracnose on pepper in Brazil and eastern Asia (Caires *et al.,* 2014; De Silva *et al.,* 2019), and more recently it has been reported in South Carolina (Toporek *et al.,* 2021). Since *C. scovillei* is considered a challenging pathogen that can result in significant yield and quality losses, and pepper is the main vegetable crop in Kosovo, our results aiming at pathogen eradication or, alternatively, to ensure an efficient disease control in the outbreak area and avoid its possible spread into new areas in Europe. The antagonistic activities of four *Streptomyces* sp. to inhibit the mycelial growth of the plant pathogen was evaluated in this study. In Figure 1, the mycelial growth inhibition of the fungus in contact with the four *Streptomyces* sp. has been presented, compared to non-treated control. The dual plate assay highlighted the reduction of *C. scovillei* mycelial growth of about 26%, 47%, 58% and 68%, for DLS1, DLS2, DLS3 and DLS4, respectively (Figure 1). The inhibition activity of DLS4 was significantly higher in comparison to DLS1, DLS2 and DLS3 ($p < 0,05$), as shown in Figure 1E.

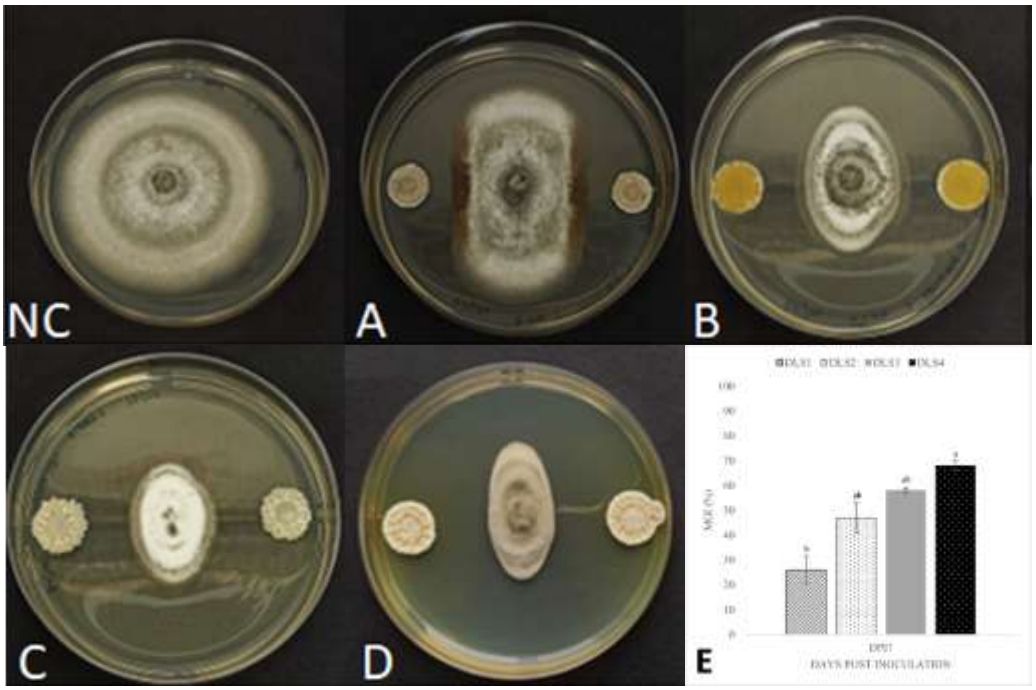

Fig. 1. Dual culture assay against *C. scovillei*: mycelial growth inhibition 7 days after inoculation. Non-treated *C. scovillei* (NC), DLS 1 (A), DLS 2 (B), DLS 3 (C) and DLS 4 (D). Graphic representing mycelial growth inhibition (%). Different letters within columns indicate significant differences according to the Kruskal-Wallis tests ($p < 0.05$) (E).

Regarding the double plate assay, Figure 2 presents the *C. scovillei* mycelial growth and the MGI (%) values in comparison to the non-treated control. The dual plate assay highlighted the reduction of *C. scovillei* mycelial growth of about 9%, 24%, 26% and 33%, for DLS1, DLS2, DLS3 and DLS4, respectively (Figure 2). The inhibition activity of DLS4 was significantly higher in comparison to DLS1, DLS2 and DLS3 ($p < 0,05$), as shown in Figure 2E.

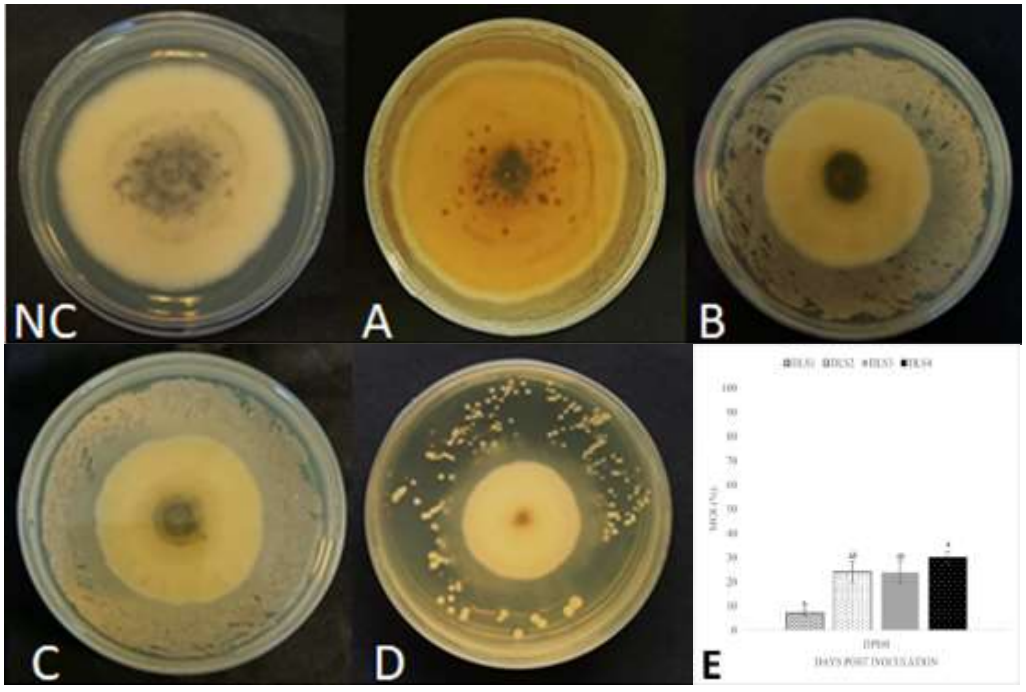

Fig. 2. Double Petri dish assay against *C. scovillei*: mycelial growth inhibition 10 days after inoculation. Non-treated *C. scovillei* (NC), DLS 1 (A), DLS 2 (B), DLS 3 (C) and DLS 4 (D). Graphic representing mycelial growth inhibition (%). Different letters within columns indicate significant differences according to the Kruskal-Wallis tests ($p < 0.05$) (E).

These results confirmed the previous studies carried out *in vitro* and *in planta* showing the antifungal activity of *Streptomyces* species against phytopathogenic fungi. Interestingly, Jinhua et al. (2010) carried out experiments exploiting an antifungal substance, the Azalomycin F complex, sprayed on soil, produced by *Streptomyces malaysiensis* to control the prevalent disease fungal agents, as *Fusarium chlamydosporum*, *Cladosporium cladosporioides*, *Rhizoctonia solani* and for *C. gloeosporioides*, reducing fungal population. It is also known the ability of *Streptomyces* to emit volatile organic compounds and antibiotics able to inhibit mycelial growth, spore germination and appressorium formation and to cause several morphological abnormalities of fungi (Palaniyandi *et al.*, 2013).

Conclusions

To our knowledge, this is the first detection of *C. scovillei* on pepper in Kosovo and Europe. Since anthracnose of pepper is considered responsible for severe economic damage due to yield and quality losses of pepper production, it is necessary to develop strategies able to ensure an efficient disease control in the outbreak area and the limitation of its spread into new areas in Europe.

In recent years, the authorities of many countries have restricted the use of pesticides due to agrochemical resistant pathogens, ecosystem pollution and accumulation in the human body of undesirable chemical residues (Mahmood *et al*., 2016), therefore alternative environment-friendly strategies for controlling pepper diseases within an integrated crop protection system are now essential. The findings obtained in this study revealed the potential use of these microorganisms, in particular of the most performing isolate DLS 4 as biocontrol agents against *C. scovillei*, as an alternative to fungicide input. Further studies are currently ongoing to confirm the antagonistic activities *in planta* of these *Streptomyces* sp. against *C. scovillei.*

In addition, the GC-MS analysis of the identification of the VOCs produced by the most active *Streptomyces* sp. DLS4 in inhibiting mycelial growth is also ongoing**.**

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FUMONISIN LEVELS IN CORN AND CORN PRODUCTS IN SERBIA IN 2022

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Abstract

Fumonisins are cancerogenic, neurotoxic, hepatotoxic and pneumotoxic metabolites of *Fusarium moniliforme*, mold fungus which grows host-specific on corn. The incidence of fumonisin contamination in corn and corn product samples collected from Serbian producers and market was investigated by using the competitive enzyme linked immunosorbent assay (ELISA) technique. In this study, a total of 60 samples of corn, corn flour, corn based snack products and other corn products were examined in 2022. FUMO was quantified in all 6 samples of corn, at levels ranging from 115 to 3550 μg/kg, all 9 samples of corn flour at levels ranging from 305 to 1970 μg/kg and in 18 samples (55 %) of corn based snack products, at levels ranging from 73 to 520 μg/kg. FUMO was not detected in 12 samples of freshly frozen sweet corn. In Republic of Serbia, maximum limit of total FUMO in corn is 4000 μg/kg and 2000 μg/kg in corn flour. Maximum limit of total FUMO in corn based snack products is 800 μg/kg. These results suggest that number of corn, corn flour and corn based snack products contaminated with FUMO is very high. Nevertheless, none of the tested samples exceeded maximum allowed limits. The occurrence of FUMO in corn and corn based products is one of the most serious problems, especially for vulnerable groups such as children and population allergic to gluten. Therefore, continuous monitoring over these products is necessary.

Keywords: *Fumonisins, Corn, ELISA.*

Introduction

In 1988, W. C. A. Gelderblom et al. reported that the fumonisins, a new class of mycotoxins, had been identified from cultures of Fusarium moniliforme and that these toxins had cancer promoting activity. This report represented a major breakthrough in nearly a century of investigation into the animal and human diseases associated with consumption of maize contaminated with Fusarium moniliforme (Munkvold et al., 1997).

Fumonisins are secondary metabolites produced in cereals by pathogenic fungi Fusarium moniliforme, Fusarium proliferatum, Fusarium verticillioides and related species (Kamle et al., 2019). Moreover, Aspergilus nigri also produces fumonisins in the crop plants of peanut, maize and grape (Mogensen et al., 2009). The maize and maize-based products are most commonly infected with fumonisins besides their presence in several other grains (rice, wheat, barley, maize, rye, oat, and millet) and grain products (tortillas, corn flask, chips) (Dall´Asta et al., 2016 Cendoya et al., 2018).

FBs are polar molecules and are usually extracted with mixtures of polar solvents, such as methanol, acetonitrile, and water in different combinations and proportions (Scudamore et al., 1997; Cortez-Rocha et al., 2003).

Several fumonisin molecules have been isolated and characterized; fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3) are the major ones produced in naturally contaminated foods (Mahnine et al., 2012).

FB1 and FB2 are diesters of tricarballylic acid and polyhydric alcohols and as they have similar structure to sphingosine, they can interfere with sphingosine metabolism, blocking the biosynthesis of complex sphingolipids and ceramides (Silva et al., 2011).
FB1 is the most frequently detected, which toxicity has been reported showing different toxicological effects in humans and animals (Galbenu et al., 2011). Fumonisins are cancerogenic, neurotoxic, hepatotoxic and pneumotoxic and FB1 was classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 2002). FB1 is known to cause equine leukoencephalomalacia (ELEM) and porcine pulmunary edema (PPE). It is also a kidney and liver carcinogen in rats and mice, respectively, causes DNA damage in human fibroblasts, and is immunotoxic (Kim et al., 2003).
The objective of this study was examination and determination of the presence of FUMO in samples of corn, corn flour, corn based snacks and other corn products.

Material and methods

Collection of samples

In this study, a total of 60 samples of corn, corn flour, corn based snack products and other corn products were examined in 2022. Before analysis, the samples were stored at cool place and protected from light.

Sample preparation

All collected samples were prepared and analyzed in accredited laboratory for food and feed safety "Jugoinspekt Beograd" in Serbia. Samples of corn, corn flour, corn based snack products and other corn products were prepared according manufacturer's instructions. Prior to analysis, 5g of each sample was pipetted into a 50ml PP-tube and mixed with 25ml of 70% methanol. This mixture was shaked for 5 minutes on shaker. After shaking, the extract was filtered through Whatman No. 1 filter. The filtered sample was diluted 1:14 with destilated water. According to manual instructions, 50 of diluted filtered sample was used in test.

Fumonisins analysis and instrumental conditions

Determinaton and quantitive analysis of ochratoxin A was done by Enzyme Linked ImmunoSorbent Assay (ELISA) method, using RIDASCREEN® Fumonisin (R-Biopharm) test kit. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s.
Analysis were performed according to manufacturer's instructions (RIDASCREEN Fumonisins Art.No.: R5602). Limit of detection (LoD) was set on 0,222 mg/kg while limit of quantification. Recovery rate was ranging from 94 to 116 %.
The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the fumonisn concentration in the sample of corn, corn flour and corn snack products.

Statistical analysis

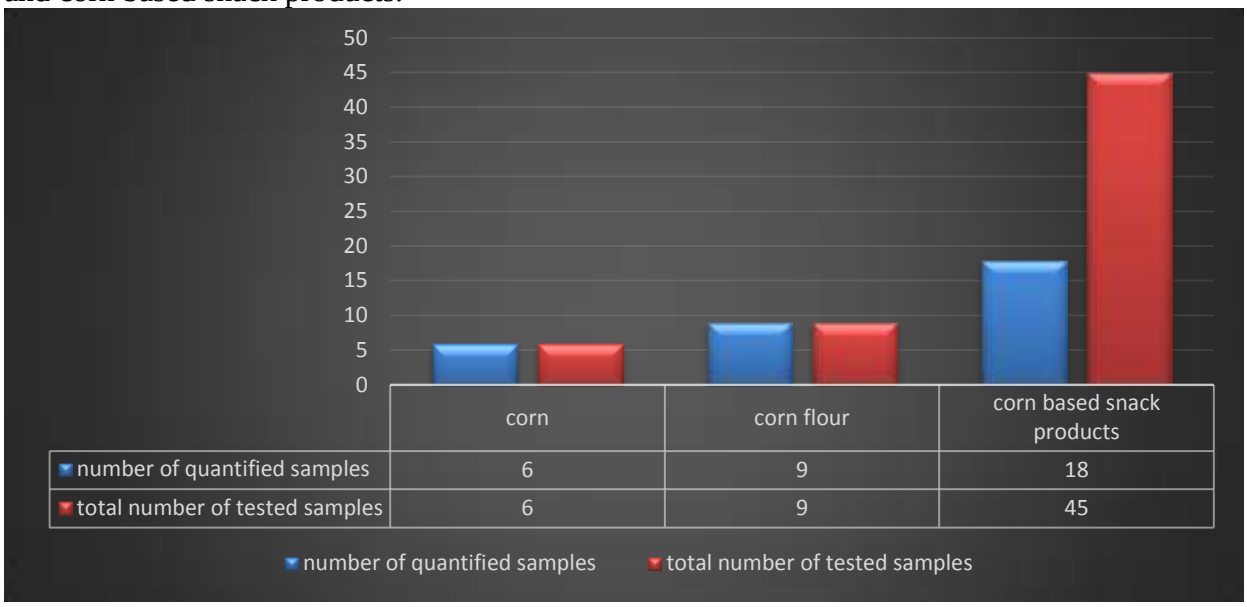
All obtained data were analyzed using SPSS 15.0 software (SPSS, IBM corporation, USA).

Results and Discussion

Fumonisins have been detected in 33 out of 60 (55%) samples of corn, corn flour and corn based snacks at levels ranging from 150 μg/kg to 1970 μg/kg. Maximum fumonisins level was 1970 μg/kg. In 45% of samples this contaminent has not been detected. FUMO was quantified in all 6 samples of corn, at levels ranging from 115 to 3550 μg/kg, all 9 samples of corn flour

at levels ranging from 305 to 1970 µg/kg and in 18 samples (55 %) of corn based snack products, at levels ranging from 73 to 520 µg/kg (Chart 1). FUMO was not detected in 12 samples of freshly frozen sweet corn. In Republic of Serbia, maximum limit of total FUMO in corn is 4000 µg/kg and 2000 µg/kg in corn flour. Maximum limit of total FUMO in corn based snack products is 800 µg/kg. These results suggest that number of corn, corn flour and corn based snack products contaminated with FUMO is very high.

Chart 1. Number of quantified samples and total number of tested samples of corn, corn flour and corn based snack products.



Comparing the results obtained in this study on the occurrence of FB1 in corn and corn based snacks the overall contamination with FB1 was much lower in study of Galbenu et al. (2011) (27.59 %). The overall contamination is higher in this study (55%).
In 2010, in Romania, Stroia et al. (2010) published a study on the incidence of *Fusarium* spp. and its mycotoxins in cereals from western Romania. Fumonisins were identified in 44 % of the maize samples with values ranging between 7.3 and 76.6 µg/kg, which is less than it was obtained in this study (100%, 150 to 3550 µg/kg).
Mahnine et al. (2012) detected fumonisins in 55.5% of corn based snack samples which is similar to results obtained in this study (40%). However, concentrations in that study were ranging from 6.2 to 152.4 µg/kg which is less than in this study (73 to 520 µg/kg).
Contaminaion of corn and corn flour was 100% of tested samples, comparing to corn based snack products (40%). One reason for the low levels found in cornflakes and other snack based cereals is that the grits have the lowest concentration of fumonisins of any other fraction produced in commercial dry-milling of maize (Kim et al., 2003). Another important reason is that free fumonisins are substantially lost during extrusion cooking of maize grits, extrusion cooking of maize flour, flaking and roasting of maize grits and the overall processing of maize flour (Kim et al., 2003; Castells et al., 2005; Castells et al., 2008). Addition of glucose results in greater reduction of FB1 on extrusion of maize grits, and during heat-processing fumonisins are also reduced because of protein binding (Kim et al., 2003).

Conclusion

Fumonisins have been detected in majority of the analysed samples. This leads to a conclusion that presence of funghi and its metabolites is natural and hardly can be avoided.

Also, these results suggest that number of corn, corn flour and corn based snack products contaminated with FUMO is very high. However, none of the tested samples exceeded maximum allowed limits. The occurrence of FUMO in corn and corn based products is one of the most serious problems, especially for vulnerable groups such as children and population allergic to gluten. Therefore, continuous monitoring over these products is necessary.

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BASIC CHARACTERISTICS, APPLICATION AND SAFETY OF STEVIA REBAUDIANA BERTONI – A LOW–CALORIE NATURAL SWEETENER IN FOOD

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Abstract

Stevia rebaudiana Bertoni, which produces diterpene glycosides belonging to the group of low-calorie sweeteners, is about 300 times sweeter than sucrose. Today, stevia is used to reduce the energy content and added sugar in foods and beverages. The basic criteria that are necessary in the application are: good taste, safety, solubility, stability and acceptable price. Stevia rebaudiana has great potential as a new agro-cultural crop because the demand of customers for plant food is growing and direct analysis showed that stevia contains folic acid (vitamin B9), vitamin C (ascorbic acid) and all essential amino acids with the exception of tryptophan. The benefits of *Stevia rebaudiana* leaves are mainly related to their nutritional composition, which is primarily a good source of carbohydrates, proteins and raw fibers, which promotes health and reduces the risk of some diseases. Numerous studies have shown that in addition to sweetness, stevioside, together with related compounds that include rebaudioside A, steviol and isosteviol, also offer a therapeutic effect as they have antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, diuretic and immunomodulatory effects. The possibilities of using stevia are great, above all in the production of dairy products, bakery, confectionery, as well as in fruit products (juices, jams, marmalades). Stevia has been shown to be safe in more than 200 studies, and JECFA has established an ADI of 4 mg/kg body weight per day, expressed as steviol equivalents, to assure consumers of this safety.

Key words: *Stevia rebaudiana Bertoni, safety, application, health benefits.*

Introduction

In order to reduce the energy value of certain food products, low-energy sweeteners are increasingly being used, and interest in natural low-energy sweeteners, such as stevia, is growing in particular. Stevia rebaudiana Bertoni is a herbaceous and shrubby plant from the Asteraceae family, which grows in the northeastern regions of South America. In its leaves, stems and flowers, it has a number of diterpene glycosides, a group of sweeteners with a common core called steviol. Stevioside and Rebaudioside-A (Reb A) are the most important glycosides, because their sweetening properties are approximately 300 times sweeter than sucrose. It was first consumed in South America more than 200 years ago, when the natives used the leaves of the plant to sweeten drinks or chewed them for their sweet taste. Stevia has always been used in Paraguay and Brazil to treat diabetes. It was first commercially accepted as a sweetener in Japan in the 1970s. (Ashwell, 2015).

Only high-purity stevia extracts containing 95% or more steviol glycosides are approved for use in food and beverages by the Joint Food and Agriculture Organization/WHO, the World Health Organization (WHO), the Expert Committee on Food Additives and the Codex Alimentarius. High purity Rebaudioside M (minimum purity 95%) was granted GRAS status

by the FDA in 2014. High purity stevia leaf extract is often referred to as stevia, steviol glycosides, stevia extracts, purified stevia leaf extract, high purity stevia, or rebiana. The sweet taste comes from steviol glycosides, especially stevioside and rebaudioside A, along with rebaudioside C and dulcoside A (Kasti et al., 2022).

The European Food Safety Authority (EFSA) (2010) assessed the safety of steviol glycosides and established ADIs for their safe use. The Commission for Food Additives (JECFA) has set a daily limit of 4 mg/kg body weight/day. In China, Japan and Korea, sweeteners derived from stevia are allowed as a food additive. The Food Safety and Standards Authority of India (FSSAI) has approved the use of the natural sweetener stevia in selected products, from the groups of milk-based beverages, soft drinks and desserts (EFSA, 2010; EFSA, 2015, Perrier et al., 2018).

Research has mainly been done on stevioside and Reb A, molecules that are converted into their aglycon steviol in the large intestine. Steviol glycosides are not subject to changes in the upper gastrointestinal tract, because they cannot be broken down by enzymes such as pancreatic α-amylase, pepsin and pancreatin found in saliva and gastric secretions, and only intestinal bacteria in the colon hydrolyze steviol glycosides into steviol by breaking off their unit of glucose. Steviol is absorbed via the portal vein and is primarily metabolized in the liver, forming steviol glucuronide, which is excreted in the urine. Since there is no accumulation of stevia (or any component or by-product of stevia) in the body, it passes through the body during metabolism, or rather it does not metabolize, so it does not contain calories (Nikiforov et al., 2013; EFSA, 2010).

Various studies have shown the health benefits of stevia against diabetes, obesity, hypertension, cancer, dental caries, oxidative stress and microbial infection (Ahmad et al., 2020; Zaidan et al., 2019). So there is a lot of interest in this sweetener. Not only is it a natural sweetener, which can help control calorie intake, but it is also a good source of carbohydrates, fiber and essential amino acids, as well as bioactive compounds such as polyphenols. The main components of polyphenols from the leaves of Stevia rebaudiana Bertoni are chlorogenic acids, a polyphenol family of esters, including hydroxycinnamic acids with quinic acid, which has excellent hydrophilic antioxidant activity and other therapeutic properties, such as the ability to prevent some allergic reaction factors that participate in inflammatory processes in the body (Liang et al., 2016; Lemus-Mondaca et al., 2012; Wölwer-Rieck, 2012).

According to the 2017 Catalog of Novel Foods of the European Commission, Stevia rebaudiana Bertoni is recognized as a traditional food when prepared with or contained in fruit, tea or herbal infusions (European Commission Novel Food, 2017).

Most often, stevia is added to products such as fruit preserves, fruit juices, energy drinks, dairy products such as ice cream, and confectionery and bakery products. In this paper, the application of stevia in the production of functional fruit products and bakery and confectionery products will be presented, and the known advantages, as well as the possible negative sides of using stevia, will be presented.

Use of stevia in the formulation of various functional fruit products

Questions such as the intensity and persistence of sweetness in the product itself, in which stevia is added, the absence of other residual influences, are constantly being asked, all with the aim of consumer acceptance. The reasons for the increasing research on the addition of stevia as a natural sweetener are also found in the confirmation of stevia's antioxidant capacity, so in the research of Bender et al. (2018) determined the effect of adding stevia on the antioxidant activity of processed raspberry juices, i.e. the possibility of producing high-quality raspberry juices sweetened with dry ground stevia leaves or their raw extract. The

addition of 1% w/v stevia increased the total phenolic content of raspberry juices between 27.8% and 48.3%. The highest content was found in raspberry juice sweetened with raw aqueous stevia extract immediately before pasteurization and was 2325 mg GAE/L ± 54. The phenol content was higher when supplemented with raw aqueous stevia extract than with stevia powder.

Positive effects were also obtained with chokeberry juice, which, due to its bitterness, requires the addition of sweetener. In comparing aronia juice sweetened with sucrose and sweetened with green stevia powder, a higher content of bioactive compounds, vitamin C (increased up to 2X), phenol content (increased up to 6%) and an increased content of antioxidant capacity by 3%, was found in the juice from aronia with the addition of stevia (Šic Žlabur et al., 2018).

Criado et al. (2014) investigated the effect of adding different concentrations of Stevia rebaudiana Bertoni to fruit mixtures incubated for 10 days at 10 and 37 °C, where there was a significant decrease in the activity of polyphenoloxidase and peroxidase enzymes, and the antioxidant capacity increased as the enzyme activity decreased. so enzyme activity can be used as an indirect index of antioxidant capacity, and stevia has significant potential for use not only as a sweetener, but also as a natural preservative.

In support of the use of stevia as a preservative, there is research by Barba et al (2014) in which, in order to preserve and avoid the use of intensive heat treatments and the use of chemical additives, in a mixture of fruit pulp (orange, mango and papaya), in addition to processing under high pressure (deactivation of enzymes, such as polyphenoloxidase and peroxidase, as well as spoilage and/or pathogenic microorganisms), as a supplement to this method, added stevia, as a natural preservative. The results show that the combination of high pressure treatment (453 MPa treatment for 5 minutes) and addition of stevia (2.5% (w/v)) is a good procedure to obtain safe fruit drinks with improved nutritional and antioxidant properties. The inactivation of min. 5 log cycles of L. monocytogenes, and the phenol content and antioxidant activity increased with minimal activity of polyphenol oxidase and peroxidase. It is also very important that this treatment had a minimal effect on the nutritional and physical chemical properties of these beverages.

One of the primary goals in the industry of jams and jellies is to reduce the sugar content in, because these are products that a large part of the population consumes on a daily basis. The total soluble solid content of the finished jam should be 60% - 65% or more, and the product should contain at least 45% fruit, but the term "jam" is also used in the literature to denote fruit preserves with a lower sugar content (low-calorie, dietary, functional) (Official Gazette of RS, 101/2015). Sugar, in addition to sweetness, also affects the increase of total soluble solid matter, affects the gelation process, which affects the color, texture and stability of jam and jelly. For example, if it is a complete replacement of sugar, the sensory characteristics such as the appearance and texture of low-calorie jam deteriorate, and these are problems facing the food industry, which need to be solved. One way is to partially replace sugar, add pectin and acidity regulator, i.e. agar-agar. It has been established that agar-agar can be used as a gelling ingredient in low-calorie jams, presenting a firmness similar to pectin (Schiato-Siso et al., 2023).

By using pectin and stevia sweetener, the amount of sucrose in the jam can be reduced and a low-calorie product with optimal quality can be produced. This was confirmed by Nourmohammadi et al. (2021), evaluated the physico-chemical, sensory and rheological properties of stevia-based cherry jam. The samples were in five levels of sucrose (50-10%), pectin (0.5-1%) and stevia (0.6-0.2%) in a constant amount of 300 g of fruit. The concentrations of sucrose, pectin and stevia had a significant influence on the color, sensory and rheological properties of the jam, because the brightness and yellowness increased with the increase in the concentration of sucrose and stevia, and the increase in pectin also increased the color (redness) of the jam. The results showed that the viscosity is a good

indicator of the effect of sucrose concentration in the jam. The optimum point of the dietary jam formulation was obtained in the amount of sugar 40%, pectin 0.4% and stevia 0.3%.
In the research of Belović et al (2017), four formulations of tomato pomace jam were developed (jam 1 - contains sucrose and no added pectin. formulations 2 and 3 - sucrose is partially (50%) replaced by stevioside, and in formulation 4 - sucrose is completely replaced fructose and stevioside). Jam formulations 1 and 2, prepared without the addition of pectin, were thermally stable in the temperature range of 25-90 °C, and can be used as fruit fillings. Jam formulations 3 and 4 were more lubricating because the tomato pomace particles are embedded in a pectin network that acts as a lubricant.
Research by de Carvalho et al (2013) presents a formulation of dietary strawberry jam with the addition of cranberry juice (for the purpose of color) and sweetened with rebaudioside A, whose physicochemical, microbiological and sensory properties were tested and a comparison was made with control samples, which were sweetened the same amount of sucralose. The physico-chemical properties of the samples that were compared did not differ, with the fact that the jam sweetened with 100% rebaudioside-A was more sensory acceptable (taste). That stronger (1.33x) sweetness of sucralose products is the likely reason, as some panelists pointed out that the sour taste of the jams determined their preference. Not a single product showed microbiological contamination after 30 days.
A tendency towards certain sensory characteristics as determinants of the intake of certain products was also shown by the research of Andersen et al (2017), which was conducted on 66 respondents, using apple-cherry fruit drinks with different levels of beta-glucan and different sweeteners, sucrose or Stevia rebaudiana. Replacing sucrose with Stevia rebaudiana did not affect hedonic and post-ingestion sensations, but the addition of beta glucan had a different result. The sensory question, not only how you like the product, but also how you feel after consuming the given product, is very important. Satisfaction as a measure of product evaluation implies knowledge about the value of food, its usefulness and appropriateness for the situation and is a good indicator of product quality. but in this trial, despite such knowledge, the addition of beta glucan was confirmed to have compromised satisfaction.
In order to obtain new food products with increased nutritional properties, mixtures of aqueous extracts of Stevia rebaudiana and fruit juice are produced, whereby processing under high pressure enables obtaining drinks that retain their characteristics similar to the fresh product.

Steviol glycosides can be used as food additives, and like all food additives, they should be stable in the food matrix as well as during each step of processing and storage under given conditions. Their lack of stability could lead to a reduction in sweet taste and thus to a lack of consumer satisfaction. Some processes can also lead to the degradation of steviol glycosides, resulting in products with an unpleasant taste or even toxic properties. Research on the application of this technology was carried out in mixtures of fruit juice (papaya (32.5%, v/v), mango (10%, v/v) and orange (7.5%, v/v) sweetened with Stevia rebaudiana Bertoni in different % (0–2.5). The physicochemical properties (color, browning index, turbidity index), bioactive compounds (ascorbic acid, total phenolic compounds, total anthocyanins, total carotenoids) and antioxidant capacity of the mixture of the mentioned fruit juices were tested, and the results showed that the best preserved physical- chemical and nutritional qualities in the beverage after the effect of high pressure at 300 MPa during 14 minutes of effect. The highest prevalence of antioxidant compounds, as well as the smallest difference in color, was with this beverage treatment Carbonell-Capella et al., 2013).
In accordance with the guidelines of the World Health Organization from 2015, which recommend limiting the consumption of free sugar in the diet to less than 10% of total energy needs and to less than 5% for additional health benefits, more and more research is being done

on other fruit products, and not only juices, jams, in which the amount of sugar is reduced (WHO Guideline: Sugars Intake for Adults and Children, 2015). One such study was conducted by Pielak et al (2020), in which they evaluated the sensory profile and reaction of consumers, as well as the physicochemical properties of apple preserves with a low sugar content (with or without a gelling agent or acidity regulator), in which is sugar replaced by different amounts of steviol glycosides. Sugar substitutes with steviol glycoside of 10% (0–0.05 g/100 g) in apple preserves without food additives and sugar substitutes up to 40% (0.20 g/100 g) in preserves, with the use of pectin and lemon acid, has shown good consumer preference. Higher percentages of sugar replacement led to deterioration of taste and smell (metallic taste and smell, bitter taste, pungent and pungent smell). The use of food additives (pectin, citric acid) in canned apples made it possible to increase the percentage of replacing sugar with steviol glycoside.

Application of stevia in functional bakery and confectionery products

Products such as cakes and biscuits contribute significantly to global sugar consumption. In theory, complete removal of sucrose from such products is impossible without affecting the quality of the final product, because the sugar content affects the dough structure, dimensions, color, hygroscopicity and surface appearance of the finished product. The crispiness I want in biscuits is obtained thanks to the crystallization of sucrose, due to the low water content in this kind of product. Sucrose is hygroscopic and competes with gluten for water, it can delay the production of the gluten network, which leads to a soft texture and retardation of starch gelatinization, providing the baked product with a more porous structure and better volume expansion. In addition, it participates in the formation of color and smell, through the Maillard reaction we get a nice brown skin color and a pronounced aroma of this product (Richardson et al., 2003; Woodbury et al., 2023).

The consumption of sugar is associated with potential health risks, and today the market is looking for low-calorie products, and the bakery and confectionery industry is also looking for solutions and optimal amounts of sugar and/or suitable sugar alternatives that will ensure that a quality product is obtained that is characterized by all sensory properties, such as and the possibility of adequate storage. Considering that stevia is a sweetener of natural plant origin, non-toxic, does not undergo chemical changes during the production process, is resistant to heat up to 200 °C, cannot ferment, flavor enhancer, is recommended for diabetics and obese people, it finds its place in production functional biscuits and cakes (Panpatil et al., 2008; Arshad et al., 2022).

A study conducted by Ali et al. (2022) found a good formulation for the production of biscuits, where dried stevia leaf powder and fresh stevia leaf juice were used as a substitute for sugar. Biscuits were made from 18 g of stevia powder and 55 ml of stevia juice per kg of flour and showed the best results in terms of sensory and microbiological evaluation. After a month of storage in a glass, plastic jar and tin can, they remain safe for human consumption

In addition to replacing sugar, researchers are focused on the development of new functional food products that, in addition to being low in calories, will also have other added value, and thus health benefits. Thus, the possibility of incorporating flour from roasted flax seeds and replacing sugar with Stevia rebaudiana leaf powder in the production of functional biscuits was investigated. A control sample and 6 biscuit formulations were made with the replacement of sucrose with stevia in amounts of 5.5%, 6%, 6.5%, 7%, 7.5% and 8%. The formulation with the incorporation of roasted flax seeds of 8% and stevia with 7%, showed improved functional properties, reduced calories and improved health benefits. The proportion of fiber, calcium, phosphorus, and iron compared to the control sample was higher (Gupta et al., 2017).

Gluten-free bakery products, which have the problem of a high glycemic index of the starch structure, are also increasingly in demand. Rice flour, corn flour and starch are ingredients that are often used in the production of gluten-free cakes, so their high glycemic index is risky for diabetics and obese people to consume. For this reason, alternative ingredients are sought in order to avoid the negative properties of gluten-free cakes, to increase their nutritional quality, and to achieve good sensory properties. In the study by Yildiz and Gocmen (2021), gluten-free cakes were produced by replacing rice flour and sucrose content with almond flour (0, 10, 20, 30%), and sucrose was replaced with stevia ratio of 0, 25 and 50%. By including almond flour and stevia in the formulation of gluten-free cakes, the content of protein, dietary fiber, phenolic compounds was enriched and the proportion of carbohydrates was reduced. Although gluten-free bakery products are generally characterized by weaker sensory properties, due to their harder structure, darker color and dry-sandy feeling that remains in the mouth, these cakes were rated as acceptable. Cookies with a high protein content and low calories prepared by replacing the wheat content with defatted soy flour at levels of 0, 10, 15, 20 and 25% and sugar with stevia leaf powder at levels of 0, 15, 20 also received good sensory acceptable ratings, 25 and 30%, using the traditional cream dough method. There was an increase in the content of protein, crude fiber and ash, and a decrease in the content of fat and carbohydrates, and cookies with 20% defatted soy flour and 20% stevia leaves had the best rated sensory properties. After 90 days of storage at room temperature, the sensory quality was reduced but was acceptable (Kulthe et al., 2014).

Conclusion

Stevia is an excellent source of nutritional elements as well as functional qualities for the purpose of producing food products with added value. Stevia can be used as a whole or partial substitute for sucrose in combination with additives. Many studies have shown that stevia has various health properties, including properties against diabetes, obesity, tumors, hypertension, microbes, caries and antioxidant properties, all of which contribute to the greater industrial application of stevia in various food products. But studies and tests still need to be done in order to get a real insight into the safety of stevia, as well as to find optimal ratios in different food products in order to avoid undesirable sensory properties.

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FIELD DIGITIZATION AND DECISION SUPPORT SYSTEM (DSS) IN PEACH CULTIVATION TO MANAGE THE MEDITERRANEAN FRUITFLY, *CERATITIS CAPITATA*IN TUNISIA

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Abstract

The Mediterranean fruit fly (MedFly), Ceratitis capitata (Wiedermann) (Diptera: Tephritidae), is a highly invasive, polyphagous species that attacks a wide range of fruits. In Tunisia, among the host species, peaches (Prunus persica (L.) are particularly susceptible, especially late-ripening cultivars. The risk of MedFly attack is related to various factors, such as the period of ripening during the season and the physical–chemical characteristics of the fruit. Traditionally, treatments consisted of cover spraying the entire crop area, with important consequences to the environment, health risks to operators and consumers, and the development of resistant pest populations. To minimize such risk, the use of bait sprays and spraying guidance, which are more economically and environmentally sound, are urgently needed. Thus, testing other methods targeting smart-precision plant protection procedures was implemented through the FruitFlynet-ii research project funded by the ENI CBC MED program.Eighteen hectares of peaches were chosen as a study site. A total of 24 conventional delta traps baited by the MedFly female attractants were set up onMay, 24, 2022, maintained till the end of fruit harvesting period (end of September 2022), and inspected weekly. Orchards and trees were digitized using google satellite imagery and the open software Qgis. To set up a decision support system (DSS) to decide when and how to apply authorized chemical treatments in cover or bait sprays such as organophosphates (malathion), pyrethroids (deltamethrin, Lambda-cyhalothrin), and spinosyns (spinosad), climatic data, harvesting times of peach cultivars, cultivar risk according to phenological stages, and risk related to the fly catches were collected. Sticky plates were photographed, and captured adults were counted weekly. The second and the third steps of the DSS aiming to determine the type of the spraying to be performed on each individual tree, based on parameters related to the tree, cultivar, field and fly catches were applied and recommendations to the owner of the site were given and discussed.

Key-words: *Field Digitization, Ceratitis capitata, Decision Support System, Peaches*

Introduction

The Mediterranean fruit fly, Ceratitis capitata (Wiedermann) (Diptera: Tephritidae), is a highly invasive, polyphagous species that attacks a wide range of horticultural crops inducing substantial reduction in both quality and quantity of fruits (Liquido et al. 1990, Bouagga et al. 2014). In Tunisia, it is considered as a key pest on citrus and several summer fruits, such as apricot, *Prunus armeniaca* peaches (*Prunus persica* Batsch and figs (*Ficus carica* L.) (Cheikh et al., 1975; Jerraya 2003; Braham et al., 2007).
In Tunisia, Ceratitis capitata has a long history, with the first attempt to control it goes back to 1885 (Fimiani 1989). Up to now, control efforts have been exclusively based on insecticidal bait spray applications. Organophosphates (e.g. malathion), and pyrethroids (deltamethrin,

Lambda-cyhalothrin) mixed with protein bait have been applied in both aerial and terrestrial treatments against C. capitata (Howell et al. 1975; Mediouni et al. 2010
In Tunisia, among the multitude of host species, peaches (Prunus persica (L.) are particularly susceptible, especially late-ripening cultivars that mature from June to late October. Despite the efforts to develop appropriate monitoring and control methods against the pest,the insect induces high fruit losses and its economic losses remain high.
Despite the efforts to develop appropriate monitoring and control methods against the pest,the insect induces high fruit losses and its economic losses remain high. In fact, this pest causes a damage rate varying from 1% to 20% even with control measures and a total loss is possible if no protection measurements are undertaken (Jerraya, 2003; Boulahia-Kheder 2012a, Boulahia-Kheder 2012b).Its control is essentially based on chemical pesticides which are applied by farmers as cover sprays frequently, up to ten times in one season and without population monitoring (Boulahia-Kheder 2021). This can be harmful for beneficial fauna, environment and human health. To reduce the amount and frequency of insecticide application, various DSSs (Decision Support System) were developed in order to manage pest field crops (Bange et al al., 2004; Pontikakos et al., 2012). As definition, the DSS is a sets of tools like computer programs, models and relevant information such as experience and knowledge of farmers, inspectors and managers, acting together to support strategic, tactical or operational decisions, and can so enable growers and their advisors to make difficult choices based on the best information on potential risks to a crop (Ramsden and Driscoll 2022).
In this context, we aim in this paper to develop a Location Aware System (LAS) using conventional traps, site digitization and field collected data to create a platform that produces maps, decision spraying tracking, insect identification, and the implementation of a proposed Decision Support System targeting spraying procedures to be used against MedFly in peaches orchards and make an evaluation of the feasibility of the system in order to reduce as much as possible number of chemical treatments in the season.

Material and method

Crop and pest field data

The experimental site consists of 18 ha peach orchards located at Khlidia locality in the North-East of Tunisia (36.6648 North, 10.1927 East, Fig 1.) belonging to "Mabrouka nursery", which is a company specialized in the production of stone fruits and plant seedlings. Ten peach cultivars were cultivated in the site in which 7 (Sagittaria;PM12; PM14; PM9; PM7; PM2; PM17) were early maturing varieties, ripening from the third week of May to late June and 3 late ripening, (Extreme 460; Extreme 568 and PM10) maturing in August – September. The location of varieties is shown in Figure 2.Trees were planted in 2017 and 2019, distanced 5 meters between the rows and 3 meters on the row, were drip irrigated. General agronomic guidelines were applied in terms of fertilization and pesticide sprays. Each year, noticeable attacks by the MedFly are reported due to high susceptibility of peach fruits (many varieties which ripe at different times).

Fig. 1. Peach experimental site

Fig 2. Layout of the experimental site

Insect Trapping

To monitor the population dynamic of the pest, prior to set up the Location Aware System and the Decision Support System, a trapping grid with conventional delta traps was used. Hence, 24 traps were set up at the beginning of May 2022 and lasted till the end of September 2022. Sixteen traps in early ripening cultivars and seven traps in late ripening cultivars. One trap was placed in the two rows of fig trees planted between PM7 and PM14 varieties. Their distribution in the site was randomly in order to cover all the peach cultivated area (Figure 3). Traps with white sticky panels were baited with the commercially female attractant (AmmoniumAcetate, Trimethylamine Hydrochloride, and putrescine). The attractants were replaced monthly, and sticky panels were inspected and replaced generally every week when they are densely filled by the insect adults.

Fig. 3. Delta trap location

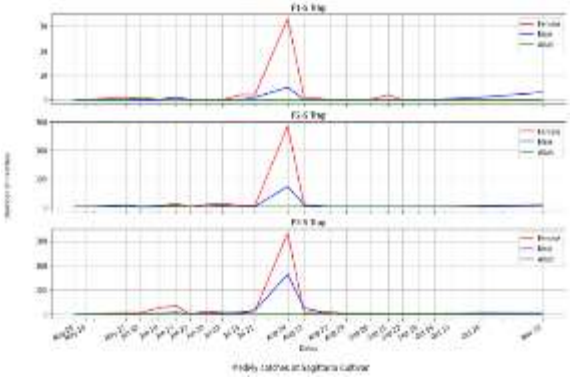

Fig. 4. MedFly catches at Sagittaria cultivar during the monitoring period (May- November 2022)

Use of mobile-GIS to collect pest data from traps

Traps were inspected weekly and plates were photographed using mobile Android Smart phones. Photos were transferred to DotDotgoose software and male and female MedFlies were counted. A database of the MedFly captures showing the number of males and females at each inspected date was prepared.

Climatic data

Climatic data were taken from the meteorological station of Mabrouka Company. This station is located about 1 km from the experimental site. Temperature, relative humidity, precipitation and wind speed were registered from January 2022 to September 2022.

Tree data **(**BBCH monitoring)

BBCH (BiologischeBundesanstalt, Bundessortenamt und ChemischeIndustrie) scale is a system for uniform coding of growth stages which has been widely used for describing phenological stages of plants (Meier et al., 1994). Fruit color or other fruit characteristics (BBCH) related to the damage or the pest control decisions were determined. The Percentage of fruit color change (veraison) of fruits was estimated visually in field. Approximately dates of reaching 20% veraison in fruits per cultivar as well as harvesting time were determined

Fruit damage data

Fruit damage was estimated visually on each visit to the experimental site by examining randomly ten trees per cultivar.

Implementation of the Decision Support System (DSS)

According to Sciarretta et al. (2019), there are 3 DDS (A, B and C).DSS A estimates the date of the MedFly presence in the field based on the calculation of degree day (DD). From this date, it is indicated to place traps in the field.

DD= $\sum_{k}^{n} Tmean - 10.2°C$ if >0; =0 if <0Tmean= $\frac{T_{max}+T_{min}}{2}$

K=1st of January

n=days starting from 1st January

T mean= daily mean temperature in °C

10.2°C = lower development threshold

620 threshold for the first emerging adults

DSS B defines the areas to be treated and the type of treatment, based on MedFly captures, harvesting time, and phenological stage of cultivars and DSS C deals with the estimation of the distribution of the spraying, defines the spraying procedures of the individual trees derived from the DSS2 output. It evaluates if the treatment at that precise time is necessary or if there is an active protection from a previous treatment. The output of DSS3 highlights on the map the trees to be treated and the spray type to be used (bait spray, cover spray, mass trapping).

Results and Discussion

Trap capture

In all orchards, MedFly adults were captured from late May to October 2022. Traps captured more females than males. For early ripening varieties (e.g.Sagittaria), the insect is present from May to August (Figure 4) and from June to October for late maturing varieties (PM10, Figure 5.)

GIS to collect pest data from the traps

Photos of plates immediately taken on the field and after processing with DotDotGoose were shown in the Figure 6. Photos of sticky plates taken in the field before (left) and after processing with DotDotGoose software (right for PM17 peach variety)

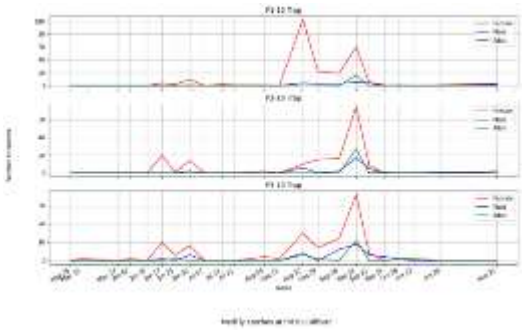

Fig.5. MedFly catches PM10

Fig. 6. Photos of sticky plate of the trap installed in PM17 peach variety immediately taken in the field and after treatment with DotDotGoose on 04 August 2022 (see red spot on *C. capitata* adults)

BBCH

The table 1 represents the estimated when fruits reached 20 % veraison

Table 1. Dates of reaching 20% veraison in fruits per cultivar

	Estimated date corresponding to 20% veraison	**Harvesting period**
Extreme 460	04-08-2022	20-31 August
Extreme 568	04-08-2022	20-29 August
PM10	21-07-2022	8-15 August
Sagittaria	26-04-2022	20-30 May
PM12	26-04-2022	14-28 May
PM14	18-04-2022	30 May-06 June
PM7	10-05-2022	01-16 June
PM9	10-05-2022	6-16 June
PM17	10-05-2022	23-30 June
PM2	10-05-2022	14-23 June

Fruit load

The total fruit load per cultivar was recorded on the dates of harvesting. The average per tree is given in the Table 2.

Table 2. Average Fruit load per cultivar in the experimental site

Cultivar	**Load / tree (kg)**
Early ripening cultivars	
Sagittaria	46
PM12	46
PM2	36
PM7	32
PM14	32
PM9	22
PM17	31
Late ripening cultivars	
Extreme 460	13
Extreme 568	14
PM10	19

Percentage of damage, stages of the pest in the fruits

Visually, the percentages of damage were ranged from 10 to 20% in early ripening cultivars and from 20 to 30% in those of late ripening. Generally, MedFlylarvae were present in some examined dropped fruits.

DSS operation

DSS A: Semi-automatic (Web based) trapping

In 2022, for Mabrouka site (Tunisia), 620 DD threshold for the first emerging adults was reached on 26 May 2022 as shown in the Figure 7. While the first MedFly adult was trapped on 10 May 2022.

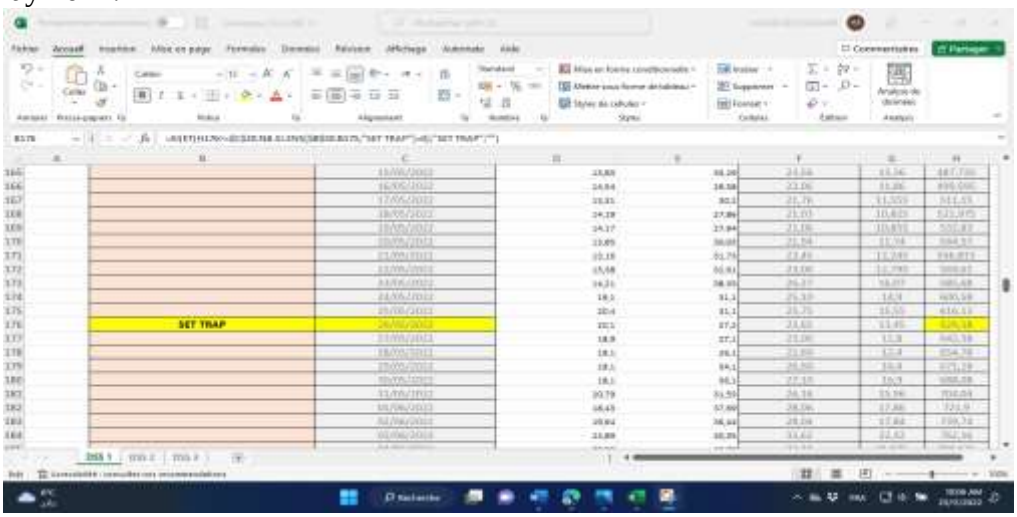

Fig.7. Running of the DSSA for Mabrouka site (Tunisia)

DSS B: Estimation of the time of spraying

Some results from the running of the DSS B per sampling date for early and late ripening cultivars are shown in the following tables(Table 3 and Table 4).

Table 3. Running of the DSS B per sampling date for early ripening cultivars

Cultivars	Dates	DSS recommendation Treatment	Actual Spraying done by the grower
Early ripening cultivars			
Sagittaria	05 May	No spray	No spray
	10 May	No spray	No spray
	27 May	No spray	No spray
	02 June	No spray	No spray
	10 June	No spray	No spray
	17 June	No spray	Cover spray (done on 18 June 2022)
PM12	05 May	No spray	No spray
	10 May	No spray	No spray
	27 May	No spray	No spray
	02 June	No spray	No spray
	10 June	No spray	No spray
	17 June	No spray	Cover spray (done on 18 June)
PM17	05 May	No spray	No spray

	10 May	No spray	No spray
	27 May	No spray	Cover spray (done on 25 May)
	02 June	No spray	No spray
	04 June	No spray	Cover spray
	10 June	No spray	No spray
	13 June	No spray	Cover spray
	16June	No spray	No spray
	17June.	No spray	Cover spray
	23 June[(1)]	No spray	No spray
	30 June[(1)]	Bait spray	No spray
	7 July[(2)]	No spray	No spray
	15 July	No spray	No spray
	21 July	No spray	No spray
PM2	05 May	No spray	No spray
	10 May	No spray	No spray
	25 May	No spray	Cover spray
	02 June	No spray	No spray
	04 June	No spray	Cover spray
	10 June	No spray	No spray
	13 June	No spray	No spray
	17 June	No spray	No spray
	23 June	No spray	No spray
	30 June	No spray	No spray

[(1)]Harvest time
[(2)]post harvest

Table 4. Running of the DSS B per sampling date for late ripening cultivar

Cultivar	DATE	DSS recommendation **Treatment**	Actual Spraying done by the grower **Treatment**
Late ripening cultivars			
PM10	7 July	No spray	No spray
	15 July	No spray	No spray
	21 July	No spray	Cover
	4 August	No spray	No spray
	11 August[(1)]	No spray	No spray
	22 August [(1)]	No spray	No spray

[(1)] harvest begins in 8 August

Conclusions

According to DSS A, B and C first adult emergence forecast will be on26 May 2022, while actual capture from the trap was on 10 May 2022.The calculation of DSS demonstrates late sprays. However, growers treat early according to a calendar basis independently of DSS recommendations. Even after fruit harvest, traps captured *C. capitata* adults in large numbers, surpassing captures before harvest.

Acknowledgement

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ORGANIC AGRICULTURE

ANTIOXIDANT PROPERTIES OF BLACK CURRANT BERRIES (*Ribes nigrum* `Titania`)

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Abstract

Fruits of black currant (*Ribes nigrum* L.) cultivar 'Titania' were taken from an urban garden in the city of Banja Luka, Bosnia and Herzegovina (B&H). This planting was established in 2010 with certified material from a reputable Italian nursery. On the same plot were also grown blackberry 'Thornfree', black chokeberry 'Nero' and jostaberry `Josta'. Their fruits were taken for a comparisons related to the antioxidant properties of black currant berries. The following parameters were analyzed: phenols, nonflavonoids, flavonoids, anthocyanins, monomeric anthocyanins, ID, ABTS and DPPH. It was found that black currant berries of cultivar 'Titania' contain, on average: phenols – 4.19 μgGAE/mg, nonflavonoids – 1.95 μgQcE/mg, flavonoids – 2.24 μgQcE/mg, anthocyanins – 2.99 μg/mL, monomeric anthocyanins – 2.69 μg/mL, ABTS – 1.33 mg Trolox/mL, DPPH – 4.39 mg Trolox/mL. This results support the opinion that black currant is a valuable small fruit crop, rich source of phenolic compounds, with substantial antioxidant capacity. Our research has shown that fruits of blackberry 'Thornfree' and jostaberry 'Josta' have slightly higher concentration of phenols compared to cultivar 'Titania'. When it comes to flavonoids, cultivar `Titania' is significantly ahead of these two species/cultivars grown under the same habitat conditions. However, the anthocyanin content in the 'Titania' is inferior to the observed species/cultivars. At the same time, the ID values indicate that there are practically no differences between the observed species/cultivars regarding the degree of anthocyanin degradation. In both tests (ABTS and DPPH), the lowest antioxidant activity was performed by black currant `Titania'.

Key words: *black currant, cultivar 'Titania', antioxidant properties*

Introduction

The genus *Ribes* includes over 160 wild shrubby species worldwide, among which is black currant *(Ribes nigrum* L.), (Cortez et Gonzalez de Mejia, 2019). The native range of this species is Europe to Russian Far East and western Himalaya (Anon., 2023). According to the same source, the species was introduced into North America and South America (Birgi et al., 2019), as well as into New Zealand (Vagiri, 2012).

In the Balkans, it is not a common species. It is much more common in northern countries. It grows as a wild plant in moist and humic places in forests, among bushes and thickets (Grlić, 1990). In the north-western Dinarides *Ribes nigrum* is present in the plant associations: *Lamio orvalae - Alnetum incanae* Dikskobler 2010 (Vukelić et al., 2017) and *Alnetum incanae* Lüdi 1921 (Douda et al., 2016). On the Rhodope Mts. in Bulgaria, along the bank of the river Devinska, a site of *Ribes nigrum* along with *Frangula alnus*, *Rubus idaeus*, *Filipendula ulmaria*, and *Urtica dioica* was described by A. Tashev (Vladimirov et al., 2011).

Practically the entire aerial part of black currant is used - fruits, including seeds, leaves, buds and stem bark. Wild black currant fruits stand out for their special, strong and somewhat unpleasant smell. Because of this, few people eat them in their fresh, unprocessed state.

Refreshing juices, liqueurs, wines, vinegar, compotes, jams, purées, jellies are prepared from berries (Grlić, 1990; Karjalainen et al., 2009; Vagiri, 2012). Residues that occur during the processing of black currant fruits can be used for the extraction of phenolic compounds (Bakowska-Barczak & Kolodziejczyk, 2011). Dried berries are an integral part of natural vitamin concentrates and vitamin teas (Grlić, 1990). In the former USSR, *Fructus Ribis nigri* was treated as a multivitamin medicinal supplement (Muravieva, 1978). Oil from black currant seeds are used in the cosmetic industry and as an ingredient in various dietary supplements (Karjalainen et al., 2009; Wójciak et al., 2022). Black currant leaves are sometimes used to flavor spirits and elixirs. Fermented leaves produce a beverage that tastes similar to Russian tea. (Muravieva, 1978; Grlić, 1990). The dried leaf (*Ribis nigri folium*) is included in the B&H Pharmacopoeia (Anon, 2022). Leaf buds, harvested during winter and early spring, serve as raw material for the production of essential oils and absolutes (Nishimura et Mihara, 1988; Le Quere et Latrasse, 1990) and phytochemicals with therapeutic benefits (Téglás et al., 2022), within the framework of modern phytoembryonic plant medicine. A decoction of the bark has been found of use in the treatment of calculus, dropsy and haemorrhoidal tumors (Grieve, 1992). Almost all parts of black currant are used in traditional medicine (Gelenčir, 1090).

In 2019, 647,815 t of black currant berries were produced (harvested) in the world. The leaders in this production are Russia with 417,600 t and Poland with 126,190 t, followed by Germany, Ukraine, Great Britain, Austria, France, and the Czech Republic (Sazonov et al., 2021).The genetic base of this production consists of a large number of cultivars, the exact number of which is not known. In Russia, in the same year, 205 were officially registered (Anon., 2021), while, for example, in Poland in 2010, 40 cultivars were mentioned (Mądry et al.). The objectives of hybridization can be different; large and stable yield, fruit quality, nutritional value, processing quality, pests and diseases tolerance, adaptability to emerging climate changes, etc. When it comes to protective moments, it should be borne in mind that *Ribes* species, including *Ribes nigrum*, are secondary hosts of the parasitic fungus *Cronartium ribicola* J.C.Fisher, which causes a disease known as "white pine blister rust". Among *Ribes* species, *Ribes nigra* is more sensitive, while red and white currants are relatively resistant (Karadžić, 2010). This pathogenic fungus was registered for the first time in B&H by Wicker & Uščuplić in 1971 in industrial plantations of eastern white pine (*Pinus strobus* L.) in the locality of Dubrave near Banja Luka (Anon., 2023/1).

There are currently no serious commercial plantations in B&H, so there are no official statistics on the volume and range of production. Black currant is *de facto* a home garden berry species in B&H. In addition, by looking at the available databases, we were unable to find information about the cultivar composition or the chemical properties of black currant berries from local cultivation. Precisely for this reason, the goal of our work is to analyze in more detail the antioxidant capacity of black currant berries on the example of the 'Titania' cultivar.

Material and method

Material

Fruits of black currant (*Ribes nigrum*) cultivar 'Titania' were taken from an urban garden in the city of Banja Luka, B&H (fig. 1). The garden is owned and maintained by the NGO "The Society for the Environment on Earth - SENSE". This planting was formed in 2010 with certified material produced in a reputable Italian nursery. The land is flat, at an altitude of about 163 m. The average annual temperature is 10.8 ^{o}C, and for the vegetation period (from March to November) 17.2 ^{o}C. The average annual rainfall is 1,017 mm/m^2 (Ljubojević et al., 2020). A drop-by-drop irrigation system from the company "Hunter" has been installed on the plot. The following are also grown on the same site: blackberry (*Rubus fruticosus*)

‘Thornfree', black chokeberry (*Aronia melanocarpa*) 'Nero' and jostaberry (*Ribes* x *nidigrolaria* `Josta', whose antioxidant capacity has already been analyzed earlier (Ljubojević et al., 2020).

Figure 1: Black currant ‘Titania’ (photo: S. Ljubojević)

The cultivar ‘Titania’ was created in Sweden in 1969 as a result of planned cross breeding between the male parent - the F hybrid of the cross: 'Consort' x 'Kayaanin Musta' and the female parent - 'Altaskaya Dessertnaya'. The cross was made by Pal Tamas, a Swedish citizen of Hungarian origin. Among other things, the cultivar is resistant to black currant rust (*Cronartium ribicola*) and mildew (*Sphaerotheca mors-uvae*) and is regarded as a high quality raw material for fruit juice production. (Anon., 2000; Vagiri, 2012).

Method

Fruits were picked in the middle of July 2022 in the stage of full ripeness. From the homogenized material, 5 g of samples was extracted with 80 % ethanol, twice at 25 ml. The solutions were refilled with 80 % ethanol up to 50 mL. Thus, 100 mg/mL concentrations were obtained and further used to determine total phenols and flavonoids, as well as for neutralization of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radicals. To determine total and monomeric anthocyanins, 20 g of samples was extracted with a 20 mL solution (85 ml of 95 % ethanol solution in 15 ml of 1.5 mol/L HCl solution) at 0 ^{o}C for 24 hours. After being left to stand, resulting mixture was filtered and used for further analysis. Total phenol content was determined by the modified Folin-Ciocalteu method. Gallic acid was used as a standard compound and the results were expressed as a phenol equivalent to the gallic acid (GAE), i.e. µg GAE/mg. The total flavonoids were determined by the method of Kumaran & Karunakaran. Quercetin was used as the standard compound, and the results were expressed as µgQcE/mg. Total and monomeric anthocyanins were determined by the spectrophotometrically modified "single" pH and by the pH differential method. The antioxidant activity in relation to the DPPH radical was determined by the method of Liyana-Pathirana and Shahidi. The modified method of Re et al. was used for the ABTS radical. The results were presented as mgTrolox/mL. The anthocyanin degradation index (ID) was calculated from the quotient: anthocyanins /monomeric anthocyanins.

The described procedure is identical to the procedure that was carried out during the analysis of material collected from blackberry 'Thornfree', black chokeberry 'Nero' and jostaberry 'Josta' in the summer of 2018 (Ljubojević et al., 2020).

Results

The fruits of black currant are considered a very rich source of phenolic compounds (Ovaskainen et al., 2008; Karjalainen et al., 2009). Our research confirmed this finding, with the fact that in the fruits of blackberry 'Thornfree' and jostaberry 'Josta' their concentration was slightly higher. The established differences came to the fore regarding the content of nonflavonoids. However, when it comes to flavonoids, blackcurrant `Titania' is significantly ahead of these two species/cultivars.

Table 1: Phytochemicals and antioxidant activity of black currant and some other fresh fruits

Parameters	Unit	**Black currant `Titania`** ($\bar{x} \pm s$)	Blackberry 'Thornfree'/1/	Black chokeberry 'Nero'/1/	Jostaberry 'Josta'/1/
Phenols	µgGAE/mg $_{F..W.}$	**4.19** ± 0.11	5.47	74.80	5.11
Nonflavonoids	µgQcE/mg $_{F..W.}$	**1.95** ± 0.05	4.59	3.39	3,18
Flavonoids	µgQcE/mg $_{F..W.}$	**2.24** ± 0.06	0.89	71.42	1.94
Anthocyanins	µg/mL	**2.99** ± 0.21	15.35	45.92	21.04
Monomeric anthocyanins	µg/mL	**2.69** ± 0.19	13.80	40.55	19.10
ID		**1.11** ± 0.01	1.11	1.13	1.10
ABTS	IC_{50} mg Trolox/mL	**1.33** ± 0.05	0.37	0.17	0.22
DPPH	IC_{50} mg Trolox/mL	**4.39** ± 0.64	1.45	0.97	1.89

/1/ Ljubojević et al. (2020)

The cultivar 'Titania' contains more than twice as many anthocyanins (2.99 µg/mL) compared to the fruits of the Serbian variety 'Čačanska Crna' (1.39 µg/mL), (Karaklajić-Stajić et al., 2023). However, the anthocyanin content in the 'Titania' is inferior to the three observed species/cultivars (tab. 1). At the same time, the ID values indicate that there are practically no differences between the observed species/cultivars regarding the degree of anthocyanin degradation, i.e. the preservation of anthocyanin monomers.

In both tests (ABTS and DPPH), the lowest antioxidant activity, i.e. the weakest ability to act as radicals scavenger was performed by black currant `Titania' (tab. 1). Compared to the fruits of this cultivar harvested in Serbia, there are practically no differences when it comes to the DPPH test. There, in the material collected during 2019 harvest, DPPH was 4.0 mg/mL, while in the material collected during 2018 harvest from the two-year-old shoots DPPH was 6.1 mg/mL, and from the three-year-old shoots, DPPH was 7.2 mg/mL (Djordjević et al., 2020).

Conclusions

While production of red currant in B&H has experienced a strong expansion in recent decades, production of black currant is limited to a small estate and has the character of a "home garden bush".

The results of our research support the opinion that black currant is a valuable small fruit crop, rich source of phenolic compounds, with substantial antioxidant capacity.

In terms of the content of phenols, black currant 'Titania' is not far behind blackberry and jostaberry cultivars, while in terms of flavonoids it is ahead of these species/cultivars grown under the same habitat conditions. Compared to some red currant cultivars, 'Titania' leads in

anthocyanin content, but lags behind blackberry 'Thornfree', jostaberry 'Josta' and black chokeberry 'Nero'. At the same time, the ID values indicate that there are practically no differences between the observed species/cultivars regarding the degree of anthocyanin degradation, i.e. the preservation of anthocyanin monomers.
Black currant ‘Titania’ has stable antioxidant capacity, which show very small differences between materials harvested in B&H and Serbia. This potential is weaker compared to the three observed species.

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ATTITUDES OF PRODUCERS' TOWARDS DISTRIBUTION CHANNELS OF ORGANIC PRODUCTS IN THE REPUBLIC OF CROATIA

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Abstract

The selection of organic product distribution channels indicates the producers' willingness to face the market challenges. The article highlights the difference between the buyers' preferences and the opinions of surveyed Croatian organic producers on the most optimal places to distribute organic products. Surveyed organic producers do not rate stores, supermarkets and specialized organic stores as the optimal place for distribution, while they see farm gate sales and specialized fairs as potential. Croatian consumers choose supermarkets and hypermarkets for purchase while buying in specialized stores of organic products is limited to larger cities. Most organic farms operate outside urban areas, so the availability of their products is conditioned by the buyer's will to come to the farm. The specialized organic fairs are limited to the days of fairs. Both may represent a limitation in the availability of organic products to a broader group of potential consumers, resulting in a lower representation of domestic products on the market. Openness to contracted production was recognized in respondents of younger age, although the number of registered organic processors limits it. Low respondents' rates about marketplaces may be aligned with the constant trend of declining their number in Croatia. Digitalization followed by the Covid crisis has opened up web sales as a new marketing niche whose potential on the domestic organic market is too early to assess. The article concludes with a discussion of buyers' purchase preferences and the importance of diversification of marketing channels, this topic being a basis for future research.

Keywords: *Organic production, Attitudes of Croatian organic producers, Customer preferences, Distribution channels, Republic of Croatia.*

Introduction

The growth trend of the organic product market is no longer the result of a holistic approach and personal choice of consumers in the use of healthy food but also the result of global policies that seek to limit the negative impact of the use of mineral fertilizers, antibiotics and pesticides used in conventional agricultural production, in order to reduce their pressure on nature, the environment, biodiversity and the sustainability of food production in the future. According to the FIBL Survey (2023), total sales of organic products amounted to almost 125 billion euros in 2021. The United States of America was the largest single market, followed by the European Union (46.7 billion euros). According to the same source, the share of organic products in total retail sales in the Republic of Croatia in 2018-2021 was 2.2 %, valued at about 99.3 million euros. The same source cites more than ten times the value of imports of organic products (EUR 34.75 million) into the Republic of Croatia than the value of exports of organic products (EUR 2.9 million). According to the Ministry of Agriculture, in the ten years from 2013 to the present, the area under organic production has grown from 40,660 hectares to 129,374 hectares (share in total arable land from 2.59% to 8.95%), and the number of organic agricultural producers from 1,789 has grown to 6,512 producers. Organic

production is dominated by permanent grassland (62,590 ha), arable land and gardens (49,069 ha) and permanent crops (17,715 ha). Although noticeable, there is still insufficient growth in the number of registered organic processors (from 181 processors in 2013 to 380 registered in 2022). The latter indicates that the growth of areas under organic production and the number of organic producers in the Republic of Croatia did not result in a larger share of domestic organic products in the Croatian market. Likewise, this growth did not result in an increase in the organic products export. In this regard, without considering other marketing mix elements, this paper indicates the importance of using different distribution channels for organic product distribution.

Materials and Methods

Data on sales experiences and attitudes of organic producers to the distribution of organic products were collected through personal interviewing of producers. For this purpose, data from the research conducted in 2019 were used to draft the doctoral dissertation. The survey examined the attitudes of organic producers about the most optimal places for organic product distribution. Attitudes were analyzed concerning the age of the respondents (20-30 years, 30-40 years, 40-50 years and more than 50 years), level of education (primary school, secondary school, higher school, college), the area in which they operate (Central Croatia, Middle and South Adriatic, Eastern Croatia, Northwestern Croatia, North Adriatic and Lika) and the gender of the producers. In order to determine the most optimal places for the distribution of organic products, respondents were asked to, according to their own experience, grade from 1 - not optimal at all, to 5 - extremely optimal, the most optimal places for sale: marketplace, shop/supermarket, specialized organic products store, specialized organic products fair, farm gate sale, contracted production or web sale. Statistical analysis of the results was carried out by the statistical program SAS Software v. 9.3 (2010). The article is also based on the literature study on the distribution channels of organic food. The research was conducted on a sample of 236 producers of organic products from five above mentioned Croatian regions. Most respondents were from Central Croatia (22.9%), followed by the Central and Southern Adriatic (20.8%), Eastern Croatia (19.5%), Northwestern Croatia (18.6%) and the Northern Adriatic and Lika (18.2%). Most respondents were aged 40-50 years (33.9%), followed by 50-60 years (33.12%), 30-40 years (27.9%) and 20-30 years (5%). The survey included 153 men (64.8%) and 83 women. According to the level of education, the highest number of respondents completed secondary school (48.3%), while college graduates (29.2%), higher education (21.2%), and the lowest number of respondents (1.3%) finished primary school.

Results and discussion

The results of the survey showed that the respondents rated as the most optimal distribution channels (sum of the percentages of grades 4 and 5): farm gate sales (83.1%), specialized organic fairs (80.9%), organic products specialized stores (49.1%), contracted production (47.8%), marketplace (44%), web sales (39.8%), stores and supermarkets (14%). The results are shown in Table 1.

Table 1. Optimal places for the sale of organic products (respondents %)

Attitudes/ Rating	Marketplace	Shops Supermarkets	Specialized shops	Specialized fairs	Farm gate	Contracted production	Web sales
1	5,1	6,4	1,7	1,3	0,8	6,4	5,1
2	14,0	30,1	14,8	3,8	3,4	12,7	22,5
3	36,4	49,2	33,9	13,1	11,9	31,8	31,8
4	18,2	9,3	18,6	14,4	8,5	13,1	16,5
5	25,8	4,7	30,5	66,5	74,6	34,7	23,3

By analyzing the research results using Pearson's chi-square test, a statistically significant difference (p.value <0.05) was found between the distribution of optimal places for organic products distribution channels by age, level of education and the area in which they operate. In contrast, for gender, we cannot claim this (p-value >0.05). Concerning the age of respondents, this difference is related to the distribution of products through specialized organic stores and contracted production. The same shows greater openness to these distribution channels in respondents aged between 30 and 40. Concerning the level of education, a statistically significant difference was found for the distribution of products through web sales. This can be explained by the insufficient IT literacy of older farmers, the non-coverage of all Croatian rural areas by broadband internet, and the willingness of younger farmers to find alternative solutions to this problem. This is also reflected in the fact that the respondents with a university degree, unlike those with a lower level of education, evaluate web sales as completely optimal. Concerning the area where organic farmers operate (work), a significant statistical difference was determined for sales channels: shops and supermarkets, specialized fairs and contracted production. In doing so, the most neutral (grade 3) responses for the shops and supermarkets were observed in respondents from Eastern Croatia. Most respondents from Central Croatia see specialized stores as entirely optimal for distribution. As specialized organic products shops mainly operate in Central Croatia, this could be the reason why they are more inclined to this type of distribution. Most respondents agree that specialized fairs are optimal for organic product distribution, especially respondents from the Middle and South Adriatic and Northwestern Croatia. The most neutral responses (grade 3) for contracted production were observed in respondents from Central Croatia. Regardless of the ongoing digitalization that opened up web sales as a new market niche, less than half of the respondents consider this distribution channel optimal. Respondents' attitudes about marketplaces are aligned with the constant trend of declining their number in the Republic of Croatia. The results are shown in Table 2.

Table 2. Distribution of optimal distribution channels for organic products according to age, level of education, area of operation and gender of the producer, results of the chi-square test.

Distribution channels	Age		Level of education		Working area		Gender	
	x^2	p =	x^2	p =	x^2	p =	x^2	p =
Marketplace	7,932	,790	10,148	,603	21,746	,152	5,382	,250
Shop, supermarket	11,878	,456	13,197	,355	35,452	,003	4,593	,332
Specialized shop	22,953	,028	14,172	,290	34,465	,005	1,922	,737
Specialized fair	14,558	,267	14,586	,265	34,320	,005	8,751	,068
Farm gate	9,585	,652	8,571	,739	16,463	,421	3,051	,549
Contracted production	27,618	,006	8,506	,744	35,232	,004	,915	,922
Web sales	13,566	,329	27,149	,007	25,583	,060	5,370	,251

The prevailing respondents' attitude on the optimal channel of distribution of organic products indicates the difference between respondents' opinions and the buyers' purchase habits, determined in the research conducted so far and in the publicly available data of the competent institutions. Data from Croatian Competition Agency data (2021) show that supermarkets and hypermarkets are retail outlets that dominate retail trade in the Republic of Croatia. More than two-thirds (71.6%) of total revenue in grocery retail is generated precisely through sales in supermarkets and hypermarkets. The two most significant organic products retail chains (Bio & Bio and Health Food Factory) operate through 35 retail stores (22 in Zagreb). Several smaller shops with organic products mainly operate in urban areas. These stores operate in larger cities, so if we consider that 60% of the population lives in Croatian urban areas and, on average, every fifth inhabitant lives in Zagreb, we can conclude that specialized stores of organic products could be a potential market niche for domestic organic products. Atănăsoaie (2011) wrote about this in his research on distribution channels, stating that in developing markets, these stores first appear in the capital or large cities, and then spread into smaller cities. From the aspect of selling organic products at fairs, it should be considered that this distribution channel is limited to the days of fairs, thus limiting availability to potential customers. When we are considering farm gate sales, it should be taken into account that they are limited by the need for the buyer to come to the farm. In addition, selling on the farm gate implies a built relationship of trust between the producer and the customer, which can be a limitation for new organic producers' market positioning. The latter, considering the expressed attitudes of respondents on the optimal distribution channel, represents a limitation in the availability of organic products to a broader group of potential buyers. In previous research Renko and Bošnjak (2009) stated that Croatian producers of organic food have not adapted to consumers with assortment and choice of location. According to the same Croatian consumers have also noticed these problems, and respondents cite the unavailability of information about places where they can buy organic products as the biggest obstacle to buying organic food. As a development priority of Croatian organic food producers, the entry of organic products into the most crucial food distribution channel - into supermarket chains - was highlighted. By entering supermarket chains, manufacturers would get a significant sales channel, making organic food more accessible to consumers. Petljak, K. (2010) points out that organic products, especially from

domestic production, are still underrepresented in supermarkets and hypermarkets, with most manufacturers distributing organic food products through direct distribution channels, at production facilities, fairs and fair exhibitions. Težak at all (2008) highlight the need to open new distribution channels to bring eco-products closer to consumers. Gugić et al. (2017) state that the domestic market for organic products is not organized, partly due to product insufficiency, limited processing capacity and insufficient consumer awareness. Rengel (2013) considers that domestic eco-products in Croatia do not have enough distribution channels, for example, eco-products can rarely be found in supermarkets, which are one of the most important distribution channels in the country. Moreover, Singh and Pandey (2012) emphasize that customers will not bother to buy green products explicitly; where possible, products should be distributed through major outlets so that they are not only available to a small market niche of green consumers (in Stoica 2020). Atănăsoaie (2011) emphasize that most consumers appreciate supermarkets because they provide the opportunity to buy organic food and food produced conventionally. Moreover, Michelsen at all (1999) emphasizes that a combination of different sales channels can result in optimal earnings and optimal consumer demand coverage, but it can also be completely the other way around if some channels are unavailable for organic products. Stoica M. (2021) also sees extensive distribution as desirable, especially in hypermarkets, supermarkets and stores in large cities or even in certain parts of the city.

Conclusions

Although a significant growth of the organic production sector in the Republic of Croatia is noticeable, the limited availability of domestic eco products on the market, as well as a negative foreign trade balance for organic products, indicates the gap between domestic organic production results and share of domestic products on the market. An effective marketing mix could bridge this gap. Although marketing channels are only one marketing mix variable, they should be seen as the first tangible exchange point in the organic food supply chain. It should be pointed out that organic product buyers' purchasing habits must play a pivotal role in producers' decisions on selecting distribution channels. The results of this research can contribute to a better understanding of producers' preferences regarding distribution channels. In that sense, we can conclude that differences in the respondents' opinions depict that distribution channel choice is influenced by the distance of specialized organic shops from the place of production, the availability of processors, the availability of marketplaces and the IT literacy of respondents. For shops and supermarkets, organic production volume and lack of distribution infrastructure should be considered, as well as the reluctance of producers to join producer organizations or cooperatives. The mentioned influences affect the choice of distribution channels to varying degrees and are also affected by the age, level of education, and the area where respondents produce organic products. Therefore, applying a unique solution for diversification of organic product marketing channels in the Republic of Croatia will not be sufficient to increase the share of domestic products on the market. In that sense, it would be helpful to tailor the marketing channels to the area where the production of organic products is carried out and work on strengthening the sales infrastructure to facilitate organic product fluctuation across the country. Finally, this research is limited by the unavailability of official data on the sale, prices and consumption of organic products in the Republic of Croatia and by the number of respondents.

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CO-CULTIVATION AS A PLANTING STRATEGY FOR QUALITATIVE AND QUANTITATIVE PLANT GROWTH AND ENVIRONMENTAL PROTECTION

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Abstract

The Mediterranean diet is a way of life. Medicinal/Aromatic plants (MAPs) have a special place in everyone's way of life, as they are a rich source of natural antioxidants. Urban co-cultivation of these plants and their ecological management are two approaches used in sustainable and environmentally friendly horticultural practices. This approaches aim is to create healthier ecosystems, increase biodiversity, conserve natural resources, protect the environment etc. In the last few years, a multi-faceted approach has been adopted in the cultivation of MAPs, both for their consumption and for their commercial marketing. However, are the benefits of co-culturing known? The present study was aimed at highlighting some of the roles played by urban MAPs, using a quantitative and qualitative approach. To this end, an experiment was carried out with six pots in which *Aloysia citrodora* and *Salvia officinalis* were cultivated with a variety of MAPs. The manual method was used to control the growth of the plants, while the Folin-Ciocalteu and DPPH methods were used to determine the total phenolic content and antioxidant activity, respectively. The results showed that an important factor in the plant growth regulation and bioactive elements of MAPs was the appropriate selection of companion plants. Therefore, future studies should investigate other combinations of co-cultivation of MAPs, even with vegetables, to increase their bioactive compounds. This would improve the daily diet of urban people.

Keywords: *Co-cultivation, sustainable horticulture, bioactive compounds, aromatic medicinal plants.*

Introduction

The concept of environmental protection is becoming increasingly important, as the continuous development of living standards while satisfying material needs shapes the image of the risk of depletion of the natural environment (Novikova et al., 2019). Also, after a long period of economic and health crises, it is necessary to promote the issue of access to fresh and nutritious food for all social groups (Macnea et al., 2021). This is because environmental degradation negatively affects weather conditions, crop production, environmental productivity, food chains, and human health (Majeed and Mazhar, 2019). In particular, food security is affected by the food chain and is highly correlated with urbanization, which is one cause of environmental degradation. Restricting the study to the Balkans, the data shows that different levels of food security are the result of different economic management and different urban development (Maknea et al., 2022).

In recent years, efforts to improve the quality of life of urban dwellers and the urban environment have led to the need for lifestyle changes. The Mediterranean diet can be considered a sustainable lifestyle that can be adapted to different cultures (Guasch-Ferré and Willett, 2021). It is based on the traditional ecological patterns and dietary choices of the countries surrounding the Mediterranean Sea. In particular, the recovery of Mediterranean plant-based diets contributes to the recovery and valorisation of traditional knowledge by

reconnecting food production and consumption (Aguilera et al., 2020). A typical example is the use of medicinal/aromatic plants (MAPs) in the daily diet, instead of salt, to improve the taste of dishes, for their natural antioxidant properties, etc. (Zafar et al., 2023).
But is there a way to improve the quality and quantity of MAPs to benefit both humans and the environment?
Intercropping can indeed be a sustainable and environmentally friendly horticultural practice. In particular, urban intercropping of MAPs involves the cultivation of many plants in a limited space; therefore, efficient land use can be cited as a key advantage. The strategy by which intercropping is carried out follows the steps of permaculture using the ethics of sustainability (Maknea et al., 2022). The main features of intercropping are the conservation of natural resources by minimizing the use of water and pesticides. In addition, the biodiversity created by intercropping MAPs through the creation of green strips provides useful habitats for pollinators and other beneficial insects. Finally, reducing the distance between the point of production and the point of consumption of edible crops, which directly contributes to the reduction of greenhouse gas emissions, are important benefits of intercropping that contribute to a local and sustainable food system (Maknea et al., 2023). In an attempt to address these issues in a city, the application of urban gardening has emerged as an alternative solution (Maknea and Tzortzi, 2019). In particular, the cultivation of potted edible plants on balconies was identified as a popular urban gardening activity during the pandemic (Maknea et al., 2021).
With these data, the subject of the present study was chosen to highlight the qualitative and quantitative benefits of intercropping edible herbs in pots. Both MAPs and phytopharmaceuticals are increasingly used either for the treatment or prevention of diseases. The Mediterranean flora of Greece is rich in MAPs, which contain important bioactive compounds (Grigoriadou et al., 2020).

Material and Methods

In the present study, 9 Mediterranean MAPs were selected and grown in pots in a house located in the center of Bucharest (altitude 64 m) in Romania. The original plant material used was derived from traditional Greek varieties and is part of academic research at USAMV Bucharest, which is monitoring the adaptation of Mediterranean herbs in the Balkan countries in the context of urban cultivation, taking into account climate change. The 9 samples used for the qualitative analysis were leaves of MAPs, collected from the pots and dried in a university laboratory.
In particular, an experiment was carried out with six pots in which *Aloysia citrodora* and *Salvia officinalis* were intercropped with different MAPs (Table 1). Four of these pots had the same dimensions and were planted with 3 plants, while the other two pots, in which one plant was planted, were reduced in size by 1/3. The substrate used in all pots was peat with perlite and the growing period of the plants was from 5/6/2022 to 26/9/2022. Table 1 shows the planting arrangement, in particular, that pots 1 and 4 were planted with one plant, while the others were planted with 3 plants. The plants to be evaluated from the 6 samples are marked in bold.

Table 1. Distribution of plants in pots

Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6
Aloysia citrodora (A1)	***Aloysia citrodora (A2)***	***Aloysia citrodora (A3)***	***Salvia officinalis (S4)***	***Salvia officinalis (S5)***	***Salvia officinalis (S6)***
	Pelargonium graveolens	*Origanum majorana*		*Thymus vulgaris*	*Rosmarinus officinalis*
	Artemisia draconiana	*Thymus vulgaris*		*Saturejia thymbra*	*Sideritis syriaca (malotira)*

The experimental design of the paper aimed to investigate some roles of urban co-location with MAPs through a quantitative and qualitative approach. The manual method was used to grow and measure the plants, while the Folin-Ciocalteu and DPPH methods were used to determine the total phenolic content and antioxidant activity, respectively.
The plants used in this experiment were selected based on criteria derived from previous research, such as degree of acclimatization in pots, ornamental use, culinary use, and others. Specifically, the following plants were used: *Aloysia citrodora, Salvia officinalis, Pelargonium graveolens, Artemisia draconiana, Origanum majorana, Thymus vulgaris, Saturejia thymbra, Rosmarinus officinalis, Sideritis syriaca (malotira)*. Apart from *Aloysia citrodora*, which belongs to the Verbenaceae family, the other plants belong to the Lamiaceae family, to which most of the popular MAPs belong.

Quantitative evaluation
For the quantitative assessment, the growth parameters of MAPs such as height and width were recorded. The crown of these plants approximates the shape of a circle, so the dimension of their width was used as the diameter. To evaluate the vertical growth of the plants, the height from the ground surface to the top of the plant was measured, while the largest dimension was chosen for the width. A ruler was used for this purpose (Zubay et al., 2021).
The data sets included 6 measurements within 111 days of cultivation. In particular, the growth of all plants was measured, but due to the needs of this study, only the measurements of *Aloysia citrodora* and *Salvia officinalis* in the 6 pots were evaluated. These data were then transferred to MS EXCEL and processed accordingly. When processing the data, it was necessary to calculate the growth rate of the plants daily, as the measurements were not taken on fixed dates. The growth rate (rate of increase in height/length) per day is determined by dividing the increase in height or diameter by the number of days since the previous measurement and is expressed in 'centimetres per growing degree day' (Horak & Loughin, 2000).

Qualitative evaluation
For the experimental part, the dry matter (DM) content was determined by the gravimetric method - water removal by evaporation and weighing. Specifically, the total dry matter was determined by weighing 1 g of the sample and drying it at 105 °C using a MAC 50 PARTNER thermal balance. The results were then expressed as percentages (Badea et al., 2022). To evaluate the total polyphenols content and the antioxidant activity of the 6 MAPs, methanolic extraction of the dried MAPs samples was applied using specific mass, volume, and extraction time (Ion et al., 2020).
The Folin-Ciocalteu method was used to determine the total polyphenol content, according to a protocol adapted from Stan et al. (2021). Specifically, 2.5 mL of Folin-Ciocalteu reagent (after 5-fold dilution) and 2 mL of 7.5% sodium carbonate were added to 500 µL of the

sample. The sample was incubated for 15 min at 50 C in a water bath, cooled in an ice bath, and read at a wavelength of 760 nm.
The DPPH method was used to measure antioxidant activity by adding 200 mL of polyphenolic extract solution and 2 μL of DPPH solution (0.2 M) in methanol. The mixture was stirred for 30 min in the dark with magnetic shaking and incubated. The results were recorded after the absorption process, which was measured at a wavelength of 515 nm (Ion et al., 2020). All measurements of the dry sample were processed using MS EXCEL and Open Document and presented in graph.

Results and Discussion

Quantitative evaluation
The results of the plant growth measurements were grouped by height/width dimensions and by date of measurement. As shown in Figure 1, among all the plants, (A1) had the highest growth in height (142 cm) and the lowest in width (17 cm), while (A3) had the lowest growth in height (22 cm) and the highest in width (46 cm). For *Salvia officinalis*, S6 had the highest growth in height and width with 58 cm and 40 cm respectively, while S4 had the lowest growth in height and width (43 cm and 30 cm). In particular, the decreasing order of growth of *Aloysia citrodora* plants based on height is A1, A2, A3, and based on width A3, A2, and A1. Similarly, for *Salvia officinalis* plants, the ranking based on height is S6>S5 >S4, and based on width is S6>S5>S4.

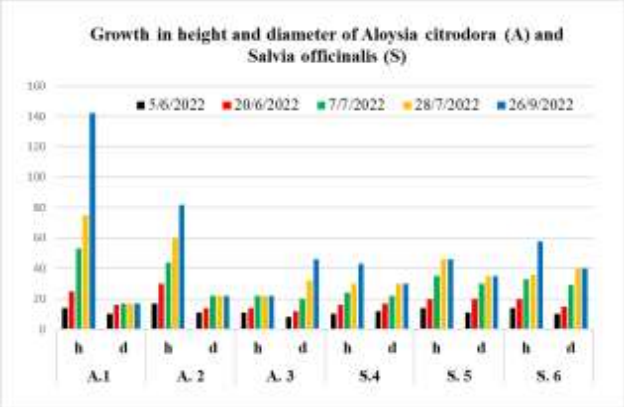

Figure 1. Total plant growth diagram

Converting the plant growth values into percentages (%), it can be seen that the growth rate (height/width) of A2 (potted plants, *Aloysia citrodora*, *Pelargonium graveolens*, *Artemisia draconiana*) is (79/50) and differs from the rate (63/75) measured for *Aloysia citrodora* with the same plant composition but grown in soil (Maknea et al., 2022a).
On the other hand, it can be mentioned that a simple way to get an initial idea of whether and how two variables (height, width) are involved - correlated - is to construct a scatter plot, i.e. the representation of the values from pairs of observations on a graph (Puniya and Sing, 2019). In this way, Figure 2 illustrates the correlation between height and width during plant growth to provide information about the overall growth and shape of the plants. By analyzing the correlation, it is checked whether the plant has a growth ratio as it is desirable to maintain the overall form and stability of the plant (Sharma et al., 2019). The correlation may also reflect the plant's strategy to optimize light fixation, overall resource use, air temperature, etc. (Tumber-Dávila et al., 2022).

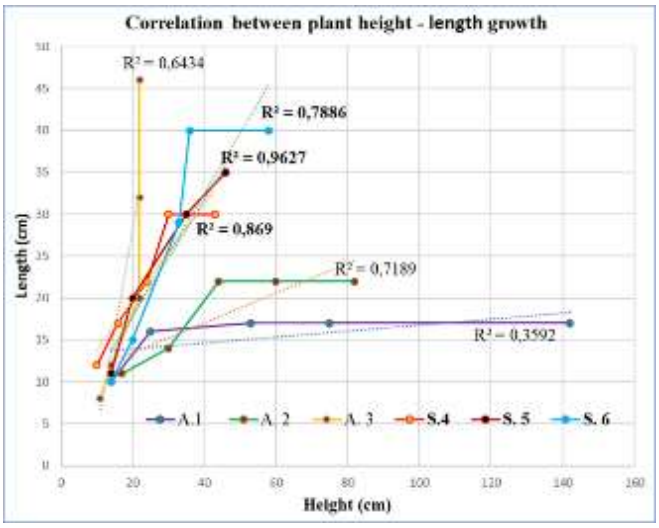

Figure 2. Correlation plot of growth variables

The scatter plot shows that some taller plants have a greater height and a greater width. There seems to be a proportional relationship between the height and width of these plants. It is therefore necessary to analyse the correlation between these 2 variables to conclude. The results showed that the regression coefficients between the width and height of the plants studied ranged from 0.36 to 0.72 for *Aloysia citrodora* and from 0.79 to 0.96 for *Salvia officinalis*. This means that the linear correlation is weak to moderate for A1, A2, and A3 plants and strong to very strong for S4, S5, and S6 plants (Bevans, 2020).

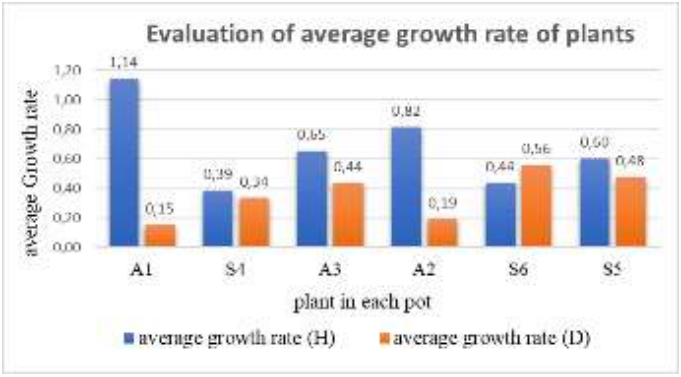

Figure 3. Comparative graph of average plant growth rate

As mentioned above, the plant growth rate per day index was used to understand the plant growth responses in each pot. Except for A1, which shows a large irregularity in the average growth rate of height and width (1.14/0.15), A2 and A3 show regularity. On the other hand, S4, S5 and S6 did not show any irregularity in the ratio of average growth rate (height/width). For example, the growth rate of the height of *Salvia officinalis* (in pots) ranged from 0.39 cm - 0.60 cm, while from experimental data of a thesis, where the culture took place in the soil, the values ranged from 0.35 cm - 0.65 cm in the same growing season (Giannousa, 2020).

Results of the qualitative assessment

The Folin-Ciocatleu method was used to determine the total phenolic content (CTP) and the DPPH method was used to determine the antioxidant capacity values of the dried leaf samples of MAP. In particular, Table 2 shows the results of the qualitative analyses of 6 samples of dried leaves of MAP. In each sample, the plant tested is marked in bold.

Table2. Results of analysing the total phenolic and evaluation antioxidant capacity of plant extracts

Pot	Sample	Cultivation country	CTP (mg GAE/g)	DPPH (mg equiv Trolox/ g)
1	*Aloysia citrodora*	Romania	16.35	387.60
2	*Aloysia citrodora, Pelargonium graveolens, Artemisia draconiana*	Romania	38.82	851.34
3	*Aloysia citrodora, Origanum majorana, Thymus vulgaris*	Romania	24.94	531.98
4	*Salvia officinalis*	Romania	46.34	731.25
5	*Salvia officinalis, Thymus vulgaris, Saturejia thymbra*	Romania	68.00	978.17
6	*Salvia officinalis, Rosmarinus officinalis, Sideritis syriaca (malotira)*	Romania	30.06	458.53

The results showed that the dried leaves of S5 contain the highest antioxidant components (978.17 mg equiv Trolox/g) and the most total phenolics (68.00 mg GAE/g), whereas A1 has the lowest antioxidant capacity (387.60 mg equiv Trolox/g) and the least total phenolics (16.35 mg GAE/g). In particular, the descending order based on DPPH and CTP values is A2, A3, and A1 for *Aloysia citrodora* plants and S5, S4, and S6 for *Salvia officinalis* plants.
To assess the suitability and reliability of the methods used, linear regression and correlation analysis was performed on the values between total antioxidant capacity and total phenolics of dried leaf samples of MAPs. Figure 5 shows the positive linear correlation between the two variables, accompanied by very high correlation coefficients (r2), for *Aloysia citrodora* (r2=0.99) and *Salvia officinalis* (r2=0.98).

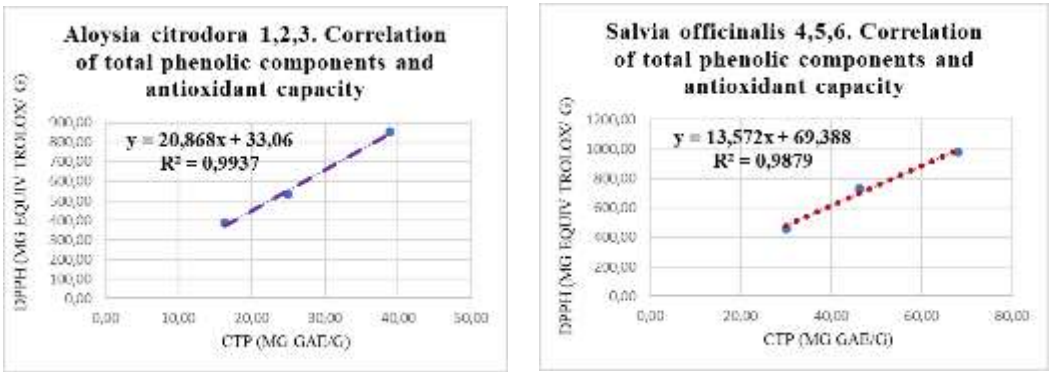

Figure 5. Correlation between CTP and DPPH for *Aloysia citrodora* and *Salvia officinalis*

Conclusions

The co-cultivation of MAPs as a planting strategy seems to be able to contribute to the qualitative and quantitative growth of plants while promoting the protection of the ecosystem, as there are indications that on the one hand, it optimizes nutrients and plant productivity, and on the other hand it also contributes to the attraction of beneficial insects, thus reducing the need for pesticides. More specifically, since the productivity of a plant is linked to its volume, an important factor in harvesting is the measurement of plant growth in height and width by checking the correlation coefficient between the 2 variables. As can be seen from the results,

the strongest coefficients were found in samples S5 (*Salvia officinalis* co-cultured with *Thymus vulgaris* and *Satureja thymbra*) and A4 (*Aloysia citrodora* co-cultured with *Pelargonium graveolens* and *Artemisia draconiana*). Another important element concerns the growth rate values since the results show that the same samples also had a balanced growth. This is an advantage for the plant as it contributes to better light absorption, aesthetic appearance, optimization of resource use, etc. Remarkably, the qualitative analysis showed that the plants with the highest antioxidant capacity and total phenolic compounds were also S5 and A2.

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ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

RELATIONSHIPS BETWEEN WILD FLORA, CROPS, APHIDS (HEMIPTERA, APHIDIDAE) AND THEIR NATURAL ENEMIES IN CITRUS ORCHARDS

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Abstract

The survey was carried out in some citrus orchards in the area of Bernalda, in the region of Basilicata, south Italy. Aphids and their natural enemies were monthly sampled from citrus tree canopies and spontaneous plants in four citrus orchards differently managed (organic and conventional with and without herb layer). Four weed species (*Rumex crispus*, *Sonchus oleraceus*, *Euphorbia peplus* and *Vicia sp*.) were reported as hosts of four non-pest aphids of citrus, which were *Myzus persciae*, *Acyrthosiphon pisum*, *Hyperomyzus lactucae* and *Aphis rumicis*, serving as preys for natural enemies including coccinellids (Coleoptera, Coccinellidae), lacewings (Neuroptera, Chrysopidae), hoverflies (Diptera, Syrphidae) and various parasitoid species (Hymenoptera, Braconidae) that were recorded on citrus canopies. Statistical analysis, using parametric tests of collected data, showed a positive correlation between weed management systems and wild plants hosting aphidophagous communities ($P < 0.05$). Moreover, organic weeding approaches have had a positive effect on aphid antagonist richness and abundance, whereas the conventional weeding method may cause scarcity of aphidiphagous, but interactions among strata (entomofaunal diversity on citrus tree canopies & wild plant cover) are still unclear. Overall, this study is a further step towards the investigation of the interaction between plants, crops and arthropods in citrus orchards in the Mediterranean basin to make weed management strategy a key for aphid management in crops.

Key words: *Citrus, wild flora, aphids, Basilicata, natural enemies.*

Introduction

Citrus orchard hosts several pests and diseases, and some of them are very harmful to citrus trees. Aphids (Hemiptera, Aphidoidea) are an important group of insects in Italy as they are throughout the world (Addante *et al.*, 2009). Aphids belong to the most important agricultural pest worldwide (Hulle et al., 2020), causing direct damage by plant feeding and indirectly as vectors of plant viruses (Conti, 1985; Blackman and Eastop, 2000; Katis *et al.*, 2007; Yokomi *et al.*, 2018). Citrus aphid pests are generally controlled by chemicals (Chouibani *et al.*, 2001). Pesticides, whether synthetically or botanically derived, are powerful tools and should be used with caution (Furk *et al.*, 1980; Sun *et al.*, 1987; Hosoda *et al.*, 1992). However pesticides decrease the biodiversity of a system, creating the potential for instability and future problems (Altieri, 1999; Altieri and Nicholls, 2004). The performance of natural enemies in agricultural systems is often limited by the absence or scarcity of essential resources (van den Bosch and Telford, 1964).Arthropod communities exploiting the soil, the grass and the canopy cohabit within the orchard and contribute to its richness (Miliczcky *et al.*, 2000). As high levels of beneficial arthropods are displayed within the herb cover, whereas a low level of predation and pest control are observed in the arboreal strata (Simon *et*

al., 2007), strong interactions among strata are not always established in field experiments (Simon *et al.*, 2010). There is therefore a need for basic research in arable systems to understand the links between biodiversity, ecosystem function and sustainability.
Due to the lack of detailed data describing the behavior of aphids on different weeds, manipulation of weedy host plants in cultivated areas may result in crop damage caused by aphid outbreaks (Perng, 2002). The objective of this study was to determine the relationship between aphids, their natural enemies and wild plants in differently managed citrus orchards and to provide information to aphid control and weed management programs.

Material and Methods

The survey was conducted from April to July 2008 in Basilicata Region. Four experimental plots were chosen in the area surrounding Bernalda in the province of Matera located at 127 meters above sea level. Two of four orchards were located in the Pantanello farm, coded as PAN1 the unweeded and as PAN2 the weeded one, together constituting the organic orchards (group A). The other two were located in the Florio farm, 5 km West of Pantanello farm (Fig. 1), coding as BER1 the weeded and as BER2 the unweeded one, together constituting the conventional orchards (group B). The selected orchards were managed differently according to the type of farming and the current situations (pest appearance, climate, etc.). Orchard characteristics are shown in Table 1.

Figure 1. Map with the location of the experimental plots.

Table 1. Characteristics of the selected citrus orchards.

	Pantanello farm		**Florio farm**	
Name of orchard	Pantanello 01	Pantanello 02	Bernalda 01	Bernalda 02
Geographical coordinates	Lat: N 40.390 Long: E016.174	Lat: N 40.390 Long: E016.171	Lat: N 40.392 Long: E016.654	Lat: N 40.392 Long: E016.653
Code of orchard	PAN1	PAN2	BER1	BER2
Variety	Navels	Navels	Navels	Navels
Type of management	Conventional With weeds	Conventional Weeded	Organic Weeded	Organic With weeds
Type of irrigation	Drip irrigation	Drip irrigation	Drip irrigation	Drip irrigation
Soil's texture	Sandy clay loam	Sandy clay loam	Clay loam	Clay loam
Age (years)	20	8	20	20
Surface (ha)	1.5	4	2	3
Planting distance (m)	5*5.5	5*6	5*5.5	5*6

Methodology of sampling

The sampling survey were done on a surface of 1 ha per orchard choosing 5 trees falling on the diagonal line of each orchard by considering one tree and skipping the next until counting 5 trees. For weed collection, we placed one quadrate of 25 x 25 cm in correspondence of each cardinal point on the soil at the base of each tree, about 50 cm away from the tree trunk and corresponding to the projection of the tree canopy.At each site, the wild plant community was assessed monthly, from April to July.

Both aphids and their natural enemies were collected from wild plants monthly on the 5 selected trees of each orchard. All the plants falling into each frame (sampling area) were collected and put into plastic bags. Collection of arthropods on citrus trees. On each of the five trees per orchard, eight shoots were monthly observed chosen at different height and orientations and collecting eight infested shoots per tree in case of infestation. All identifications have been carried out at the Laboratory of Zoology, by Dr Rocco Addante, Università degli Studi di Bari, Department of Soil, Plant and Food Sciences. Arthropods were identified up to family, genus or species (as far as possible). To compare the effect of management on the tested variables One Way Completely Randomized ANOVA has been used.

Results and Discussion

Wild plants in the selected citrus orchards. In the four citrus orchards, 32 plant species were recorded, which belonged to 20 botanical families. Two of these families were monocotyledonous (including 6 species) and 18 were dicotyledonous (including 26 species). Identification of aphids and related antagonists. During the investigation on the four orchards, many aphid species and antagonists were found associated with wild plants and citrus trees. Table shows the aphid guild recorded on citrus and weeds. In citrus orchards, *A. gossypii* Glover, 1877 and *A. spiraecola* Patch, 1914 were the most commonly recorded aphid species, in addition to *A. fabae* Scopoli, 1763 which has been encountered on citrus in April only. While, in plants naturally occurring in the citrus crop, *Rumex crispus* served as host for *Aphis rumicis* (Linnaeus, 1758), whereas *Sonchus oleraceus*, *Euphorbia peplus* and *Vicia spp*. hosted *Acyrthosiphon pisum* (Harris, 1778), while *Myzus persciae* (Sulzer, 1776) and *Hyperomyzus lactucae* (Linnaeus, 1758) were found on *Sonchus oleraceus.*

Table 2. Aphids and their natural enemies associated with wild plants and citrus in the four citrus groves.

Categories	**Species, genus or family**	**Host plant**
Aphids on citrus	*Aphis gossypii* *Aphis spiraecola* *Aphis fabae*	*Citrus* spp.
Aphids on weeds	*Myzus persicae*	*Sonchus oleraceus*
	Hyperomyzus lactucae	*Sonchus oleraceus Euphorbia peplus*
	Acyrthosiphon pisum	*Vicia* sp.
	Aphis rumicis	*Rumex crispus*
Beneficials	*Coccinella septempuctata* *Adalia bipunctata* *Adonia variegata* *Scymus* sp. *Chrysoperla carnea* *Syrphus* sp. Braconidae	*Citrus* *Rumex crispus Euphorbia peplus* *Sonchus oleraceus* *Vicia* sp.

The systematic sampling method with random collection of citrus shoots and herb layer species that was adopted for this research work in addition to the irregular distribution of both naturally occurring plant cover and arthropods either on citrus or under canopies have led to the data values heterogeneity translated by the standard deviation high values. As shown in Fig. 4B, ANOVA pointed out a clear effect of farming management on "host plants" colonized by non-pest citrus aphids ($P>0.05$). The mean number of "host plants" colonized was 2 for BER2, and 1.2 for BER1, whereas in PAN1 and PAN 2 no "host plant" was found colonized by aphids, so the two conventional managing orchards were completely free from primary consumers (aphids) which can support communities of natural enemies. ANOVA didn't show a positive correlation between neither farming systems nor weeding systems ($P>0.05$), although the infestation rate of citrus aphids A. spiraecola and A. gossypii was different at each citrus orchard, independent of management approaches as shown in Fig. 4C. ANOVA performed to compare the effect of the farming management types on the mean number of predators on citrus did not show a significant difference between the four fields (Fig. 4D).

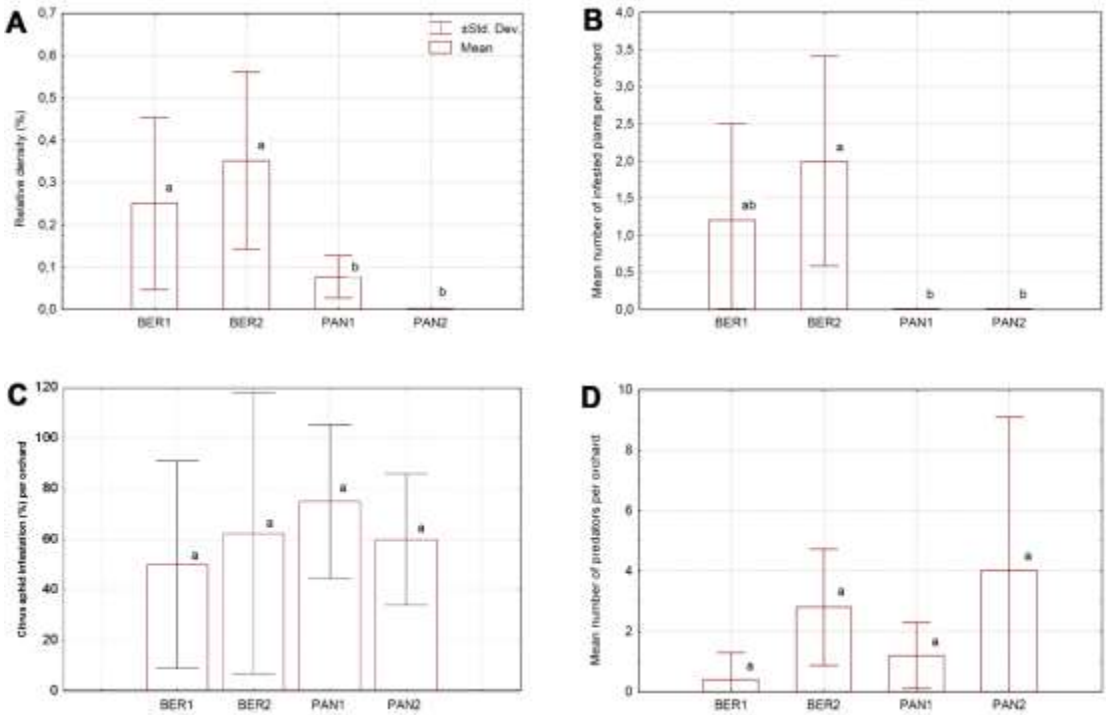

Figure 4. Results of ANOVA applied to: A. Mean relative density of host plant; B. Mean number of colonized host plants; C. Percentage of citrus aphid infestation; D. Mean number of predators on citrus, in the four citrus orchards (organic weeded BER1, unweeded organic BER2; and (unweeded conventional PAN1 and weeded conventional PAN2).

Floristic diversity in the studied areas was high with a specific richness of 32 species. Among them four species: R. *crispus*, *S. oleraceus*, *Vicia sp.*, and *E. peplus* might provide habitats, food sources, reproduction sites, or over- wintering shelters for several species of aphids and many beneficial belonging to several orders (Kozàr *et al.*, 1994; Simões *et al.*, 2004). During the sampling dates, four aphid species and several aphidophagous species including predators and parasitoids and belonging to four different orders have been recorded on naturally occurring plants in the studied citrus orchards.

Aphis gossypii and *A. spiraecola* were the dominant aphid species in all studied citrus orchards. According to Blackman and Eastop (2006), the majority of species belonging to the genus Aphis were reported on herbaceous plants, more rarely are the species that live on perennial plants. *A. gossypii* and *A. spiraecola* are part of this minority. They are the main aphid species on citrus in Algeria (Labdaoui *et al.*, 2018; Labdaoui, 2019). While Aphis fabae which is a very polyphagous species, anholocyclic on secondary hosts in Northern Africa and the Middle East, found particularly on Solanaceae (Müller, 1982), it is a secondary pest on citrus (Blackman and Estop, 2000). On wild plant cover, several aphid species not harmful to citrus have been identified. They were recorded from four spontaneous plants (Table 2). These aphid species may in fact be providing some control of the weeds, serving as an alternate resource for aphid predators and parasitoids (Jadot and Roland, 1971; Wyss, 1995). Several aphid natural enemies have been recorded during this work on both citrus and host plants. Aphidophagous ladybeetles (Coleoptera, Coccinellidae) were the most numerous species that have been found living on "host plants" under several stages (larvae, pupae, and adults). These generic predators were the most important aphid natural enemies occurring on citrus, *R. crispus*, and *S. oleraceus* in BER1, BER2, however in PAN2, citrus and *E. peplus* were harbouring most aphidophagous.

Although ANOVA didn't show a significant effect of both farming and weed management on the density of aphid predators (ladybeetles, hoverflies, and green lacewings) on citrus, the

mean number of predators occurring in the organic unweeded citrus orchards BER2 was higher compared to the conventional unweeded citrus orchard PAN1, with 2.8 and 1.2 predators per orchard respectively (Fig. 4D). In contradiction to the mean number of predators in the weeded citrus orchard either organic or conventional was low, which can be probably explained by the hypothesis of resources concentration. In fact, the infestation of A. gossypii and A. spiraecola in BER2 and PAN1 was high enough to attract predators on the trees (Fig. 4C). These predators prefer to occupy an area only if prey density is sufficiently high (Honek, 1994; Dixon, 2000). The "host plants" and aphid populations feeding on them might have attracted the predators occurring within or outside the orchards. Many authors have strengthened this hypothesis revealing that weeds influence the abundance of coccinellids in the fields, including the timing of colonization of the field by the predators in addition to the density of aphids (Coderre and Tourneur, 1986; Cottrell and Yeargan, 1998b; Wu *et al.*, 2010). While, others reported that as high levels of beneficial are displayed within the herb cover, whereas low level of predation and pest control are observed in the arboreal strata (Simon *et al.*, 2007). A similar observation was observed in the organic unweeded organic citrus orchard BER2, where the severe attack of aphid pest species was recorded on citrus new shoots, although, various aphidophagous species (coccinellids, lacewing and hoverflies) were abundant on host plants (Fig. 4C).

Conclusion

The survey work has ended revealing some interesting conclusions. 32 plant species have been recorded in the four orchards belonging to 20 families. *Cynodon dactylon* was the most abundant and it is the most harmful among the weed species range occurring in the citrus orchards. *Aphis gossypii* and *Aphis spiraecola* were the main aphid pest species occurring in citrus orchards whereas three aphid species absolutely harmless on citrus *Aphis rumicis*, *Acyrthosiphon pisum*, and *Hyperomyzus lactucae* were serving as natural limitation agents of four weed species currently occurred in citrus orchards *Rumex crispus, Sonchus oleraceus, Euphorbia peplus* and *Vicia sp*. The latter have provided habitats for several aphidophagous insects. Furthermore the comparative study on the effect of different farming management has shown that organic farming is more able to provide habitats and preys for beneficials in the citrus agroecosystem. Citrus agroecosystem showed an interesting biodiversity potential hence this work is a further step toward the investigation of the interactions between plants, crops and arthropods in citrus orchards of the Mediterranean Basin.

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SOIL WETNESS AND VEGETATION TYPES OF SEMBERIJA REGION (BOSNIA AND HERZEGOVINA) ACCORDING TO COPERNICUS DATABASE

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Abstract

The aim of the research is to precisely determine different types of soil moisture and vegetation at high density population region of Semberija. Semberija region is a lowland in the northeast of Bosnia and Herzegovina (B&H). It is characterized by Sava and Drina rivers that form the border from the north (Croatia) and the east (Serbia). Those rivers are the most important cause of the occurrence of temporary wetland, which occupies as much as 18.2% of the region. On the other hand, grassland covers 16.3% and forest occupy 21.7% of the territory. Regarding forests, the most common is broadleaved vegetation. In this research we used three of Copernicus' database, Water and Wetness (WaW), Forest - Dominant Leaf Type (DLT) and Grassland (GRA). All are based on the photointerpretation of satellite images Sentinel-1 and Sentinel-2, in resolutions 10 m and 20 m. Wetlands are divided into four categories, based on the registered percentage of water and moisture during the year: permanent water, intermittent water, permanent wetland and temporary wetland. Compared to other B&H regions, Semberija belongs to one of the wettest areas because of the following factors: low groundwater level, proximity to the Drina and Sava, and lowland flat terrain. Bearing in mind the aforementioned factors the risk of flooding is higher than in other parts of B&H. At the same time, Semberija belongs to one of the poorest areas when it comes to vegetation generally, especially considering the fact that grassland and forest vegetation cover about 80% of the territory of B&H.

Keywords: *wetness, vegetation, Semberija, Copernicus, database*.

Introduction

Semberija is mainly agricultural region in the northeast of Republic of Srpska (RS) political entity within Bosnia and Herzegovina (B&H). The region covers a lowland area of 734 km^2, with Sava river at north, Drina river at east and southeast and Majevica mountain towards southwest and west. The climate is moderate-continental, with the average annual air temperature is 10.5 ^{o}C and precipitation is 737 mm for the period 1961-1990. (RHI RS; Bajic & Trbic, 2016). The capital of the region is Bijeljina, city located at the centre of the region, with about 114.000 inhabitants according to the 2013 census (AS B&H, 2016).

Soil moisture plays a pivotal role in vegetation dynamics, considering that soil water availability is a crucial limiting factor for plant photosynthesis (Proietti et al., 2019). Consequently, soil moisture maps provide an invaluable resource to quantify the effects of rainfall deficits on vegetated lands (Cammalleri et al., 2015). The vegetation structure and plant species composition are influenced by soil moisture, soil nutrient availability, strong biogeographical, and climatic gradients (Škvorc et al., 2020). The floristic composition of grass vegetation depends on the ecological conditions of the habitat, geological base, soil type, terrain slope, and exposition (Aćić, 2019). When it comes to forests, the same conditions are important for its density and biodiversity. Among other important functions, forest

vegetation prevents the occurrence of torrents and floods, and slow down soil erosion. This is the best biofilter for drinking water. The total area under forest in B&H in 2018 was 30498.57 km^2 or 59.55% of the territory, which is a decrease of 2.94% compared to 2012 (Drašković et al, 2021).

The importance of the research can be figured out in the possibility of agrotechnical zoning and determination of soil preference for certain agricultural crops having in mind soil types and its properties. The objectives of the study are to define the degree of soil moisture by zones and consequently dominant vegetation types as well. The paper could potentially have a function in inventorying, monitoring, and managing wet areas, grassland and forest vegetation in Semberija region.

Materials and methods

The Copernicus Land Monitoring Service (CLMS) provides geographical information on land cover and its changes, land use, vegetation state, water cycle and Earth's surface energy variables to a broad range of users in Europe. Pan-European High Resolution Layers (HRL) are raster-based datasets which provide information on specific land cover characteristics produced from satellite imagery through a combination of automatic processing and interactive rule based classification.

The WaW is a classified layer, which contains defined classes of permanent water, temporary water, permanent wet, temporary wet, and dry areas, derived from water and wetness occurrences in the period 2012-2018. It is complemented by the Water and Wetness Probability Index (WWPI) which indicates the occurrence of water and / or wet areas (with saturated soil moisture content) during a prolonged part of the year over a number of years (CLMS, 2020b). Permanent water comprises e.g. rivers and lakes while temporary water include e.g. temporarily inundated areas. Among the wet zones, permanent wet areas comprises e.g. areas of permanently high soil moisture while temporary wet areas include e.g. areas of changing soil moisture
(Langanke, 2016).

The GRA database includes natural, semi-natural and managed grasslands (according to their origin and utilization) as well as all types of grassland (permanent or seasonal) under highly heterogeneous biogeographic conditions (wet or dry climate, fertile or poor soil). Grass cover within the context of the product is understood as herbaceous vegetation with at least 30% ground cover and with at least 30% graminoid species such as *Poaceae, Cyperaceae and Juncaceae*. Additional non woody plants such as lichens, mosses and ferns can be tolerated (CLMS, 2020d).

Primary status forest layer Dominant Leaf Type (DLT) provides information about leaf type (broadleaved/coniferous). Apart from mapping the current status, it is possible to monitor change, which can be a result of manifold influences, e.g. related to climate or forestry (CLMS, 2020f).

The WaW 2018, GRA 2018 and DLT 2018 comprise the products in full spatial resolution of 10 m. The region of Semberija has been extracted and calculated by using the GIS tool Extract and Clip. The obtained data have been exported to Excel and individual areas have been calculated (according to spatial resolution). By using the tool Raster Calculator, the areas under grassland and forests have been calculated by wetness zones types.

Results and Discussion

According to WaW 2018 database (CLMS, 2020a), temporarily wet soil covers 133.7 km^2 (18.2%). It is area of changing soil moisture which means that moisture appears depending on

the season, amount of precipitation, water level of rivers, etc. Generally, in Semberija there are the following types of soil: brown soil, wetlands, alluvial soils, and podzols. The pedological composition of the soil in the region was created under the influence of various pedogenetic factors: geological composition, relief, water, climate, vegetation, man and weather factors. Brown meadow soil are the most valuable and abundant soils considering it has a high content of organic matter. They were created by the drifts of the Drina and Sava rivers (Pašalić, 1994). Permanent water cover 0.9% and refers to surface water bodies, primarily the Drina and Sava rivers, while permanent wet soil cover 0.1% of the territory, mostly in the alluvial plain around the rivers, Table 1.

Table 1. Water and wetness types at Semberija region (according to WaW 2018)

Class name/Area	km^2	%
Dry	593.2	80.8
Permanent water	6.4	0.9
Temporary water	0.2	0.0
Permanent wet soil	0.4	0.1
Temporary wet soil	133.7	18.2
Total	733.9	100.0

When it comes to vegetation, grasslands and forests cover a total of 278.8 km^2 or 38% of the territory. Based on the analysis of the GRA database for the year 2018 (CLMS, 2020c), grass vegetation in the area of Semberija covers a total of 119.6 km^2 or 16.3% of the territory, Table 2 In addition to classic meadows and lawns, park grass areas, uncultivated and grassed agricultural zones, etc. are also included. According to Copernicus DLT database in 2018 (CLMS, 2020e), forests in Semberija cover 159.2 km^2, i.e. 21.7% of the territory, of which 21.3% is broadleaved and 0.4% coniferous forest, Table 2. Conifers appear only in the peripheral hilly-mountainous zone in the southwest of the region.

Table 2. Grassland and forests in Semberija region (according to GRA 2018 and DLT 2018)

Classes/Area	km^2	%
No vegetation	455.2	62.0
Grassland	119.6	16.3
Broadleaved	156.6	21.3
Coniferous	2.6	0.4
Total	734.0	100

Compared to other regions in B&H Semberija has less vegetation percentage cover, considering it is the country with the highest forest share and the highest diversity of forest types in the Western Balkans (FAO, 2015), primarily due to the lowland relief, high population density and agriculture.

Using Raster Calculator option we can monitor the pixels of vegetation overlapping the pixels that represent wet and water areas. In this case, the results show that grassland cover temporary wet zones about 6 km^2 or 5% of the total grassland in Semberija, while forests, as can be expected, are very few on wetland, less than 0.1 km^2.

Speaking about spatial distribution, in areas closer to the rivers, the percentage of WWPI increases, the terrain is lower and the groundwater level is shallower. In some locations, swamp and marsh vegetation appears. Grassy vegetation is more common if you are closer to the rivers, while with increasing altitude towards the southwest, broadleaved forest prevails

(Figure 1). There is almost no coniferous forest. It appears only at the Majevica mountain on southwest of the region.

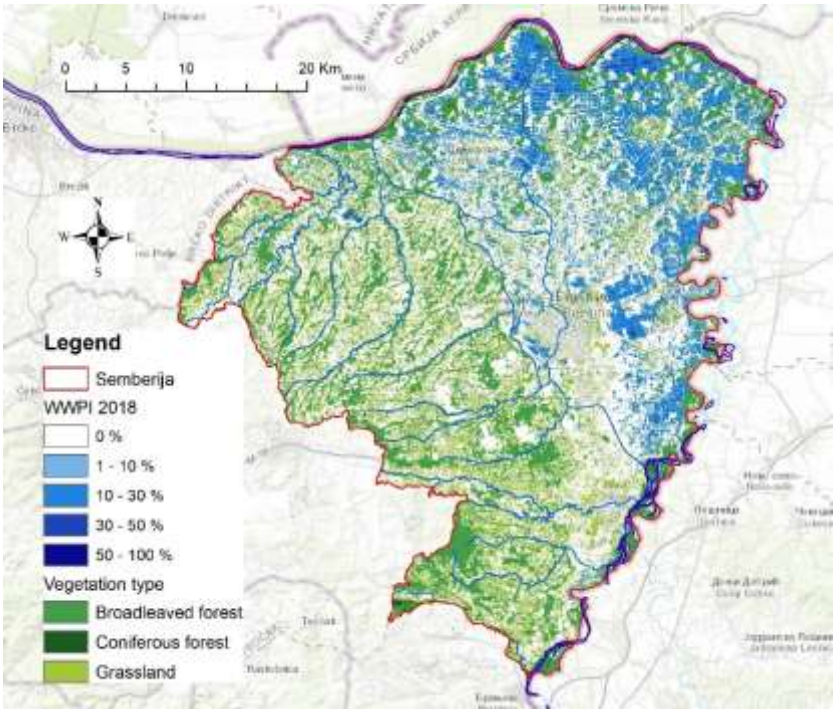

Figure 1: Map of WWPI and vegetation at Semberija region

Conclusions

According to the Copernicus WaW database 18.2% of the territory of Semberija region is covered by temporary wet soil, 0.9% is permanent water and 0.1% permanent wet area. Wetlands are located mainly around the Sava and Drina rivers and these zones are exposed to higher risk of floods. As you make distance from the rivers, the soil becomes drier and more suitable for agricultural crops. In lower zones natural vegetation is dominated by grasslands with 16.3%. With increasing of altitude grassland is replaced by broadleaved forest that covers 21.3% of the territory.

The Copernicus WaW, GRA and DLT databases are focused on the visual interpretation of satellite images and show the types of land cover and changes that occur on the ground over time. This is important in agricultural regions due to agroclimatic zoning and the definition of zones that are suitable for the cultivation of certain plant species. Also, in the case of Semberija region, mapping soil moisture and vegetation is important for flood prevention, especially considering the proximity of Sava and Drina rivers.

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POLLINATION AND SEASONAL DYNAMICS OF ANEMOPHILIC FLORA IN THE AREA OF THE CITY OF MOSTAR IN BOSNIA AND HERZEGOVINA

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Abstract

Allergenic plants are characteristic in relation to certain areas, depending on meteorological factors, vegetation cover and geographical-climatic area. Plant pollen is one of the most important airborne allergens for humans. Pollen grains cause allergic reactions (bronchitis, conjunctivitis, dermatitis, hay fever) in more than 20% of the human population (every fifth person). The negative impact on human health caused by the pollen of certain plant species classifies these particles as "natural" air pollutants. The concentration of plant pollen in the air depends on a number of factors that prevail in natural habitats and urban environments. Measurements of the concentration of pollen grains in the air in the area of the City of Mostar begin in mid-February and last until the end of October, that is, until the end of the growing season. The analysis covers three groups of allergenic plants: grasses, weeds and trees. By collecting and analysing data in the area of the City of Mostar during 2022, 56 types of pollen grains were identified in the air, of which grass, weed, cypress and pine pollen dominate. Pines and cypresses belong to the group of allergenic plants whose allergic potential is expressed at the beginning of the growing season, while grass pollen is present throughout the year. Data on pollen concentration are extremely important in assessing the level of exposure of sensitive persons to allergens, and it is necessary to continuously monitor such research.

Keywords: *Allergenic plants, Pollen, Ragweed, Grasses, Mostar*

Introduction

In recent decades, interest in urban flora research has been on the rise. Urban areas are extremely rich in plant species, primarily due to the great diversity of habitats (Jašprica et al., 2010), as well as due to the large amount of artificially or accidentally inhabited foreign species, some of which are invasive (Pyšek, 1998). Allergenic plants are characteristic in relation to certain areas, depending on meteorological factors, vegetation cover and geographical-climatic area.

Mostar is located at 43°21' north latitude and 17°49' east longitude (Gelo, 2020). It is located at an altitude of 40m to 70m. In terms of climate, Mostar belongs to the sub-Mediterranean zone of the eastern Adriatic coast (Horvatić, 1963). The mean monthly temperature of the hottest month is less than 22°C, while the mean monthly temperature of the coldest month is between 4°C and 13°C (FHZM).

Pollen causes seasonal allergies, the so-called. pollinosis, most often in spring. The most common causes of allergies are tree, grass and weed pollen. There are important differences in the number of pollen grains in a cubic meter of air that will cause the appearance of allergic symptoms and complaints. In principle, the concentration of grass pollen in the air is considered very high already at 200 pollen grains per cubic meter of air. Trees, on the other hand, will cause the appearance of symptoms in all allergic persons only at very high concentrations of pollen in the air (>1500 pollen grains/m3) (Sertić et al., 2012).

Hypersensitivity to pollen depends on the dominant type of pollen grains in the air for a particular region, depending on the season. The identification and quantification of pollen grains in the air enables the objectification of the condition and contributes to the timely treatment and prevention of symptoms (Prus and Čuljak, 2004). Measurements of the concentration of pollen grains in the air in the area of the City of Mostar begin in mid-February and last until the end of October, i.e. until the end of the growing season. Plant pollen is one of the most important airborne allergens for humans. Pollen grains cause allergic reactions (bronchitis, conjunctivitis, dermatitis, hay fever) in more than 20% of the human population (every fifth person). The negative impact on human health caused by the pollen of certain plant species classifies these particles as "natural" air pollutants. The concentration of plant pollen in the air depends on a number of factors that prevail in natural habitats and urban environments.
The aim of this work is to identify allergenic plants of the city of Mostar based on pollen collection, quantify them and compare their representation based on the period of flowering and the proportion of pollen grains in the air during pollination, in order to reduce the risks of allergies and accompanying diseases.

Material and methods

Measurements of the concentration of pollen grains in the air in the area of the City of Mostar begin in mid-February and last until the end of October, that is, until the end of the growing season. The analysis covers three groups of allergenic plants: grasses, weeds and trees. The measurement and analysis of pollen from the air was carried out at the Faculty of Agriculture and Food Technology, where the pollen collection device is located (Picture 1.).

Picture 1. Location of the pollen collection device

The collection is done with Burkard's volumetric spore and pollen collector with a built-in vacuum pump with a flow rate of 10 l/min (Figure 2). The device is adapted to collect particles from the air such as spores or pollen grains. The device is placed approximately 6 m above the ground, away from tall buildings or other obstacles. Reading is done by counting pollen grains under a light microscope (Olympus Bx41) at a magnification of 400 times. The obtained data are processed and displayed as the number and type of pollen grains per cubic meter of air (pz/m3). The processing of the obtained data determines the total daily concentration and in which period of the day the concentration of pollen grains was the highest.

Picture 2. Burkard's volumetric pollen and spore collector

Results and discussion

By collecting and analyzing data in the area of the City of Mostar during 2022, 56 types of pollen grains were identified in the air, of which grass, weed, cypress and pine pollen dominate. The allergenic properties of different plant species vary from weak to strong. (Table 1).

Table 1.

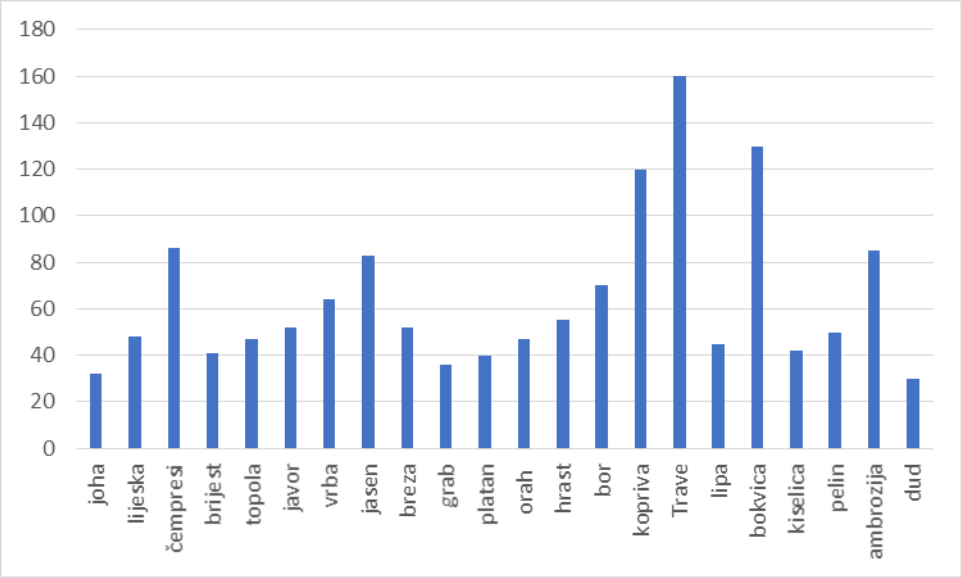
Species	**Family**	**Total**	**Allergic potential**
Sambucus nigra L.	*Adoxaceae*	287	Low
Amaranthus retroflexus L.	*Amaranthaceae*	320	Mild
Ambrosia artemisiifolia L.	*Asteraceae*	155	Very high
Artemisia absinthium L.	*Asteraceae*	225	Very high
Taraxacum officinale F. H. Wigg	*Asteraceae*	511	Low
Alnus spp.	*Betulaceae*	177	Mild to high
Carpinus betulus L.	*Betulaceae*	98	High
Corylus avellana L.	*Betulaceae*	753	Mild to high
Capsella bursa-pastoris (L.) Medik.	*Brassicaceae*	370	Low
Chenopodium album L.	*Chenopodiaceae*	820	Low to mild
Cupressus sempervirens L.	*Cupressaceae*	6720	Low to mild
Juglans regia L.	*Juglandaceae*	490	Low to mild
Robinia pseudacacia	*Fabaceae*	266	Low

L.			
Castanea sativa L.	*Fagaceae*	28	Mild to high
Quercus robur L.	*Fagaceae*	294	Mild
Morus alba L.	*Moraceae*	367	Low
Morus nigra L.	*Moraceae*	367	Low
Fraxinus excelsior L.	*Oleaceae*	854	Mild to high
Fraxinus angustifolia Vahl	*Oleaceae*	490	Mild to high
Olea europaea L.	*Oleaceae*	374	Mild
Pinus sylvestris L.	*Pinaceae*	4225	Low
Plantago major L.	*Plantago*	211	Low to mild
Plantago minor L.	*Plantago*	184	Low to mild
Platanus spp.	*Platanaceae*	1208	Mild
Avena fatua L.	*Poaceae*	243	Very high
Bromus erectus Huds.	*Poaceae*	185	Very high
Cynodon dactylon Pers.	*Poaceae*	168	Very high
Dactylis glomerata L.	*Poaceae*	284	Very high
Digitaria sanguinalis (L.) Scop.	*Poaceae*	213	Very high
Echinochloa crus-galli (L.) P.Beauv.	*Poaceae*	265	Very high
Elymus repens (L.) Gould	*Poaceae*	249	Very high
Hordeum bulbosum L.	*Poaceae*	86	Very high
Hordeum murinum L.	*Poaceae*	315	Very high
Secale cerale L.	*Poaceae*	106	Very high
Setaria viridis L.	*Poaceae*	330	Very high
Reynoutria japonica Houtt.	*Polygonaceae*	273	Mild
Rumex acetosa L.	*Polygonaceae*	148	Mild
Populus spp.	*Salicaceae*	1865	Low
Salix spp.	*Salicaceae*	479	Low
Acer campestre L.	*Sapindaceae*	140	Low to mild
Tilia cordata L.	*Tilia*	594	Low
Tilia platyphyllos L.	*Tilia*	394	Low
Ulmus spp.	*Ulmaceae*	510	Mild
Urtica dioica L.	*Urticaceae*	740	Low

During the period of grass pollen measurement, the total amount of pollen grains per m³ of air during the year was 2444, and the maximum concentration in one day was 260 grains per m³ of air. During the pollination period of weed plants, a total of 1740 nettle pollen grains were recorded with a maximum daily concentration of 98 grains per m³ of air. Nettle pollen does not belong to strong allergens, but due to its large amount it can cause increased allergic reactions in cross-reactions with other weed pollen.

Also, pines and cypresses dominate during tree pollen measurements, the total amount of cypress pollen grains per m³ of air is 6720, while the total amount of pine pollen grains is 4225 per m³ of air. By analyzing the results obtained from measuring the type and number of

pollen grains in the air in the City of Mostar, the length of pollination can also be seen (Graph 1).



Graph 1. Length of pollination

From the attached, the length of pollination is distinguished by grasses (about 160 days), followed by nettles (a little over 120 days), buckthorns (about 130 days), while all other species and families pollinate for less than 100 days.

Conclusion

During the measurement of pollen concentration in the City of Mostar, grass roots, weeds and pine and cypress pollen predominate. Tree pollen appears in the air in spring, grass and weed pollen in early summer, while weed pollen dominates during summer and autumn. Pines and cypresses dominate during tree pollen measurement, the total amount of cypress pollen grains per m³ of air is 6720, while the total amount of pine pollen grains is 4225 per m³ of air. During the period of grass pollen measurement, the maximum concentration in one day was 260 grains per m³ of air. In the season of pollination of weed plants, the maximum daily concentration of nettles was recorded with 98 grains per m³ of air. The increased number of people with allergic problems, especially to pollen, is directly conditioned by the modern way of life (sterile food, vaccinations, antibiotics, etc.), as well as significant changes in the environment. Regular monitoring of the concentration of pollen grains in the atmosphere is of great importance, especially for those who are allergic to some type of pollen. People who are allergic to pollen should have precise information about the type and concentration of pollen, in addition to their daily activities and when they go on a trip. With this information in mind, they will be able to keep the symptoms of the disease under control and certainly improve their quality of life.

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STATE OF VASCULAR FLORA IN THE ENVIRONMENT OF BORAČKO LAKE IN BOSNIA AND HERZEGOVINA AND SUSTAINABLE DEVELOPMENT

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Abstract

Bosnia and Herzegovina represents one of the most unique regions in Europe in terms of biological diversity. One such natural resource is Boračko Lake. Research was carried out in the area of Boračko Lake in the period May - June 2022, with the aim of determining the diversity and distribution of plant species ing the coastal part of Boračko Lake. Using the method of Braun-Blanquet (1964), phytocenological recording was carried out in selected localities. The goals of the research were: to carry out field research and determine plant species in the researched area, and to determine life forms and flora elements for each plant species.

In the coastal part of the Lake, the vegetation of low forests and thickets has been developed, where 51 plant species have been identified, located in 23 families, the most numerous of which are Poaceae, Asteraceae and Fabaceae. Seven life forms were determined (H, G, T, P, H(Ch), H(G) and Ch) of which hemicryptophytes were the most numerous with 33 plant species.

Twenty-six flora elements are present for the largest number of species belonging to the europeansub-oceanic-central Mediterranean, sub-Atlantic central Mediterranean, and central Mediterranean. Most human activities lead to the rapid disappearance of rare and ecologically specialized species as well as the fragmentation of their habitats. Environmental protection ensures the complete preservation of the quality of the environment, the preservation of natural communities, and the rational use of natural resources in the best way for the environment, as a basic condition for healthy and sustainable development.

Key words: *Boračko Lake, phytocoenological recordings, floristic composition of vegetation, anthropogenic factor, sustainable development.*

Introduction

The Boracko Lake, situated near the town of Konjic, is an outstanding refuge of tertiary flora and vegetation which distinguishes itself with striking diversity of species. Due to a wide spectrum of ecological niches, many vascular flora species display indicator values as compared to the condition and load capacity of the adoption of life communities and eco system. As such, they can serve as a reliable biological-ecological indicator in assessment of the conditions and quality of the environment of mainland eco systems and landscapes.

It should be emphasized that, in terms of biodiversity, the Bosnia and Herzegovina area is among the most interesting in Europe (Ademović, 2018).

This area, situated at the foot of the Prenj mountain north of Konjic, is one of those poorly explored natural resources. The Boracko Lake is a unique natural resource, which is constantly exposed to negative impact of man. This is especially evident in terms of illegal construction which takes place in this area. The degree of manifestation of such activities questions the very existence of this colorful outing spot (Mihić,1985).

All, but especially refuge eco systems of the Prenj mountain (and thereby the Boracko Lake area), possess unique geomorphologic forms, variety of soil types, hydrological networks and eco climates. These have been generators of ecological and species diversity in Bosnia and Herzegovina (and the Dinarides as well).
This part of Bosnia and Herzegovina has true forms of nature, both living and inert, whereas the abundance of steno-endemic and relict plant and animal species gives importance to this area in terms of global biodiversity. The Rakitnica Canyon is among the most beautiful canyons in this area, while the refuges of Prenj, Cvrsnica and Cabulja are heartlands of development and survival of endemic species. Endemic species of deciduous, light-conifer and hygrophilic forests and shrubs, including those of willows, aiders, poplars and planes, and especially unique plant species in the ruptures of carbonate rocks and rock creeps give special importance to these colorful refuge biotopes (Redžić, Barudanović, Radević, (eds.), (2008).

Geographical position

The Boracko Lake is situated among the Prenj, Visocica and Bjelasnica mountain. It is a glacial lake which lies on the north-east foot of Prenj, 397 meters above sea level. It is surrounded by steep and wooded peaks of Crna gora (1343 m above sea level) to the west, and Tranjine (1055 m above sea level) to the east. The lake basin was formed in the Boracka creek through the process of glacial erosion. This elliptic-shaped lake is 786 meters in length and 402 meters in width. Its size is 0.26 square kilometers, and the lake shore is 2.4 kilometers long (Mihić,1985), (Picture 1.).

Picture 1. Position of research sites at the Boračko Lake

Material and Methods

Field research was conducted in 2022 during the vegetation season - spring (May-June) in the area of the Boračko Lake. The survey covered the coastal part of the Boračko Lake at sites: Izviđački dom, Kamp Boračko jezero, Staro selo Boračkog jezera i Blizina eko sela where phytocoenological analyses were done. Braun-Blanquet (1964) methodology was used, adapted to the research needs. Most plant species were identified on site, during fieldwork. When it was not possible, the samples were identified in the laboratory. Nomenclature of plant taxa was determined in accordance with Tutin et al. (1964-1985), Mucina (1997), Rodwell et al. (2002), Šilić (1984), Beck (1903-1927), and Hayek (1927-1933). The affiliation to the floral element and life form was assigned according to the data by Oberdorfer (2001) and Raunkiaer (1905).

Results and Discussion

The assessment of vegetation was conducted during May and June 2023 at the Busko Lake. These surveys covered four sites in total: Izviđački dom, Kamp Boračko jezero, Staro selo Boračkog jezera i Blizina eko sela Phytocoenological analyses and identification of plant species were conducted at each site (Picture 2., 3., 4 i 5.).

Picture 2.: Izviđački dom (foto: Knežević A.)

Picture 3. Kamp Boračko jezero (foto: Knežević A.)

Picture 4.: Staro selo Boračkog jezera (foto: Knežević A.)

Picture 5.:. Blizina eko sel(foto: Knežević A.)

Elements that are directly or indirectly related to determination of the shape and the level of biodiversity are: geological and pedological characteristics, altitude, terrain inclination and the action of anthropogenic factor, manifested through various forms.

Taxonomic analysis

Taxonomic analysis of riparian flora at selected sites of the Boračko Lake was performec at the family level. All taxa were sorted into 23 families. Out of 51 taxa identified at selected sites, majority of species were from the family Poaceae (11),Asteraceae (7), Fabaceae (4), Scrophulariaceae (3) and Plantaginaceae i Oleaceae (2), with all other families represented by one taxon each (Table 1).

Table 1. Taxonomic affiliation of plant species in the researcharea of Boračko Lake

Familia	**Species**	**Familia**	**Species**
Poaceae	*Bromus erectus* Hudson	Scrophulariaceae	*Rhinanthus minor* L.
	Cynosurus cristatus L.		*Veronica chamaedrys* L.
	Lolium perenne L.		*Verbascum pulverulentum* Vill.
	Poa pratensis L.	Apiaceae	*Daucus carota* L.
	Anthoxanthum odoratum L.		*Anthriscus sylvestris* (L.) Hoffm.
	Poa trivialis L.	Lamiaceae	*Salvia pratensis* L.
	Holcus lanatus L.		*Ajuga reptans* L.
	Dactylis glomerata L.	Brassicaceae	*Alyssum montanum* L.
	Agrostis capillaris L.		*Lepidum campestre* (L.) R. Br.
	Arrhenatherum elatius (L.) J. et K. Presl.	Plantaginaceae	*Plantago media* L.
	Agropyron repens (L.) P. Beauv.		*Plantago lanceolata* L.
Asteraceae	*Leucanthemum vulgare* Lam.	Oleaceae	*Syringa vulgaris L.*
	Bellis perennis L.		*Fraxinus angustifolia* Vahl
	Taraxacum officinale Web.	Asparagaceae	*Ornithogalum umbellatum* L.
	Erigeron annua (L.) Nees	Vitaceae	*Vitis vinifera* L.
	Achillea millefolium L.	Ranunculaceae	*Ranunculus repens* L.
	Crepis biennis L.	Sapindaceae	*Acer campestre* L.
	Centaurea jacea L.	Rubiaceae	*Galium mollugo*
Fabaceae	*Trifolium pratense* L.	Cornaceae	*Cornus mas* L.
	Trifolium repens L.	Junglandaceae	*Juglans regia* L.
	Trifolium campestre Schreb.	Geraniaceae	*Geranium molle* L.
	Lotus corniculatus L.	Betulaceae	*Alnus glutinosa* L.
Rosaceae	*Rosa canina* L.	Malvaceae	*Malva sylvestris* L.
	Prunus domestica L.	Caryophyllaceae	*Silene vulgaris* (Mch.) Garcke
	Sanguisorba minor Scop.	Dipsacaceae	*Knautia arvensis* L.
		Polygonaceae	*Rumex crispus* L.

Spectar of Life Forms

A range of life forms was determined for the identified plant species at researched sites, amounting to a total of 7, with transitional forms.

Analysis of the structure of hemicryptophytes (H) in the coastal part of the Boračko Lake's flora showed that they were represented by a largest number of taxa (28). If we also include hemicryptophytes of transitional forms – H, G i H (Ch), it can be concluded that hemicryptophytes make up a total of 33 species. Phanerophytes (P) are the second most numerous life form (7 species), followed by terophytes (T) (3), hamephytas (Ch) (2) and geophytes (G) –- (1 species) (Chart 1.).

Spectrum of floral elements

Considering the climatic, geo-morphological, pedological and other diversities of the researched area, it was expected that vascular floral species present in the area would show a significant diversity of floral elements, which was found to be 27. The most numerous floral elements are: eurassubozean-smed (6), smed (4), no-euraassubozean, subatl-smed, subatl-smed, in ozean-temp. and osmed (3). Finally, other floral elements were represented by two or one species (Chart 2).

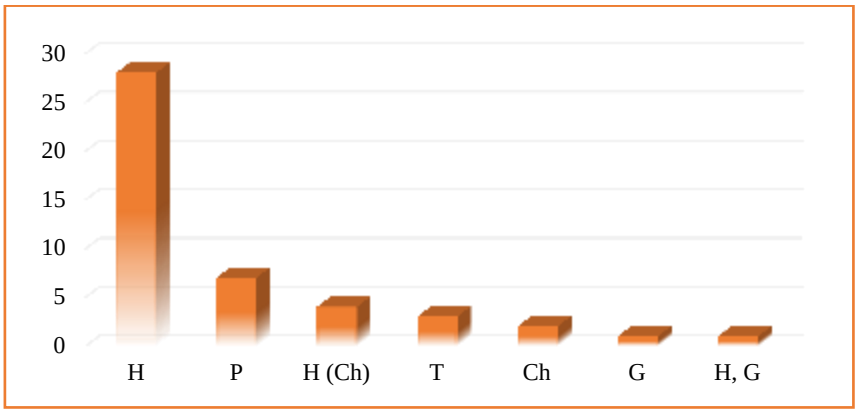

Chart 1.Total representation of life forms in the researched area of Boračko Lake

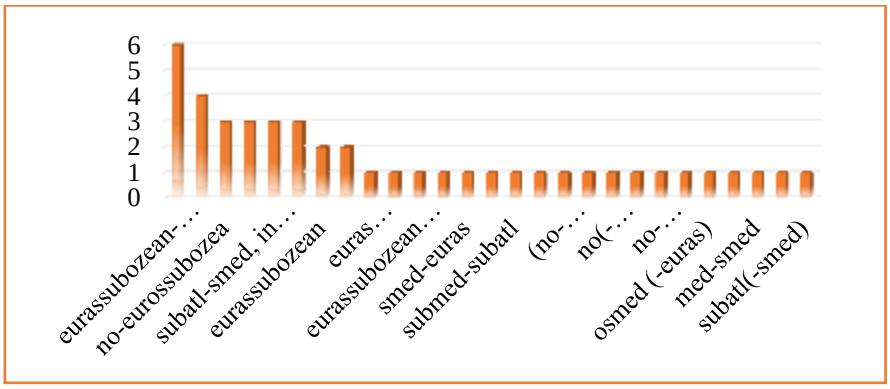

Chart 2. Total representation of floral elements in the researched area of Boračko Lake

Influence of pollution on flora and vegetation

The Boračko Lake is an area rich in natural beauty, however we also have to take into consideration a significant influence of an anthropogenic factor, which has created various problems on the lake due to human ignorance. Most human activities lead to the rapid disappearance of rare and ecologically specialized species as well as the fragmentation of their habitats. General long-term preventive measures against biotic and non-biotic agents, which could endanger the stability of the lake and its environment, will be based on water management that takes into account natural processes, as well as the maintenance and appropriate improvements of biodiversity conservation. Fundamental biological resources, including the Boračko Lake, must be effectively preserved, without further internal fragmentation, significant reduction in size or degradation through development and construction, in order to preserve the natural resources and ecosystem processes that depend on them. Environmental protection guarantees the complete preservation of environmental quality, the preservation of natural communities, the rational use of natural resources and

energy in the best way for the environment, as a basic condition for healthy and sustainable development.

Conclusions

The research results that included sampling sites: Izviđački dom, Kamp Boračko jezero, Staro selo Boračkog jezera i Blizina eko sela on the Boračko Lake, indicate that 51 taxa were identified within 23 families. The total spectrum of life forms in the researched area is 7 with transitional forms, while the spectrum of floral elements is 27.

These findings point to the need to organize additional comprehensive field research on parts of the Busko Lake area, which to date have been partially or almost never explored. These activities would certainly lead to new knowledge that would to a great extent update the present understanding of the biodiversity of flora in the Busko Lake area.

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ADVANCEMENTS IN ROBOTICS, SMART FARMING, AND COMPUTER VISION: REVOLUTIONIZING AGRICULTURE

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Abstract

The use of robotics, smart farming, and computer vision technologies is revolutionizing agriculture by making it more efficient and cost-effective. These advancements have transformed traditional farming practices and opened up new opportunities for agricultural productivity. Computer vision algorithms play a crucial role in the monitoring and management of crops in real-time. By leveraging sophisticated image processing techniques, these algorithms enable farmers to obtain timely and accurate information about the growth and development of their crops. This empowers them to make quicker and more informed decisions regarding essential farming practices such as fertilization, irrigation, and the application of pesticides. Robotics and automation systems have significantly contributed to the development of crop monitoring robots. These robots are equipped with advanced sensors, enabling them to perform tasks such as precision planting, targeted spraying of chemicals, and efficient harvesting. With modern technology, farmers can remotely monitor and control the growth and health of their crops. Deep learning algorithms, particularly convolutional neural networks, have shown immense promise in agricultural applications. These algorithms excel in image classification, object detection, and image segmentation, facilitating the identification of plant diseases, weeds, and other anomalies. The integration of robotics, smart farming, and computer vision technologies holds great potential to enhance resource use efficiency and productivity in controlled environment agriculture systems. This paper explores the recent advancements in robotics, smart farming, and computer vision technologies, highlighting their positive impact on the agricultural sector. These technologies are driving a transformative shift in agriculture, empowering farmers with actionable insights.

Keywords: *Smart farming, Automation, Computer vision, Robotics, Technology.*

Introduction

The realm of robotics is vast and encompasses a multitude of diverse technological domains. In recent decades, there has been a surge in the use of robotic systems across various fields, thanks to the advancements in visual identification, 3D reconstruction, positioning, and fault tolerance technologies (Yang, et al., 2016). Computer vision is a branch of technology that focuses on enabling machines to "see" (Tang, et al., 2020). It relies on cameras and computers, rather than the human eye, to detect, track, and measure objects for subsequent image analysis. The emergence of computer vision has led to its widespread application in the field of agricultural automation, where it has played a crucial role in driving the industry's progress (Hongkun, et al., 2019). In the world of agriculture, keeping a close eye on the growth and development of plants is essential to ensure optimal production processes. Traditional methods of monitoring plants, such as visually inspecting them or taking manual measurements, can be quite tedious and costly, resulting in less efficient production. Fortunately, the application of computer vision in agriculture provides a more effective way of monitoring the growth and development of plants. By analyzing images or videos of plants, computer vision algorithms can automatically extract plant characteristics and track their

progress over time. This enables farmers to monitor their plants in real-time and make quicker, more informed decisions regarding fertilization, irrigation, pesticides, and other farming practices.

While robots were mainly utilized for harvesting crops in the past, their applications in agriculture now extend to planting, spraying, harvesting, and monitoring. Of these applications, crop monitoring is particularly crucial for the efficient and stable performance of tasks such as planting, spraying, and harvesting. Therefore, various studies are underway to enhance the development of crop monitoring robots.

In a relatively brief amount of time, the vision community has made significant advancements in object detection and semantic segmentation accuracy. With the integration of deep learning techniques, the goal of computer vision is to train artificial intelligence systems to go beyond mere process automation and achieve greater levels of sophistication and accuracy. Thanks to these technological advancements, farmers no longer have to spend long hours in the field manually grading and scanning fruits and vegetables, which can be done effectively and remotely with the help of artificial intelligence.

Agricultural revolutions

The initial stages of the digital revolution primarily focused on automation techniques with limited computational capabilities (Khelifa, et al., 2015). Subsequently, intelligent agricultural systems incorporated sensors to gather climate and environmental data. However, in recent years, the landscape has undergone significant changes, with artificial intelligence and machine learning research concentrating on agriculture-related areas such as irrigation, livestock, and farms.

In the realm of irrigation, the emphasis has been on developing monitoring, control, and decision-making solutions to conserve water and enhance production (Goap, 2018; Mahalakshmi, et al., 2018; Nageswara Rao & Sridhar, 2018; Navarro-Hellín, et al., 2016; Rajalakshmi & Mahalakshmi, 2016; Zhao, et al., 2017). The advancement of devices and communication technologies has paved the way for increased integration of computational resources into agricultural systems. This integration aims to cater to diverse demands in agricultural automation, farm management, and precision farming (Ray, 2017). Solutions are evolving to encompass comprehensive management systems rather than mere monitoring, presenting new challenges and opportunities (Zanella, et al., 2020).

Implementation of agricultural robots

As the field of robotics advances at a rapid pace, there is ongoing innovation in the realm of agricultural robots, leading to their extensive utilization across diverse areas of agricultural production (Cheng, et al., 2023; Sales, et al., 2015). Agricultural robots, commonly referred to as machines specifically designed for farming production purposes (Reddy, 2016), are an essential component of the robotics family. These robots typically exhibit advanced perception capabilities, autonomous decision-making abilities, precise control, and accurate execution capabilities. Agricultural robots can be categorized into three types based on their application scenarios, including fields, orchards, and farms. Agricultural production follows a long-term cycle that involves essential steps such as seeding, planting, nurturing, harvesting, and processing. These steps play a crucial role in advancing agricultural industrialization. Additionally, agricultural robots can be classified based on their position within the industrial chain. This is shown in Figure 1.

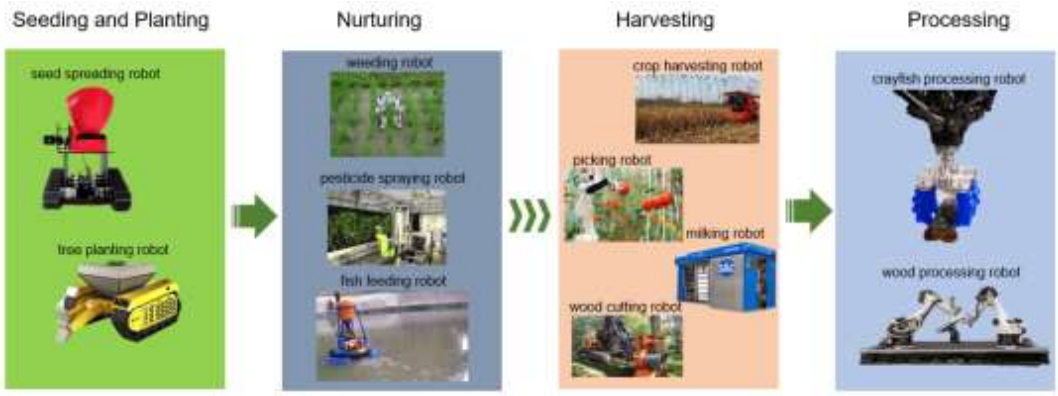

Figure 1. Agricultural robots along the industrial chain.

Sources: (Sori, et al., 2018; Oberti, et al., 2016; Pribadi, et al., 2020; Geng, et al., 2022; Wagner, et al., 2020)

The diverse and complex conditions of agricultural production necessitate the integration of exceptional adaptability, precise navigation, and obstacle-avoidance capabilities in agricultural robots. Consequently, these robots are typically composed of four key components essential for their operation: a vision system, a control system, mechanical actuators, and a mobile platform. These components work in harmony to ensure the successful execution of their tasks, as illustrated in Figure 2.

Figure 2. Components of agricultural robots

Sources: (Raj, et al., 2019; KATZSCHMANN, et al., 2018)

These four components have a significant impact on agricultural production. Firstly, the vision system utilizes a variety of cameras, including thermal, RGBD, TOF, and multi-spectral cameras, to capture data and transform it into images. Thermal images., are particularly effective in detecting hidden vegetables (Hespeler, et al., 2021). Secondly, the control system acts as the central processing unit of the robot, playing a crucial role in decision-making and motion planning. Thirdly, advanced mechanical actuators are essential for precise operations, especially when handling delicate fruits and vegetables. Lastly, mobile platforms enable robots to navigate, avoid obstacles, perform detection tasks, and carry out their assigned operations.

Advancements in computer vision for agriculture

Artificial vision systems serve as highly effective tools for automating the inspection process of fruits and vegetables (Cubero, et al., 2011). With the increasing advancements in computer vision, the application of this technology in crop monitoring is becoming increasingly significant.

Achieving agricultural sustainability can be accomplished by harnessing the power of vision-enabled autonomous machines working collectively to guarantee global food security. With the growing need for efficient and dependable food production methods, there has been a significant focus on leveraging computer vision and machine learning approaches to analyze and comprehend input images from various sources such as humans, robots, drones, sensors, and satellites. The fusion of computer vision, machine learning, and deep learning techniques enhances agricultural productivity (Uddin, et al., 2021).

Computer vision enables the automatic analysis of images and videos to obtain valuable information about crops. One key aspect of applying computer vision in crop monitoring is the identification and analysis of phenotypic changes in plants. This is achieved through the use of advanced machine learning algorithms, including models based on convolutional neural networks. These models enable high-throughput plant phenotyping, accelerating research on genetic functions associated with plant characteristics.

The use of multiple sensors to collect various spectra allows for the non-invasive acquisition of a range of data on plant development and physiological responses throughout their life cycle. These data are used for the analysis and modeling of genotype-phenotype relationships, enabling more precise predictions of plant characteristics. Another trend is the use of remote sensing through image collection platforms such as unmanned aerial vehicles and tractors for large-scale field phenotyping in crop breeding and precision agriculture.

This technology allows farmers to accurately monitor the growth, health, and development of plants, helping them make informed decisions regarding fertilizer application, irrigation, plant protection against pests, and other agricultural practices.

Automation and robotics in controlled environment agriculture

Automation technology has been in use for over 20 years in controlled production environments (Grift, et al., 2008). Automation and robotics play a vital role in the field of controlled environment agriculture (CEA). CEA refers to the practice of growing crops in indoor environments such as greenhouses, vertical farms, and plant factories, where various environmental factors like temperature, humidity, light, and nutrient levels are precisely controlled. The integration of automation and robotics in CEA systems has revolutionized the way crops are grown and managed. In CEA, automation systems are responsible for controlling and regulating environmental conditions, such as temperature, humidity, and lighting. They ensure that crops receive optimal growing conditions throughout their lifecycle. Robotic systems have also found extensive application in CEA. These robots are designed to perform various tasks, including seeding, planting, irrigation, nutrient delivery, crop monitoring, and harvesting. They are equipped with advanced sensors and imaging technologies to assess crop health, growth, and quality. Robotic systems offer precise and targeted operations, reducing the risk of human error and ensuring uniformity in crop management. As technology continues to advance, the integration of automation and robotics is expected to further revolutionize the field of controlled environment agriculture.

Challenges and future directions

Recent advancements in computer hardware and the availability of big data have opened up new opportunities for applying artificial intelligence (AI) in agriculture. AI algorithms heavily rely on the quantity and accuracy of data (Zhao, et al., 2023).

While the integration of automation, robotics, and computer vision technologies in agriculture has brought significant advancements, there are still challenges to address and future directions to explore:

- Scalability and affordability: Ensuring the affordability and cost-effectiveness of automation, robotics, and computer vision systems for farmers of all scales is crucial for widespread adoption.
- Integration and interoperability: As these technologies continue to evolve, it is important to focus on integrating and interoperating different systems and devices. Creating standardized protocols and interfaces that allow seamless communication and collaboration between different automation, robotics, and computer vision systems will promote compatibility and enable the development of integrated solutions.
- Adaptability to diverse environments: Developing adaptable technologies that can handle the diverse and dynamic nature of agricultural environments is essential. This includes developing robust algorithms and sensor technologies that can account for variations and uncertainties in real-world farming conditions.
- Data management and analysis: Automation, robotics, and computer vision technologies generate vast amounts of data. Developing advanced data analytics and machine learning techniques tailored for agricultural applications will enable farmers to leverage the full potential of the data collected by these technologies.
- Human-machine collaboration: While automation and robotics aim to reduce labor requirements, there is still a need for human involvement and expertise in agricultural operations. This includes developing user-friendly interfaces, intuitive control systems, and training programs that enable farmers and operators to work alongside automated systems.
- Ethical and social implications: The adoption of automation, robotics, and computer vision technologies in agriculture raises ethical and social considerations. These include concerns about job displacement, privacy, data security, and the impact on rural communities. Addressing these concerns and ensuring responsible implementation of these technologies is necessary to ensure their acceptance and long-term sustainability.
- Sustainability and environmental impact: Developing energy-efficient systems, optimizing resource usage, minimizing waste generation, and reducing reliance on agrochemicals are critical goals for future advancements.
- Education and training: To fully realize the potential of these technologies, there is a need for education and training programs that equip farmers, agronomists, and technicians with the necessary skills to understand, operate, and maintain automation, robotics, and computer vision systems. Building capacity and providing support for technology adoption will be vital in driving the successful implementation of these technologies.

While automation, robotics, and computer vision technologies have already made significant strides in agriculture, addressing challenges related to scalability, integration, adaptability, data management, human-machine collaboration, ethics, sustainability, and education will drive further advancements.

Conclusion

The integration of robotics, smart farming, and computer vision technologies in agriculture has brought about a transformative shift in the industry. The use of computer vision algorithms has played a crucial role in real-time monitoring and management of crops. By analysing images and videos, these algorithms provide farmers with timely and accurate information about the growth and development of their crops, enabling them to make informed decisions regarding fertilization, irrigation, and pesticide application. Robotics and automation systems have greatly contributed to the development of crop monitoring robots. Equipped with advanced sensors, these robots can perform tasks such as precision planting, targeted spraying of chemicals, and efficient harvesting. Deep learning algorithms, particularly convolutional neural networks, have shown immense promise in agricultural applications. These algorithms excel in image classification, object detection, and image segmentation, facilitating the identification of plant diseases, weeds, and other anomalies. The integration of robotics, smart farming, and computer vision technologies holds great potential for enhancing resource use efficiency and productivity in controlled environment agriculture systems. However, several challenges need to be addressed for further advancements. Ensuring the scalability and affordability of these technologies for farmers of all scales, promoting integration and interoperability, developing adaptable solutions for diverse environments, and addressing data management and analysis challenges are essential. Additionally, fostering effective human-machine collaboration, addressing ethical and social implications, prioritizing sustainability and minimizing environmental impact, and providing education and training programs are crucial for successful implementation and acceptance.

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THE CALCULATION OF GHG EMISSIONS OF CULTIVATED CROPS AND USAGE OF THESE CROPS IN FEED MIXTURES IN ANIMAL NUTRITION

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Abstract

In accordance with EU directives emerges the necessity to update the typical carbon footprint values of selected grown crops and, simultaneously, to develop a convenient calculator to quantify the carbon footprint for the main agricultural crops according to the local conditions of the farms. The carbon footprint value corresponds to the typical greenhouse gases (GHG) emission factor expressed in CO_2eq per unit product weight related to various local natural soil and climatic conditions of the Czech Republic. The emission factor of the main crops reflects the cropping technology with all crop cultivation stages as a whole; it is expressed as a result of a mathematical formula. For specific mathematical terms, values of emissions of GHG were used from: a) the production, storage and transport of seeds (seedlings); b) production and transport of fertilisers (organic, organo-mineral, mineral), sewage sludge and soil additives; c) production and transport of pesticides; d) fuels to ensure field operations, mainly used tillage systems; e) field emissions (i.e. GHG emissions from volatilization and leaching – mainly from applied fertilisers). The study is focused on the effect of different factors and conditions (mainly soil conditions, i.e. type and soil texture) and various farming systems (e.g. tillage systems, fertilising, etc.) on final value of emissions (emissions coefficients) from cultivated crops, which are further used for production of feed mixtures for animal nutrition. Determined emission coefficients (values) of crops will be used in common agricultural practice in real soil and climatic conditions of farms and, in the same time, to reduce GHG and ammonia emissions in animal nutrition and husbandry, respective.

Key words: *carbon footprint, GHG, soil conditions, animal nutrition.*

Introduction

Agriculture is estimated to produce 19 - 29 % of so-called greenhouse gases (GHG) (The World Bank, 2021). The 2022 CEMA European Agricultural Machinery Industry Association report points out that the greenhouse gas emissions of the agricultural sector in the EU27 correspond to 10% of the total greenhouse gas emissions of the EU27. On the other hand, in agriculture, at the same time, CO_2 is incorporated back in the process of photosynthesis. To the greenhouse gases contributing to the increasing of the greenhouse effect are included CO_2, N_2O, CH_4, water vapor (Eitzinger et al., 2013).

In July 2021, the European Commission published a set of measures called Fit for 55, which is intended to lead Europe to a 55% reduction in greenhouse gas emissions by 2030. These measures also include Directive (EU) 2018/2001/EU of the European Parliament and of the Council on supporting the use of energy from renewable sources ("RED II directive"), which is the main instrument of the European Commission for achieving an increase in the share of renewable sources in the energy mix. The objective of the RED II directive was to achieve a

share of renewable energy sources of 32% by 2030. In view of the energy crisis linked to the war in Ukraine, the European Parliament adopted a strategy called REpowerEU in 2022 and in May 2023 adopted the final draft revision of RED III setting out the achievement share of renewable energy sources in the amount of 45%. Technically, this requirement is to be ensured, among other things, by the production of new types of renewable fuels with a low carbon content. According to the Land Use, Land Use Change and Forestry (LULUCF) Regulation, in the period 2026-2030 each Member State will have a binding national 2030 target for the absorption of greenhouse gases. Commission Implementing Regulation EU 2022/996 on the rules for the verification of sustainability criteria defines improved agricultural practices, thanks to which the saving of greenhouse gas emissions, originating from the cultivation of agricultural crops, can be taken into account. For the above-mentioned reasons, it is necessary to gradually establish suitable innovative methods of management and farming, effective agrotechnical and cultivation measures, including carbon farming (carbon farming, regenerative farming), leading to the right to offset savings in greenhouse gas emissions.

The source of these emissions for determining the emission (carbon) footprint within agricultural production are practically all work operations applied within agricultural production. Attention is mainly focused on the consumption of energy (fuels) during individual agrotechnical operations (there is big effect of soil conditions, i.e. kind and soil texture for soil cultivation), the use of fertilizers and pesticides, waste management, etc. GHG emissions and carbon footprint will differ when converted to area (ha) or yield ($t.ha^{-1}$) and also according to the soil type and soil texture, culture (arable land, meadows and pastures, etc.) and type of agriculture/farming (conventional, integrated, organic, precision), etc.

Materials and methods

Project No OK21020121 focuses to specify in the years 2021 - 2023 all related procedures, database expert systems and selection of necessary inputs of cultivated crops.

This study focuses on the effect of different conditions (mainly soil conditions, i.e. type and soil texture) and various farming systems (e.g. tillage systems, fertilising, etc.) on final value of emissions from cultivated crops, which are further used for production of feed mixtures for animal nutrition and future determination of possibilities to reduce emissions in livestock production (consequential project No QK23020085).

The emission factor (i.e. typical greenhouse gas emission factor expressed in CO_2eq per product weight unit) for the main crops reflects the cropping technology with all crop cultivation stages (soil tillage, sowing, cultivation, fertilisation, harvesting, post-harvest processing of crops, handling and storage) as a whole.

Results and discussion

The specific mathematical formula was developed for the typical carbon footprint determination (in different local natural soil and climatic conditions of the Czech Republic).

The procedure for calculating specific emissions from cultivation of crops and the effect of all inputs: $\mathbf{e_{ec} = e_s + e_{fer} + e_{pe} + e_{fuel} + e_{N2O}}$ (in kg of CO_2eq/ha/year)

where:

$\mathbf{e_{ec}}$ = sum of GHG emissions

$\mathbf{e_s}$ = emissions of GHG from the production, storage and transport of seeds (seedlings);

e_{fer} = emissions of GHG from production and transport of fertilisers (organic, organo-mineral, mineral), sewage sludge and soil additives;

$\mathbf{e_{pe}}$ = emissions of GHG from production and transport of pesticides (plant protection products)
$\mathbf{e_{fuel}}$ = emissions of GHG from fuels to ensure field operations (mainly used tillage systems;), transport and electricity and fuels for post-harvest operations processing and storage;
$\mathbf{e_{N2O}}$ = field emissions of GHG (volatilization and leaching – mainly from applied fertilisers);

For individual members of this formula different values of CO_2eq per unit can be used – it means average normative values or specific values determined by producers.
Example for fertilisers:
The production and transport of mineral fertilizers represent a very significant contribution to the production of GHG emissions (Brentrup et al., 2016) from the cultivation of crops:

1) Standard emission factor for "general" mineral nitrogen fertilizer is 5.8806 kg CO_2eq/kg N, phosphorus fertilisers 1.0107 kg CO_2eq/kg P_2O_5 and potassium fertilisers 0.5761 kg CO_2eq/kg K_2O without further distinction represents the average European estimate of GHG emissions for these types of mineral fertilizers.
2) For specific mineral fertilizers, the standard values are considerably lower, e.g. ammonium nitrate 3.4687 kg of CO_2eq/kg of N, ammonium nitrate with phosphate 1.9082 kg of CO_2eq/kg of N, urea 1.916 kg of CO_2eq/kg of N, triple superphosphate 0.5437 kg of CO_2eq/kg of P_2O_5, compound NPK fertilisers (15-15-15) 5.0133 kg of CO_2eq/kg of NPK.
3) The emission factors of farm fertilizers of animal and plant origin, some organic, organo-mineral fertilizers and sewage sludge as substances usable for fertilization are considered to be zero throughout its life cycle until they are obtained. However, their further processing requires electrical energy and heat.

Example for fuel:
Fuel consumption used for soil cultivation during field operations, as well as the type and number of field operations have a significant impact on GHG calculation. So, it is necessary to take into account the differences in the energy required for soil operations according to different soil types and textures (diesel consumption for ploughing, harrowing, combined seeding/harrowing, etc.). There are a lot of various soils in the Czech Republic.
Selection of proper soil operations on different soil types is also influenced by soil erosion level of fields and directly influences fuel consumption (Table 1):

Table 1. Fuel consumption used for cereals growing on soils with different erosion levels

Crop	**Fuel consumption (in l . ha^{-1})**		
	CON	**MEO**	**SEO**
wheat	90.0	66.5	58.7
barley	89.2	66.1	58.5
rye	87.2	64.2	57.8

CON – no soil erosion – conventional way of soil management with deep ploughing
MEO – medium soil erosion – shallow soil tillage, combined sowing
SEO – significant soil erosion – no tillage, sowing in crop residues

Based on these two examples above there is evident, that for farmers is preferable during cropping system to use generally farm (organic) fertilisers or specific mineral fertilisers with zero and lower carbon footprint.
Simultaneously it is preferable apply kinds with minimum tillage or no tillage systems of soil cultivation with lower fuels consumption.
These results have logically positive effect on carbon footprint of final product (e.g. grain, meat, milk, etc.).

Of course there is set of other management practices that can be used to reduce greenhouse gas emissions from cultivation of crops – according to formula: $\mathbf{e_{ec} - e_{sca} - e_{ccs} - e_{ccr}}$
where:
$\mathbf{e_{ec}}$ = sum of GHG emissions
$\mathbf{e_{sca}}$ = emissions savings from soil carbon sequestration through improved agricultural practices:

- better agricultural management, e.g., gradual efficient use of biomethane, biodiesel to drive machines and equipment;
- preference for fuel, heat, electricity energy with a lower carbon footprint;
- switching to minimal tillage technologies or soil protection technologies with significant fuel savings, e.g. savings of up to 22 litres.ha^{-1} of diesel when growing wheat and oil-seed rape, 28 litres.ha^{-1} for corn;
- suiatable crop rotations;
- using cover crops to improve soil carbon storage;
- management of crop residues;
- use of organic fertilisers, e.g. compost, digestate, fugate, soil additives (activated biochar, biostimulants).

$\mathbf{e_{ccs}}$ = emission savings due to CO_2 collecting and geological storage - CO_2 storage when growing crops is excluded
$\mathbf{e_{ccr}}$ = emissions savings due to collecting and replacement CO_2
For both factors ($\mathbf{e_{ccs}}$, $\mathbf{e_{ccr}}$): it requires energy, mostly to collect them, but also to transport and store them, causing additional GHG emissions. The credit should be based on the net amount of CO_2 - preventing the release of CO_2 rather than storage it.

Conclusion

Agriculture produces greenhouse gases (CO_2, N_2O, CH_4), and therefore it is appropriate to monitor or calculate the carbon footprint, but the question remains where to start. If already during production or at the farmer level in terms of application of fertilizers, calcium substances, pesticides into the soil, etc. The carbon footprint will vary according to soil types and soil texture, selected crops, types of agriculture/farming (conventional, organic, integrated, precision), cultivation technology (tillage) and post-harvest processing. However, agriculture (and forestry) has the only ability to incorporate the increasing concentration of CO_2 in the process of photosynthesis into the biomass (yield) of plants while simultaneously producing oxygen, which should also be expertly even socially considered, especially with the growing global population explosion. The value of emissions savings is important factor on the market of agricultural commodities and, simultaneously, it is hidden tool in frame of competitive fight.

Acknowledgements

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IMPROVING LANDSCAPE RESILIENCE IN THE MEDITERRANEAN AGAINST CLIMATE CHANGE THREATS: THE "RESALLIANCE" PROJECT

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Abstract

Climate change is increasing the frequency and intensity of extreme weather events. The Mediterranean basin, which is warming 20% faster than the global average, is particularly vulnerable to climate change. Still, solutions exist and today there is a growing consensus among academics, experts and practitioners that these hazards must be addressed proactively by building resilience at the landscape level. However, today the application of solutions to improve landscape resilience is still slow and faces many barriers. Against this background, "ResAlliance" (complete name "Landscape resilience knowledge alliance for agriculture and forestry in the Mediterranean basin") is a thematic network project, developed by an international consortium of 16 partner organizations with funding from the Horizon Europe Programme of the European Union and the Swiss State Secretariat for Education, Research and Innovation, that aims to improve information and knowledge flow and increase the capacity of foresters and farmers on landscape resilience. Focusing on Mediterranean countries, ResAlliance gathers and assesses the gaps, barriers, knowledge, and good practices available to achieve resilient landscapes against the hazards of climate change, with particular emphasis on landscape fires and drought. This is made effective across 4 thematic areas that will help set specific learning objectives to better address knowledge and implementation gaps: Governance, Management practices, Technology, and Finance. To achieve its objectives, ResAlliance promotes multi-stakeholder interactive initiatives at two levels: a LandNet (an open Mediterranean thematic network on landscape resilience for forestry and agriculture) and five LandLabs, a tool for supporting knowledge transfer and activating regional landscape resilience solutions in 5 different Mediterranean countries: Portugal, Spain, Italy, Greece, and Cyprus.

Keywords: *Landscape Resilience, Climate change, Landscape fires, Drought, Mediterranean.*

Introduction

Climate change is increasing the frequency and intensity of extreme weather events. The Mediterranean basin, which is warming 20% faster than the global average, is particularly vulnerable to climate change (Lange 2020). The results have already started becoming evident through a host of problems and threats. These are either directly related to weather or indirectly linked to it and they range from natural risks that may cause severe damages to properties and even threaten lives, to challenges in agricultural production with subsequent economic and social effects.

The most important weather influences of climate change in the Mediterranean include higher temperatures that take the form of heat waves, prolonged droughts, and extreme precipitation events. The frequency and intensity of these phenomena are expected to increase in the future,

affecting agricultural and forest production, natural hazards and the health and well-being of people. One such hazard of great importance in the Mediterranean region are forest fires.
In recent decades, forest fires are becoming increasingly difficult to control as a result of changes in weather extremes due to climate change but also due to changes in the quantity and continuity of the fuels on the landscape that have followed a steep reduction of rural populations in many parts of the world. Both these causal factors are present in the Mediterranean basin which is particularly vulnerable to climate change and has lost an alarming percentage of its rural population in the last few decades (Figure 1).

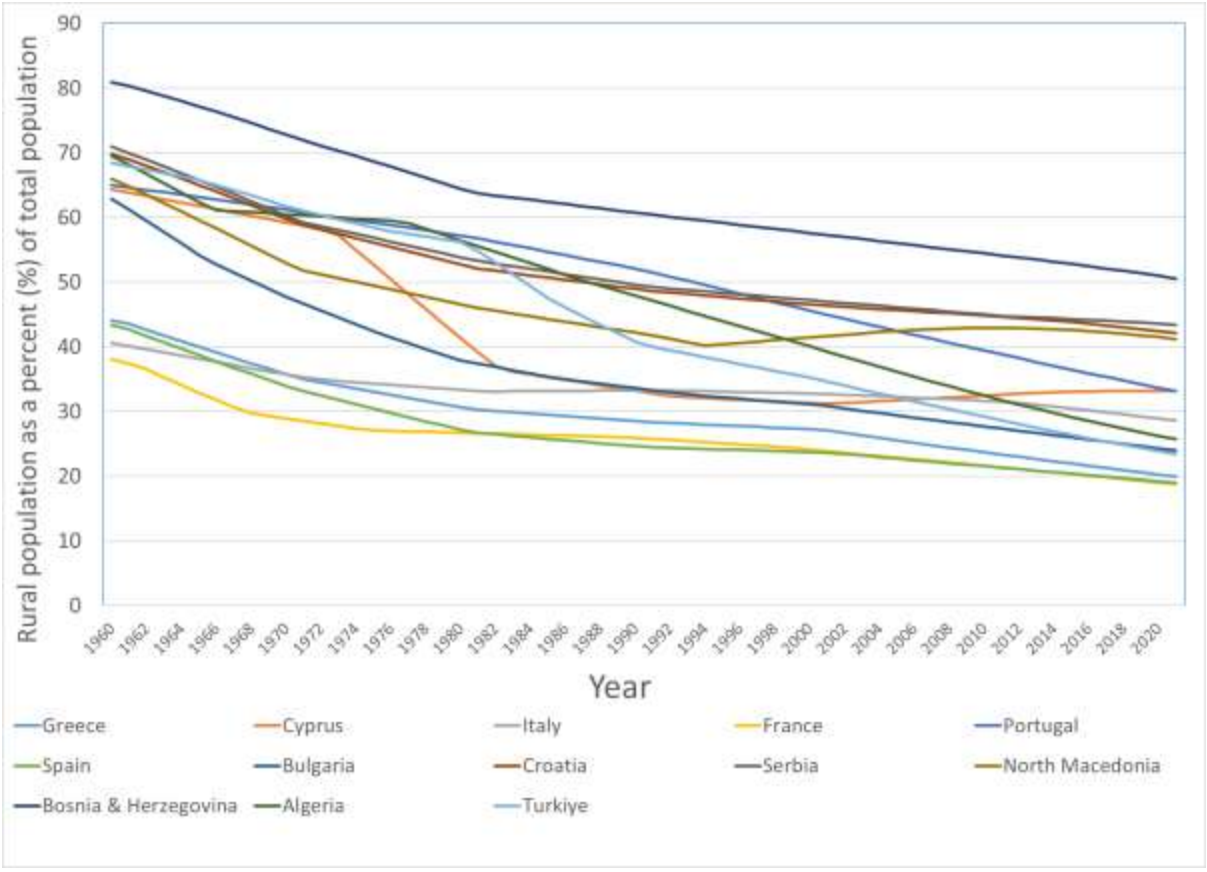

Figure 1. The decline of rural population, as a percent of the total population, in the countries around the Mediterranean Sea, between 1960 and 2020 (Source: Gavriil Xanthopoulos, based on data from IndexMundi.com 2021).

A continuous but short-sighted effort to improve the national firefighting capacity by increasing aerial firefighting fleets and the number of firefighters and through the use of new technologies, which has taken place across all Mediterranean countries, has not solved the problem. Nowadays, while most starting fires are detected and suppressed quickly, a few fires, under very adverse weather conditions and burning in a continuum of heavy forest fuels, reach intensities well beyond the capacity of the firefighting resources, becoming megafires, that cause devastation and often result in the loss of lives. This reality, that the more effective we are at suppressing wildfires, the worse they behave and the harder to fight they become, is termed "the fire-paradox" phenomenon, and has manifested itself repeatedly in recent years. Currently, there is a lot of skepticism about this growing problem and there are numerous calls for paradigm change, away from current short-sighted policies (Moreira et al. 2020).
Another very negative influence of climate change is drought. Whereas drought spells have happened numerous times in the past, their frequency is increasing under the current climate change. Current projections by the EURO-CORDEX regional climate model (RCM)

ensemble, show that in Italy, Portugal, Spain, and parts of Greece and Turkey, rainfall during the warm, dry season of April through September will decrease by as much as 10 percent by 2030 and as much as 20 percent by 2050 (Woetzel et al. 2020). This is highly distressing in many ways since it signals the potential for significant conflicts regarding the use of water (drinking versus irrigation and other uses), as well as many secondary effects. Drought is likely to affect agriculture in many ways, such as damaging crops and reducing production, which in turn may affect the ability of rural populations to maintain production and make a living in their place of stay in the countryside. It will also affect the occurrence and characteristics of forest fires. Burned areas are expected to increase, in some countries even to double by 2050 (Turco et al. 2018, Woetzel et al. 2020). Secondary flooding and soil loss are also likely after large fires. These influences may further act to reduce agricultural populations if no action is taken to prevent or mitigate these risks, further increasing fuels quantity and continuity on the landscape.

The bleak outlook described above should clearly not be seen as an inevitable fate, a future of suffering, without security, and without hope. The answer can only be found in improving the capacity of societies to mitigate, to withstand or to recover quickly from the manifestation of adversities such as those described above. This is the definition of resilience. Today, there is a growing consensus among academics, experts and practitioners that solutions exist, and that the adverse effects of these hazards must be addressed proactively by building resilience at the landscape level (Moreira et al. 2020, Woetzel et al. 2020).

Building resilience starts from realizing the problem, understanding it and identifying its causes. This is not easy and requires open minds and forward thinking. Resilience promotion at the landscape level is quite complex as there are many elements that must be examined in order to develop solutions, focusing on the things that can be changed or controlled instead of dwelling on what cannot be changed in the foreseeable future. The fact that some natural elements, such as drought, change relatively slowly while others, such as forest fires, can manifest themselves abruptly, further complicate the picture. Currently, science has identified many opportunities and has proposed various solutions (Damianidis et al. 2021, Xanthopoulos 2023). Furthermore, numerous initiatives have started being developed in practice in the agriculture and forestry sectors across the Mediterranean (Ascoli et al. 2023). However, the generalized application of solutions to improve landscape resilience is still slow and faces many barriers. Obviously, this needs to be changed. The need has been identified by the European Commission which has specifically set it as a priority in some of its recent calls for proposals for research projects in the frame of its Horizon Europe Programme. One such project is ResAlliance. The objective of this paper is to present the project, its aims and the methods it will employ to contribute towards the development of resilient landscapes to the impacts of climate change.

The ResAlliance project

Against this background, "ResAlliance" (complete name "Landscape resilience knowledge alliance for agriculture and forestry in the Mediterranean basin") is a thematic network project, developed by an international consortium of 16 partner organizations (Table 1), coordinated by the European Forestry Institute, that aims to improve information and knowledge flow and increase the capacity of foresters and farmers on landscape resilience.

Methods

Focusing on Mediterranean countries, ResAlliance focuses on gathering and assessing gaps and barriers, and collecting and transferring existing knowledge and good practices to achieve

resilient landscapes against the hazards of climate change, with particular emphasis on landscape fires and drought. This will be made effective across four thematic areas that will help set specific learning objectives to better address knowledge and implementation gaps: Governance, Management practices, Technology, Finance.

Table 1. The 15 organizations participating in the ResAlliance thematic network project.

	Organization	**Short name**	**Country**
1	European Forest Institute	EFI	Finland
2	ETIFOR SRL	ETIFOR	Italy
3	Fundacio d'Ecologia del Foc i Gestio d'Incendis Pau Costa Alcubierre	PCF	Spain
4	Ellinikos Georgikos Organismos - DIMITRA	ELGO - DIMITRA	Greece
5	European University - Cyprus	EUC	Cyprus
6	Societa Cooperativa Agricolo Forestale - D.R.E.AM. Italia	DREAM	Italy
7	Center For International Forestry Research	CIFOR-ICRAF	Indonesia
8	Instituto Superior de Agronomia	ISA	Portugal
9	Consorci Centre de Ciencia i Tecnologia Forestal de Catalunya	CTFC	Spain
10	Institut de Recerca i Tecnologia Agroalimentaries	IRTA - CERCA	Spain
11	Jouzour Loubnan Association	JL	Lebanon
12	Institut National de Recherches en Genie Rural, Eaux et Forets	INRGREF	Tunisia
13	Mediterranean Agronomic Institute of Chania	CIHEAM - MAICH	Greece
14	Agenzia Forestale Regionale per lo Sviluppo del Territorio e dell'Ambiente della Sardegna	FORESTAS	Italy
15	Federation Europeenne d'Agroforesterie (EURAF) Association	EURAF	France
16	International Union for the Conservation of Nature	IUCN	Switzerland

The project started in December 2022. To achieve its objectives, ResAlliance promotes multi-stakeholder interactive initiatives at two levels: Development and operation of a LandNet and five LandLabs.

The LandNet

The LandNet, is an open thematic Mediterranean Network on landscape resilience for forestry and agriculture. It has partners in Europe, Middle East (Lebanon) and Northern Africa (Tunisia), allowing the consortium to easily collect solutions and best practices from all around the Mediterranean. It is an international network of professionals from the agriculture and forestry sectors. Its aim is to link practitioners and representatives from a variety of institutions, spanning from land managers to decision-making bodies working at different levels, and to foster cooperation and knowledge sharing among them regarding innovative solutions and good practices. More specifically, the LandNet may be joined by individuals such as farmers, foresters, researchers, experts, etc., private enterprises and organizations interested in innovative landscape resilience solutions, as well as public administrations, such as rural and regional administrations, dealing with landscape and green areas planning in the Mediterranean area.

Through exchanges between LandNet members, and the support and contribution of the ResAlliance partners, the LandNet network will:

- Prepare context-specific knowledge in the four thematic areas that the project focuses on (Governance, Management practices, Technology, Finance), through:
    - Development of a knowledge repository of scientific and grey literature
    - Review of the literature, distilling it in practical form
    - Identifying good practices and innovative solutions, documenting them, and producing practice abstracts, factsheets and videos
    - Uploading the main products to the EU-FarmBook platform for broader dissemination and long-term storage
- Engage and train farmers, foresters and other key stakeholders for knowledge transfer regarding these best practices and solutions.

The motivation to join the LandNet includes:

- To have access to and learn from the wealth of valid information and good practices that will be compiled by the LandNet
- To pose questions and discuss about concerns on the subject
- To contribute own observations, findings, ideas and examples of good practices that will be discussed and ultimately included in proposals for the future, towards EU and national policymakers and the public
- To develop synergies that will lead to truly making a difference

The LandLabs

The LandLabs are a tool for supporting knowledge transfer and activating regional landscape resilience solutions in 5 different Mediterranean countries: Portugal, Spain, Italy, Greece, and Cyprus. They are context-specific knowledge transfer and engagement programs that aim to connect and engage farmers and foresters with a range of key stakeholders involved, regarding the four thematic areas, in order to achieve effective uptake of knowledge and to learn from examples.

The LandLab in each country will encompass a particular area/region. Participants in each LandLab will contribute to the formulation of good management practices or will demonstrate existing ones, for adaptation to climate change mainly in regard to the problems of drought and wildfires in agriculture and forestry landscapes.

The LandLabs will be developed in five Mediterranean regions that are highly exposed to climate change hazard occurrence (Figure 2): Sardinia (Italy), Catalonia (Spain), Peloponnese (Greece), Cyprus and Regiao Norte (Portugal). An initial investigation by the ResAlliance partners led to a preliminary detection of needs and challenges that led to the selection of the five regions. Even though the five regions share the threats posed by typical Mediterranean hazards aggravated by climate change – especially wildfires and droughts – each region has specific social needs and agricultural and forestry challenges that call for tailored solutions.

Several activities will be developed in each LandLab region aiming to elicit experiences, visions, and expectations of participants, to eventually create solutions with the potential to overcome the current challenges:

- **LandLab Launch and exploitation workshop:** project presentation, and first dialogue with stakeholders to establish the challenges and barriers that arise in the context of climate change.
- **LandLab landscape resilience workshop:** a framework for dialogue and to explore desired future scenarios, find possible facilitators and governance solutions to achieve it, and discover and adapt solutions existing elsewhere in the Mediterranean.

- **Showrooms:** innovation and knowledge solutions and best practices for improving the resilience against climate hazards.
- **Field trip:** showcase selected good practices by local stakeholders.

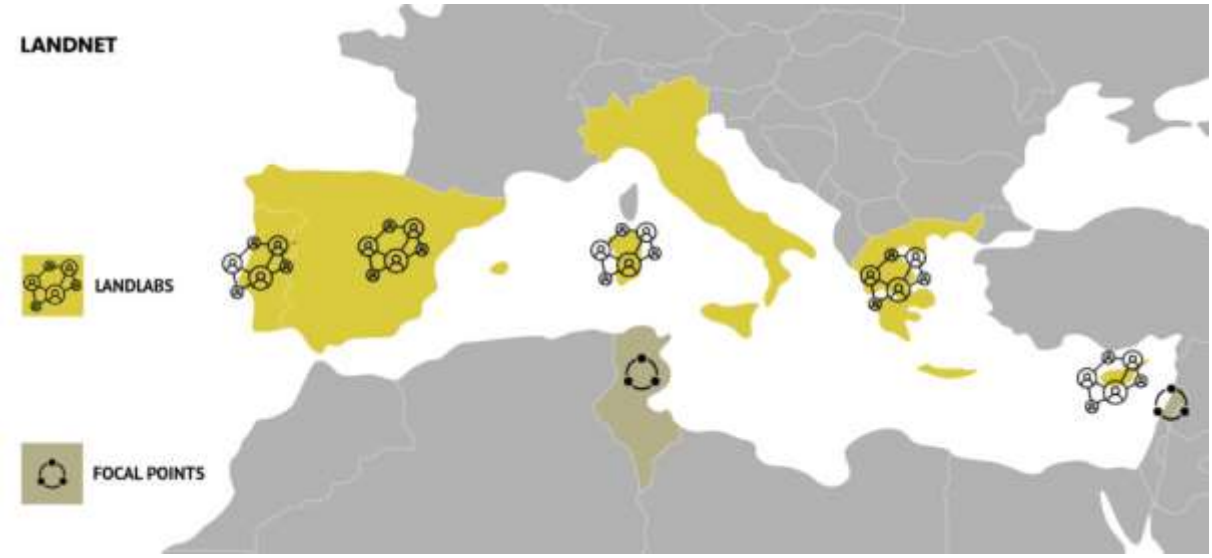

Figure 2. The five Mediterranean regions where the ResAlliance LandLabs will be developed.

Specialized "Resilience Ambassadors" will facilitate the activities of the Landlab in each region. They have been selected within the corresponding partner teams in each country and have received training in communication, in order to carry out their tasks in the best possible way. It should be noted that because the LandLabs have a specific regional focus and the activities will be in-presence and in local language, it is best for interested stakeholders to join the LandLab in their country or closer to their location.

Registration to the LandNet and the LandLabs

Registration to the LandNet and the LandLabs is straightforward. It is available through the ResAlliance site: www.resalliance.eu where the "Join the "LandNet" button is immediately available. It is also available under the sections "LandNet" and "LandLabs". The registration form is common and starts with a privacy statement and request for consent to the use of personal data, as it will be needed to keep registered stakeholders informed of the activities of the project.
A member of the LandNet may choose not to participate in a LandLab, as these are country and area specific. On the other hand, a member of a LandLab is automatically registered as a member of the LandNet.

Discussion and Conclusions

The need to work on the mitigation of the consequences of climate change is imperative. The accumulated experience in practice shows that the approach adopted so far is usually directed to respond to the problems after they arise, without due attention to prevention of the problem and its consequences. This can be described by the metaphor of the "firefighting trap", which is well known in the business management domain, where "putting out fires" allegorically expresses the concept to deal with problems (fires). It is used by managers "to describe a short-sighted cycle of problem-solving, dealing with problems, as they arise, mainly by

suppressing their symptoms, rather than understanding and addressing the factors that cause the problem" (Xanthopoulos et al. 2020).

As pointed out here, a significant part of the solution will need to be sought in the development of resilient landscapes. However, even if the impetus to act in this direction is developed, solutions can be quite complex as they have to address many policy, governance, environmental, financial, technical, financial and, of course, social considerations. The path is to a large extent uncharted. Knowledge has to come from many different sources, with an emphasis on tried and tested solutions, especially in the form of bottom-up initiatives (Ascoli et al. 2023). The ResAlliance project offers the mechanisms, its LandNet, its LandLabs and the combined knowledge of its 16-partner consortium, to support efforts towards achieving landscape resilience in the Mediterranean against climate change threats.

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"ON EITHER SIDE OF THE ELECTRIC FENCE" (A BOTANICAL STUDY OF THE AUTUMNAL ASPECT OF THE DRAINED LAKE BED OF A DOMESTIC WATER BUFFALO PASTURE IN THE ZÁMOLYI BASIN)

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Abstract

Our studies were carried out in a water buffalo pasture of the Zámolyi basin, in the area of the Csíkvarsai meadow, in the bed of a former lake, which was drained in 2022, then dried up by autumn and it was grazed by domestic water buffalo (*Bubalus bubalis*). The grazed area was surrounded by an electric fence. The vegetation of this part of the area and part of the outside was surveyed on 19 of November 2022. We carried out 3-3 coenological surveys in both separated areas. Based on the results, there were significant floristic and physiognomic differences in the vegetation. Among the clover species, species with ground-running stems (*Trifolium repens* and *T. fragiferum*) were dominant in the grazed area, but in the ungrazed area, there was a coherent plant population of 30-40 cm high, densely located alsike clover (*Trifolium hybridum*) in the lake bed. In addition, the occurrence of swamp pricklegrass (*Crypsis schoenoides*) was a floristic new discovery. Due to the loss of water coverage, several *Trifolium* species have become abundant in the area, which is a result of the germination properties of the species. The thick seed coats were cracked by the loss of the effect of the water coverage on temperature equalisation, and germination could have begun, resulting in a dominant species in the area. During the present study, we have seen a very impressive result of the analysis of the vegetation of the lake bed in autumn aspect in the Zámolyi Basin, which is the result of the impact of water buffalo grazing. The number of species was more significant in the grazed area. Moreover, floristic rarities have been found and as an indicator of grazing, species of different life form types were dominant within the same genus.

Keywords: *vegetation of lake bed, Trifolium sp., grazing.*

Introduction

The survey was carried out exactly one day after the electricity fence had been removed from the area (Figure 1). The track of the electricity fence is clearly visible.
The conservation of native grasslands needs human nature conservation management, mowing or grazing (Török *et al*., 2010, 2011, 2012, 2014; Pywell *et al*., 2002; Valkó *et al*., 2012, 2014; Bajor *et al*., 2016). In order to increase grassland area, there are also replanting activities that contribute significantly to the management, conservation and establishment of grassland (Kiss *et al*., 2011). In nature conservation practice, grazing is one of the most common practices which has economic outcomes and might be even a driving force (Deák *et al*., 2016, 2020; Török *et a*l., 2014, 2018; Fűrész *et al*. 2023; Magyar et al., 2017).

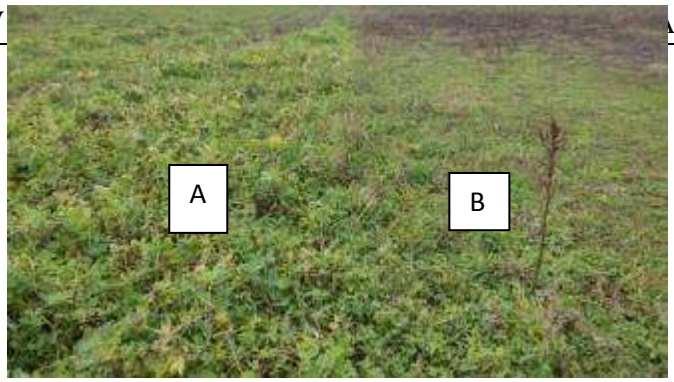

Figure 1. The former lake bed, where area „A” is the ungrazed and area „B” is the grazed area

The majority of grazing is conducted with grey cattle, but grazing is also carried out with beef cattle (Járdi *et al*., 2021; T-Járdi *et al*., 2022; Hajnáczki *et al*., 2021). Grazing with grey cattle is generally preferable for the conservation of grassland biodiversity due to its low selectivity, which can lead to the development of semi-natural habitats, in contrast to grazing with horses, sheep or goats (Póti *et al*., 2007). Recently, importance of grazing with domestic water buffalo has been increasing and the practice so far was beneficial in terms of the turf management in different vegetation types (Fűrész *et al*., 2023).
Among the sustainable landscape management practices, the stimulation of grazing can play a role not only in the conservation of ecological values but also in the control of invasive plant species that are spreading increasingly, in which the water buffalo can be applied (Fűrész *et al*., 2023).
In the present study, we found a particular vegetation example where water buffaloes were grazed in a bed of a lake, and there was also an ungrazed area directly alongside as a control. The water buffaloes grazed only the northern area, which was interspersed with the electric fence. The research question was: is there a detectable difference between the two areas, and if so, what is the difference?

Materials and methods

Sample collection

Our works were carried out in the Csíkvarsai meadow, which is located in the Zámoly basin. The former lake in the basin was grazed with water buffaloes in the northern part of the area, whereas the southern part of the lake bed was not grazed. In each sample area, 2×2 m quadrats were used for coenological surveys (Braun Blanquet, 1964). Sample areas were assessed based on the nature conservation value categories (Simon, 2000) and social behaviour of the species (Borhidi, 1995). In addition to Raunkiær's (1934) life form types, the life form analysis was carried out based on Pignatti's (2005) life form types, considering the morphological characteristics of the species in addition to the location of the overwintering organ. The species names follow the work of Király (2009).

Results and discussion

On both sides of the electric fence, the grazed and ungrazed sides, the vegetation was completely different. On the ungrazed side, we found a 30-40 cm high continuous dense vegetation, dominated by alsike clover (*Trifolium hybridum*) (Figure 2, side A of the lake bed).

Figure 2. The coherent population of dense alsike clover (*Trifolium hybridum*) in the former lake bed ("Area A")

In the grazed area (Figure 1 B), the plants were only a few cm tall and the continuous patches of vegetation were also formed by Trifolium species, but there was no presence of the moorhen. The two dominant Trifolium species were white clover (Trifolium repens) and strawberry clover (Trifolium fragiferum) (Figure 3, side B of the lake bed).

Figure 3. The coherent population of white clover (*Trifolium repens*) and strawberry clover (*Trifolium fragiferum*) in the former lake bed ("Area B")

Due to grazing by buffaloes, species of the genus Trifolium have become dominant only with creeping stems (Pignatti, 2005). Other papilionaceous also occurred in the area, such as the narrowleaf trefoil (*Lotus tenuis*) and the black medick (*Medicago lupulina*).
In the middle part of the area, 2 species of *Rumex* were also found, the bitter dock (*Rumex obtusifolius*) and the seashore dock (*Rumex maritimus*).
On the dried lake bed, a rare grass species, the swamp pricklegrass (*Crypsis schoenoides*), also occurred on the grazed part (Figure 4), the nearest data for which were available so far from the southern coast of Lake Balaton (Király, 2009).
We also took coenological surveys in the area; the results clearly showed that the number of species is significantly higher in the grazed area and the number of papilionaceous species is much higher due to the grazing of animals.
As a result of livestock grazing, the dominant species of genus clover became the creeping stem species, so much so that the numerous alsike clover on the "other side" of the pasture were not found in the area. There was good evidence from the vegetation that creeping stem species were present in larger proportions, and that perennial or annual species would increase in abundance, as species with rosette, and that the Pignatti (Pignatti, 2005) life form types of species were a good indicator here.

Figure 4. The swamp pricklegrass (*Crypsis schoenoides*) is easily recognisable for its characteristic appearance and unique inflorescence, even when dry

Conclusion

The characteristic properties of the seeds of the species lurk behind the cover of clover (Trifolium) in the lake bed (Csontos 1998, Purgar et al., 2008). The seed coats of the species are very hard and resistant to water.

This means they can survive years of being covered in water, and the water covering keeps them at a constant temperature. When the water cover is eliminated, temperature changes in the seed coat may cause the thick seed coat to crack and germination may start, resulting in being dominant in the area.

In conclusion, we have seen a very interesting result of grazing in the Zámolyi basin, analysing the autumn aspect of the lake bed. The number of species was much more significant in the grazed area and floristic rarity was also found, and as an indicator of grazing, within the same genus, species of different life form types became dominant.

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EFFECT OF DIFFERENT SHRUB SPECIES ON SOIL: SEED BANKS CHEMICAL AND BIOLOGICAL PROPERTIES

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Abstract

Little information is available about the effects of different species of shrubs on soil parameters such as seed bank composition, chemical and biological properties and how the soil seed bank (SSB) buried under the shrub canopies and other soil parameters could contribute to restoration of degraded grasslands. We determined the role of three different shrubs on soil characteristics and evaluated the SSB potential for their possible use in rangeland restoration projects. Ten sites, each containing three shrub species (*Onobrychis cornuta*: cushion and deciduous, *Berberis integerrima*: open canopy and deciduous and *Juniperus sabina*: procumbent and evergreen) and a herbaceous patch (control) in close proximity, were sampled and their SSB characteristics (density and richness), chemical (total organic carbon and total nitrogen) and biological (basal and substrate-induced respirations) were determined. The results showed that SSB density was lowest under *J. sabina* and highest under herbaceous control, but did not differ between *B. integerrima* and *O. cornuta*. Species richness of SSB was not significantly different between the three shrubs. Topsoil total organic carbon content was significantly highest under *J. sabina* and *B. integerrima* (3.30% and 3.07% respectively) and lowest under *O. cornuta* and herbaceous vegetation (2.61% and 2.23%, respectively). This study revealed that the extent to which vegetation affected soil characteristics depended on the species of shrub in the grasslands. We conclude that *B. integerrima* is a priority species in restoration projects due to its significant positive influences on SSB density.

Keywords: *shrub Canopy architecture, Basal soil respiration, Substrate-induced soil respiration, Shrub patches, Iran.*

Introduction

Arid and semi-arid ecosystems occupy 36% of the land area of the globe and shrubs function as foundation species within these ecosystems (Yang and Williams, 2015). A foundation species in ecology was described as species with significant impacts on the structure and functioning of an ecosystem (Lortie et al., 2017). It has been frequently called shrubs as fertile islands, since, they have significant influences on habitat conditions. Shrubs have been shown to increase soil microbial functions (Chandregowda et al., 2018), enhance mycorrhizal colonization (Armenta Calderón et al., 2019), alter runoff and sediment yields (Lu et al., 2019) and affect the soil seed bank (SSB) (Funk et al., 2019). Study on SSB is important, since, it is one of the most important functional part of any plant community and can be significant component in the process of rehabilitating degraded lands (Mohammed and Denboba, 2020). In semiarid regions, shrubs are able to change SSB characteristics. According to some reports, SSB density was much higher under the shrubs than the surrounding areas (Marone et al., 2004). Shrubs accumulated large and diverse SSBs beneath their canopy which were different in composition from seed banks of the open matrix. Knowledge of the SSB and its spatial variation under shrubs would be helpful for restoration efforts in degraded areas. In addition, it has often been reported that vegetation composition, particularly woody plants, can influence soil properties, and that the nature and composition

of the vegetation community are determinant of the soil properties. For instance, Urbina et al. (2020) and Ding et al. (2019) highlighted the role of woody plants in the sequestration of soil C and nutrients through the allocation to the aboveground biomass. However, none of previous studies focused on the effect of different woody plant species with focusing on different feature and structure of woody plant species on soil properties in the mountainous grasslands.

Different species of shrubs are different in their canopy architectures. Some shrub species have raised stems and some are attached to the ground. The canopy is dense in some and open in others. Therefore, it can be supposed that shrubs may have different performances in producing and trapping seeds and plant materials and could affect differently seed production by smaller species in their sub-canopy, trapping sediment and plant materials. As a result, different species of shrubs may alter SSB characteristics and soil chemical, physical and microbial properties, differently. Nevertheless, our literature review showed that none of the studies has compared the effect of different species of shrubs, differently in architectures and features, on SSBs and other soil properties in the semiarid regions.

Material and Methods

Study area

The study was conducted in Baladeh Watershed in Mazandaran province, Iran (36° 16′30″N - 36° 18′19″N; 51° 49′30″E -51° 51′17″E). The elevation is between 2844 to 2963 m above sea level with an average annual rainfall of 394 mm. The average annual temperature is 5.5 °C with average annual maximum temperature 16.4 °C and average annual minimum temperature of 2.6 °C.

Woody plant selection

Three dominant woody species with different features and landforms together with herbaceous vegetation as control were selected in the study area, viz. a) *Berberis vulgaris* L. (family Berberidaceae) is an upright deciduous woody plant with an average 180 cm tall in the study area.

b) *Juniperus sabina* L. (family Cupressaceae), is a woody plant by evergreen dwarf conifer that is native to mountain areas. It is usually seen as a wide-spreading woody plant to an average of 90 cm tall in the study area. C) *Onobrychis cornuta* (L.) Desv. (family Fabaceae), perennial, deciduous, branched spiny woody plant, forming cushions or tufts, up to 60 cm in height in the study area. The canopy structure is low stature with dense compact form. d) Herbaceous vegetation dominated by perennial grasses such as *Festuca ovina* and *Bromus tomentellus* as control treatment.

Site selection

We selected 10 sites in which all three shrubs were found closed to each other in each site (Fig. 1). Soil sampling was conducted in early autumn, after seed dispersal. In each sampling area, in each individual patch, after removing coarse litter (> 2 cm) 10 soil cores (subsamples) were collected at random, to a depth of 10 cm, with a 5 cm diameter auger. The soil samples were transported to the laboratory and were stored at ca. 4 ºC for cold stratification for a period of one month. Subsequently, soil samples were transported into the greenhouse for soil seed bank studies through seed germination method. In addition, under each individual shrub, 10 soil cores were collected at random to a depth of 10cm (Baskan et al. 2016), with a 5cm diameter auger. Then the soil cores pooled per each shrub (totally 40 soil samples). Then the soil samples were transported to the laboratory and stored at 4ºC until they were processed. The samples were sieved, the roots and coarse gravel (>2mm) were removed by sieving, and the <2mm soil was used to examine the effects of shrubs on soil properties (Liu et al. 2014)

including wet aggregate stability (WAS), pH and electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN) and microbial respiration.

Figure 1. Positions of the three studied shrubs and and herbaceous patch (as control) in each site.

Results and Discussion

The density of SSB was significantly different among patch types and between depths (Table 1; Figure 2). Seed density in the upper soil layer (0-5 cm) was lowest under *J. sabina* (3,898 seeds m-2) and highest under the herbaceous patches (9,737 m-2). At 5-10 cm depth, seed bank density was highest under *B. integerrima* (2823 seeds m-2) (Table 1; Figure 2). Total density (0-10 cm) was highest in the herbaceous and *B. integerrima* and lowest in *J. sabina* patches (df = 3, F = 3.01 and P = 0.04) (Figure 2). Species richness of the SSB was significantly different among patch types at 5-10 cm (highest under *B. integerrima*), but not at 0-5 cm (Table 2; Figure 3). At 0-10 cm it was higher under *B. integerrima* than under the rest (df = 3, F = 3.8, P = 0.04; Figure 3). The values of H' were significantly different between depths (Table 3; Figure 4) but not among patches; no differences were found at 0-10 cm.

Table 1 Results of a split-plot ANOVA comparing effects of patch (three different shrub species and herbaceous vegetation) and depth of sampling on soil seed bank density. ANOVA was conducted using square root transformed values of soil seed bank densities.

Source of variation	SS	df	MS	F
Main-plot (Patch)	3904.89	3	1301.63	2.94*
Main-plot error	15917.04	36	442.14	
Sub-plot (depth)	43684.65	1	43684.65	212.65**
Main-plot and sub-plot interaction	3705.83	3	1235.28	6.01**
Sub-plot error	7395.49	36	205.430	
Total	74607.91	79		

*, significant at α=0.05; **, significant at α=0.01

Table 2 Results of a split-plot ANOVA comparing effects of patch (three species of shrubs and herbaceous vegetation) and depth on species richness of soil seed bank.

Source of variation	SS	df	MS	F
Main-plot (patch)	90.05	3	30.02	2.89*
Main-plot error	373.5	36	10.38	
Sub-plot (depth)	1344.8	1	1344.80	146.04**
Main-plot and sub-plot interaction	35.7	3	11.90	1.29ns
Sub-plot error	331.5	36	9.21	
Total	2175.55	79		

*, significant at α=0.05; **, significant at α=0.01; ns, not significant

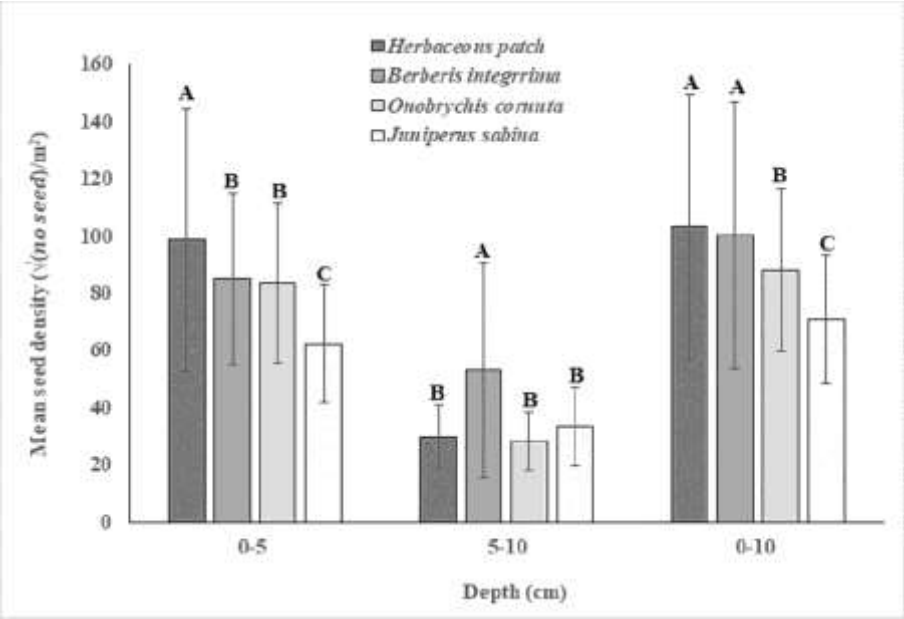

Figure 2. Mean densities ($\sqrt{X \pm SE}$) of seeds that germinated under three shrubs: Onobrychis cornuta, Berberis integerrima and Juniperus sabina and herbaceous vegetation. Upper case letters indicate statistically significant differences (P<0.05) among patch types within each soil layer.

This study showed differences in the richness and abundance of the SSB associated with the different shrub species in a semiarid montane rangeland. Combined the fact that *B. integerrima* is a palatable shrub and has a larger and more diverse SSB than the other two species argue for its prioritisation in the restoration of degraded areas of the rangeland. We expected that the open canopy in *B. integerrima* would give an enhanced opportunity to herbaceous species for growing and producing seeds in the empty spaces inside the shrub patches. Indeed, our results showed that SSB density under *B. integerrima* was higher than under *O. cornuta* (not significant at 0-5 cm) or *J. sabina*. We argue that probably the open canopy of *B. integerrima* appears to increase the density of SSB, particularly the persistent component in the deeper soil layer. Comparing with *B. integerrima*, less divers and smaller SSB under *O. cornuta* indicated that the procumbent, dense, and compact canopy of *O. cornuta* should restrict seed inputs, and limit the growth and reproduction of herbaceous species under it. Therefore, seed density and richness beneath *O. cornuta* became less than beneath *B. integerrima* and less than outside its canopy as well. Finally, *J. sabina* with its open canopy had a lower SSB density (0-5 cm and 0-10 cm) than the other two shrubs and outside the canopy. Thus, in the case of *J. sabina*, factors other than canopy architecture might be involved in having a less dense SSB in relation to the other species. Some species of

Juniperus and other conifers (e.g. *Pinus halepensis*) have been shown to have allelopathic effects on herbaceous species (e.g. Young and Bush 2009) by possessing phenolic compounds, monoterpenes, benzoic and cinnamic acid (Kil, 1992).
There were no significant differences of WAS values between four woody plant species (Table 3, Fig. 3). Soil moisture content was highest understory the *B. integerrima* and *J. sabina* with 5.91% and 5.78%, respectively (Table 3, Fig. 3). The lowest amount of soil moisture content was observed in the control area (3.88%). Electrical conductivity and pH showed a non-significant difference between different species of woody plants. Soil TOC content was significantly highest under *J. sabina* and *B. integerrima* (3.30% and 3.07% respectively) and lowest under *O. conuta* and herbaceous vegetation (2.61% and 2.23%, respectively) (Table 3). Similarly, the highest values of POC were found under *J. sabina* and *B. integerrima* (both 4.99 g/kg) and lowest under herbaceous vegetation (3.14 g/kg) (Table 3). Soil TN content was significantly highest under *J. sabina* and *B. integerrima* (0.18% and 0.17%, respectively) and lowest under *O. conuta* and herbaceous vegetation (0.16% and 0.15%, respectively) (Table 3).

Table 1. The effect of woody plant species on some physico-chemical soil properties (one-way ANOVA).

Soil properties	df	F-value	P-value
Total organic carbon	3	5.15	**0.003****
Particulate organic carbon	3	4.96	**0.003****
Total nitrogen	3	2.65	**0.048***
C/N	3	0.59	0.623ns
pH	3	0.21	0.88ns
EC	3	0.14	0.93ns
Moisture	3	2.90	**0.046***
Wet aggregate stability	3	0.37	0.77ns
Microbial respiration	3	6.25	0.002

** significant at P<0.01; * significant at p<0.05; ns: not significant

Our research has presented the topsoil differences and heterogeneity under the woody plants in the grasslands. Overall, our results showed that the presence of woody plants improves the values of understory topsoil nutrient. Previous studies reported that nutrient accumulation beneath woody plants in the grasslands is common and provides opportunities for carbon and nitrogen sequestration (Jackson et al. 2002). McClaran et al. (2008) showed that TOC and TN accumulation was ca. 80-750% greater beneath *Prosopis velutina* than in open grassland. Soils under woody canopies are more fertile than soils from the surrounding grassland (Noumi 2015). The increase of nutrient content in soil can be a result of an increase in the plant litter amount, on the one hand, and an enhancement in soil microorganisms, on the other hand. However, among the three woody plants, *J. sabina* and *B. integerrima* have a stronger effect with higher values of topsoil N and C contents, respectively, compared to the other woody plant and control. The first species with highest soil carbon and nitrogen amount in the understory compared with two other species and open areas is a needle-leaved and evergreen species. Probably, the high litter production due to being ever green resulted to the highest C storage in the understory. Coniferous species are able to increase the content of carbon in soil (Bu et al. 2012). Moreover, organic carbon accumulation may be mainly a result of not only higher organic matter production by the woody plant (Ruwanza and Shackleton 2016) but also by the slower rate of litter mineralization. The second species with highest values of carbon and nitrogen under the canopy was *B. integerrima*. These high values of TN, TOC and POC might be a result of litter input by this species as well as by herbaceous species growing in the understory of the woody plant. Numerous studies have shown that the establishment

and fecundity of herbaceous plants are higher in the woody plant patch than in the surrounding areas (e.g. Badano et al. 2009).

Conclusion

This study showed that shrubs, overall, played an important role in reserving of herbaceous species seeds and soil quality parameters under their canopies. However, the extent to which shrubs affects SSB and other soil characteristics is dependent on the species of shrub. These different roles of shrubs on soil are advised to be considered in the restoration of areas through the conservation of endemic shrubs in the semiarid regions.

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THE COLLECTION OF *HOSTA* Tratt FROM "AL. CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE)

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Abstract

The genus *Hosta* Tratt. (hostas, plantain lilies or giboshi) belongs to the subfamily *Agavoidae*, family Asparagaceae Juss., order Aspargales Link., class Monocotyledoneae. It is native to the Eastern Asiatic floristic region: Japan, China, Sakhalin and Kuril Islands. These wonderful ornamental plants, which have been known and cultivated in Japan since ancient times, were brought to Europe by the end of the 18th century, becoming more popular in the 1990s. Currently, 38-40 species are known. In the wild, plantain lilies grow on riverbanks, cliffs, mountain slopes and swampy areas and even on sand dunes. They prefer shady and humid habitats. Currently there are many varieties of *Hosta*. According to some sources, about 3,000 varieties and a much larger number of cultivars have been recorded. The article reflects the current state of the collection of the Ornamental Plant Laboratory of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI). The taxonomic analysis points out the presence of 50 species and cultivars, which impress the visitors with the decorative character of their leaves and flowers. The specimens were obtained from seed and rhizomes through: the international exchange of germplasm (*Index Seminum);* the work trips taken by collaborators from other botanical gardens; purchases; donations; exchanges with ornamental plants amateurs etc. For a successful cultivation of the Japanese lily are presented information about: the botanical and horticultural classification; seasonal development rate and multiplication techniques.

Keywords: *Hosta Tratt., collection, description, phenology, cultivation.*

Introduction

For today's society, ornamental plants have become consumer goods, indispensable in everyday life. The green spaces combine a varied assortment of trees, shrubs and herbaceous plants, but the flowering plants are the ones that, through their grace, delicacy and vast color palette, give color, shape and texture to the landscape and represent a visual element of maximum appeal.

The landscape of any garden, even with species that display the most beautiful flowers, will not be complete without plants with ornamental foliage. The queen of decorative leafy plants is rightly considered *Hosta* Tratt. The Japanese lily, in addition to the decorativeness of the leaves, is appreciated for the color and abundance of pleasantly smelling flowers, the long period of flowering, and thus ennobles the assortment of sciadophilous plants, being irreplaceable for shady places. From large-scale attractions like *Hosta* 'Blue Angel' to miniature specimens like *H.* 'Blue Mouse Ears', hostas are the "*little black dress*" – a must-have for individual gardens and public green spaces. The grace and splendor of hostas has divided plant lovers into two camps, those who adore them and those who have yet to discover them, but in most cases it is "*love at first sight...*"

Representatives of the *Hosta* Tratt. genus are native to Japan, Korea and China, where they have been cultivated for hundreds of years. In nature, autumn lilies grow on riverbanks, on rocks, mountain slopes, marshy places and even on sand dunes. It prefers shady and moist

habitats (Lord, 2003; Sîrbu, 2008). Currently there are many varieties and forms of *Hosta*. According to some sources, about 3000 cultivars are registered (Grenfell and Shadrack, 2009; Hosta Library, *n.d.*). These wonderful decorative plants, which in Japan have been known and cultivated since ancient times, were brought to Europe towards the end of the 18th century, becoming more popular only in the 90s of the last century (Lord, 2003; Sfeclă *et al*., 2017).

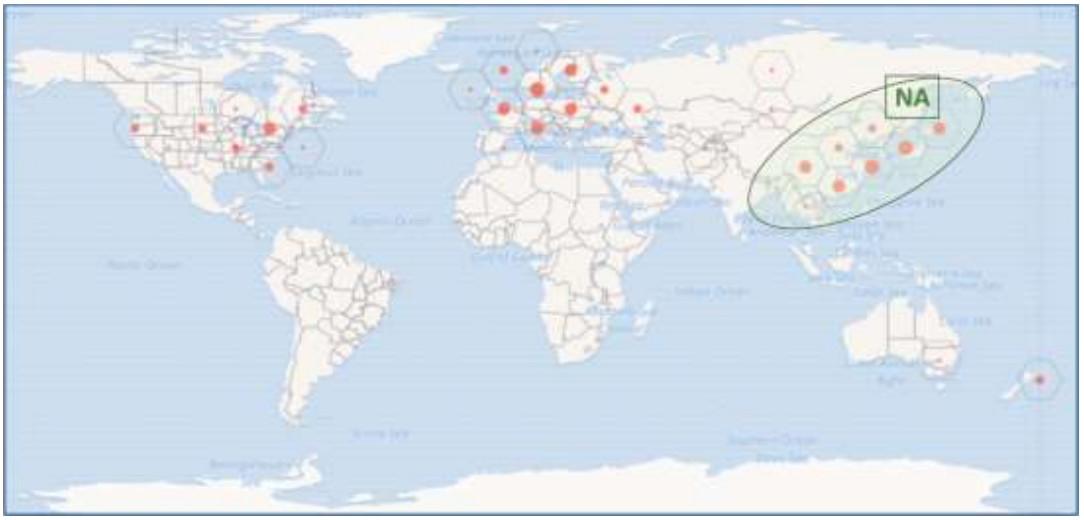

Figure 1. The natural area (Na) and cultivation areas of the genus *Hosta* Tratt. (WFO, 2013).

The genus *Hosta* Tratt. (Japanese Lily or Autumn Lily) belongs to the subfamily *Agavoidae*, family *Asparagaceae* Juss., order *Aspargales* Link., class *Monocotyledoneae* (Sfeclă *et al*., 2017). It originates from the East-Asian floristic region: Japan, China, the Kurile Islands and Sakhalin (Figure 1). About 38-40 species are known and described (Lord, 2003; WFO, 2013). Some authors assign this genus to the H*ostaceae* family. The name was given in honor of the Austrian botanist and doctor N. Hosta (1761-1834). For a long time, hostas were considered representatives of the genus *Hemerocallis* L., and later, of the genus *Funkia* Spreng. The last one remained as a synonym (Sîrbu, 2008; Cuciurcă *et al*., 2010, Sfeclă *et al.*, 2017).
Hostas are perennial herbaceous plants, geophytes, with short, slightly lignified rhizomes. Root system - very bushy and branched. The leaves are ovate-lanceolate, cordiform, with prominent veins, they are green, emerald-green or variegated, with sometimes wavy edges. It forms lush, very decorative bushes. Some species, varieties have glaucous leaves. The infundibuliform, pendant, lavender, purple or white flowers consist of six arched stamens, a filamentous pistil and perigone with six lacinia. Inflorescences of the raceme type, arched, which can be higher or almost as tall as the bush. The fruit is a tricarpellar, polyspermous capsule.
This paper presents *Hosta* species and cultivars existing in the "Alexandru Ciubotaru" National Botanical Garden (Institute) (GBNI), which can be successfully used in the design of public green spaces and individual gardens.

Material and Methods

The research was carried out in the Ornamental Plants Laboratory of the NBGI. The binary nomenclature of the species is in accordance with current international nomenclature, such as *The World Flora Online* (2013). Intraspecific taxa verified according to the "*Hosta library*" database. Author citation of species was performed after the paper "*Authors of plant names*" de R. Brummitt and C. Powell (1992). Hosta taxa were classified and grouped by height, flower shape, flower color, and leaf color (Table 1). The study of the development rate was

carried out according to the "*Methodology of phenological observations in botanical gardens*" (1975). Phenological spectra were constructed and examined.

Table 1. Classification of *Hosta* Tratt according to characteristic features.

Height categories					
H_1	H_2	H_3	H_4	H_5	H_6
Very large (70 cm & her)	Large (45-70 cm)	Medium (25-45 cm)	Small (15-25 cm)	Very small (10-15cm)	Miniature (up to 10)
Flower shape					
F_{sh}		B_{sh}		S_{sh}	
Funnel-shaped		Bell-shaped		Spider-shaped	
Flower color					
W		L		P	
White		Lavender		Purple	
Base Leaf Color					
L_{c1}		L_{c2}		L_{c3}	L_{c4}
Green		Blue-green		Yelow	White
Leaf Variegation					
L_{vM}		L_{vm}		L_{vs}	
Marginal		Medial		Streaked	
Variegation Color					
1	2	3	4	5	6
White	Creamy white	Greenish white	Yelow	Blue-green	Green

*Source: Author's elaboration.

Results and Discussion

Within the Ornamental Plant Collection of the NBGI, in addition to other generic collections, such as: *Paeonia* L., *Eremurus* Bieb., *Kniphofia* Moench. and other, the genus *Hosta* Tratt. (Aspargaceae) currently lists 56 specific taxa, 5 species and 51 cultivars (Figure 2), which impress visitors with the decorativeness of the leaves and flowers (Sîrbu *et al*., 2019). The specimens were obtained from seeds or rhizomes, through the international exchange of germplasm (*Index Seminum*), the trips made by collaborators to botanical gardens abroad, procurement, donations, exchange with ornamental plant lovers, etc The passage of the phenological phases represents an important index in the process of introducing plants. It indicates the level of adaptation of the species in *ex situ* conditions. The normal development, fruiting and stability of the phenological phases demonstrate the concordance of the species with the climatic rhythm of the place of introduction and its stability in culture (Sfeclă, 2018). Examining aspects of the seasonal rhythm of development, following the method of phenological observations in botanical gardens (1975), hostas go through the following phenological phases: initiation of vegetation; budding; blooming; fructification; the end of vegetation.The initiation of vegetation, in the studied species, takes place in the 3rd decade of April, the first decade of May. The first leaves appear wrapped in a pointed bud of green or green-purple color, corresponding to the species. Within 10-15 days the bud grows in height and the first leaves unfold. Throughout the vegetative period, the hostas decorate with the shape, size and color of the leaves, which are preserved until the specimens enter the latent period (October-November). Of all the *Hosta* taxa in the NBGI collection, only *H.* 'White Feather' is an exception. This cultivar develops the first 2-3 pairs of white leaves, which dry up at the end of May. After a rest of 10-12 days, it develops a rosette of green leaves with white striations (Figure 2). Budding is the moment that determines the passage of plants into generative phases. This includes on average 15-20 days.

Specific taxon	Phenological phases							
	*	04	05	06	07	08	09	10
Hosta kiyosumiensis	H_3 F_{sh} L L_{c1}							
H. lancifolia	H_4 F_{sh} L L_{c1}							
H. plantaginea	H_2 B_{sh} W L_{c1}							
H. sieboldii	H_3 B_{sh} L L_{c1} L_{vm1}							
H. ventricosa	H_3 B_{sh} P L_{c1}							
H. 'Abiqua Drinking Gourd'	$H_{2\text{-}3}$ B_{sh} W L_{c2}							
H. 'American Halo'	H_2 B_{sh} W L_{c2} L_{vM2}							
H. 'American Switheart'	H_2 F_{sh} L L_{c1} L_{vm1}							
H. 'August Moon'	$H_{2\text{-}3}$ B_{sh} W L_{c1}							
H. 'Behemot'	H_1 B_{sh} W L_{c1}							
H. 'Big Daddy'	H_2 B_{sh} W L_{c2}							
H. 'Blue Angel'	H_1 B_{sh} W L_{c2}							
H. 'Blue Mamut'	H_1 B_{sh} L L_{c2}							
H. 'Blue Mouse Ears'	H_5 B_{sh} L L_{c2}							
H. 'Brim Cup'	H_3 F_{sh} L L_{c2} L_{vM2}							
H. 'Capitan Kirk'	H_2 B_{sh} L L_{c2} L_{vm4}							
H. 'Cherry Berry'	$H_{3\text{-}4}$ B_{sh} L L_{c2} L_{vM1}							
H. 'Dream Queen'	H_2 B_{sh} W L_{c2} L_{vm1}							
H. 'El Nino'	H_3 B_{sh} P L_{c2} L_{vM1}							
H. 'Elegans '	H_1 F_{sh} W L_{c2}							
H. 'Enterprise'	H_3 B_{sh} L L_{c1} L_{vm1}							
H. 'Fire & Ice'	H_3 B_{sh} L L_{c1} L_{vm1}							
H. 'Fragrant Bouquet'	H_2 F_{sh} L L_{c1} L_{vM4}							
H. 'Francee'	H_3 F_{sh} L L_{c1} L_{vM1}							
H. 'Fortunei Albopicta'	H_2 F_{sh} L L_{c1} L_{vm4}							
H. 'Francens Williams'	H_2 B_{sh} W L_{c2} L_{vM4}							
H. 'Gold Standard'	H_2 F_{sh} L L_{c1} L_{vM4}							
H. 'Great Expectation'	H_3 B_{sh} W L_{c2} L_{vm4}							
H. 'Halcyon'	H_3 B_{sh} L L_{c2}							
H. 'June'	H_3 F_{sh} L L_{c3} L_{vM5}							
H. 'Lemon Lime'	H_5 F_{sh} P L_{c3}							
H. 'Liberty'	H_2 B_{sh} L L_{c2} L_{vM4}							
H. 'Little Sunspot'	H_3 B_{sh} W L_{c3} L_{vM6}							
H. 'Love Pat'	H_2 B_{sh} W L_{c2}							
H. 'Minuteman'	H_2 B_{sh} L L_{c1} L_{vM1}							
H. 'Night Befor Christmas'	H_2 B_{sh} L L_{c1} L_{vm1}							
H. 'Niko'	H_2 B_{sh} L L_{c1} L_{vM4}							
H. 'On Stage'	H_2 B_{sh} L L_{c1} L_{vm4}							
H. 'Paradise Joyce'	H_3 B_{sh} W L_{c2} L_{vm4}							
H. 'Patriot'	H_2 B_{sh} L L_{c1} L_{vM1}							
H. 'Piligrim'	H_3 B_{sh} P L_{c1} L_{vM4}							
H. 'Praying Hands'	H_3 B_{sh} P L_{c1} L_{vM4}							
H. 'Purple Heart'	H_3 B_{sh} P L_{c1}							
H. 'Raspberry Sundae'	H_4 B_{sh} P L_{c1} L_{vm1}							
H. 'School Mouse'	H_5 B_{sh} L L_{c2} L_{vM4}							
H. 'So Sweet'	H_2 F_{sh} L L_{c1} L_{vM2}							
H. 'Stained Gass'	H_2 B_{sh} L L_{c1} L_{vm4}							
H. 'Stiletto'	H_3 B_{sh} P L_{c2} L_{vM2}							
H. 'Sum and Substance'	H_1 B_{sh} L L_{c1}							
H. 'Tokudama Flavocircinalis'	H_3 B_{sh} W L_{c2} L_{vM2}							
H. 'Tom Schmid'	H_1 B_{sh} L L_{c2} L_{vM2}							
H. 'Undulata'	H_3 B_{sh} L L_{c1} L_{vm1}							
H. 'Venus'	H_3 F_{sh} W L_{c1}							
H. 'Wide Brim'	H_3 B_{sh} L L_{c2} L_{vM2}							
H. 'White Feather'	H_3 F_{sh} L L_{c1} L_{vs1}							
H. 'Winter Snow'	H_1 B_{sh} L L_{c1} L_{vM1}							

	Vegetation phase		Budding phase		Blooming phase		Fructification phase
*	Classification		Early flowering period		Middle flowering period		late flowering period

Figure 2. Ritmul sezonier al hostelor în condițiile climaterice ale Republicii Moldova.

The flowering phase is one of the most important in the annual development cycle of ornamental plants, determining both the floral decorativeness and the ability of the plant to multiply generatively. For representatives of the genus *Hosta*, the decorativeness of the flower is a complementary character to the decorativeness of the leaves. The flowering period of hostas is 20-30 days. A long flowering period is characteristic for *H. lancifolia* (Thunb.) Engl., *H. ventricosa* Stearn, *H.* 'Stiletto', *H.* 'Winter Snow', and other. Flowering occurs gradually from the base of the inflorescence to the top. The flowers ((♀♂ *$P_{(6)}A_{(6)}\underline{G}_{(3)}$) are white, lavender or purple in color, which does not change during the flowering phase. The duration of flowering for one flower is, on average, 3-4 days. Depending on the beginning of the flowering phase, hostels are divided into three groups:

- early flowering hostas, which initiates the flowering phase in June (*Hosta kiyosumiensis* F.Maek., *H.* 'Behemot', *H.* 'Big Daddy', *H.* 'Blue Mamut' and others);
- mid-flowering hostas, which initiates the flowering phase in July (*Hosta sieboldii* (Paxton) J.W.Ingram, *H.* 'August Moon', *H.* 'Blue Angel' and others);
- late flowering hostas, which initiate the flowering phase in August-September (*Hosta lancifolia* (Thunb.) Engl., *H. plantaginea* (Lam.) Asch., *H.* 'Fragrant Bouquet' and others).

Out of all the *Hosta* taxa existing in the NBGI, only 11 recorded fruiting phases (Figure 2). The initiation of the fruiting phase for early flowering species is marked in the 2nd-3rd decade of July, those with middle and late flowering - in the months of August-September. The duration of the fruiting phase is about 20-50 days. The fruit of the studied species is a dehiscent capsule, which opens into three valves.

The end of the vegetation period occurs with the first frosts, which is manifested by the withering and drying of the leaves characteristic of the initiation of biological dormancy.

The horticultural classification of hostas refers to characters related to habitus, leaf and flower.

Figure 3. The number of *Hosta* Tratt taxa by height categories.
H_1 - very large; H_2 - large; H_3 - medium; H_4 - small; H_5 - very small; H_6 - miniature.

Depending on the height in the *Hosta* collection, medium-sized taxa predominate (*Hosta kiyosumiensis*, *H.* 'Fire & Ice' and others) followed by large ones *(Hosta* 'American Switheart', *H.* 'Capitan Kirk', *H.* 'Gold Standard' and others). Unfortunately, there are currently no taxa of miniature hosts in the collection (Figures 2 and 3).

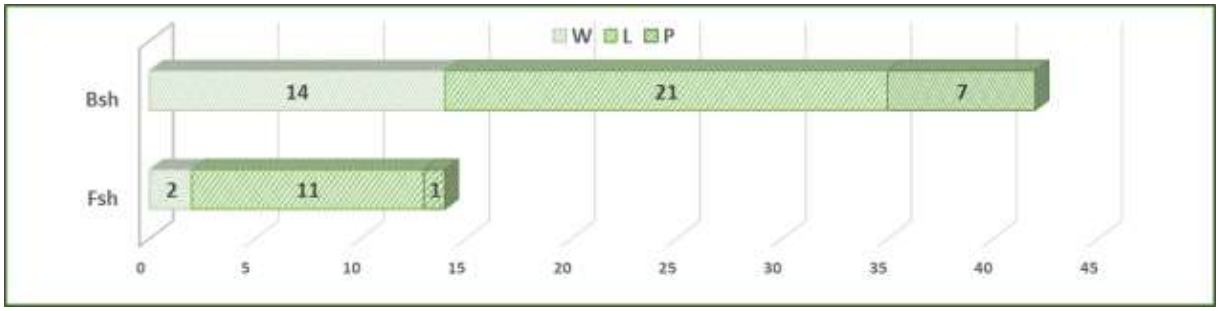

Figure 4. The number of taxa according to the shape and color of the flower.
F_{sh} - funnel-shaped; B_{sh} - bell-shaped; *W* - white; *L* - lavender; *P* - purple.

Depending on the shape and color of the flower, the collection predominates in lavender bell-shaped flower hostels (21 taxa), followed by white bell-shaped flower hostels (14 taxa) and lavender funnel-shaped flower (11 Toll). Depending on the shape and color of the flower, in the collection predominates hostas with bell-shaped flower of lavender color (21 taxa), followed by hostas with bell-shaped flower of white color (14 taxa) and with funnel-shaped flower of lavender color (11 taxa). Out of all the taxa, only 7 have a purple bell-shaped flower, 2 taxa – white funnel-shaped flower and 1 taxa – purple funnel-shaped flower (Figures 2 and 4).

The *Hosta* collection includes 18 taxa with non-variegated green or blue-green leaves and 38 taxa with variegated green, blue-green or yellow leaves (Figures 2 and 5). Specimens with variegated leaves are grouped into:

- Marginal variegation – 23 taxa (*Hosta* 'American Halo', *H.* 'Fragrant Bouquet', *H.* 'Gold Standard', *H.* 'Patriot', *H.* 'Piligrim', *H.* 'Wide Brim' and others);
- Medial variegation – 14 taxa (*Hosta* 'Fortunei Albopicta', *H.* 'Dream Queen', *H.* 'Raspberry Sundae', *H.* 'Stained Gass', *H.* 'Undulata' and others);
- Streaked variegation – 1 taxa (only *Hosta* 'White Feather').

The variegation of hosta leaves has the following colors (Figures 2 and 5):

- White variegation color – 15 taxa (*Hosta* sieboldii, *H.* 'El Nino' and others);
- Creamy white variegation color – 7 taxa (*Hosta* 'Brim Cup', *H.* 'Stiletto' and others);
- Yelow variegation color – 14 taxa (*Host*a 'Capitan Kirk', *H.* 'Niko' and others);
- Blue-green variegation color – 1 taxa (only *Hosta* 'June');
- Green variegation color – 1 taxa (only *Hosta* 'Little Sunspot').

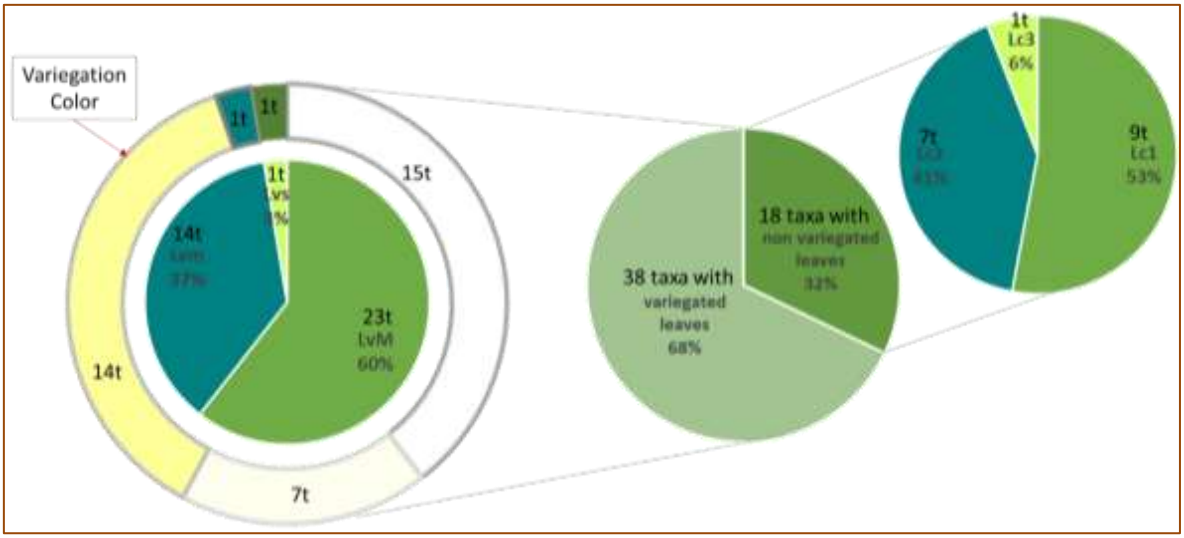

Figure 5. The number of taxa according to the shape and color of the leaf.
Base leaf color *:* L_{c1} – green; L_{c2} – blue-green; L_{c3} – yelow;
L_{vM} – marginal leaf variegation; L_{vm} – medial leaf variegation; L_{vs} – streaked leaf variegation;

Hosta species that produce viable seeds in the new living conditions can be multiplied both generatively and vegetatively(*Hosta plantaginea*, *H. sieboldii*, *H. ventricosa*). Species that do not develop fruit in ex situ conditions and cultivars can be multiplied by dividing the bushes and micropropagation. Sowing is carried out in late autumn or winter, in greenhouses. Propagated by seed, the Japanese lily reaches maturity in 3-4 years. Thus, vegetative propagation is more cost-effective for multiplying these plants, because we get specimens with flowers the following year. Dividing and planting is recommended to be done in autumn

(September–October), when there is no danger of frost, or in spring, before the beginning of the growing season. Vegetative propagation is achieved by fragmenting with a sharp tool the mother plant into portions that must have both buds and roots. The division can be applied once every 2-3 years.

Conclusions

Within the Collection of Ornamental Plants of the "Alexandru Ciubotaru" National Botanical Garden (Institute) includes 56 specific taxa, 5 species and 51 cultivars. In the climatic conditions of the Republic of Moldova, they keep their biomorph, a fact that demonstrates their plasticity of adaptation. The beginning of vegetation is highlighted by a small variability, while the other phases vary a lot. According to the terms of the initiation of the generative phases, the studied species can be grouped into three categories: with early flowering, with middle flowering and late flowering. Vegetative propagation, by dividing plants, is more cost-effective for Japanese lily cultivation. The decorativeness of the leaves, the long duration of flowering, the vital form, the size and the habitus of the plants represent important indicators of decorativeness and allow the use of hostas in the arrangement of green spaces, as a container culture and in the art of bouquets.

Acknowledgement

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REMOVAL OF THE WATER FROM FENAMIPHOS PESTICIDE IN THE SOIL USING MORINGA OLEIFERA

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Abstract

The development of a sorbent low cost-effective to improve the removal of Organophosphate pesticides from contaminated water solutions continues to inspire. in the present display study, different sorbents such as Moringa oleifera. (FENAMIPHOS). was studied. Effect of temperatures (10 to 40 °C) affecting the adsorption process, such as concentration of FENAMIPHOS (2 to 30 ppm), sorbent dose (0.05 to 0.4)g, solution pH (2 to11), exposure time (10 to 80) Minutes. The adsorption of FENAMIPHOS sorbents follows the Freundlich model is more suitable than the Langmuir model. Maximum absorption capacities of F FENAMIPHOS about 49 mg/g were found. , M.oleifera removal was achieved at pH 7 at initial concentrations of FENAMIPHOS 20ppm in a contact 30 minutes period. The kinetic data fit well with the energy functions and linear models. Thermodynamic parameters reveal the spontaneous and endothermic nature of the FENAMIPHOS adsorption process. Sorbents can be introduced as an efficient and inexpensive alternative for removing FENAMIPHOS-contaminated water.

Keywords: *Water, Environmental health, pollution, pesticides,Gaza.*

Introduction

Pollution risks and develops plans for improving management practices for reducing these risks. Groundwater from deep and confined aquifers is usually microbially safe and chemically stable in the absence of direct contamination; however, shallow or unconfined aquifers can be subject to contamination from discharges or seepages associated with agricultural practices (e.g. pathogens, nitrates, and pesticides, chemicals responsible for large-scale health effects through drinking-water (RS2, 2012). The most commonly used methods for the treatment of pharmaceutical industry wastewater can be broadly classified into physicochemical processes, and biological processes (Shah & Shah, 2020). Treatment of wastewater by using the adsorption derived. Which from agricultural waste was carried out by Mohan. (Kulkarni & Goswami, 2013). Pesticides have been widely and increasingly used to control insect pests, plant pathogens, and weeds over the past 50 years (Prasertsup & Ariyakanon, 2011). Pesticides such as chemical substances enter the ecological cycle and pose many threats to life forms. (Shanmugam, 2013). Organophosphate pesticides are highly toxic to freshwater invertebrates (Zaranyika et al., 2010). Non-biodegradable compounds may be toxic, carcinogenic, or mutagenic at very low concentrations (to plants, animals, and humans). Some may bioaccumulation in food chains (Hisham et al., 2012). Research in developed and developing countries is designed to find inexpensive and simple technologies for treating domestic and agricultural wastewater contaminated with pesticides to protect human health and the environment from adverse side effects caused by these substances and their derivatives. (Qurie et al., 2016). The main advantages of using natural plant-based coagulants as POU water. earth's natural resources and widespread environmental degradation, the application of these coagulants is a vital effort in line with the global

sustainable development initiatives comparable to their chemical counterparts in terms of treatment efficiency(Yin, 2010). WasteOrganic substances, inorganic substances, and microbiological classifications in wastewater are based on the chemical and natural composition of their active substances, which depend on the efficacy, and physical and chemical properties of the pesticides. The information on the chemical and physical characteristics of pesticides is useful in determining the mode of application, and precautions that need to be taken during application. The classification of chemical compounds is divided into four main groups: argon chlorine, organic phosphorus, carbamate, pyrethrin, and pyrethroids. The inorganic compound is also used as a pollutant. The basic of these problems is discharging sewage without suitable treatment by wastewater treatment plants in the Gaza strip. Pesticides are one of the very important pollutants that have negative effects. In Gaza Strip, insecticides (Kaur et al., 2019). Fenamiphos an organophosphorus pesticide is primarily used to control nematodes in a wide range of horticultural crops and in turf. Fenamiphos is highly toxic via the oral route, with reported LD50 values of 2 to 19 mg/kg in the rat and 56 to 100 mg/kg in guinea pigs. Various treatment procedures have been reported for the removal of Fenamiphos from water to treat toxic organic pollutants in water. (Abu Ghalwa & B Farhat, 2016) Fenamiphos in Soil Half-life Soil (278, 91.8, 79.2 days) (Zaranyika et al., 2010). Of everyone produced almost the annual consumption of the Gaza Strip was estimated to be more than 144.68 tons of pesticides. the curve shows fig 1 the amounts of pesticides (insecticide/ton) used in the Gaza Strip between (2002 and 2010) ministry of agriculture MOA Palestine, 2011(Musabeh, 2016)

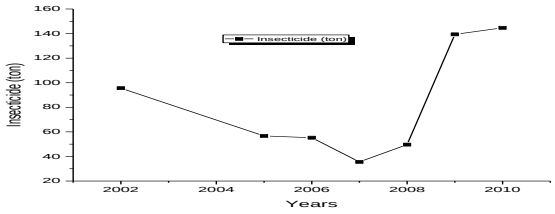

Figure 1.

Environmental pollution is one of the major problems being faced in the world through the 21st century. Environmental pollution is of global concern due to the drastic effects they have on human health, air, water, soil, and ecosystems. Therefore, pollution in the Mediterranean from land-based sources are organic pollution, biological contaminants in addition to nutrients, and toxic substance. (Alnady, 2013). Although polluted by agriculture or households causes damage to human health or the environment. This water pollution affects the health and quality of soils and vegetation. Some water pollution effects are recognized immediately, whereas others don't show up for months or years(Mashhood Ahmad Khan & Arsalan Mujahid Ghouri, 2011). The impact of different pollution as a general on the health of farm animals are cancer, neurobehavioral disorders, cardiovascular problems, reduced energy levels, premature death, asthma, asthma exacerbations, headaches and dizziness, irritation of the eyes, nose, mouth, and throat, reduced lung functioning, respiratory symptoms, respiratory disease, disruption of endocrine and reproductive and immune systems. Exposure to environmental pollutants affected reproductive functions and in particular, produced adverse effects on pregnancy outcomes, fertility, and fetal health. Exposures to ambient levels of pollutants are associated with low birth weight, intrauterine growth retardation, prematurity, neonatal death, decreased fertility, and a decrease in sperm quality in males and indicated also that female fertility was also disturbed. (A. A. M & EL-Tarabany, 2018) Conventional wastewater treatment, typically, consists of a combination of physical, chemical, and biological processes and operations to remove solids, organic matter, and, sometimes, nutrients from wastewater (Alsahhar, 2015). Fenamiphos is highly toxic via the oral route, with reported LD50 values of 2 to 19 mg/kg. The inhalation toxicity of the compound is also

high, with reported inhalation of 2 to 19 mg/kg LC50. (Abu Ghalwa & B Farhat, 2016)Fenamiphos (Nemacur ®),IUPAC name ethyl 3-methyl-4-(methylthio) phenyl (1-methyl- ethyl) phosphoramidite.(Lee et al., 1986). Properties of Fenamiphos(Abu Ghalwa &b frahat,2016).

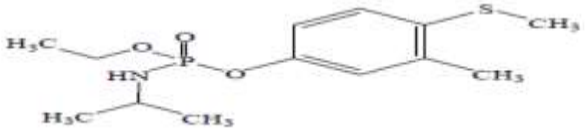

Figure 2. Chemical structure of Fenamiphos.

Coagulants used The seeds of M. oleifera, were harvested when they were fully matured. This is determined by observing if there are any cracked pods on the plants. The pods that were plucked were cracked to obtain the seeds which were air-dried at 40°C for two days. The shells surrounding the seed kernels were removed using the knife and the kernels were pounded using laboratory mortar and pestle into powder and sieved using a strainer with a pore size of 2.5 mm 2 to obtain a fine powder. This was the coagulant prepared from M. oleifera. The study A new method of removing FENAMIPHOS from contaminated water using molecularly imprinted polymeric microspheres for FENAMIPHOS is described which were prepared by the emulsifier-free polymerization method. The removal efficiency and selective recognition ability of the molecularly imprinted polymeric microspheres were studied. The highest removal efficiency was observed at pH 7. Moreover, molecularly imprinted polymeric microspheres can be re-used at least 10 times without losing any removal efficiency. A selective, simple, reliable, and practicable solution to remove FENAMIPHOS from contaminated water (Zhang et al., 2011).

Material and Methods

coagulant prepared size of (2.50 mm) from M.oleifera, which is used in the study.Preparation of Stock solutions of FENAMIPHOS (Nemacur ®), (100 ppm) were prepared by dissolving 20 ml of concentration (400g/L) of FENAMIPHOS in 100 ml of deionized water. The stock solution was used to prepare different solutions having different concentrations range from 1 ppm to 30 ppm by suitable dilution Then The absorptivity coefficient (Ɛ) of each was determined UV-vis spectrophotometrically according to Beer- Lambert law Eq. (1)

A = Ɛ C L ---------- (1)

Where, A: Absorbance, Ɛ: Absorptivity coefficient, C: concentration, and L: cell width. The absorptivity constants (Ɛ) of FENAMIPHOS determined from the slops of linear plots of absorbance versus concentration.Removal The FENAMIPHOS process was performed using batch stirring process at certain temperature. In each experiment, 100 ml of known amount of FENAMIPHOS solution was added to a certain amount of M.oleifera,. 0.05, ,0.1, 0.2, 0.3 ,0.4 gram and add (2, 5, 10, 15, 20, 25, 30 ppm) of FENAMIPHOS was used for each experiment, the optimum values of variables such as stirring time (interval time 30 minutes) and temperature 25 were kept constant. The FENAMIPHOS adsorbed by M.oleifer , seeds settled at the is termed as. The FENAMIPHOS content left out in treated water sample was measured spectrophotometrically at the corresponding maximum wavelength of the FENAMIPHOS utilizing their pre-constructed calibration curves. The remaining aliquot of the FENAMIPHOS solution were withdrawn for absorption measurements using UV-vis spectrophotometer. Using Eq. (2).

$$qt = \frac{V(C0 - Ct)}{m} \quad -----(2$$

The adsorption capacity of the adsorbent FENAMIPHOS were determined. where, C0 is the initial FENAMIPHOS concentration (ppm), Ct is the FENAMIPHOS concentration in solution at a pre-defined time (ppm), t is the time by seconds (s), V is the volume of the FENAMIPHOS (L), and m is the mass of the FENAMIPHOS and adsorbent (g). Moreover, the percent removal of pesticides (R%) was determined from Eq. (3)

$$R\% = \frac{C0 - Ct}{C0} \; x\,100 - - - - - - - - (3)$$

Where, C0 and Ct have the previously mentioned meaning. Additionally, by utilizing Eq. (4), the equilibrium adsorption capacity of the FENAMIPHOS adsorbent qt (mg/g), can be determined.

$$qt = \frac{V(C0 - Ce)}{m} - - - - - - - - (4$$

Where, Ce (ppm) is the FENAMIPHOS concentration at equilibrium in the supernatant after separation of the adsorbent; and V, m, as well as C0 have the meaning mentioned before. Ultraviolet-Visible absorption spectra were obtained using a Shimadzu- 1601 UV-vis spectrophotometer in the wavelength range from 270 to 320 nm for FENAMIPHOS. Removal of FENAMIPHOS from water contamination water solution by adsorbents by use Five samples were chosen which FENAMIPHOS solution Samples of solution of water were taken for determine FENAMIPHOS concentration, effect of weight, temperature, and pH for each sample. All of that to reach an appropriate amount of M.oleifera with a suitable volume of water

Results and Discussion

The absorption spectra of FENAMIPHOS were shown with an absorption peak at 249 nm Fig.4.1 whereas the absorption spectra Calibration curves have been obtained spectrophotometrically by measuring the absorbance of distinctive concentrations of FENAMIPHOSat same conditions (contact time 35 min, room temperature, pH 7.4 and concentration= variety of (2 to 30) ppm. Figs. (4.2) show the absorption spectra of FENAMIPHOS. The absorption band of FENAMIPHOS occurs at 249 nm. It was enacted by the Lambert-Beer law Eq. (1) that the absorptivity. coefficient was calculated from the slope and compiled with the correlation coefficient R2. The obtained absorptivity coefficients are given for each FENAMIPHOS in Absorptivity coefficient, correlation coefficient, and maximum wavelength for FENAMIPHOS Absorptivity coefficient (Ɛ)(FENAMIPHOS /ppm*cm ±0.01897, Correlation coefficient R-Square = 0.99242 in wavelength 200 to 300 nm.

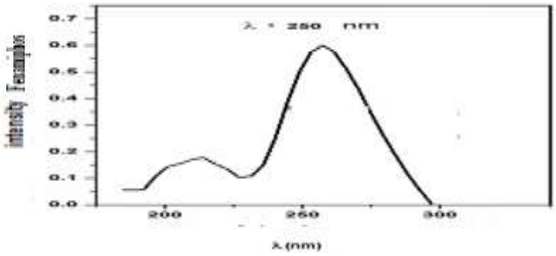

Fig. 4.1 Absorption spectra of FENAMIPHOS

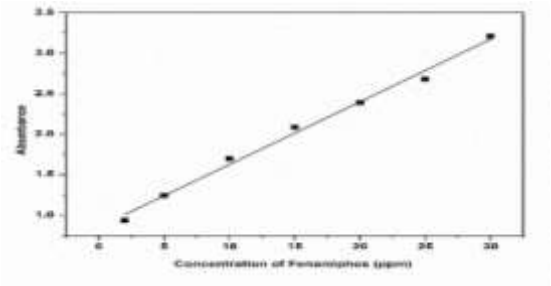

Fig.4.2 Calibration curve FENAMIPHOS

The effect of the different masses of M.oleifera –adsorbent was studied at 25 ºC . Different amount of M.oleifera adsorbent was used (0.05, 0.1, 0.2, 0.3, and 0.4 g) of adsorbents with known initial FENAMIPHOS, concentration was used from (2, 5, 10, 20, and 30)ppm of aqueous solution and calculate the percentage of removal (R%) according to (Eq. (3) as

shown in The effect of contact time, pH, Temperature, and rotation on the adsorbent of each FENAMIPHOS in the different mass of M.oleifera adsorbent, Also read Absorbance of UV spectra of FENAMIPHOS with the different mass of M.oleifera adsorbent. The effect of FENAMIPHOS initial concentration on the adsorption capacity of the different doses of adsorbents was examined using different initial concentrations of FENAMIPHOS (10 to 30 ppm) are shown in Figs (4.3), but all other conditions (contact time 35 min, room temperature, pH 7.4 and concentration equal 2 to 30 ppm) remain constant. The maximum efficiency of FENAMIPHOS removal about 94%, 92% 89%, 88%, 86% with the adsorbent dose 0.05, 0.1, 0.2, 0.3 and 0.4g respectively. On the other hand, the maximum removal efficiency of respectively to an initial concentration of both 30 ppm. The results show increases in the quantity of adsorbed FENAMIPHOS per unit mass with an increase in FENAMIPHOS initial concentrations due to the escalation in the mass driving force that permits more FENAMIPHOS molecules to pass from solution to adsorbent surface. This is consistent with (Lu et al 2006). The impact of solution pH on FENAMIPHOS removal using 0.2 g of different adsorbents was investigated at pH values of 2 to 11 and presented in Figs. (4.4) while all other conditions (adsorbent dosage equal 0.2 g, contact time 35 min, room temperature, and concentration equal 30ppm) were kept constant. The highest percentage of removal of FENAMIPHOS was about (84 %) for Moringa FENAMIPHOS removal percentage decreased for Moringa.Therefore, the optimum pH chosen for the maximum removal of FENAMIPHOS was pH 7 but Fenamiphos removal for all adsorbents increase with an increase degree of pH

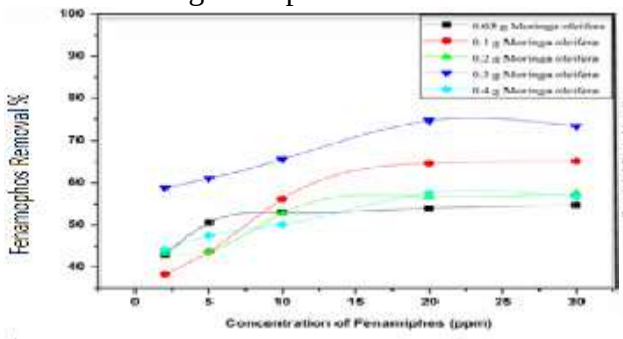

Fig.4.3 Effect weights on M.oleifera on the removal of FENAMIPHOS %

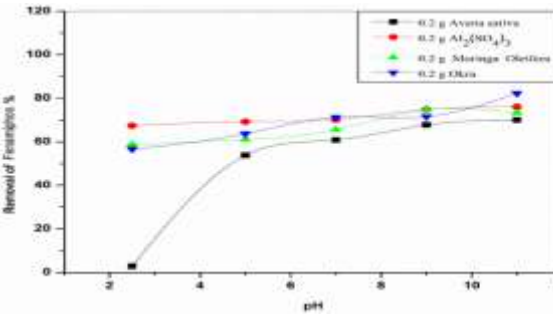

Fig 4.4 Effect of pH on the removal of FENAMIPHOS

farms, Adsorption isotherms explain the relationships between the concentration of the adsorbate (C_e) in the liquid phase and its concentration on the adsorbent at equilibrium and constant temperature (M.Y. Nassar, et al., 2016). The data experiments depended on known initial FENAMIPHOS 2 to 30 ppm and different amounts of – M. Oleifera adsorbent. They were checked using adsorption isotherms models Langmuir and Freundlich isotherm. That's explains the Formation of a monolayer adsorbate where there is no any interactions between the adsorbed the adsorbate molecules. Langmuir isotherm model can be presented in Eq. (5) (M.Y. Nassar, et al., 2016). Also using a dimensionless constant called equilibrium parameter RL (Eq.(6)) the efficiency and characteristics of the Langmuir isotherm model can be evaluated.

$$\frac{Ce}{qe} = \frac{1}{qm} KL + \frac{Ce}{qm} \quad -----(5)$$

$$RL = \frac{1}{1 + KLC0} -------(6)$$

Where, C_e is the equilibrium concentration of the FENAMIPHOS solution (ppm), q_m constant is the maximum adsorption capacity (mg/g), K_L is Langmuir constant (L/mg), C_0 is the initial concentrations of FENAMIPHOS (mg/L), and R_L value gives an indication of the

adsorption process. Either RL is an irreversible (RL equal 0), favourable (0 <RL< 1), linear (RL equa 1), and unfavorable (RL> 1). The results show that RL values present between zero and one, and this means that the adsorption of FENAMIPHOS by M.oleifera adsorbent is favorable. The plot of 1/qe versus 1/Ce values for the adsorption of the FENAMIPHOS results in a is not straight line and unfavorable (RL> 1) for each FENAMIPHOS and the KL and qq values can be determined from the intercept and slope of the plots as shown in Fig. 4.5(c) and Table (4.1)respectively.

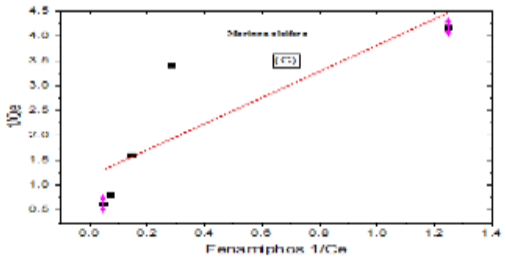

Fig.4.5 Langmuir isotherm model of (c) M.oleifera in removal of FENAMIPHOS

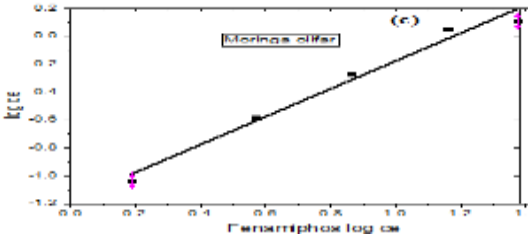

Fig 4.6 Freundlich isotherm model of FENAMIPHOS

Table (4.1) The obtained isotherm parameters from Langmuir model

Isotherm model	Constants	R^2	RL	K_L	q_m(mg/g)
Langmuir	M.oleifera adsorption	0.69	4.20	0.13453	3.5

In this model, Freundlich depends on the assumption that the adsorption takes place on a heterogeneous surface can be given by Eq.(7) (Nassar et al., 2016). The maximum adsorption capacity can also be determined using the Freundlich isotherm model by employing Eq. (8).

$$\ln qe = \ln KF + \frac{1}{n}\ln Ce \quad -------(7)$$

$$KF = \frac{qm}{C0\frac{1}{n}} \quad ----------(8)$$

Where, Ce is the equilibrium concentration of the FENAMIPHOS (ppm), KF is the Freundlich constant[(mg/g)(L/mg)1/n], 1/n is the heterogeneity factor which indicates the adsorption strength ,and qm is the maximum adsorption capacity (mg/g). The plot of lnqe versus lnCe values for the adsorption of the FENAMIPHOS results in a straight line M.oleifera as shown in Fig. 4.6. The KF and 1/n values can be obtained from the intercept and slope of the plots as shown in Fig. (4.21,4.22) for both of FENAMIPHOS.The value of 1/n= (0–1) can be used as an indication of the surface heterogeneity. The values that closer to zero are the higher to the heterogeneity (Nassar, et al., 2016). According to the results using the Freundlich isotherm model, the adsorption of the FENAMIPHOS by M.oleifera adsorbent tends to place on a heterogeneous surface since the 1/n value was in the range of 0–1.The calculated isothermal parameters of two models are reviewed in Tables (4.2) .The obtained isotherm parameters explain that the adsorption of these FENAMIPHOS by M.oleifera -adsorbent -adsorbent serve better the Langmuir isotherm model. Furthermore, the adsorption is monolayer coverage because the correlation coefficient (R2) values are closer to unity comparing to the Freundlich

Table (4.2) The obtained isotherm parameters from Freundlich mode(FENAMIPHOS)

Isotherm model	Constants	R^2	KF	1/n	qm (mg/g)
Freundlich	M.oleifera adsorption	0.99	0.009171	0.8973	48

Conclusions

In the present display study, the low-cost adsorbent substance was evaluated for remediation of FENAMIPHOS from solutuion. fitted well to Freundlich and power function models. had the highest FENAMIPHOS removal rates at about (80%), M.oleifera removal was achieved at pH 7 at initial concentrations of FENAMIPHOS 20 ppm in contact. 30 minutes' period. The kinetics and thermodynamic studies revealed that the FENAMIPHOS adsorption reaction onto the different adsorbents is exothermic in nature and the associative mechanism is involved in the FENAMIPHOS sorption process. Thermodynamic parameters reveal the spontaneous and exothermic nature of the FENAMIPHOS sorption process. Adsorbents can be introduced as an effective and inexpensive alternative for removing FENAMIPHOS from contaminated water. The governing factors for FENAMIPHOS adsorption onto M.oleifera seed include H bonding, hydrophobicity, and electrostatic and π–π interactions. The obtained results revealed that the newly sorbent produced is an efficient adsorbent and exhibits high capability as removal contamination of water.

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PURIFICATION OF WATER FROM CHLORPYRIFOS IN THE SOIL BY USING MORINGA OLEIFERA

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Abstract

The development of cost-effective sorbents to improve the removal of pesticides from contaminated water solutions continues to inspire. In this study, different sorbents such as Moringa oleifera. Chlorpyrifos (CPF). was studied for the first time. Effect of temperatures (10-40 °C) affecting the adsorption process, such as concentration of CPF (2-30 ppm), sorbent dose (0.05-0.4)g, solution pH (2-11), exposure time (10-80) Minutes. The adsorption of CPF sorbents follows the Freundlich model is more suitable than the Langmuir model. Maximum absorption capacities of F Chlorpyrifos 53 mg/g were found. Moringa oleifera removal was achieved at pH 7 at initial concentrations of CPF 20ppm in a contact 30 minutes period. The kinetic data fit well with the energy functions and linear models. Thermodynamic parameters reveal the spontaneous and endothermic nature of the CPF adsorption process. Sorbents can be introduced as an efficient and inexpensive alternative for removing CPF-contaminated water (ΔS0) and standard free energy (ΔG0). The adsorption was carried out by an endothermic process because the obtained (ΔH0) values for removal were less than 40 KJ/mol. Negative value Standard free energy values (ΔG0) indicate that the adsorption process is irreversible. Removal of (CPF) from contaminated water solution.

Keywords: *wastewater , Environmental health , pollution, pesticides, Palestine.*

Introduction

There are a lot of kinds of pollution like water pollution, air pollution, and land and solid waste pollution (Tariq & Salim, 2021). This water pollution affects the health and quality of soils and vegetation. One effect of water pollution effects is recognized immediately, whereas others don't show up for months or years (Mashhood Ahmad Khan & Arsalan Mujahid Ghouri, 2011). Therefore, to understand the suitability of the adsorbent for any particular adsorption process and the efficiency of the process. it is necessary to know the steps involved in the adsorption of a pollutant from an aqueous solution (Georgieva et al., 2017). It seems to be a promising technique due to its economic feasibility, simplicity of design, recycling of adsorbent, and nonexistence of harmful residues. Although conventional adsorbent like activated carbon is effective, the high cost associated with the material has discouraged its practical applications(Tariq & Salim, 2021). The detected concentrations of Chlorpyrifos in both surface and groundwater are in the range of 0.13–0.24 μgl−1. Existing wastewater treatment plants (WWTPs) are often inefficient in removing these pollutants from wastewater. This leads to the accumulation of toxic substances in the water body, thus causing human health risks and damage to the ecosystem (Qurie et al., 2016). The main advantages of using natural plant-based coagulants as POU water. earth's natural resources and widespread environmental degradation, the application of these coagulants is a vital effort in line with the global sustainable development initiatives comparable to their chemical counterparts in terms of treatment efficiency. These natural coagulants function by means of an adsorption mechanism followed by a charge neutralization or polymeric bridging effect. Frequently

studied plant-based coagulants include Moringa oleifera (Yin, 2010). The Gaza Strip contains many pollutants such as suspended solids, biodegradable organics, pathogens, nutrients, refractory organics, heavy metals, and dissolved inorganic compounds. Direct discharge of untreated wastewater into the natural (Program & Jamee, 2014). The wastewater quality from GWWTP is not sufficiently treated to be used for direct irrigation and plantation. The final effluent discharged to the sea is estimated at 75000 m3 per day (Program & Jamee, 2014). Chronic human exposure to this agrochemical and to the consumption of water contaminated with it may cause long-term mutagenic effects, neurological effects neuropathies, encephalopathies, and visual disturbances affecting the cardiovascular and respiratory systems and different stages of the female reproductive cycle,(Agudelo et al., 2010). Organophosphorus pesticides (OPs), developed in the 1950s as insecticides for fruit, vegetables, and other crops, are one major class of typical chiral pesticides. The OPs, which pose potential human health risks, were detected in food in China and other countries (Synthesis, 2011). (OP) induced neuro- toxicity remains a significant occupational health concern. (Farahat, 2018). Chlorpyrifos was found to undergo degradation in natural environmental waters (Qurie et al., 2016). When using the organophosphate insecticide Chlorpyrifos most were directly exposed to Chlorpyrifos, potentially leading to illnesses (Rattanaselanon et al., 2018).Chlorpyrifos (Dursban ®) [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate is used worldwide as an agricultural insecticide. Chlorpyrifos IUPAC(O,O-diethyl-O-3,5,6-trichloropyridin- 2-phosphorothioate) and Chemical structure of Chlorpyrifos show in Fig.1 (Qurie et al., 2016).

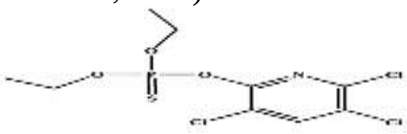

Fig.1 Structure of Chlorpyrifos

Occupational health concern. Chlorpyrifos has many adverse human health effects and can be efficiently absorbed by dermal contact, inhalation, and ingestion Chlorpyrifos residue can be detected on the skin, in blood, and through metabolites in urine (Lappharat et al., 2014). It has been reported that rural communities in African countries utilize its crude seed extracts to clear turbid river water were among the researchers to study the use of M. oleifera as a natural coagulant, conducted to optimize its usage as low-cost POU water treatment technology, with emphasis on the application within the African continent(Yin, 2010).

Material and Methods

This was the coagulant prepared size of (2.5 mm) from Moringa oleifera, which is used in the study. Preparation of Stock solutions of Chlorpyrifos (Drusban ®) (100 ppm) were prepared by dissolving 20 ml of concentration (480g/L) of Chlorpyrifos in 100 ml of deionized water. The stock solution was used to prepare different solutions having different concentrations range from 1 ppm to 30 ppm by suitable dilution Then The absorptivity coefficient (Ɛ) of each was determined UV-vis spectrophotometrically according to Beer- Lambert law Eq. (1)

$A = Ɛ\ C\ L$ ---------- (1)

Where, A: Absorbance, Ɛ: Absorptivity coefficient, C: concentration, and L: cell width. The absorptivity constants (Ɛ) of Chlorpyrifos determined from the slops of linear plots of absorbance versus concentration.Removal The Chlorpyrifos process was performed using batch stirring process at certain temperature. In each experiment, 100 ml of known amount of Chlorpyrifos solution was added to a certain amount of Moringa oleifera, 0.05, ,0.1, 0.2, 0.3 ,0.4 gram and add (2, 5, 10, 15, 20, 25, 30) ppm of Chlorpyrifos was used for each

experiment, the optimum values of variables such as stirring time (interval time 30 minutes) and temperature 25 were kept constant. The Chlorpyrifos adsorbed by Moringa oleifera, seeds settled at the is termed as. The Chlorpyrifos content left out in treated water sample was measured spectrophotometrically at the corresponding maximum wavelength of the Chlorpyrifos utilizing their pre-constructed calibration curves. The remaining aliquot of the Chlorpyrifos solution were withdrawn for absorption measurements using UV-vis spectrophotometer. Using Eq. (2).

$$qt = \frac{V(C0 - Ct)}{m} \quad ------ (2$$

The adsorption capacity of the adsorbent Chlorpyrifos was determined. where, C0 is the initial Chlorpyrifos concentration (ppm), Ct is the Chlorpyrifos and Fenamiphos concentration in solution at a pre-defined time (ppm), t is the time by seconds (s), V is the volume of the Chlorpyrifos (L), and m is the mass of the Chlorpyrifos and adsorbent (g). Moreover, the percent removal of pesticides (R%) was determined from Eq. (3)

$$R\% = \frac{C0 - Ct}{C0} \; x\,100 \; ------- (3)$$

Where, C0 and Ct have the previously mentioned meaning. Additionally, by utilizing Eq. (4), the equilibrium adsorption capacity of the Chlorpyrifos adsorbent qt (mg/g), can be determined.

$$qt = \frac{V(C0 - Ce)}{m} ------- (4$$

Where, Ce (ppm) is the Chlorpyrifos concentration at equilibrium in the supernatant after separation of the adsorbent; and V, m, as well as C0 have the meaning mentioned before.Ultraviolet-Visible absorption spectra were obtained using a Shimadzu- 1601 UV-vis spectrophotometer in the wavelength range from 270 to 320 nm for Chlorpyrifos. Removal of Chlorpyrifos from water contamination water solution by adsorbents by use Five samples were chosen which Chlorpyrifos solution Samples of solution of water were taken for determine Chlorpyrifos concentration, effect of weight, temperature, and pH for each sample. All of that to reach an appropriate amount of Moringa oleifera, with a suitable volume of water.

Results and Discussion

The absorption spectra of Chlorpyrifos was showed with absorption peak at290 nm Fig.4.1 whereas the absorption spectra Calibration curves have been obtained spectrophotometrically through measuring the absorbance of distinctive concentrations of Chlorpyrifosat same conditions (contact time 35 min, room temperature, pH 7.4 and concentration= variety of 2-30 ppm). Figs. (4.2) show the absorption spectra of Chlorpyrifos. The absorption band of Chlorpyrifos occurs at 290 nm. It was enacted by the Lambert-Beer law Eq. (1) the absorptivity coefficient was calculated from the slope and compiled with the correlation coefficient R2. The obtained absorptivity coefficients are given for each Chlorpyrifos in Absorptivity coefficient, correlation coefficient, and maximum wavelength for Chlorpyrifos Absorptivity coefficient (Ɛ)(Chlorpyrifos /ppm*cm ±0.01897,Correlation coefficient R-Square = 0.99242 in wave length 290nm.

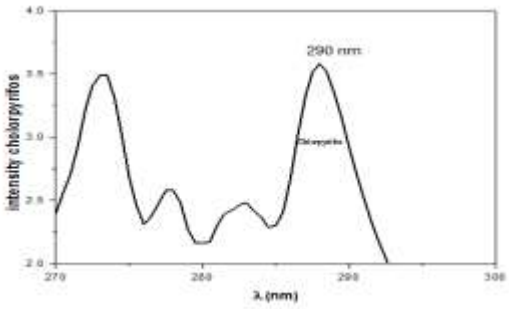

Fig. 4.1 Absorption spectra of Chlorpyrifos

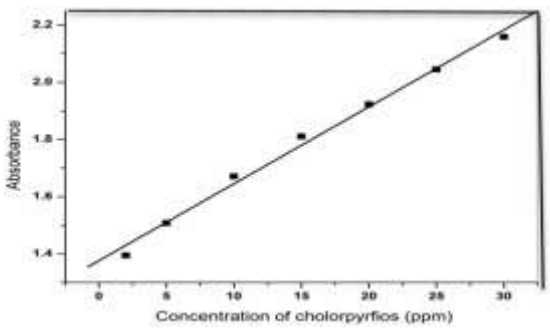

Fig.4.2Calibration curve Chlorpyrifos

The effect of different mass of Moringa oleifera, –adsorbent was studied at 25 ºC .Different amount of Moringa oleifera, adsorbent was used (0.05, 0.1, 0.2, 0.3, and 0.4 g) of adsorbents with known initial Chlorpyrifos ,concentration was used from (2, 5, 10, 20 and 30)ppm of aqueous solution and calculate percentage of removal (R%) according (Eq. (3) as shown in The effect of contact time,pH,Temperature and rotation on the adsorbent of each Chlorpyrifos in different mass of Moringa oleifera, adsorbent , Also read Absorbance of UV spectra of Chlorpyrifos with different mass of Moringa oleifera, adsorbent .The effect of CPF initial concentration on the adsorption capacity of the different doses of adsorbents was examined using different initial concentrations of CPF (10–30 ppm) are shown in Figs (4.3), but all other conditions (contact time 35 min, room temperature, pH 7.4 and concentration= 2-30 ppm) remain constant. The maximum efficiency of Chlorpyrifos removal 95%, 93% 90%, 88%, 86% with the adsorbent dose 0.05, 0.1, 0.2, 0.3 and 0.4g respectively. On the other hand, the maximum removal efficiency of respectively to an initial concentration of both 30 ppm. The results show increases in the quantity of adsorbed CPF per unit mass with increase in CPF initial concentrations due to the escalation in the mass driving force that permits more CPF molecules to pass from solution to adsorbent surface. This is consistent with (Lu et al2006). The impact of solution pH on Chlorpyrifos removal using 0.2g of different adsorbent was investigated at pH values of 2-11 and presented in Figs. (4.4) while all other conditions (adsorbent dosage =0.2g, contact time 35 min, room temperature and concentration= 30ppm) were kept constant. The highest percentage removal of CPF (85 %) for MoringaChlorpyrifos removal percentage decreased for Moringa. Therefore, the optimum pH chosen for the maximum removal of Chlorpyrifos was pH 7 but Fenamiphos removal for all adsorbent increase with increase degree of pH farms, Adsorption isotherms explain the relationships between the concentration of the adsorbate (C_e) in the liquid phase and its concentration on the adsorbent at equilibrium and constant temperature (M.Y. Nassar, et al., 2016). The data experiments depended on known initial Chlorpyrifos2-30 ppm and different amounts of – Moringa oleifera, adsorbent. They were checked using adsorption isotherms models Langmuir and Freundlich isotherm. That's explains the Formation of a monolayer adsorbate where there is no any interactions between the adsorbed adsorbate molecules. Langmuir isotherm model can be presented in Eq. (5) (M.Y. Nassar, et al., 2016). Also using a dimensionless constant called equilibrium parameter RL (Eq.(6)) the efficiency and characteristics of the Langmuir isotherm model can be evaluated.

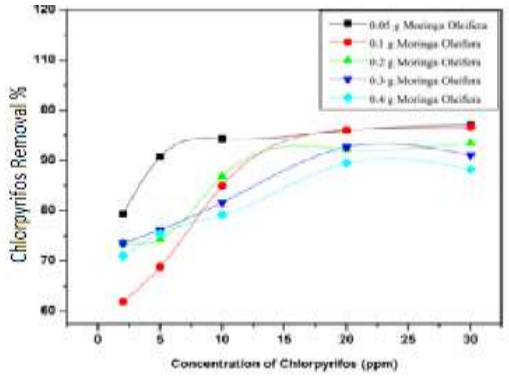

Fig.4.3 Effect weights on Moringa oleifera on the removal of Chlorpyrifos %.

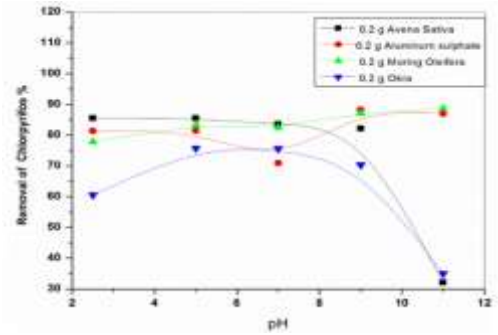

Fig 4.4 Effect of pH on the removal of Chlorpyrifos

$$\frac{Ce}{qe} = \frac{1}{qm}\, KL + \frac{Ce}{qm} \quad -----(5)$$

$$RL = \frac{1}{1 + KLC0} --------(6)$$

Where, C_e is the equilibrium concentration of the Chlorpyrifos solution (ppm), q_m constant is the maximum adsorption capacity (mg/g), K_L is Langmuir constant (L/mg), C_0 is the initial concentrations of Chlorpyrifos (mg/L), and R_L value gives an indication of the adsorption process. Either R_L is an irreversible (R_L=0), favourable (0 <R_L< 1), linear (R_L = 1), and unfavorable (R_L> 1). The results show that RL values present between zero and one, and this means that the adsorption of Chlorpyrifos by Moringa oleifera, adsorbent is favorable. The plot of 1/qe versus 1/Ce values for the adsorption of the Chlorpyrifos results in a is not straight line and unfavorable (R_L> 1) for each Chlorpyrifos and the K_L and qq values can be determined from the intercept and slope of the plots as shown in Fig. 4.5(c) and Table (4.1)respectively.

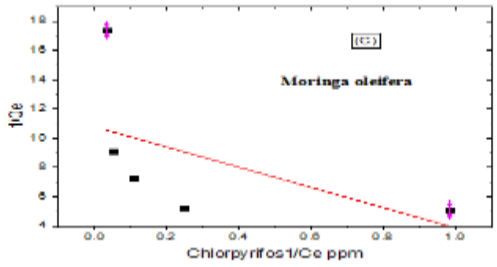

Fig.4.5 Langmuir isotherm model of (c) Moringa oleifera in removal of Chlorpyrifos

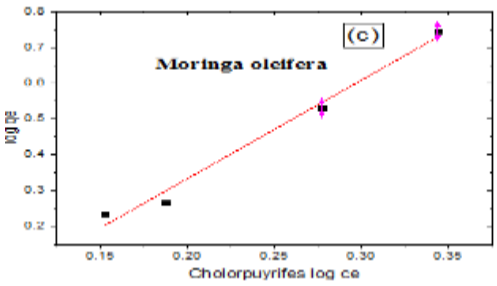

Fig 4.6Freundlich isotherm model of cholorpuyrifes

Table (4.1) The obtained isotherm parameters from Langmuir model

Isotherm model	Constants	R^2	R_L	K_L	q_m(mg/g)
Langmuir	Moringa oleifera adsorption	0.23	7.11	0.0203	2.19

In this model, Freundlich depends on the assumption that the adsorption takes place on a heterogeneous surface can be given by Eq. (7) (M.Y. Nassar, et al., 2016). The maximum adsorption capacity can also be determined using the Freundlich isotherm model by employing Eq. (8).

$$\ln qe = \ln KF + \frac{1}{n}\ln Ce \quad ------- (7)$$

$$KF = \frac{qm}{C0\frac{1}{n}} \quad ----------(8)$$

Where, Ce is the equilibrium concentration of the Chlorpyrifos (ppm), KF is the Freundlich constant[(mg/g)(L/mg)1/n], 1/n is the heterogeneity factor which indicates the adsorption strength ,and qm is the maximum adsorption capacity (mg/g). The plot of lnqe versus lnCe values for the adsorption of the Chlorpyrifos results in a straight line Moringa oleifera as shown in Fig. 4.6. The KF and 1/n values can be obtained from the intercept and slope of the plots as shown in Fig. (4.21,4.22) for both of Chlorpyrifos.The value of 1/n= (0–1) can be used as an indication of the surface heterogeneity. The values that closer to zero are the higher to the heterogeneity (M.Y.Nassar, et al., 2016). According to the results using the Freundlich isotherm model, the adsorption of the Chlorpyrifos by Moringa oleifera, adsorbent tends to place on a heterogeneous surface since the 1/n value was in the range of 0–1.The calculated isothermal parameters of two models are reviewed in Tables (4.2) .The obtained isotherm parameters explain that the adsorption of these Chlorpyrifos (by Moringa oleifera, -adsorbent -adsorbent serve better the Langmuir isotherm model. Furthermore, the adsorption is monolayer coverage because the correlation coefficient (R2) values are closer to unity comparing to the Freundlich isotherm model

Table (4.2) The obtained isotherm parameters from Freundlich mode(Chloropyrifos)

Isotherm model	Constants	R^2	KF	1/n	qm (mg/g)
	Moringa oleifera adsorption	0.95	1.121295	0.91741	53

Research on adsorption kinetics is an important parameter for predicting the adsorption mechanism and the removal rate of Chloropyrifos. It also helps in designing practical systems for treating water from foreign matter. In this sense, kinetic results were tested using pseudo-first order. (Eq. (9)) and pseudo-second-order (Eq. (10)).

$$\log(qe - qt) = logqe - \left(\frac{K}{2.303}\right)t \quad ----(9)$$

$$\frac{t}{qt} = \left(\frac{1}{K2qe2}\right) + \frac{t}{qe} - - - -(10)$$

Where, qe is the quantity of the adsorbed Chlorpyrifos equilibrium (mg/g), qt is the quantity of the adsorbed Chlorpyrifos time (mg/g), t is the time in seconds (s), k1 is the pseudo-first-order rate constant of the adsorption process (min^{-1}), k2 is the pseudo-second-order rate constant of the adsorption process (g mg-1 min-1). The initial sorption rate (h) that is nursery for pseudo-second-order was calculated using Eq. (11).

$$h = K2qe2 - - - - - -(11)$$

Adsorption rate at temperatures of 25ºC, known initial concentrations of chlorpyriphos, with a known mass of 0.05 g of Moringa oleifera, adsorbent with applying of the equation (9) The process parameters were estimated by applying log (qe-qt) to t (s), as shown in Figure 4.23(a through c). We estimated the pseudo-first-order parameters using the slopes and intercept of the straight lines in the graph. Calculation using equation (10), that's explain the plot t/qt versus t (s) as shown in Figs (4.7 , 4.8) .The parameters of pseudo-second-order model were estimated from the slope and intercept of the straight line

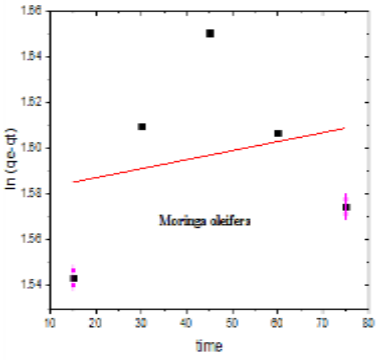

Fig. 4.7 Pseudo-first-order model of Moringa oleifera Chlorpyrifose

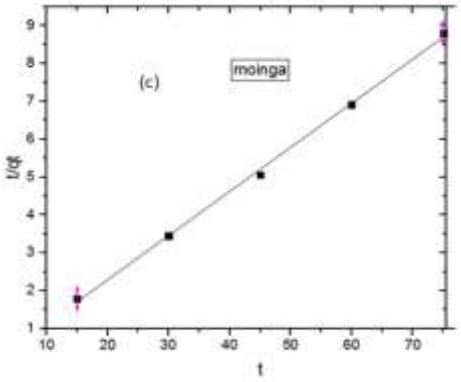

Fig. 4.8 Pseudo-second-order model of Moringa oleifera Chlorpyrifose

The results showed that the Chlorpyrifose closer to the model pseudo-second-order because the R2 is not closer to unity compared to pseudo-first-order, but the range is so far between the two models adsorption based on pseudo of these Chlorpyrifos as shown in tables (4.5, 4.6)

Table (4.5) The obtained parameters of -first-order model

Isotherm model	Constants	R^2	k1	qe (mg/g)
	Moringa oleifera adsorption	0.26148	0.0526	4.851413

Table (4.6) kinetic parameters of adsorption based on pseudo-second-order model

Isotherm model	Constants	R^2	k2	qe (mg/g)
	Moringa oleifera	0.99821	-1726.58	73.60392

The effect of temperature on the adsorption process of chloropyriphos on Moringa oleifera, adsorbent at different temperatures (25, 35, 45, and 55 °C). The adsorption capacity (qe) of the Chlorpyrifos under the adsorption conditions at initial concentrations 2-30 ppm for Chlorpyrifos with Moringa oleifera, -adsorbent 1mg for each was investigated. The thermodynamic parameters were obtained by using The Van't Hoff relationship Eq.(12) and Gibbs Law Eq.(13) (Dargo H, et al., 2014 ; M.Y. Nassar, et al., 2016).

$$\ln Kc = \frac{\Delta S0}{R} - \frac{\Delta H}{RT} - - - - - - - - - - - (12)$$

$$\Delta G0 = \Delta H0 - T\Delta S0 - - - - - - (13)$$

Where, ΔH^0 is a change in enthalpy, ΔG^0 is a change in free energy, ΔS^0 is a change in the entropy that can be obtained from Eq. (13), and K_C is the thermodynamic equilibrium constant (L/g). That can be calculated from Eq. (14).

$$Kc = \frac{qe}{ce} - - - - - - - - - - (14)$$

Where, C_e is the equilibrium concentration of the Chlorpyrifos solution (ppm), $q_e$is the quantity of the adsorbed Chlorpyrifos at equilibrium (mg/g), R is universal gas constant that equal (8.314 J/mol.K), and K is content temperature. The Van't Hoff plot acording to equation (12) results in a straight line, and ΔH^0 and ΔS^0 constants can be estimated from the slope and intercept of the straight line for each Chlorpyrifos . The results showed that the adsorption of Chlorpyrifos on the Moringa oleifera, adsorbent is irreversible because (ΔG^0) values are negative. Also, it is endothermic process duo to (ΔH^0) values are positive for this process. The adsorption process is more favored at higher temperatures due to increasing the negative values of (ΔG^0) with increasing the temperature. The positive values of ($\Delta S0$) show that adsorption increases with the rise in system randomness. The (ΔH^0) values are less than 40 kJ/mol and the (ΔG^0) values are between −20 and 0 kJ/mol, so the adsorption processes of all Chlorpyrifos by M Moringa oleifera, (adsorbent -adsorbate) is physisorption (M.Y. Nassar, et al., 2016). As shown in Tables (4.7, 4.8).

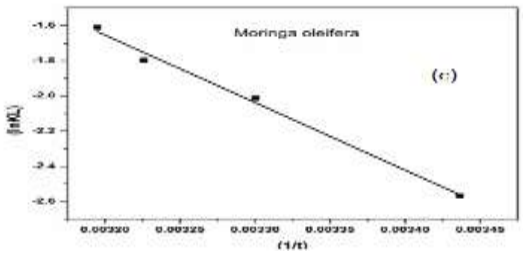

Fig. (4.25) Thermodynamic parameters (Van't Hoff plot) of Moringa oleifera

Table (4.7) The obtained values of ΔH0 and ΔS0 for adsorption of Chlorpyrifos

Adsorbent	**ΔH0**	**ΔS0**
Moringa oleifera	3.795168	3.606016

Table (4.8) The obtained values of -ΔG0 for removal process of Chlorpyrifos at different temperature for different adsorbent.

(-ΔG0)				
Temperature / Adsorbent	**18 C^0**	**30 C^0**	**37 C^0**	**40 C^0**
Moringaoleifer	6.21181	5.069851	4.886947	3.923 27

Conclusions

In the present study, the low-cost adsorbent substance evaluated for remediation of CPF from solutuion. fitted well to Freundlich and power function models. had the highest CPF removal rates (90%), Moringa oleifera removal was achieved at pH 7 at initial concentrations of CPF 20 ppm in contact. 30 minutes period. The kinetics and thermodynamic studies revealed that the CPF adsorption reaction onto the different adsorbent is exothermic in nature and the associative mechanism is involved in the CPF sorption process. Thermodynamic parameters reveal the spontaneous and exothermic nature of the CPF sorption process. Adsorbents can be introduced as an effective and inexpensive alternative for removing CPF from contaminated water. The governing factors for CPF adsorption on to Moringa seed include H bonding, hydrophobicity and electrostatic and π–π interactions. The obtained results revealed that the newly sorbent produced is an efficient adsorbent and exhibits high capability as removal contamination of water

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SEASONAL VARIATIONS OF FLOWERING OF Amorpha fruticosa L. AS AN INDICATOR OF GLOBAL WARMING IN BLUE-GREEN INFRASTRUCTURE

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Abstract

The research of blue-green infrastructure (BGI) is of utmost importance in the design of local landscapes with a mixture of human and natural pressures. The design refers to the set combining forest communities, agricultural land, meadows and green areas interconnected by environmental corridors. BGI plays a decisive role in protecting and enhancing biodiversity, as it can counteract fragmentation and build and strengthen relationships and interactions between isolated elements. The paper investigates the seasonal variations in the flowering phenophase of Amorpha fruticosa L. (indigo bush) acting as an element of the BGI of Belgrade between the main road M19 and the right bank of the Sava River from Ada Ciganlija to Umka. It describes the phenomenon of indigo bush reblooming, with the first and repeated flowering being recorded and analyzed. Quantitative characteristics of its inflorescence and flowers differ in several ways. Reblooming is an ancestral trait but also an indicator of global warming. It has not yet been used in indigo bush breeding because it rarely occurs in blue-green infrastructure. The analysis of the spatial grid and seasonal variations of indigo bush flowering phenological patterns proved to have practical use in monitoring BGI performance and support to decision-making, management and landscape design.

Keywords: *climatic factors, indigo bush, blue-green infrastructure, landscape design.*

Introduction

Blue-green infrastructure is a system characterised by structure, function and changes (Zipperer, 2002). The interaction between social, environmental, economic and institutional spheres shapes the structure and functioning of BGI over time. These processes can rapidly transform spatial patterns and create different configurations of elements, corridors and matrices in a landscape mosaic. Furthermore, they strongly affect ecological processes (survival of populations, maintenance of biodiversity, ecosystem health, etc.) and the provision of ecosystem services (Sukopp, 2004). Nowadays, there are numerous examples of suburban BGIs that require policies aimed at preserving biodiversity. A strategy that can meet these needs implies the preservation and management of BGI, i.e. systems of green areas interwoven with corridors. In addition, a larger number of papers (Chailakyan, 1988; Ocokoljić et al., 2023, etc.) on the *reblooming* of woody plants encourages the need to analyze information on the flowering phenophase and the causes that lead to deviations from the usual flowering patterns. *In many cases, local governments have successfully adopted programs based on the BGI approach to halt biodiversity loss and facilitate the reintroduction of specific plant and animal species under climate change conditions (Yli-Pelkonen & Niemelia, 2005).*
Besides the aspects of the spatial analysis of the suburban BGI in Belgrade, the research aims include (1) identification and state of Amorpha fruticosa L. in mosaic landscapes, (2) regeneration of green areas using indigo bush, (3) seasonal variations of its flowering under

the conditions of global warming and (4) measures of environmental protection and preservation of spatial elements.

Material and Methods

The research area encompasses the BGI of the southwestern territory of the Belgrade municipality of Čukarica (Figure 1) with a total area of 2,160,011m2. Čukarica is situated on the right bank of the Sava River and includes the settlement of Ostružnica where three locations were studied. Ostružnica is also known for numerous finds of ancient Roman pottery, sculptures and coins. Archaeologists claim that the Sava riverbed near the settlement contains an abundance of the remains of mammoths, bison, extinct species of rhinoceros and deer from ancient times (Mala Enciklopedija, 1985). Spatial arrangement and fragmentation of the blue-green infrastructure were analysed using Google Earth images (ArcGis/ArcMap 20), cartographic and planning documents and field research.

Figure 1. *Amorpha fruticosa* L. sample points in blue-green infrastructure

In order to determine the impact of climate change on this invasive but honey-bearing species, flowering phenology was monitored at five locations where the second full flowering was recorded from 1 September 2022 to the end of the flowering phenophase in June 2023. Using the BBCH scale (Meier, 1997), visual phenological observations were made at an interval of two days by noting the dates of the beginning of flowering (BF), i.e. the day with more than 10% of flowers in bloom, full flowering (FF), i.e. the day with more than 50% of flowers in bloom and the end of flowering (EF), i.e. the day by which more than 80% of flowers had opened. Dates were expressed as DOY, with 1 January being DOY 1, which was also the first day to start adding temperature sums to BF, FF and EF. The sum of growing degree days (GDD) was determined according to Lalić et al. (2021). A temperature threshold of 5°C was used for humid continental climate conditions. GDDs were determined by adding the active temperature sums for each location and presented as the arithmetic mean of the investigated BGI. Climatological data were obtained from the Main Meteorological Station of Surčin (44º49'27.37" N; 20º17'27.82" EGr; altitude: 99m) https://www.hidmet.gov.rs/index.php (accessed on 24 June 2023), RHMS, and not from the main Belgrade-Vračar station due to environmental conditions that are closer to the conditions in which *indigo bush* grows.

Inflorescence samples of *Amorpha fruticosa* L. were collected in autumn 2022 and spring 2023. The coordinates and other characteristics of the selected locations are shown in Table 1. A total of 20 inflorescences were taken from each location, in the full phenophase of flowering, in the autumn of 2022 and the spring of 2023. The length and width of the inflorescence, the number of flowers in the inflorescence, the length of the petal and the number of stamens in the flower were determined on a total sample of 100 inflorescences in

autumn and spring. Quantitative parameters were processed using descriptive statistics, one-way ANOVA and Spearman Rank Test between variables in the XLSTAT 2020 software package.

Table 1 Description of the study areas

Location	Latitude φ	Longitudeλ	Altitude H (m)	Aspect	Slope*
1.	44º42'11.30" N	20º18'32.92" EGr	74	W	1.58
2.	44º42'59.98" N	20º18'32.25" EGr	75	W	5.23
3.	44º43'22.54" N	20º18'39.57" EGr	75	W	3.005
4.	44º44'14.93" N	20º19'26.43" EGr	77	N	0.58
5.	44º46'30.83" N	20º22'25.31" EGr	73	NW	0.08

* Slope angle: 0-1° (flat terrain), 1-3° (very slightly sloping terrain), 3-5° (slightly sloping terrain), 5-8° (quite sloping terrain)

Results and Discussion

According to the collected data and field research of BGI elements, occasional flooding of the Sava River is the main feature of the study area. The analyzed locations were west and north-west oriented, and the slope was from completely flat terrain to quite sloping terrain, which confirms that repeated flowering, in addition to other factors, was most influenced by climatic factors. In spring, when the water level is high, the river overflows and floods the surrounding area. It has led to the development of distinctive ecosystems in the BGI. All the uninhabited narrow strips along the banks of the Sava River are covered with low vegetation or forests, and there is a distinctive water ecosystem in Pećanska Bara. Right next to the bank, there are pioneer communities of almond willow on moist, gley soil. They are poor in the shrub and ground layer due to high groundwater levels and prolonged inundation (Tomić 1992, WRB, 2015). A layer of shrubs with species adapted to large fluctuations in the wetting regime has developed on undeveloped alluvial deposits and clay soils (WRB, 2015). They include *Frangula alnus* Mill., *Cornus sanguinea* L. and *Rubus caesius* L. (Tomić, 1992). White willow forests are found right along the river or in the depressions of the alluvial plains (Tomić, 1992). On the recent alluvial deposits (WRB, 2015), there are pioneer coppice forests with the occasional presence of European white elm, field elm and narrow-leaved ash (Tomić, 1992). The shrub presence varies with the duration of high water. Black poplar (Tomić, 1992) grows on slightly higher terrain on the soil with a more favourable water-air regime that gives way to drier clay soils, and then to moist and transitional alluvial pararendzine (WRB, 2015). As the soil gets drier (WRB, 2015), white poplar forests occur in mosaics as monodominant communities (Tomić, 1992). The shrub layer comprises common privet, Hungarian thorn, hawthorn and common dogwood (Tomić, 1992). In such conditions, *Amorpha fruticosa* L. is the most common of all shrub species on all types of soil from Ada Ciganlija (φ 44º42'11.30" N, λ 20º18'32.92" EGr) to the settlement of Umka (φ 44º41'6.57" N, λ20º18'27.89" EGr). We identified five locations (Figure 1) where one to three individuals per location recorded the second flowering in autumn 2022. The percentage of plants with repeated flowering is from 51 to 74 at the location level and 62.5 at the level of the research area. These five locations are the study area of the research related to the BGI.

Considering the need for unifying concepts for urban and rural land at a local and regional scale aimed to defragment BGI (Lindholm, 2019) by applying indigo bush, we determined its phenological flowering patterns that can provide a deeper insight into ecosystem functioning. Figure 2 shows the phenological flowering patterns of indigo bush in autumn 2022 and spring 2023, as well as for the period 2007-2022, expressed as DOY and GDD. The average length of the spring flowering phenophase was 22 days in a sixteen-year period (Ocokoljić et al., 2023), and in spring 2023, three days fewer (19). The BF DOY was 139 in spring 2023, which means that flowering started 7 days later than in 2007-2022. During the autumn of 2022, the length of the flowering phenophase was 37 days, i.e. 15 days longer compared to the monitored multi-year spring series and 18 days longer than the flowering in spring 2023.

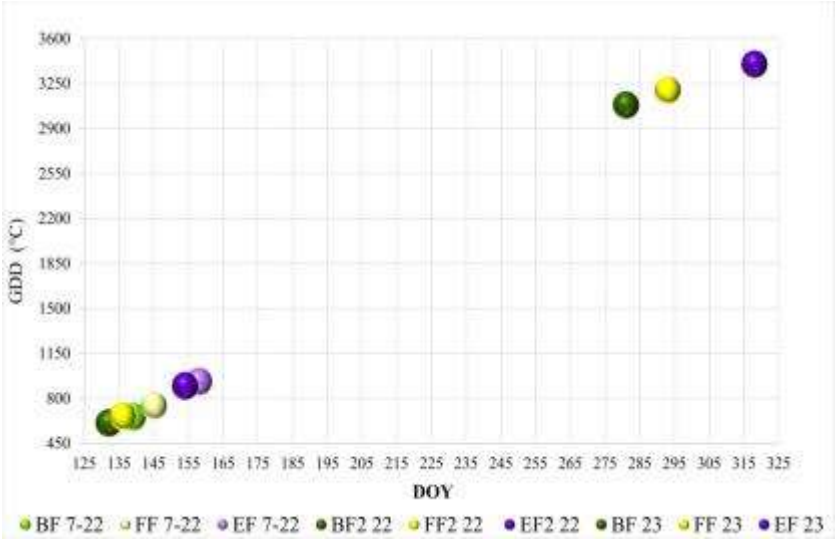

Figure 2. Phenological observations: GDD and DOY for spring flowering phases in the period 2007-2022 (BF 7-22, FF 7-22 and EF 7-22), repeated flowering in autumn 2022 (BF2 22, FF2 22 and EF2 22) and flowering in spring 2023 (BF 23, FF 23 and EF 23).

According to the case study for 2007-2022, a temperature sum of 504.9°C was necessary for the spring BF (Ocokoljić et al., 2023), while a sum of 655.8°C was required in spring 2023 (Figure 2). The obtained results are in line with previous literature reporting that indigo bush flowers in late spring (Ocokoljić & Petrov, 2022) and the growth is slow after the bud opening as it is affected by climatic parameters (Menzel, 2000). Namely, the winter of 2022/23 was the third warmest winter in Belgrade since 1888 and had the lowest number of frost days on record (RHMS), which led to a higher GDD accumulated for BF. However, due to the rainy spring with average air temperatures on the one hand and the above-average spring cloudiness (6/10 for Belgrade) and a small number of clear days (RHMS) on the other hand, the flowering phenophase started later, and the accumulated sum was higher by 150.9°C. The values of the Spearman coefficient (ρ) obtained for the analysed GDD and DOY of the corresponding flowering phenophases are statistically significant (at the $p<0.05$ level) and confirm the direct correlation between these two variables. Given that the indigo bush is a honey-bearing species important in beekeeping and an attractive source of food for birds in cold seasons, morphological flower and inflorescence traits were investigated in the BGI of Belgrade (Figure 3). A comparative analysis of the first and repeated flowering reveals several data on survival traits and general characteristics of the species. One-way ANOVA test confirmed statistically significant differences ($p<0.0001$) for three out of five characters, with F of 50.692 (inflorescence length), 94.665 (number of flowers in inflorescence) and

100.719 (corolla length), while inflorescence width and the number of stamens in a flower had insignificant values of F (12.211 and 0.812). The validity of the results was confirmed by the low values of the standard deviation and coefficients of variation for all variables. The main elements of descriptive statistics are shown in Figure 3. The positive values of the Spearman coefficient (ρ), at the $p<0.05$ level, for the length of the inflorescence and the length of the petals confirm that the number of flowers increases with an increase in the length of the inflorescence, while the length of petals decreases with an increase in the number of flowers in the inflorescence and the width of the inflorescence. Distinctly changed conditions during the repeated flowering affected the morphology of the inflorescence, which was confirmed by the comparative analysis. Namely, the autumn of 2022 was warm and rainy, with a heatwave in late October and early November at the time of repeated flowering (RHMS).

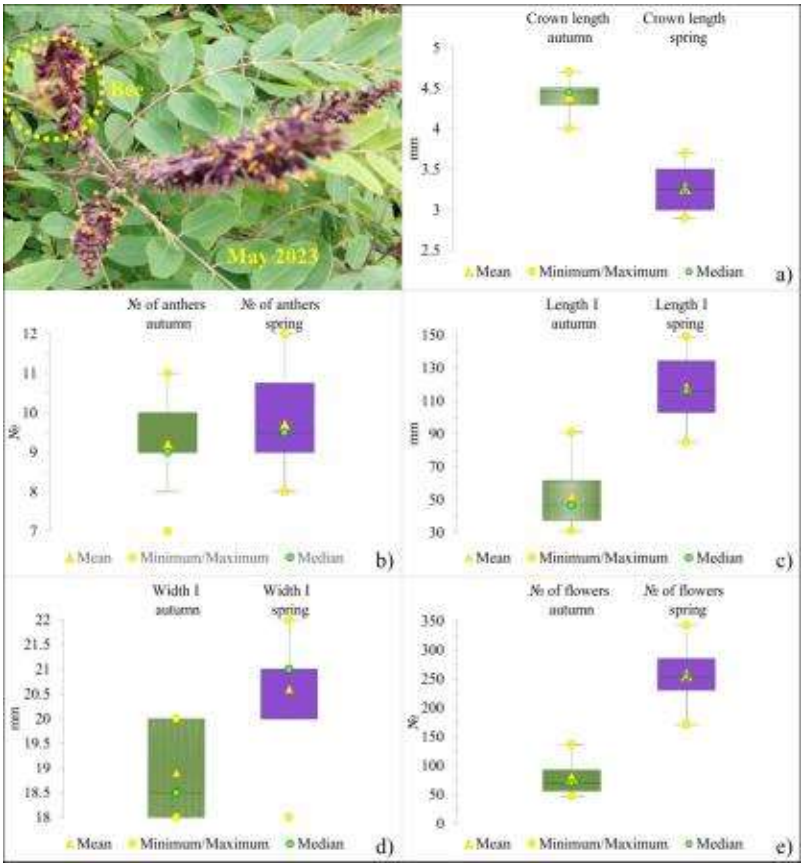

Figure 3. Floral morphological and metric characteristics: inflorescence length (graphic label: Length I), inflorescence width (graphic label: Width I), number of flowers in the inflorescence, corolla length and number of stamens in a flower for Indigo Bush for autumn (2022) and spring (2023) flowering

The recorded variability points to the effects of the disturbance, stabilisation and the possibility of directed use of indigo bush depending on the variety of environmental conditions of the BGI. A range of different ecosystems makes the research area important for bees and birds and as a winter habitat for amphibians. Due to the broken continuity of green spaces and the area urbanisation (Figure 1), it is not rich in animal species, but there are still squirrels, common buzzards, great tits, blackbirds, common starlings, barn owls, long-eared owls and other permanently protected species (Batrićević and Batanjski). Therefore, the investigated species can have a prominent role in the defragmentation of the analysed BGI.

Conclusions

The paper points to the fragmentation of natural areas due to environmental disturbances that affect the movement of species, the diversity, structure and distribution of vegetation, and the design, construction and maintenance of BGI. Increasing isolation, perforation and degradation of ecosystems is a critical threat to biodiversity that overlaps with other anthropogenic disturbances, causing cumulative effects in conjunction with global warming. In such conditions, successful colonisation and adaptation of indigo bush in the research area were recorded, as well as repeated or second flowering, which is typical of warm region species. This phenomenon confirms that seasonal variations of flowering are an indicator of global warming. However, the multiple flowering of some indigo bush individuals has not been sufficiently used in species breeding or the landscape design of BGI. It is recommended that the species should be used in the regeneration and preservation of the analysed BGI and similar environmental conditions, with adequate care measures.

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EFFECTS OF CLIMATE PARAMETERS ON BLACKTHORN IN AGROFORESTRY ECOTONES AS GREEN ROAD INFRASTRUCTURE ELEMENTS

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Abstract

Phenological flowering patterns of *Prunus spinosa* L. (blackthorn) are indicators of climate change and help us understand the impact of climate change on agroforestry ecotones as elements of green infrastructure along the Belgrade-Obrenovac roads on the right bank of the Sava River. We studied the response of the blackthorn flowering phenophase to climatic factors (temperature and precipitation) in the period from 2007 to 2023. The results confirm that: (1) the beginning of the growing season, which in the case of blackthorn coincides with the start of flowering, is affected by climate change; (2) the beginning, as well as the end of the flowering phenophase, significantly correlates with air temperature and precipitation and (3) the abrupt stop of flowering in April 2023 was caused by a sudden drop in air temperature and heavy snowfall. The response of blackthorn phenological flowering patterns to climate change in the study area also confirms that the greater the accumulation of heat, the smaller the negative effect of daily temperatures on the beginning of flowering. The obtained results are a basis for studying non-systematic phenological shifts as responses to climate change in agroforestry ecotones that are elements of green road infrastructure. They are also a valuable contribution to the research in the field of landscape ecology and landscape architecture, setting out landscape design guidelines to form place identity, and pointing to the necessity of introducing the values of the physiognomic landscape composition into the land use policy.

Keywords: *climatic factors, flowering period, agroforestry ecotones, landscape design, landscape ecology.*

Introduction

Ecotones are found in most biomes where they regulate the movement of organisms and the flow of matter and energy and have a significant impact on the dynamics of populations and communities (Fagan et al., 1999). They are often known as "zones of tension", sensitive to climate change or other external factors. It is a challenging task to investigate why and how ecotones change over time and what the changes imply for other ecological attributes or functions. Answers to these questions are fundamental for predicting future vegetation changes at both local and regional scales (Hufkens et al., 2009). Therefore, the research focuses on climate parameters (temperature and precipitation) that define the formation of agroforestry ecotones, along with relief, hydrology and soil (Virtanen et al., 2010). Harmonious planning of agroforestry landscapes has an increasingly important role in achieving social satisfaction arising from environmental quality in suburban and rural areas (Arnot, 2004). It is also a way to create a unique identity of green road infrastructure.

The study aims to determine the impact of climate change on the phenological patterns of flowering and development of Prunus spinosa L. (blackthorn) in the operationalization of the landscape design of agroforestry ecotones. The research deals with blackthorn because in landscape architecture it is known as a species native to Serbia that achieves good vitality at both lower altitudes (along canals and field roads) and altitudes up to 1500m (the Balkan Mountains). It typically has a well-developed root system, which makes it a valuable bio-ameliorative species. It is also an ornamental species that attracts animals, especially birds

(Ocokoljić and Petrov, 2022). Phenological patterns applied in landscape design can: (1) influence the selection of species, (2) determine the suitability of the selected plant material for the place and purpose, (3) contribute to the floristic richness in agroforestry ecotones, (4) enable a better visual perception of ecotones as elements of green road infrastructure and (5) optimize maintenance costs.

Material and Methods

The research area extends northeast-southwest from Ada Ciganlija at φ 44º47'9.42"N and λ 20º24'49.45"Egr (altitude 75m) to the settlement of Ostružnica at φ 44º44'8.89"N and λ 20º19'19.77"Egr (altitude 75m)), on 23,979,339 m^2 on the right bank of the Sava River, suburban zone of Belgrade (Republic of Serbia). To study the spatial planning of green elements in the road infrastructure network, including M-19 national road (Belgrade-Obrenovac-Šabac-Loznica), E-75 highway (southern bypass around Belgrade city, part of the corridor X), E- 763 national road of IA order (Belgrade-South Adriatic highway, Belgrade-Požega section), and a network of local roads), cartographic materials, planning documents, and field research were used.
Aiming to determine the stability of ecotones that had been changing or migrating under conditions of climate change in the period from 2007 to 2023, authors used the BBCH scale (Meier, 1997) to conduct visual phenological observations of blackthorn at an interval of 2 days and note the date of the beginning of flowering -BF (more than 10% of flowers), full flowering -FF (more than 50% of flowers opened) and the end of flowering -EF (more than 80% of flowers ended flowering). Dates were converted to the day of the year (DOY). The DOY range is defined by the first (BF) and the last date (EF), whereby DOY 1 corresponds to January 1, which is also the starting date for adding temperature sums from which the sum of degree days, i.e. growing degree days (GDD), was determined according to Lalić et al. (2021). A temperature threshold of 5°C was used for humid continental climate conditions. GDDs were determined by the active temperature sums for each ecotone and presented as the mean value for each of the 17 years. Photographic material was also collected during the phenological observations. The data were processed using descriptive statistics, and the relationships between climatic parameters and phenological events were studied using Spearman's correlation coefficient (ρ) in the XLSTAT 2020 software package. The effects of temperature and precipitation were studied based on the data obtained from the RHMS: https://www.hidmet.gov.rs/index.php (accessed on May 29th, 2023) from the Meteorological Station of Belgrade (44º 47' 54.44" N; 20º 27' 53.35" EGr). The term "normal" is defined as the climatological standard, i.e. mean, maximum, and minimum values of air temperature and total precipitation for March and April for the period 1991-2020. Statistical climatological methods of percentiles and associated terciles (RHMS) were applied, where the n-th percentile describes the value below which n% of the data sorted in an ascending series fall.

Results and Discussion

Ecosystems as elements of green road infrastructure, whose characteristics are shaped by the Sava River, were identified based on data obtained from satellite maps according to Simović et al. (2022). The riparian zone and alluvial plains are exposed to flooding and groundwater, and the aquifer in the alluvium is in direct hydraulic connection with the Sava River: https://www.srbijavode.rs/images/dokumenti/vodoprivredna_osnova_republike_srbije.pdf.
Woody plants include species tolerant to prolonged retention of surface water, such as the character species of the swamp forests of black alder and bush willows (Teofilović et al. 2008). Hygrophilous forests are also present in some zones. Still, the research area includes

only the mosaic-distributed agroforestry ecotones with a more significant number of species and denser populations compared to neighbouring ecosystems (Wang et al. 2022). Ecotones are found around the boundaries of abandoned land and forests, and their identification *in situ* complements the conventional landscape pattern as remote sensing leaves out transition zones (Arnot et al. 2004). The presence of woody species pointed to the uneven distribution of trees in ecotones, and seven agroforestry ecotones were identified in the research area, with the blackthorn being dominant. The structure, composition and diversity of ecotones changed significantly over the 17 years of research, with forest and meadow habitats remaining stable, which is why our work analyses the influence of climatic elements on the phenological patterns of blackthorn. Figures 1a and 1b show the mean monthly air temperatures and precipitation for March and April of the study period (2007-2023) compared to the reference period of 1991-2020.

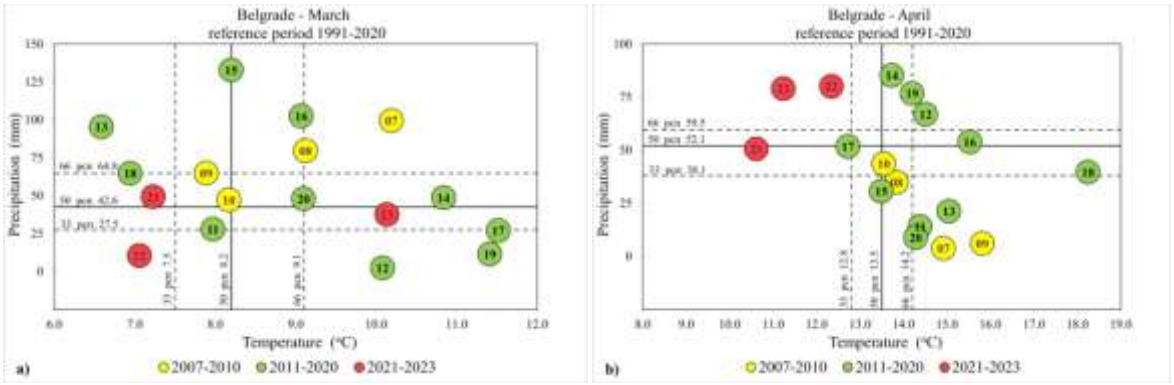

Figure 1. Mean monthly air temperature and precipitation sums for March (a) and April (b), and their corresponding terciles for Meteorological Station of Belgrade in the research period, compared to the reference period 1991-2020.

The earliest beginning of flowering was recorded in 2007 and 2008 (DOY: 71 and 63), when air temperatures and precipitation sums In March were above the upper tercile limit (Figure 1a), and the flowering phenophase was the longest (28 days) in seventeen consecutive years. The same years had April temperatures above the limit of the upper tercile (2007), and precipitation at the lower tercile limit (Figure 1b). The latest BF was recorded in 2013 (DOY: 98) when air temperatures were significantly below the lower tercile, and precipitation was above the upper tercile. On the other hand, the April temperatures were above the upper tercile and precipitation below the lower tercile (Figures 1a and 1b). This year had the shortest flowering phenophase (12 days) in the phenological flowering pattern of the 2007-2023 period. As can be seen in Figure 1a, March 2023 had air temperatures above the limit of the upper tercile and the precipitation sums within the limits of average values, which initiated the BF on the 80th DOY. March 2023 was warm and with average rainfall according to the percentile and tercile method (RHMS). On the other hand, April 2023 was very cold and the sixth rainiest (RHMS). As shown in Figure 1b, temperatures in April 2023 were well below the lower tercile and the precipitation sums were above the upper tercile. The sudden EF was caused by snowfall and a drop in temperature on the 98th DOY. Namely, in early April, the maximum height of the snow cover ever recorded in Belgrade was exceeded (Figure 2). The specific impact of climate change was also reflected in the maximum temperatures, which from the 3rd to the 7th of April were below the minimum temperatures for the same dates in the reference period 1991-2020 (Figure 2). The flowering phenophase lasted for 19 days, which was the average length of the blackthorn flowering in the study ecotones over seventeen consecutive years.

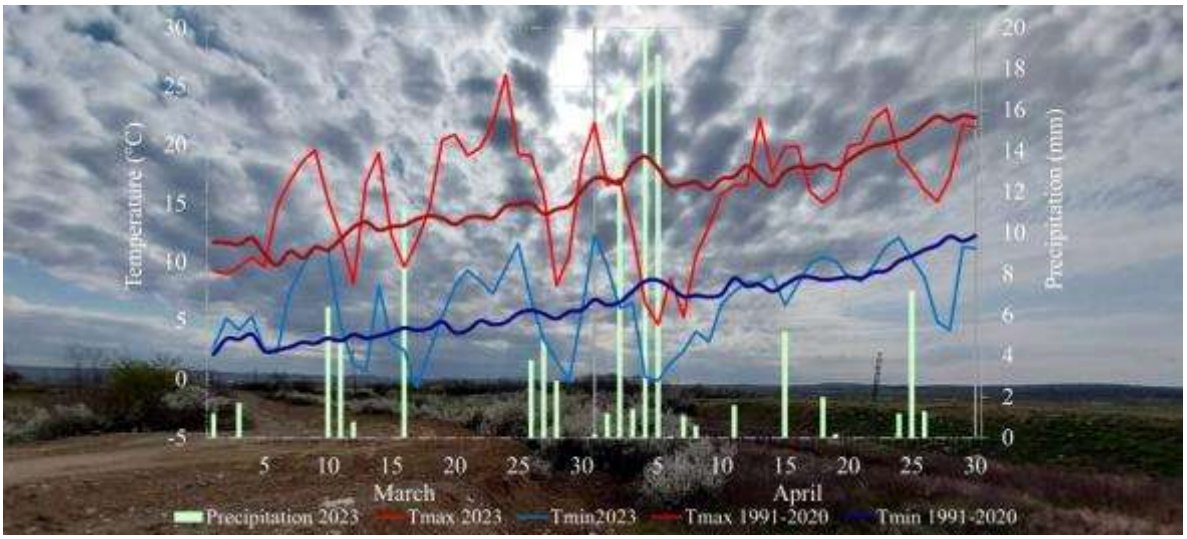

Figure 2. Agroforestry ecotone on April 3rd, 2023, showing daily precipitation sums, maximum and minimum daily air temperatures in March and April 2023 and their normal for the reference period 1991-2020.

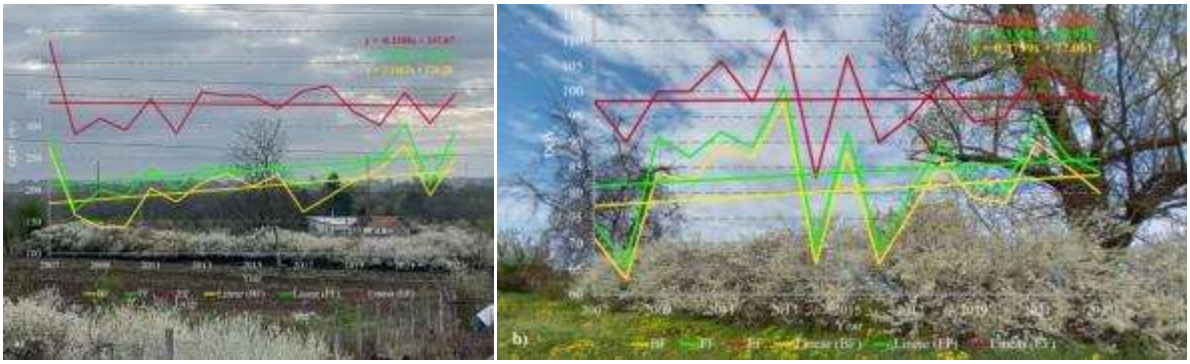

Figure 3. Agroforestry ecotones (spring 2023) showing linear trends of the observed blackthorn phenophases based on GDD (a) and DOY (b), over the period 2007-2023, for the beginning of flowering (BF), full flower (FF) and the end of flowering (EF).

The BF and the FF had growing GDD trends, the EF trend was negative, but the trends were statistically not significant, which confirmed that the blackthorn phenophases took place within the expected temperature sums over the 17 years of research (Figure 3a). Linear trends for all three elements of the phenological flowering pattern were positive and statistically significant (Spearman correlation coefficient values amounted to 0.917 to 0.980), which confirmed the shift and extension of the flowering phenophase caused by the analysed climate parameters. Accumulated heat sums of 141.3°C (2009, DOY: 84) to 270.0°C (2021, DOY: 90) were required for the BF. In the current 2023, the BF started after an accumulation of 251.7°C (DOY: 80). The mean value of the accumulated heat for the BF was 204.8°C in the period 2007-2023. The GDD values determined were variable but close, regardless of the DOY. So it is necessary to conduct a comparative analysis of temperatures and precipitation. This need was further confirmed by Spearman's coefficients which indicated a significant correlation of the GDD with air temperature and precipitation. Our research coincides with the findings of Mota and Salatha (2009) and Elsner et al. (2009) that climate change modifies the dynamics (composition, presence and phenology of plant species) of agroforestry ecotones of the green road infrastructure in alluvial sites. Current scenarios (WMO, 2021) foresee warmer and drier summers and warmer and wetter winters, as well as changes in the form of precipitation, which indicates the potential for prolonged summer droughts and longer

growing seasons. Climate change can cause indirect or secondary effects such as increased frequency and intensity of insect attacks or forest fires (Littell et al. 2009), which can completely change the characteristics of existing ecotones or create new boundaries. Given that our research confirmed the stability of blackthorn phenological patterns and the increase of its coverage in the researched agroforestry ecotones, its results can be used to lay down guidelines for landscape design and landscape context in order to create a place identity by predicting future changes in biological diversity and to point to the necessity of introducing values of the physiognomic composition of the landscape in the land use policy.

Conclusions

Seventeen years of research into agroforestry ecotones and phenological patterns of blackthorn has placed climate change in a broader context. The studied ecotones have remained structurally different from hygrophilous forests and retained elements of meadows with the dominance of blackthorn. Vegetation dynamics in ecotonal habitats result from the aggregate effect of ecological interactions. Quantification of landscape patterns and recording of changes should include biodiversity and ecotone ecosystem protection in order to achieve sustainable planning and management of green road infrastructure. Permanent research and monitoring will provide deeper insights into the biological processes of restoration of abandoned agricultural land and forests through the approach of ecotone extraction.

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PHYSICAL, CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF DRINKING WATER ON THE TERRITORY OF PROKUPLJE (SERBIA)

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Abstract

Water represents one of the most valuable natural resources. The demand for clean water is growing continually due to population growth and industrial development. In order to keep drinking water safe for human use, it is necessary to keep up the monitoring and the maintenance of the distribution network. The aim of this work is to examine the physical, chemical and microbiological parameters of drinking water in order to determine whether it is safe for the health of users and to prevent water pollution in the distribution network on the territory of Prokuplje. The six-month analysis was carried out retrospectively and it included physical, chemical and microbiological analysis performed according to the Regulations concerning the hygienic quality of drinking water. The analysis of the physical and chemical properties included the following parameters: colour, odor, turbidity, pH, $KmNO_4$ consumption, residual chlorine, chlorides, ammonia, nitrates, nitrites, iron, manganese, vapor residue at 105°C and electrolytic conductivity. Microbiological analysis dealt with the total coliform bacteria, coliform bacteria of faecal origin, the total number of aerobic mesophilic bacteria, streptococci of faecal origin, *Proteus spp*, the number of sulphite-reducing clostridia and *Pseudomonas aeruginosa*. A total of 160 samples of drinking water were tested at the Public Health Institute in Niš. All the values obtained from the tested samples were in accordance with the Regulations concerning the hygienic quality of drinking water.

Keywords: *water, analysis, monitoring, microbiology, Prokuplje.*

Introduction

Water resources can be used for supplying the population with clean water, as well as for dumping the waste water, which poses a risk of water quality deterioration. Humans and their activities have the greatest influence on the change of water quality. Lack of water is one of the most acute problems today (UNDP, 2006). Agriculture and urban population are the two primary users of water (Ye et al, 2018). Water supply systems depend on water sources, and in this regard basins affect the quality of raw water (McDonald et al, 2016). The quality of water is determined by the value of certain indicators (chemical, physical and biological) that give us information about the composition, concentration and properties of certain substances present in the water.Preservation of good quality of drinking wateris considered to be of utmost importance for preserving human health (Moyo et al, 2004). Water is a potential source of infectious diseases, as well as of chemically induced intoxication (Madigan et al, 2010). The World Health Organization (WHO) estimates that 4 billion cases of diarrhea and 2.2 million deaths each year are the result of consumption of impure drinking water. Different types of diarrheal diseases make up almost 3.6% of all global diseases, which again stresses the importance of good quality drinking water (Clasen et al, 2015). Some waterborne diseases can cause cognitive impairment and stunted growth in infants and children under the age of five. Also, the existence of biological and chemical pollutants causes great concern because of the ecological impact on biota in the environment (Bruce et al, 2015). Drinking water must be

"produced", because raw water, due to its characteristics and quality, is usually not acceptable (Klašnja, 1998). The generally accepted term "water factory", meaning a water treatment plant, is justified in the case of large capacities and more complex processes of water treatment. The greatest attention is paid to the removal of undesirable ingredients such as iron, manganese, ammonia, also of toxic ingredients (heavy metals and organic micropollutants), pathogens (bacteria and viruses), as well as ingredients that spoil the organoleptic properties of water (turbidity, color, smell, taste). With the development of analytical equipment for testing water, the processes of detection and monitoring of disinfection products, especially chlorination, have been significantly improved (Ćirić & Petrović, 2017). In order to obtain good quality drinking water, it is necessary to monitor water quality at key water supplypointsby determining the most significant physico-chemical and microbiological parameters. The aim of this work is to examine the physico-chemical and microbiological parameters of drinking water in order to determine whether it is safe for the health of users and to prevent water pollution in the distribution network on the territory of the town of Prokuplje.

Material and methods

The drinking water samples from the central water supply on the territory of the town of Prokuplje in Serbia were examined at the Institute of Public Health in Niš during the period from June 1st 2022 to December 31st 2022. Water was sampled four times from eight selected places of the distribution network, in accordance with the legal regulations (Regulations concerning the sampling and methods of laboratory analysis of drinking water - Official Gazette of SFRY No. 33/87)). The analysis of physico-chemical properties included the following parameters: colour, odour, turbidity, pH value, KmNO4 consumption, residual chlorine, chlorides, ammonia, nitrates, nitrites, iron, manganese, vapour residue at 105°C and electrolytic conductivity. Microbiological analysis included the analysis of total number of coliform bacteria, coliform bacteria of fecal origin, total number of aerobic mesophilic bacteria, streptococci of fecal origin, *Proteus* spp, sulphite-reducing clostridia and *Pseudomonas aeruginosa*. All the aforementioned analyzes were carried out in accordance with the Regulations concerning the hygienic quality of drinking water (Official Gazette of RS No. 28/19).

Results and discussion

The results of testing the physico-chemical parameters of the drinking water samples are given in Tables 1 and 2. These results are presented as the monthly mean value of all the tested samples taken from selected places at the distribution network.

Table 1. Results of physico-chemical testing of water for the months of June, July, August and September 2022.

Parameter	Measuring unit	Testing method	June	July	August	September
Colour	°Co-Pt	P-IV-5/B	<5	<5	<5	<5
Odour		P-IV-2	bez	bez	bez	bez
Turbidity	NTU	P-IV-4/B	<0,05	0,10	0,15	0,10
pH value		P-IV-6/A	7,0	7,0	7,0	7,0
Consumption of $KMnO_4$	mg/L	P-IV-9a	1,3	1,2	2,5	1,9
Residual chlorine	mg/L	P-V-18/B	0,40	0,40	0,40	0,40
Chlorides	mg/L	P-V-19/B	15,5	16,0	15,5	15,5
Ammonia	mg/L	P-V-2/B	<0,05	<0,05	<0,05	<0,05

Nitrites	mg/L	P-V-32/A	<0,005	<0,005	<0,005	<0,005
Nitrates	mg/L	P-V-31/C	6,2	6,9	6,5	6,3
Iron	mg/L	P-V-17/A	<0,05	<0,05	<0,05	<0,05
Manganese	mg/L	DM 121	<0,01	<0,01	<0,01	<0,01
Vapour residue at 105°C	mg/L	P-IV-7	412	418	408	414
Electrolytic conductivity	mg/L	P-IV-11	687	703	691	690

Table 2. Results of physico-chemical testing of water for the months of October, November and December 2022.

Parameter	Measuring unit	Testing method	October	November	December
Colour	°Co-Pt	P-IV-5/B	<5	<5	<5
Odour		P-IV-2	bez	bez	bez
Turbidity	NTU	P-IV-4/B	0,15	0,15	0,20
pH value		P-IV-6/A	7,1	7,3	7,1
Consumption of $KMnO_4$	mg/L	P-IV-9a	0,9	2,0	2,0
Residual chlorine	mg/L	P-V-18/B	0,40	0,40	0,40
Chlorides	mg/L	P-V-19/B	16,5	17,0	15,0
Ammonia	mg/L	P-V-2/B	<0,05	<0,05	<0,05
Nitrites	mg/L	P-V-32/A	<0,005	<0,005	<0,005
Nitrates	mg/L	P-V-31/C	6,6	7,2	6,5
Iron	mg/L	P-V-17/A	<0,05	<0,05	<0,05
Manganese	mg/L	DM 121	<0,01	<0,01	<0,01
Vapour residue at 105°C	mg/L	P-IV-7	420	434	400
Electrolytic conductivity	mg/L	P-IV-11	703	723	700

The results of microbiological testing of drinking water are given in the Tables 3 and 4.

Table 3. Results of determining microbiological parameters of drinking water samples for the period from June to September 2022.

Standard methods	Measuring unit	June	July	August	September
Total number of coliform bacteria,	per 100 ml of sample	/	/	/	/
Coliform bacteria of fecal origin	per 100 ml of sample	/	/	/	/
Total number of aerobic mesophilic bacteria	per 100 ml of sample	<1cfu	<1cfu	<1cfu	<1cfu
Streptococci of fecal origin,	per 100 ml of sample	/	/	/	/
*Proteus*species	per 100 ml of sample	/	/	/	/
Sulphite-reducing clostridia	per 100 ml of sample	/	/	/	/
Pseudomonas aeruginosa	per 100 ml of sample	/	/	/	/

/ - presence of a given bacteria not recorded

Table 4. Results of determining microbiological parameters of drinking water samples for the period from October to December 2022.

Standard methods	Measuring unit	October	November	December
Total number of coliform bacteria,	per 100 ml of sample	/	/	/
Coliform bacteria of fecal origin	per 100 ml of sample	/	/	/
Total number of aerobic mesophilic bacteria	per 1 ml of sample	<1cfu	<1cfu	<1cfu
Streptococci of fecal origin,	per 100 ml of sample	/	/	/
*Proteus*species	per 100 ml of sample	/	/	/
Sulphite-reducing clostridia	per 100 ml of sample	/	/	/
Pseudomonas aeruginosa	per 100 ml of sample	/	/	/

/ - presence of a given bacteria not recorded

The results of all 160 tested samples are in accordance with the Regulations concerning the hygienic quality of drinking water (Official Gazette of the RS, No. 28/2019). However, although all the parameters are in accordance with the Regulations, if we monitor the changes over the months we can see that the valuesfor the turbidity parameter have changed. Turbidity represents the capacity of suspended particles to absorb heat from solar radiation, which makes the water warmer and thus reduces the concentration of oxygen in the water. Measuring turbidity is important, because these particles protect pathogenic organisms from the effects of disinfection (Tamaš, 2000). The highest turbidity was measured in December and the value was 0.20 NTU. As Ćirić (2009) proved during the examination of certain points in the water supply of the city of Kruševac, greater variations in the number of bacteria are accompanied by greater changes in water turbidity.

According to Grašić (2000), beside turbidity, the consumption of $KMnO_4$ is most often usedto evaluate the efficiency of water treatment. In our research, the highest measured value was in August and it was 2.5 mg/L. Slightly lower values were recorded for the months of November and December and they amounted to 2.0 mg/L. The residual chlorine values recorded during the whole period were 0.40 mg/L.

The results of microbiological tests showed that all samples were microbiologically correct. The presence of pathogenic microorganisms was not determined.

Conclusion

The quality and quantity of drinking water is great importance for public health. For this reason, water analysis is carried out in order to determine the health suitability of drinking water and it represents one of the chief measures for the prevention and control of infectious diseases. The subject of our research was monitoring the physico-chemical and microbiological parameters of drinking water from the territory of the town of Prokuplje. All the results, obtained during the six-month study, indicate that the drinking water is safe for use.

Taking into account the obtained results, the recommended measures are constant further monitoring along with pollution prevention.

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CHARACTERIZATION OF DIFFUSE PHOTOSYNTHETICALLY ACTIVE RADIATION IN BURGOS (SPAIN)

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Abstract

Photosynthetically active radiation (PAR) is the component of solar radiation with a wavelength range between 400 and 700 nm that exerts the greatest influence on photosynthesis and plant growth. Vegetation acts as a CO_2 sink, mitigating the effects of climate change, so knowing the influence of PAR on plant growth is paramount. In addition, the diffuse component of PAR is a key factor in ecosystem productivity models, as it can improve light use efficiency in the canopy by increasing carbon sequestration.
Due to a shortage of PAR sensors at ground meteorological stations, this component of solar radiation is often studied in relation to other more common meteorological variables. Therefore, in this work, a statistical analysis has been carried out analysing the ratio between diffuse PAR (DHPAR) and Global Horizontal Diffuse Irradiance (DHI).
The experimental data used in this work were measured between September 2020 and June 2022 in Burgos (Spain). DHPAR was measured from Diffuse Photosynthetic Photon Flux Density data, Q_{pd} ($\mu mol \cdot s^{-1} \cdot m^{-2}$), and subsequently converted to DHPAR data ($W \cdot m^{-2}$) using McCree's conversion factor (4.57 $\mu mol \cdot J^{-1}$) using a EKO quantum sensor. DHI was measured in $W \cdot m^{-2}$ using a Hukseflux pyrheliometer. All data were collected every ten minutes (averages from 30 seconds). This study analyses the relationship between the DHPAR/DHI ratio to all sky types and the three sky types classified based on the CIE standard classification (clear, intermediate and overcast skies) are analyzed according to different time scales: seasonal, monthly and hourly.

Key words: *Diffuse Photosynthetically Active Radiation, Global Horizontal Diffuse Irradiance, vegetation growth, sky types.*

Introduction

PAR, is the component of solar radiation with a wavelength between 400 and 700 nm (Alados-Arboledas et al., 2000). PAR is a key factor in several processes as plant growth, photosynthesis, biomass production and climate change (Hu et al., 2016; Qin et al., 2019; Sudhakar et al., 2013). Although PAR measurement is essential for canopy growth, agricultural yields and other environmental variables, sensors that measure it are not found at most weather stations (López et al., 2001). As a result, PAR is usually obtained from solar radiation measurements (Monteith and Reifsnyder, 1974; Moon, 1940; Tsubo et al., 2005).
The global incident PAR is made up of the direct and diffuse components. The relative amount of these components, direct and diffuse, depends on geography and atmospheric parameters. The proportion of direct and diffuse radiation in the global radiation is also influenced by sky conditions (Han et al., 2019). Diffuse PAR (DHPAR) at the Earth's surface depends mainly on clouds, aerosols and solar zenith angle (Choosri et al., 2017), increasing with the presence of clouds and aerosol contamination (Trisolino et al., 2016; Yamashita and Yoshimura, 2019). DHPAR is usually measured from Diffuse Photosynthetic Photon Flux

Density data, Q_{pd} (μmol·s^{-1}·m^{-2}) and, subsequently converted to DHPAR data (W·m^{-2}) using the McCree's conversion factor (4.57 μmol·J-1) (Akitsu et al., 2015).
The main objective of this study is to establish DHPAR/DHI ratio in Burgos (Spain) in at different time scales, and to analyse this ratio as a function of sky type (clear, intermediate and overcast) according to the CIE standard sky classification.

Materials and methods

The weather station is situated on the flat roof of the Higher Polytechnic School building (EPS) of Burgos University, Spain, as shown in Figure 1. A complete description of the station has been made in previous works (García-Rodríguez et al., 2020).

Figure 1. Location of the experimental equipment on the roof of the Higher Polytechnic School building at University of Burgos, Spain.

DHPAR was measured from Diffuse Photosynthetic Photon Flux Density data, Q_{pd} using a EKO quantum sensor. DHI was measured in W·m^{-2} using a Hukseflux pyrheliometer.
In order to carry out this work, the experimental campaign took place from September 2020 to June 2022. All data were collected every ten minutes (averages from 30 seconds) and filtered according to stringent quality criteria (Gueymard and Ruiz-Arias, 2016). The sky classification was determined through experimental sky scanner measurements.

Results and discussion

Sensitivity of the ratio DHPAR/DHI using ten -minutes data basis.

In a first analysis, the DHPAR/DHI ratio has been studied. Using a ten minutes data interval of the DHPAR/DHI ratio, a positive correlative has been obtained between both variables, (R^2=0.992), with a slope of 0.403 W·m^{-2}, as shown in Figure 2. This value is close of the mean value 0.447 W·m^{-2} with a standard deviation of ±0.05 W·m^{-2}.

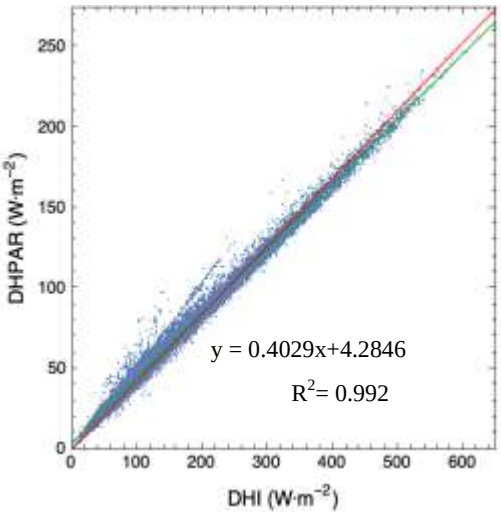

Figure 2. Relationship between the Diffuse Photosynthetically Active Radiation, (DHPAR, $W \cdot m^{-2}$), and Global Horizontal Diffuse Irradiance (DHI, $W \cdot m^{-2}$), measured in Burgos, from September 2020 to June 2022.

Variability of the ratio DHPAR/DHI for CIE standard sky type.

Previous studies (García-Rodríguez et al., 2020) have shown that the predominant skies in Burgos are clear. In spite of this, this study has considered the sky types classified according to the CIE standard, grouped in clear, intermediate and overcast. These results are represented in Figure 3. As can be seen, the highest DHPAR/DHI ratio was obtained for clear skies, with a mean of 47.967 ± 2.025 %, while for intermediate and overcast skies, the mean is slightly above 40%. In contrast, the interquartile range and standard deviation is lower for intermediate skies (1.624 and 2.05, respectively). These data are much higher for clear skies (7.832 and 5.264, respectively).

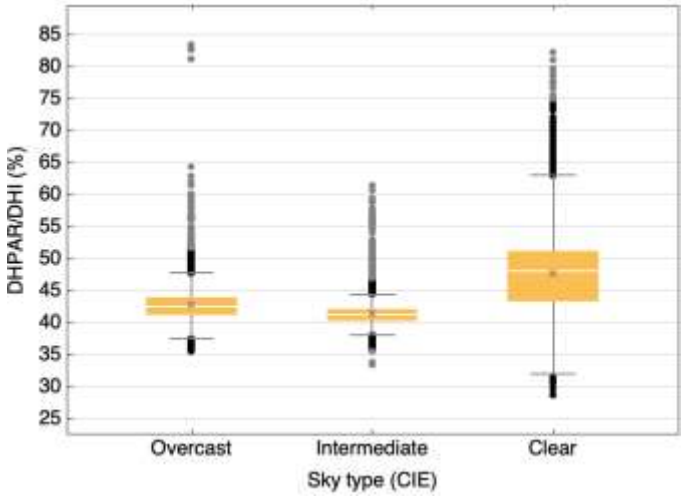

Figure 3. Box-plot ratio of Diffuse Photosynthetically Active Radiation, and Global Horizontal Diffuse Irradiance DHPAR/DHI for CIE cloudiness sky classification.

Variability of the ratio DHPAR/DHI seasonally.

The seasonally study (Figure 4) shows that summer is the season where the highest ratio has been reached. For the rest of the seasons, the ratio presents a similar value, around 44%. The interquartile ranges are similar for winter, spring and autumn (around 4%), while for summer, it is almost double (7.8%).

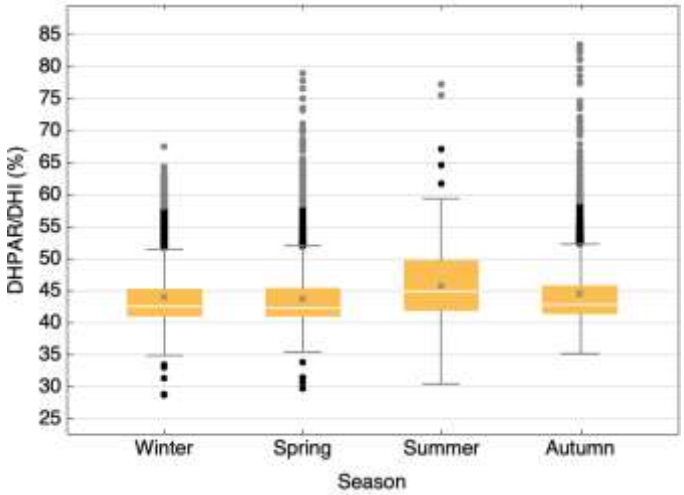

Figure 4. Box-plot ratio of Diffuse Photosynthetically Active Radiation, and Global Horizontal Diffuse Irradiance DHPAR/DHI for each season.

Conclusion

The ratio of Diffuse Photosynthetically Active Radiation, and Global Horizontal Diffuse Irradiance (DHPAR/DHI) obtained in Burgos during the experimental campaign, from September 2020 to June 2022, at 10-minute data basis, has a dependence on both sky type and season.

The highest data of the DHPAR/DHI ratio was obtained for clear skies, while for intermediate skies, less scattered and smaller values were obtained. The global mean value is $0.447 \pm 0.05\ W \cdot m^{-2}$.

When analysing the DHPAR/DHI ratio seasonally, it has been observed that the highest ratio was obtained in summer, while for spring and summer, the data were less scattered and smaller.

Acknowledgements

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CHARACTERIZATION OF ERYTHEMAL ULTRAVIOLET RADIATION IN BURGOS, SPAIN

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Abstract

Solar ultraviolet radiation represents a small fraction of the total radiation (5-7%). It is a highly energetic component of the solar spectrum that must be monitored, as it can be damaging to life on Earth. Ultraviolet (UV) radiation is the region of the solar spectrum with wavelengths between 100 and 400 nm, in shorter wavelengths it has deleterious effects in many biological systems. Excessive radiation can produce serious adverse effects: premature aging of the skin, skin cancer, as well as damage to ecosystems, crops and the biosphere. However, in moderate doses it is beneficial to health: it reduces blood pressure, stimulates the synthesis of vitamin D, improves mental health, etc. The effects of UV radiation on skin that has not previously been exposed to solar irradiance are usually studied through Erythemal Ultraviolet Radiation (UVER). The experimental campaign used in this work covered period from September 2020 to June 2022. Data were collected every 30 seconds and were recorded every 10 minutes. The main objective of this study is to analyze the variation of UVER on the horizontal plane GHUVE with respect to the global solar irradiance on the horizontal plane GHI, (GHUVE/GHI), as a function of the sky type classified according to the ISO/CIE standard at different time intervals. During the experimental campaign carried out in Burgos (Spain), clear skies were predominant, and intermediate skies were less frequent. The highest value of the ratio GHUVE/GHI was obtained in the summer months. GHUVE/GHI gradually increased from sunrise to noon and then decreased until sunset.

Keywords: *Ultraviolet radiation, Erythemal Ultraviolet Radiation, Global Solar Irradiance.*

Introduction

Solar ultraviolet radiation UV represents about 5 to 7% of total radiation (Ahmed et al., 2022). Is the region of the solar spectrum with wavelengths between 100 and 400 nm. The UV radiation emitted by the sun does not receive entirely on the earth´s surface. UVC component (100-280 nm) is completely absorbed by ozone and atmospheric oxygen, a fraction of UVB (280-315 nm) and UVA (315-400 nm) components reaches the Earth's surface as ozone partially absorbs only a portion of this components (Alados-Arboledas et al., 2003). UV radiation varies greatly on the ground, depending mainly on latitude, solar elevation, cloud characteristics, total ozone, aerosol pollution, temperature, and surface albedo (Hu et al., 2010; Murillo et al., 2003).

Ultraviolet radiation can induce serious adverse effects: premature skin aging, skin cancer (Human and Bajic, 2000; Modenese et al., 2020) immune deficiencies and cataracts as well as damage to ecosystems, crops and the biosphere (Al-Aruri, 1990). However, in moderate doses it is beneficial to health: it reduces blood pressure, stimulates the synthesis of vitamin D, improves mental health, among other advantages (Serrano et al., 2017). To reduce the adverse

effects associated with exposures that are too high or too few, ideally there should be a balance in UV radiation exposure (Vuilleumier et al., 2021). The effects of UV radiation on skin that has not previously been exposed to solar irradiance are usually studied through Erythemal Ultraviolet Radiation (UVER).

Materials and methods

The experimental campaign used to carry out this study was conducted at the meteorological facility (Figure 1) located on the Higher Polytecnic School building at the University of Burgos, Spain (42º21′04″N, 3º41′20″W, 856 m.a.s.l.) from September 2020 to June 2022. A complete description of the meteorological facility can be found in Ref. (García-Rodríguez et al., 2022).

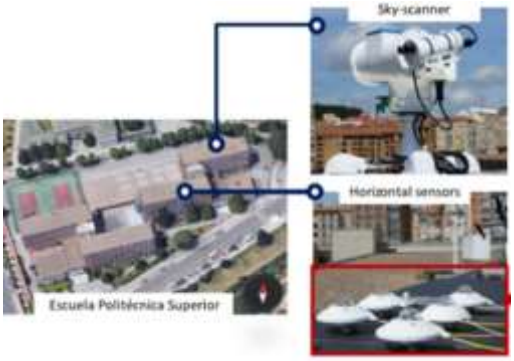

Figure 1. Location of the experimental equipment on the Higher Polytecnic School building at the University of Burgos, Spain.

The atmospheric variables measured at the weather station and the sensors used to measure radiation are shown in Table 1. The data are recorded every 30 seconds and 10 minute average values are stored, the data obtained are analysed and filtered according to quality criteria (Gueymard, 2018; Gueymard and Ruiz-Arias, 2016).

Table 1. Characteristics of the sensors located in the installation.

Type	**Units**	**Model**
Temperature	ºC	Campbell Scientific - CS215
Precipitation	mm	Campbell Scientific - 52202 Electrically Heated Rainand Snow Gage
Pressure	mbar	VAISALA - PTB110
Wind	m s^{-1}	Campbell Scientific - 03002 Wind Sentry Set
Irradiance	W m^{-2}	Hukseflux - SR12-T205
UV E	W m^{-2}	K&Z - SUV E

A Kipp&Zonen SUVE sensor is used to measure UVER on the horizontal plane(GHUVE) and horizontal global solar irradiance (GHI) data are measured with a pyranometer (Hulseflux, model SR12) (Table 1). The sky type is determined from the luminance data collected by a Sky-Scanner (EKO MS-321 LR) (Table 2) according to the CIE classification (ISO 15469:2004(E)/CIE S 011/E:2003; Spatial Distribution of Daylight—CIE Standard General Sky. ISO: Geneva, Switzerland; CIE: Vienna, Austria, 2004). The Sky-Scanner takes measurements of the sky from sunrise to sunset by performing a complete sweep of the sky for 4 minutes, and starts a new sweep of the sky vault every 10 minutes.

Table 2. Model of the installed Sky-Scanner.

Model	MS-321LR Sky Scanner
Dimensions (W × D × H)	430 mm × 380 mm × 440 mm
Mass	12.5 kg
FOV	11°
Luminance	0 to 50 kcd/m2
Radiance	0 to 300 W/m2
A/D Convertor	16 bits
Calibration Error	2%

Table 3 shows the annual and seasonal mean values of GHI, GHUVE, air temperature (T), relative humidity (RH), and wind speed (WS), which were calculated from the 10 minutes records. These values were obtained for the whole year and they were classified according to each sky type (overcast, intermediate, and clear sky).

Table 3. Seasonal 10 min mean values (N) between September 2020 and June 2022 for GHI, GHUVE, T, RH and WS, for overcast, intermediate, and clear sky conditions.

Sky Type	**Parameter**	**All Data**	**Season**			
			Winter	**Spring**	**Summer**	**Autumn**
All Sky	N	25101	5423	8302	5635	5741
	GHI ($W \cdot m^{-2}$)	446.44	342.24	490.91	596.01	333.74
	GHUVE ($W \cdot m^{-2}$)	0.06	0.03	0.07	0.10	0.04
	T (°C)	14.34	8.24	14.36	22.21	12.25
	RH (%)	64.45	72.26	62.97	51.44	71.99
	WS ($m \cdot s^{-1}$)	2.25	2.47	2.28	2.05	2.22
Overcast skies	N	7197	1883	2521	785	2008
	GHI ($W \cdot m^{-2}$)	163.88	146.27	183.53	198.57	142.14
	GHUVE ($W \cdot m^{-2}$)	0.03	0.02	0.03	0.04	0.02
	T (°C)	10.71	6.61	11.82	18.60	10.07
	RH (%)	80.32	84.50	77.64	67.13	84.93
	WS ($m \cdot s^{-1}$)	2.42	2.70	2.32	1.86	2.50
Intermediate skies	N	6458	1521	2107	1196	1634
	GHI ($W \cdot m^{-2}$)	392.12	343.57	443.41	440.48	335.77
	GHUVE ($W \cdot m^{-2}$)	0.06	0.04	0.07	0.07	0.04
	T (°C)	13.67	8.35	14.58	20.99	12.08
	RH (%)	66.14	72.77	62.50	55.42	72.50
	WS ($m \cdot s^{-1}$)	2.33	2.64	2.28	2.03	2.31
Clear skies	N	11446	2019	3674	3654	2099
	GHI ($W \cdot m^{-2}$)	654.75	523.99	729.07	732.29	515.44
	GHUVE ($W \cdot m^{-2}$)	0.09	0.05	0.11	0.12	0.06
	T (°C)	17.01	6.69	15.97	23.54	14.48
	RH (%)	53.52	60.47	53.17	46.76	59.20
	WS ($m \cdot s^{-1}$)	2.11	2.13	2.25	2.09	1.88

As not all ground meteorological stations are equipped with UV radiation sensors, UV components are usually estimated from radiometric or meteorological parameters.

Results and discussion

To determine the frequency of occurrence (FOC, %) of each CIE sky type group (overcast, intermediate and clear) a Sky-Scanner has been used (Granados-López et al., 2021; Suárez-García et al., 2020). In figure 2 it can be observed clear skies being predominant in Burgos

during the experimental campaign (FOC higher than 45%) and intermediate skies were the least predominant (FOC lower than 25%).

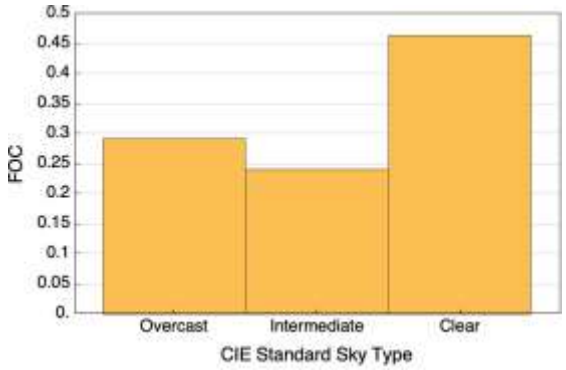

Figure 2. Frequency of Occurrence (FOC, %) of each CIE standard sky type group in Burgos (Spain).

This study has analysed the variation of UVER on the horizontal plane (GHUVE) with respect to the horizontal global solar irradiance (GHI) at different time intervals during the experimental campaign.

Figure 3 shows the statistical analysis of the hourly averages of the GHUVE/GHI ratio. It can be observed that GHUVE/GHI gradually increased since sunrise until noon and then, decreased until sunset. Between 10:00 a.m. and 2:00 p.m., there was the higher dispersion of data (interquartile range: $0.7 \cdot 10^{-2}$% - $0.9 \cdot 10^{-2}$%). The maximum value ($4.2 \cdot 10^{-2}$%) was reached at 12.00h.

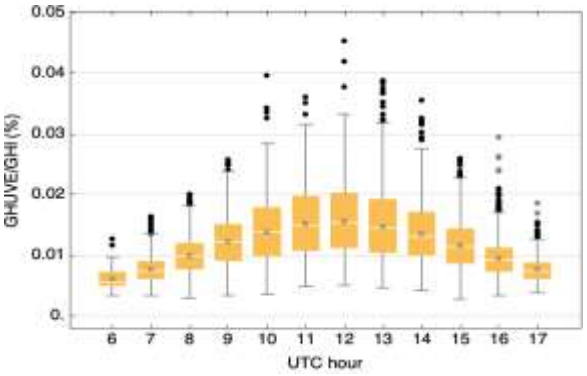

Figure 3. Box and whisker plot of the GHUVE/GHI hourly.

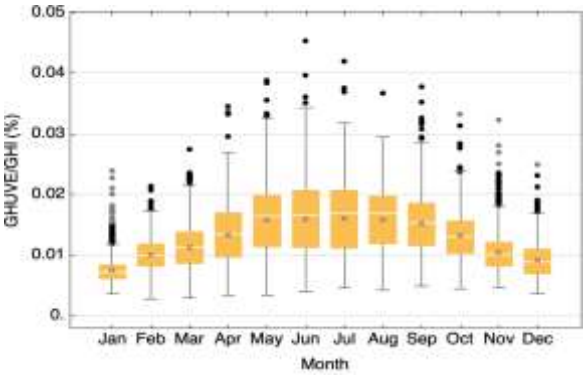

Figure 4. Box and whisker plot of the GHUVE/GHI monthly.

Box and whisker plot of Figure 4 shows the monthly values of GHUVE/GHI. The ratio gradually increased from January to May, remained almost constant until August and decreased until December. The greatest dispersion of data took place between May to August, the interquartile ranges were between $0.8 \cdot 10^{-2}$% and $1 \cdot 10^{-2}$% and standard deviations around $0.6 \cdot 10^{-2}$%. The minimum value of the ratio occurred in the months between February and May ($0{,}3 \cdot 10^{-2}$%), while the maximum value was reached in June ($4{,}5 \cdot 10^{-2}$%).

In this study, winter has been considered from December to February, spring from March to May, summer from June to August and autumn from September to November. Figure 5 shows the greater value of the ratio was obtained in the summer months, while the lowest value of GHUVE/GHI was reached in the winter months. The highest dispersion of the data occurred

in summer, and the lowest in winter. The interquartile ranges were between $0.9 \cdot 10^{-2}$% and $1.5 \cdot 10^{-2}$% with standard deviations of $0.5 \cdot 10^{-2}$% and $0.3 \cdot 10^{-2}$%, respectively.

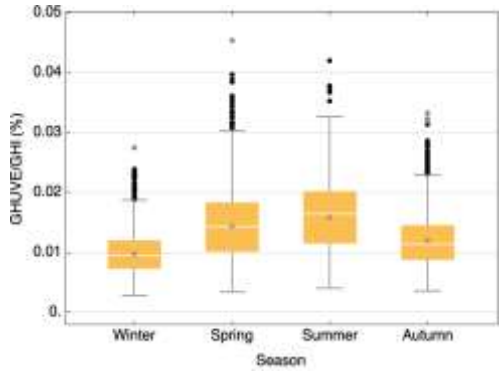

Figure 5. Box and whisker plot of the GHUVE/GHI seasonal based on 10 min data.

Figure 6 analyzes the GHUVE/GHI ratio based on the 3 sky types. It is shown that in all cases (overcast, intermediate and clear skies) the data dispersion (interquartile range: $0.7 \cdot 10^{-2}$% - $0.9 \cdot 10^{-2}$%, standard deviation $0.05 \cdot 10^{-2}$%) and the mean value were very similar ($1.3 \cdot 10^{-2}$% - $1.4 \cdot 10^{-2}$%).

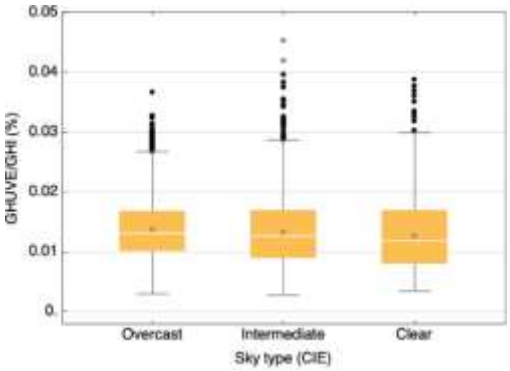

Figure 6. Box and whisker plot of the GHUVE/GHI ratios for each CIE sky type group using the 10 min data.

Conclusion

GHUVE and GHI data were recorded at 10-minute intervals in Burgos, Spain, between September 2020 and June 2022 and the GHUVE/GHI ratio was analysed under all sky conditions.

In the city of Burgos during the experimental campaign, clear skies being predominat, FOC higher than 45%, and intermediate skies were the least predominant, FOC lower than 25%.

After analysing the ratio at different time intervals, it is observed that the ratio gradually increased from January to May, remained almost constant until August. The minimum value of the ratio occurred in the months between February and May ($0{,}3 \cdot 10^{-2}$%), while the maximum value was reached in June ($4{,}5 \cdot 10^{-2}$%). Analyzed the ratio based on all seasons, the highest value of GHUVE/GHI was obtained in summer and the lowest in winter. The data dispersion (interquartile range: 0.7·10-2% -0.9·10-2%) and the mean value ($1.3 \cdot 10^{-2}$% - $1.4 \cdot 10^{-2}$%), on the 3 sky types (overcast, intermediate and clear skies), were very similar.

Acknowledgement

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INFLUENCE OF COVER CROPS ON SOIL BIOLOGICAL QUALITY

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Abstract

Healthy soil is a foundation for agriculture and an essential resource to ensure human needs. Several studies have been conducted to quantify the impacts that agriculture has on the ecosystems, and have shown that the use of conservative practices, such as cover crops, can have positive effects on the soil fauna and its ecological services. The essential part of this fauna, the soil arthropods, has been used as bioindicators of soil quality. This study aimed to monitor soil Arthropod biodiversity, in an intensive horticultural system, where a cover crop was installed and compared with a control modality. It has been conducted since 2021, in a test field, located in Chamusca - Portugal, where a mixture of legumes and grasses was installed as cover crop, preceding a main crop of tomato. Two monitoring methods were used: (I) arthropod capture with pitfall traps and (II) deep soil collection, applying the QBS-ar methodology. In the laboratory, the arthropods collected by the traps were quantified, organized by morphotypes, and taxonomically identified. The soil samples were placed in a Berlese-Tüllgren extractor for arthropod separation and posterior classification. In the first year, the cover crop plot revealed a greater amount and diversity of arthropods, as well as a higher soil quality index. Data regarding the current cultural campaign has been demonstrating the same tendency, so far.

Keywords: *Biodiversity, Cover Crops, Pitfall traps, QBS-ar, Soil arthropods.*

Introduction

The foundation of a healthy and productive cropping system relies on a healthy soil environment (Wang & Hooks, 2021). Despite this, human related land use is the primary cause of accelerated soil erosion, which has substantial implications in nutrient and carbon cycling, land productivity and in turn, worldwide socio-economic conditions (Borreli et al., 2017). A "healthy soil" is a stable soil that can overcome stress, usually high in biological diversity, and capable of maintaining functions such as nutrient cycling. The greater the biodiversity within the soil, the quicker the soil ecosystem can return to initial conditions after exposure to disturbances (Wang & Hooks, 2021). Organic matter provided by cover crops, support and regulates most microbial activities and soil organisms. Thus, cover crops can be used as an effective tool to help regulate soil fauna composition and ecosystems services, increasing functional diversity, and its associated nutrient cycling (Wang & Hooks, 2021). The ability of the soil to correctly perform its functions, is designated by "Soil quality" (Karlen et al., 1997), and one of the main tools to evaluate it is the use of biological indicators (Bastida et al., 2008). Among them, some soil invertebrates have been included in monitoring programs as soil quality bioindicators. Being an important part of soil fauna, arthropods are involved in many processes such as organic matter decomposition and translocation, nutrient cycling, microflora activity regulation and bioturbation. Many studies have reported the use of soil arthropods to define soil quality (Menta & Remelli, 2020). Although a large variety of arthropod taxa are suitable to be used as bioindicators, a base group that best reflect the ecosystem in study should be selected, considering their sensitivity to alterations of the local

environmental conditions. A ground-layer indicator set should include ants, millipedes, ground beetles (Carabidae e Staphylinidae families), harvestmen and spiders (Gerlach et al., 2013). Microarthropods groups such as Acari and Collembola should be considered as well (Menta & Remelli, 2020). For this paper, the previous mentioned will be referred as "main bioindicators groups". For the last three-quarters of a century, pitfall traps have proved to be one of the most versatile, useful, and widely used invertebrate sampling techniques. This technique has been used in practically every terrestrial habitat to provide information such as the structure of invertebrate communities, habitat associations, activity patterns, spatial distribution, relative abundances, total population estimates and distribution ranges (Woodock, 2005). One more recent index, the QBS-ar (Soil Biological Quality-arthropod), joins the biodiversity of soil microarthropods community with the degree of soil vulnerability; basing his criterion on the concept that the number of well adapted microarthropod groups is higher in soils characterized as "good quality". This method has been used in numerous studies so far (Menta et al., 2018). This study aimed to evaluate the influence of cover crops on the soil biological quality, through the monitoring of soil arthropod bioindicators. It has been conducted since 2021, in a test field in Portugal, where a mixture of legumes and grasses was installed as cover crop, preceding the main crop. Two modalities were considered in the field: a) Cover Crop [CC] and b) Control modality [CO]; as well as two monitoring methods: I) arthropod capture with pitfall traps and II) deep soil collection applying the QBS-ar methodology.

Materials and Methods

Test field

The field was located in the Ribatejo region of Portugal, which is characterized by the predominance of intensive monocultural crops systems, mainly for industrial processing, with a heavy use of mechanization and chemical inputs (Nunes, 2021). The long-term effects of these practices have led to a high incidence of pests and diseases, as well as a decrease of soil quality and crop productivity (Pinto, 2022). As an attempt to introduce simple solutions for the land managers to overcome these issues, a selected mixture of grasses and legumes was implemented as an intercrop, in a plot with an approximate area of 0.5 ha (CC); an equal plot was chosen as control modality (CO), that followed the usual crop profile (unattended soil during the off season).

Pitfall Traps

Sampling was performed in both plots, at 5 time periods: 3 during the main tomato crop of 2022 (June and July); 1 during the cover crop of 2023 (March), and 1 during the main tomato crop of 2023 (June). For each period modality, 4 pitfall traps were installed, following a linear transect, starting at 10 meters from the margin and 10 meters apart. The traps consisted in glass containers, buried in the soil, containing ethylene glycol as preserver and a cover to serve as rain guard. After 7 days in the field, the traps were transported to the laboratory, where its contents were filtered, separated into morphotypes, catalogued, photographed, and taxonomically identified, up to the family level.

QBS-ar Soil sampling

Sampling was performed in both plots, at 6 time periods: 1 during the cover crop of 2022 (February); 3 during the main tomato crop of 2022 (June and July); 1 during the cover crop of 2023 (February), and 1 during the main tomato crop of 2023 (June). For each period, 15 random soil sub-samples were obtained with a soil probe, at the dept of 20 cm, starting at 10 meters from the margin and 10-15 meters apart. Sampling was performed during the morning, to avoid the periods of higher soil temperature which can cause the edaphic arthropods to

migrate vertically as described by Menta et al. (2018). The samplings were transported in plastic bags to the laboratory, and placed in a *Berlese-Tüllgren* extractor, equipped with 40W incandescent lamps, where the heat produced by the former, promotes the soil micro-arthropods vertical migration and collection in Erlenmeyer flasks with a solution of 70% ethanol. The extractions were carried out during a period of 7 consecutive days. The extracted specimens were observed in a stereoscope and taxonomically classified at the order level. To each morphotype, an *Eco Morphological Index* (EMI) was assigned, ranging between 1 (no adaptation to soil) and 20 (maximum adaptation to soil), according to a dedicated key, as described by Menta et al. (2018). The final QBS-ar index, was obtained by the sum of the previous EMI values, as described by Parisi et al. (2005).

Results and Discussion

Pitfall Traps

During this assessment, a total of 1259 arthropods were captured in both plots, separated in 12 orders and 150 morphotypes. Most of the specimens belonged to the Insecta class (59.9%), followed by the Arachnida (27.4%), Entognatha (11.8%), Malacostraca (0.5%), and Chilopoda (0.4%) classes.

The obtained results mainly showed higher values, regarding the total quantity and number of arthropod morphotypes (biodiversity), in the cover crop plot, in relation to the control modality (Figure 1).

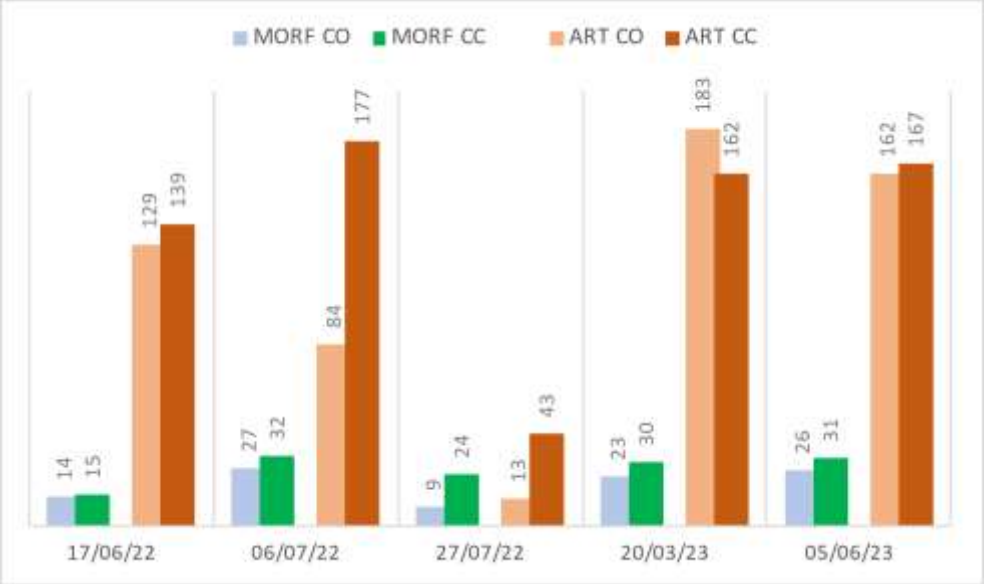

Figure 1 – Total number of Arthropods [ART] and morphotypes [MORF] by sampling period and plot (Control [CO]; cover crop [CC]).

An exception was noted in the sampling period of 20/03/2023, where the total quantity of captured arthropods was higher in the control modality, although the detailed analysis (Figure 2a) revealed that 63% of those specimens belonged to the super-order Parasitiformes (ticks), considered disease-spreading vectors to humans and animals, as stated by Hillary and Ceasar (2021). In the cover crop plot (Figure 2b), the higher value of biodiversity found, as well as a higher presence of Hymenoptera (mainly Formicidae), possibly contributed to the decrease of the previous group dominance, due to the strong predator-prey relation between ants and ticks, as referred by Samish and Alekseev (2001).

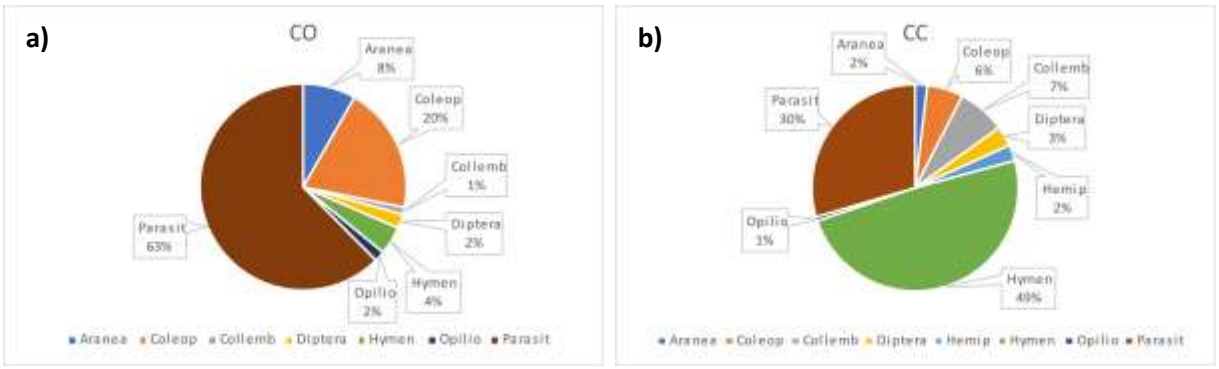

Figure 2 – Arthropod distribution by taxonomic order, in the sampling period of 20/03/2023: a) control plot [CO], b) cover crop plot [CC].

In relation to the main bioindicator groups (Figure 3), the cover crop plot consistently shown higher values, both in amount and diversity of arthropods, in comparison to the control modality.

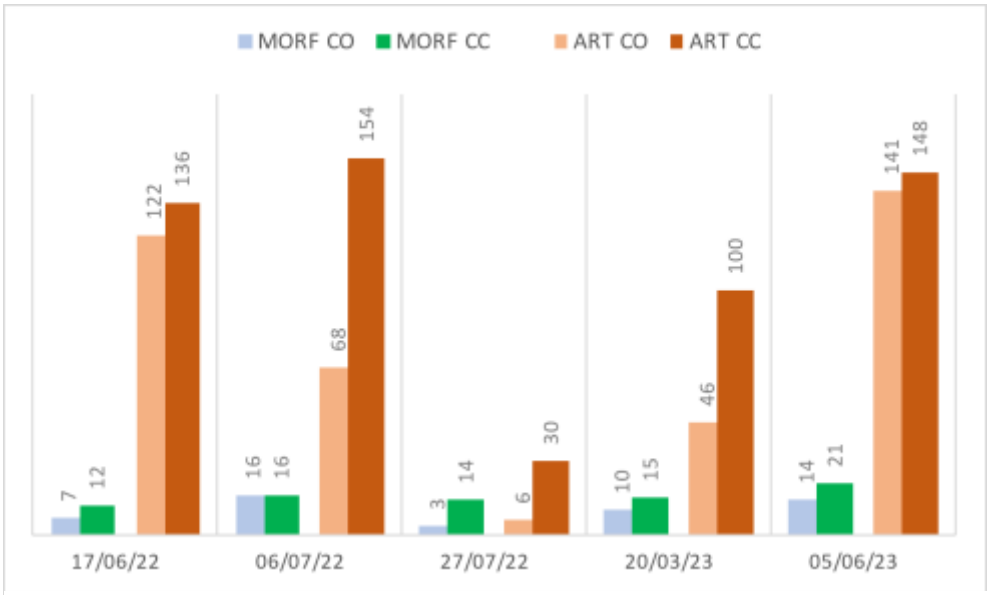

Figure 3 – Total number of main bioindicator Arthropods [ART] and morphotypes [MORF] by sampling period and plot (Control [CO]; cover crop [CC]).

Overall, the pitfall traps assessment, revealed a tendency for a greater presence of overall and main bioindicator Arthropoda, in the plot where the cover crop was installed, which is coherent with the results obtained by Jacobsen et al., (2022), that refers a tendency for greater density and diversity of arthropods in plots managed with conservative practices, which according to the author, can lead to more resilient cropping systems with fewer input needs.

QBS-ar Index

The obtained EMI and QBS-ar index (Table 1), consistently demonstrated a higher index of soil biological quality in the cover crop plot, in relation to the control modality. Considering the mean value of QBS-ar (93.7) proposed by Menta et al. (2018), to distinguish between high quality soils from typical poor soils, the test field showed an overall degraded soil condition, which is consistent with its history of intensive agricultural practices.

Nonetheless the implementation of a cover crop in the agricultural off-season, revealed an improvement in the soil biological quality, which is coherent with the results obtained by

Menta et al. (2018), Jerez-Valle et al. (2014) and Sapkota et al. (2018), who found superior biodiversity indexes in plots where conservative agricultural practices were applied, that can lead to positive effects on the ecosystems regulating services as stated by Balvanera et al. (2006).

Table 1 – EMI values and resultant QBS-ar Index, by sampling period.

	2022								2023			
	Cover Crop		Main Crop						Cover Crop		Main Crop	
	16/02		17/06		06/07		27/07		01/02		05/06	
	CC	CO	CC	CO	CC	CO	CC	CO	CC	CO	CC	CO
Acari	20	20	20	20	20		20		20	20	20	20
Araneae		5										
Collembola	10	2	10	4	10		10	10	10	8	20	10
Coleoptera	5				5	10	1					
Diptera			1	1	1	1	1	1	1			
Hemiptera		1			1	1		1				
Himenoptera					1	5						
Lepidotera					1	1						
Neuroptera						1						
Larvae:												
Coleoptera	10		10									
Diptera									10			
Other							10	10				
QBS	45	28	41	25	39	19	42	22	41	28	40	30

Conclusions

Both monitoring methodologies have demonstrated a similar tendency, showing an improvement in soil Arthropoda presence, in the plot where a cover crop was installed, in relation to the control modality.

The option to install a cover crop in the crop profile, proved beneficial in the improvement of the soil biological quality, and the maintenance of this practice along with its monitoring is advised, to determine if in the long-term further improvements can be obtained in the soil faunal communities.

Between both monitoring methodologies, the QBS-ar index have demonstrated, in the authors opinion, to be a more comprehensive tool to evaluate the soil biological quality. While the Pitfall traps can provide information about the relative presence of arthropod groups, the results can prove less concise than the index provided by the QBS-ar methodology. Furthermore, the pitfall traps proved to require more material, time expended in field and laboratory analysis, as well as in taxonomic identification expertise. Considering the growing need to implement comprehensive and easy-to-use methodologies to allow land managers and technicians to evaluate the impact of agricultural practices in the soil quality, the authors suggest the QBS-ar method as a baseline tool to evaluate soil biological quality.

Acknowledgments

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STATUS OF RADIONUCLIDES IN THE SOILS FROM VOJVODINA PROVINCE, NORTHERN SERBIA

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Abstract

This study investigated the activity concentrations of ^{238}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in 11 locations throughout Vojvodina, northern part of Serbia. The samples were collected at a depth of 10-20 cm. Activity concentrations of radionuclides were analyzed by gamma spectrometry. The activity concentrations of ^{238}U was found to be in the range from 36.4 to 54 Bq kg^{-1}, ^{226}Ra in the range of 41.2-51.2 Bq kg^{-1}, ^{232}Th in the range of 42.6-54 Bq kg^{-1}, ^{40}K in the range of 49-669.8 Bq kg^{-1} and ^{137}Cs concentrations ranged from 4.1-9.1 Bq kg^{-1}. Obtained activity concentrations of radionuclides do not indicate any risk and concern to public health and the environment.

Keywords: *gamma spectometry, radionuclides, soil, Vojvodina Province*.

Introduction

Not only is nuclear radiation a part of the natural environment, it is also a huge public concern all over the world. Soil is an important source of nuclear radiation, mainly containing natural radioisotopes of uranium and thorium series, together with ^{40}K. The most abundant fission product in soil is ^{137}Cs, which has been uniformly spread and adsorbed on the surface layer. Its major releases have come from nuclear weapons testing and nuclear accidents such as the Chernobyl disaster in 1986. Soil acts as a source of continuous radiation exposure to humans due to radionuclides which can be transferred to biological systems causing radiological contamination in the environment. The concentrations of natural radioactivity in soil vary from one region to another (UNSCEAR 2008). Surveys of terrestrial radionuclides have attracted great interest throughout the world (Karahan et al. 2000; Kanan et al. 2002; Hafezi et al. 2005; Tahir et al. 2005; Abbaspour et al. 2010; Mehra et al. 2010; Bikit et al. 2011; Rashed-Nizam et al. 2015; Forkapic et al. 2017). Monitoring of Vojvodina's soil is especially important because of the bombarding of Serbia in 1999, and the possible contamination by depleted uranium. The major purpose of this study was to determine the activity concentrations of ^{238}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in Vojvodina's soil from 11 locations and to access the current status of investigated compounds which are of prime importance for environmental protection.

Materials and methods

Study area: Serbia lies between latitudes 41° and 46° N and longitudes 18° and 23° E (STAT 2012). The Vojvodina region is situated in the northern part of Serbia and the southern part of the Panonnian plain, a region with favorable soil and climate conditions for agricultural production. Based on 1961-1990 observed daily weather data, according to Koppen climate classification (Kottek et al., 2006), the climate is characterized as a moderate continental

climate - *Cfwbx"* (Lalić et al. 2011; Mihailović et al. 2014). The first letter in the formula is *C* – the average temperature of the coldest month is between -3.0 °C to -18 °C (January – 0.7 °C), the average temperature of the hottest month is higher than 10 °C (July 21.4 °C), those are the characteristic temperatures for a moderate continental climate. The second letter from the formula is *f,* which indicates that the maximum of precipitation appears in the summer period (in our conditions in June, 80 mm), while *w* – is described as the minimum precipitation that appears during winter season (in our conditions in March, 37 mm). The third letter in the climate formula is *b* - expressing the average temperature of the hottest month during the year and varied between 10 °C and 22 °C (July, 21.4 °C). The last letter in the formula is *x* – which means that the secondary maximum in precipitation appears in late autumn (in our conditions during November and December, 53 mm for both months) (Lalić et al. 2011; Mihailović et al. 2014).

It is necessary to mention that the distance between the most northern and southern point of Vojvodina region is 2 latitude degrees and is simple, there is no significant difference between temperatures among smaller regions in Vojvodina (Lalić et al. 2011).

In Vojvodina, the most usually represented soils are chernozem, alluvial soils, smonitza, saline and alkali soils (Jovanović et al. 2013). The most widespread soil type, on 43% arable land in the Vojvodina Province is a chernozem soil type with its varieties (WRB 2007). In WRB classification a chernozem soil type is soil with a thick black surface layer that is rich in organic matter. This soil type is developed under a moderate continental climate, vegetation, orography and mostly on loess base. With a $CaCO_3$ presence and carbonate humus active layer, this soil type has a favorable mechanical and chemical composition for agricultural production (WRB 2007).

Sample collection and preparation: Soil samples were collected from 11 locations in Vojvodina: Bač, Zmajevo, Despotovo, Temerin, Čenej, Lovćenac, Čurug, Kisač, Bački Brestovac, Bački Jarak and Rumenka. The soil classifications of the chosen locations are presented in the soil map (Benka and Salvai 2000). The soil type at chosen locations was chernozem, except in one location, Bač, was alluvium type, the soil formed beside the river basin. Samples were collected from June 2015 to April 2016 years. The soil was sampled at a depth of 10-20 cm. From each location of an approximately 100 m^2 of cultivated area, 10-15 subsamples exclusively from the plain surfaces were collected, mixed and homogenized. Most samples belong to chernozem-like soil. The soil samples were dried at 105 °C to constant mass (IAEA 1989). All mechanical contaminants, mainly small stone pieces and plant material were removed. The samples were then sieved and homogenized as fine powder samples of about 300 g which were prepared and packed in cylindrical geometry-Marinelli beakers (V=250ml) and measured on the cap of detectors. Typical measurement time was 100 ks.

Analysis of radionuclides**:** Activity concentrations of radionuclides gamma emitters were determined by the method of low-level gamma spectrometry on high resolution HPGe coaxial detector system produced by ORTEC with nominal efficiency of 28%, resolution (FWHM) at 1.33 MeV ^{60}Co of 1.67 keV and Peak-to-Compton ratio 67:1. The detector was shielded with a cylindrical lead shield of 10 cm wall thickness. The lead case is coated on the inside with an absorber of cadmium, copper and plexiglass. Through ORTEC type pre-amplifiers and amplifiers spectra were channeled to multichannel analyzer MCA with analog-digital converter of 16384 channels total memory. MCA was directly connected to a PC in which measured spectra were stored and analyzed. The gamma spectra were acquired and analyzed using the GammaVision® software. The program calculates the activity concentration of an isotope from all prominent gamma lines after peaked background subtraction. All measurement uncertainties are presented at 95% confidence level. That means that probability of errors in repeated measurement of the same sample would be less than 5%. The precision

and accuracy of the method was tested using a certified reference material LR 320 (Deutscsher Kalibrierdienst, Germany).
Statistical analysis: Data analysis was performed using Statistica 13 software and Excel (Microsoft Excel, 2010) to determine the descriptive statistic parameters.

Results and discussion

The average radioactivity concentrations (mean ± SD) of ^{238}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in soil samples are given in Table 1. The activity concentrations of ^{238}U were found to be in the range of 36.4 to 54 Bq kg^{-1}, ^{226}Ra in the range of 41.2-51.2 Bq kg^{-1}, ^{232}Th in the range of 42.6-54 Bq kg^{-1}, ^{40}K in the range of 49-669.8 Bq kg^{-1} and ^{137}Cs concentrations ranged from 4.1-9.1 Bq kg^{-1}. In comparison with corresponding world average values of 33, 32, 45 and 420 Bq. kg^{-1} given for ^{238}U, ^{226}Ra, ^{232}Th and ^{40}K respectively by the United Nations Scientific Committee on the Effects of Atomic Energy radiation (UNSCEAR 2000), the activity concentrations of ^{238}U, ^{232}Th and ^{226}Ra are slightly higher, while ^{40}K mean concentration is lower than the world average values reported in UNSCEAR. The radionuclide ^{137}Cs was identified in all the soil samples. This radionuclide originates from nuclear weapons test fall-out and nuclear accidents. If we compare ^{137}Cs activity concentration results with measurements of Bikit et al. (2011) in Vojvodina region also, which results ranged from 2.3-42.6 Bq kg^{-1}., we note a slight decrease. This can be explained by its washing out and redistribution after the last accident in 1986 (Chernobyl). The concentration levels of natural radioactivity of the soil samples have a wide range of values due to the soil formation and transport processes.

Table 1. Activity concentrations of radinuclides in soil samples fromVojvodina Province

No.	Locations	Activity concentration Bq kg^{-1} X ± SD				
		^{137}Cs	^{226}Ra	^{232}Th	^{40}K	^{238}U
1.	Bač	9.1 ± 0.3	41.2 ± 0.9	44.8 ± 0.9	66.1 ± 9.2	39.8 ± 8.5
2.	Zmajevo	4.5 ± 0.3	43.9 ± 0.9	45.5 ± 0.9	53.3± 8.6	36.4 ± 7.3
3.	Despotovo	4.8 ± 0.2	50.6 ± 0.8	52.3 ± 0.9	56.1 ± 4.3	54.0 ± 8.8
4.	Temerin	5.5 ± 0.2	42.4 ± 0.9	46.5 ± 0.9	49.0 ± 9.1	39.1 ± 7.5
5.	Čenej	6.8 ± 0.1	45.1 ± 0.8	42.6 ± 0.8	602.4 ± 11.2	48.7 ± 7.4
6.	Lovćenac	5.1 ± 0.1	49.5 ± 0.8	50.2 ± 0.9	60.8 ± 5.5	51.6 ± 7.1
7.	Čurug	4.2 ± 0.1	46.0 ± 0.7	43.5 ± 0.8	646.9 ± 11.3	44.7 ± 6.4
8.	Kisač	5.0 ± 0.2	46.0 ± 0.9	54.0 ± 1.1	669.8 ± 12.4	38.3 ± 6.3
9.	Bački Brestovac	4.1 ± 0.1	47.3 ± 0.8	46.9 ± 0.8	57.7 ± 4.6	48.5 ± 6.7
10.	Bački Jarak	4.6 ± 0.1	46.9 ± 0.8	48.0 ± 0.9	57.4 ± 6.8	49.3 ± 9.4
11.	Rumenka	5.8 ± 0.2	51.2 ± 1.0	52.1 ± 1.3	198.9 ± 11.7	50.1 ± 8.1
Mean (X±SD)		5.4 ± 1.4	46.4 ± 3.2	47.8 ± 3.8	228.9 ± 267.6	45.5 ± 6.1
Range		4.1 – 9.1	41.2 – 51.2	42.6 – 54.0	49.0 – 669.8	36.4 – 54.0

The soil of Vojvodina may contain radioactive contaminants from different sources. There are nuclear power plants in the South East Europe region that could contaminant this soil through the release of radionuclides into air and water. During the year 1999, Serbia was bombarded and potentially contaminated by depleted uranium. The application of phosphate fertilizers with high uranium concentration may also cause a gradual increase in the uranium series activity concentration in soil. The importance and significance of natural radionuclides as potential polluters of agricultural and livestock production is also reflected in the latest „Rulebook on the limits on the content of radionuclides in drinking water, foodstuffs, animal feed, medicines, general use objects, building materials and other goods placed on the market" (Official

Gazette, Republic Of Serbia, 2018.). This amendment was introduced by the new article, which prescribes the limit of the content of natural radionuclides (^{238}U, ^{226}Ra and ^{40}K) in mineral phosphate fertilizers, as possible sources of radioactive contamination to the soil. For Vojvodina, as an area with great opportunities for the production of healthy and safe food, it is extremely important to systematically monitor the level of radioactivity of the agricultural land, because if it is contaminated with wet or dry atmospheric precipitates as well as substances with a technologically elevated level of natural radioactivity, the soil can be a permanent reservoir of radionuclides that significantly contribute to the overall radiation exposure and total population radiation dose.

Conclusion

The activity concentration levels of ^{238}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in the soil samples collected from the Vojvodina region, Serbia are at the normal environmental levels and within the recommended safety limits. Taking into account the mean value of ^{238}U of 5.4 Bq kg^{-1} is at a natural environment level, there is no indication for contamination by depleted uranium during the bombarding of Serbia in 1999 at the investigated localities. It is therefore important to monitor the presence and concentrations of radionuclides from soil and to reduce environmental contaminants input into the food chain. The obtained results may provide useful information for the exposure assessment in risk assessment of radinuclides from soil.

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SHEEP FARMERS' PERCEPTION OF CLIMATE CHANGE: CASE OF KONYA PROVINCE, TÜRKİYE

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Abstract

The aim of the study is to determine the perception of climate change in sheep breeding enterprises, to determine their attitudes and behaviors. It was carried out in Konya, one of the driest provinces of Turkey. In the study, the sample size was calculated as 151 according to Neyman's Stratified Random sampling method. In determining the level of knowledge and application level of farmers about climate change perception, attitudes, and behaviors, a 5-point Likert-type scale was used, and the data were interpreted by creating tables and graphs according to weighted averages and importance index. Although the rate of farmers who heard about climate change in sheep farms is 69.54%, only 17.88% believe that this change is human induced. While 46.36% of the farmers define climate change only as an increase in temperature. Again, 31.79% of the farmers state that agriculture is dependent on climate change. Farmers declare that they receive information about climate change mostly from visual media such as television and radio. A total of 47.02% of the farmers are aware that climate change is important in terms of protecting the environment and natural resources. Also, 97.40% of the sheep enterprises are aware that climate change is an important global problem, 95.60% that it will increase input costs due to climate change, 94.20% that it will increase the agriculture and farmers of Konya region in the next 10 years starting from 2022. It has been determined that they know agriculture will be greatly affected by climate change.

Key words: *Sheep farming, climate change, perception, Konya, Türkiye*.

Introduction

The climate is an important component of agricultural productivity. Considering the fundamental role of agriculture in human nutrition and well-being, concerns about the future arise (Oğuz et al., 2023). Various scenarios are being developed and strategies are being formulated by world countries to assess the potential impacts of climate change on agricultural productivity. There are numerous definitions of climate change in the literature. Some of them describe it as a phenomenon resulting from greenhouse gas emissions caused by factors such as fuel combustion, deforestation, urbanization, and industrialization. Climate change can significantly affect agricultural production and thus food security (availability of food). Climate, in its simplest form, refers to the average conditions of all-weather phenomena experienced or observed in a particular location on Earth over many years. Climate change, on the other hand, can be defined as statistically significant changes occurring over decades or longer periods in the average state or variability of climate.
Agriculture is not only affected by climate change but also contributes to it. It is important for producers to have knowledge, recognition, and awareness of climate change and its effects to mitigate these impacts (Masud et al., 2017; Somda et al., 2017; Chedid et al., 2018). Because the more knowledge the producer has about climate change and its effects, the more effort they will make to mitigate the negative impacts. Konya province is one of the driest regions in Turkey and ranks 20th in terms of development level. Therefore, Konya province has been

selected as the study area. Konya province accounts for 5% of Turkey's agricultural production value, 5.08% of the small ruminant population, and 5.88% of the sheep population (1,432,705). The aim of the study is to determine the perception of climate change in sheep farming enterprises. In fact, in all national reports on climate change in Turkey, as well as in the climate change strategy document and the climate change action plan, the identification of the impacts of climate change on agriculture at the national, regional, and local levels is emphasized (Anonymous, 2013).

Materials and methods

The primary data for the research was compiled through surveys conducted with sheep farming enterprises in Konya province. The collected data pertains to the production period of the year 2021. Accordingly, there are a total of 9,228 sheep farming enterprises in Konya province. The districts with the highest sheep population in the province are Karapınar, Ereğli, Cihanbeyli, Meram, Karatay, and Çumra, which constitute 54.92% of the sheep population in Konya province. Therefore, these districts form the main framework of the research. The number of surveyed enterprises was determined as 151 using Neyman's "Stratified Random Sampling Method" according to the formula below.

$$n = \frac{[\sum(N_h S_h)]^2}{N^2 D^2 + \sum[N_h (S_h)^2]}$$

$$D^2 = d / z$$

In formula; n = sample size, N = total unit number belonging to the sampling frame, N_h=the number of enterprises in the hth stratum (frequency), S_h= the standard deviation of the hth stratum. D = d / z, d = deviation from the average, z = standard normal distribution value (Yamane, 1967).

The perception of climate change among farmers, their level of knowledge, and the level of implementation were determined using a 5-point Likert scale. The data were analyzed by creating tables and graphs based on weighted averages and interpreted accordingly. The Likert scale is the most suitable technique for measuring perceptions, attitudes, and behavioral patterns. Furthermore, the importance index was calculated using the following formula to measure the perception of climate change among sheep farming enterprises:

$$\text{The importance index} = \frac{\sum_{i=1}^{5}(piqi)}{\sum_{i=1}^{5} qi} * 10$$

Where, p1, p2, p3, p4, p5 represent the corresponding response frequencies, while q1, q2, q3, q4, q5 represent the response labels or categories (Oğuz and Karakayacı, 2017; Masud et al., 2017). The rating scale is as follows: q1: Strongly Disagree, q2: Disagree, q3: Partially Agree, q4: Agree, q5: Strongly Agree.

Results and discussion

Reducing the adverse effects of climate change can only be possible through adaptation to these impacts. Despite farmers' awareness of climate change, it is noted that they may not recognize these changes as climate change itself. The awareness levels of sheep farming enterprises regarding climate change in the research area are presented in Table 1. According to the table, the total number of sheep farming enterprises that have heard about climate

change is 105, which represents 69.54% of the total enterprises. Furthermore, within the research area, 18.42% of the enterprises in the first group, 44.24% in the second group, and 36.84% in the third group interpreted climate change as sudden weather changes.

Table 1. Awareness of sheep farming enterprises regarding climate change.

Farmers' Definitions of Climate Change	Farm Size Groups								
	1-100		101-250		251 - +		Total		
	Number	%	Number	%	Number	%	Number	Total	%
Number of farmers who have heard about climate change	12.00	11.43	56.00	53.33	37.00	35.24	105.00	151	69.54
Changing seasons	5.00	8.06	32.00	51.61	25.00	40.32	62.00	151	41.06
Result of human activities	1.00	3.70	18.00	66.67	8.00	29.63	27.00	151	17.88
Extreme temperatures, drought, and global warming	9.00	12.86	36.00	51.43	25.00	35.71	70.00	151	46.36
Adverse effects of greenhouse gases	2.00	13.33	9.00	60.00	4.00	26.67	15.00	151	9.93

The number of enterprises that define climate change as changing seasons is 62, with 8.06% in the first group, 51.61% in the second group, and 40.32% in the third group. Among the 70 enterprises that define climate change as extreme temperatures, drought, and global warming, 12.86% are in the first group, 51.43% are in the second group, and 35.71% are in the third group. In the research area, while all farmers acknowledge the existence of climate change, some farmers express the presence of uncertainty due to the lack of conclusive evidence. The farmers' sources of information on climate change were calculated based on a five-point Likert scale average of 4.29 out of 5. This means that 68.50% of farmers receive information on climate change from visual media such as newspapers, television, and radio daily. The perceptions of farmers regarding climate change are crucial in the research area and are presented in Table 2.

Table 2. Perceptions of farmers regarding sensitivity to climate change

Factors	5	%	4	%	3	%	2	%	1	%	Avg.	Importance Index %
It is a serious problem on a global scale.	92	85.19	14	12.96	1	0.93	0	0.00	1	0.93	4.81	96.20
Humans have a significant role in climate change.	74	68.52	24	22.22	5	4.63	2	1.85	3	2.78	4.52	90.40
It will have negative consequences in the long term.	79	73.15	23	21.30	6	5.56	0	0.00	0	0.00	4.68	93.60

1=Strongly disagree, 2=Disagree, 3= Partially agree, 4=Agree, 5=Strongly agree.

According to the five-point Likert scale, the importance indices related to the overall perception of sheep farming enterprises about climate change range from 28.80% to 96.20%. In the first group, 96.20% of farmers express that climate change is a serious global problem, emphasizing the significant role of humans, the occurrence of more negative consequences in the long term, and its greater impact on agricultural enterprises. In a similar study conducted in Malaysia, farmers were asked about their attitudes toward climate change sensitivity, and when addressing the challenges of climate change, the positive attitudes of farmers were considered critical (Masud et al., 2017). The importance index was calculated as $62.5 < SI < 87.5$, with an importance index of 69.54% indicating that "Adaptation is necessary for

everyone." Furthermore, 69.02% of farmers expressed that they believe climate change is happening. Since our farmers are aware of climate change, it is necessary to work together with them on mitigating the impact of climate change and promoting adaptation measures.

The perceptions of farmers regarding the extent to which climate change affects agriculture and their importance index are given in Table 3.

Table 3. Farmers' perceptions and importance index on the degree of climate change impact on agriculture.

Factors	5	%	4	%	3	%	2	%	1	%	Avg.	Importance Index %
I acknowledge human-induced climate change.	79	73.15	20	18.52	4	3.70	2	1.85	3	2.78	4.57	91.40
Climate change will result in lower productivity on my farm due to diseases and pests.	82	75.93	21	19.44	3	2.78	0	0.00	2	1.85	4.68	93.60
Climate change will affect agriculture in Konya in the next 10 years.	86	79.63	17	15.74	2	1.85	2	1.85	1	0.93	4.71	94.20
Input costs have increased/will increase due to climate change.	91	84.26	12	11.11	4	3.70	0	0.00	1	0.93	4.78	95.60
Climate change is a significant global issue.	98	90.74	8	7.41	1	0.93	0	0.00	1	0.93	4.87	97.40

1=Strongly disagree, 2=Disagree, 3= Partially agree, 4=Agree, 5=Strongly agree.

After experiencing severe drought in agriculture between 2006-2009, farmers in the region have become more sensitive to climate change. We can see this sensitivity in Table 3. According to the table, 97.40% of farmers are aware that climate change is a significant global issue, and 95.60% believe that input costs will increase due to climate change. From these findings, it is evident that farmers are well aware of the significant impact of climate change on agriculture. In a study conducted specifically in the United States, Calil et al. (2012) estimated that the costs of energy, feed, and heat stress would increase by 2.7% under the most optimistic scenario, 8% under the moderate scenario, and 15% under the most adverse conditions by the year 2050. In Australia, due to climate change, a decline in milk production of approximately 5-15% across the country is expected on dairy farms by the year 2050 (Hanslow et al., 2014; Rojas-Downing et al., 2017).

Table 4 presents the perceptions of farmers in the region who are highly sensitive to the impact of climate change on agriculture regarding whether livestock is affected by climate change. The importance indices were calculated by attempting to measure farmers' perceptions of the extent to which livestock is affected by climate change in the research area. According to the findings, 91.60% of farmers believe that because of climate change, grain production will decrease, leading to much higher feed prices. Additionally, 91.40% believe that there will be an increase in heat stress on animals.

Table 4. Farmers' perception of the impact of climate change on livestock.

Factors	5	%	4	%	3	%	2	%	1	%	Avg.	Importance Index %
Livestock contributes negatively to climate change.	20	18.52	14	12.96	19	17.59	18	16.67	37	34.26	2.65	53.00
Water consumption by animals will increase	71	65.74	25	23.15	9	8.33	1	0.93	2	1.85	4.50	90.00

by 2-3 times due to climate change.												
There will be an increase in heat stress on animals due to climate change.	78	72.22	19	17.59	8	7.41	1	0.93	2	1.85	4.57	91.40
Feed prices will significantly rise due to a decrease in grain production caused by climate change.	79	73.15	19	17.59	6	5.56	2	1.85	2	1.85	4.58	91.60

1=Strongly disagree, 2=Disagree, 3= Partially agree, 4=Agree, 5=Strongly agree.

In the research area, the agricultural activity-related factors (both plant-based and animal-based) that farmers believe can contribute to climate change are provided in Table 5. As evident from the table, based on the importance index of farmers' perceptions regarding the plant-based and animal-based factors they believe can contribute to climate change, farmers emphasize that the factors predominantly originate from plant-based sources. The most significant factor, with a percentage of 96.20, is identified as crop residue burning caused by human activities.

Table 5. Farmers' perceptions on agricultural activity-related factors that impact climate change.

Agricultural activity-related factors	Factors	5	%	4	%	3	%	2	%	1	%	Avg.	Importance Index %
Animal-based	Methane gas produced by animal digestion	20	18,52	23	21,30	12	11,11	10	9,26	43	39,81	2,69	53.80
Animal-based	Long-term storage of fertilizer	26	24,07	13	12,04	22	20,37	8	7,41	39	36,11	2,81	56.20
Animal-based	Greenhouses	25	23,15	5	4,63	38	35,19	14	12,96	26	24,07	2,90	58.00
Plant-based	Fertilization practices	60	55,56	31	28,70	7	6,48	4	3,70	6	5,56	4,25	85.00
Plant-based	Agricultural waste	88	81,48	7	6,48	3	2,78	4	3,70	6	5,56	4,55	91.00
Plant-based	Crop residue burning	97	89,81	7	6,48	1	0,93	1	0,93	2	1,85	4,81	96.20

1=Strongly disagree, 2=Disagree, 3= Partially agree, 4=Agree, 5=Strongly agree.

Agricultural waste is perceived as a significant factor by 91%, followed by agricultural pesticide applications at 89.60%, excessive irrigation at 88.80%, and fertilizer applications at 85%. Greenhouse activities are considered influential by 58% of farmers. Among the animal-based factors, 56.20% of farmers believe that long-term storage of manure has an impact, while 53.80% attribute the production of methane gas from animal digestion as a contributing factor.

Conclusion

The research area is one of the driest provinces in Turkey and is widely used for sheep farming activities. Small ruminant husbandry holds significant importance, particularly in arid or semi-arid regions. Although farmers in the research area are aware of climate change, it is also a fact that they may not necessarily recognize these changes as climate change. Indeed, the percentage of those who have heard about climate change in the research area accounts for

69.54% of the total enterprises. Among them, 46.36% primarily define climate change as an increase in extreme temperatures and drought. It is crucial to provide farmers in sheep farming enterprises in the research area with informative training on climate change awareness and adaptation, aiming to develop suitable strategies for farmers' conditions. Both farmers and governments should address climate change adaptation measures within an institutional framework, effectively planning production activities.

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RESEARCH ON RECREATIONAL VALUE OF FOREST PARKS BASED ON CHOICE EXPERIMENT METHOD: A CASE STUDY OF WUYI MOUNTAIN IN CHINA

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Abstract

The growing recognition of the importance of forest resources in social and economic development has led to increased interest in studying their value, especially in regions like Fujian Province, China. Wuyi Mountain National Forest Park, as a key area, offers a relevant case study. However, the park has faced challenges due to rapid tourism development and competition with other famous forest parks. Evaluating the recreational value of such non-market goods has become crucial for policymaking. Our study focuses on assessing the main values of forest recreation, using Wuyi Mountain National Forest Park as the subject and involving college students in a questionnaire survey. Employing the choice experiment method, the research estimates tourists' willingness to pay for improving the park's environmental resources. The results highlight the positive impact of factors like forest coverage, infrastructure development, and visitor flow on the park's recreational economic value. Based on the findings, the study proposes practical strategies and recommendations for effective environmental management of the forest park. By considering ecological, societal, and managerial perspectives, the aim is to promote sustainable and responsible tourism while preserving the park's ecological integrity and recreational appeal. These insights can guide local governments and relevant authorities in developing optimal resource management strategies, contributing to a deeper understanding of the recreational value of forest parks and their role in socio-economic development.

Key words: *ecological civilization, forest resources, willingness to pay, environmental management.*

Introduction

The recreational value of forests and their significance in supporting human survival and development have gained increasing attention in light of environmental challenges like global warming and deforestation. Fujian Province, a key forest area in China, offers a relevant case study with its abundant forest resources and Wuyi Mountain National Forest Park, designated as one of the first national forest parks in 2017. However, the rapid growth of the tourism industry and competition with other renowned forest parks have led to a decline in Wuyi Mountain's tourist awareness.

Evaluating the value of non-market goods, such as public goods and quasi-public goods, is challenging due to their non-rivalry and non-exclusivity. The choice experiment method has emerged as a valuable tool to analyze and rank the relative importance of different environmental resource attributes based on consumers' willingness to pay. This study employs the choice experiment method to assess the environmental management of Wuyi Mountain National Forest Park and provide reference suggestions for administrative departments and resource development strategies.

Research on the valuation of environmental resources started earlier in foreign countries. Xu Songling (2002) pointed out that as early as the 1960s, environmental economists proposed the concept of non-market goods' value and developed a preliminary framework for calculating forest values. Subsequently, scholars continued to analyze individual cases of timber economic value in various regions. For example, Peters (2000) focused on non-timber forest products in the Amazon rainforest, while Adger (1991) and others conducted evaluation studies and policy recommendations for Mexican forests, yielding meaningful research results. In addition, countries have conducted extensive discussions on various social values of forest resources. Hanlev (2003) and others focused on the cultural, landscape, and aesthetic values of forest resources, while Tobias et al. and Maille et al. (1999) delved into the economic value of tropical rainforest recreational resources. Subsequently, in April 2013, during the 10th session of the United Nations Forum on Forests, the "Resolution" emphasized that member countries should attach importance to the contribution of forest products and services to the national and regional economy, fully recognize the impact of forest social, cultural, and environmental values on urban and rural communities, and incorporate various values of forest resources into the national economic accounting system.

Compared to foreign countries, domestic scholars started researching the valuation of environmental resources relatively late, and there is a relative lack of literature employing the choice experiment method for quantitative analysis. Liu Lili et al. (2010) reviewed the development theory and methods of the choice experiment method, specifically comparing literature on the evaluation of forest resource values domestically and internationally. Based on the calculation characteristics of various evaluation methods, they summarized the three commonly used methods in the practical application of forest resource value evaluation: direct market valuation, substitute market valuation, and hypothetical market valuation. Zhang Shengling and Zhou Yexin (2012) analyzed the application of experimental economics in resource and environmental issues and reviewed the latest research progress on social dilemmas and cooperative behavior, pointing out that further research is needed in policy evaluation and experimental studies in overlapping areas.

Compared to relevant research literature both domestically and internationally, the current research on the influencing factors of the economic valuation of non-market resources is relatively narrow. The economic value of forest recreation not only depends on natural factors but also on social factors, management factors, cost factors, and other socio-economic factors. There are few studies that comprehensively evaluate the value of forest environmental resources using multiple factors. This study takes utility theory as the modeling foundation, uses Wuyi Mountain National Forest Park as an example, recruits college students as subjects through the choice experiment method, and combines multiple economic, social, and natural attributes of the park's environmental resources. It establishes conditional logit models and random parameter logit models to estimate tourists' marginal willingness to pay for improving the environmental resources of Wuyi Mountain National Forest Park. The study aims to provide decision-making references and guidance for local governments in the scientific conservation and rational development of forest resources.

Materials and methods

In recent years, experimental economics has been increasingly applied in social sciences, particularly in the study of resources and the environment. Its research characteristics are based on Lancaster's consumer choice theory and random utility theory. Participants evaluate and assess the value of different attributes of resources based on stated preferences. The experimental process requires participants to make choices regarding environmental value attributes for various alternative scenarios provided in virtual settings. Econometric models

are then applied to analyze the marginal willingness to pay for different resource attributes of forest products, thus evaluating the recreational economic value of various alternative scenarios grouped by different attributes and levels.

Choice Experiment Design

In the process of designing the choice experiment, the researchers first divided the attributes with different levels into different groups of alternatives. Then, they paired each alternative to form corresponding choice sets. Based on the previous literature review and pre-surveys on the preferences of college students towards different attributes of forest parks, the researchers finally selected the following four elemental attributes as the main indicators influencing college students' perceived environmental value of forest parks. The explanations of each attribute are as follows:

1. Vegetation Coverage: The higher the vegetation coverage, the greater the ecological and recreational value provided by the vegetation, leading to lower visitor travel time and expenses.
2. Biodiversity: Biodiversity plays a crucial role in maintaining ecological balance, and the higher the biodiversity, the greater the aesthetic value it provides.
3. Infrastructure and Specific Facilities: The more complete the public infrastructure and facilities related to safety and wellness in the scenic area, the higher the ecological and recreational functional value it can provide.
4. Park Visitor Flow: The park visitor flow reflects the park's capacity to attract tourists. The higher the visitor flow, the greater the potential recreational and economic value it can offer.

During the experiment, the four elemental attributes were presented to the participating students through two videos. The first video was taken from the Chinese government's official website, specifically from the promotional video of Wuyi Mountain National Forest Park. The second video was sourced from a travel video created by a Bilibili user, showcasing their journey in Wuyi Mountain National Forest Park. These two videos provided different perspectives, one from the official promotional angle and the other from a traveler's personal experience, showing the ecological and socio-economic environment of Wuyi Mountain National Forest Park. After watching the videos, the participating students completed a questionnaire survey.

Considering the feasibility of the choice experiment, the core factors that influence the four attributes were selected as their status levels. For each attribute, the "maintain the current status" level was chosen as the baseline. The status levels for vegetation coverage were set as "increase the current vegetation coverage area by 5% and 10%". For biodiversity, the status levels were "increase the current animal and plant species by 5% and 10%". For infrastructure and specific facilities, the status level was "increase the current facilities by 5%". And for park visitor flow, the status levels were "increase the current visitor flow by 5% and 10%". In terms of visitors' willingness to pay, monetary payment was used, and the status levels were set as "paying 0-50 yuan, 50-100 yuan, 100-150 yuan, 150-200 yuan" annually, with tax payment as the alternative method.

According to the number of attributes and levels in the research design, theoretically, the four choice experiments can be combined into 324 different alternatives ($3\times3\times3\times3\times4$). However, to make it more convenient for the participants to choose, besides providing sufficient and effective information in the experiment, this study specifically used a fractional factorial design to screen 12 options. Then, based on the D-efficiency principle, the alternatives were combined and paired in choice sets. Additionally, in each choice set, an opt-out option (Option 4) was included and used as a baseline to avoid forced choices. An example of a choice set is shown in Table 1. Ultimately, each choice experiment group was designed with four choice sets, and participants were required to choose their most preferred option in each set.

Table 1. Choice Set

	Vegetation Coverage	**Biodiversity**	**Infrastructure and Specific Facilities**	**Park Visitor Flow**	**Willingness to Pay**
Option 1	increase by 10%	increase by 5%	increase by 5%	increase by 10%	100-150RMB
Option 2	increase by 5%	maintain the current status	increase by 10%	maintain the current status	150-200RMB
Option 3	maintain the current status	increase by 10%	maintain the current status	increase by 5%	0-50RMB
Option 4	The three options are not selected				

The questionnaire survey was conducted in December 2021. The participants were college students, and the survey was carried out online through the distribution of questionnaires. As a 10 yuan compensation was provided to the participants, the questionnaire response rate was high, and the quality of responses was also good. A total of 44 questionnaires were distributed, and 44 valid observations were collected, resulting in a total of 528 valid observations.

Results and discussion

Multinomial Logit Model and Conditional Logit Model The Multinomial Logit model forms the basis of discrete choice models and can provide a robust and optimized model for explaining choices and determining factor levels in cases with multiple alternatives. It is suitable for scenarios with multiple available options and can be considered as a simultaneous estimation of multiple binary Logit models for each pair of choice behaviors in the dependent variable (Simultaneously estimation). However, the model itself has inherent theoretical limitations, such as the assumption of independence of irrelevant alternatives (IIA), which makes it easier to estimate and widely used for statistical purposes. In this study, the purpose is to explore the influence of environmental attribute levels, participants' socioeconomic characteristics, and willingness to pay on the environmental value. "Choice Group" is taken as the dependent variable, and living expenses, forest coverage, biodiversity, infrastructure development, park visitor flow, and willingness to pay for ticket prices are taken as explanatory variables. The regression results of the model are shown in Table 2, it can be seen that the regression coefficients of both the Multinomial Logit model and the Conditional Logit model are significant at the 10% level.

Table 2. Results of Multiple Logit Regression & Conditional Logit model

Variables	Multinomial Logit model		Conditional Logit model	
	Coeffcient	Standard error	Coeffcient	Standard error
Living expenses	-2.975*	-1.666	0.147	-0.711
Vegetation Coverage	2.637	-1.914	-0.477	-0.966
Biodiversity	-3.338*	-1.962	-0.841	-0.933
Infrastructure and Specific Facilities	3.234*	-1.96	1.198	-0.902
Park Visitor Flow	0.382	-1.15	-0.897	-0.692
Willingness to Pay	-0.14	-1.176	-1.562*	-0.837
Log-likelihood	-38.155		-14.576	
Pseudo-R^2	0.3745		0.3296	
N	44		44	
Standard errors in parentheses				
*** p<0.01, ** p<0.05, * p<0.1				
Resource: Survey data				

Based on the calculation method used by Wang Xigang in his study of Fujiazhuang Coastal Park, this study utilized effective survey data to calculate the odds ratios of various attribute levels in Wuyi Mountain National Forest Park. From the table, it can be observed that the odds ratio for the variable "living expenses" is 1.159. This means that, with all other conditions unchanged, an increase of one level in total living expenses will lead to a 17% increase in the probability of choosing this forest park for travel. The variable "infrastructure development" has the greatest impact on option selection, indicating that respondents are most sensitive to changes in the level of service facilities at the forest park. A decrease in park visitor flow and forest coverage will reduce the probability of being chosen by respondents, while an increase in biodiversity will increase the probability of being chosen. Furthermore, for every 50 yuan increase in ticket prices, the probability of respondents choosing the option will decrease by 33%, highlighting that visitors are very sensitive to price changes when making choices, which is consistent with economic principles.

According to the calculations, the average per capita daily recreational economic value of the sample college student population is 2.63 yuan in the current state, while the optimal average per capita daily recreational economic value is 11.54 yuan. This indicates that there is still a significant room for improvement in the recreational economic value of Wuyi Mountain National Forest Park in the current sample area.

Conclusion

Overall, visitors tend to prioritize management factors such as infrastructure development over natural environmental factors. Moreover, preferences for willingness to pay for ticket prices and park visitor flow are significantly higher than those for forest coverage and biodiversity. Therefore, in comparison with various park resources, the impact of willingness to pay for ticket prices is the greatest, followed by park visitor flow and infrastructure development, and finally, forest coverage and biodiversity. These empirical findings are consistent with previous assumptions in the literature. Therefore, managers should take appropriate measures to enhance the level of park infrastructure, increase efforts in forest resource conservation, and also implement reasonable visitor flow management and ticket price planning to enhance the recreational economic value of Wuyi Mountain National Forest Park.

In conclusion, the recreational economic value of Wuyi Mountain National Forest Park can be significantly enhanced through the implementation of precise strategies for key factors such as forest coverage, infrastructure development, and park visitor flow. Diversifying the recreational value of quality forestry resources, enhancing biodiversity protection, and continuously exploring natural and scenic landscapes are vital steps in creating a positive and healthy tourism environment that attracts more visitors.

Social and management factors play a crucial role in shaping the recreational value of forest parks. To ensure sustainable development, it is essential to promote the concept of ecological civilization and implement effective supervision during park development. Prohibiting extreme development practices that prioritize short-term benefits over environmental conservation is vital for the long-term success of the park. Encouraging civilized and responsible forest tourism practices among visitors will create a healthy and environmentally friendly atmosphere within the park.

Comprehensive infrastructure development is key to providing humanized services to park visitors. By formulating comprehensive plans for the park and its surrounding tourism, improving transportation networks, and ensuring fully equipped public service facilities, the overall service level of the park can be enhanced.

Furthermore, successful marketing and promotion are crucial for expanding the influence of Wuyi Mountain as a forest tourism destination. Creating a new and distinctive label for Wuyi Mountain tourism will attract a broader audience and increase tourist satisfaction with the forest tourism and recreational experience.

By embracing these strategies, Wuyi Mountain National Forest Park can reclaim its leading position in the realm of forest tourism, providing not only material support but also spiritual nourishment for visitors. The careful balance of nature, society, and management aspects will contribute to the sustainable development of this precious natural resource, ensuring that it continues to enrich the lives of both current and future generations.

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PREDICTIVE CLASSIFICATION OF AQUACULTURE FISH MORTALITY USING DATA MINING CLASSIFIERS

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Abstract

A data-driven approach utilizing data mining classifiers has been proposed to predict and classify instances of fish mortality, addressing the critical issue of increased mortality rates in fish populations, due to unsustainable fish farming practices and environmental factors. The goal of the project is to improve the competitiveness of Greek farming fish through the individual development of an intelligent system for the diagnosis of fish diseases in fish farms with a simultaneous response to the issues of medication and dosage referred to the project "*Improving Competitiveness of the Greek Fish Farming through Development of Intelligent Systems for Disease Diagnosis & Treatment Proposal and Relevant Risk Management Supporting Actions*", funded by the Operational Program for Fisheries & Maritime 2014–2020, grant number (MIS) 5067321. A comprehensive dataset from diverse aquaculture, including geographical locations and husbandry methods, was leveraged, encompassing indicative factors (i.e., water quality parameters, weather conditions, and biological characteristics of fish). The primary objective of this research was to classify fish mortality instances using predictive models. State-of-the-art data mining classifiers, including decision trees were employed, allowing for comparison to identify the most suitable method with high precision and recall rates for predicting fish mortality. To ensure result reliability, a rigorous evaluation process involving k-fold cross-validation and performance metrics like accuracy and precision was adopted. Additionally, feature importance analysis was performed to gain insights into the factors influencing fish mortality, aiding the development of targeted conservation and management strategies. The research findings have far-reaching implications for sustainable management efforts, enabling stakeholders to take proactive measures and monitoring aquaculture practices to protect farmed fish stocks while meeting global seafood demands. The data mining classification approach presented contributes to the broader UN sustainability goals by mitigating seafood production losses due to climate change.

Keywords: *Data mining classifiers, Fish mortality prediction, Sustainable aquaculture management, UN sustainability growth indicators, Environmental factors.*

Introduction

Fish mortality is a significant concern in both wild fisheries and aquaculture. Factors such as overfishing, pollution, habitat destruction, and climate change contribute to increased mortality rates. Overfishing has led to the depletion of many fish stocks, threatening the sustainability of global fisheries (FAO, 2020; Pauly *et al*., 2002). Pollution from industrial and agricultural activities has degraded water quality, affecting fish health and survival (Cheung *et al*., 2009).

Unsustainable fish farming practices, including overcrowding, poor water quality, and excessive use of antibiotics, can lead to disease outbreaks and high mortality rates. The rapid

growth of aquaculture has sometimes been accompanied by environmental degradation and the spread of diseases, leading to significant fish losses (Naylor *et al*., 2000; Stentiford *et al*., 2012).

This section provides an overview of a specific project aimed at improving Greek fish farming through innovative technologies and sustainable practices. The project focuses on developing intelligent systems for disease diagnosis and treatment, enhancing the competitiveness of Greek aquaculture (Klinger & Naylor, 2012).

Details about the grant and funding, including the Operational Program for Fisheries & Maritime 2014–2020 and grant number (MIS) 5067321, are outlined here. The funding supports research and development initiatives to promote sustainable fisheries and aquaculture in Greece. Project Overview: Improving Greek Fish Farming Competitiveness. This project aims to enhance Greek fish farming by developing an intelligent system for diagnosing fish diseases, considering factors such as medication, food, temperature, and volume. The initiative is part of a broader effort to improve the competitiveness of Greek fish farming through technological innovation and sustainable practices (www.fishai.upatras.gr).

Materials and methods

The research leveraged a comprehensive dataset, including geographical locations, husbandry methods, and other factors such as water quality and weather conditions. This dataset encompasses diverse aquaculture practices, providing a rich source of information for analysis (Bostock *et al*., 2010; Tacon & Metian, 2008). The data were collected in fisheries in Ionian Sea in Greece. The data contains one nominal and four numerical factors. Any rows containing at least one missing value were excluded from the analysis. After excluding rows with any missing values, the dataset consists of 37,203 complete rows. The factors were analyzed to understand their influence on fish mortality. Water quality parameters, weather conditions, and biological characteristics of fish were considered as indicative factors influencing mortality rates (Table 1) (Boyd & Tucker, 1998).

Table 1. Summary statistics for the "Deaths" variable

Statistic	Value
Count	37,203
Mean	-44.34
Standard Deviation	83.75
Minimum	-995.00
Maximum	-1.00
Count	37,203 non-missing values

Referring to the dataset it contains information about fish with variables such as the median atomic weight of the fish (MAB), the volume of the cell occupying the fish (Vol), the concentration of fish inside the cell (i-f), the temperature of the water (Temp), and the number of "Deaths". In order to predict the potential correlation between deaths and each one of the other variables the Pearson correlation coefficient was used to measure the linear relationship for each pair of continuous variables (Table 4) (Matplotlib, 2023; NumPy, 2023; Pandas, 2023; Scikit-learn, 2023; Seaborn, 2023). The "Deaths" variable ranges of values are converted into discrete classes, allowing classification algorithms to apply. This process is referred to as binning/discretization Table 2).

Table 2. Binned "Deaths" classes

Statistic	Value
Unique Bins/Classes	5
Most Frequent Bin	(−199.8−1.0 (35,536 occurrences)

Table 3. Equal width binning strategy for the "Deaths" variable

Range	Instances
Class 1 (−995−796]	74
Class 2 (−796,−597]	139
Class 3 (−597,−398]	301
Class 4 (−398,−199]	1153
Class 5 (−199,−1.0]	35,536

Following, a series of data mining classifiers was used allowing for comparison to identify the most suitable method with high precision and recall rates for predicting fish mortality. Data mining represents a method for extracting valuable insights by employing a collection of analytical tools and algorithms. These tools enable the uncovering of important correlations that can significantly inform decision-making and predictive processes. The technique frequently utilized in data mining is Decision Trees. The application of data mining fulfills the objective of unearthing novel knowledge, forging new connections and correlations, and unveiling intriguing patterns (Karim & Rahman, 2013; Kohavi, 1995).

Optimizing the binning strategy to achieve maximum classification accuracy requires understanding the underlying patterns and relationships between the "deaths" variable and other features. One common approach to this task is to explore different binning strategies and evaluate their impact on classification performance using cross-validation or other model validation techniques. For this experiment Equal Width Binning was applied to the "deaths" variable. It splits the data into training, validation, and testing sets (70%, 20%, 10%), trains a simple model (e.g., Decision Trees) on the training set for each binned target, it evaluates the model on the validation set, it applies it to the entire dataset on the training set. A decision tree, a commonly used tool in data mining, functions as a classification mechanism. It interprets data, differentiates classes, and assigns values to each class, often following an 'if-then-else' sequence. Datasets consist of attributes, each possessing properties and multiple instances, and these attributes may be either numeric or nominal. Within a decision tree, nodes symbolize the dataset's attributes, while branches signify the values of those attributes. The top node acts as a super-class, and the leaf nodes serve as sub-classes. The process involves dividing examples into training, validation, and testing sets. After training the algorithm with the largest set of examples (the training set), a hypothesis is generated, and the percentage of correctly classified examples in the validation sets is computed. This procedure continues as the size of the training set changes. The testing set then validates the outcome with entirely new data. A challenge in this process is overfitting, which occurs when the tree's training reaches a point where it must guess values due to a lack of data, or when deep noise affects the classification. To mitigate this, tree pruning techniques are employed. In the referenced research, to gauge the success rate of the data mining method, the average success rate of the ten experiments was calculated. The model's training and evaluation involved a 10-fold cross-validation approach. A pruned Decision Tree Classifier with a maximum depth of 3 was used to prevent overfitting, maintaining the model's simplicity and interpretability. The 10-fold cross-validation provided a robust assessment of the model's performance, yielding a mean accuracy of 95.43% (Guyon & Elisseeff, 2003; Kohavi, 1995; Quinlan, 1986).

Results and discussion

According to the correlations results with "Deaths", MAB (Median Atomic Weight of the Fish): Correlation = 0.1638. This suggests a weak positive relationship between the median atomic weight of the fish and the number of deaths. A higher median atomic weight may be associated with an increased number of deaths, although the correlation is weaker in the

binned data. Vol (Volume of the Cell Occupying the Fish): Correlation = 0.1331. A weak positive correlation here indicates that a larger cell volume may lead to more deaths. This could imply that fish in larger cells are more prone to mortality, possibly due to environmental factors within the cell. However, the relationship is not strong. i-f (Concentration of Fish Inside the Cell): Correlation = 0.1449. The weak positive correlation implies that higher concentrations of fish inside the cell could lead to more deaths. This might be related to competition, stress, or other factors affecting fish health, but the correlation is not strong in the binned data. Temp (Temperature of the Water): Correlation = -0.0305. The negligible negative correlation with temperature suggests that water temperature has little to no direct effect on the number of deaths (Table 4). (Matplotlib, 2023; Scikit-learn, 2023; Seabornl; 2023).

Table 4. Binned correlations of factors using the discretized "deaths" classes

Feature	Binned Correlations to “Deaths”
MAB	0.1638
temp	-0.0305
vol	0.1331
i-f	0.1449

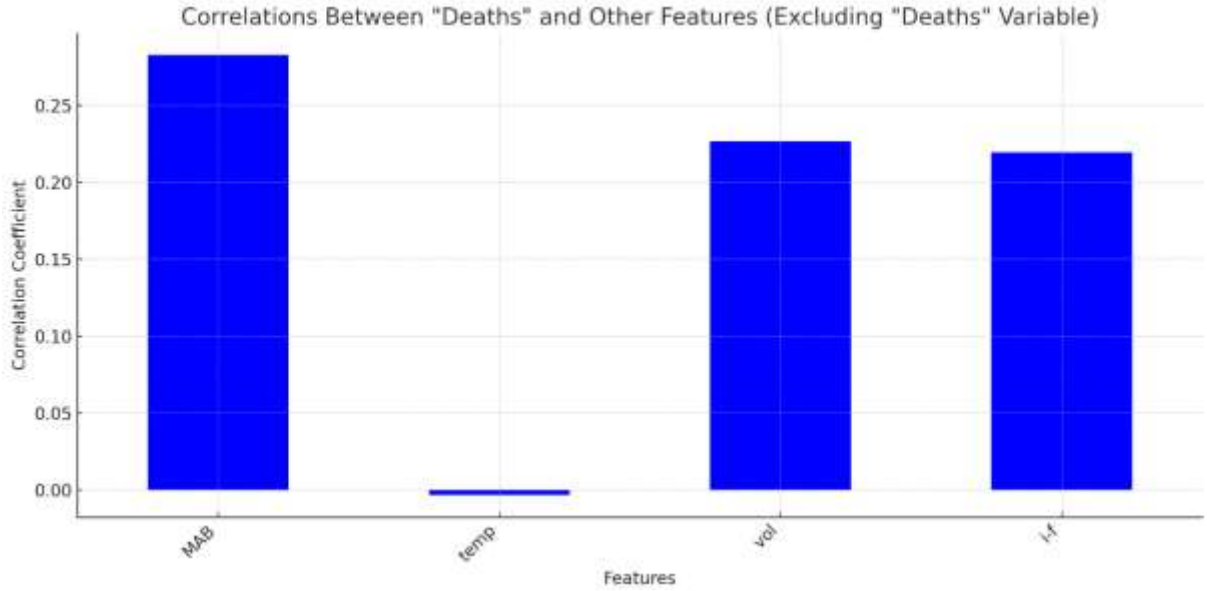

Figure 1. Correlations between the target variable "deaths" and the other features in the dataset.

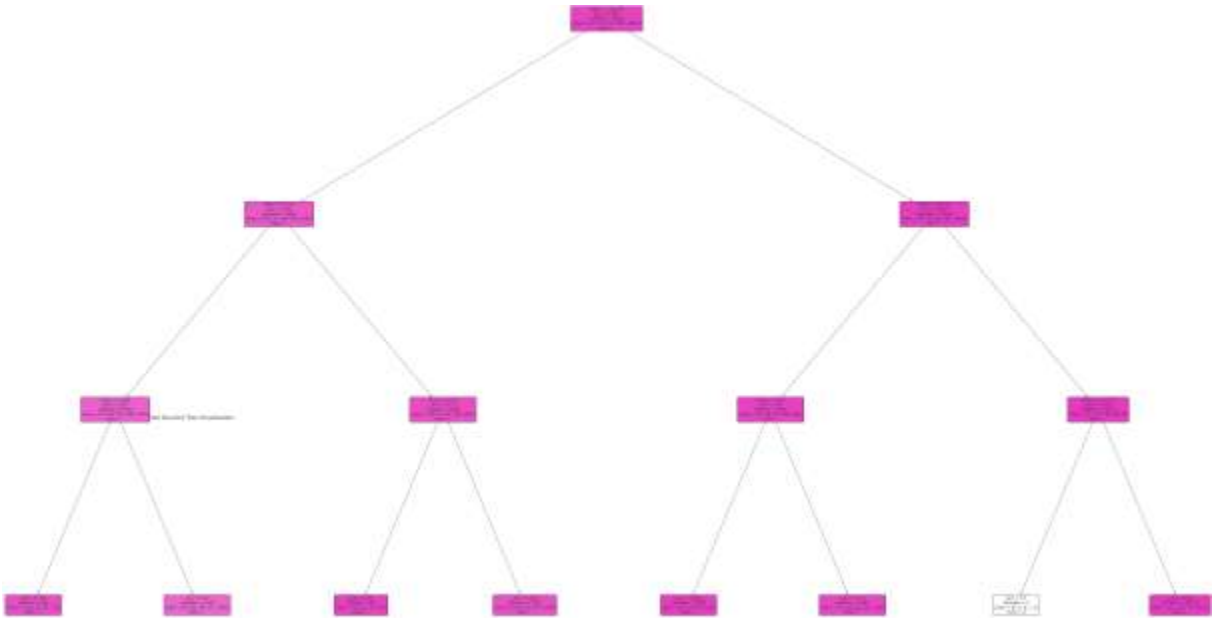

Figure 2. Decision Tree representation for “Deaths” prediction.

Based on the decision tree structure presented above, when MAB is less than or equal to 111.85, it leads to different branches of the tree, mainly resulting in classes 3 and 4. When MAB is greater than 111.85, most outcomes are classified as class 4, with occasional occurrences of class 3. Temperature plays a significant role, with different thresholds like 16.90, 12.65, 17.90, and 18.90 leading to various branches and classifications. For example, when MAB is less than 25.55 and the temperature is less than 16.90, different temperature thresholds further classify the outcomes into classes 3 and 4. Volume thresholds such as 2155.00 and 1160.50 appear in the tree, helping to classify outcomes when combined with other variables like MAB and temperature. The concentration of fish inside the cell also influences the outcomes, with thresholds like 2.61, 0.20, and 1.29. In combination with MAB and temperature, this variable further refines the classification into specific classes. The Decision Tree classifier was trained on a dataset after applying preprocessing steps such as handling missing values and discretizing the "deaths" variable into five equal-width bins. The model was pruned with a maximum depth of 5 to avoid overfitting, and 10-fold cross-validation was used to assess the model's performance. The results demonstrate strong consistency across the training, validation, and testing sets, with accuracies of 95.47%, 95.43%, and 96.26%, respectively. The visualization of the pruned Decision Tree provides insight into the decision-making process used by the model to classify the data based on the given features. The model's high accuracy and generalization performance indicate its potential effectiveness in predicting the discretized "deaths" variable, based on the selected features in the dataset. Predictive models were developed to classify fish mortality instances, considering various factors. These models utilized state-of-the-art data mining classifiers, including decision trees, to identify the most suitable method for prediction (Hastie *et al*., 2009). The models achieved high precision and recall rates, indicating their effectiveness in predicting fish mortality. The success of these models demonstrates the potential of data-driven approaches in fisheries management (Powers, 2011). Feature importance analysis was performed to gain insights into the factors influencing fish mortality. This analysis aided the development of targeted conservation and management strategies, providing valuable insights for stakeholders (Kohavi, 1995). Based on the analysis, targeted conservation and management strategies were developed to address the identified issues. These strategies aim to reduce fish mortality through proactive measures, informed by data-driven insights (Garcia *et al*., 2014). The research findings have far-reaching implications for sustainable

management efforts, enabling stakeholders to take proactive measures. The insights gained from this research contribute to broader efforts to protect fish stocks and promote sustainable fisheries (FAO, 2016; Worm *et al*., 2009). The study emphasizes the need for proactive measures and monitoring in aquaculture to protect farmed fish stocks. Implementing best practices and continuous monitoring can enhance the sustainability of aquaculture operations. The research contributes to the broader UN sustainability goals by mitigating seafood production losses due to climate change. By addressing fish mortality and promoting sustainable practices, the project aligns with global efforts to ensure responsible consumption and production (United Nations, 2015; IPCC, 2018).

Conclusion

Future Directions and Global Seafood Demand Consideration. The conclusion summarizes the key findings and discusses future directions and considerations related to global seafood demand. The research highlights the importance of data-driven approaches in addressing complex challenges in fisheries and aquaculture, paving the way for future innovations and sustainable growth (FAO, 2018). The project offers a promising direction for improving fish farming practices, considering various factors that influence fish mortality. Continued research and collaboration are essential to meet global seafood demands sustainably. The lessons learned from this study provide a valuable foundation for future efforts, guiding the development of innovative solutions and evidence-based strategies to enhance the resilience and sustainability of the aquaculture industry. In the face of mounting challenges in fisheries and aquaculture, this research underscores the pivotal role of data-driven methodologies in predicting and managing fish mortality. By employing advanced data mining classifiers, including decision trees, the study has not only demonstrated the potential effectiveness of such models in the realm of aquaculture but has also set a benchmark for future endeavors in the field. The findings from this study are not just academic achievements; they resonate with the broader objective of sustainable fisheries and aquaculture management. As the global demand for seafood continues to grow, so does the pressure on fisheries and aquaculture systems to meet this demand without compromising on sustainability. The research provides a beacon for such efforts, emphasizing the importance of technological interventions, rigorous data analysis, and informed decision-making. This project, with its emphasis on data-driven strategies, provides a robust foundation for such future endeavors, heralding a new era of sustainable growth in the aquaculture industry.

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A MONTHLY STUDY OF INTERCEPTED PHOTOSYNTHETICALLY ACTIVE RADIATION (IPAR) FOR VINEYARDS IN CASTILLA Y LEÓN

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Abstract

Agriculture is of outstanding economic, environmental, and social importance in the Autonomous Region of Castilla y León (Spain). Within the variety of crops grown in the region, vineyards are internationally recognized for the high quality of their wine production. In 2022, the area destined for grapevines was almost 65.000 ha. Most of them are included in a Denomination of Origin (D.O) established for wines, making up the nine that are located in Castilla y León. The Denomination of Origin is a system that recognizes a product's distinctive quality. Climatic conditions strongly determine the production and quality of the grapes and, consequently, of the wine. Intercepted Photosynthetically Active Radiation ($IPAR$) is one of the least known but one of the most influential variables on vegetative development. $IPAR$ is estimated with the Beer-Lambert Law for all vineyard crops in Castilla y León. For its calculation, it is necessary to consider the Photosynthetically Active Radiation (PAR), the light extinction coefficient (k), and the Leaf Area Index (LAI). Then, monthly $IPAR$ values of vineyards are calculated and spatially represented with Geographical Information Systems (GIS) as a monitoring method of the different seasonal growth variations of grapevines. The maximum values of $IPAR$ are reached in July and from October onwards these values decrease considerably because the grape harvest begins in this month. Knowledge of the spatio-temporal distribution of $IPAR$ provides valuable information about a wine-growing region and could optimize the use of the resources employed in wine production.

Keywords: *Denomination of Origin, Intercepted Photosynthetically Active Radiation, Leaf Area Index, vineyards.*

Introduction

Viticulture is an important economic activity in the Castilla y León region (Spain), the role of this activity also has a significant impact on the territory as it is helping to keep active many areas that were becoming depopulated. In 2022, the vineyard area was 64,972 ha (ITACyL, 2022), which represents almost 7% of the total area destined to this crop in Spain (OIV, 2022).

Some of the viticultural regions of Castilla y León are listed as Denominations of Origin (D.O). The D.O is the Spanish system to recognise a product originating from a specific place. These products have a quality and characteristics that are exclusive to that specific geographical location and its environmental and social factors. In addition, the production process must take place entirely in the defined geographical area (Vélez *et al.*, 2021a). In Castilla y León there are nine D.Os: Arlanza, Arribes, Bierzo, Cigales, Ribera del Duero, Rueda, Tierras de León, Tierra del Vino de Zamora y Toro (Figure 1).

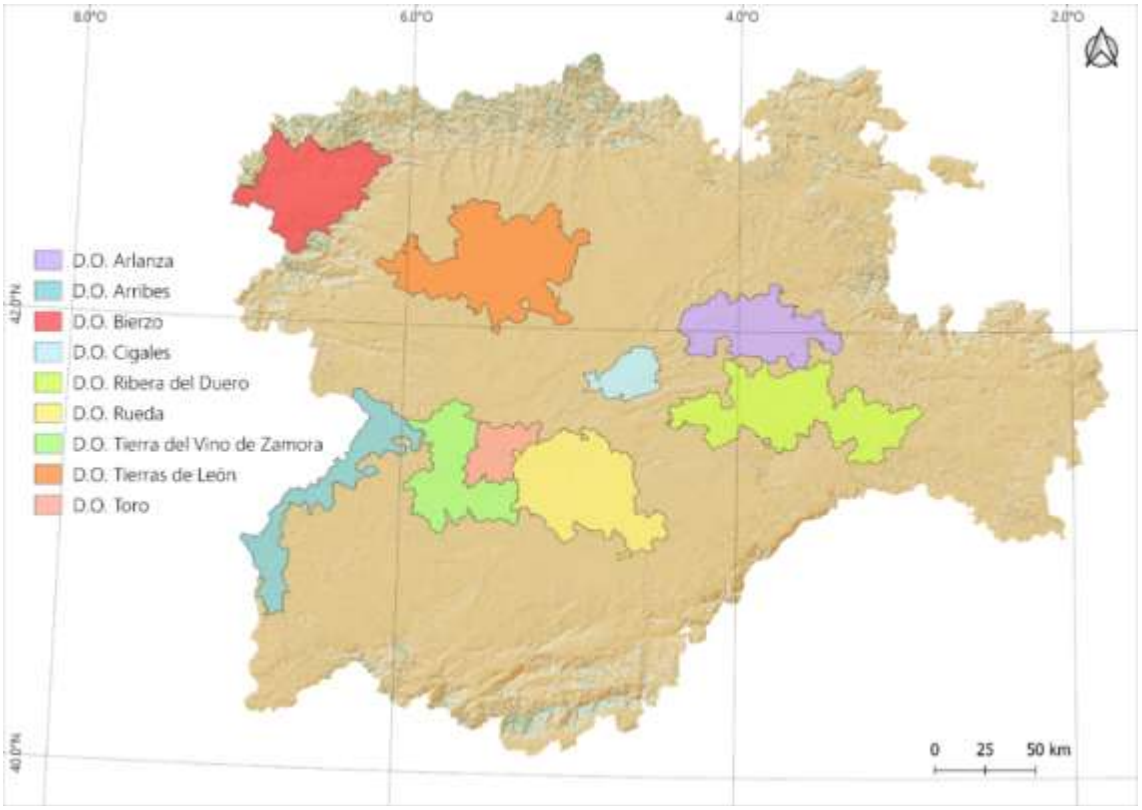

Figure 1. Denominations of Origin (D.O) in Castilla y León.

Vineyards are characterised by a strong spatial structure stable over time (Vélez *et al.*, 2021b) and are affected by several climatic factors such as air temperature, solar radiation and precipitation. Grapevines grown in semi-arid regions, as most of the D.Os of Castilla y León (Beck *et al.*, 2018), are often exposed to high temperatures and solar radiation during summer. Photosynthetically Active Radiation (PAR) is the component of solar radiation that is directly involved in photosynthesis and has a strong influence on vine growth and, thus, on wine quality and production.

Intercepted Photosynthetically Active Radiation ($IPAR$) is usually estimated from an approach of Beer's Law (Monsi and Saeki, 2005) (Eq. 1), which, in addition to PAR, also requires the light extinction coefficient (k) and the Leaf Area Index (LAI):

$$IPAR = PAR \cdot (1 - e^{-k \cdot LAI}) \quad (1)$$

The light extinction coefficient (k) is a parameter that describes the efficiency of light interception in canopies. It varies among vegetation types, canopy structures and styles (Ilniyaz *et al.*, 2022).

The Leaf Area Index (LAI) is the ratio of one-sided leaf area per unit ground area (Ilniyaz *et al.*, 2022). It can be estimated by direct (harvested leaves) or indirect methods (commercial devices or remote sensing data).

This study aims to calculate and represent the monthly mean $IPAR$ values of the vineyards during their growing season. In this way, it is possible to compare the values obtained in each of the D.Os of Castilla y León.

Materials and methods

Castilla y León covers an area of 94,000 Km^2, which makes it the largest region of the Iberian Peninsula. Its orography is very distinctive, as it has three mountain systems (the Cantabrian Mountains to the North, the Central System to the South, and the Iberian System to the East) that surround a wide central plateau.

The "Mapa de Superficies Naturales de Castilla y León 2022" (MSNCyL), published annually by the "Instituto Tecnológico Agrario de Castilla y León" (ITACyL, 2022), was used to determine the area destined to vineyards.

Fourteen years of Global Horizontal Irradiance (GHI) data were collected at 93 agroclimatic stations provided by several meteorological networks. The initial data were analysed and filtered to obtain the monthly average of the daily GHI (MJ/m²). These values were interpolated using the Universal Kriging method and graphically represented with QGIS 3.16 software. As PAR databases are spatially and temporally limited, it was calculated by establishing a constant ratio of 0.48 to GHI data (Harbo *et al.,* 2022). The light extinction coefficient (k) was set by default to 0.7, as this value has been described to be the most accurate for grapevine canopies (De Bei *et al.,* 2016; Pichon *et al.,* 2020). LAI data were obtained from satellite imagery provided by the MODIS/Terra + Aqua Leaf Area Index/FPAR 4-Day L4 Global 500 m SIN Grid (MCD15A3H) (NASA) (Myneni *et al.,* 2015).

Finally, monthly $IPAR$ maps in a pixel size of 10 meters were created applying the Beer-Lambert Law for vineyards in Castilla y León (Eq. 1).

Results and discussion

Monthly mean $IPAR$ values were estimated for each month of the growing season (April-October in the Northern Hemisphere) (Fraga *et al.,* 2014). The maximum monthly mean $IPAR$ values are shown in Table 1.

Table 1. Maximum $IPAR$ (MJ/m^2) values for each D.O during the growing season.

	April	**May**	**June**	**July**	**August**	**September**	**October**
Arlanza	6.80	8.85	6	6.23	5.20	3.88	1.23
Arribes	7.02	7.48	7.47	7.57	6.48	4.26	1.68
Bierzo	7.89	9.71	10.61	11.20	9.83	7.67	3.32
Cigales	7.39	8.90	4.89	5.42	4.39	2.92	0.82
Ribera del Duero	7.92	9.85	10.85	11.53	10.24	7.92	3.42
Rueda	7.50	8.31	6.44	8.05	6.43	4.43	1.45
Tierras de León	6.48	7.66	9.75	11.74	9.86	4.65	1.37
Tierra del Vino de Zamora	5.94	6.27	7.08	7.22	6.46	3.57	2.48
Toro	6.20	7.11	6.45	7.74	5.94	4.83	2.50

It shows that most of the D.Os reach the maximum monthly mean $IPAR$ values in July, except Arlanza, Cigales and Rueda, which register them in May. Ribera del Duero registered the highest values during the entire growing season, followed by Bierzo. Monthly mean $IPAR$ values of September are represented in figure 2.

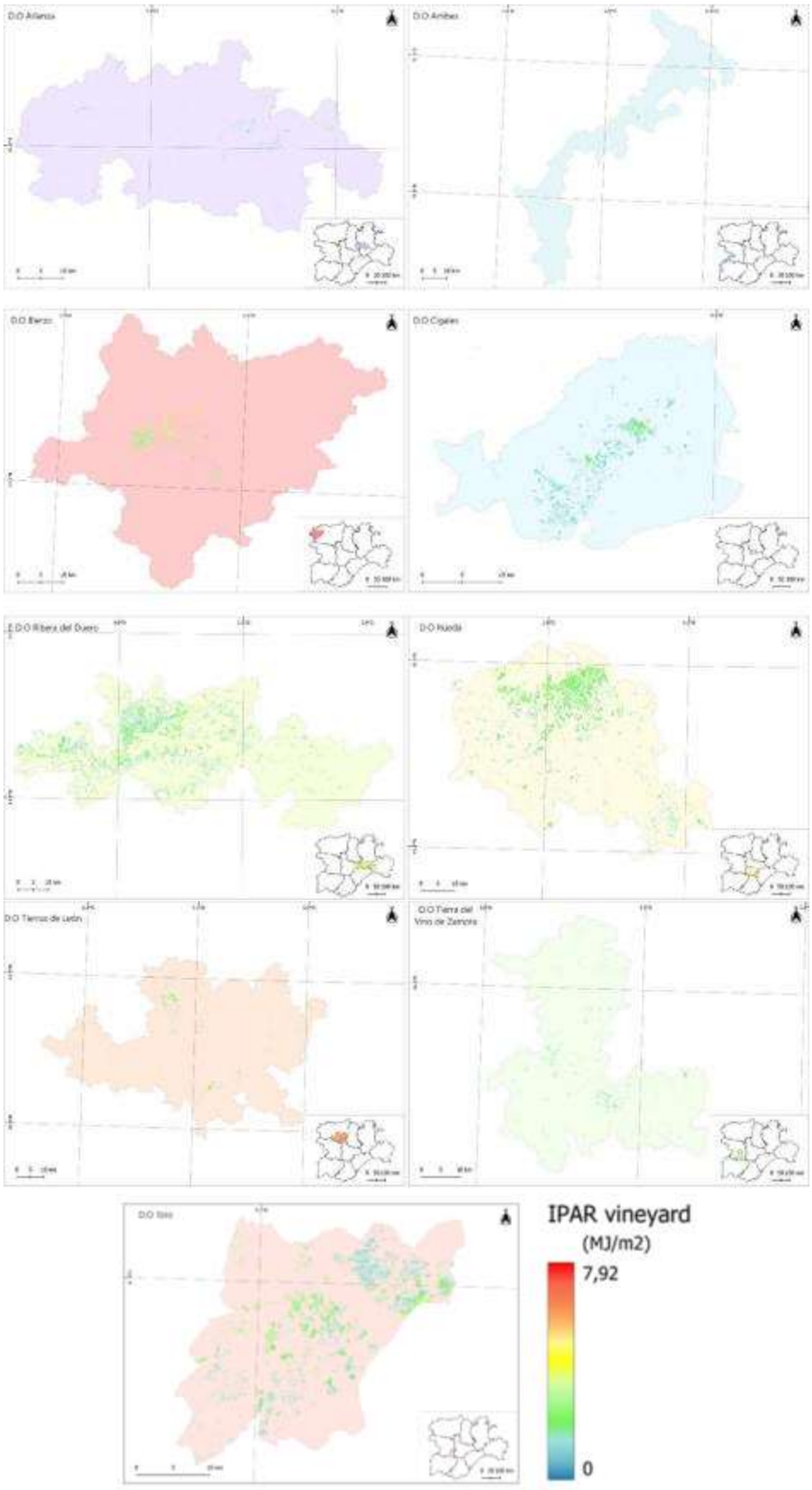

Figure 2. Monthly mean *IPAR* values (MJ/m2) in each D.O in September.

Bierzo reaches its maximum *LAI* value (6.46) in July, while Ribera del Duero and Arribes register it in April, 4.06 and 3.46 respectively. After that month, the *LAI* values in both D.Os began to decrease progressively.
Most of the area included in Ribera del Duero has a cold semi-arid steppe climate (BSk). This climate is predominant in the majority of the D.Os, except in Bierzo and in Arribes, whose prevailing climates are temperate with warm dry summer (Csb) and temperate with hot dry summer (Csa), respectively (Beck *et al.*, 2018).
Maximum *LAI* values in June in the three most northern D.Os of the region (Arlanza, Bierzo and Tierras de León) are higher than in the rest of the D.Os. The maximum *LAI* values of the other six D.Os range from 1.8 – 2.84 and are similar to those obtained in the same month in a Csb climate (Vélez *et al.*, 2021b) and in a BSk climate (López-Lozano and Casterad, 2013).

Conclusion

The application of Beer-Lambert Law has provided the monthly $IPAR$ evolution of vineyards in each D.O of Castilla y León. Besides their climate classification, the viticultural regions present different characteristics, which explains the diversity of wines produced. Ribera del Duero and Bierzo are the two D.Os with the highest $IPAR$ values.
This study highlights the importance of studying in detail variables such as $IPAR$ and its influence on crops such as vineyards. In addition to this monthly estimation, future work can conduct more specific analyses at vineyard scale, considering other factors such as environmental conditions, grape varieties or vineyard disposition.

Acknowledgement

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ANIMAL HUSBANDRY

CHARACTERIZATION AND IDENTIFICATION OF POTENTIAL PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM EGYPTIAN TRADITIONAL FERMENTED DAIRY PRODUCTS

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Abstract

This study involved the identification and characterization of potential probiotic strains of lactic acid bacteria (LAB) isolated from fifteen samples of fermented dairy products, specifically Laban Rayeb (fermented milk), Laban Khad (buttermilk), and Kariesh cheese, which are commonly consumed in Egypt. Out of the total of 25 isolates, a mere 14 (equivalent to 56%) were identified as Gram-positive, endospore-negative, non-motile, catalase-negative. Furthermore, these isolates shown the ability to thrive within a temperature range of 15-45°C, as well as in media containing 4% and 6.5% NaCl. The majority of isolates exhibited antibacterial activity against the investigated bacterial pathogens. The most optimal LAB isolates (LAB-3, LAB-4, LAB-13, and LAB-14) were chosen, identified using 16S rDNA gene sequencing, and afterwards assessed for their probiotic characteristics. The phylogenetic tree revealed that isolate LAB-3 exhibited a genetic match with *Enterococcus durans* MT545074.1, while LAB-4 demonstrated similarity with *Enterococcus lactis* MH348130.1. Additionally, LAB-13 displayed homology with *Lactobacillus acidophilus* MT604714.1, while LAB-14 exhibited similarity with *Leuconostoc mesenteroides* MT538672.1. All selected strains shown susceptibility to antibiotics. Both *Lb. acidophilus* and *Leu. mesenteroides* exhibited resistance to vancomycin. Two of the four bacteria, namely *E. durans* and *Lb. acidophilus*, exhibited notable resistance in their ability to survive under low pH. Moreover, the aforementioned two strains exhibited resilience at both acidic conditions of pH 2.0 and 3.0. Moreover, *Lb. acidophilus* is regarded as a strain that exhibits resistance to bile, as seen by its notable survival rates when exposed to varying concentrations of bile salt, specifically 0.1%, 0.3%, and 0.5%.

Keywords: *Isolation, identification, fermented dairy, probiotic, Lb. acidophilus*

Introduction

Traditional Egyptian fermented dairy products, Laban Rayeb (sour defatted fermented milk), Laban Khad (Buttermilk) and Kariesh cheese have an important place in the rural communities due to their flavors, nutritional and therapeutic attributes. The production of these products has been based on the spontaneous fermentation, resulting from the development of lactic acid bacteria (LAB) microflora naturally present in the raw milk and its environment (Abou-Donia, 2008; Abd-El-hamid *et al*., 2015; Abd-Alla *et al*., 2020).

Specific LAB are termed probiotic strains contribute to the liberation of health-enhancing bioactive peptides that improving absorption in the intestinal tract, prevention of diarrhea from various causes, stimulating the immune system, exerting antihypertensive, antithrombotic effects, increased nutritional value of foods, reduction of serum cholesterol levels, improvement of digestion of proteins and fats, synthesis of vitamins and detoxification and protection from toxins (Wan & El-Nezami, 2018 ; Moens *et al*., 2019; Tridip *et al*., 2022).

The isolation and identification of new potentially probiotic lactic acid bacteria stains from traditional fermented products, to be used as starter cultures is the major concern in food and beverages industry (Ricci *et al*., 2019). Toward that goal, several studies on isolation and screening of probiotics from traditionally fermented dairy products were undertaken by many researchers (Mulaw *et al*., 2019, Azzam, 2021, Fouad *et al*., 2021). The commonly used probiotic in many functional food products was from the genus *Lactobacillus, Streptococcus, Leuconostoc,* and *Pediococcus, and Enterococcus* (Pessione, 2012; Mathur *et al*., 2020).
Therefore, the aim of this study were to isolate new LAB as potential probiotics from Egyptian traditional fermented dairy products and identify LAB isolates via 16S rDNA sequence analysis and evaluate *in vitro* the growth characteristics of potentially probiotic strains isolated.

Materials and methods

Sampling:
Fifteen samples of traditional fermented dairy products, including Laban Rayeb, Laban Khad, and Kariesh cheese, were randomly selected from local markets of Fayoum, Egypt. These products are usually made at home utilizing spontaneous fermentation. The samples were aseptically collected and placed in sterile containers and then, quickly brought to the lab at 5°C. The samples were microbiologically analyzed within 1–4 hours after arrival.

Reference pathogens:
The Faculty of Agriculture, Fayoum University, Egypt, Microbiology Department provided *Staphylococcus aureus* ATCC 15923, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis* ATCC 13076, and *E. coli* O157:H7.

Isolation of lactic acid bacteria (LAB):
Aseptic homogenization was performed by mixing 1 g of each sample with 9 ml of sterile physiological solution (0.85% w/v NaCl). The mixture homogenised for 5 minutes. The appropriate 0.1 ml of each sample was serially diluted and placed to MRS agar plates in duplicate. For 48 hours, MRS plates were incubated at 37°C. Randomly choosing colonies from each sample considered colour, shape, and size. Replating on fresh MRS agar plates purified them. Pure isolates were obtained by reselecting the colony twice in MRS broth at 37°C for 24 hours. Gram staining, spore, catalase, and motility were next tested microscopically on the isolates. Only Gram-positive and catalase-negative isolates were saved for research. Strains were stored at -20°C in tubes.

Morphological and biochemical features of LAB isolates:
LAB isolates were largely identified by their visual and biochemical features, according to Bergey's Manual of Systematic Bacteriology. Bacterial colony morphology was examined under a light microscope. Form, colour, and shape were evaluated. Each isolate was tested for milk coagulation at 37°C for 12, 24, and 38 hours. After coagulation pH was determined (Sowmya *et al*., 2016). Omar *et al*. (2006) isolated and categorised microorganisms by testing their growth at 10, 15, 37, and 45°C and in different NaCl concentrations (2, 3, 4, and 6.5%, w/v).

Antibacterial activity:
The isolates were tested for antibacterial activity against *Staph. aureus, B. cereus, P. aeruginosa*, *Sal. enteritidis*, and *E. coli* using the nutrient agar well diffusion method published by Tagg *et al.* (1976).

Molecular identification of LAB isolates:
The selected isolates were grown in sterile test tubes with 10 ml nutrient broth. The test tubes were incubated at 28ºC for 48 hours (Zhang *et al.*, 2016). Cultures were transported to Assiut University's Molecular Biology Research Unit for DNA extraction. The Patho-gene-spin DNA/RNA extraction kit from Intron Biotechnology Company in Korea was used. SolGent Company in Daejeon, South Korea, performed PCR and gene sequencing on the DNA samples. The universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GTTACCTTGTTACGACTT3') were used for PCR. Electrophoresis on a 1% agarose gel with a 100-base-pair nucleotide marker confirmed PCR amplicons. Adding dd NTPs to the reaction mixture sequenced the amplicons using 27F and 1492R primers (White *et al*.1990). Using the NCBI website's Basic Local Alignment Search Tool (BLAST), the sequences were further analyzed. According to Madeira *et al*. (2019), Clustal Omega multiple sequence alignment algorithms were used for phylogenetic analysis.

Probiotic properties of selected LAB:
Antibiotic susceptibility
The identified strains were tested for five antibiotic resistance. The NCCLS (2016) standard disc diffusion method was used for this. This study used Penicillin (10 units), Chloramphenicol (30 mg), Erythromycin (15 mg), Tetracycline (30 mg), and Vancomycin (30 mg).
Low pH and bile salts tolerance tests
The strains were inoculated in MRS broth with pH values of 2, 3, and 4 for 24 hours to test growth. In MRS broth with 0.1%, 0.3%, and 0.5% bile salt, LAB tolerance was tested. The experiment lasted 24 hours at 37°C, according to Grosu-Tudor and Zamfir (2012).
Statistical analysis:
Triple testing on all experimental samples yielded means in the statistical analysis. The statistical analysis used SPSS 17.0. The significance of distinct groups was assessed using a one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.

Results and discussion

Probiotics study on lactic acid bacteria (LAB) affects food preservation and human health. This issue remains popular with researchers. In order to find probiotic lactic acid bacteria from traditional Egyptian fermented dairy products, key functional and technological criteria have been used to select them.
Laban Rayeb, Laban Khad, and Kariesh cheese, which are popular in Egypt, were used to select 14 from 25 isolate. The isolates' morphological, physiological, and biochemical tests are summarised in Table 1. Fourteen isolates (56%) were Gram-positive bacteria without endospores, motility, or catalase activity. Their colonies were tiny, circular to oval, and white. Isolates are straight rods or cocci. Previous research has identified and isolated probiotic LAB genera like *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus,* and *Bifidobacterium* spp. from raw milk, cheese, yoghurt, and other fermented dairy products (Thirabunyanon *et al.*, 2009; Bin Masalam *et al.*, 2018; Huang *et al.*, 2021 and Taye *et al.*, 2021).

After 16 hours at 37°C, all 14 LAB isolates except LAB-1 and LAB-7 coagulated milk. The milk coagulation ability of various strains varied. Only eight of 14 isolates coagulated milk in 8 hours. All 14 isolates' coagulation pH values ranged from 6.4 to 4.2.
Eleven of the 14 isolates when tested at 15, 37, and 45°C were found to thrive in this temperature range. However, Table 1 shows that 13 isolates grew best at 37°C. Egypt's mild climate was expected to favour thermophilic and mesophilic lactic acid bacteria (LAB) in dairy products and raw milk samples. Jagadeeswari *et al*. (2010) discovered similar species in fermented foods. Franciosi et al. (2009) found that mesophilic cocci and rods were more common in fermented food than thermophilic cocci and rods. In addition, Refay *et al*. (2020) collected 30 lactic acid bacteria (LAB) strains from Luxor-region raw milk and traditional dairy products. Of these strains, 76.1% were lactic acid bacilli that could thrive at 45 °C, whereas the rest grew best at 37 °C.
Table 1 further shows that all 14 pure isolates grew well (++) at 4% NaCl. Salt levels above 10% decreased bacteria viability. At 10% NaCl, ten isolates grew. Four rod-shaped bacteria that thrived in 4% NaCl were isolated by Mulaw *et al.* (2019). Three of the four lab isolates grew in 6.5% NaCl. According to Azadnia and Khan Nazer (2009), conventional drinking yoghurt lactic acid bacteria thrived at 4% NaCl but not 6.5%.

Table 1: Morphological, biochemical and physiological characteristics of LAB isolates from fermented dairy products.

characteristics	**LAB-1**	**LAB-2**	**LAB-3**	**LAB-4**	**LAB-5**	**LAB-6**	**LAB-7**	**LAB-8**	**LAB-9**	**LAB-10**	**LAB-11**	**LAB-12**	**LAB-13**	**LAB-14**
Source	k	R	K	BM	R	k	R	R	BM	BM	k	R	R	BM
Cell shape	cocci	rod	cocci	cocci	cocci	rod	cocci	rod	cocci	rod	rod	rod	rod	cocci
Colony color	white	white	white	white	white	white	white	white	white	white	white	white	white	white
Colony size	small	small	small	small	small	small	small	small	small	small	small	small	small	small
Gram staining	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spore staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Milk coagulation														
4 h	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8 h	-	+	-	-	+	+	-	+	-	+	-	+	+	+
16 h	-	+	+	+	+	+	-	+	+	+	+	+	+	+
pH after coagulation	6.4	4.0	5.2	4.9	4.8	4.1	6.2	4.6	5.3	4.4	5.4	4.6	4.2	4.3
Growth temp.														
15°C	++	-	+	+	+	-	+	-	+	+	+	+	+	+
37°C	++	++	++	++	+	++	++	++	++	++	++	++	++	++
45°C	+	++	+	+	++	+	++	++	++	+	+	++	+	+
Growth in NaCl														
4%	++	++	++	++	++	++	++	++	++	++	++	++	++	++
6.5%	+	+	++	++	-	+	+	++	+	+	+	+	++	++
10%	+	-	+	+	-	+	-	+	-	+	+	+	+	+

*K: Karish cheese; R: Rayeb milk; BM: Buttermilk (Laban khad); G+: Gram positive

The results of antibacterial activity test showed that crude extracts from each isolate inhibited all food-borne pathogens except LAB-5, which did not inhibit *B. cereus*. The study found a substantial ($p < 0.001$) correlation between inhibition zone diameter and extract antibacterial activity. According to Table 2, LAB-3, LAB-4, LAB-13, and LAB-14 have high *Staph. aureus* antagonistic activity. The inhibition zones for these strains were 17.00, 17.33, 15.66, and 14.66 mm. These findings were significant (p-value < 0.001). Table 3 shows statistical analysis of all 14 LAB isolates and tested pathogens' primary effects. Study results show that

isolate LAB-13 has the highest antibacterial activity, followed by LAB-3. The 13.73 and 13.40 mm inhibitory zone sizes showed this. LAB-4 and LAB-14 had 12.46 and 11.86 mm inhibition zones, respectively, and showed antibacterial activity. The smallest inhibition zone diameter showed the lowest antibacterial activity in LAB-5.

Several studies have shown that some LAB strains can produce antibacterial chemicals. Several mechanisms of action make these medicines powerful inhibitors of many microorganisms, including harmful ones. LAB synthesises antimicrobial metabolites like lactic and acetic acid, hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoin, carbon dioxide, and bacteriocins. Organic acid produced by LAB decreased pH and produced hydrogen peroxide. Organic acids and similar compounds have been shown to be antibacterial against a variety of Gram-negative microorganisms (Al-Gamal *et al*., 2019; Mulaw *et al*., 2019; Refay, 2020).

Table 2: Effect of interaction between LAB isolates and main effects of LAB isolates pathogenic strains on inhibition zone diameter.

Isolate No.	**inhibition zone diameter (mm)**					**Main effect of LAB**
	B. cereus	*E. coli*	*Pseudomonas sp.*	*Sal. enteritidis*	*Staph. aureus*	
LAB-1	12.00^{ijkl}	10.00^{mnop}	12.00^{ijkl}	11.00^{jklmn}	13.00^{fgh}	11.60^{d}
LAB-2	12.33^{hijk}	12.66^{fghi}	12.00^{ijkl}	11.00^{jklmn}	10.66^{klmno}	11.73^{cd}
LAB-3	14.00^{ef}	12.00^{ijkl}	13.00^{fgh}	11.00^{jklmn}	17.00^{ab}	13.40^{a}
LAB-4	10.00^{mnop}	11.00^{jklmn}	12.00^{ijkl}	12.00^{ijkl}	17.33^{a}	12.46^{b}
LAB-5	0.00^{t}	14.00^{ef}	11.00^{jklmn}	12.00^{ijkl}	11.00^{jklmn}	9.60^{e}
LAB-6	7.66^{rs}	12.66^{fghi}	11.00^{jklmn}	11.00^{jklmn}	12.00^{ijkl}	10.86^{d}
LAB-7	9.33^{opq}	11.00^{jklmn}	11.66^{ijklm}	11.33^{ijklm}	13.66^{efg}	11.40^{d}
LAB-8	8.66^{pqr}	10.00^{mnop}	11.33^{ijklm}	11.00^{jklmn}	13.00^{fgh}	10.86^{d}
LAB-9	6.66^{s}	11.33^{ijklm}	10.33^{lmno}	9.66^{nopq}	10.66^{klmno}	9.73^{e}
LAB-10	9.66^{nopq}	9.66^{nopq}	12.00^{ijkl}	11.33^{ijklm}	14.00^{ef}	11.33^{d}
LAB-11	7.66^{rs}	12.66^{fghi}	11.33^{ijklm}	11.00^{jklmn}	11.33^{ijklm}	10.80^{d}
LAB-12	8.33^{qr}	12.66^{fghi}	11.00^{jklmn}	11.33^{ijklm}	11.66^{ijklm}	11.00^{d}
LAB-13	12.00^{ijkl}	12.00^{ijkl}	16.00^{bc}	13.00^{fgh}	15.66^{cd}	13.73^{a}
LAB-14	9.66^{nopq}	11.00^{jklmn}	12.00^{ijkl}	12.00^{ijkl}	14.66^{de}	11.86^{c}
Sig.			***			***
SE±			0.43			0.19

a, b, ….. and t: Means having different superscripts within each column are significantly different ($p< 0.001$).
SE: Standard error

After reviewing the findings, the best four isolates were selected to be analysis by PCR amplification and sequencing of 16S ribosomal RNA and study their probiotic properties. The procedure of molecular identification involves 16s rDNA sequencing and phylogenetic tree reconstruction.

The 16s rDNA was amplified and sequenced, then compared to GenBank sequences. Table 5 displays the data. Compared to GeneBank Database sequences, all isolates had 99.7% to 100% homology. A phylogenetic tree (Figure 1) was created to show the isolates' closeness. *Enterococcus durans*, *Enterococcus lactis*, *Lactobacillus acidophilus,* and *Leuconostoc mesenteroides* were the four outgroups in the phylogenetic tree. According to the data, Accordingly, LAB-3 showed 100% match with *Ent. durans* MT545074.1, LAB-4 showed 99.93% similarity with *Ent. lactis* MH348130.1, LAB-13 showed 100% homology with *Lactobacillus acidophilus* MT604714.1, and LAB-14 showed 99.7% homology with *Leuconostoc mesenteroides* MT538672.1.

Four effective probiotic LAB isolates were found by Mulaw *et al.* (2019). 16S rDNA gene sequence comparisons designated these isolates as Lactobacillus strains. *L. plantarum* CIP 103151 and *L. paracasei* subsp. *paracasei*, and *L. plantarum* NBRC 15906, 15889, and 1149. A 16S rRNA gene comparison was performed by Shokryazdan *et al*. (2014) on LAB isolates

L. acidophilus, fermentum, buchneri, and *casei*. To find probiotic Lactobacillus strains, Cho *et al*. (2009) examined 16S rRNA genes. Breast-feeding pigs' faeces yielded these strains. In another study, Dowarah et al. (2018) found numerous probiotic lactic acid bacteria strains. This strain was isolated from particular substrates and identified using 16S rDNA phylogenetic estimate.
Certain strains of lactic acid bacteria (LAB) have been studied for their probiotic properties.

Table 3: Bacterial strains of the isolates and their sequence homologies (%) compared to that in the GeneBank Database

	LAB-3	LAB-4	LAB-13	LAB-14
Identification	Ent. durans 4562	Ent.lactis F38	Lb.acidophilus 2218	Leu. mesenteroides 3825
Accession	MT545074.1	MH348130.1	MT604714.1	MT538672.1
Homologies (%)	100	99.93	100	99.78

+: Positive, −: Negative; *Ent.: Enterococcus; Lb.: Lactobacillus; Leu.: Leuconostoc*
*Homology was recorded according to that shown in the BLAST

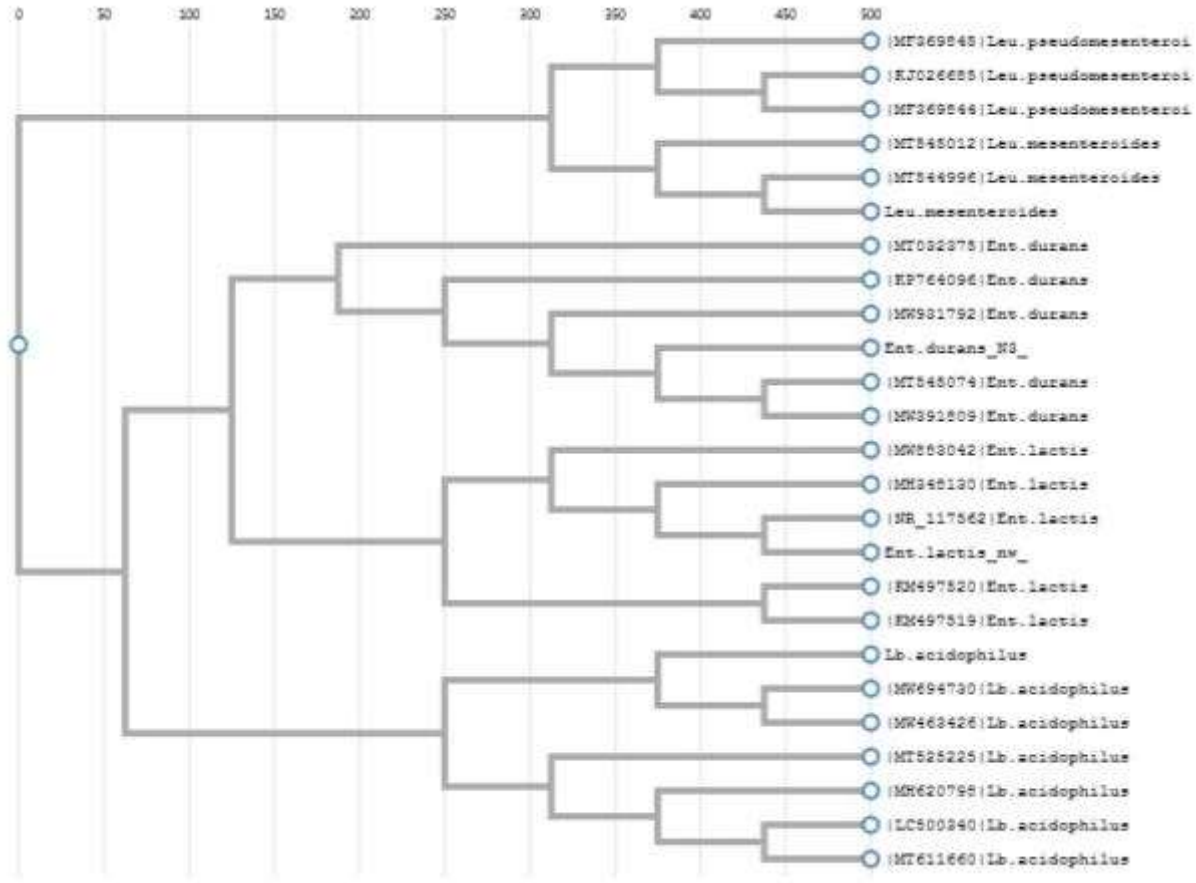

Figure (1): Phylogenetic tree based on 16S sequences of rDNA of the bacterial strain isolated in the present study aligned with closely related sequences accessed from the GenBank. Isolate No. 1 showed 100% identity and 100% coverage with several strains of *Enterococcus durans*. Isolate No. 2 showed 99.93% - 100% identity and 100% coverage with several strains of *Enterococcus lactis*. Isolate No. 3 showed 100% identity and 100% coverage with several strains of *Lactobacillus acidophilus*. Isolate No. 4 showed 99.7% identity and 100% coverage with several strains of *Leuconostoc mesenteroides.*

The four identified bacteria' susceptibility to five antibiotics is shown in Table 4. Clarithromycin, Erythromycin, Penicillin, and Tetracycline work on all isolates. Lacking an

inhibitory zone, *L. acidophilus* and *Leu. mesenteroides* were Vancomycin-resistant. Nunziata *et al*. (2022) found that dairy-derived Lactobacillus, Streptococcus, and Bifidobacterium strains are resistant to gentamycin, kanamycin, chloramphenicol, and tetracycline.
Tetracycline-resistant LAB strains from fermented food were less common than those from animals in earlier studies. LAB strains have low rates of tet genes, which provide tetracycline resistance. The ribosomal protective protein (tet) gene was found in *L. salivarius*, *Ent. faecium* and *L. acidophilus* which isolated from fermented food in India (Dec *et al*., 2018; Erginkaya *et al*., 2018; Dec *et al*., 2020; Stefańska *et al*., 2021). In addition, Nawaz *et al*. (2011) found this gene in *L. plantarum, L. salivarius, L. animalis*, and *L. brevis*. LAB strains were rarely erythromycin-resistant. However, earlier investigations have found that LAB strains, particularly *Lb. plantarum* and *Ent. durans* .are resistant to these antibiotics (Dec *et al*., 2017; Erginkaya, 2018). Only *E. durans* 1586 was erythromycin-resistant, while *L. plantarum* 2021p was susceptible. Portillo *et al*. (2000) discovered the gene as an *Ent. faecium* trait. It was then found in many different Enterococcus species, including durans, lactis, and casseliflavus, as well as *L. fermentum* strains (Thumu and Halami, 2012).

Table 4: Antibiotic susceptibility of selected LAB strains.

Isolate	Inhibition zone diameter (mm)				
	Clarithromycin	**Erothromycin**	**Penicillin**	**Vancomycin**	**Tetracycline**
Ent. durans	22.33^{defg}	26.00^{bc}	22.33^{defg}	21.00^{gh}	32.00^{a}
Ent. lactis	24.00^{cdef}	21.66^{efg}	24.33^{cde}	20.33^{gh}	31.66^{a}
Lb. acidophilus	16.66^{i}	21.66^{efg}	31.00^{a}	0.00^{j}	24.66^{cd}
Leu. mesenteroides	18.33^{hi}	28.00^{b}	20.00^{gh}	0.00^{j}	21.33^{fg}
Sig.	$P \leq 0.001$				
SE±	0.91				

a, b, ….. and j: Means having different superscripts within each column are significantly different (p< 0. 001).
SE: Standard error

The selected four strains with different acid tolerance patterns were tested. Two of four isolated lactic acid bacteria were resistant to low pH. At pH 4.0, *Ent. durans* and *L. acidophilus* survived above 99%. The other two isolates survived over 60% at pH 4.0. *Ent. durans* and *Lb. acidophilus* showed good tolerance at both pH 2.0 and 3.0 compared to *Ent. lactis* and *Leu. mesenteroides* At pH 2.0, LAB strains (3, 1) exhibit the highest acid tolerance, with survival rates of 72.33% and 64.56%. As pH drops, all four isolates' viable counts decrease, as indicated in Table 7.
In his 2020 investigation on isolated LAB's acid tolerance at pH 2.0 and 3.0, Azzam found a similar pattern. A large majority of the 28 LAB isolates examined were resistant to low pH. At pH 2.0, Tulumoglu *et al.* (2013) found 45% survival. According to Zhang *et al.* (2016), 21 strains survived over 90% at pH 2.0 and 17 at pH 3.0. The study also found that 30% and 25% of strains had survival rates above 90% at pH 2.0 and pH 3.0. In addition, Rajoka and colleagues (2017) found an above-80% survival rate at pH 2.0. When pH dropped to 2.0, Mourad and Nour-Eddine (2006) found survival rates of 55%, 49%, 65%, and 57%.
All strains showed different levels of inhibition for bile salt tolerance. Its survival rates of 145.00%, 120.33%, and 94.66% at 0.1%, 0.3%, and 0.5% bile salt concentrations show that *L. acidophilus* is highly hardy. With increasing media bile salts, all four cultures became tolerant. As demonstrated in Table 8, all isolates were resistant to 0.1% bile salts. This investigation found 2 of 21 isolates bile-resistant, matching Zhang *et al*. (2016). All three fermented maize lactobacillus acidophilus strains were bile-resistant, indicating a higher percentage of resistant isolates, according to Jacobsen *et al.* (1999). In addition, Azzam (2020) found one laboratory isolate that was resistant and four that were tolerant. Additionally, twelve strains show restricted bile tolerance. A sensitive biliary response was

seen in two laboratory isolates. LAB isolates were tested for bile salt tolerance by Wu *et al.* (2021). At 0.3% and 0.5% bile salt concentrations, eight isolates survived. Isolates include *L. rhamnosus* with virtually 100% viability grew the most. In addition, *L. plantarum* R7 and *Leu. mesenteroides* AC11.1 had the second greatest viability in MRS medium with 0.3% and 0.5% bile salt.

To resist bile salts, gut microorganisms must have innate resistance mechanisms. LAB have several proteins that transport bile salts or protons, modify sugar metabolism, or prevent protein misfolding (Ruiz *et al*., 2013). The amino acids produced by bile salt deconjugation can provide carbon, nitrogen, and energy. Because glycine and taurine can be metabolised into ammonia, carbon dioxide, and sulphate. Thus, deconjugating bile salts may benefit hydrolytic lactic acid bacteria (LAB) strains nutritionally. Tanaka *et al*. (2000) saw bile salt hydrolysis (BSH) encodes a nitrogen control cascade glutamine synthetase adenyltransferase in *B. longum*.

Table 5: effect of different pH numbers and bile salt concentrations on survival rates of LAB isolates

Isolate NO.	% Survival rates of LAB					
	pH			Bile salt concentration (%)		
	4	**3**	**2**	**0.1**	**0.3**	**0.5**
Ent. durans	100.00^{a}	76.00^{c}	64.56^{e}	85.60^{f}	74.66^{g}	70.33^{i}
Ent. lactis	57.38^{f}	54.36^{g}	51.46^{h}	94.33^{d}	88.00^{e}	73.66^{gh}
Lb. acidophilus	99.98^{a}	97.00^{b}	72.33^{d}	145.00^{a}	120.33^{b}	94.66^{d}
Leu. mesenteroides	54.66^{g}	47.00^{i}	42.33^{j}	100^{c}	85.33^{f}	72.66^{h}
SE±		0.65			0.26	
Sig.		***			***	

a, b, ….. and j: Means having different superscripts within each column are significantly different (p< 0. 001).
SE: Standard error

Conclusion

The current investigation involved the identification of four strains of lactic acid bacteria derived from traditional Egyptian fermented dairy products, namely Kariesh cheese, Laban Rayeb, and Laban Khad. These strains exhibited promising attributes that suggest their potential as probiotics. These strains are recommended as potential probiotic cultures with additional health benefits for use in dairy industries. However, additional research is required to assess the in vivo probiotic properties of these putative lactic acid bacteria, in addition to their use as starter cultures.

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HIPPOTHERAPY IN THE CONTEXT OF MODERN METHODS OF REHABILITATION

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Abstract

The analysis provides information about the history of the treatment by horse-riding – hippotherapy, its positive effect on health of adults and children, especially patients with cerebral palsy (CP). The positive outcomes of hippotherapy depend a lot on the correct selection of horses and the requirements to them (disposition, bodybuild type, exterior, endurance, etc.). The article considers the system of measures for selection of horses intended for hippotherapy, and their training, taking into account animal psychology and interaction horse -patient-instructor-doctor. It is concluded that the mechanism of interaction "man-horse" should be thoroughly examined, and to maintain the health of the nation we should receive government financial assistance for hippotherapy development, rehabilitation centers and equestrian sport for disabled people.

Keywords: *Hippo therapy, horse, horse riding treatment, rehabilitation centers, animal psychology, special training.*

Introduction

Positive effects on the health of adults and children are reached with the help of "therapeutic" abilities of pets: cats, dogs, rabbits, guinea pigs, hamsters, and horses. Hippotherapy (ride therapy), a treatment mode based on the interaction of humans and horses specially trained to meet the needs of the patient, is increasingly spreading in the treatment of patients. Today, hippotherapy is used to treat gastrointestinal disorders, diseases and injuries of the musculoskeletal system, cardiovascular disorders, neurological disorders, multiple sclerosis, prostatitis, oligophrenia, infantile cerebral palsy, and others [2, 3]. Medical professionals have proved that communication with a horse calms people well, boosts their self-esteem, gives them a sense of confidence and safety. And the best recovery results are achieved with the use of hippotherapy in the treatment of musculoskeletal disorders and problems with movements' control. During the therapeutic riding, the body swings in three dimensions: left-right, forward-back, and up-down. The affected muscle groups and nervous structures start working. It develops mobility, a sense of balance, and motion coordination in patients suffering from paralysis [4, 8].

In the process of horse riding, all the major groups of body muscles are engaged. This happens at the reflex level, as the rider, moving along with the horse, instinctively seeks to maintain a balance not to fall, thereby inducing both healthy and affected muscles to work actively, without even noticing it. Hippotherapy acts upon the human body in the same way as any other form of physical therapy – under the influence of physical exercises the functions of vegetative systems are enhanced. Hippotherapy has received a well-deserved recognition. Children confined to wheelchair due to their illness, which traditional medicine was powerless to cope with, were up and about again thanks to training with horses. No special simulator has a look in with what a contact with a live horse gives to a child. While the horse is on the move, the rider's body performs the same movements as during their independent walking. Riding a rhythmically moving horse, the rider instinctively seeks to maintain balance. At the

same time, all muscles of their body, both healthy and affected by the disease, are engaged. It is achieving the synchrony of the movements of the child and the horse, in other words, creating a unified biological system that is the basis of self-correction of the curved spine. With each hippotherapy session, the patient's back muscles get stronger, and legs muscles and tendons warm up, increasing blood circulation in the limbs and blood supply to the brain. This contributes to the creation of a strong muscle corset around the spine. Positive results in hippotherapy in many respects depend on the correct selection of horses. There are certain requirements to the character, temperament, endurance, type and exterior which the horse that is used for therapeutic riding should meet. In addition, each horse must undergo a special training, which will develop and enhance the necessary qualities, for example, a horse should be able to ease the possible fear of a rider and dampen down their aggression [9-14]. Therefore, the purpose of the study was to examine the selection parameters for horses intended for hippotherapy and to explore the existing training systems, taking into account the mechanism of "human-horse" interaction.

Material and methods

This paper is based on extensive literature research.

Results and discussion

But what makes horse riding so useful, you may ask. Many things! The horse passes the rider more than 100 different body vibrations per minute: up and down, back and forth, leftward and rightward, etc. This forces the human body to adjust its movements very physiologically. When riding, all major muscle groups are included in the work. In order not to fall down of the horse's croup, the rider is forced to make various movements using his body, as well as with both arms and legs - this effect cannot be achieved with any other types of movement. Moreover, the efforts of the rider to maintain balance in the saddle encourage both healthy and affected muscles to be active. And what is most important, it happens at the level of reflexes – a person does not notice such a complicated work of his body, but simply gets pleasure from movement and communication with a magnificent animal. Hippotherapy is very useful for treatment of musculoskeletal system disorders, scoliosis, arthrosis of the joints. It normalizes muscle tone, improves coordination, strengthens muscles, and even improves gait. At optimal load, a strong muscular corset develops along the spinal column, blood circulation becomes better, the metabolism in the intervertebral discs get normalized, therefore, with the beginning osteochondrosis, riding is just the thing. The horse's body temperature is 1.5-2º C higher than that of a human and that is why the horse is both a live fitness simulator and a "massager with heating" at the same time. When riding in the saddle, the rider receives massage of the leg muscles, blood flow turns faster and pelvis area warms up, which is good for people of both genders. Men - prevention and treatment of prostatitis; women - getting rid of gynecological diseases. It is hippotherapy and therapeutic (adaptive) horse riding that requires horse's maximum discipline and responsibility, since it's not just about children, but about the children working with whom there is no room for mistake. However, it should be remembered that even a horse with an ideal temper can be spoiled by negligence of an unskilled groom, their rudeness and indifference, harshness of a coach or doctor.

Fig. 3: Hippotherapy - a complex multifunctional method of rehabilitation of patients

In the stables meant for hippotherapy, all those basic humanitarian principles that have been implemented and mastered in the course of many centuries in the relationship between humans and horses must be respected. In view of therapeutic specifics, such relationships are crucial. Horses should have optimal conditions for a full-fledged manifestation of kindness and mercy. Therefore, the most important criterion for selecting a horse is its temperament. Horses used in the therapy should be friendly, trusting, calm, patient and balanced animals. It is important that the horse remains frisky and vigorous, since only such horses may have an appropriate therapeutic step, and it is difficult to work with feeble and apathy animals. Of course, the horses that bite, kick and are aggressive to people cannot be used for the therapy. In hippotherapy, mares and gelded horses are mainly used, which is due to the fact that they have the most appropriate temperament. Stallions, as a rule, are not used, but there are no severe restrictions here. It happens that stallions also work effectively, but in the case there is always a risk factor.

Horse's exterior is the next criterion for assessing its suitability. Preferably, it should not be too height (145-160 cm in the withers), as high horses complicate the patient's safeguarding. Being not high, the horse should have a wide and rather long back with well-developed muscles, middle or low withers, and its legs should have the correct position.

It has been proved that in view of these characteristics the best breeds are the following: Huzul horses, Arabic horses, Novooleksandrivska Heavy Draft horses and others with a strong balanced type of higher nervous activity. The horses of the elder age (7-10 years old) are selected, mainly gelded horses and mares with a characteristic exterior: short, stretched, with a wide back, with a step at a trot of 80-90 cm, a step of 68-76 cm, with strong limbs, but the main thing is their well-balanced nature [1]. In addition, a horse possessing all the

necessary therapeutic qualities should be well prepared for certain exercises during therapy. Therefore, the horse-doctor, whatever happens, should be calm, obedient, and steady, accustomed to be touched everywhere, attentive to the voice of the instructor and to what is happening. Thus, the horse should accept any human action absolutely positively. A comprehensive study of the interaction of all participants of animal-assisted therapy: animals, patients, therapists (instructors, psychologists, doctors) is carried out according to the methodology of animal psychology. Ideally, the instructor and the horse should form a coordinated team, and not only during hippotherapy sessions.

Duration of hippotherapy sessions varies depending on the illness and physical fitness of the rider. The loads are given purposefully. It would be wrong to say that horses are recommended only if there is some kind of physical problem. A healthy person will be really amused and delighted after the interaction with these cute and graceful creatures. Taking care of the animal develops a person's communicative skills, helps them overcome emotional barriers, and promotes social rehabilitation. Horse riding has a particularly strong healing effect on children. Communication with a horse solves such complex problems as hyperactivity and attention deficit disorder. But besides this, horseback riding fully develops the child, makes him bold, agile, resilient, determined and resourceful.

Horse riding classes are also useful for elderly people. Scientists have calculated that the work of the muscles when riding in steps is similar to fast walking, and at a gallop to running. And at the same time there is almost no load on the joints. Riding a horse leads to an improvement in fine motor skills, which helps elderly people to more easily cope with household. In addition, the development of fine motor skills is closely related to the speech apparatus.

Fig. 2: Iinteraction "human-horse"

Each horse is a personality. During hippotherapy they are expected to do almost an impossible thing: to become a reliable and safe simulator, to clearly and unconditionally carry out our orders. This can be achieved only if we treat the horse as a partner, with due respect, and give them time and space to satisfy our requirements.

Conclusions

Thus, the "human-horse" interaction mechanism needs to be thoroughly investigated, and in order to preserve the health of the nation, the government should provide financial assistance for the development of hippotherapy, rehabilitation centers and equestrian sport for the disabled.

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EFFECT OF DIFFERENT STOCKING DENSITIES IN THE OUTDOOR AREA ON BROILER PERFORMANCE CHARACTERISTICS

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Abstract

The aim of this study is to determine the effect of different stocking densities in the outdoor area on the performance characteristics of broilers. Fast-growing broiler chickens were used in the study. In the outdoor area, two groups were formed with a stocking density of 1 m^2 per chicken and 2 m^2 per chicken. Each group was formed from four replications. A total of 160 broiler chickens, 20 in each replicate and 80 in each group, were used. Feed and water were given ad libitum. The body weight value on the 42nd day was determined as 2815 g in the S1 group and 2806 g in the S2 group, and the differences between the groups were statistically insignificant. Feed consumption was found to be 152 g/day/hen in the S1 group and 151 g/day/hen in the S2 group, with no statistically significant differences between the groups. The average rate of carcass on the 42nd day was found to be 75% in the S1 group and 74% in the S2 group (P>0.05).As a result, it would be more economical to arrange the settlement frequency in the outdoor area at 1 square meter per chicken.

Keywords: *Broiler, stocking density, Outdoor area, Free-range system.*

Introduction

Broiler production around the world is mostly done in barn systems. However, in recent years, broiler production has started to be carried out in the free-range system, depending on many factors as well as the preferences of the consumer. Indoor rearing of animals, restricting the natural behavior of animals, and depriving them of sunlight are limiting factors for animal rights. The opinion of consumers that the products of animals raised in natural environments are healthier also highlights broiler breeding in the free-range system. Consumers prefer free-range products because they are more flavorful, have a higher nutritional value, have a lower fat level, and include a higher concentration of vitamins and minerals (Fanatico *et al.,* 2005; Wang *et al.,* 2009; Michalczuk *et al.,* 2014). Stocking density should be considered together with indoor and outdoor areas in free-range systems. Stocking density refers to the number of animals per unit area. The unit of stocking density is also stated as unit chicken/m^2, m^2/chicken, and kg CA/m^2. In the free-range system, it is necessary to optimize the stocking density in the outdoor area.

Fast- and slow-growing genotypes are used in free-range broiler production. In general, the stocking density in the indoor area is designed at 2.4 broilers per m^2 and 12 broilers per m^2, while the stocking density in the outdoor area is 0.75 m^2 /broiler and 5 m^2 /broiler (Sauveur 1997; Bogosavljevic-Boskovic *et al.,* 2010; Połtowicz and Doktor 2011; Eleroğlu *et al.,* 2013; Ipek and Sozcu 2017; Skřivanová *et al.,* 2017; Akyar and Yeter 2021). The objective of this study is to examine the impact of different stocking densities on the performance parameters of the outdoor area.

Materials and Methods

This study was carried out at Selçuk University, Faculty of Agriculture, Department of Animal Science (Turkey). The study was conducted between May-2023 and July-2023. A total of 160 one-day old of Ross-308 chickens were randomly classified into 2 experimental groups with different stocking densities, each of 4 replicates. In the outdoor area, two groups were formed with a stocking density of 1 m^2 per chicken and 2 m^2 per chicken. Each group was formed from four replications. The stocking density in the indoor area was arranged as 10 broilers/m^2 in all groups. All broilers had ad libitum access to water and commercially-formulated broiler chicken feed (starter day 0–10, grower day 11–26, and finisher day 27–42). Broilers had access to one lamps/pen and 24 h light in the first 3 days, followed by a light:dark schedule of 16L:8D. Each pen was supplemented with one tube feeders and 3 adjustable water nipple systems. The experimental period was 6 weeks (42 days). The humidity, temperature, litter, and other management conditions were regulated based on standard instructions for Ross-308 strain (Ross 2014). The body weights of the animals were weighed at the beginning of the trial and at the end of each period on a subgroup basis. Feed intake within each subgroup was calculated simultaneously with determination of BW by subtracting residual feed from the offered feed. The feed conversion ratio (FCR) was calculated as the ratio of FI to BWG (g feed/g gain). Mortality was recorded daily and expressed as a percentage of the initial number of broilers.

Statistical analysis

One-way analysis of variance was applied to the data obtained from the study. Tukey's multiple comparison test will be used to identify differences between treatments. All hypothesis tests will be performed at a significance level of 0.05 and the Minitab 16 package program will be used for statistical analysis.

Results and discussion

The body weight value on the 42nd day was determined as 2815 g in the S1 group and 2806 g in the S2 group, and the differences between the groups were statistically insignificant (Figure 1.)

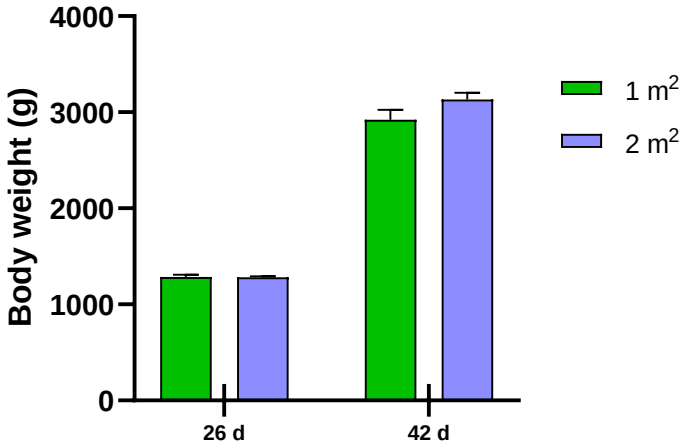

Figure 1. The mean body weight of the experimental groups was determined on the 42nd day.

Feed consumption was found to be 152 g/day/hen in the S1 group and 151 g/day/hen in the S2 group, with no statistically significant differences between the groups (Figure 2).

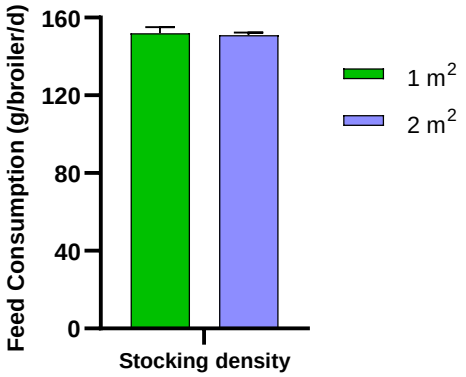

Figure 2. The feed consumption values of the experimental groups for the period from the 26th to the 42nd days.

The average feed efficiency throughout the period from the 26th to the 42nd days was determined to be 1.29 in the S1 group and 1.31 in the S2 group. The observed differences between the two groups were not found to be statistically significant (Figure 3).

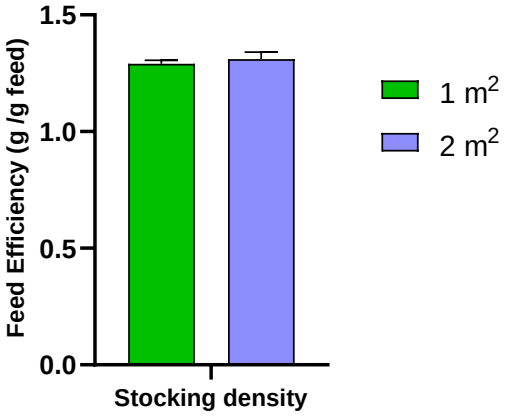

Figure 3. The feed consumption values of the experimental groups for the period from the 26th to the 42nd days.

The average rate of carcass on the 42nd day was found to be 75% in the S1 group and 74% in the S2 group. The observed differences between the two groups were determined to be statistically insignificant (Figure 4).

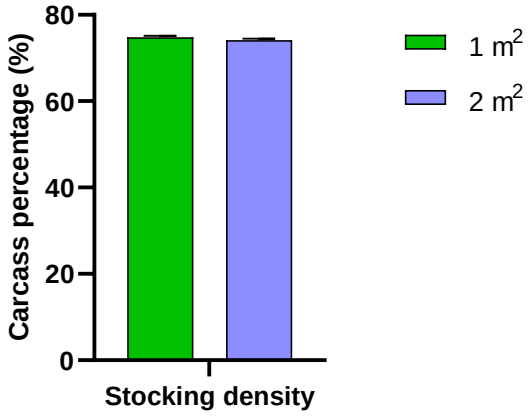

Figure 4. The mean carcass weight of the experimental groups was determined on the 42nd day.

The fact that the density of settlement did not have a significant effect on performance characteristics in our study may be due to the inadequate use of the animal's outdoor area. Rodriguez-Aurrekoetxea *et al.* (2014) reported that 63% of the animals did not go to the outdoor area. Despite having access to the outdoors, birds frequently stay inside or choose to stay near to the house, without using the range (Fanatico *et al.*, 2016). Based on the findings of our research, increasing the stocking density in the outdoor area did not have negative effects on performance characteristics. Phillips and Heins (2021) stated that differences between groups in terms of body weight values of chickens with low (2.5 m^2/broiler) and high stocking density (0.5 m^2/broiler) are statistically insignificant. On the other hand, Sanchez-Casanova *et al.*, (2019) stated that kept at low density were heavier on weeks 5 (2.710 vs. 2.600), compared to those housed at high density ($P<0.05$). The carcass yields obtained from our study (75% and 74%, S1 and S2 groups, respectively) were found to be compatible with the studies obtained from the 74–75% carcass yields obtained from broilers (Grigore *et al.*, 2023).

Conclusion

Consequently, the implementation of a higher stocking density in the outdoor area does not adversely impact performance attributes and serves to optimize cost efficiency in production. In future studies, it would be valuable to explore the impact of stocking density in outdoor areas on behavior and meat quality.

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COMPARISON OF THREE DIFFERENT LAYER GENOTYPES RAISED IN A FREE-RANGE SYSTEM FOR EGG QUALITY

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Abstract

One of the factors affecting egg quality is genotype. Generally, genotypes producing white and brown shell eggs are used in commercial egg production. In recent years, genotypes developed for egg production in the free-range system and producing beige-shelled eggs have been developed. The aim of this study is to compare the egg quality characteristics of three different layer genotypes (Lohmann Sandy; L, Lohmann Brown; B, and ATAK-S; A) reared in the free-range system. In this the study, a total of 120 eggs were used, with 40 from each genotype. Egg weight, eggshell color, eggshell strength and Haugh unit were investigated as egg quality characteristics. The L genotype (80.3) had the highest eggshell L value, while the B genotype had the lowest eggshell L (60.2; $P<0.05$). The lowest egg weight was detected in the A genotype (57.2 g), and the difference in egg weights between the L (62.1 g) and B (62.1 g) genotypes was found to be statistically insignificant. The L genotype (5.041 kg) had the highest eggshell strength value, while the A genotype had the lowest eggshell strength value (3.725 kg; $P<0.05$). The highest Haugh unit was detected in the B genotype (95.3), and the difference in Haugh unit between the L (89.1) and A (86.8) genotypes was found to be statistically insignificant. As a result, it was found that genotype significantly influenced egg weight, eggshell color, eggshell strength, and Haugh unit.

Keywords: *Genotype, egg weight, eggshell color, eggshell strength, and Haugh unit. free-range system.*

Introduction

Conventional cage egg production has been banned in European Union countries since 2012 and egg production has been suggested to be done in alternative systems (EU, 1999). One of these production systems is the free-range system. It has become increasingly important to identify the layer genotypes that will be used in egg production in the free-range system. Many studies have been conducted for this purpose by researchers (Türker *et al.,* 2017). Lohman Brown is a foreign layer hybrid that is grown in Turkey using the free-range system. The Ankara Poultry Institute developed the ATAK-S layer hybrid, which produces brown-shelled eggs (Goger *et al.,* 2016). It is preferred in free-range systems and small family breeding production models in Turkey (Tutkun *et al.,* 2018). Egg quality attributes can be influenced by a number of factors, including genotype (Tůmová *et al.,* 2007; Bozkurt and Tekerli 2009; Rajkumar *et al.,* 2009; Zita *et al.,* 2009; Obike *et al.,* 2014; Hayirli *et al.,* 2015; Sokołowicz *et al.,* 2018; Kraus *et al.,* 2020). According to Bozkurt and Tekerli (2009), white layers produce eggs with higher Haugh units than brown layers. However, Hayirli *et al.,* (2015) reported that genotype had no significant effect on any other quality characteristics besides egg shape index and yolk index. According to Kraus *et al.,* (2020), there were statistically significant differences found in the egg weight, shape index, shell break

resistance, Haugh unit, and shell thickness of eggs from different genotypes. The goal of this study is to compare different layer genotypes in the free-range system in terms of egg quality.

Materials and Methods

This study was carried out at Selcuk University, Faculty of Agriculture, Department of Animal Science (Turkey). The study was conducted between April-2022 and November-2022. Three different genotypes, commercial (Lohmann Sandy; (L), Lohmann Brown; B), and domestic (ATAK-S, A) at 39 weeks of age, were used in the study.
The hens were reared in a free-range system. The stocking density in the in-door area is 7 birds /m^2, while the out-door area provides 4 m^2 per hen. Water and feed are given as ad-libitum. Quality analyses were carried out on 20 randomly taken eggs from eggs produced for two consecutive days of each genotype. Egg weight, shell color, breaking strength and Haugh unit were investigated as egg quality characteristics. Egg weight was measured using a balance and was recorded to the nearest 0.01 g. Colour measurement was performed using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The egg shell color was measured at the large pole of the egg (Aygun, 2014).
Eggshell strength (kg) was measured with an Egg Force Reader (Hong Kong, China). The height of the albumen was measured using a height gauge. The Haugh unit was calculated using the following formula: Haugh unit = $100 \times \log(H + 7.57 - 1.7W^{0.37})$, where H is the albumen height (mm) and W is the egg weight (g) (Haugh 1937).

Statistical analysis

In the study, the variance analysis technique was used to compare the groups for continuous data obtained from chickens in four replication poultry houses with three different genotypes. Kolmogorov Smirnov and Levene tests will be applied to determine that the data meet the parametric test assumptions. Data that do not show normal distribution will be transformed into a state suitable for parametric test assumptions by rank transformation method. After it is determined that the data have normal distribution and the variances are homogeneous, one-way analysis of variance will be applied. Tukey's multiple comparison test will be used to identify differences between treatments. All hypothesis tests will be performed at a significance level of 0.05 and the Minitab 16 package program will be used for statistical analysis.

Results and Discussion

Egg shell color values of genotypes are given in Figure 1. Egg shell color was significantly affected by genotype. The eggs with the lightest brown eggshell color were obtained from the L genotype, while the eggs with the darkest brown egg color were obtained from the B genotype ($P<0.05$). This finding is consistent with the research by Kraus and Zita (2019), which found that genotype had a significant impact on eggshell color. Egg shell color is an important criterion in terms of consumer preference. Joseph *et al.,* (1999) stated that genotype has a significant effect on eggshell color.

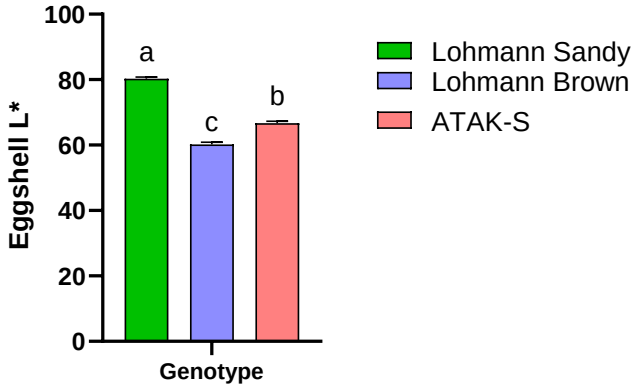

Figure 1. Eggshell color of different genotypes.
[a-c] Differences between groups indicated with different letters are statistically significant (P<0.05).

The effects of genotype on egg weight are given in Figure 2. While the lowest egg weight was obtained in the A genotype, no statistical difference was found between the L genotype and the B genotype in terms of egg weights. Egg prices are determined by egg weights in the egg market. Egg weight is positively related to body weight but negatively related to egg production (Du Plessis and Erasmus 1972). According to Kraus *et al.,* (2020), the genotype had a significant effect on egg weight and that the Lohmann Brown eggs (65.18 g) were heavier than the Hisex Brown eggs (63.73 g).

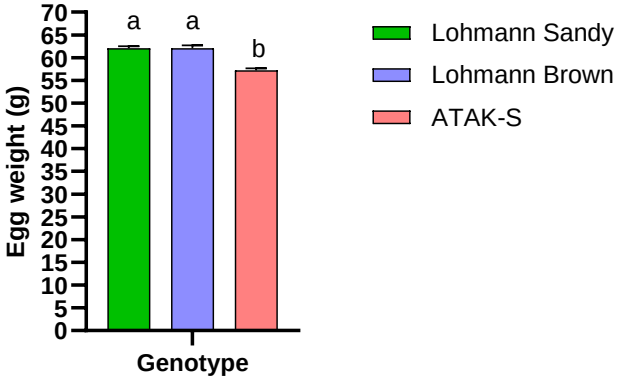

Figure 2. Egg weights of different genotypes.
[a-b] Differences between groups indicated with different letters are statistically significant (P<0.05).

The highest eggshell strength was obtained in the L genotype, while the lowest fracture resistance was obtained in the A genotype (Figure 3; P<0.05). The findings are similar to those of Türker *et al.,* (2017), who found that the ATAK-S genotype's eggshell strength is worse than the foreign genotype's. In contrast, Petričević *et al.*, (2017) found that genotype had no effect on eggshell strength.
While the highest Haugh unit value was obtained in the B genotype, no statistical difference was found between the L genotype and the A genotype in terms of Haugh units (Figure 4). Similar result, Bozkurt and Tekerli (2009) reported that the Haugh unit value of the eggs derived from the Lohmann White genotype (85.92) was higher than that of the eggs derived from the Isa Brown (79.72). The obtained result contradicts the findings of Türker *et al.,* (2017), who found no significant difference in Haugh unit value between eggs obtained from the A genotype and eggs obtained from the foreign genotype.

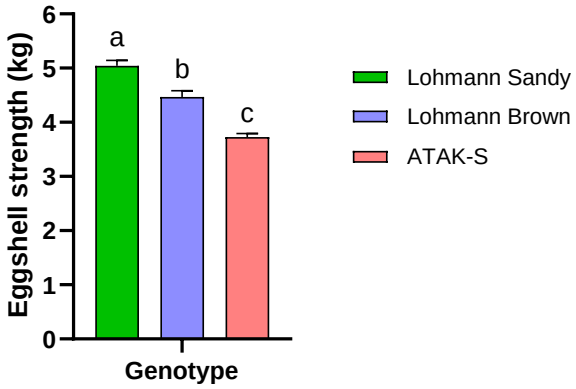

Figure 3. Eggshell strength of different genotypes.
[a-c] Differences between groups indicated with different letters are statistically significant (P<0.05).

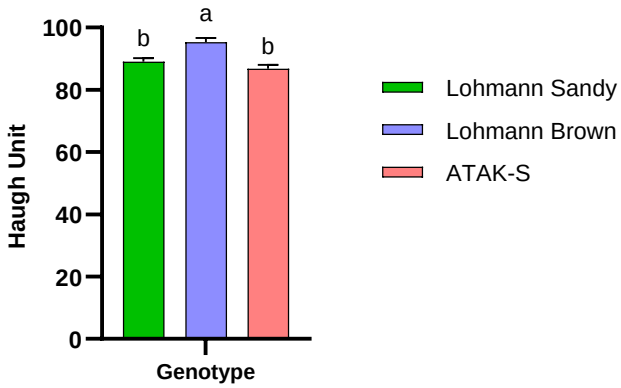

Figure 4. Haugh units of different genotypes.
[a-b] Differences between groups indicated with different letters are statistically significant (P<0.05).

Conclusion

While the eggs of the Lohmann Sandy genotype are better in terms of egg shell quality, the eggs of the Lohmann Brown genotype are better in terms of internal quality characteristics. The egg quality of the ATAK-S genotype is worse than other genotypes.

Acknowledgement

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MICROBIOLOGICAL STATUS AND MOLD CONTENT IN MILK AND FEED MIXTURES WITH THE ADDITION OF ADDITIVES

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Abstract

The research was carried out on the milk of three groups of 15 dairy cows each and on the feed mixtures that were given to the cows. Tufozel type zeolite was added to animal food. Three groups of fodder mixtures were tested. No zeolite was added to the control feed mixture (K-group). While 2% Tufozel was added to the first test mixture (I-O group). In the second sample group of mixtures (II-O group), 4% of zeolite was added. Tufozel is the commercial name of a natural adsorbent of mycotoxins, produced by the zeolite manufacturer Nemetali from Vranjska Banja. It was obtained by surface mining from excavations in Zlatokop near Vranje. The requirements of the European Union regarding the microbiological correctness of feed mixtures and milk are far ahead of the domestic legislation, which states that the hygienic correctness of milk and feed mixtures must be raised to a higher level. Modern milk purchase is based on milk hygiene. On the other hand, a large number of bacteria and molds can be introduced into the animal's organism through animal feed, and for this reason a parallel must be drawn between the hygienic correctness of animal feed and the quality of milk. This research deals directly with the content of bacteria and molds in animal feed, more specifically in concentrated feed for dairy cows, as well as milk. It was established that the addition of zeolite to the feed mixtures given to dairy cows had an effect on the microbiological correctness of the feed mixtures themselves, and consequently on the milk. the milk.

Key words: *bacteria, zeolite, feed mixture, milk, cow.*

Introduction

Each new rulebook on the quality of raw milk or feed mixtures is increasingly rigorous in terms of the microbiology of milk and animal feed, but little is done to remove the very factors that affect the microbiological quality of animal feed and milk.
Feed mixtures are a very suitable medium for the development of harmful microorganisms. They contain starch that microorganisms use as food. Microorganisms break down nutrients, whereby metabolites are released that reduce the nutritional value of the concentrate or completely neutralize it.
The analysis of feed mixtures for bacteria included the analysis of coliform bacteria, streptococci, staphylococci, proteus spp., salmonella spp., clostridia, moulds and TBN 48 hours and 30 days after sowing on the nutrient medium.
Milk is one of the most favourable substrates for the development of bacteria. It contains all the necessary substances for the growth and reproduction of microorganisms. Under favourable temperature conditions, microorganisms reproduce very quickly and reduce the quality of milk or completely neutralize it.

The milk was analysed for coliform bacteria, streptococci, staphylococci, proteus species, salmonella, sulphite-reducing bacteria, moulds and the total number of bacteria. Milk analyses were done 48 hours and 5 days after the day of sowing.
Microorganisms or bacteria can enter the milk in different ways, through the teat duct, during milking or in some other way. Unhygienic conditions of storage, transport or conservation can lead to the appearance of bacteria in milk. Quality and microbiologically correct milk can only be obtained from the milk of healthy, well-cared and properly-fed cows, Jovanovic (1998).
According to the research cited by Katić (2003), later Petrović et al. (2006), increasing attention will be paid to the hygiene of raw milk, which will later affect the quality of the final milk product. The price of milk is determined according to the percentage of fat in milk but also according to the total number of microorganisms in it as well as the number of somatic cells. Prolić et al. (2003) assert that increasing the number of cows to at least 20 will increase the hygienic quality of milk by introducing modern milking and cooling systems into production. Tucović et al. (2002) write about organizing large milk-receiving stations to improve the quality of raw milk through the use of various stationary lactofreezers as well as modern milk transport tanks.
According to the research conducted by Niketić et al. (2003), in Germany, the number of microorganisms/ml in raw milk for cow's milk is ≤ 50,000, and the number of somatic cells is ≤ 400,000, while in Croatia it is ≤ 100,000, and the number of somatic cells is ≤ 400,000. The Rulebook on the Quality of Raw Milk (Official Gazette of the RS, No. 21/2009) for Serbia states that raw milk containing up to 100,000 microorganisms belongs to the E class. Class I milk is the milk with a microorganism content of 100,001-400,000/ml and Class II milk has a microorganism content of over 400,000/ml.

Material and methods

Microbiological analysis was performed on samples of feed mixtures as well as on milk samples. Samples of feed mixtures were taken in sterilized nylon bags, and milk in specially sterilized containers. Collective samples were made from all individual samples and then analysed. Three groups of 15 dairy cows of the Simmental breed, whose milk was analysed, were fed with feed mixtures. The cows came from private farms in the village of Veliki Šiljegovac in the municipality of Kruševac in Serbia. All microbiological analyses were carried out in the chemical and microbiological laboratory of the DPPK "Delišes" dairy in Vladičin Han.
The medium used in the experiment came from the Torlak Institute of Immunology and Virology in Belgrade, and the Mac Conkey Broth (MAK) medium from Hungary. Other media used are EA (endo agar), SH (Chapman's medium), SSA (dry agar), SA (sulphate agar), and SMA (Saburo maltose agar).
The total number of bacteria (TNB) represents the total number of living bacteria (1 bacteria = 1 colony). A colony represents a group of individual bacteria or a group of bacteria formed through reproduction. Colonies can be seen with the naked eye but are best viewed with a magnifying glass or by utilizing a microscope.
The TNB was determined with a 10^{-1} dilution. This means that 1 ml of milk is taken from the milk sample and placed in a test tube containing 9 ml of sterilized water. The test tube is shaken for 1 minute and in this way the dilution is obtained. The number of colonies is multiplied by the degree of dilution and thus the number of bacteria in 1 ml is determined.

Results and discussion

Microbiological analysis was performed on feed mixtures 48 hours after sowing on the substrates, as well as 5 and 30 days after sowing. The content of mould after 5 and 30 days after sowing on the substrates is shown. The total number of bacteria was controlled after 48 hours, as well as after 30 days.
The media used in the experiment are Endo Agar (EA), Mac Conkey Broth (MAK), Saburo Maltose Agar (SMA), Chapman's medium (SH), SS Agar (SSA) and Sulphate Agar (SA).
Table 1 shows the analysis of feed mixtures for coliform bacteria, streptococci, staphylococci, proteus spp. and Salmonella spp. 48 hours after sowing on the nutrient media.

Table 1. Analysis of feed mixtures for bacteria 48 hours after sowing

Test	**48 hours after planting sowing**			
Foundation	**EA**	**SH**	**SSA**	**SSA**
Bacteria	Coliform bacteria	Staphylococci Streptococci	Proteus spp.	Salmonella spp.
K- Group	coliform bacteria	no	No	no
II-O (2%)	coliform bacteria	no	No	no
I-O (4%)	coliform bacteria	bacilli	No	no

Coliform bacteria were found in all three groups of cows in the analysis of feed mixtures 48 hours after taking the sample, on the EA medium.
The testing confirmed the presence of staphylococcus and streptococcus after 48 hours, on the SH substrate. They were not found in the K-Group and II-O group, while the appearance of bacilli was recorded in the I-O group.
After 48 hours, no proteus species were identified on the SSA substrate in any of the samples of the feed mixture in any group of cows.
The presence of salmonella was not confirmed on samples of feed mixtures, on SSA substrates, 48 hours after sowing.
All analyses were repeated after 30 days on the same substrates and on the same bacteria. Coliform bacteria were present, while proteus species and salmonella did not appear even after 30 days. There were small white cocci in the K-Group and II-O group, while in the I-O group, there were some even after 48 hours and their number was at the level of the other two groups, which means that the feed mixtures of the I-O group of cows had streptococci and staphylococci even before the first test and that the zeolite influenced that number did not increase and did not differ after 30 days from the mixtures that were without these bacteria.
Table 2 shows the analysis of feed mixtures for coliform bacteria, streptococci, staphylococci, proteus spp. and Salmonella spp. 30 days after sowing on nutrient media.

Table 2. Analysis of feed mixtures for bacteria 30 days after sowing

Test	**After 30 days**			
Foundation	**EA**	**SH**	**SSA**	**SSA**
Bacteria	Coliform bacteria	Staphylococci Streptococci	Proteus spp.	Salmonella spp.
K- Group	Coliform bacteria	Small white cocci	No	No
II-O (2%)	Coliform bacteria	Small white cocci	No	No
I-O (4%)	Coliform bacteria	Small white cocci	No	No

In addition to the aforementioned analyses, tests were carried out 48 hours and 5 days after sowing on nutrient media. After 5 days, there were no sulphite-reducing bacteria in the SA medium. On the SMA substrate, in a test after 5 days, mould was detected in 1 ml. In the K-Group there was the least amount of mould (7), there was some more in the II-O group (18) and the largest amount was in the I-O group (23).
The analysis of the feed mixtures for the total number of bacteria in them after 48 hours, on the medium for the total number of bacteria, showed that the mixture of the control group had the most bacteria (5320), a smaller number was recorded in the II-O group (4820) and the smallest number was present in the I-O group (2850).
These analyses are presented in Table 3.

Table 3. Analysis of feed mixtures for clostridia, moulds and TNB

Test	**After 5 days**		**After 48h**
Foundation	**SA**	**SMA**	**TNB foundation**
Bacteria	sulphite-reducing clostridia	Mould in 1ml	TNB in 1ml
K- Group	no	7 mould	5320
II-O (2%)	no	18 mould	4820
I-O (4%)	no	23 mould	2850

Analyzes for sulphite-reducing clostridia, moulds and TNB were also performed 30 days after sowing on the substrates.
The results are shown in Table 4.

Table 4. Analysis of feed mixtures for clostridia, moulds and TNB after 30 days

Test	**After 30 days**		**After 30 days**
Foundation	**SA**	**SMA**	**TNB foundation**
Bacteria	sulphite-reducing clostridia	Mould in 1ml	TNB in 1ml
K- Group	no	25 mould	6200
II-O (2%)	no	20 mould	4200
I-O (4%)	no	26 mould	3100

By analysing sulphite-reducing clostridia, moulds and TNB 30 days after sowing on nutrient media, it was determined that there were no sulphite-reducing clostridia on the SA media.
After 30 days, on the SMA substrate, 25 moulds were found in the feed mixtures of the K-Group, 20 moulds in the II-O group and 26 moulds in the I-O group. It is noticeable that the presence of mould is much higher in the feed mixtures fed to the K-Group and I-O group compared to the mixture fed to the II-O group of cows.
Comparing the tests after 5 days and after 30 days, it is noted that there were no clostridia even after 30 days from sowing. The highest increase in the mould was recorded in the K-Group mixture (from 7 to 25). A slight increase in mould (from 18 to 20) was noted in the mixture of group II-O. An increase in the number of moulds (from 23 to 26) was recorded in the mixtures of the I-O group. A significantly faster increase in the number of moulds was observed in cows that did not receive zeolite.
TNB in the mixtures fed to cows of the K-Group was the highest (6200). It was lower in the cows of the II-O group (4200), while the lowest number of bacteria was recorded in the mixtures fed to the cows of the I-O group (3100). A significantly higher increase in the number of bacteria was recorded in mixtures that did not contain zeolite. In the K-Group mixtures, the number of TNB increased from 5320 to 6200, in the I-O group from 2850 to 3100, while in the II-O group, the number of bacteria decreased from 4820 to 4200.
Tables 5 and 6 show the results of milk analysis for coliform bacteria, streptococci, staphylococci, proteus species, salmonella, sulphite-reducing bacteria, moulds and the total number of bacteria. The media on which bacteria and moulds were tested were EA, SH, SSA, SA and SMA. Table 5 shows the bacteriological analysis of milk 48 hours after sowing.

Table 5. Analysis of milk for bacteria 48 hours after sowing

Test	**48 hours after sowing**			
Foundation	**EA**	**SH**	**SSA**	**SSA**
Bacteria	Coliform bacteria	Staphylococci Streptococci	Proteus spp.	Salmonella spp.
K- Group	yes	yes	No	no
II-O (2%)	yes	yes	No	no
I-O (4%)	yes	yes	No	no

In the tests 48 hours after sowing, on nutrient media EA, SH and SSA, the presence of coliform bacteria, streptococci and staphylococci, proteus species and salmonella was not determined.

After this analysis, the milk analysis was done after 5 days on SA, SMA and TNB media. The presence of sulphite-reducing clostridia, moulds and TNB was determined.
Table 6 shows the analysis of milk after 5 days.

Table 6. Analysis of milk for clostridia, moulds and TNB after 5 days

Test	**After 5 days**		
Foundation	**SA**	**SMA**	**TNB foundation**
Bacteria	sulphite-reducing clostridia	Mould in 1ml	TNB in 1ml
K- Group	no	72	1.450.000
II-O (2%)	no	12	960.000
I-O (4%)	no	36	850.000

The analysis of milk after 5 days, on the SA medium, did not reveal the presence of sulphite-reducing bacteria.
In the test after 5 days, on SMA in 1 ml of content, the largest number of moulds was determined in the milk of cows obtained from the K-Group of cows (72), i.e. cows that did not receive zeolite. Milk from cows of the I-O group had a smaller number of moulds (36). Milk from the II-O group of cows had the lowest mould content (12).
The analysis of milk after 5 days, on the TNB medium in 1 ml of content, showed the largest amount of mould in the milk of K-Group cows (1,450,000), i.e. milk of cows that did not receive zeolite. Lower content of TNB (960,000) was recorded in the milk of the II-O group of cows, i.e. milk of cows that received 2% zeolite in feed mixtures. The lowest number of TNB was recorded in the milk of cows of the I-O group of cows, i.e. the milk of cows that received 4% zeolite in their meals (850,000).
According to the obtained results, the milk of cows that did not receive zeolite showed the lowest quality because it had the highest content of mould and TNB.
Milk from the II-O group of cows showed the lowest mould content but had more TNB than milk from the I-O group of cows. The milk of the I-O group of cows contained more mould than the milk of cows of the II-O group, but a lower number of TNB.
The Rulebook on the quality of milk, dairy products, composite dairy products and starter cultures (Official Gazette of the SRY No. 26, 2002) determines the amount of the total number of microorganisms and somatic cells in milk. According to this Rulebook, the total number of microorganisms must not exceed 1,000,000/ml of milk and somatic cells up to 400,000/ml of milk. According to the Rulebook on the quality of raw milk (Official Gazette of the RS, no. 21/2009), the TNB at a temperature of 30°C can be up to 400,000.
The regulations of the European Union, according to Howells (2004), are significantly more rigorous. The total number of microorganisms should be less than 100,000, and the number of somatic cells less than 400,000. The SMBM content should be greater than 8.50%, while the protein content should be greater than 2.80%.
According to Đukić (1994), there are usually over 100,000 saprophytic microorganisms in 1 g or 1 ml, and if that number exceeds a million, the durability of the product is reduced, and there is a possibility of poisoning. Petrović D. M. et al. (2006) report the average number of microorganisms in milk as 844,878/ml, while the number of somatic cells is 3,578.85/ml.
The problem of the content of bacteria, yeasts and moulds in feed mixtures was dealt with by a small number of researchers, including Pešev et al. (2006), Ilić et al. (2006) and Ilić et al. (2007). They point out the importance of adding zeolite to feed mixtures because research has

shown a greater occurrence of bacteria, yeasts and moulds in feed mixtures without zeolite. Similar results were obtained in these studies, which show that in Serbia great attention must be paid to milk hygiene in order to meet the EU standards, which would contribute to easier milk export and more correct milk for consumption and processing.

Conclusion

Based on the investigation of the influence of different concentrations of the Tufozel-type zeolite on the microbiological quality of feed mixtures and milk and the obtained results, we can conclude the following:

Feed mixtures with the addition of zeolite had a better microbiological composition compared to mixtures that did not contain zeolite. Milk from cows that did not receive zeolite showed the lowest quality because it had the highest content of mould and TNB. It was established that there is a connection between the quality of feed and the quality of milk. The results of the research justify the use of the Tufozel-type zeolite in the diet of dairy cows because its use led to a better microbiological performance of feed and therefore milk compared to cows that did not receive zeolite or Tufozel. In addition to the microbiological quality of food, the hygiene of milking, transport, storage and processing of milk must also be harmonized so that the quality of the final product is at a satisfactory level.

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REFERENCE INTERVALS OF HEAVY METALS IN THE MUSCLE TISSUE OF CATTLE CONSIDERING PARATYPIC FACTORS

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Abstract

The aim of the study was to calculate reference intervals for the concentration of heavy metals in the muscle tissue of bull-calf, taking into account the influence of paratypic factors. The research object was the evaluation of concentration of manganese, copper, and lead in the muscle tissue of Hereford bulls raised in the territories of Krasnozersky, Maslyaninsky, and Novosibirsky districts of the Novosibirsk Oblast, as well as the Tselinny district of the Altai Territory. Muscle tissue samples were collected from 31 clinically healthy animals aged 16-18 months. The determination of chemical element concentrations in the samples was performed using atomic absorption spectrometry with electrothermal atomization. The robust mean values of copper, manganese, and lead contents in the muscle tissue of cattle in the studied regions ranged from 0.91 to 1.11, 0.15 to 0.32, and 0.01 to 0.11 mg/kg, respectively. The level of phenotypic variability in lead content among the bulls was significantly higher than that of other heavy metals. Group dispersions for lead and manganese concentrations in the muscle tissue of bulls were heteroskedastic (χ^2 = 9.1 and 12.1; p = 0.03 and 0.005, respectively). The influence of paratypic factors on the levels of these metals in the animal's muscle tissue was revealed (H = 22.1 and 11.0; p < 0.001, respectively). Bulls from the Maslyaninsky district had a statistically significantly higher concentration of lead in skeletal muscle compared to animals from the Novosibirsky and Tselinny districts, and the level of manganese was higher than that of cattle from the Krasnozersky district. The reference range for manganese, copper, and lead contents in the muscle tissue of Hereford cattle raised in the conditions of southern Western Siberia is 0-0.545, 0.70-1.36, and 0-0.237 mg/kg, respectively.

Keywords: *Hereford breed, Muscle tissue, Heavy metals.*

Introduction

From an ecological perspective, all metals that can be toxic are often classified as heavy metals (Alengebawy *et al*., 2021) They accumulate in soil, plants, and animals. Contamination of the food chain with heavy metals has become a significant issue in recent years due to their potential accumulation in biosystems as a result of increased anthropogenic impact (Akarsu *et al*., 2022; De Palma *et al*., 2022; Heidari *et al*., 2021). Heavy metals are always present in the body of cattle, including the muscle tissue, in certain concentrations. Imbalances in chemical elements can disrupt the functions of organs and systems (Pardo *et al*., 2020).

The establishment of reference intervals for copper, manganese, and lead content in the muscle tissue (meat) of cattle is of significant importance due to their relevance for food safety and human health (Emami *et al*., 2023). Reference intervals serve as valuable tools for evaluating the levels of these trace elements in meat products and assessing their nutritional adequacy. Moreover, reference intervals provide a basis for identifying potential contamination and establishing regulatory limits to ensure consumer safety.

Copper, manganese, and lead are trace elements present in the muscle tissue of cattle and play critical roles in various physiological processes. While copper and manganese are essential for enzymatic functions, lead is a toxic heavy metal with detrimental effects on human health (Daniel *et al*., 2023; Haskovic *et al*., 2021; Palomares, 2022). Establishing reference intervals for these elements in cattle meat enables accurate assessment of their concentrations and facilitates comparisons with established safety guidelines. This ensures that meat products meet quality standards and do not pose health risks to consumers (Silva *et al*., 2022; Sorbo *et al*., 2022).

The aim of this study is to calculate the reference concentrations of microelements in bull calf muscle tissue, considering the influence of paratypical factors.

Material and Methods

For the study, samples of muscle tissue (m = 100 g) from diaphragm muscles were selected from Hereford breed bulls aged 16-18 months, raised in the Zelinny District (n=4) of Altai Krai and in the Novosibirsky (n=6), Krasnozersky (n=4), and Maslyaninsky (n=17) districts of the Novosibirsk Oblast (Russia). The animals were kept under typical conditions of an industrial complex, adhering to veterinary requirements. According to pre-slaughter inspection data, the animals were clinically healthy. Sample collection for analysis was performed immediately after slaughter. The determination of copper (Cu), manganese (Mn), and lead (Pb) concentrations was carried out using atomic absorption spectrometry with electrothermal atomization on an MGA-1000 spectrometer.

Statistical analysis was performed using the R statistical programming environment. Robust statistics were used to describe the variables, including median (Me), minimum and maximum values (Min, Max), first and third quartiles (Q1, Q3), and interquartile range (IQR). Calculation of the mean and standard deviation was performed using a universal method regardless of the distribution of the variable (Wan *et al*., 2014). Homogeneity of variances was assessed using the Fligner-Killeen test. The impact of the factor was evaluated using the Kruskal-Wallis test. Post-hoc analysis was conducted using the Dunn method with Holm adjustment. Calculation of reference intervals was performed using a robust method with bootstrapping to obtain adjusted and unbiased intervals (Efron and Tibshirani, 1994).

Results and Discussion

The data on the content of heavy microelements in the muscle tissue of Hereford breed bulls, depending on the region, are presented in Table 1 and Figure 1. Despite the fact that the distribution of observations for the levels of heavy metals in the muscle tissue did not always follow a normal distribution, the mean values were close to the medians, indicating a symmetric nature of the estimation proposed by Wan *et al*., (2014). The concentration of heavy metals in the cattle from the Maslyaninsk district was higher than in animals from other districts. The level of phenotypic variability in bulls from the Maslyaninsk district was significantly higher than in cattle from other districts. For copper levels, a lower phenotypic variability was observed compared to other investigated heavy metals. The minimum variability was observed for manganese concentration in animals from the Novosibirsk district, while the maximum variability was recorded for lead levels in cattle from the Zelinny district.

Table 1. Content of microelements in the muscle tissue of Hereford breed bulls, mg/kg.

Heavy metal	District	Mean	SD	Me	Min	Max	Q1	Q3	IQR	SW*	p_{sw}**
Mn	Krasnozyorsk	0.15	0.07	0.15	0.10	0.20	0.10	0.20	0.10	0.729	0.024
	Maslyaninsky	0.32	0.17	0.30	0.20	0.80	0.20	0.42	0.22	0.784	0.001
	Novosibirsky	0.2	0.02	0.20	0.19	0.21	0.19	0.21	0.02	0.853	0.167
	Tselinny	0.17	0.04	0.20	0.10	0.20	0.14	0.20	0.06	0.630	0.001
Cu	Krasnozyorsk	1.05	0.32	1.00	0.80	1.30	0.88	1.18	0.29	0.926	0.572
	Maslyaninsky	1.11	0.13	1.10	0.80	1.30	1.03	1.20	0.17	0.930	0.301
	Novosibirsky	0.91	0.04	0.90	0.80	1.30	0.89	0.93	0.04	0.667	0.003
	Tselinny	0.94	0.23	0.95	0.80	1.10	0.84	1.06	0.22	0.993	0.972
Pb	Krasnozyorsk	0.024	0.006	0.026	0.010	0.032	0.016	0.030	0.014	0.888	0.372
	Maslyaninsky	0.119	0.082	0.082	0.06	0.210	0.071	0.160	0.089	0.838	0.012
	Novosibirsky	0.019	0.025	0.010	0.004	0.029	0.005	0.021	0.016	0.905	0.402
	Tselinny	0.011	0.003	0.005	0.005	0.022	0.005	0.015	0.010	0.630	<0.001

*Shapiro–Wilk test; **p-value for Shapiro–Wilk test

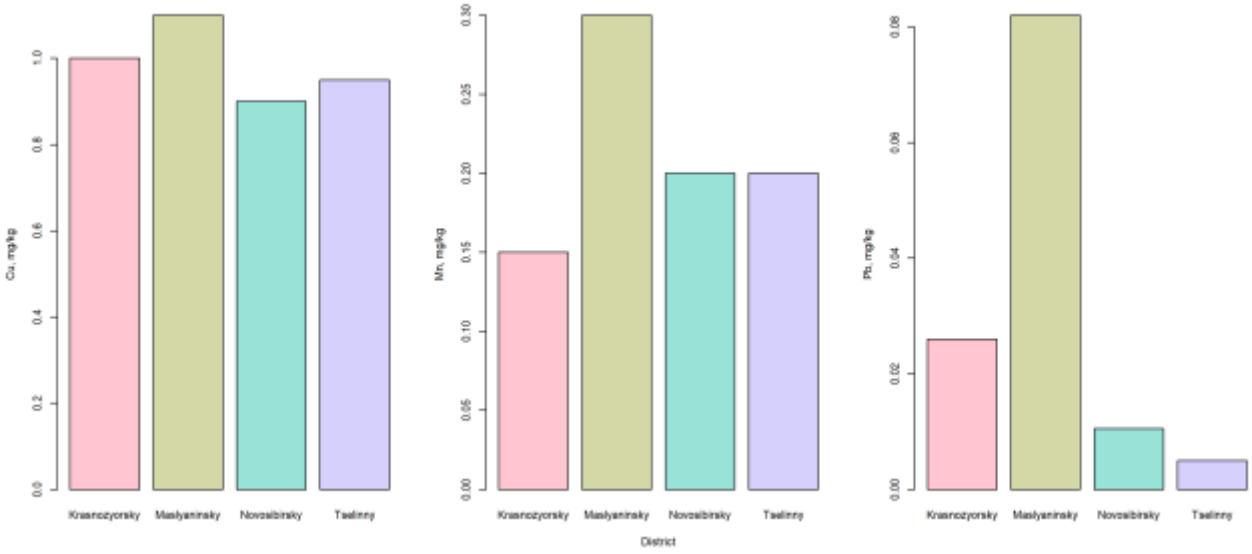

Figure 1. Median values of heavy metal levels in animal muscle tissue, mg/kg.

The levels of heavy metals in the muscle tissue of cattle can vary significantly depending on various factors, including paratypical and genetic factors. It has been observed that the concentration of copper in muscle tissue is influenced by the age of the animals. For young bulls aged 6-12 months, the average copper content was 1.83 mg/kg, while for adult bulls aged 24-36 months, the average copper content was 1.42 mg/kg, which is nearly 1.5 times higher than our study (Domaradzki *et al*., 2016). The influence of genetic factors on the copper level in muscle tissue of cattle has also been identified, with higher copper concentrations observed in Angus breed compared to Simmental breed (Gooneratne *et al*., 1994; Mullis *et al*., 2003).

Several studies have shown that the manganese level can vary (up to 6 times) depending on the muscle type (Alonso *et al*., 2002; Cabrera *et al*., 2010; Pilarczyk, 2014). The manganese levels in our study were slightly higher compared to cattle from other European countries (Alonso et al., 2002; Pereira *et al*., 2018). In the study by Ramos et al. (2012), the influence of age and breed on manganese accumulation in muscle tissue was evaluated. According to their findings, age did not have a significant impact on the manganese level in muscle tissue. However, inter-breed differences were observed, with higher manganese levels in Hereford cattle compared to Braford. Overall, the manganese level in bulls of both breeds ranged from 0.07 to 0.14 ppm, depending on age and muscle type.

The average lead levels in our study ranged from 0.011 to 0.119. These data are consistent with other studies (Miranda *et al*., 2003; Lopez-Alonso et al., 2000; Khalafalla *et al*., 2011). However, there have been some studies reporting lead concentrations that are 2-10 times higher (Korenekova *et al*., 2002; Pilarczyk, 2014; Nwude *et al*., 2011). For instance, Haskovic et al. (2021) found lead levels in muscle tissue reaching 1.24 mg/kg, which is more than 12.4 times higher than the maximum permissible concentration established by the EU (Commission Regulation, 2006). However, in the study by Ward et al. (1995), the copper level in Simmental cattle was higher than in Angus and Charolais breeds.

These findings indicate that the variability in the levels of heavy metals in cattle meat is influenced by factors such as muscle type, breed, and productivity direction.

One of the objectives of the study was to assess the impact of paratypical factors on the levels of heavy metals in muscle tissue. Since the distribution of heavy metal levels in animals sometimes deviated from the Gaussian distribution, and heteroscedasticity of variances was detected in the groups based on lead and manganese levels, the influence of paratypical factors was evaluated using the Kruskal-Wallis test (Table 2). The analysis results showed that the copper level did not differ across the studied regions, while the manganese and lead concentrations varied significantly among animals in the examined regions.

Table 2. Assessment of homoscedasticity of variances and the impact of paratypical factors on the levels of heavy metals in cattle muscle tissue.

Heavy metal	df	*χ^2	p	**H	p
Mn	3	12.1	0.007	11.0	0.011
Cu	3	0.55	0.908	5.4	0.145
Pb	3	9.1	0.03	22.1	<0,001

* Fligner-Killin chi-square; ** Kruskal-Wallis statistics

A posteriori analysis showed that the level of manganese differed only in animals from the Krasnozersky and Maslyansky regions (Table 3). In turn, the level of lead in the Tselinny and Novosibirsk regions is much lower than in Maslyaninsky. In order to identify individual factors influencing its variability in lead and manganese, it is necessary to continue monitoring the level of heavy metals in livestock in large populations.

Table 3. Intergroup comparison of regions by the content of zinc and lead in the muscle tissue of bulls

District	Mn		Pb	
	Z statistic	p	Z statistic	p
Krasnozyorsky - Maslyaninsky	-2.715	0.020	-2.301	0.043
Krasnozyorsky - Novosibirsky	-0.996	0.479	0.775	0.438
Maslyaninsky - Novosibirsky	1.823	0.136	3.716	<0.001
Krasnozyorsky - Tselinny	-0.454	0.325	0.978	0.492
Maslyaninsky - Tselinny	2.137	0.081	3.529	0.001
Novosibirsky - Tselinny	0.498	0.619	0.296	0.383

Copper levels did not differ significantly between animals in the studied areas, so they can be combined into one group to calculate reference intervals. If animals from areas with excellent levels of lead (Novosibirsky and Tselinny districts) and manganese (Krasnozersky district) are excluded from the general pool, reference values for metals can be calculated from the remaining groups. The resulting ranges with confidence intervals for the lower and upper limits are presented in Table 4.

Table 4. Reference intervals with 90% confidence intervals (CI) for the content of heavy metals in the muscle tissue of Hereford cattle (mg, kg)

Heavy Metal	Reference interval	Low confidence intervals 90%	High confidence intervals 90%
Cu	0.70 – 1.36	0.61 – 0.78	1.28 – 1.44
Pb	0 – 0.237	0 – 0	0.185 – 0.301
Mn	0 – 0.545	0 – 0	0.412 – 0.700

Since the level of heavy metals in muscle tissue is influenced by both genetic factors (breed, direction of productivity, individual characteristics) and paratypical factors (conditions of keeping, feeding and exploitation of animals and other environmental and geographic factors), they must be taken into account when developing reference intervals.

Conclusions

In the course of the study, a significant influence of paratypic factors on the concentration of manganese and lead in the muscle tissue of Hereford cattle was established. In animals bred in the Maslyaninsky district, the concentration of heavy metals is higher than in cattle from other areas. The level of copper is characterized by a lower phenotypic variability in contrast to other studied heavy metals. The obtained reference values for the content of manganese, copper and lead can be considered as a physiological norm for Hereford cattle. In the future, it is necessary to continue studying the content of heavy metals in the organs and tissues of cattle and develop reference values for other chemical elements.

Acknowledgments

The obtained values can be used as physiological norms for Hereford cattle. The study was supported by the Russian Science Foundation grant No. 22-76-00003, https://rscf.ru/project/22-76-00003/.

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STUDY ABOUT THE MODE OF IMPLEMENTATION OF THE BREEDING PROGRAM ON ABERDEEN ANGUS BREED IN ROMANIA

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Abstract

In recent years, as a result of the decreasing number of dairy cows, the development of specialized beef cattle farms has seen a rapid development in our country. Aberdeen Angus is the main beef breed wich was imported in our country and its development on national level is carried out by the Aberdeen Angus Association. Aberdeen Angus breeding program on national level, represents the basic structure for managing the official performances control (O.P.C.), the herd book, the sistem and the methodoly of breeding and selection, bull testing results and the general disemination of the recorded data. The main objective of the Aberdeen Angus Breeding Program is to raise on national level the standard of this breed, both quantitative but also qualitative. The breeding program it will take place on the period of 10 to 15 years, during which the value of the coefficients of the indicators established in this program remains unchanged, even active population of cattle will grow, only the number of cow per bull will change. Through this paperwork the specific results according to the: national evolution of the flock divided by physiological categories and according to the heerdbook framing, animal performances accroding to the data from O.P.C., breeding values and the breeding bull evalution from Aberdeen Angus test station will be followed.

Keywords: *Aberdeen Angus, Romania, beef breeds, breeding program, herd book.*

Introduction

Aberdeen Angus breed on the Romanian national level is actually a breed with short and young historical status. The first contact of this breed with Romanian animal husbandry was made in the year of 2000, when Dr. Pachițanu Vasile and dr. prof. Robertson Edwin realized with succes the first embryo transfer program on local cattle with genetic material from Aberdeen Angus breed (Gociman *et al*.,2019). Later in the year of 2008, the first cattle import was made by Karpaten Meat company from Sibiu, when around 120 heifers were brought from Germany (Gociman *et al*.,2020). The destiny of this breed on national level from 2015 is coordinated by the Romanian Aberdeen Angus Association, which is the main responsible for the heerbook and the official performances of this breed population (Grosu and Gociman, 2018; www.aberdeenangus.ro). The national Aberdeen Angus Bredding Program is the main structure on how to carry on the control of performances (O.P.C), the methods of disseminating of the results achieved, the organization of the heerdbook, breeding system and the methodology of selection and testing of the bulls (Ivancia, 2007). The main specific objective of the national breeding program is to raise the quantitative and qualitative standards of the Aberdeen Angus breed in Romania (Maciuc, 2006). Through this paperwork it will be followed the specific results according the: national evolution of the flock divided by physiological categories and according to the herd book framing, animal performances according to the data from O.P.C., breeding values and the breeding bull evaluation from Aberdeen Angus test station.

Material and Methods

In order to monitor the specific results achieved within the cattle population of the Aberdeen Angus breed at the national level, the following specific tools were used:

- **OFFICIAL PERFORMANCES CONTROL (O.P.C.)**. Through the O.P.C. program, information regarding performances of the animals born in Romania are gathered from all the farms, respectively measurements of young calf between 3 months and 14 months, both females and males. Also, information about breeding bulls are managed togetter, about the measurable aspects of the lengths (length of the rump grip the tail), depth (chest depth), heights (height at the rump croup), scrotal circumference, live weight (Grosu and Gociman, 2018).
- **PEDIGREES**. These official papers contain general information about the biological material in form of maternal and paternal ascendant and their breeding values (Huțu *et al*., 2020).
- **BIDAA** (Aberdeen Angus Informatic Database) is the official computer application managed by Romanian Aberdeen Angus Association which summarize the information regarding holdings, animals, official performance control results, genealogical register, breeding values and breeding bull evaluation of Romanian Aberdeen Angus cattle population (Grosu and Gociman, 2018).

Results and Discussion

According to the data recorded in the computer application BIDAA (Aberdeen Angus Informatic Database), developed by the Romanian Aberdeen Angus Association and processed by the department of C.P.P.C. at the end of 2022 were registered a total of 128,011 heads from the Aberdeen Angus breed structured by physiological categories as follows:

- *cows: 43.123 heads;*
- *heifers: 65.610 heads;*
- *young males: 17.907 heads;*
- *breeding males: 1.371 (table 1)(*www.bidaa.ro).

Table 1. Aberdeen Angus breed population structured by physiological categories

TOTAL HEADS ON FARM	NUMBER OF FARMS	COWS			YOUNG BULLS			HEIFERS			BREEDING BULLS	LIVESTOCK
		TOTAL	A	B	TOTAL	A	B	TOTAL	A	B	N.B.	TOTAL
1 - 10	55	28	23	5	72	55	17	177	140	37	5	282
11 - 20	132	491	429	62	213	181	32	1374	1225	149	24	2102
21-50	601	7092	6080	1012	2055	1442	613	10923	8847	2076	319	20389
51-100	415	10099	8235	1864	3552	2176	1376	15141	11537	3604	353	29145
101-250	253	13172	10089	3083	5321	3388	1933	19716	14318	5398	394	38603
Above 250	90	12241	9869	2372	6694	4447	2247	18279	13983	4296	276	37490
TOTAL	**1546**	**43123**	**34725**	**8398**	**17907**	**11689**	**6218**	**65610**	**50050**	**15560**	**1371**	**128011**

The capacity of the total number of heads on the farm is also highly variable, this being an influencing factor from the point of view of the degree of technology and intensification can apply, as well as the level of economic profitability recorded annually. That's how it can be notice that farms with a herd between 21-50 heads have a share of 38.9% at the level

nationally, while at the opposite pole are the farms that have in the total structure of the livestock included between 1-10 heads have a share of 3.6% (fig. 1).
From the total Aberdeen Angus flock in terms of the sex ratio at the end of 2022 there were registered 108,733 (84.95 %) female and 19,278 (15.05 %) male. At the county level in terms of the flock structure of the total population by physiological categories, the county with the most livestock is Sibiu with 16,941 heads, and Ilfov with the fewest livesctock, respectively 197 heads.

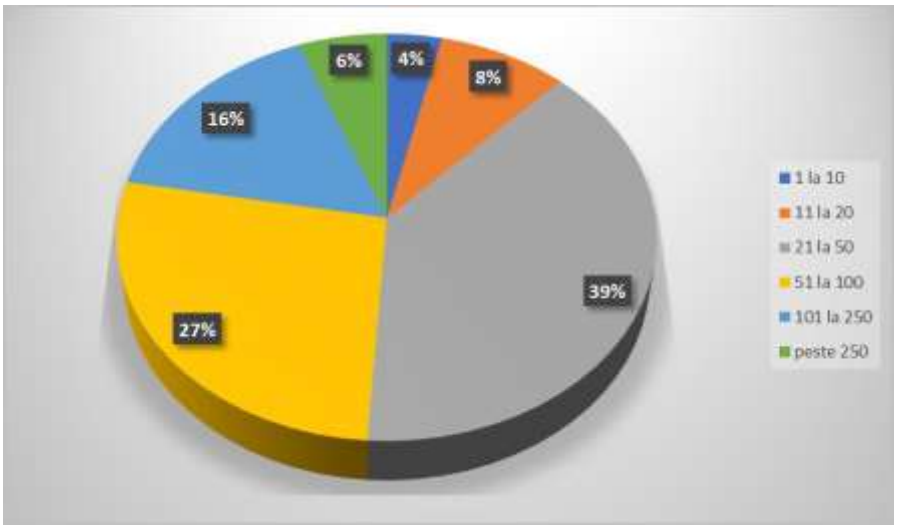

Figure 1. Share of farms at national level according to total number of heads by farm

Official Performances Control (O.P.C.), is one among the tools of improving an animal population, being the starting point in organization of the genetic improvement of the population of the Aberdeen Angus breed (Acatincăi, 2004). This workflow is carried out by specialized staff (animal husbandry engineer/ veterinarian), with mobile cattle crush equipment and scales metrologically approved annually (fig. 2).

Figure 2. Official performances control tools

Beef recording requires recording schemes that can accommodate beef production as implemented in practice. The recording procedures must account for all important effects including the existence of genotype by environment interactions (Georgescu, 1998). Beef recording maybe undertaken in:

- Breeding farms;
- Finishing farms;
- Individual test stations (fig. 3) (www.icar.org).

Figure 3. Official performance control technique for beef cattle

The interval between two weightings is at least 60 days and at most 210 days. At the end during the finishing period, the development of the musculature and the skeleton is assessed through scoring (notes) as well as functional capacity. The registered data are: animal ID; live weight at birth; live weighing at weaning; post-weaning weight, finishing weight and test center weights (ICAR, 2019).
Thus, from the year of 2018 to 2021, the average of the recorded performances for the 4 categories of age achieved are showed through the values expressed in the table nr.2.

Table 2. Evolution of average weight and daily gain between 2018 and 2021

Specification	UNIT	2018	2019	2020	2021
B.W.	kg	28	29	30	30
G7	kg	208	208	214	215
G10	kg	278	278	281	280
G12	kg	327	315	311	313
A.D.G. G7	g	892	887	917	929
A.D.G. G10	g	816	828	880	834
A.D.G. G12	g	791	780	770	775

At the level of the herd book department, during the year 2022 there were issued a total of 6442 pedigrees, only for animals of the Aberdeen Angus pure breed, registered in the main section A, according to the C.E. Regulation and European Parliament law 1012 from 8.june.2016.
In order to improve the populations of bulls from the Aberdeen Angus breed in Romania, am evaluated the reproductive and productive capacities of the bulls, and following the testing, bulls were ranked according to calculated breeding value, followed by bulls poorly ranked to be excluded from the herd, those with average results to be introduced to local farms, and finally, those with performances above the population average should be used as semen donors (fig.4).

Figure 4. Growth performance of bulls from 2022 testing lot

Nr.	ID	Birth date	02.08.2022			29.09.2022			10.11.2022		
			A.W.D. (days)	W.1 (kg)	AD.G. (gr.)	A.W.D. (days)	W.2 (kg)	AD.G. (gr.)	A.W.D. (days)	W.3 (kg)	AD.G. (gr.)
1	RO503006666280	11.02.2021	537	664	1167,12	595	708	1128,77	637	748	1115,07
2	RO504011133277	17.02.2021	531	638	1136,99	589	696	1123,29	631	706	1065,75
3	RO504011202382	24.02.2021	524	660	1194,52	582	664	1082,19	624	718	1095,89
4	RO509009964811	01.04.2021	488	598	1150,68	546	682	1183,56	588	708	1142,47
5	RO504011199620	02.04.2021	487	512	972,6	545	586	1005,48	587	630	1008,22
6	RO502011199637	05.04.2021	484	520	1002,74	542	566	980,82	584	596	961,64
7	RO505011173627	07.04.2021	482	566	1095,89	540	626	1087,67	582	646	1043,84
8	RO504011173637	12.04.2021	477	540	1049,32	535	606	1060,27	577	654	1065,75
9	RO502011702886	14.04.2021	475	538	1052,05	533	602	1057,53	575	635	1038,36
10	RO501011703055	19.04.2021	470	592	1178,08	528	636	1131,51	570	682	1128,77
11	RO501010485860	23.04.2021	466	588	1197,26	524	658	1197,26	566	684	1156,16
12	RO502011280407	28.04.2021	461	642	1339,73	519	706	1312,33	561	754	1298,63
13	RO503009685026	08.05.2021	451	508	1054,79	509	578	1073,97	551	598	1027,4
14	RO503011702623	01.06.2021	427	596	1317,81	485	634	1238,36	527	652	1175,34
16	RO508011145810	12.07.2021	386	574	1419,18	444	614	1323,29	486	648	1279,45
17	RO502011702620	20.07.2021	378	662	1668,49	436	686	1501,37	478	710	1419,18

In order to improve the populations of cattle from the Aberdeen Angus breed in Romania, there were evaluated the reproductive and productive capacities of the bulls, and following the testing period, bulls were ranked according to the estimated breeding values, followed by bulls poorly ranked to be excluded from the herd, those with average results to be introduced to local farms, and finally, those with performances above the population average should be used as semen donors. In order to evaluate the growth performances as objectively as possible, the tested bulls were introduced in individual stalls equipped with specific resting and feeding areas, they being feed twice a day with the same amount of feed, water being at discretion. In order to monitor the growth performances were weighed at the entrance to the test, during the test period and at the exit from the testing period (fig. 5).

Figure 5. Romanian Aberdeen Angus test station farm

Thus, we were able to observe the results of the bulls at the exit from the test station regarding: the performances of growth expressed in A.D.G. (average daily increase), the estimated breeding values calculated relative to the average of contemporary (fig. 6) as well as relative only to the average of individuals are in testing station, we performed the body condition score and took the DNA samples of the bulls for confirmation of maternity and paternity.

I.D.	A.V.B.	R.V.B.	ACC.B	A.V.W.	R.V.W.	ACC.W.	A.V.10M.	R.V.10M.	ACC.10M.	A.V.12M.	R.V.12M.	ACC.12M.	T.A.V.	T.R.V.	T.ACC.
RO503006666280	0,80	102,69	51,60	32,00	108,95	51,60	0,00	99,00	0,00	0,00	99,00	0,00	12,80	102,98	20,64
RO501011703055	1,68	105,64	60,70	5,30	101,48	60,60	0,00	99,00	0,00	24,25	108,58	59,20	9,39	102,87	42,00
RO502011280407	-3,52	88,28	60,70	26,90	107,53	60,60	0,00	99,00	0,00	-1,35	99,52	59,20	10,36	102,57	42,00
RO509009964811	0,88	102,97	60,70	20,50	105,74	60,60	0,00	99,00	0,00	3,45	101,22	59,20	9,23	102,36	42,00
RO508011145810	-3,12	89,62	60,70	24,10	106,74	60,60	0,00	99,00	0,00	0,00	99,00	0,00	9,64	102,10	24,24
RO505011173627	1,68	105,64	60,70	16,50	104,62	60,60	0,00	99,00	0,00	-2,55	99,10	59,20	5,83	101,28	42,00
RO504011202382	-0,40	98,69	51,60	-10,80	96,98	51,60	0,00	99,00	0,00	15,00	105,31	44,70	0,18	100,09	34,05
RO504011199620	1,68	105,64	60,70	2,90	100,81	60,60	0,00	99,00	0,00	0,00	99,00	0,00	1,16	99,72	24,24
RO502011199637	0,48	101,63	60,70	-0,70	99,80	60,60	0,00	99,00	0,00	0,00	99,00	0,00	-0,28	99,32	24,24
RO502011702620	-1,15	96,19	60,70	0,00	99,00	0,00	0,00	99,00	0,00	0,00	99,00	0,00	0,00	99,00	0,00
RO502011702886	1,68	105,64	60,70	-5,10	98,57	60,60	0,00	99,00	0,00	0,00	99,00	0,00	-2,04	98,83	24,24
RO503011702623	-0,48	98,41	60,70	-31,10	91,30	60,60	0,00	99,00	0,00	24,25	108,58	59,20	-5,16	98,79	42,00
RO503009685026	-0,72	97,63	60,70	-11,50	96,78	60,60	0,00	99,00	0,00	-16,55	94,14	59,20	-9,57	96,65	42,00
RO504011133277	-0,40	98,69	51,60	-21,20	94,07	51,60	0,00	99,00	0,00	-15,00	94,69	44,70	-12,98	95,74	34,05
RO504011173637	2,08	106,97	60,70	-16,70	95,33	60,60	0,00	99,00	0,00	-20,95	92,59	59,20	-12,97	95,61	42,00
RO501010485860	-1,52	94,96	60,70	-31,10	91,30	60,60	0,00	99,00	0,00	-10,55	96,27	59,20	-15,61	95,10	42,00

Figure 6. Estimating breeding values of bulls from 2022 testing lot

Conclusions

The high standard of living, the constantly growing population, as well as the consumer preferences for high quality animal food, it is unanimously necessary to raise good and healthy animals translated in animal products, as the demand at european level is increasing and growth opportunities in other european countries are low, fact which puts Romania in a favourable position for developing animal husbandry sector (Velea and Mărginean, 2012). The sum of several of some favourable factors such as the natural grazing potential of the country, national and European subsidies programs, government programs for the purchase of purebred animals, extensive-intensive breeding technology, the price of meat per kg, population trends towards consumption of meat from animals raised in ecological conditions (Maciuc et al., 2015).

Thus, the beef cattle sector on national level can grow and perform but in order to archive this goals is unanimously necessary to constantly monitor the evolution of animal performance through the heerd book values (average daily gain, estimating breeding values, body condition score, DNA testing).

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ASSESSMENT OF THE ECONOMIC VIABILITY OF SUCKLER COW BREEDING MODEL

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Abstract

The weaning phase is one of the most important in the production cycle of a beef cattle farm, as it is the moment when the maternal capacity of the mothers is identified. The tendency will always be to sell the calves as heavy as possible, which may eventually delay weaning too much and overload the mother's milk production. In this study, a sample of 285 animals was taken from the ACOS Auction Service, in Beja, Portugal, in order to obtain real market numbers. The objective was to understand the economic results obtained from the sale of calves aged between 6 and 8 months, that is, around the weaning period, which can vary depending on the exploited breed, the intercalving interval and the daily cost of maintaining the suckling cow. In view of the results obtained, it was concluded that with financial support to the suckling cow, the productive efficiency depends on the daily cost of the cow, which should ideally be kept below 2,0€ and the intercalving interval below 395 days. Hypothetically, without financial support the intercalving interval must be maintained at 365 days, which obliges the breeder to optimize the efficiency of feeding and reproduction, at the same time that he will have to consider the exploited breed.

Keywords: *Weaning, efficiency, weight, suckling cow, calf.*

Introduction

The weaning phase is one of the most important in the production cycle of a beef cattle farm, as it is the moment when the maternal capacity of the suckler cows is identified, on the one hand, and, on the other, because it directly reflects the final value of the calf, on which the remuneration of the activity will depend.

On farms where the objective is to sell calves at weaning, it is the suckler cows that support the entire efficiency of the reproductive system, as increasing expenses with them decreases the efficiency of meat production (Henriques, 1998). This objective often has random results, as traditional management is the most practiced and consists of leaving the bull with the cows throughout the year. This option means that cows often give birth during unfavorable pasture season and if breeders do not supplement these animals adequately, the lactation is done at the expense of the cow's body reserves, which will later harm its return to estrous cyclicity. For these reasons, animals that come from the same breeding farm are sometimes very heterogeneous, which contributes to the devaluation of lots.

These problems become worse when, in addition to bad feeding seasons, the effects of genetics resulting from crossbreeds are added (often F1's are left as breeding stock) and the fact that males and females are valued differently at auctions.

For these reasons, it was decided to find out the selling price of calves aged between 6 and 8 months, using the Auction Service of the Association of Sheep Breeders of the South (ACOS) in the southern region of Baixo Alentejo in Portugal, and collecting the values of the animals auctioned at those ages on the days January 19th, February 16th, March 16th, April 6th, May 25th of 2023, in a total of 285 animals (161 males and 124 females), in order to understand if this breeding model is adequate to remunerate the activity.

1 - Brief overview of current knowledge

The main objective of a beef cattle farm is to obtain one weaned calf per cow per year (Valeria et al, 1999). Being a simple objective in appearance, it is one of the most difficult to achieve due to its low heritability and the interference of environmental factors that are very difficult to control and define. According to the authors Hamilton & Stark (1997), one of the key components for the success of this objective is the high level of fertility, which depends on the combination of the genetic potential of the suckler cow with several environmental factors, including the nutrition and management of the cows and of the bulls.

The shorter the average intercalving interval of a suckler cow, the greater the number of calves weaned and sold (Carolino et al. 2000). For this to occur, an intercalving interval of 365 days must be achieved, and the females must be pregnant up to 85 days after calving, never exceeding 115 days. If this does not happen, there may be several factors contributing to this situation, namely genetics, nutrition, environment and health status. The negative effects exerted on fertility are difficult to minimize, because the mentioned factors do not act independently, but interact with each other (Walker et al. 1994, *cit in* Bento, 2013).

The differences that were observed for the different ages also illustrate how data on weights and real prices can mask the genetic value of animals, environmental conditions and, above all, management, with obvious repercussions on the daily profitability of the suckler cow, since it is the sale of the weaned calf that pays its expenses.

According to Valeria et al. (1999) the reproductive traits are ten times more important in the productivity of beef cattle than the productive traits themselves. Among these, the intercalving interval stands out, which, together with the age at first calving and the duration of the productive life, will determine the total number of calvings throughout the cow's life.

Unfortunately, the economic results of extensive beef cattle farms are not very profitable, as this analysis published in the Report on CAP Strategic Plan - Portugal (2023 – 2027) on pages 658, suggests:

"*Regarding annual net income, beef cattle farms have on average (in the years 2016, 2017 and 2018) a net income of 720 € per suckler cow and per year.*

However, this income is reached taking into account the income from the activity combined with the dissociated support (556 €) and the associated support (132 €).

By analyzing the average profitability level of activities in the market, measured by the capacity of the holding's activities, within a framework of support totally disconnected from production, to subsist by itself, through the income they obtain only in the market, it appears that cattle holdings of meat would have an income from the market of around 32 € per suckling cow, that is, an income almost nil, which represents only 4.4% of the total net income.

It follows that without the existence of associated support, these agricultural systems would not exist in most cases, and without the associated support, in most cases there would be no economic reasons for maintaining a herd of cattle.

We also took into account the experience of applying this support in the current period, which allowed the maintenance of the staff without exceeding the established maximum thresholds, although registering a slight increase. Therefore, it is justifiable to reduce the payment amount per normal head from 130 € to 103 €, which will lead to an income of more than 700 € in the period 2023-27, with no risk of overcompensation."

But the main question remains: is there any economic viability of the system if, by hypothesis, there was no longer any support?

Material and methods

After consulting the elements made available by ACOS on its official website, the following data were collected: lot, number of animals/lot, total weight of the lot, average weight of animals in the lot, breed, sex and price/animal.
The following data were collected from the lots:
- 50 individual lots (35 males; 15 females. Total: 50)
- 33 lots of 2 animals (34 males; 32 females. Total: 66)
- 17 lots of 3 animals (24 males; 27 females. Total: 51)
- 27 lots of 4 animals (68 males; 40 females. Total: 108)
- 2 lots of 5 animals (10 females)

Total: 285 animals
Once all the elements were collected the animals were grouped by age and sex, and the average weight of each and respective price was determined. The maximum price reached was for a male with 1144.26 € and the minimum for a female with 273.34 €; the overall average price of the 285 animals was 693.70 €.
In order to obtain the average daily cost of the suckling cow, an average daily cost of 1.5€, 2.0 € and 2.5 € was arbitrarily assigned to it, to be applied over 3 possible intercalving intervals: 365, 395 and 425 days (12, 13, 14 months). Logically, the longer the intercalving interval, the greater the total cost attributable to the cow.
The income per cow was calculated based on the payment to the suckler cow of 103€/year, that is 103€/365 days, which represents a daily income of 0.28 €. To this value is added the value of the calf's sale, which divided by the number of days between calvings, will also result in a daily income. Obviously, the longer the intercalving interval, the more diluted the economic return from selling the calf will be. As a whole, the daily value of the premium (0.28 €) added to the daily value of the sale of the calf, must cover at least the daily expenses of the cow (from 1.5 € to 2.5 €). For each animal individually the results were observed and the dissociated support was not considered.

Results and discussion

Observing the data, the weight of the animals is not always decisive in the final price achieved, with the crossbred animals obtaining the highest prices, with the undefined designation of "crossbred beef". Examples include the results of the May 25th auction, in which an animal weighing 183kg at 7 months old, was sold for 550.83 € and another weighing 249kg at 8 months old was sold for 596.80 €, which means 45.97 € more for an increase of 66 kg in weight. In the same auction, the heaviest animal was a 7 month old male weighing 326kg, which reached 772.62 €, while another male of lesser weight (288kg), also aged 7 months, reached the highest auction price: 881.28 €. Furthermore, all animals were classified as "crossbred beef".
The sex of the animals was also an issue: in general the males were worth more than the females - 724.37 € against 648.29 €, on average - but there were females that reached very high prices; for example in the auction of March 16th 2 females exceeded the value of 1000 € as well as 3 females at the auction on February 16th. In both cases the animals were "crossbred beef".
When breed is considered, the following results are obtained: pure Limousine animals reached the highest sale price on average (863.37 € – 4 animals), followed by Charolais (784.86 € – 2 animals), Limousine crossbred (766.17 € – 26 animals), Angus crossbred (730.44 € – 3 animals) and finally "crossbred beef" (679.82 € - 250 animals), which means that purebred animals achieve higher sales values than crossbreds. However, as always there are exceptions

and as previously mentioned, the highest price obtained (1144.26 €) was for a "crossbred beef" male.

It is important to note that in the soil and climatic conditions of Baixo Alentejo, breeding herds should preferably be of indigenous breeds due to their hardiness and adaptation to the environment, despite their poor productive performance. However, these breeds are capable of giving birth and suckling a calf of a select breed, larger and heavier, which means that many animals appear at auctions with the designation "crossbred beef". Unfortunately, the degree of crossbreed is not always known, as there are animals that have genetics from 2, 3 and sometimes 4 breeds, in a variable degree and in a totally random way, just like the results of the weights at weaning.

When all calves are analyzed individually at their average selling price, it appears that with a minimum daily cost (1.5 €) per suckler cow, most cows produce a calf that covers their annual expenses, regardless of sex and breed of the calf produced. The exception occurred at the auction on May 25th, in which the females did not obtain sales prices that covered the expenses with their mothers, making it necessary to take from the associated support to compensate for the losses and keep de balance positive. This happened even with an intercalving interval of 395 days (see table 1).

When the value of the daily cost of a suckler cow rises to 2.0 €, the values clearly worsen, with negative results beginning to appear for animals that have intercalving intervals equal to or greater than 365 days. Even males are unable to obtain sales prices (with the exception of the auction on February 16th) that compensate for their mother's costs, with the positive balance in the accounts being ensured by the associated support of 103€. When we consider that in the Baixo Alentejo region intercalving intervals are normally longer than 395 days, we realize the difficulties that these results represent for the majority of beef cow farms (see table 1).

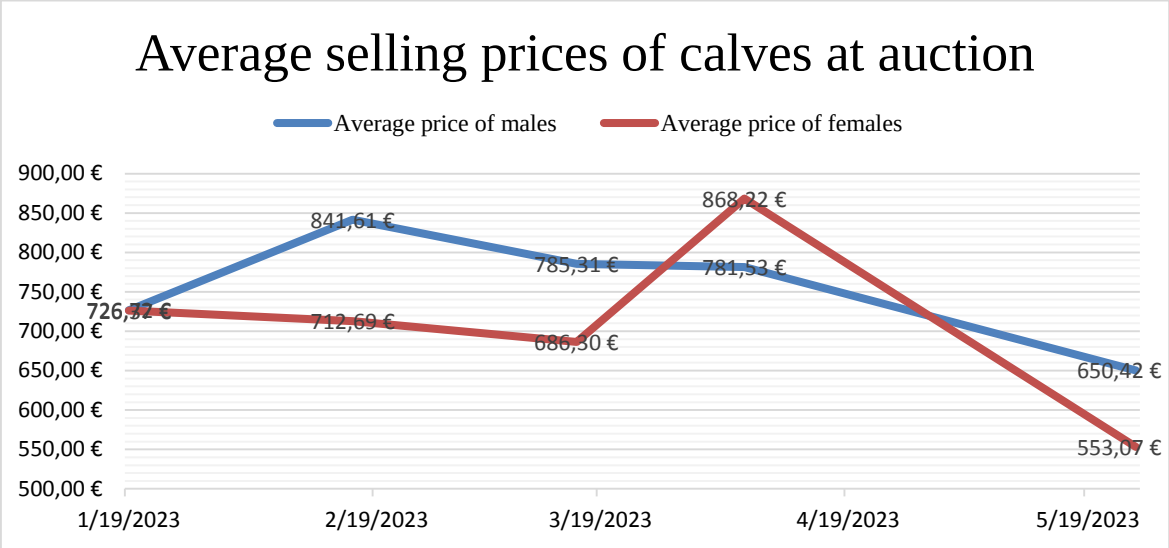
In the case of males, losses are smaller than in females, but sometimes there are exceptions such as the one that occurred in the April 6th auction, when the females obtained the best results from the 5 auctions as seen in graph 1.

Table 1. Average economic results depending on the daily cost of the suckler cow and the intercalving interval

Daily cost of the suckler cow		1,50 €	1,50 €	1,50 €	2,00 €	2,00 €	2,00 €	2,50 €	2,50 €
Intercalving interval		365 days	395 days	425 days	365 days	395 days	425 days	365 days	395 days
Balance zero at		547,5 €	592,5 €	637,5 €	730 €	790 €	850 €	912,5 €	987,5 €
Auction date	**Average selling price of males**	**Average net income**			**Average net income**			**Average net income**	
19-01-2023	726,72 €	384,72 €	237,22 €	192,22 €	99,72 €	39,72 €	-20,08 €		
16-02-2023	841,61 €	397,11 €	352,11 €	307,11 €	214,61 €	154,61 €	94,61 €	32,11 €	-42,89 €
16-03-2023	785,31 €	340,81 €	295,81 €	250,81 €	158,31 €	98,31 €	38,31 €		
06-04-2023	781,53 €	337,03 €	292,03 €	247,03 €	154,53 €	94,53 €	34,53 €		
25-05-2023	650,42 €	205,92 €	160,92 €	115,92 €	23,42 €	-36,58 €			
	Average selling price of females								
19-01-2023	726,37 €	281,87 €	236,87 €	191,87 €	99,37 €	39,37 €	-20,63 €		
16-02-2023	712,69 €	268,19 €	223,19 €	178,19 €	85,69 €	25,69 €			
16-03-2023	686,30 €	138,80 €	196,8 €	151,8 €	59,30 €	-0,70 €			

06-04-2023	868,22 €	423,72 €	378,72 €	333,72 €	241,22 €	181,22 €	121,22 €	58,72 €	-16,28 €
25-05-2023	553,07 €	108,57 €	63,57 €	18,57 €	-73,93 €				

Green squares: revenue generated from the sale of the calf plus 103 € of the associated support
Yellow squares: the balance remains positive thanks to the associated support of 103€
Red squares: net loss



Graphic 1 – Average prices of calves at auction

As would be expected, with a daily cost of 2.50 € at any calving interval, the vast majority of calves sold do not generate enough income to cover the costs of the suckler cow, even with the support of 103€. Due to the drought that was felt in Baixo Alentejo during the 2022/2023 agricultural year and the need to feed the animals with food purchased outside the farms which also rose a lot, the costs associated with suckler cows rose noticeably. This situation can lead to the lack of interest on the part of breeders and abandonment of the activity.

Conclusions

Given the need to conserve the genetic diversity of national species, the allocation of financial support to livestock species in general and to indigenous cattle in particular, is extremely important to encourage breeders to preserve national breeds wich are capable of crossing with selected breeds and producing calves at low cost, due to their low feed requirements.
Even so, to guarantee the income generated by the sale of calves at weaning plus the support of 103 €, it is necessary that the breed or crossbreed used must be well defined and boost the selling price of calves to values equal to or greater than 790 €, as long as the daily cost of the suckler cow does not exceed 2.0 € and the intercalving interval remains equal or below 395 days. In order to respect these values, the breeder must maintain strict control over reproductive and nutritional management, replacing the random results that come from traditional management.
Without the supplementary income of 103 €, the extensive production of beef cattle will be residual, as it will not generate sufficient returns to justify the activity. Due to the drought that was felt in Baixo Alentejo during the 2022/2023 agricultural year and the need to feed the animals with food purchased outside the farms which also rose a lot, the costs associated with suckler cows rose noticeably. This situation can lead to the lack of interest on the part of breeders and abandonment of the activity.

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SENSORS DATA IN AUTOMATIC MILKING SYSTEM AND MASTITIS

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Abstract

In this subject focus will be on sensors that Automatic Milking System (AMS) can collect during milking proces , funcition of the sensors that could help farmers indicate mastitis and other disises. Most common is detecting mastitis by measuring somatic cell count (SCC) and electrical conductivity (EC). For every 100,000 increase in cell count above 200,000, there will be a reduction in yield of 2.5%. When lactose concentrations in the udder fall, sodium and chloride levels increase to maintain the osmotic pressure of the milk. These changes can also be used to help assess mastitis status by electrical conductivity (EC) measurements, since sodium and chloride are much better conductors of electricity than lactose. Sensor systems are also available to measure a change in color, which is a visible aspect of abnormal milk, mostly due to cronic mastitis. By using computational algorithms, these data can be connected to events that are of interest to herd managers and thus can be converted to useful management information. When mastitis occurs it has affects on various milk components, yield of lactose and casein is reduced substantially and there are increased levels of the enzymes lipase and plasmin which break down milk fat and casein. Farms with milking robot have the possibility to monitor the physiological state of the cows on the farms by applying the results of milk analysis during milking, which enables an insight into the production/health status of each cow.

Key words: *Sensors, Automatic Milking system, Somatic cell count, eletrical conductivity, milk components*

Introduction

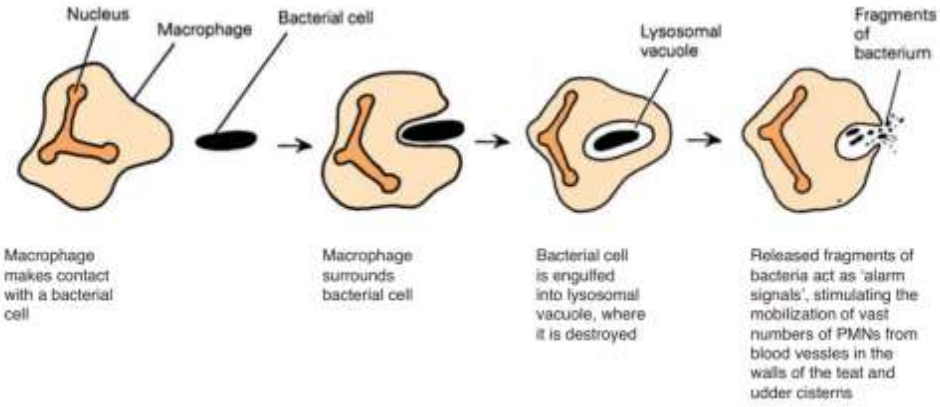
Automatic milking systems collect information on milk quantity and quality and cow health, which helps farmers better manage their herd. More recently sensor systems measuring somatic cell count (SCC) became commercially available. Most research where using milk quality variables, such as electrical conductivity, SCC, and color, to detect mastitis in Automatic Milking System (AMS). By using computational algorithms, the data from AMS can be connected to events that are of interest to herd managers and thus, can be converted to useful management information. Electrical conductivity (EC) is the measure of the resistance of a material to an electric current and is linked to a change in blood capillary permeability due to mastitis. For decades, this change in EC allso has been used as indicator for mastitis.

Somatic Cell Count

The somatic cell count (SCC) is the number of cells present in milk ('body' cells as distinguished from invading bacterial cells). It is used as one indicator of udder infection. Somatic cells are made up of a combination of white blood cells and epithelial cells. White blood cells enter milk in response to inflammation, which may occur due to disease, or occasionally to injury, they make up the majority of the somatic cells, especially when the cell count is raised. Individual cow cell counts are the best way to identify high cell count. A count of over 200,000 indicates subclinical infection. The majority of the losses in high cell count herds are from subclinical infection resulting in depressed production and reduced yields of lactose, casein and butterfat. It is generally accepted that herds with a cell count of 200,000 or less will have no significant production losses due to subclinical infection. For every 100,000 increase in cell count above 200,000, there will be a reduction in yield of 2.5%.

Sensor Systems

There is a variety of different types of cells in normal milk, but by not all of them can kill bacteria. The total number of cells can be counted and is expressed as the somatic cell count (SCC). The main function of macrophages and lymphocytes is to recognize bacteria in udder and then trigger alarm systems that induce a more vigorous host response, eventually leading to huge numbers of PMNs (polymorphonuclear leucocytes, mainly neutrophils) entering the milk. These alarm systems are the inducible defence mechanisms in mammary gland. PMNs are important bacteria-killing cells that originate from blood. However, in normal milk they are present in such low numbers as to be ineffective against a heavy bacterial challenge. Macrophages and PMNs engulf bacteria and destroy them. One of the methods of destruction is the release of lysozymes (destructive enzymes) within the PMN vacuole, with the resultant production of hydrogen peroxide. The hydrogen peroxide produced that way needs to be destroyed immediately by the action of glutathione peroxidase, a selenium-dependent enzyme. Failure to destroy the hydrogen peroxide can quite rapidly result in the death of the phagocytosing cell itself. Vitamin E reduces the rate of hydrogen peroxide formation within the PMN and stabilizes its cell membranes against its attack, while selenium increases the activity of glutathione peroxidase (Blowey and Edmondson, 2010).



Picture 1. Phagocytosis (Blowey and Edmondson, 2010)

An important compositional change consists of the influx of polymorphonuclear neutrophil leukocytes into the mammary tissue, making up a large proportion of the SCC (Kelly et al., 2000; Wickstrom et al., 2009), forming the basis of the use of SCC as a test to monitor udder

health (Harmon, 1994; Dufour and Dohoo, 2013; Damm et al., 2017). More recently sensor systems measuring SCC became commercially available. Two systems are measuring SCC indirectly, either based on gel formation of the milk (comparable to the California Mastitis Test; Deng et al., 2020) or physical measurements in the milk flow. The third SCC sensor is based on staining of a milk sample and optical counting of the number of cells by fluorescence (Dalen et al., 2019). In addition to sensor systems directly aimed at measuring mastitis indicators, many other sensor systems are on the market that measure one or more aspects that may serve as support to more specific mastitis sensors (Caja et al., 2016), activity monitors or behavior sensors (Van Hertem et al., 2016), milk production and constituent sensors, such as the commonly available electronic milk meters and fat and protein sensors, location sensors (Barker et al., 2018), rumination measurement (Grinter et al., 2019; Hamilton et al., 2019), temperature sensors (Kim et al., 2019), rumen pH sensors (Doroodmand et al., 2016) and automated body condition scoring systems (Spoliansky et al., 2016; Mullins et al., 2019). A sensor system based on inline measurements of l-Lactate dehydrogenase is commercially available. Following mastitis enzymatic reactions, l-lactate dehydrogenase will appear as part of the cow's innate immune response against infection (presence of udder pathogens) and changes in cellular membrane (Chagunda et al., 2006; Friggens et al., 2007), reflecting the host response to an intramammary infection (IMI) (Jorgensen et al., 2016). Sensor systems are also available to measure a change in color, which is a visible aspect of abnormal milk, mostly due to cronic mastitis. By using computational algorithms, these data can be connected to events that are of interest to herd managers and thus, can be converted to useful management information. Regarding mastitis management, algorithms are aimed at the identification of a deviation from normality that could be predictive of mastitis. As a further step, diagnosis can be carried out, defined as judgment about the presence of a particular illness after an appropriate diagnostic procedure has been performed.

Glucose is produced in the liver, primarily from propionate, a product of rumen fermentation. After it is transferred to the udder, part of the glucose is converted into another simple sugar, galactose. Next, one molecule of glucose combines with one of galactose to produce lactose. Lactose is known as a disaccharide (i.e. two monosaccharide sugars conjoined). When mastits occurs and lactose concentrations in the udder fall, sodium and chloride levels increase to maintain the osmotic pressure of the milk. This is one of the causes of the bitter and slightly salty taste of mastitic milk. These changes can also be used to help assess mastitis status by electrical conductivity measurements, since sodium and chloride are much better conductors of electricity than lactose (Blowey and Edmondson, 2010). Electrical conductivity (EC) is the measure of the resistance of a material to an electric current and is linked to a change in blood capillary permeability due to mastitis. For decades, this change in EC has been used as indicator for CM (Nielen et al., 1992;Hamann and Zecconi, 1998).

	Components	Effect of subclinical mastitis
Desirable	Total proteins	Decreased slightly
	Casein	Decreased between 6 and 20%
	Lactose	Decreased between 5 and 20%
	Solids not fat (SNF)	Decreased by up to 8%
	Butterfat	Decreased between 4 and 12%
	Calcium	Decreased
	Phosphorus	Decreased
	Potassium	Decreased
	Stability and keeping quality	Decreased
	Taste	Deteriorates and becomes bitter
	Yogurt starter cultures	Inhibited
Undesirable	Plasmin (degrades casein)	Increased
	Lipase (breaks down fat)	Increased
	Immunoglobulins	Increased
	Sodium	Increased – hence the 'bitter' taste

Picture 2. Effect of subclinical mastitis (i.e. raised cell count) on various milk components (Philpot and Nickerson, 1991.)

Picture 2 indicates that the yield of lactose and casein is reduced substantially when mastitis occurs, while the total protein level remains little changed, the level of casein is decreased by up to 20%. This is of great significance to dairy manufacturers, especially cheese makers. The changes in butterfat and lactose levels are of great economic significance to the farmer as they make up the basis of his milk price. Mastitis may cause a reduction in butterfat and protein, lowering the price of milk by up to 15%. Mastitis also produces increased levels of the enzymes lipase and plasmin, which break down milk fat and casein respectively and therefore have a significant effect on manufacturing yield and keeping quality (Blowey and Edmondson, 2010). The majority of protein in milk is in the form of casein. Amino acids are transported to the udder via the bloodstream and transformed into casein by the mammary alveolar cells. Once formed, casein is extruded from these cells in a mechanism similar to the fat droplets. Mastitic milk has a reduced casein content but increased levels of albumin and globulin. The total protein content of the milk may remain constant therefore, but the milk is of much poorer quality, particularly for manufacture. Mastitic milk contains increased levels of the enzyme plasmin, which decomposes casein in stored milk. Unfortunately, plasmin is not destroyed by pasteurization and it remains active even at 4°C. Mastitic milk will therefore continue to be degraded even following pasteurization and storage at 4°C. Milk fat is formed in the udder secretory cells when fatty acids are combined with glycerol and converted into a neutral form of fat called triglyceride. Besides the enzyme plasmin, mastitic milk also has an increased level of the enzyme lipase. This leads to degradation of the milk fat into its fatty acid components and thus imparts a rancid flavour to the milk. (Blowey and Edmondson, 2010). An important advantage of sensor systems is the availability of multiple measurements per day from Automatic Miliking System (AMS). By clearly defining the need for detection of subclinical mastitis (SCM) and clinical mastitis (CM) from the farmers management perspective, detection and management of SCM and CM may be improved. Sensor systems may also be used for other aspects of mastitis management. Although never studied, it is expected that because of the effects of severe CM, such a high detection performance is feasible. Algorithms to detect events of interest may use data from one or more sensors of AMS, potentially combined with data from other (farm) sources (Dominiak and Kristensen, 2017; Slob et al., 2021). The algorithms are expected to detect deviations from normality that could be predictive of a specific status that is of interest to the decision maker. Basic evaluation measures of a sensor system are repeatability and reproducibility. Repeatability can be defined as the closeness of the agreement between the results of successive measurements of a sensor in the same sample. The repeated measurements should be done under circumstances as equal as possible. Reproducibility can be defined as the closeness of the agreement between the results of successive measures of the sensors under varying circumstances, using another sensor and measuring under different circumstances. New research has appeard based on behavior and production measures to detect locomotion and metabolic disorders such as lameness, hoof disorders, ketosis, subclinical ketosis (SCK), displaced abomasum (DA), metritis, and pneumonia, in addition to briefly discussing mastitis detection. New cases of lameness in AMS were generally associated with lower milk yield, rumination time, milk temperature, supplement intake, and milking and refusal frequencies compared with healthy cows. for lame cows, milk temperature was the only variable that deviated negatively from the baseline. A key feature of AMS is data collection and subsequent generation of management reports and alert lists. Data can be used to create management reports and task lists, as well as attention lists of cows with potential health problems. Despite some inconsistencies in the literature, the potential certainly exists to

combine the use of several behavior and production variables to create highly sensitive and specific health detection models. The goal should be to achieve 99% specificity and 80% sensitivity, as recommended by the International Organization for Standardization, to evaluate model performance for automated detection of abnormal milk (Rutten et al., 2013). One study investigated the potential for accurate detection of clinical mastitis (CM) in an automatic milking system (AMS) using electronic data from the support software. Data from cows were used to develop the model, which was then tested on 2 independent data sets, one with 311 cows (same farm but from a different year) and other with 568 cows (from a different farm). In addition, the model was used to test how well it could predict CM 1 to 3 days before actual clinical diagnosis. Logistic mixed models were used for the analysis. Twelve measurements were included in the initial model before a backward elimination, which resulted in the following 6 measurements being included in the final model: quarter-level milk yield (MY; kg), electrical conductivity (EC; mS/cm), average milk flow rate (MF; kg/min), occurrence of incompletely milked quarters in each milking session (IM; yes or no), MY per hour (MYH; kg/h), and EC per hour (ECH; mS/cm/h) between successive milking sessions. Many commercial brands supplying AMS already incorporate a variety of milk monitoring or sensing equipment (electrical conductivity, milk yield, milk flow rate, incomplete milking, kick-off), and some researchers have been working to develop algorithms that use and integrate data captured during the milking process to find the most accurate mastitis alert guideline (Hogeveen et al., 2010; Rutten et al., 2013). To prevent further animal suffering and potentially the death of the cow, the purpose of a sensor system to detect cows with severe CM is to detect virtually all severe cases of CM within hours after, and definitely not more than 12 h after, the onset of the severe CM. A very high (close to 100%) sensitivity is needed to ensure the trust of farmers in the system to be able to detect cows that would definitely be detected visually. Because of the clear consequences of severe CM (toxic mastitis) on SCC, EC, and milk production, it is expected that a very high sensitivity is possible. As a result, Tse et al. (2017) reported that, after transitioning to AMS, 66% of producers changed their health management strategy and 80% of producers found illness detection to be easier than before transitioning because of the AMS and associated health-monitoring software.

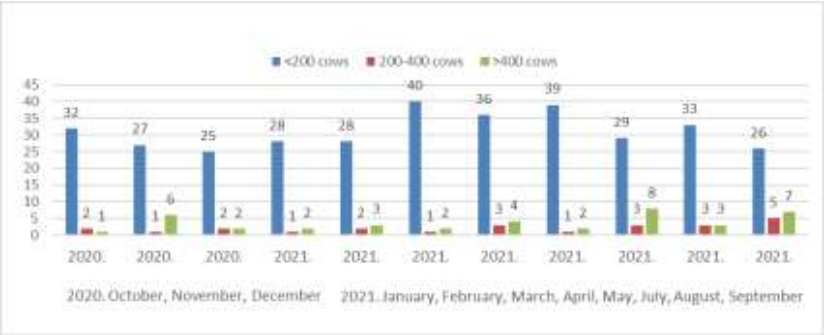
SOMATIC CELLS ACCORDING TO THE STAGE OF LACTATION

Day of control: 28.4.2021.

	First calves			Other cows		
Lactation days	**Cows**	**> 400.000**	**%**	**Cows**	**> 400.000**	**%**
until 60	5	1	20,0	5	0	0,0
60-120	4	0	0,0	6	1	16,7
121-240	0	0		8	0	0,0
240 and more	3	0	0,0	11	2	18,2
	12	**1**		**30**	**3**	

Movement of the number of somatic cells during the year

		do 200.000		od 200.000 do 400.000		od 400.000	
Month of control	**All cows**	**Cows**	**%**	**Cows**	**%**	**Cows**	**%**
10.2020	35	32	91,43	2	5,71	1	2,86
11.2020	34	27	79,41	1	2,94	6	17,65
12.2020	29	25	86,21	2	6,90	2	6,90
1.2021	31	28	90,32	1	3,23	2	6,45
2.2021	33	28	84,85	2	6,06	3	9,09
3.2021	43	40	93,02	1	2,33	2	4,65
4.2021	43	36	83,72	3	6,98	4	9,30
5.2021	42	39	92,86	1	2,38	2	4,76
7.2021	40	29	72,50	3	7,50	8	20,00
8.2021	39	33	84,62	3	7,69	3	7,69
9.2021	38	26	68,42	5	13,16	7	18,42
	407	**343**	**84,31**	**24**	**5,90**	**40**	**9,80**



Picture 3. SCC according to lactation stage (CAAF, 2022.)

Cows in the herd are shown according to the number of somatic cells (BSS) in different stages of lactation, separately for heifers and other cows. The limit value of BSS is the industrial value of 400,000 somatic cells in ml of milk. The display also provides insight into the movement of the number of cows according to BSS classes (up to 200,000, 200,000 – 400,000 and more than 400,000) during the last 12 months (CAAF, 2022.).

Conclusions

Automatic Milking System have been among the most important technological solutions and innovations in recent years because they have shown a number of advantages compared to conventional milking, which can be seen in relation to higher daily milk production per cow in AMS systems, saving of human labor, increased length of the productive life of the cow and better farm management. Using all the sensors that milking robots have, farmers claim that they have improved the cow's health, whether it is reproductive problems, mastitis or hooves. Farms with milking robot have the possibility to monitor the physiological state of the cows on the farms by applying the results of milk analysis during milking, which enables an insight into the production/health status of each cow.

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TOP-DOWN AND BOTTOM-UP REGULATIONS AT THE ALGERIAN COASTS (1°W – 8°E): MODELING OF COPEPODA - MEDUSOZOA RELATIONSHIPS

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Abstract

The interrelations between two major components (Copepoda and Medusozoa) of the food web were analysed from a series of interannual samples (2014 to 2017) in several ecosystems of the Algerian coasts (Western, Central and Eastern). The prey (Copepoda) and predator (Medusozoa) relationships were approached by the prey-predator model of Lotka – Volterra. The results from the modelling showed the dependence of predators (Medusozoa) on their prey (Copepoda). The latter are subject to strong predation pressure qualified as Top-Down regulation. The regression of stocks in Copepoda induces a regression of Medusozoa abundances (Bottom – Up regulation). A notable observation is the influence of seasonal variability on both types of regulation, encompassing top-down and bottom-up processes. Seasonal shifts, driven by factors such as water temperature and prey availability, play a pivotal role in shaping the interactions between these two crucial marine organisms.
This study's significance lies in its contribution to our understanding of the intricate ecological dynamics along the Algerian coasts. It illuminates how Copepoda and Medusozoa interact within the food web and how their populations are meticulously regulated through both top-down predation (Medusozoa on Copepoda) and bottom-up prey availability (Copepoda) mechanisms. Furthermore, the study underscores the crucial role of seasonality in governing these marine ecosystems, emphasizing the need to consider these seasonal fluctuations when managing and preserving these delicate environments. These insights are invaluable for marine ecologists, conservationists, and policymakers aiming to protect and sustain these coastal ecosystems.

Keywords: *Copepoda, Medusozoa, Algerian coasts, Prey and Predators interactions, seasonal variability.*

Introduction

Zooplankton play a major role in marine ecosystems: they are responsible for the flux of nutrients in trophic chains (Anderson and Lucas, 2008), they exercise top-down control over primary producers (Banse, 1995), and actively participate in the remineralization of organic matter (Ducklow et al., 2000). Through their relationship with producers, copepods are of considerable importance within the microbial chain (Turner, 2004). They also serve as prey for large carnivorous organisms (gelatinous zooplankton, ichthyoplankton) (Ara & Hiromi, 2007; Cotté et al., 2009; Kherchouche & Hafferssas, 2020; Mellak et al. 2023 *in press*).
Among these, the Medusozoa play an important role in controlling the population size of copepods. This mesozooplankton group is considered a significant prey item for jellyfish and siphonophores (Colin et al., 2005). This trophic relationship has been previously documented in the Mediterranean (Colin et al., 2005; Fernandez de Puelles et al., 2007), the Atlantic Ocean (Sanvicente-Aňorve et al., 2007). According to Purcell (1997), medusozoans can consume 13 to 94% of the copepod stock per day. Indeed, when they are abundant, the prey population can be significantly affected (Purcell, 1982; Sabatés et al., 2010). An example of

this occurred in the Adriatic Sea in 1997 when an invasion of *M. atlantica* modified the structure and the quantitative distribution of copepod fauna (Kršinić & Njire, 2001).
In the Algerian coast, the majority of studies have primarily focused on the biodiversity and abundance of zooplanktonic groups (Seridji and Hafferssas, 2000; Hafferssas and Seridji, 2010; Hafferssas et al., 2010; Ounissi et al., 2016; Chaouadi and Hafferssas, 2018; Khames and Hafferssas, 2018, 2019; Kherchouche and Hafferssas, 2020; Mellak and Hafferssas, 2023). This study aims to provide supplementary information on the interactions between copepods (prey) and medusozoans (predators), examining the implications of both types of regulation (Top-Down and Bottom-Up) in relation to seasonal variability.

Material and Methods

The planktonic samples were collected along the Algerian coast between 1°W and 7°E including several years (from 2012 to 2017). More than 96 samples were taken there: 12 at western (1°10' W - 1°10'W); 77 at central (2°30'E - 4°30'E) and 7 at eastern (6°13'E-6°53'E). The zooplankton populations were sampled by vertical hauls between 0 and 100 meters' depth. The WP2 net type with a 200 µm mesh size was used (SCOR - UNESCO, 1968).
In order to describe the trophic interactions between two zooplankton groups (Medusozoa and Copepoda) within the food web, the prey–predator Lotka–Volterra model was used by Past ® software. This describes the effects of upward (bottom-up) and downward (top-down) forces, governed respectively by producers and consumers (Hunter & Price, 1992). Copepods were considered such as prey and Medusozoa such as predators. The simultaneous evolution of prey and predator populations was described below (Fig 1): high predation of prey and increased abundance of predators (quadrant I); rarefaction of predators due to the decrease in the abundance of prey (quadrant II); low predator numbers and increasing prey stock (quadrant III) and regeneration of prey and predator abundances (quadrant IV).

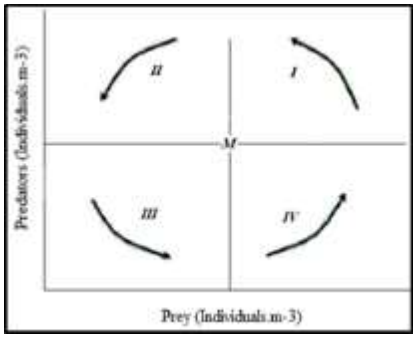

Figure 1. Lotka – Volterra trophic interactions between two zooplankton groups (Medusozoa and Copepoda) within the food web (Bertrandias & Bertrandias, 1994)

Results and Discussion

Within this region, the model describes four different states (Fig. 2). The first is linked to the communities of station 1.1 (quadrant I of the model). The Copepods during Spring are heavily exploited by Medusozoa. These exert top-down control over prey. Nevertheless, during the summer season, this pressure is non-existent due to the decline in the abundance of predators. The second state (quadrant II) relates to the spring communities sampled in station 1.2. The predation pressure is low; the abundances of Medusozoa are decreasing. This state is due to a low availability of Copepoda populations. In the summer season, the numerical stocks of the two planktonic groups regenerate (quadrant IV). The third state (quadrant III) corresponds to

an increase in the spring abundance of copepods, linked to the low presence of gelatinous zooplankton. This situation is described at stations 1.3 and 2.3. In Summer, the communities of the first station describe the same tendency. Nevertheless, the interactions from the communities of station 2.3 show a strong predation pressure on copepods. Medusozoa exert top-down control (quadrant I).
Finally, in the spring situation, the last state (Type IV) is linked to station 2.1. The abundances of prey and predator populations jointly begin to increase. This is also the case for the summer communities from station 2.2. The quantitative reduction of predators is correlated with the extinction of prey (station 2.1, in Summer).
Overall, the change in the types of interactions from one season to another is a major characteristic (80%) within Habibas ecosystem (case of stations (1.1, 1.2, 2.1, 2.3).

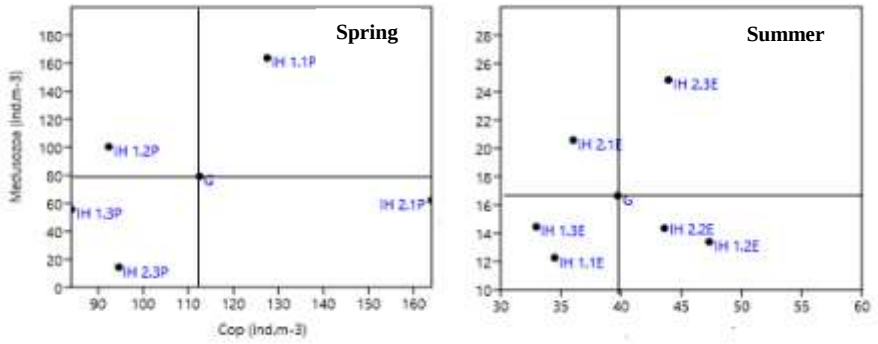

Figure 2. Variation of the Medusozoa - Copepoda seasonal interactions at the level of the Habibas archipelago

Ecosystem of the coasts of Sidi Fredj (Summer 2015)
According to the model, the Copepod species located within station 1.2 are subject to strong predation pressure where bottom-up type regulation is evoked (Fig. 3). This promotes an increase in the abundance of macrozooplankton populations. On the contrary, the reduction of this pressure is clearly favorable to the regeneration of the abundance of Copepod species relating to stations 1.1, 2.1, 2.2 and 2.3. Within station 1.3, the quantitative regression of copepods has a negative impact on the abundances of gelatinous zooplankton.
Overall, the dominant trophic interactions (50%) are mainly of type 3 where the low predation pressure of Medusozoa favors the numerical increase of Copepods.

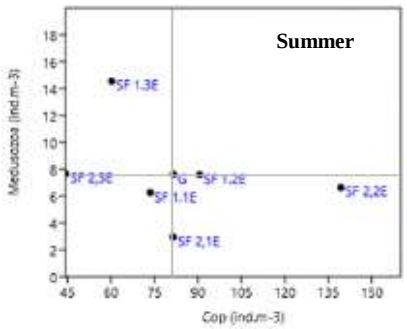

Figure 3. Variation of the Medusozoa - Copepoda interactions at the level of the Sidi Fredj coasts

Ecosystems of Bou Ismail Bay (Automn 2014, Spring 2016 & Autumn 2016)
At the level of this ecosystem, Medusozoa – Copepoda type 3 interactions are almost – dominant (more than 70%) (Fig 4). Indeed, low numerical levels of predators and increase in the stock of prey characterize stations BB1, BB2 (Autumn 2014), BB.1.1, BB.1.2, BB.1.3, BB.1.4, BB.1.5, BB.2.1, BB .2.2, BB.2.3, BB.2.4 (Spring 2016), BB6, BB.4.1 and BB.4.2

(Autumn 2016). Medusozoa exert predation pressure on Copepods at station BB5, in the Autumn of 2016. During this same season, the 2 zooplanktons' communities are in the quantitative regeneration phase (stations BB7, BB.3.2, BB.3.3) see in extinction (station BB.3.1).

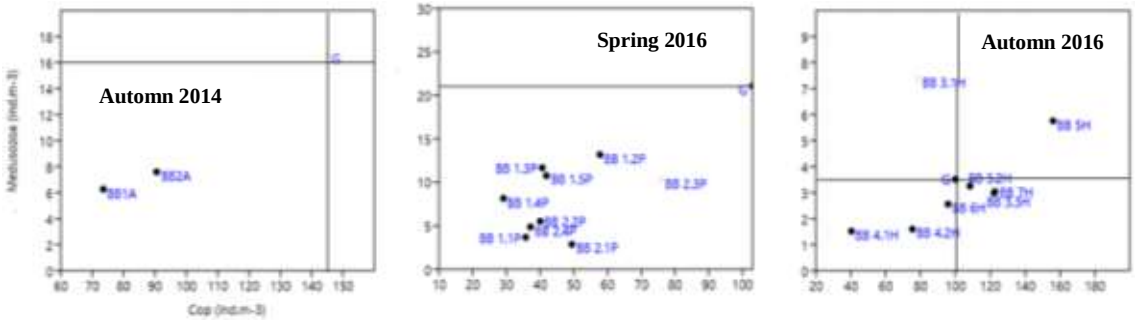

Figure 4. Variation of the Medusozoa - Copepoda interactions at the level of the Sidi Fredj

Ecosystems of Algiers Bay (Autumn 2014, Winter 2015, Winter 2016, Spring 2016, Summer 2016, Spring 2017, Winter 2017, Spring 2017)

From one season to another, the Lotka – Volterra model extracts 2 major trends: i) stability of trophic interactions and ii) dominance of one type of interaction to the detriment of the 3 others (Fig. 5)The first trend characterizes the trophic interactions between Medusozoa and Copepods from many stations. Indeed, stage 4 (regeneration of the abundances of the 2 zooplankton groups) is constant between Winter 2015, Spring 2016 and 2017 (station BA.3.4). Stage 3 (low predator numbers and increased prey stock) is maintained at station BA.2.1 during the spring (2016 and 2017) and summer (2016) seasons. Stage 1, linked to the optimal exploitation of Copepods, appears during Summer (2016) and Spring (2017). Type 2 interactions (rarity of predators due to the decrease in abundance of prey) are stable at the level of two trophic components of the station (BA.4.2) during the winter and spring periods of the year 2017.

The second tendency (dominance of one interaction to the detriment of others) is frequently reported (67% to 50% of cases) by the communities:

- the BAP port station where the scarcity of predators due to the decrease in prey abundance is constantly observed between the 6 sampling seasons (Winter 2015 and Spring 2016, 2017 and Summer 2016);
- station BA.3.3 (Winter 2016 and Spring 2017) whose copepod communities show a strong increase. This situation is favoured by the rarefaction of the abundance of Medusozoa;
- from station BA.3.2 (Spring 2016 and 2017) where a regeneration of prey abundance is followed by that of predators;
- station BA.3.1, where Medusozoa predation pressure on Copepods is dominant and spreads out between Winter and Spring 2017.

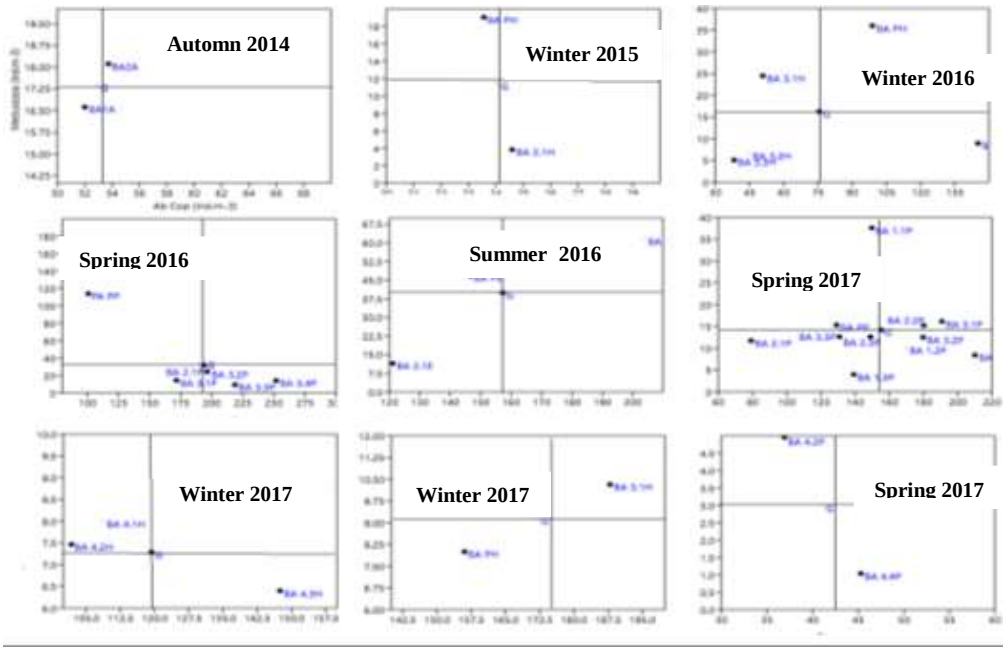

Figure 5. Variation of the Medusozoa - Copepoda seasonal interactions at the level of the Algiers bay ecosystems

Ecosystems of the coasts of Ain Chorb (Winter 2016, Spring 2016 & Autumn 2016)
The Copepods hosted by stations AC.2 and AC.3 are in the regeneration phase (stages 3 or 4); their predators also describe low abundances during the 3 sampling seasons (Winter 2016, Spring 2016 and Autumn 2016) (Fig 6). This characteristic extends to the interactions within the communities of station AC.1 in the spring or autumn periods. Nevertheless, on the latter, Medusozoa are rare (stage 2). Conversely, the winter communities linked to station AC.1 show strong predation by Copepods (stage 1). This situation is corroborated by the level of abundance of Medusozoa.

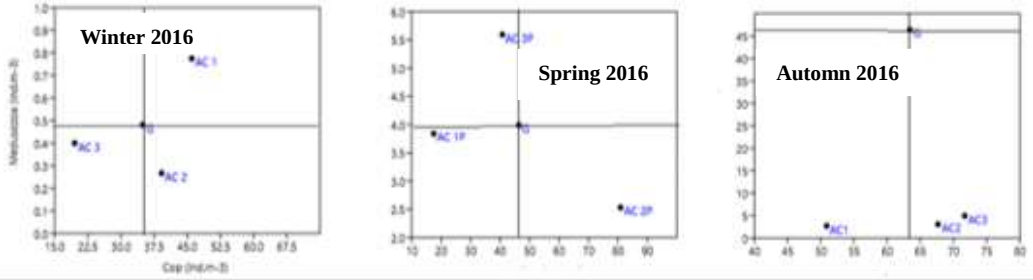

Figure 6. Variation of the Medusozoa - Copepoda seasonal interactions at the level of the coasts of Ain Chorb

Tizi Ouzou coastal ecosystems (Autumn 2014)
Two types of trophic interactions are described at the level of this ecosystem during the autumn period of 2014 (Fig 7). Type 2 relates to the communities sampled in the ETA station where the predation pressure is low; the abundances of Medusozoa are decreasing. This state is due to a low availability of copepod populations. Type 3 corresponds to an increase in Copepod abundance, linked to the low presence of gelatinous zooplankton. This situation is described at the level of the EAA station.

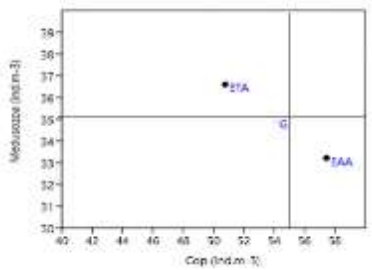

Figure 7. Variation of the Medusozoa - Copepoda interactions at the level of the Tizi Ouzou Ecosystems of the coasts of the Jijel – Skikda (Autumn 2014)

During the fall of 2014, the Medusozoa – Copepoda interactions show three trends (Fig. 8):

- Copepod predation by predators (case of EBJA & BBS3A stations);
- Predation pressure is low (case of stations BC2.A & BBS1.A); predator abundances are declining. This state is due to low availability of prey populations;
- An increase in copepod abundance, linked to the low presence of gelatinous zooplankton. This situation is described at stations BC1.A & GSA.

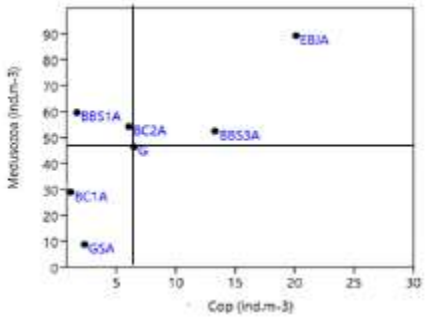

Figure 8. Variation of the Medusozoa - Copepoda interactions at the level of the Jijel – Skikda coasts

Our study is confirmed by many results (Benović et al., 2005; Colin et al., 2005; Fernandez de Puelles et al., 2007). Copepods are preferred prey for top predators (Mellak et al. 2023 *in press*). Being selective (small stomach size), the food component of Siphonophores is dominated by small Copepods (Purcell, 1982; Pestorić et al., 2012; Licandro et al., 2012).

Conclusion

A study of six ecosystems along the Algerian coast has shown that Medusozoa populations are linked to copepod populations. These populations interact trophically, leading to: i) an increase in Medusozoa; ii) a reduction in copepod populations; iii) a rarefaction of Medusozoa and iv) an increase in Copepod populations, or to the regression of both populations at the same time.

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THE HUMAN ANIMAL RELATIONSHIP IN DAIRY FARMING

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Abstract

This study was carried out in dairy farms in two different areas on a number of breeds (Holstein and Montbeliarde) at different ages (< 1 year and > 1 year). The total number of the first farm was 34 individuals, the latter was located in the village of Sidi Omar Al –Abadia d'Ain defla city. The total number of the second farm was 36 individuals, this farm was located in EL hamoul communal of the village of Rouina, Ain Defla city. The objective of this study was to determine the situation of the human-animal relationship in cattle farming. The results showed that the avoidancetest in the first farm and the second farm was 12.02-70.30 cm, respectively(p=0.0001 , proving that the farmer on Farm 1 had a good relationship with his flock compared to the farmer on Farm 2. The young cattle showed a distance of avoidance shorter than the adults at the value of 1.68-132.27 cm, respectively, (p=0.00001), and this was due to the adaptation of the young cows to a man, It was also noted that the Montbeliarde and Holstein cows had a leakage distance of 119.5-81.92, respectively, (p=0.03164). In conclusion, this study showed that cattle's behaviour towards humans is directly related to age, race, how the breeder treats them and the welfare conditions they provide.

Keyword: *human-animal relationship, avoidance distance, welfare, behavior, psychological freedom.*

Introduction

The quality of the human-animal relationship plays a central role in defining the welfare of the animals (Waiblinger, 2019 ; Hemsworth *et al.*, 2000), and undoubtedly, in dairy animals the human-animal interactions are more frequent and more intensive than in the other farm species as some procedures are performed daily (handling, milking). (Boivin *et al*., 2012) This relationship can range from a predator/prey-like response (high flight distance and high fear of humans) to dominance-like interactions (medium to short flight distance and submissive approach to humans) and affiliative-like relationship (no flight distance and humans perceived as social partners) (Rushen *et al*., 2001). The fear of man therefore affects the safety of the breeder but also on the welfare of animals. It is thus the cause of significant problems within the livestock: handling difficulties and risks increased accidents to animals and breeders (Le Neindre *et al*., 1996), product quality (Hemsworth, 2003 ; Mota-Rojas *et al*., 2020) and immunosuppression due to stress (Breuer *et al*.,2000). However, the breeding of large herbivores is now experiencing deep changes in the world.The number of animals per breeder increases strongly and the time spent by humans with animals decreases proportionally. Contact with humans could become even more challenging with increasing use of technologies. In this way, it is possible to reduce the opportunities for animals to become accustomed to humans**.** (Waiblinger *et al*., 2006).
The objective of this study was to determine the situation of the human-animal relationship in the dairy farms of the city of Ain Defla.

Materials and methods

To study the quality of the human-animal relationship in cattle farming, authors visited two breeding cattle farms. The first farm was in region village of Sidi Omer municipal of EL Abadia city of Ain defla, and the second farm was in El hamoul municipal of Rouina city of Ain defla. In order to study the psychological freedom of the cattle during the spring season of 2022, we conducted the avoidance test by measuring the distance of escape or fear for the whole cattle, according to the method (Waiblinger *et al*., 2003) on 70 cattle of two breeds (Montbeliard, Holstein-Frisian) and of different ages. All statistical analyses were performed with the "XL stat" program, by applying the Student test (comparison of two means).

Results and Discussion

1/ Farmer's effect on the human animal relationship

It can be seen from table 1 that the avoidance distance was lower at farm 1 than at farm 2 at the value of (12,02- 70,30) cm respectively (p=0.0001). This means that the farmer on farm 1 have a good relationship with his animals compared to the farmer on farm 2.

In breeding, certain interventions (insemination, veterinary care) are perceived as negative by animals: they correspond to unpleasant manipulations imposed by man. The animal associates man with these unpleasant manipulations and can then manifest escape or defense reactions (Pajor *et al.*, 2000). The nature of contact is indeed an important determinant of human fear (Hemsworth, 2003) and conditions the response of animals to humans.

Gentle contact during the intervention (Lange *et al*., 2020), as well as the usefulness of food distribution as a reward after the intervention (Mounaix *et al*., 2007*)* allowed a better contact between the breeder 1 and his animals. Waiblinger *et al*., 2002 sayed that for improving human–animal interactions on small dairy farms farmer must changing their attitudes and behaviour towards animals.

Table 1. The comparison of the avoidance distance according to different factors

Factors	farmer1	farmer 2	Montbeliard	Holstein	young	adults
M	12,02	70,30	119,5	81,92	1,68	132,27
STD	34,49	66,22	38,41	75,15	2,14	23,48
SEM	5,91	11,35	10,27	20,09	0,45	5
p	**0,0001**		**0,03164**		**0,00001**	

M= Mean of the avoidance distance (cm) ; STD= *standard deviation ; p= p value <0.05 ; SEM= Standard error of the average*

2/ Age's effect on the human animal relationship

It can be seen from table 1 that the avoidance distance in young animal compared to adults was lower at a value of (1,68- 132,27) cm respectively (p=0.00001).

The reason why is because of the habituation of the early contact between the breeder and the calves has create a positive harmony for their relationship (Ujita *et al.*, 2021). Tactile contacts and distribution of food by man shortly after birth decrease the escape distance, facilitate the approach by reduce fear and improve the future docility of the animal (Mounaix *et al.*, 2007**)** On the other hand, man is associated with food, he can be considered a social substitute for the mother (Boivin *et al.*, 2003).

3/ Race's effect on the human animal relationship

It can be seen from the table (1) that avoidance distance of the Montbelirad cows was higher than in Holstein cows at value of (119,5- 81,92) cm respectively (p=0.03164).. Genetic

dispositions contribute to explaine differences in human animal relationship. (Rimbaud, 2015). Breeds subjected to intensive selection had negative relationshipe toward humans than breeds which had not been subjected to such selection. (Lankin, 1997) As a result, our work is in contradiction with two studies which have not shown a link between the animal's escape response in relation to humans and the genotype of the cattle tested (Waiblinger *et al*., 2003 ; Boivin *et al*., 1992).

Conclusion

The fear reaction in cattle is due to the negative interaction or lack of human contact with the animal, and this impedes the development of a good human-animal relationship. The human-animal interactions can be also related to age and race

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PERIPHERAL INDICATORS OF ACIDOSIS IN DAIRY COWS

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Abstract

The welfare of dairy cows is critical for ensuring the highest production, therefore it's crucial to maintain the ruminal environment in excellent condition so that it can operate efficiently. The threat of rumen acidosis is permanent, particularly for cows with high dairy potential. The acute form is the most dangerous, whereas the chronic or latent form is the hardest to identify because there are no indicative symptoms during a clinical examination. Our objectives were to develop a method to diagnose chronic acidosis in animals without harming them based on peripheral acidosis indicators and to research the bentonite's therapeutic benefits. The study was conducted on 20 Holstein and Montbéliard dairy cows at the farm level of Sidi Mhamed Benali, Wilaya de Relizane. The parameters that were examined were the rumen filling score, the chewing index, the measurement of the amount of undigested food, the measurement of the consistency of the dung, and the determination of its pH. A diet containing (5 Kg of Miscellaneous Concentrate, 7 Kg of alfalfa hay (Sec), 17 Kg of Sorghum green + 1.5% bentonite or 250g/cow/day) was distributed to Lot 1 and 2, except that lot 2 received (1.5% bentonite or 250 g/cow/day) in addition. The results revealed a significant difference between the dung pH, dung consistency, and chewing index of our sample in comparison to the usual values of (6.42, 2.78, and 55.53, respectively) (p 0.05), while lot 2 receiving bentonite showed an improvement in dung pH and chewing index in comparison to lot 1 by (6.55, 6.29), and (61.5, 49.74), respectively (p 0.05). In conclusion, chronic acidosis can be diagnosed using peripheral indicators, and the usage of bentonite has prevented this pathology.

Keywords: *animal welfare, dairy production, bentonite, acidosis, food management.*

Introduction

National milk production has undergone remarkable changes in recent years. Nevertheless, the quantities produced are still insufficient to meet the needs of the growing population. This obliges the state to use imports to cover the gap between production and consumption (Kaouche-Adjlane, 2019). Acute acidosis in ruminants is fatal, rare and accidental. This type of acidosis gives way to a subclinical or latent acidosis state characterized by a greater number of affected animals and low mortality. This type of acidosis is to be feared because its installation in a herd has a negative effect on production (Galyean and Rivera, 2003). To meet the very high nutritional needs of animals, farmers distribute a higher-grain and lower-fibre diet to maximize energy intake in early lactation, This type of rationing causes a disorder of ruminal fermentation or digestion. A rapidly fermentable diet with low chewing time and insalivation (Sauvant *et al.*, 2006) jeopardizes the control of acidity in the rumen. There is no secure rationing system that prevents acidosis apart from the use of buffers (Peyraud and Apper, 2006). Monitoring of latent acidosis has been less studied (Martin *et al.*, 2006). The parameter to best characterize the state of acidosis is ruminal pH. In addition, this measurment in the field has limitations in both practical and animal welfare terms (Nejash, 2016). The diagnosis of the disease must be supported by parameters other than ruminal pH. The

objective of this study is to diagnose latent acidosis using peripheral indicators and to study the effect of bentonite on these indicators.

Materials and methods

Our study was carried out at the level of the municipality of Sidi Mhamed Benali wilaya of Relizane, on a number of 20 cows of breed Prim'Holstein and Montbéliard of which 8 cows were used to evaluate the effect of bentonite on peripheral indicators. These 8 cows were divided into two lots (lot 1= the control lot and Lot 2= the lot that received the bentonite). All cows were in second lactation and were followed during the calving period. The equipment used is as follows :

- One sieve diameter (2 and 5) mm
- Bomann balance (made in China)
- 100 mL capacity beaker
- Laquatwin pH meter, Horiba
- Distilled water
- Bentonite

Records were made based on the following parameters:

1. Assessment of the dung's pH: The determination of the dung's pH was carried out using a PH meter, according to the method of (Sakouvogu et al., 2018).
2. Evaluation of the dung's consistency : Dung is the final product of digestion, their evaluation allows to assess digestibility and food efficiency. Their colour, consistency, presence and size of the fibres, presence and shape of the grains are evaluated according to the method of (Guedon, 2017).
3. Rumen Fill Assessment: The rumen fill note is scored 1-5 by observing the cow's left flank using the method of (Burfeind et al ., 2010).
4. The undigested food fraction was made using standards established by (Kleen et al., 2003 ; Vagneur, 2007).
5. The evaluation of the mastication index : it was carried out when the cows are at rest (during the rumination phase), We counted the number of mastications per minute (sauvant et al., 2008).

The average daily ration per cow distributed twice a day was (5 kg of various concentrates, 7 kg alfalfa hay (dry), 17 kg of green sorghum combined with 1,5 % of bentonite according to Kaboul and Ouchem in (2012) for batch 2, is 250 g / cow / day

The statistical analysis was performed with the SPSS software using the student's test.

Results and Discussion

The average production of cows during the first month of the share was (20.5± 4.31) l/j. This result is insufficient according to Kadi *et al.* (2007), for farms which breed the two breeds Prim Holstein and Montbéliarde. It was observed in Table 1 that there is a significant difference between the dung's pH, the dung's consistency and the chewing index of our cow sample compared to the usual values of (6.42, 2.78, 55.53) respectively ($p<0.05$). While there was no significant difference between the undigested fraction and rumen filling in our sample compared to the usual values of (16.92, 3.03) respectively ($p<0.05$).

Table 1. Comparison between standard and experimental pH values, CD, RF, UFF, MI

Parameter	pH	CD	RF	UFF %	MI numbre M/mn
Mean	6.42	2.78	3.03	16.92	55.53
Standard deviation	0.22	0.33	0.41	4.36	4.16
p	0.005	0.01	0.25	0.10	0.0005
Usuel value	6.8-7.5 (Sakouvogu *et al.*, 2018)	3 (Guedon, 2017)	3 (Burfeind *et al.*, 2010)	18 (Kleen, Hooijer, Rehage and Noordhuizen 2003)	80-100 (sauvant *et al.*, 2008a)
CD= dung's consistency, RF= Rumen Fill, UFF= undigested food fraction, IM= mastication index					

We observed in Table 2, the dung's pH and mastication index of the bentonite receiving lot were higher than those of the control lot with $p<0.05$, without reaching the usual values (6.55, 6.29); (61.5, 49.74), respectively.

Table 2. Comparison of peripheral acidosis indicators between lot 1 and lot 2

Parameter	pH	CB	RF	UFF %	IM nombre M/mn
Lot1	6.29	2.62	2.87	14.52	49.74
Lot 2	6,55	2,87	2,62	11,15	61.5
p	0.012	0.48	0.48	0.16	0,0005
Usuel value	6.8-7.5 (Sakouvogu *et al.*, 2018)	3 (Guedon 2017)	3 (Burfeind *et al.*, 2010)	18 (Kleen, Hooijer, Rehage and Noordhuizen 2003)	80-100 (sauvant *et al.*, 2008a)
CD= dung's consistency, RF= Rumen Fill, UFF= undigested food fraction , IM= mastication index					

Sub-acute acidosis has been described as a chronic latent acidosis, low in specific clinical signs and more prevalent than acute lactic acidosis. (Kleen *et al.*, 2012). Subacute Ruminal Acidosis (ARSA) is a disease of nutritional origin that results from disruption of microbial fermentations and abnormal ruminal compartment acidity. The chronic installation of this digestive dysfunction might have a severe impact on animal production efficiency and health. The main cause of ARSA is improper nutrition management for dairy cows, They often relate to high-production cows which are at a higher risk of acidosis during early lactation. This is a phase where cows have a limited capacity to ingest while their energy requirements for milk production increase (Stone, 2004). Dung's pH can be used as a peripheral indicator (Sakouvogu et al., 2018). Indeed our results showed that the dung's pH was lower than the usual value (6.8-7.5) at a rate of 6.42. Dung is a reflection of digestion and its examination provides valuable information on the ration's balance (Vagneur, 2007). During acidosis the fermentations will move to the colon resulting in the formation of gas giving the dung a bubbly appearance. This is caused by decreased rumination. Indeed the mastication index was low compared to the usual values. This may be explained by the fact that lactic acid does not accumulate particularly in animals with subacute ruminal acidosis, the decrease in ruminal pH is rather related to the accumulation of volatile fatty acids (Oetzel *et al.*, 2000), the latter when they exceed a certain threshold activate epithelial chemoreceptors located in the wall of the rumen, the stimulation of these receptors cause a central inhibition of motor skills (Martin *et al.*, 2006). Dung is an excellent indicator of the digestion profile of the herd. A decrease in the score of consistency of dung (< 3) is an adaptation of the animal to the digestion imposed on

it by the ration, the latter is characterized by the influx of water into the digestive system (Enemark *et al.*, 2004; Lessire *et al.*, 2017; Mulligan *et al.*, 2006; O'Grady *et al*., 2008). Indeed our results showed that the dung's consistency is lower than the usual value at the rate of 2.78. Bentonite is used as a digestive aid in the dairy industry, A Yugoslav study (Rajcevic, 1989) compared over a three-month period the addition of 300g of bentonite in two different rations, one rich in forage and silage, the other rich in concentrates. This study found that milk production was significantly higher in the last two months for cows fed a high concentrate ration receiving bentonite. This can be explained by the bentonite's neutralizing action on rumen pH, thus improving the digestibility of the ration (Ouachem and Nouicer, 2006 ; Kaboul and Ouchem, 2012). Indeed we noticed a better chewing index and a slight improvement in the dung's pH. Subclinical acidosis is difficult to diagnose. It was the combination of several peripheral indicators that led to a diagnosis and the use of bentonite improved some peripheral indicators.

Conclusions

It can be concluded that the use of peripheral indicators to determine latent acidosis is of interest in order to diagnose diseased cows early and ensure better prevention. These techniques are easy to make and interpret even if the breeder has a low level of school culture. We must encourage the use of bentonite because it is an organic product, abundant in nature and cheap.

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EVOLUTION OF BIOCHEMICAL PARAMETERS IN POST PARTUM COWS AFFECTED WITH BRUCELLA

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Abstract

Brucellosis is a major zoonosis of concern in Algeria. This pathology (sometimes also called Malte fever) is an animal disease transmitted to humans (zoonosis). It is due to bacteria of the genus Brucella. Three species predominate: Brucella melitensis, the most pathogenic, invasive and widespread species in the world, B. abortus, and B. suis. Brucellosis is a serious economic injury for the State and for the Breeder because it causes the loss of brucellic dairy females, Loss of newborns by abortion and Reduction of the herd by slaughter.
This study was conducted to determine the biochemical parameters of brucella cows during the four months post partum at some farms in Sidi Mhamed Benali (Algeria). We followed and analyzed the blood biochemical profile of 77 cows. One farm had 26 brucella cows. During the four months, we observed that the cows had brucella serum cholesterol, serum calcium, phosphorus and a normal creatinine at a rate of 1.09 to 1.33 g/l, 87.05 to 94.52 mg/l, 58.52 to 65.57 mg/l and 9.60 to 10.99 g/l, respectively. However, these cows showed hypertriglyceridemia in parallel during the four months of the study, ranging on average between 0.71 and 0.92 g/l. Regarding blood glucose, we observed hyperglycemia around calving period at a rate of 0.79 g/l, which was stabilized during the four months after calving. In conclusion, hypertriglyceridemia is common in brucellosis. These results could eventually be used for more extensive studies in this area.

Keywords: *Dairy cow, biochemical profile, brucellosis*.

Introduction

Bovine brucellosis is considered as an endemic infectious disease that is widespread at all the Algerian territory. It is a highly contagious disease, whose economic impact on the development of animal industries is considerable. Moreover, being considered as the most common major zoonosis worldwide, it represents a serious threat to human health (OMS, 1986). The various control programs implemented by the Algerian Veterinary Services have not yet paid off, for not being applied, because of the many constraints (Lounes, 2009). The etiological agents of the disease are members of the genus Brucella (Rajashekara *et al*., 2006). Previously, six species Br. melitensis, Br. abortus, Br. suis, Br. ovis, Br. canis and Br. neotomae were identified in the genus Brucellae (Rajashekara *et al.*, 2006). Animals are infected after either an abortion or full term parturition. (Aqasthya *et al.*, 2007). During the post partum biochemical parameters in dairy cows undergo significant changes caused by high milk production (Brugère-Picoux, 1995). The main objective of this study was to determine the biochemical parameters of affected cows with brucella.

Materials and methods

This study was conducted at the farms located in Sidi Mhamed Benali, Relizane city (North-West of Algeria). The study was done on four farms; one farm contained 26 infected cows with brucella, after confirmation by the laboratory "Kharoubi" of Mostaganem. We collected our blood samples during the peri partum period: at calving, 1 month, 2 months, 3 months and 4 months later, in order to assess the following blood parameters: blood glucose, creatinine, phosphate, calcium, cholesterol and triglycerides. Plasma were obtained by centrifuging heparinized blood immediately, and were placed at a temperature of 4°C. Assays were performed after 24 h of collection enzymatically with an automatic spectrophotometer multi analyzer. The blood glucose testing was performed on site, using a glucometer of ACCU-CHEK type. Statistical analysis of collected data was performed using the software "XL stat", applying the student test (comparison of two means).

Results and discussion

The obtained results of biochemical parameters are summarized in Tables 1 to 6. Our results show that cows affected with brucella presented at calving hypertriglyceridemia, which extended into the fourth first months of the postpartum period compared with healthy cows; 0.78, 0.92, 0.71, 0.87, and 0.89 g/l respectively, $p < 0.05$. This means that there was a significant difference between the rates of blood triglycerides in affected cows with brucella compared with healthy cows. With regard to serum calcium, creatinine and phosphate, they showed no significant difference between the two samples $p<0.05$, We also noted an hyperglycemia at calving in affected cows with brucella (0.79 g/l), which was stabilized in the following months, $p <0.05$. For cholesterolemia, we observed that it did not exceed the normal values at calving up to four months later. However, we noted a significant difference between the averages in brucellic cows compared with healthy ones at calving during the first and the fourth postpartum months, $p<0.05$.

Table 1. Comparison of the average triglyceridemia (g/l) of cows affected with brucella and cows not affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=28 x=0.59 v=0.027 E=0.164	n=24 x=0.52 v=0.033 E=0.183	n=37 x=0.49 v=0.033 E=0.181	n=27 x=0.56 v=0.045 E=0.211	n=14 x=0.53 v=0.025 E=0.158	0.60-0.65 (Laizeau, 2003)
Cow B	n=25 x=0.78 v=0.104 E=0.322	n=10 x=0.92 v=0.013 E=0.115	n=5 x=0.71 v=0.131 E=0.362	n=4 x=0.87 v=0.022 E=0.148	n=5 x=0.89 v=0.087 E=0.295	
P	0.008	<0.0001	0.031	0.009	0.003	
t o	2.778	6.419	2.237	2.807	3.92	
t c	2.008	2.037	2.021	2.045	2.110	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation; to = value observed by Student test; tc = Student critical value.

Table 2. Comparison of the average blood cholesterol (g/l) of cows affected with brucella and cows not affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=45 x=0.99 v=0.044 E=0.211	n=41 x=0.94 v=0.031 E=0.176	n=54 x=1.04 v=0.112 E=0.334	n=27 x=1 v=0.034 E=0.184	n=13 x=0.95 v=0.028 E=0.167	
Cow B	n=25 x=1.11 v=0.045 E=0.213	n=10 x=1.33 v=0.076 E=0.275	n=5 x=1.32 v=0.139 E=0.373	n=4 x=1.09 v=0.009 E=0.093	n=5 x=1.26 v=0.132 E=0.363	0.5 - 1.35 (Gautier , 1979)
P t o t c	0.022 2.346 1.995	<0.0001 5.557 2.010	0.086 1.747 2.002	0.341 0.969 2.045	0.023 2.521 2.120	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation; to = value observed by Student test; tc = Student critical value.

Table 3. Comparison of the average blood glucose (g/l) of cows affected with brucella and cows not affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=51 x=0.70 v= 0.009 E=0.092	n=41 x=0.64 v=0.010 E=0.101	n=54 x=0.64 v=0.008 E=0.087	n=28 x=0.66 v=0.006 E=0.079	n=13 x=0.68 v=0.006 E=0.075	
Cow B	n=26 x=0.79 v=0.044 E=0.209	n=12 x=0.66 v=0.010 E=0.101	n=5 x=0.7 v=0.007 E=0.081	n=4 x=0.65 v=0.014 E=0.118	n=5 x=0.71 v=0.004 E=0.062	0.5 - 0.7 Verriel and Bedouet (1999)
P t o t c	0.018 2.416 1.992	0.544 0.610 2.008	0.146 1.473 2.002	0.688 -0.405 2.042	0.435 0.800 2.120	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation, to = value observed by Student test; tc = Student critical value.

Table 4. Comparison of the average blood creatinine (mg/l) of cows affected with brucella and cows not affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=29 x=10.11 v=4.205 E=2.051	n=24 x=10.10 v=2.647 E=1.627	n=38 x=10.33 v=4.278 E=2.068	n=27 x=10.60 v=5.683 E=2.384	n=13 x=9.74 v=4.247 E=2.061	
Cow B	n=21 x=10.60 v=2.941 E=1.715	n=10 x=10.99 v=3.985 E=1.996	n=4 x=10.95 v=7.074 E=2.660	n=4 x=9.60 v=1.187 E=1.089	n=5 x=9.82 v=2.718 E=1.649	10 - 15 Lorin (et al. 2009)
P t o t c	0.370 0.905 2.011	0.182 1.363 2.037	0.579 0.559 2.021	0.423 -0.813 2.045	0.940 0.077 2.120	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation; to = value observed by Student test; tc = Student critical value.

Table 5. Comparison of the average blood phosphorus (mg/l) of cows affected with brucella and cows not affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=27 x=64.93 v=140.218 E=11.841	n=25 x=60.87 v=20.052 E=4.478	n=38 x=61.26 v=32.812 E=5.728	n=27 x=59.59 v=27.931 E=5.285	n=14 x=60.09 v=13.408 E=3.662	40 - 86 Verriel and Bedouet (1999), Brugère and picoux (1995)
Cow B	n=25 x=61.24 v=93.691 E=9.679	n=10 x=65.57 v=34.585 E=5.881	n=5 x=59.92 v=19.874 E=4.458	n=4 x=58.52 v=11.036 E=3.322	n=5 x=61.36 v=50.98 E=7.140	
P	0.226	0.015	0.619	0.700	0.610	
t o	-1.225	2.561	-0.502	-0.389	0.519	
t c	2.009	2.035	2.020	2.045	2.110	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation; to = value observed by Student test; tc = Student critical value.

Table 6. Comparison of the average blood calcium (mg/l) of cows affected with brucella and cows no affected with brucella.

Parameter	Calving time					Standard
	At calving	1 month	2 months	3 months	4 months	
Cow N	n=31 x=87.75 v=300.866 E=17.346	n=40 x=94.84 v=101.412 E=10.070	n=53 x=99.04 v=118.469 E=10.884	n=27 x=100.16 v=68.209 E=8.259	n=14 x=95.16 v=90.654 E=9.521	
Cow B	n=11 x=90.63 v=45.887 E=6.774	n=10 x=94.52 v=25.715 E=5.071	n=4 x=90.51 v=9.309 E=3.051	n=4 x=87.05 v=10.671 E=3.267	n=5 x=92.40 v=54.887 E=7.409	80 - 120 Verriel et Bedouet (1999), Brugère picoux (1995)
P	0.597	0.924	0.127	0.004	0.567	
t o	0.533	-0.096	-1.550	-3.102	0.584	
t c	2.021	2.011	2.004	2.045	2.110	

N = cow not affected with brucella; B = cow affected with brucella; n = number; x = mean; v = variance; E = standard deviation; to = value observed by Student test; tc = Student critical value.

Postpartum period is under the control of physiological, metabolic and endocrine changes accompanying a process of adaptation developed by dairy cows in response to nutritional needs, essentially based in growing energy. These changes are necessary to maintain the constancy of the internal environment (homeostasis) and to properly conduct a new round of dairy production metabolically more advantaged (Chilliard *et al.*, 1980; Bauman and Currie, 1980). The carrying out of this study during this period and on brucella cows allowed us to note interesting data on bovine brucellosis. The characteristics that differentiate brucella cows and the other cows revolve around the level of triglyceride in the blood. We found that the cows affected with brucella had very high levels of blood triglyceride from the first to the 4th postpartum month, compared with healthy cows, based on research conducted on human beings (Apostolou *et al.*, 2009). The authors found that Brucella infection is associated with an atherogenic lipid profile, which is not fully restored after four months of treatment. Hypertriglyceridemia was found in a child with brucellosis (Erduran *et al.*, 2010). This is explained by the fact that Brucella abortus is a gram-negative intracellular bacterium; it induces the production of tumor necrosis factor (TNF) (Bruce *et al.*, 2002; Huang *et al.*, 2003) and the production of TNF inhibits lipoprotein lipase, which results in elevated plasma triglycerides (Feingold *et al.*, 1989; Creput *et al.*, 2005). Glycemia was highest at calving and then declines during the first two months after calving (Miettinen, 1991). This confirms our result on the hyperglycemia observed at calving, and this is explained by the fact that dairy

cows around this period present a very important energy metabolism for the synthesis of lactose to increase milk production (Vagneur, 1992).

Conclusion

Evaluation of these various biochemical parameters in brucellic cows allowed us to conclude that brucellosis is associated with a hypertriglyceridemia that is not fully restored during the four months after calving. This change in blood triglyceride levels requires further studies even more specific in this area.

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INFLUENCE OF ULTRASONIC TREATMENT ON THE FERMENTATION OF GOAT'S MILK SUPPLEMENTED WITH MILK PROTEIN ISOLATES

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Abstract

Total solids content, especially protein content, has a significant effect on the rheological characteristics of yogurt. To achieve adequate viscosity and texture of goat milk yogurt, the non-fat solids content must be increased, which is usually achieved by adding various milk products such as milk protein isolates (MPI). High-power ultrasound can be used in dairy industry to improve some properties of milk and dairy products. In addition, ultrasound hydrolyzes lactose and accelerates acid production, so it could be assumed that the fermentation time of milk subjected to ultrasonic treatment would be shorter. In order to investigate the effect of ultrasonic treatment on fermentation, goat milk supplemented with 1% MPI was subjected to ultrasonic treatment: samples B - 200 W/10 min and samples C - 400 W/10 min. As a control, samples A were prepared by standard treatment without the application of ultrasound. During yogurt production physico-chemical characteristics of milk prior to inoculation were investigated. The course of fermentation was monitored by viscosity and pH measurements. Ultrasonic treatment of samples C had a favorable effect on the fermentation time, which was 30 min shorter than for samples A, and 60 min shorter compared to samples B. However, the lowest viscosity values were measured for these samples at the end of fermentation - 172.3 mPas. The results of this study show that in the production of yogurt from goat milk supplemented with 1% MPI, ultrasonic treatment can be used to shorten the fermentation time.

Keywords: *goat milk, milk protein isolates, ultrasound, fermentation.*

Introduction

Many good characteristics of goat's milk, such as nutritional value, better digestibility and lower allergenic properties compared to cow's milk, have led to its increasing processing and consumption in the last couple of decades. Goat milk has a specific odor and taste that is unacceptable to many consumers. These characteristics are significantly reduced by the fermentation of the milk during yogurt production. However, the lower proportion of casein nitrogen and the size of casein micelles affect the poorer structure of fermented goat milk beverages (Antunac et al., 2000; Park et al., 2007). Desirable textural properties of goat's milk yogurt can be achieved by increasing the content of non-fat dry matter, which is obtained by concentrating the milk using different membrane processes, adding gelatin and pectins, or various milk products such as whey and milk protein concentrates and isolates (Lucey, 2004). In addition, milk-based protein concentrates added to milk during yogurt production cause less syneresis and higher viscosity of yogurt. Compared to cow's milk, the fermentation of goat's milk is slower due to its higher buffering capacity, which is a consequence of its specific protein composition (Park and Guo, 2006).

In dairy industry high intensity ultrasound is used for the inactivation of enzymes and microorganisms, assistance in membrane processes, homogenization of milk, etc. (Herceg et al., 2009; Riener et al., 2009, 2010). Chemical and physical changes that occur during the application of ultrasound are result of cavitations that lead to the formation of a larger number

of milk fat globules of smaller diameter and larger total surface area, on which the casein is adsorbed. The resulting pseudocasein micelles cause better water binding, less syneresis, higher viscosity and more compact structure of the casein gel (Bermúdez-Aguire and Barbosa-Cánovas, 2011; Wu et al. 2001). In traditional yogurt production, lactose is hydrolyzed during fermentation only by the lactic acid bacteria present. Therefore, the degree of lactose hydrolysis depends on the number of viable cells and their activity. On the other hand, ultrasonic treatment of the milk damages the cell membranes of the bacteria so part of the β-galactosidase enters the milk and hydrolyzes the lactose (Wang et al, 1997).
Considering all the changes that ultrasound induces in milk, the aim of this study was to determine whether the applied ultrasonic treatments have an influence on the composition of goat milk supplemented with milk protein isolates, as well as on the course and duration of its fermentation.

Material and method

Goat milk from the farm "Beocapra", Kukujevci (Serbia), was used for the experiments conducted between September and December in the Dairy Laboratory of the Faculty of Agriculture, University of Belgrade, Serbia. One hour before heat treatment goat milk was supplemented with 1% milk protein isolate Promilk 852 A2 (Ingredia, France) and three series of samples were produced. Yogurt samples A were produced according to the standard technological production process, while for the production of yogurt samples B and C ultrasonic treatments were applied prior to inoculation. Ultrasonic treatments were performed using an ultrasonic bath (Raypa, Spain) for 10 minutes at a frequency of 35 kHz, a temperature of 43°C, and an output power of 200 W for samples B and 400 W for samples C. Fermentation was performed at 43°C using starter culture FD-DVS YFL812 Yo-Flex Chr. Hansen, Danmark.
The chemical composition of raw milk, milk with an addition of 1% MPI, heat-treated milk and milk after ultrasonic treatment was investigated by the following methods: total solids (Carić et al., 2000), fat (IDF 105:1981; Carić et al., 2000), proteins (IDF 20B:1993), lactose (IDF 28:1974), ash (Carić et al., 2000), titrable acidity (°SH) (Carić et al., 2000), and pH value with pH meter (Consort C 931). Each analysis was performed in triplicate.
During fermentation, the change in viscosity was monitored at constant spindle speed (60 rpm) using Visco Basic+R Viscometer (Fungilab, Spain). Viscosity was measured every 30 min starting from the time of inoculation (zero measurement) until the end of fermentation, i.e., until a pH of 4.6 was reached. The tests were performed in two replicates, using a new sample for each measurement. At the same time interval, the change in pH was measured using a pH meter with a combined electrode (Consort C 931).
Obtained data were analyzed with Statistica 6.0 software (Stat Soft. Inc., Tulsa, USA) using analysis of variance (ANOVA). Mean comparisons of parameters were performed using the t-test, at the 0.05 level of significance.

Results and discussion

Fermentation of milk is the most important process in the production of yogurt, during which a gel whose basis is a protein matrix is formed. Accordingly, the physico-chemical and sensory properties of the gel obtained depend to a large extent on the characteristics of the milk used and the course of fermentation.
For all samples, the composition of raw milk, milk after addition of 1% MPI, heat-treated milk, and milk after ultrasonic treatment (samples B and C) was examined. The results are presented in Table 1.

Table 1. Physico-chemical properties of goat milk used for yogurt production

Sample	Parameter	Raw milk	Milk supplemented with 1% MPI	Heat treated milk	Ultrasonic treated mik
A –Standard treatment	TS (%)	11.31±0.61bA	12.02±0.54aA	12.34±0.47aA	-
	MF (%)	3.17±0.38 aA	3.18±0.39 aA	3.28±0.42 aA	-
	TSNF (%)	8.15±0.24bA	8.84±0.15aA	9.06±0.09aA	-
	Proteins (%)	2.75±0.12 bA	3.46±0.03 aA	3.57±0.11 aA	-
	Lactose (%)	4.62±0.12bA	4.70±0.11abA	4.78±0.12aA	-
	Ash (%)	0.81±0.04aA	0.82±0.02aAB	0.84±0.02aB	-
	Acidity (^{o}SH)	5.29±0.39bAB	5.64±0.38abA	5.84±0.37aA	-
	pH	6.74±0.01 aA	6.71±0.01 aA	6.62±0.04 aA	-
B – US 200W/10 min	TS (%)	11.30±0.43cA	12.01±0.28bA	12.22±0.21aA	12.26±0.18aA
	MF (%)	3.10±0.30 aA	3.08±0.27 aA	3.26±0.31 aA	3.24±0.30 aA
	TSNF (%)	8.20±0.15bA	8.93±0.08aA	8.97±0.11aA	9.02±0.15aA
	Proteins (%)	2.84±0.14 bA	3.53±0.14 aA	3.53±0.12 aA	3.61±0.11 aA
	Lactose (%)	4.52±0.16bA	4.56±0.17bA	4.74±0.16aA	4.77±0.13aA
	Ash (%)	0.83±0.02cA	0.85±0.01bcA	0.87±0.03abA	0.88±0.03aA
	Acidity (^{o}SH)	5.10±0.15bB	5.17±0.19bB	5.41±0.22aB	5.49±0.32aB
	pH	6.72±0.06 aA	6.70±0.07 aA	6.61±0.08 aA	6.61±0.07 aA
C – US 400W/10 min	TS (%)	11.09±0.77cA	11.94±0.74bA	12.10±0.66aA	12.10±0.71aA
	MF (%)	2.93±0.44 aA	2.93±0.44 aA	3.07±0.38 aA	3.07±0.38 aA
	TSNF (%)	8.15±0.35bA	9.00±0.33aA	9.03±0.27aA	9.03±0.30aA
	Proteins (%)	2.70±0.12 bA	3.44±0.03 aA	3.52±0.04 aA	3.51±0.02 aA
	Lactose (%)	4.60±0.24aA	4.68±0.21aA	4.72±0.26aA	4.76±0.28aA
	Ash (%)	0.81±0.04bA	0.82±0.03bB	0.87±0.04aA	0.87±0.04aA
	Acidity (^{o}SH)	5.31±0.08cA	5.68±0.00bcA	5.72±0.08bA	5.80±0.10aA
	pH	6.67±0.01 aA	6.64±0.02 aA	6.58±0.03 aA	6.58±0.02 aA

a, b,c: means in the same row followed by different letters were significantly different ($p < 0.05$); A, B, C: means in the same column followed by different letters significantly different ($p < 0.05$); TS – total solids, MF – milk fat, TSNF – total solids non fat

From the data presented in Table 1, it can be seen that an increase in total solids content (TS), total solids non fat (TSNF) and protein content was observed in all the samples studied after the addition of milk protein isolate. Moreover, during the thermal treatment, due to the evaporation of part of the water from the milk, an increase in the content of TS and TSNF was observed in samples B and C. Minor changes in protein content due to exposure to ultrasound were observed, which is consistent with the results of Vučić et al., 2014. Furthermore, according to Wrigley and Lorca (1992), ultrasonic treatment at lower temperatures (below 50°C) does not lead to a change in the casein content.

Samples C had higher titrable acidity after ultrasonic treatment. The increase in titrable acidity is the result of lipolysis (Walstra et al., 2006), i.e., the formation of free fatty acids under the action of ultrasound. In addition, ultrasonic treatment of milk can lead to the formation of nitrates, nitrites, and H_2O_2, which can affect the increase of titrable acidity (Bermúdez-Aguirre et al., 2009).

Comparing goat milk before heat treatment and after the application of ultrasound, a decrease in pH can be observed in all samples, but these differences are not statistically significant ($p > 0.05$). According to Walstra et al. (2006), cavitations cause the hydrolysis of esters and the

release of free fatty acids, which leads to a decrease in pH. In addition, cavitations can cause the formation of free radicals, which contribute to the lowering of pH after ultrasonic treatment (Bermúdez-Aguirre and Barbosa-Cánovas, 2011).

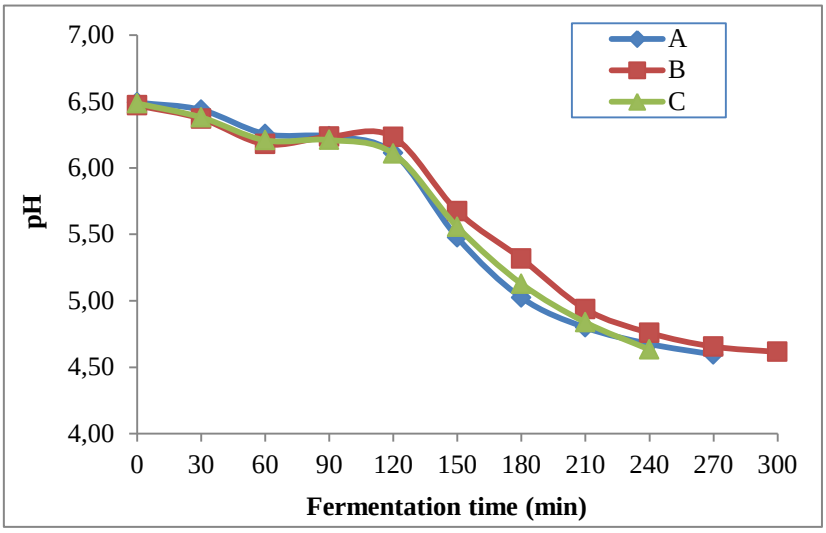

Figure 1. pH change during fermentation

The change in pH value during the fermentation of goat's milk with the addition of 1% IPM is shown in Figure 1. The samples treated with 400 W ultrasound had the shortest fermentation time (240 min), even though they had the lowest protein and milk fat content immediately before inoculation (Table 1). Samples B had the longest fermentation - 300 minutes. In terms of chemical composition, milk samples A and B were very similar, indicating the negative influence of 200 W ultrasound on the fermentation of milk to which 1% IPM was added. In samples B, an increase in pH from 6.16 to 6.24 was observed during fermentation in the period from the 60th to the 90th minute. During the same period, the pH in samples C remained at the same level - 6.2 - while in samples A there was a constant decrease in pH during fermentation. The resistance of samples B to pH changes can be attributed to the buffering capacity of goat milk, as well as to the positive effect of ultrasonic treatment on the properties of whey proteins (Režek Jambrak et al., 2009).

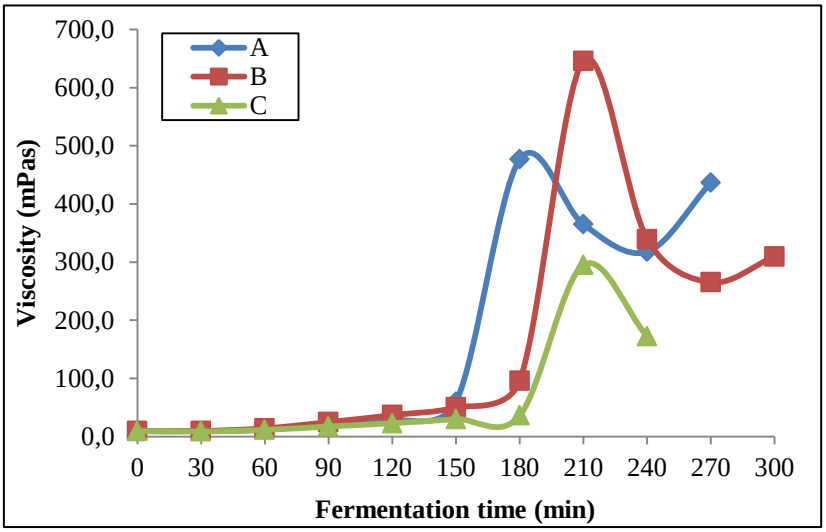

Figure 2. Viscosity change during fermentation

Although total solids content has a significant effect on the rheological characteristics of yogurt, the increase in milk viscosity during fermentation is attributed to the increased water binding capacity of proteins (Tammime and Robinson, 2000). The change in viscosity during fermentation is the result of casein micelles aggregation and gel formation. Milk fermentation in yogurt production is characterized by an induction phase (stationary viscosity), a logarithmic phase (maximum viscosity increase) followed by a metastable equilibrium phase in which the viscosity is constant and a syneresis phase in which the viscosity decreases (Tammime and Robinson, 2000; Robinson et al., 2006; Macej et al., 2007).
The change in viscosity during fermentation of goat's milk with the addition of 1% IPM is shown in Figure 2. In samples produced in the standard way (A), the induction phase was recorded in the first 150 min of fermentation when casein micelles are uniform in size and distribution (Jumah et al., 2001; Maćej et al., 2007). The maximum value of the viscosity of samples A was recorded after 180 min of fermentation and was 476.8 mPas. On the contrary, in the samples produced with the application of ultrasound (B and C) after 180 min significantly lower viscosity values were recorded - 96.1 mPas and 36.1 mPas, respectively. The highest viscosity was recorded after 210 min of fermentation in samples B and was 646.0 mPas, which is 281.2 mPas and 350.9 mPas ($p<0.05$) higher compared to the values recorded in samples A and C. The lowest viscosity during fermentation was observed in samples C, which is a consequence of the reduction in the size of casein micelles and the formation of aggregates in milk serum due to the action of ultrasound. The maximum viscosity of samples C was 295.1 mPas after 210 min of fermentation. A decrease in viscosity was observed in the syneresis phase in the period from the 210^{th} to the 240^{th} min, when there is a partial disintegration of casein micelles and the formation of smaller casein aggregates characterized by a more porous structure (Jumah et al., 2001), so the viscosity of samples C at the end of fermentation was 172.3 mPas, which is 264.4 mPas ($p < 0.05$) and 137.3 mPas ($p > 0.05$) lower than the viscosity of samples A and B at the end of fermentation.

Conclusion

Based on the results obtained, it was found that fermentation took the shortest time in samples treated with 400 W ultrasound, while 200 W ultrasound resulted in an increase in fermentation time by 30 min. At the end of fermentation, samples produced in the standard way (A) had the highest viscosity - 436.7 mPas, while the lowest viscosity was recorded in samples C - 172.3 mPas. In general, the application of ultrasonic treatment with a power of 400 W can be used to shorten the fermentation time of goat milk supplemented with 1% MPI.

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TWO GENETIC VARIANTS IN *EBF1* GENE EFFECT ON LIVE WEIGHT IN SHEEP

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Abstract

Live weight is a very important economic trait for meat sheep breeding industry. Here we searched for the new genetic variants associated with the live weight trait in two Russian sheep breeds. Volgograd breed is a double-purpose breed both for meat and fine wool. A semi-fine wool Southern meat breed was bred for meat production. 48 Volgograd ewes and 48 Southern meat ewes were genotyped on Illumina OvineSNP50 array. Filtering and QC conducted in Rstudio2023.03.0 and plink1.9. Animals of both breeds were divided into subpopulations (by choosing extra high and extra low live weight) and fixation index (F_{st}) were calculated in plink. SNPs with the highest F_{st} values were selected as significant. In both breeds we detected significant SNPs within the *EBF1* gene. Volgograd ewes of the GG genotype had mean live weight 3.55 kg less than animals of AG genotype (p=0.0035) on rs404567493. The difference in mean live weight between animals of AA and GG genotype in Southern meat breed was about 13 kg (p=0.0045) on rs427770093. The *EBF1* gene genetic variants have demonstrated significant associations with the live weight in two breeds of different origin. Therefore, it is very interesting to investigate it in detail.

Keywords: *Sheep, Live weight, Fixation indices, Candidate genes.*

Introduction

Live weight is a very important economic trait for meat sheep breeding industry. The polymorphic traits, as live weight, are under control of the environment and the set of genes, which involve in the process of animal's growth on different stages of ontogenesis. The second characteristic of quantitative traits is their low inheritance (h^2=0.15-0.21 for birth weight) (ICAR Guidelines, 2021). Many works are devoted to the investigation of the growth traits and its genetic background. It is interesting to note that candidate genes revealed quite often are breed specific (Gholizadeh et al., 2015; Raadsma et al., 2009).

The most common method for detecting new genetic variants associated with productive traits as live weight, fertility etc. last 15 years became GWAS method. Due to this method now the Sheep QTL database contains information on 270 loci associated with live weight in sheep. Unfortunately, the main limitation of GWAS is that you need to analyze quite big samples to obtain the significant results. In case of small samples, the F_{ST} method could help to overcome this limitation (Weir and Hill, 2002).

The aim of our work was to find the new genetic variants associated with the live weight trait in two Russian sheep breeds. Volgograd breed was created in the middle of XX century in USSR. This breed was made on the basis of local coarse wool ewes with the merino rams imported from Europe. It's a double-purpose breed both for meat and fine wool. Rams and ewes are polled. Live weight of adult ram is about 110 kg, ewes – 60 kg. The live weight of 9 months lambs was measured. Ewe lambs of this age usually have 85% of adult live weight.

The second breed Southern meat breed is semi-fine wool and was bred for meat. Live weight of adult ram is 120 kg and more, ewes – 70 kg. It was registered in the 2001; the 'mother breed' was local semi-fine wool ewes and the 'sire' breed was European Texel rams, later East Friesian breed was added. For this breed we have data on adult ewes live weight (elder than 24 months).

Materials and methods

Firstly, all the data were checked on the normality of distribution and animals were arranged on the live weight trait. After we choose 48 Volgograd ewes and 48 Southern meat ewes for genotyping, animals were genotyped on Illumina OvineSNP50 array. QC and filtering on --geno, MAF≥0,05, HWE (p<10-6), indep-pairwise (50_5_0.8) were completed in plink 1.9. Then animals with extra high extra low live weight (breeds were analysed separately from each other) were chosen. Animals of both breeds were divided into subpopulations and fixation indices (F_{st}) were calculated in plink1.9. SNPs with the highest F_{st} values were selected as significant. Using Ensembl VEP tool candidate genes were found where significant SNPs are localized.

Results and discussion

In both breeds significant SNPs within the *EBF1* gene were detected (Table 1).

Table 1. SNPs within *EBF1* gene

Breed	SNPs	F_{st} value	Consequence type	Rs
Volgograd	OAR5_74249563.1	0.579542	downstream_gene_variant	rs404567493
Southern meat	OAR5_74606882.1	0.537658	intron_variant	rs427770093

The effects of detected SNPs on the live weight in Southern meat and Volgograd breeds were checked, the mean live weight for every genotype, standard deviation and the significance of live weight difference between genotypes were calculated.

In Volgograd breed rs404567493 was located in downstream gene variant. We detected 2 ewes of AA genotype and equal weights, 15 ewes had AG and 31 had GG genotype. As we can see on figure 1 ewes of the GG genotype had mean live weight 3.35 kg less than animals of AG genotype (p=0.0035). It was impossible to complete significant calculations for ewes of AA genotype, as this group included just two animals with the same weights.

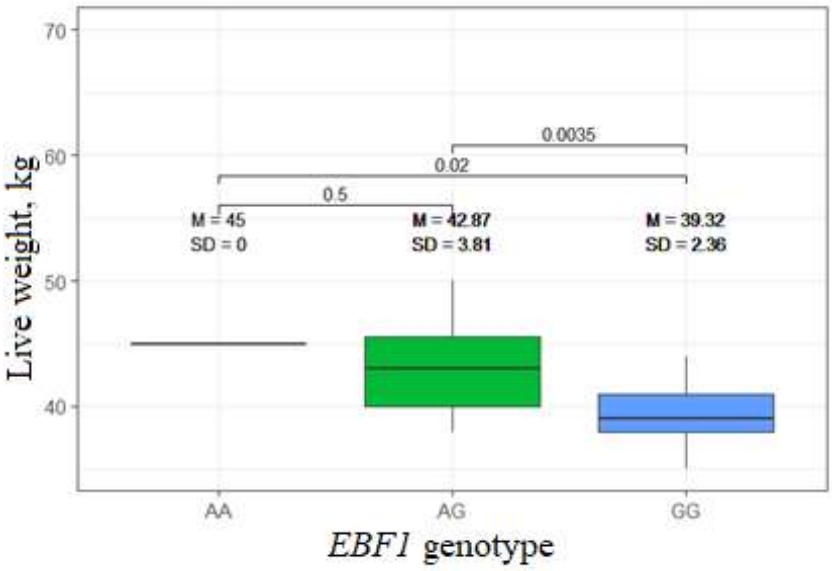

Figure 1. Boxplots of Volgograd breed ewes' live weight due to different genotypes on rs404567493

In Southern meat breed we found three different genotypes for rs427770093 which located in intron region of *EBF1* gene. There were 8 ewes of AA genotype, 26 animals of AG genotype and 13 of GG genotype. The difference in mean live weight between animals of AA and GG genotype was about 13 kg (p=0.0045).

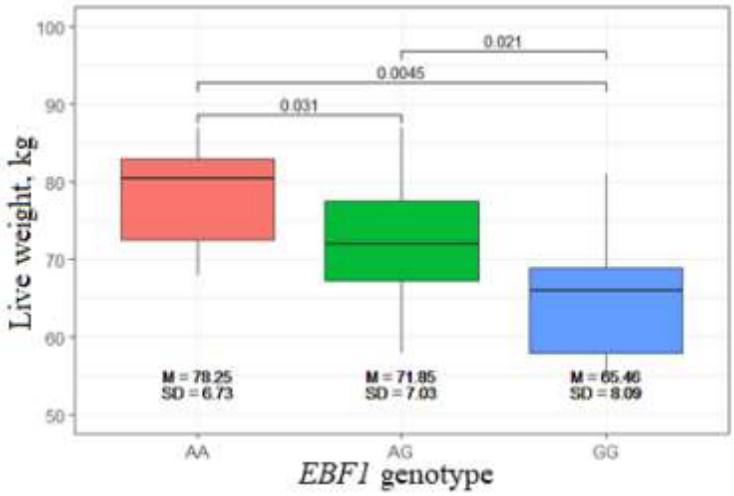

Figure 2. Boxplots of Southern meat ewes' live weight due to different genotypes on rs427770093

From the boxplots it can be seen that the preferable genotype in Southern meat breed is AA. But for Volgograd breed it was more difficult to choose the desirable genotype.

Conclusions

By the F_{st} method two significant SNPs in *EBF1* gene which effect on live weight in two Russian sheep breeds were found. The rs404567493 influences on live weight in Volgograd sheep and provides the prevalence in this trait for heterozygous genotype in ≈3.5 kg comparing to GG genotype. Southern meat breed ewes with AA genotype on rs427770093 have the live weight on ≈ 12 kg more than GG animals. It should be mentioned that two breeds of different goals (*meat* and *meat+fine wool)* were not compared. As these breeds are

local, they have adapted very well to the conditions of the South of Russia. It’s very important to know more about their genetic structure both for preserving their gene pool and for successful breeding.

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IN OVO α-TOCOPHEROL MODIFIES SERUM LIPID PROFILE BY GENDER OF BROILER CHICKS AT 72TH-HOUR OF POST-HATCHING STAGE

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Abstract

This study was aimed to examine the effect of *in ovo* α-tocopherol injection into yolk sac on the 7th day of embryonic age on the blood serum lipid profile of broiler chicks at 72th hour after hatching. The trial used total of 160 hatching eggs was conducted with non-injected group (control) and injection group, which was injected 37.5 mg of α -tocopherol (250 µl vitamin E) into yolk sac on day 7 of incubation. Total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) levels were measured in serum obtained from blood samples of 10 male and female chicks at the 72th hour post-hatching period. *In ovo* α-tocopherol injection did not affect serum lipid profile of broiler chicks on day 3 of post-hatching stage. However, the significant interactions between *in ovo* α-tocopherol administration and gender on serum total cholesterol, LDL and HDL were determined. Serum total cholesterol level of female chicks in the α-tocopherol group was higher than control group (P=0.011). Serum LDL level of the female chicks was higher than male chicks in the α-tocopherol group and female chicks in the control group (P=0.023). The reason of significant interaction was that female chicks had the lowest serum HDL concentration compared to male chicks in the control group (P=0.039). Results of the study indicated that *in ovo* α-tocopherol administration at pre-hatching stage can modify serum total cholesterol and LDL concentrations of female broiler chicks at early post-hatching stage.

Keywords: *α-tocopherol, Vitamin E, Lipid profile, In ovo injection, Broiler chick.*

Introductıon

Maternal effects are defined as non-genetic effects of the environmental conditions provided by the maternal on phenotype of the offspring (Nager et al., 2006; Müller et al., 2007; Mousseau et al., 2009). Maternal effects are indirect genetic effects and indirect environmental effects, which include interaction between genotype and environment effects of phenotypic variation (Groothuis et al., 2005; Groothuis and Schwabl, 2008; Love et al., 2013). Maternal effects in poultry are in three different periods. The first one is pre-ovulation maternal effects (pre-zygotic maternal effects): egg weight, egg quality, maternal antibodies, maternal hormones and maternal antioxidants which are the subject of this study. Second period maternal effects are post-zygotic maternal effects. The third period of maternal effects is the early developmental period after hatching, especially the developmental period of about one week after hatching. In this study, the characteristics of the third period maternal effects were examined in blood serum samples from 72-hour-old chicks. Maternal antioxidants have long-term effects on development and growth of embryo/chick (Babacanoğlu et al., 2018). Since the immune system of the chick is not functional at hatching, maternal antioxidants are very important in terms of reducing the harmful effects of free radicals on immune system activity (Babacanoğlu and Özelçam, 2013). Fat-soluble maternal antioxidants such as vitamins A, D, E, K and carotenoids are transferred to the yolk by the breeder hen during the development of the yolk follicles. Moreover, antioxidants such as vitamins A, E and

carotenoids are transferred from the yolk sac to the tissues of the embryo during embryo development (Surai et al., 1996). Vitamin E is the first line of defence that protects the polyunsaturated fatty acids in cell membrane phospholipids from free radicals and is a non-enzymatic vitamin of lipid origin. α-Tocopherol is the most important active isomer of vitamin E and maternal origin α-tocopherol stored in yolk sac is the major source of vitamin E for the hatched chicks during the 1st week of age. The average vitamin E level in egg yolk of different poultry species is reported to be 216.4 µg (Remeš et al. 2011). The vitamin E contents of the yolk sac of chicken embryos at days 14, 19 and 20 of incubation are 162.1, 211.5 and 223.4 µg, respectively (Gaal et al. 1995). It has been found that *in ovo* injection method, which is a biotechnological application, reveals the effectiveness of antioxidants more effectively and rapidly compared to some applications based on maternal effects (Hossain et al., 1998; Babacanoğlu et al., 2018; Babacanoğlu and Cellak, 2019). Therefore, the aim of this study was to investigate the effect of *in ovo* α-tocopherol injection into the yolk sac of the embryo on the lipid profile in the blood serum of 72-hour-old broiler chicks.

Material and method

The experimental material consisted of 160 hatching eggs obtained from broiler breeder females of Ross 308 genotype at 43 weeks of age. The first group was served as control (no antioxidant addition and no injection group) and the other group was injected 37.5 mg α-tocopherol (0.25 ml vitamin E/egg) into the yolk sac of the embryo on the 7th day of embryonic age using *in ovo* method.

After the hatched chicks were classified to gender, standard rearing conditions were applied to day-old chicks until 72nd hour. Blood samples taken from 10 male and 10 female chicks from each group at day 3 of chick age (72nd hour) were centrifuged at 3750 rpm for 10 minutes at 4 ºC in the SIGMA 3 30 K centrifuge device and serum were obtained. Serum total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) levels were measured in the Roche CREJ2 Cobas INTEGRA 400 plus and Cobass E 411-USA auto-analyzer instruments using commercial kits at 512/659 nm wavelength (Babacanoğlu et al., 2013; Babacanoğlu, 2018). Serum very low density lipoprotein cholesterol (VLDL) level was calculated by adding serum triglyceride level to the formula.

Statistical analysis

In ovo treatment and gender effects and the interaction of these two effects were included in the model using ANOVA in JMP 7.0.1 (SAS Institute Inc., 2007). Tukey HSD test was used for comparisons of means.

Results and Discussion

In ovo α-tocopherol treatment had no effect on serum total cholesterol, triglyceride, HDL, LDL and VLDL levels on the day 3 of chick age and similarly, the effect of gender on serum lipid profile was found to be insignificant (Table 1). The interaction between *in ovo* α-tocopherol treatment and gender was insignificant on blood serum triglyceride and VLDL levels, while it was significant for serum total cholesterol, HDL and LDL levels. However, this significant interaction shown that serum HDL level of both sexes in the control group was similar to *in ovo* α-tocopherol group (Table 1). These results for the blood serum triglyceride, HDL and VLDL levels revealed that the application based on the second period maternal effect during the embryo development was not reflected in the third period maternal effect.

At the day 3 of chick age, total cholesterol level of female chicks treated *in ovo* α-tocopherol to the yolk sac at the 7th day of incubation was higher than female chicks in the control group

(Table 1). This result was similar to the result of a study which reported that blood serum total lipid level of the female chicks in the vitamin E group was higher found than chicks of both sexes in the control group at day 3 of age (Babacanoğlu and Karageçili, 2022). Fatty acids are the main energy substrates stored as lipids in the yolk sac of the embryo (De Oliveira et al., 2008) and are transported from the yolk sac to the tissues of the embryo, especially towards hatching (Herman et al., 2000). Most of the lipids in the tissue of developing chick embryo are polyunsaturated fatty acids (Speake et al., 1998). Since an effective antioxidant system is required in the embryo (Surai, 1999), polyunsaturated fatty acids can be rapidly oxidized compared to saturated fatty acids (Leyton, 1987). According to the results of this study, female chicks treated *in ovo* α-tocopherol had higher serum cholesterol level than male chicks at the early developmental period, which is an indication that it reflects transported cholesterol from tissues to blood serum. Serum LDL level was higher in female chicks than male chicks in the *in ovo* α-tocopherol treatment group at day 3 of age and this result was due to the significant interaction between IO treatment and gender. The effect of *in ovo* administration of α-tocopherol indicated that the transfer of lipids from the tissues to blood serum is more efficient in female chicks compared to male chicks. The main reason for this effect is reduced lipid peroxidation in the tissues due to increase the vitamin E concentration in the chick tissues (Surai, 1999). Vitamin E is transported from the egg yolk to the tissues during the development of the embryo (Noble et al., 1993; Gaal et al., 1995; Surai et al., 1996) and shows antioxidant properties by breaking the chain reaction of lipid peroxidation (Yamauchi, 1997; Surai et al., 2016).

Table 1. Means and standard errors of the effects of *in ovo* (IO) treatment on serum total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) levels

	Cholesterol	**Triglyceride**	**HDL**	**LDL**	**VLDL**
	mg/dl				
In ovo treatment (IO)					
Control	201.8±7.8	106.4±11.5	137.2±4.4	43.3±4.1	21.3±2.3
α-tocopherol	206.8±7.9	94.25±11.7	135.9±4.5	51.9±4.2	18.8±2.3
P value	0.661	0.471	0.847	0.162	0.471
Gender					
Female	203.8±8.2	102.1±12.2	134.1±4.6	49.3±4.4	20.4±2.4
Male	204.7±7.5	98.6±11.0	139.1±4.21	46.0±3.9	19.7±2.2
P value	0.934	0.836	0.441	0.580	0.836
IO * Gender					
Control * Female	185.4±11.0^{b}	100.6±16.3	127.7±6.2^{b}	37.6±5.8^{ab}	20.1±3.3
Control * Male	218.2±11.0^{ab}	112.2±16.3	146.7±6.2^{a}	49.02±5.8^{ab}	22.4±3.3
α-tocopherol * Female	222.2±12.4^{a}	103.5±18.2	140.5±6.9^{ab}	61.0±6.5^{a}	20.7±3.6
α-tocopherol * Male	191.3±10.1^{ab}	85.00±14.9	131.4±5.7^{ab}	42.9±5.33^{b}	17.0±2.9
P value	0.012	0.374	0.039	0.023	0.374

^{a,b} Means in the same column are significantly different (P<0.05).

Conclusion

This study showed that increased vitamin E level in the yolk sac by injection of α-tocopherol into the yolk sac of the embryo can associate with an increase in the transfer of some lipids from the tissues to the blood serum of 72-hour-old female chicks. As a result of this association can improve the efficiency of the antioxidant system of female broiler chicks during the early developmental period after hatching.

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CURRENT SITUATION OF CATTLE HUSBANDRY AND BREEDING POSSIBILITIES IN BİNGÖL PROVINCE OF TÜRKİYE

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Abstract

The purpose of this survey is to put forward the current situation and the potential of cattle husbandry and the breeding opportunities in Bingöl province of Eastern Anatolia in Türkiye. Many reasons such as the development of the industry based on animal-derived raw materials, the creation of employment in rural areas, the development of priority regions for development, the increase of productivity in agriculture, and the establishment of foreign trade balances are of strategic value for Türkiye. Bingöl is one of the most prominent cities of this area in which its population sustain themselves by animal production. For this reason, it is very important to define condition, potential, and problems of breeding sector especially with respect to cattle stock in this region. Due to the interest of private sector representatives in this field in recent years, cattle breeding has started to gain importance. The fact that a very large private integrated enterprise started to operate in the province was effective in this development. Indigenous breeds raised in this region need to be genetically improved, because genetic improvement offers solutions for satisfaction of needs in livestock husbandry. In Bingöl province, cattle husbandry participates with 0.73% in general livestock production in Türkiye with high share. Characteristics of cattle husbandry in this region are small farms with 3-4 cows per farm (75-80% of total number of farms). The most important of native cattle breeds are Eastern Anatolia Red and Native Black breeds. The cow milk from these breeds is either consumed by the household as raw milk or processed as cheese. As a result, preservation of native cattle breeds as a gene source for milk and meat production is very important.

Keywords: *Cattle husbandry, Milk production, Breeding system, Bingöl province.*

Introduction

The most critical sub-sector for entry into the European Union is livestock. Many reasons such as the development of the industry based on animal-derived raw materials, the creation of employment in rural areas, the development of priority regions for development, the increase of productivity in agriculture, and the establishment of foreign trade balances are of strategic value for our country.

Livestock contributes to the development of the country's economy, creates employment in rural areas and provides significant added value. Adequate and balanced nutrition of the people ensures the production of products such as meat, milk and eggs, which are absolutely necessary for a healthy mental and physical development, especially in children and young people. It provides raw materials to various industries such as meat, milk, textile and leather, helps the establishment and development of sub-industry branches such as feed, medicine and equipment, and contributes to the country's export revenues at varying rates. Animal manures are effective in improving the physical structure of the soil and increasing soil fertility in plant production. Livestock allows the use of unemployed labor in agricultural enterprises, especially in the period when there are no plant production activities in winter (Aygün, 2020).

Cattle breeding in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications. With the inquiry works, results are determined as yield of cattle is low, the number of animals raised are small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Lack of food, especially lack of protein is still basic problem in Turkey. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry (Aygün, 2020; TOB, 2023).
Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). The first practices that should be done to increase the profitability in livestock enterprises should be directed to environmental regulations. As the environmental factors improve, the genotype needs to be improved accordingly (Öztürk, 2009).
The aim of this survey is to put forward the current situation and the potential of cattle husbandry and breeding opportunities in Bingöl province of Eastern Anatolia in Turkey.

Demographic Structure of Bingöl Province

Bingöl province is located in the Upper Euphrates section of the Eastern Anatolia Region. It is surrounded by Muş in the east, Erzurum and Erzincan in the north, Tunceli and Elazığ in the west, and Diyarbakır in the south. Bingöl Province is located between 41º - 20 and 39º - 56º east longitudes and 39º - 31 and 36º - 28º north latitudes. Its area is 8.125 km^2 (Anonymous, 2023). Economy of Bingöl province is an economy mainly based on agriculture and animal husbandry, and aquaculture. The livestock sector in Bingöl province has been developing and gaining importance day by day.
There are 7 districts of the province, namely Adaklı, Genç, Karlıova, Kiğı, Solhan, Yayladere, and Yedisu. The city center is located at an altitude of 1151 meters from the sea, in the northwest corner of the Capakçur plain, on a plain overlooking a branch of the Göynük stream, which meets the Murat water around Genç District.
Bingöl, which has an ideal structure for forestry in terms of climate and land structure, is one of the provinces with the richest forest area in the Eastern Anatolia Region. However, the use of forests to meet the need for fuel for a long time and to be used in animal husbandry has resulted in it becoming a degraded coppice (Anonymous, 2023). Different plant species, distributed in meadow and pasture areas in Bingöl province, are seen as the main food source for nutrition of cattle, sheep, and goat.
Bingöl city is sixty-second the most crowded city in Türkiye. As of the begin of 2023, its population is 282.556 people. The educational status of the animal breeders and families of Bingöl province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country (Anonymous, 2023).

The Importance of Cattle Breeding in Bingöl Province

In the province of Bingöl and its surroundings, the presence of meadow and pasture areas in areas where animal husbandry is carried out especially in the form of pasture livestock is very important in terms of making livestock more economically as it will provide suitable natural environment conditions for livestock. For this reason, the existence of meadow and pasture

areas in any place provides the development of animal husbandry and the first source of livelihood as an economic activity (Kılıç, 2018).
In this region, animal husbandry is mostly done with traditional methods. In the animal husbandry activities carried out in the region, animals are taken to the pastures with the removal of snow from the ground in the spring and stay in the pastures for a total of 6-7 months until the end of autumn. During this period, animals meet their nutritional needs from meadow and pasture areas. In the winter season, a significant part of the nutritional needs of the animals is met by the dried grass piles that are collected and dried from the pastures in the summer period. However, sometimes straw and ready-made fodder are used in cases where the pastures are not sufficient (Kılıç, 2018).
Cattle husbandry is an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Cattle husbandry is indispensable and an important source of income for farmers in Bingöl province. Bingöl province is suitable for both small ruminant breeding and cattle husbandry in terms of large pasture areas, water resources, and climate characteristics.
The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001). Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level.
Although goat husbandry used to be very common in this province, its population has gradually been declined in the last 20 years. Because, roughage and concentrate feed costs have gradually increased. However, goat breeders in Bingöl still consider the state's incentives insufficient. The sheep and the goats have been raised together in the region. Small ruminant husbandry in the study area is carried out with traditional methods. Goat production is an animal sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Goat production is indispensable and an important source of income for farmers in Bingöl province. Actually, this province is suitable for both the small ruminant breeding and the cattle and the water buffalo husbandry in terms of large pasture areas, water resources, and climate characteristics (Esen, 2017).
It can be said that the province has an important dairy cattle breeding potential. The existence of a private dairy farm is a result of this potential. The enterprise makes a significant contribution to the animal husbandry and economy of the province.

Number of Cattle in Bingöl Province

Characteristics of cattle husbandry in Bingöl province are small farms with 3 to 4 cows per farm (75-80 % of total number of farms). The most important of native cattle breeds of Turkey is the Eastern Anatolia Red breed. It is very important to define the condition, potential and problems of breeding sector especially with respect to the cattle, sheep, and goat stock in this city. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. The most important of these problems is roughage. Inventory studies constitute the basis of all kinds of studies that are planned to be carried out at national or regional level. Therefore, important suggestions for the Bingöl region will be presented with this study. Number of cattle and buffalo is presented in Table 1 in Turkey and in Table 2 in Bingöl province.

The numbers of indigenous cattle are low and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Bingöl province of Eastern Anatolia in Turkey, the indigenous cattle were found more in numbers. Therefore, the milk productivity of the indigenous cattle is very low. These large number of non-descript indigenous cattle have to be improved by upgrading with suitable indigenous high milk yielding breeds such as Simmental and Kilis breed. Improvement in productive and reproductive performance is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers.

Table 1. Number of cattle and buffalo in Turkey (head) (TÜİK, 2023).

Year	Cattle	Buffalo	Total
2018	17042506	178397	17220903
2019	18070500	180826	18251326
2020	17965482	192489	18157971
2021	17850543	185574	18036117
2022	16851956	171835	17023791

Table 2. Number of cattle and buffalo in Bingöl province (head) (TÜİK, 2023).

Year	Cattle	Buffalo	Total
2018	141331	164	141495
2019	140559	215	140774
2020	141736	194	141930
2021	135825	136	135961
2022	134100	76	134176

Animal Production from Cattle in Bingöl Province

It is considered that the size of the establishment has no effect on cattle breeding practices (Özyürek et al., 2014). Unfortunately, in most of the enterprises studied in the region, there are no suitable environments for animal husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to country's economy. Meat and milk products from cattle in Turkey is presented in Table 3.

Table 3. Meat and milk products from cattle in Turkey (tons) (TÜİK, 2023).

Year	Meat	Milk
2018	1281234	20036877
2019	1330169	22960379
2020	1341446	23503790
2021	1460719	23200306
2022	1572747	239500345

In Turkey, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of dairy cattle farms. In large-scale researches conducted in 2019, it is observed that the bodies of cows in milk processing are unacceptably dirty (up to 70%), thus causing frequent foot, breast and reproductive health and milking hygiene problems (TOB, 2023).

It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks (Kılıç, 2018).

Zoonotic diseases are one of the most important problems of workers in cattle husbandry. Workers and animals must be vaccinated against various zoonotic diseases. The rules of order and hygiene must be taken into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of workers, and a separate toilet in each household to facilitate personal hygiene. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered (Aygün, 2021).

It is known that the most important problem of milk producers in the province is the lack of organization. Accordingly, it is understood that the inputs used in milk production are expensive and of poor quality, the producer is not financed under sufficient and appropriate conditions, and the producer has to accept the market conditions in the marketing of milk. It can be said that in order to develop dairy cattle breeding in the province, it is necessary to organize the producer and to be supported continuously by the state.

Conclusion

The main problems of cattle breeding in the region are the lack of infrastructure and organization. In recent years, it is a danger for the sector that the domestic producer cannot make money due to the lack of animal husbandry, insufficient policies and the import of animals and animal products from abroad. Whereas Bingöl province has ecological conditions suitable for animal production. This potential of the province should be turned into an opportunity for breeders. As a result, preservation and development of native cattle breeds as a genetic source is very important. If the current potential especially in the Eastern Anatolian Region and in Turkey is evaluated, it can become very important in this region. For cattle presence and animal production in Bingöl province, it can be said that it has an important place in terms of its features.

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OCCUPATIONAL AND ZOONOTIC DISEASES IN LIVESTOCK SECTOR

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Abstract

In this review study, it is aimed to provide sensitivity of breeders about the occupational and the zoonotic diseases in the sector of animal production. It is also aimed to provide suggestions for the occupational and the zoonotic diseases in animal husbandry. One of the most important health problems encountered in every field and stage of the livestock sector is the zoonotic and the occupational diseases. What should be the preventive health measures regarding these diseases is one of the most important issues. Animal production is associated with a variety of occupational illnesses and injuries. In general, possible dangers of workers in the livestock sector are ergonomics, noise, air conditioning, chemicals, pesticides, animal attacks, bites, injuries, accidents in transport, psychological stress, and skin-borne diseases etc. Especially, the animal hitting and the zoonotic diseases are very important problem in animal husbandry. It is seen that the economic losses of accidents, which can cause disability or cost to the life of the workers in the livestock business as a result of work accidents and occupational diseases, are at very serious levels. Therefore, the precautions related to the occupational and the zoonotic diseases must be taken for the workers at livestock sectors. It is the duty of the owner of the business to provide healthy and safe working environments where the risks of disability, illness and death will be minimized for those working in livestock. For this purpose, it is necessary to focus on the zoonotic and the occupational diseases, and the occupational accidents and to train employees in terms of occupational health and safety.

Keywords: *Animal husbandry, Occupational health and safety, Illness, Zoonosis.*

Introduction

One of the most important issues encountered in the livestock sector in Türkiye is occupational diseases and zoonotic diseases transmitted from animals to humans. The livestock sector has different health problems compared to other sectors. In livestock enterprises, the employees in this group should be treated according to the work they do. Special attention should be paid to the use of appropriate personal protective equipment for occupational diseases and diseases transmitted from animals to humans, which can be seen in farmers engaged in animal husbandry, and training should be provided for this.

Zoonoses and occupational diseases, which are among the most important health problems encountered in every field and stage of the livestock sector in Türkiye, and what should be the preventive health measures related to these diseases are among the most important issues. It is seen that the economic losses of these accidents, which can cause disability or cost to the life of the workers in the livestock business as a result of work accidents and occupational diseases, are at very serious levels (Aygün, 2021).

Workers working in the livestock sector take part and play an important role in all phases of animals from breeding to birth and rearing, from feeding to obtaining products. In addition to the high working potential in the animal husbandry sector, it is understood that their efforts cannot be rewarded despite the intense need for workers, and therefore, their share of

production is quite inadequate due to the excess of unregistered activities in the sector. Due to its unique characteristics in the livestock sector, problems in terms of security, salary, accommodation, health, transportation, education, social and cultural aspects can be seen quite often, despite intense working hours and high performance (Aygün, 2017; 2022).

One of the most important risk factors in animal husbandry is animal shelters. Regular ventilation is one of the most important issues. It is emphasized that especially workplace ventilation should be done to control exposure to pollutants in the air (Eğri et al., 2014). In general, it is used to remove impurities such as smoke, dust and steam in order to provide a healthy and safe working environment.

The livestock activities in Türkiye has an important place. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of occupational health and safety in animal production is very important as it is in many other areas. Occupational diseases and accidents that can be encountered by workers at business have caused the losses of very serious economic and the qualify person in animal production. In addition, the sustainability of production is negatively affected. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for the workers at the livestock enterprises, the field and, the factories such as the feed, the skin and the meat (Aygün et al., 2019).

It is true that more occupational health and safety intervention research focusing on preventing illness and injury needs to be conducted. Conducting this type of research is difficult and time-consuming; however, without increasing the number and methodological rigor of these studies, it will be difficult to identify effective intervention methods and confidently encourage their use (Goldenhar and Schulte, 1996). Animal production is associated with a variety of occupational illnesses and injuries.

In this review, information about occupational diseases and zoonotic diseases observed in animal husbandry was compiled. And brief information about the possible risks in livestock production and the measures to be taken against them were presented. In addition to, it is aimed to provide sensitivity of breeders about the occupational and the zoonotic diseases in the sector of animal production. It is also aimed to provide suggestions for the occupational and the zoonotic diseases in animal husbandry.

The Place and Importance of Occupational and Zoonotic Diseases in Livestock Sector

The livestock activities in Türkiye have been characterized by the different regional applications. The activities of animal husbandry are especially an important source of income for the indigenous people in Türkiye. The issue of occupational health and safety has become more important today than in previous times, and it has begun to be seen as a measure of the importance given to people. In fact, zoonotic and occupational diseases, the number of which has reached an undeniable size, have become a very important issue for employees, businesses, the state and increasingly for the society. In this term project, it was emphasized that zoonotic and occupational diseases can be reduced significantly by giving the necessary importance. For this, it is necessary to raise awareness by giving training seminars for those working in the livestock sector. While these studies are being carried out, it is necessary to create an occupational health and safety culture adopted within a system understanding by taking the views of the group, including the employers, as well as being aimed at those dealing with animal husbandry in the country (Aygün et al., 2018). One of the biggest problems for agricultural sector workers arises at this stage. It is essential to ensure the hygiene of these environments in order to prevent the lack of clean drinking and utility water, unsuitable conditions for personal hygiene, and to prevent diseases caused by pests and

deterioration. The agricultural sector is unique in nature. It has serious differences from other business lines in terms of working conditions, living standards, business environment. It is one of the rare sectors that is generally carried out as a family business and where business and home life cannot be separated from each other with certain lines. Unfortunately, all the legal arrangements made to increase the welfare and safety of workers in the agricultural sector, which is among the sectors with the highest rate of informality and child labor, unfortunately did not affect the living standards of the agricultural sector workers in their active working life. Due to long working hours, working tempo, lack of competent personnel, socio-economic and political factors, low earnings, widespread employment of child workers and most importantly, the integration of agriculture as a way of life rather than a profession, thus the lack of social security. Insecurity against work-related accidents is among the problems that await solutions (Çamurcu and Seyhan, 2015).

Studies made by some researchers in order to achieve their political goals on political and ideological studies relating to a multi-ethnic structure and stockbreeding activities that is the main source of income of the politicized tribes were the main theme of this study. One of the most important issues to be taken into account for sustainable small ruminant breeding in the region is no doubt nomadic living culture of tribes that they presented this culture and breeding system to nowadays. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds (Aygün et al., 2013).

The Most Common Occupational and Zoonotic Diseases in Livestock Sector

Animal production is associated with a variety of occupational illnesses and injuries. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses. Zoonotic (zoonosis) diseases; it can be defined as diseases that humans and animals can transmit to each other and that are shaped jointly in individuals belonging to both groups. In addition to zoonotic diseases caused by farm animals such as cattle, sheep, cats, dogs, chickens, birds, etc. poultry, monkey, mouse etc. The zoonotic disease of many animal species, such as wild mammals and rabbits, is transmitted to humans and causes serious problems.

An "occupational disease" is any disease or disorder contracted primarily as a result of an exposure to risk factors arising from work activity. "Work-related diseases" have multiple causes, where factors in the work environment may play a role, together with other risk factors, in the development of such diseases. The World Health Organization emphasizes the following: Carrying our estimates of the global burden of disease from major occupational risks, such as injuries, airborne exposures, carcinogens, ergonomic stressors, noise and other specific risks. Incorporating occupational diseases and their causes in the 11th revision of the International Statistical Classification of Diseases and Related Health Problems. Working with ILO to develop diagnostic and exposure criteria for occupational diseases and to enable primary and secondary health care providers to detect and report such diseases (WHO, 2020).

The World Health Organization (WHO) defines zoonotic diseases as 'any diseases or infections that are naturally transmitted between vertebrate animals and humans.' Agents causing zoonotic diseases may be bacteria, fungi, viruses, parasites or any other communicable agents, for example prions. Currently there are over 200 recognized zoonoses, some of which have a worldwide distribution and others which are localized to specific regions. The situation is not static, and emerging zoonotic diseases are continually being recognized, both animal diseases which have spread to humans for the first time and existing zoonoses spreading to new geographical areas. Occupational zoonotic diseases are most common where there is close contact between animals and humans at work, for example in

animal husbandry and agricultural occupations, although workers in a wide range of other occupations may also be exposed to zoonotic agents, including those employed in the outdoor leisure industry or the waste water industry and laboratory workers. There are many occupational zoonotic diseases in the world, many of which occur very rarely, although some do pose a significant health risk for workers in certain occupations. While the incidence of specific zoonoses varies from country to country, there are many occupational zoonoses that occur across Europe, although not every disease is present in every country (Cook and Farrant, 2020). The most common hazards at small ruminant husbandry in Türkiye are the ergonomics, the noise, the air conditioning, the chemicals, the occupational diseases, the zoonotic diseases, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress and, the skin-borne diseases etc. Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in small ruminant husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field (Aygün, 2017). Other health and safety risks include skin problems, hearing loss, stress, and mental well-being issues particular to farming and the rural way of life. Occupational skin disorders are common in livestock workers. The effects of sun exposure are an important cause of morbidity in shepherds group.

Farmers' lung is one of many forms of *hypersensitivity pneumonitis*. This problem is becoming rare, which is likely due to the reduction of exposure to organic dust from the increasing mechanization of agriculture and the effect of livestock health and safety programs. Another danger for breeders is the waste of animals. Animal wastes are frequently stored underground and are a source of toxic gases. Entering confined spaces used for manure storage can lead to fatalities, which are often caused by hydrogen sulfide exposures (Von Essen and McCurdy, 1998). Occupational diseases can go undiagnosed and untreated and worst of all, effective preventive measures are not taken because of lack of awareness to the problem. It is therefore believed that with the knowledge of Occupational Health and Safety and the ability to apply this knowledge in recognizing potential accident situations (hazards) would assist in decision making and the taking remedial measures (Demba et al., 2013).

The number of farmers in the Türkiye affected by pesticides is unknown. Little is known about the extent or magnitude of chronic health problems related to occupational exposure to pesticides. Although difficult, it is important to carry out further studies on the adverse health effects associated with pesticides among farm workers. Migrant farm workers have exposure to other hazards that may increase their risk of health problems: climate-dependent problems, such as heat stroke or cold shock, and occupationally caused infections such as *anthrax, ascariasis, encephalitis, leptospirosis, rabies, salmonellosis, tetanus,* and *coccidioidomycosis*. Sensory problems are common: eye problems, caused by irritation, infection, or injury from the wind, sun, dust or soil, agricultural chemicals, debris ejected from farm machinery, and allergic reactions to plants, and hearing problems due to noise from farm machinery and cannery work.

Some Suggestions and Possible Preventions for Breeders

It can be easily said that work accidents and occupational diseases will increase if occupational safety and occupational health are not given importance. Those who will be most affected by this will be the first-degree workers. First of all, the income level of the worker and naturally the worker's family will decrease. This will have some negative effects on the worker and his family. The worker who is disabled or who loses certain limb or limbs may also suffer from some psychological disorders. This will negatively affect both workers and society (Yiğit, 2005; Karacan and Erdoğan, 2011).

In animal husbandry, precautions should be taken by providing adequate training to all employees regarding the dangers and risks they encounter in terms of occupational health and safety, regardless of the type of animal husbandry. It is very important to raise awareness of farmers about occupational accidents, occupational diseases and zoonotic diseases transmitted from animals to humans. Here, it is very important to take precautions against adverse climatic conditions and mechanical risk factors that may affect especially the farmers who are engaged in animal husbandry (Aygün, 2021).

It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of animal husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.

Zoonotic diseases are one of the most important problems of farmers in animal husbandry. Workers and animals must be vaccinated against various zoonotic diseases.

With regard to the control of occupational zoonoses, there are some general control measures which reduce the risk of infection for a wide range of zoonoses. These include the following:

- good personal hygiene practices, especially washing with soap and warm water;
- covering cuts and scratches with waterproof dressings;
- wearing of appropriate PPE (Personal Protective Equipment), for example gloves, overalls, respiratory protection – this must provide relevant protection, while also being suitable for carrying out the required task;
- good hygiene practices for animal husbandry; and
- use of an appropriate disinfectant to clean potentially contaminated areas (Aygün, 2021).

For certain zoonoses there is an effective vaccine available and it may be appropriate to administer this to individuals in high risk occupations, for example laboratory workers handling infected animals. In many cases there are effective prophylaxis and treatments available. For these to be used to maximum advantage, it is necessary for workers to be aware of any diseases they may be at risk from and to be able to recognize early symptoms of these diseases. For certain occupations it may be required for workers to inform their employer if they have a weakened immune system (Cook and Farrant, 2020).

Taking precautions for occupational health and safety are very difficult, costly and time consuming. Among the difficulties is the varied nature of agriculture, the many ethnic groups engaged in the activities, the traditionalist view of farming families, and rapidly changing technology. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered (Aygün, 2022).

Conclusion

In animal husbandry, precautions should be taken by providing adequate training to all employees regarding the dangers and risks they encounter in terms of occupational health and safety, regardless of the type of animal husbandry. It is very important to raise awareness of farmers about occupational accidents, occupational diseases and zoonotic diseases transmitted from animals to humans. Here, it is very important to take precautions against adverse climatic conditions and mechanical risk factors that may affect especially the farmers who are engaged in animal husbandry. Occupational diseases and zoonotic diseases that can be encountered by workers have caused the losses of very serious economic and the qualify

persons in livestock sector. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field.

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USE OF SERUM LEVELS OF FSH AND LH AS INDIRECT SELECTION CRITERION IN GENETIC IMPROVEMENT OF REPRODUCTION IN SHEEP

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Abstract

The aim of this review is to emphasize the importance of gonadotropic hormones (FSH and LH) in genetic improvement of reproduction in sheep. One of main objectives of sheep breeding in terms of contemporary sense is to increase the fertility of sheep. While doing this, it is necessary to take advantage of animal material and breeding opportunities at the highest level. Increasing fertility of sheep gives advantage of providing a higher income by selling the non-breeding animals as well as the chance to make a more effective selection. Achieving these goals is possible with the use of advanced technological methods and approaches as well as physical measures such as shelter and care. With the introduction of biotechnological processes, hormones and the more effective use of immunization methods, it has been possible to present different options for controlled reproduction. Since the reproductive activity in sheep is seasonal, it imposes great limitations on studies aimed at improving reproduction. Until today, fertility measures based on direct lambing results such as lambing number and tummy width have been used in studies on genetic improvement of fertility in sheep populations. However, in addition to these known criteria, which we can be called classical, more effective physiological and indirect fertility criteria have become up-to-date. In selection studies, indirect and physiological variables related to fertility are ovarian activity, ovulation rate, characteristics of estrus activity, testicular characteristics, production age of gonadotropic hormones, the change of these hormones, and serum or plasma levels at pituitary. It would be beneficial to define such criteria, which are so related to reproductive performance, and to clarify their usability as indirect selection criterion.

Keywords: *Endocrinology, Genetic improvement, Gonadotropic hormones, Selection, Sheep.*

Introduction

Research on the determination of hormone levels in the blood as an indirect selection criterion for the improvement of fertility in sheep remains up-to-date in terms of genetic improvement. When the studies on the improvement of fertility by using physiological criteria in countries where sheep breeding is developed are examined, it is seen that there are positive relationships between gonadotropic hormone concentration and fertility. For this reason, it is very important to determine hormone levels such as FSH and LH as indirect selection criteria in the improvement of fertility on sheep breeds (Peters, 1991; Karaca et al., 1992).

As in other farm animals, one of the ways to increase the yield per animal in sheep is genotypic improvement of populations. Quantitative characters can be determined more in the later stages of life. This situation affects the selection efficiency negatively by prolonging the time between generations. The probability of genotypic superiority of individuals determined to be phenotypically superior determines the degree of accuracy in selection. One of the ways used to increase the degree of accuracy in selection and to shorten the time between generations is to determine the yields and various physiological events related to each other (Aygün and Karaca, 1999).

Although there are many studies conducted in this direction in highly fertile sheep breeds, gonadotropic hormone (FSH and LH) levels that are effective in increasing fertility performance and ovulation rate of animals have not been defined in domestic sheep breeds of our country. It would be beneficial to define such criteria, which are so related to reproductive performance, in sheep breeds and to clarify their usability as indirect selection criteria (Karaca et al., 1992; Aygün and Aşkın, 1999).

In sheep, there are important criteria that determine intra- and inter-racial variation in terms of seasonal mating efficiency. Among these, attention is drawn to the levels of estradiol and testosterone hormones, as well as FSH and LH. However, it has been reported that these criteria cannot be used alone as an indirect selection criterion in selection programs, since the gonads must be taken in the measurement of estradiol and testosterone hormones (Haresign and McLeod, 1985).

It is clear that it is necessary to determine the level of LH hormone together with FSH in the blood, which is directly related to the number of ovulation, and to make estimations of genetic and phenotypic parameters related to these hormones, and also to define the fertility performances of sheep breeds in a more sensitive way. In this review, the usability as indirect selection criterion and the importance of gonadotropic hormones (FSH and LH) in genetic improvement of reproduction in sheep have been emphasized.

Role and Release of Gonadotropin Hormones (FSH and LH) in Reproduction of Sheep

Follicle-stimulating hormone (FSH) is a glycoprotein hormone synthesized and secreted by the pituitary gland. The pituitary gland, as one of the endocrine organs of the animal, plays a crucial and pivotal role in different physiological processes of mammals due to the secretion of various hormones. In addition to FSH, the other hormones secreted by the pituitary gland are growth hormone (GH), prolactin, adrenocorticotropic hormone, melanocyte-stimulating hormone, thyroid-stimulating hormone (TSH) and luteinizing hormone (LH) (Hong et al., 2016; Wang 2021). Among these hormones, FSH, as one of the important gonadotropins involved in mammalian reproductive development, is secreted into the blood after synthesis by the gonadotrophic cells (a type of basophilic cell) in the anterior pituitary gland (adenohypophysis). Then, it acts on the corresponding target organs of the mammals, namely the testes and ovaries, to exert its biological functions through the peripheral blood circulation. Since FSH is a key regulator in the hypothalamic-pituitary-gonadal (HPG) axis, it plays an indispensable role in mammalian reproductive activities (Wang 2021).

Gonadotropin hormones, luteinizing hormone and follicle-stimulating hormone are synthesized by the anterior pituitary gonadotropes and secreted into the circulation to regulate gonadal function. Every secretory pulse of LH from the pituitary corresponds to a pulse of GnRH from the hypothalamus (Levine et al., 1982; Coss, 2018). On the other hand, FSH secretion is not entirely regulated by GnRH and most of FSH is constitutively released (Levine and Duffy, 1988).

Pulsatile LH secretion maintains basal level of this hormone in the circulation and steroidogenesis throughout the cycle. LH increases steroid hormone production via induction of steroidogenic enzymes in interstitial leydig cells and theca cells in males and females, respectively. In females, LH exhibits a 5–10 fold mid-cycle increase that causes ovulation. FSH in males induces androgen binding protein and contributes to spermatogenesis through interaction with testosterone. In females, FSH stimulates aromatase expression in follicle granulosa cells, which increases estrogen production and follicular growth. FSH increases together with LH during the pre-ovulatory surge and exhibits a separate, second increase that is necessary for folliculogenesis (Coss, 2018).

Both gonadotropins are synthesized in the same gonadotrope cells, but differences in the relative amounts occur at specific stages of the menstrual or estrous cycle. As illustrated above, LH exhibits a single increase during the pre-ovulatory surge, while FSH increases two times during the menstrual or estrous cycle. The question of how are these two hormones differentially regulated in the same gonadotrope population, by the same hormonal milieu, has been an area of intense investigation. Differential levels of LH and FSH may stem from differences in secretion, since LH is secreted strictly through the GnRH-regulated secretory pathway while FSH is constitutively secreted (Levine and Duffy, 1988).

Relationships between FSH and LH levels and fertility performance in sheep

The normal reproductive function and reproductive ability of animals depend on the precise regulation of various reproductive hormones, including FSH. Therefore, it may help us better understand the physiological and pathological processes, such as spermatogenesis, ovulation, the menstrual cycle, puberty, and even reproductive system diseases, if the molecular mechanisms that regulate the synthesis of gonadotropins can be determined. In recent decades, we have clarified the molecular mechanism of the overall regulation of FSH secretion. Every step of FSH synthesis and secretion is strictly controlled by the signals that mediate initial synthesis to the signals required to successfully perform biological functions. Therefore, the regulation of FSH secretion is a highly complex and multilevel network. All of these phenomena further increase the complexity of the molecular mechanism regulating FSH secretion. There are still more kinds of apparent genetic modifications that play unknown functions, although we have clarified the regulation of the FSH molecular basis and signaling pathways. Additionally, more comprehensive studies are needed to decipher the intertwined potential molecular mechanisms that regulate the synthesis and secretion of FSH in different physiological systems. These studies will help us have a clearer understanding of the internal processes regulating animal reproduction, improve the artificial regulatory system of animal reproductive processes, and even provide deeper theoretical support for the exploration and development of potential therapeutic targets and effective therapies related to reproductive diseases or other diseases affected by FSH (Wang et al., 2021).

FSH and LH hormones play an important role in spermatogenesis in male farm animals (Kumar et al., 1997; Araki et al., 2000). However, studies in male mice suggest that a mutation occurring during FSH production adversely affects both testicular and semen characteristics. In addition, growth retardation in reproductive organs negatively affected Gn-RH production of spermatozoa capable of fertilization in mice (Cattanach et al., 1977; Abdelrahman et al., 2019). FSH and LH hormones regulate the function of reproductive organs by a feedback mechanism. The issue of how the inhibin hormone regulates FSH secretion in domestic mammal female animals is still a research topic (Kandemir et al., 2023).

FSH and LH are important hormones in the reproductive physiology of both male and female organisms (Şenok et al., 2020). However, in male animals, testosterone is the most important gonadal hormone regulating both FSH and LH secretion. As known, the role of the inhibin hormone, which acts as a regulator of FSH secretion, is largely dependent on the species and age of the animal. However, the importance of inhibin hormone in the regulation of FSH secretion in adult male animals varies between species (McKeown et al., 1997; Kandemir et al., 2023).

It has been reported that the level of FSH hormone at the time of birth is low in ewes (Chamley et al., 1974). Bodin et al. (1988) reported that there was a positive phenotypic correlation between fertility performance and FSH levels in Lacaune brood lambs. In this study, besides the age of the animal at the time of blood sampling, the effect of birth type and blood collection time on plasma FSH and logFSH was found to be insignificant ($p>0.05$). On

the other hand, the effect of herd and fathers was found to be quite significant ($p<0.01$). The mean FSH and logFSH levels determined in the study were estimated to be lower in singleton females compared to those giving birth to twins or more, but it was reported that this difference was not significant (Table 1).

Table 1. Least Square means and standard errors of plasma FSH and logFSH levels corrected for flock and lambing age in Lacaune brood lambs with different birth types (Bodin et al., 1988).

Litter Size	FSH (ng/ml)	logFSH (ng/ml)
1	7.45±0.16	1.89±0.016
≥ 2	7.74±0.19	1.89±0.016

Some genetic parameter estimates for FSH and LH hormone

Studies in this area were first compiled by the researcher Land (1974). Land (1974) stated that it is essential to know the genetic parameters in the comparison of various herds and breeds and in selection practices.

Studies on the heritability of FSH levels in sheep are very limited. One of the first studies conducted in this context was the study carried out by Bodin et al. (1986) on Lacaune brood lambs. In this study, in which herd and paternal effects were seen to be very significant ($p<0.01$), heritability was estimated as 0.38 for plasma FSH and 0.47 for plasma logFSH. The effect of the width of the abdomen on the FSH level was found to be insignificant.

Bodin et al. (1988) estimated the heritability of plasma FSH and logFSH and litter size in 5-week old brood lambs. Heritability estimates were significantly positive, 0.44 ($p<0.05$) for FSH, 0.50 ($p<0.01$) for logFSH, and 0.37 ($p<0.05$) for abdominal width. The phenotypic and genetic correlations between FSH, logFSH and abdominal width were also predicted as positive).

Studies on the degree of recurrence of measurements of FSH level in sheep are very limited. However, researchers named Findlay and Bindon (1976) reported that the degree of recurrence of FSH level was higher than that of LH. In a study by Elsen et al. (1988) in Booroolax Romanov females, 3-10 days after birth. In the measurements made between weeks, the rate of recurrence for weekly FSH measurements was reported to be 0.49.

Increasing lamb productivity should be the main goal in sheep breeding. This is possible by obtaining more offspring from one sheep per unit time. It is possible to increase the fertility of sheep by using newly developed methods. Genetic improvement efforts will gain a new dimension with more sensitive studies on the detection of hormone concentrations in the blood, which emerged as a new approach. It will be useful to make genetic and phenotypic parameter estimations of these hormones by re-detection of the LH hormone concentration in the blood, which is directly related to the number of ovulation, with more frequent inspection intervals in different physiological periods (Aygün and Karaca, 1999).

Usability of Gonadotropic Hormones as Selection Criteria

The fertility rate in sheep can be evaluated as a natural result of lambing rate, the number of lambs born per ram and the survivability of the lambs. Research on these basic characteristics in sheep populations is quite extensive and advanced. Within the framework of progressive knowledge of reproductive physiology, some contemporary techniques for improving fertility can be put into practice with a better understanding of genetic principles and environmental impact mechanisms such as care-feeding and management. There are even molecular

manipulation and gene transfer studies related to seasonality, number of ovulation, pre- and postnatal viability (Wilmut, 1990).

Radioimmunoassay (RIA) methods have been developed for the detection of reproductive hormones. These methods have contributed greatly to a better understanding of reproductive physiology and to the implementation of fertility programs in this field. In addition, RIA methods have found wide application in examining the biochemical and physiological mechanisms that control reproductive functions in detail. For example, the determination of FSH and LH gonadotropins, estradiol-17ß, progesterone, prostaglandins, and later inhibin by RIA has been a basis for understanding the control of ovarian function (Peters, 1991; Aygün and Karaca, 1999).

Conclusion

In this review, the usability as indirect selection criterion and the importance of gonadotropic hormones (FSH and LH) in genetic improvement of reproduction in sheep have been discussed. Investigations on biology of reproduction have provided that both some new physiological criteria and litter size, and some criteria of reproduction put into practice. There are criteria such as ovarian activity, ovulation rate, age of sheep when gonadotrophins reach level to effect on gonads, change and levels of these gonadotrophins during year, traits of oestrus activity among physiological criteria for reproductive activity. Using as an indirect selection criterion of change of gonadotrophins during year and level in certain physiological periods is fairly new. Genetic improvement works for reproduction will take a new level if investigations on determining hormone concentrations in blood are made sensitively.

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THE INFLUENCE OF THE NUTRITIONAL STATUS OF DAIRY COWS ON THEIR WELL-BEING

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Abstract

The milk yield of cows depends on a number of factors, of which the most important are genetics and nutrition of cows, which are related to the way of nutrition and the quality of the food consumed by the animals, which affects the well-being of dairy cows. The welfare of dairy cows can be observed through the control of milk yields, which is important for determining the nutritional health and reproductive status of the herd. The content of milk fat and protein, the level of urea and their mutual relationship, which indicates metabolic problems caused by nutrition, help in determining the nutritional status. For this reason, it is very important to follow the daily report of the milk yield control, which shows the results of laboratory analysis in which the values of individual cows are shown, such as the stage of lactation, the daily amount of milk, content of milk fat, protein and lactose, number of somatic cells, fat/protein index, urea, and the last recorded insemination. On the basis of individual results from the report, decisions are made on the improvement of damaged values, thereby increasing the welfare of dairy cows.

Keywords: *Nutritional status, Report, Dairy cows, Welfare.*

Introduction

Animal welfare is one of the most important areas of the European Union's common agricultural policy, which is often included in the support system as compensation for increased costs incurred due to reaching generally accepted standards. The need for new tools and ways of assessing animal welfare is continously growing especially on dairy farms. Many livestock developed countiries within the framework of ICAR (International Committee for animal recording), of which CAAF is aslo member, develop specific welfare assessment models, which include the results of specific activities (registration of domestic animals, control of productivity, etc.)

The analyzed model (Lactanet Canadian network for dairy excellence and the Bavarian model) evaluates well-being through several areas and indicators such as: longevity (number of lactations, cows exuded and dead), relationship between nutrition and production (urea in milk, management, cows in transition, cows in production), calves and youth (calf mortality and age of heifers at calving), and health and reproduction (abortion, beta-hydroxybutyrate level, number of somatic cells in milk, the ratio of protein and fat in milk, and lameness and injuries of cows), etc.

Relationship between feeding and mlik production of cows

The quality of raw fresh milk shows us how much influence the way of feeding has on the well-being of dairy cows. Namely, it is very important to monitor the values of the composition of milk, that is, the fat and protein index, through which we can see if the nutrition is adequate or if it needs to be adjusted. It is important to look at the milk fat content

which can indicate metabolic problems, because due to lower food consumption, the body's tissues are depleted

„Protein content in the first (up to 60 days) and second stage of lactation (60-120 days) can reveal the cow's readiness for fertilization. Due to lower performance, it is not recommended to inseminate cows with a protein content of less than 3%. The fat/protein index (IMB) should be between 1,1 and 1,5. (CAAF, 2020.).

Inadequate nutrition, diseases or harmful environmental influences cause a change in the ratio of fat and protein, thereby impairing the animal welfare of dairy cows. If the IMB is greater than 1.5, it is most often the result of excessive pumping of body reserves in the first phase of lactation and it tells us that the cows have a health problem. As a higher IMB is not good, neither is a lower IMB, which most often occurs due to inappropriate feeding, that is feeding with an excessive amount of concentrated feed, which leads to diseases such as acidosis and paw disease, and a decrease in the ability to consume feed. Deviation from the recommended value of the IMB index reduces the quality of fresh raw milk, and thus the well-being of cows. Feeding nutrient-dense foods can result in the accumulation of organic acids in the rumen and reduced buffering of the rumen. (Kleen et.al., 2003, Stone, 2004, Rustomo et.al., 2006a, Rustomo et.al, 2006b, Rustomo et al., 2006c). A combination of these changes can lead to a drop in rumen pH. When the pH of the rumen is lowered for a long time each day, eg <5.6 for >3 h/day, subacute ruminal acidosis occurs (SARA) (Kleen et al., 2003., Stone, 2004., Gozho et al., 2005.) The amount of proteins in the meal and their degradability in the rumen affect the ammonia content in the rumen juice. Released ammonia, which is formed during the breakdown of proteins in the rumen, and which microorganisms cannot use for their development, passes into the main bloodstream and is converted into urea in the liver, which is mostly excreted in urine and the other part in milk. "The content of urea in milk should be from 15 to 30 mg/100 ml of milk. Low urea content in milk is an indicator of insufficient ammonia content in the rumen juice (CAAF, 2020.). It can indicate problems that arise due to unsuitable conditions for the development of rumen microorganisms. There can be a decrease in the digestibility of organic substances, most often cellulose, but also a decrease in feed consumption. In dairy cows, this results in a reduced amount of milk, in which the content of fat, protein and sugar is reduced. Contrary to a low urea concentration, a high content is an indicator of excessive ammonia content in the rumen juice, which causes disorders related to the use of a significant part of energy for the excretion of nitrogen from the body through the metabolism of ammonia in urea, which damages the liver and leads to frequent reproductive disorders. „"There is an indicative relationship between the content of urea in milk and fertility, because insemination is most successful if urea is approximately 25 mg/100 ml of milk (CAAF, 2020.).

Subacute ruminal acidosis (SARA) is a condition of particular concern to the dairy industry. The susceptibility of dairy cows to SARA appears to be highest for cows in early lactation (Fairfield et al., 2007; Penner et al., 2007; DeVries et al.,2009). A drop in the pH-value of rumen content below the critical point of 5.5 leads to a complex disorder that significantly disrupts the normal course of metabolism, called subacute rumen acidosis. If we use a large amount of carbohydrates in the rations of dairy cows, there will be a change in the micropopulation of the rumen content. By increasing the amount of bacteria that release lactic acid, the concentration of which increases in the rumen content. Lactic acid damages the rumen wall, and at the same time passively passes into the bloodstream, causing changes in the liver, lungs, heart valves, kidneys and joints.

Report of nutritional status

Based on the ratio of urea and proteins, it is possible to estimate the supply of the organism with degradable and metabolic proteins that are synthesized in the rumen, and since energy is necessary for the growth of microorganisms, it is possible to conclude on the basis of this data the energy supply of cows. If the ratio of urea and protein is not ideal, there is a disturbance in the supply of energy and protein, and it is necessary to investigate the causes of such a condition and adjust the ration to the actual needs of the cows and thus achieve an optimal ratio.

The content of urea in milk should range from 15 to 30 mg urea/100 ml of milk.). Deviation from the recom (CAAF, 2020.) mended values leads to the fact that the degradability of proteins, and thus the supply of metabolic proteins, is insufficient. In addition to the lack of protein in the meal, the cause can also be an insufficient energy level. The excretion of excess urea has a negative effect on the environment, but it can also damage the liver and lead to metabolic and reproductive problems, while with reduced urea content, there is a decrease in the amount of milk, and a decrease in the content of fat, protein and sugar. Research has shown that insemination was most successful in cows that had approximately 25 mg of urea/100 ml of milk" (CAAF, 2020.).

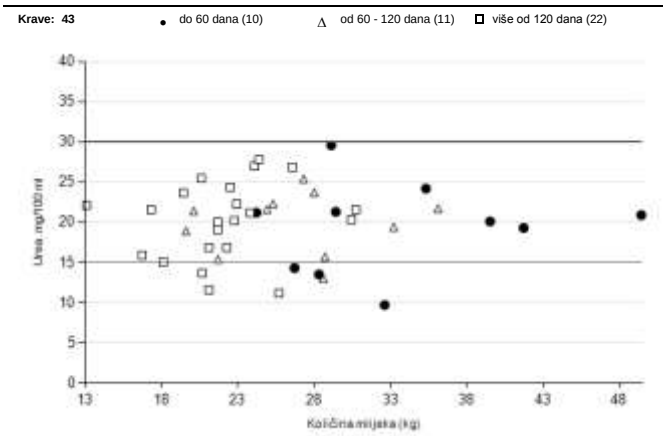
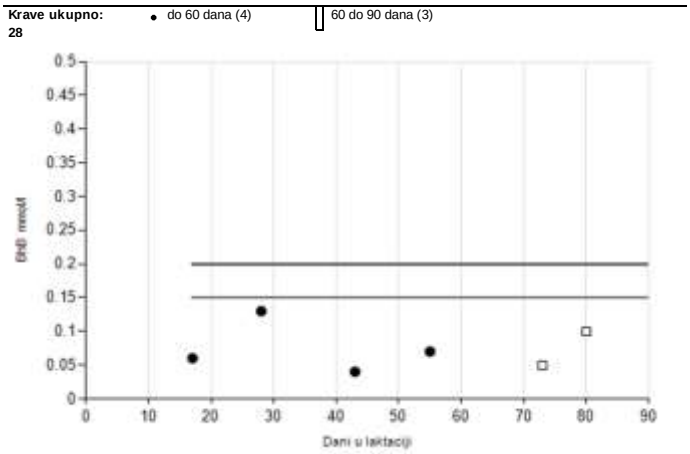


Figure 1. Ratio of urea: daily amount of milk (CAAF, 2020.)

"The relationship between IMB and daily milk volume makes it possible to detect cows that are at risk of developing metabolic diseases. IMB greater than 1.5 indicates the possible occurrence of metabolic problems such as ketosis. There are two mechanisms responsible for the increase in IMB in milk) (CAAF, 2020.). The first mechanism is an increase in milk fat due to the mobilization of body reserves caused by a negative energy balance, while the second is a decrease in the protein content of milk as a result of a lack of energy in the meal and (or) reduced intake of dry matter. "When the IMB is less than 1.1, then the cow is at risk of developing subacute ruminal acidosis (the reason may be an excessive amount of concentrate in the ration) (CAAF, 2020.). A decrease in milk production and quality occurs in any case, so that is why it is extremley important to monitor the nutritional status to improve the health of cows, and thus their welfare.



< 60 dana

< 0,15 (nizak	DE 0360411262 (152), HR 4201092221 (141)

>= 60 dana

< 0,15 (nizak	DE 0360482224 (153), HR 0201888784 (150)

Napomena: na izvještaju su prikazane krave do 90. dana laktacije kojima je poznata vrijednost za BhB.

Figure 2. Risk assessment and presentation of ketosis (CAAF, 2020.)

Ketosis requires extensive and expensive treatment, and it causes a decrease in milk production by 50-70%, a decrease in reproductive capacity and a greater number of excretions of cows from production, which leads to large economic losses. Ketosis leads to reduced milk production, decreased fertility and increased incidence of other diseases (Djoković et al.2019). Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows(Ospina et al., 2010; Brunner et al., 2019; Djoković et al 2019.). Subclinical ketosis was more likely to be found in cows experiencing metritis than in unaffected cows (Dohoo, I.R., and S. W. Martin. 1984.). Ketone body levels in blood, urine and milk can be monitored to detect SCK in cows, and to increase their chances of successful lactation (Geishauser et al., 2001; Seifi et al., 2011; Zhang et al., 2012). The gold standard test for ketosis is blood BHB. This ketone body is more stable in blood than acetone or acetoacetate (Tyopponen and Kauppinen, 1980). Clinical ketosis generally involves much higher levels of BHB, about 3.0 mmol/L or more (Oetzel, 2007; Brunner et al.,2019.).
Metabolic predisposition of ruminants, nutrition (obesity or thinness) and irrational nutrition are the main reasons for the appearance of ketosis in the herd. We distinguish between three types of ketosis cows, but due to overlapping causes, it is difficult to determine a sharp and clear boundary between ketosis. Differences between: „ "Spontaneous or starvation ketosis, Fat cow syndrome, Acid silage ketosis (Acid ketosis, Acidosis ketosis)." (CAAF, 2020.). Ketosis can be recognized by the smell or by the breath of sick animals, which can be detected by acetone. Unbalanced milk production in cows, a tendency to lose weight with a good appetite and normal rumen function, and the appearance of ketones in the urine are signs of latent ketosis. In addition, there is also a weaker reporductive power appearance, and the best prevention in ketosis is a timely reaction to the appearance of ketone bodies in the blood, urine or milk. Prevention depends on several factors, including proper feeding of the transition cows, body condition management, and the use of certain feed additives such as niacin, propylene, glycol and ionophores (Duffield, T. 2000.)
With the help of the MilkoScan 7RM analyzer, it is possible to test the concentration of beta-hydrobutyric acid (BHB) and acetone in milk samples in the Central Laboratory for Milk Quality Control. With this type of laboratory test at SLKM, using a rapid test, it is possible to

monitor the entire herd and reduce the risk of ketosis through milk yield control. Balanced meals, adjusted microbiological habits and proper dryness of animals prevent the occurrence of ketosis, as well as regular monitoring of the occurrence of elevated concentrations of BHB in milk in risk groups of animals leads to a reduction in the risk of ketosis.

In order to avoid possible inflammatory processes in the udder with regard to the level of lactose digestibility (due to the presence of microorganisms) and possible mastitis, it is recommended to measure the ratio of lactose to the daily amount of milk, where the usual level of lactose should not be below 4.5 (usually 4.8%), and if the values are lower, a mastitis test is also recommended.

Depending on the composition of the meal, milk fat can increase or decrease. It often happens that two opposite metabolic disorders combined with feeding problems appear within the same group of cows. For example due to insufficient food consumption, cows tend to mobilize body reserves at the beginning of lactation. The cause of the decrease in milk fat is a lack of digestible fiber, while the mobilization of body fat tends to increase. Since the assessment of milk fat is done independently of milk proteins, it mainly shows the effects of digestible fiber in the meal.A high value of milk fat at the beginning of lactation is a signal of a metabolic disorder.

Through whole year, it is necessary to monitor the relationship between protein and the daily amount of milk, because the protein content in milk can be an indicator of the utilization of genetic and production potential. If meal changes occur, with this approach, a comparison can be made between different meals. While using production data of a group of cows, the effect of factors such as stage of lactation is reduced. Cows are most often found within the interval 3 – 3.2%. The interpretation of the report should be approached with caution, especially if the number of cows is less than 10 and if most of the cows are in the same stage of lactation. The protein content of milk can also be an indicator of a cow's readiness for insemination.

Detecting cows that are at risk of developing metabolic diseases is possible based on the ratio of IMB to the daily amount of milk." The preferred ratio of IMB is 1-1.5. A IMB greater than 1.5 is considered a risk factor for metabolic problems such as ketosis. There are two mechanisms responsible for the increase in IMB in milk. (CAAF, 2020.) An increase in milk fat due to the mobilization of body reserves caused by a negative energy balance is the first mechanism, while the second is a decrease in the protein content of milk as a result of a lack of energy in the meal and/or a decrease in dry matter intake.„ “When the IMB is less than 1.1, the cow is at risk of subacute ruminal acidosis (CAAF, 2020.). A decrease in milk production and quality occurs both when the protein content in milk increases and when it decreases.

Conclusion

Based on the collected data, we can conclude that more and more attention is being paid to the well-being of animals, and thus to dairy cows. Studying certain indices and relationships between different IMB values, we saw that their deviation is greatly influenced by the way of feeding and the nutritional status of dairy cows, whereby optimal IMB values are achieved with controlled feed intake, and thus the quality and daily amount of milk show a high level of well-being of dairy cows. The high welfare of cows reduces the morbidity of animals, increases reproductive efficiency and reduces the use of antibiotics, thus improving the general health of animals that enter the food chain, thus reducing the transmission of diseases from animals to humans. With the increase in the yield of quality fresh milk, there is a higher profit for the farmer, and mutual satisfaction of both the farmer and the welfare of the dairy cows.

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EVALUATION OF BIRTH WEIGHT BY BANDED MEASURE IN SELECTED BREEDING HERD OF SLOVAK SPOTTED BREED

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Abstract

Birth weight is a characteristic of great economic importance in the cattle industry. The relatively more accurate methods are measuring the circumference of the forelimb in the wrist area, measuring weight on a hanging scale and weighing on a digital scale. A total of 270 Slovak Spotted calves born between 2020 and 2023 were used for estimation of parameters for birth weight by banded measure based on wrist circumference. The basic statistical and variability characteristics were evaluated using the SAS version 9.4. The mixed procedure was used for the final analysis. The value of statistical significance, the value of the F-test and the coefficient of determination of the above mentioned growth traits were evaluated with its relation to: year and month of birth, sex of and sire. The average value of birth weight of calves was 39.64 kg and circumference of the wrist was 18.29 cm. The results of 270 measures during the years 2020 and 2023 were as follows: the highest number of calves was analysed during 2021 (118), where the average birth weight of calves was 42.0±10.65kg and circumference of the wrist was 18.56±7.6cm. The linear model to represent coefficient determination R^2 = 0.4671% (P<0.001) for birth weight of calves all fixed effects. The analyses by the effect on birth weight of calves were most influenced by the sire R^2 = 0.3852% and year of birth R^2 = 0.2876% (P<0.001). The correlation among the birth weight of calves and circumference of the wrist was r= 0.6684 (P<0.001).

Keywords: *Slovak Spotted breed, calves, birth weight, circumference of the wrist, correlation and coefficient of determination.*

Introduction

Birth weight is a characteristic of great economic importance in the cattle industry as it was showed by different authors (Bailey and Mears, 1990; Utsunomiya et al., 2013; Hickson et al., 2015). The relatively more accurate methods are measuring the circumference of the forelimb in the wrist area, measuring weight on a hanging scale and weighing on a digital scale.

Genetic part of the phenotypic variation (heritability) of birth weight only counts for about 45 percent with the remaining 55 percent being caused by environmental conditions (Nelson-Licking, 2018). Direct heritability forbirth weight from different age points reported in literature were in a moderate to high range 0.22 to 0.26 (Johanson et al., 2011) and 0.58 (Brotherstone et al., 2007) to 0.60 (Coffey et al., 2006), indicating the potential for genetic improvements.

As stated by Atil et al. (2005),phenotypic and genetic correlations between birth weight (BW) and weaning weights (WW) are 0.89 and 0.80, respectively. Bures et al. (2008) reported

phenotypic correlations between different measurements and calf birth weights as well as the course of parturition were calculated. The correlation coefficients for the relationship between the body measurements and the birth weight of the calves were from 0.6to 0.72.
Many genetic factors have an effect on the birth weight of calves such as the effect of the father, the mother, the year and month of birth(Szabó et al., 2006; Strapák et al., 2000 and 2013; Bujko et al., 2020 and 2022; Coleman et al., 2021). Also, significantly non-genetic factors namely nutrition (Martin et al., 2007; Klein et al., 2022) and breeding conditions (Hozáková et al., 2019). Factors affecting on birth weight shows in many publications (Krupa et al., 2005; Ozluturk et al., 2007; Shahzad et al., 2010;Hozáková et al., 2020; Vavrišínová et al.,2020; Filipčík et al. 2020; Ducháček et al., 2022).The relationship of birth weight assessment has an impact on further growth and later also on production and reproductive indicators, as reported by Bayou et al. (2015); Shoup et al. (2017) and Lopez et al. (2020).
The aim of this study was to evaluate the birth weight of the Slovak Spotted calves by banded measure based on wrist circumference in selected breeding herd in Eastern Slovakia.

Material and Methods

The material for evaluation of breeding herd of Slovak Spotted cattle was the database of Breeding Service of Slovak Republic (S.E., 2023) and the database of agricultural enterprise Agrofarma in Eastern Slovakia. A total of 270 Slovak Spotted calves born between 2020 and 2023 were used for estimation of parameters for birth weight by banded measure based on wrist circumference.We divided calves only breed-type S_0 - calves with genetic proportion of pure Slovak spotted blood into 87.5 %.The basic statistical and variability characteristics (least square means, standard deviations) were evaluated using the Statistical System (SAS) version 9.4(TS1M2) Enterprise Guide 7.1 (SAS, 2016). The mixed procedure was used for the final analysis. The value of statistical significance, the value of the F-test and the coefficient of determination of the above mentioned growth traits were evaluated with their relation to: year and month of birth, sex, breeding type and sire. For actual computation a linear model with fixed effects was used:

$$y_{ijklm} = \mu + YB_i + MB_j + S_k + F_l + e_{ijkl}$$

where: μ = mean value of depended variable, YB_i = fixed effect of years of birth (1 - 4), MB_j= fixed effect of month of birth (1 - 12), SC_k= fixed effect of sex of calves (1 - 2), F_m = fixed effect of sire (1 - 26), e_{ijkl} = residual error. Statistical evaluations of the differences between traits were tested at the levels of statistical significance: $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ or $^{-}P>0.05$.

Results and Discussion

The average value of birth weight (BW) of calves was 39.64±5.78kg and circumference of the wrist (CW) was 18.29±1.33cmare presented in Table 1. The results of 270 measures during the years 2020 and 2023 were as follows: the highest number of calves was analysed during 2021 (118), where the average birth weight of calves was 42.0±10.65kg and circumference of the wrist was 18.56±7.6cm.
The statistical parameters of BW and CW in selected breeding herd of Slovak Spotted calves were similar to the national average for evaluated traitsof growth in Slovak Republic (The Breeding Service of the Slovak Republic S.E., 2023).

Table1. Statistical characteristic of BW and CW of Slovak Spotted calves

Traits	Statistical parameter					
	n[1]	$\bar{x}$ [2]	SD[3]	CV[4]	MODE[5]	MEDIAN[6]
Birth weight (BW)	270	39.64	5.78	14.58	41.0	41.0
Circumference of wrist (CW)		18.29	1.33	7.28	19.0	18.5

[1]number of observation, [2]average, [3]standard deviation, [4]coefficient of variation, [5]mode (value that appears most often in a set of data), [6]median (value separating the higher half from the lower half of a data sample)

In Figure 1 the differences for evaluated traits (BW and CW) of calves by years 2020 to 2023 were shown. In case of birth weight the values were between 43.86kg (in 2020) to 33.41 kg (in 2023), the difference could be caused by the small number of evaluated calves and also by the higher number of female calves in the given evaluated period. These results conform to the breed standard Slovak Spotted breed (2023) and conclusions results Strapák et al. (2013) and Vavrišínová et al. (2020).

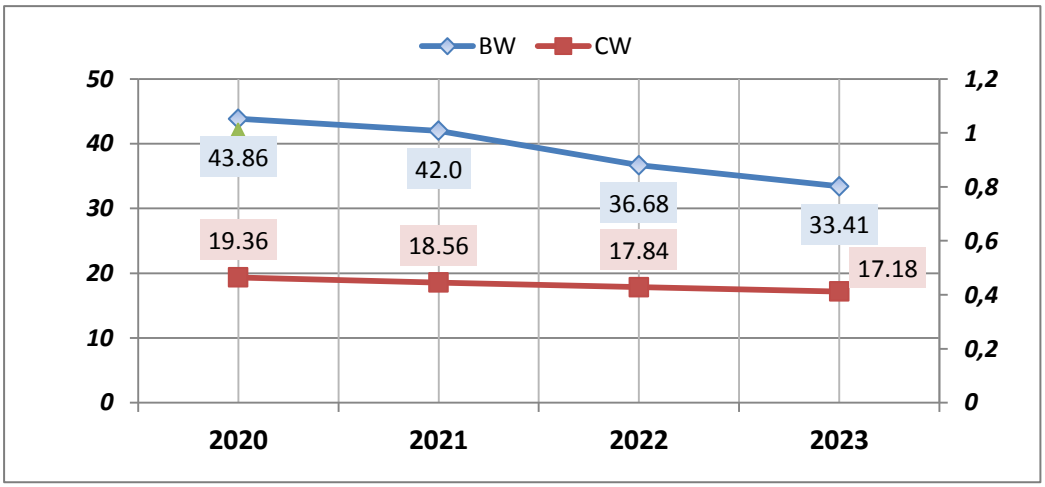

Figure1. Birth weight (BW) and circumference of the wrist (CW) of calves on breeding herd in individual years

Regarding the influence of the sex of the calves (Table 2.), male calves was heavier than female calves in all monitored birth weight + 2.8 kg. Differences between the sex of calves in CW was + 0.22cm for bull. The highest number of male calves was born in 2020 (70), female calves in 2022 (58), while the highest average birth weight for male calves was 44.50±5.24 kg and female calves 43.27±3.94 kg (in 2020).

Table2. Statistical characteristic of BW and CW by sex of calves

Sex	n	Traits	Mean	Std Dev
male calves	144	Birth weight (BW) in kg	40.95	5.13
		Circumference of wrist (CW) in cm	18.39	1.33
heifer	126	Birth weight (BW) in kg	38.15	6.13
		Circumference of wrist (CW) in cm	18.17	1.33

The correlation between birth weight (BW) and circumference of wrist (CW) in the evaluated calves in the selected breeding farm of Agrofarma in Eastern Slovakia, for example BW with

CW, was found as follows r= 0.6684 (Table 3). This coefficient was statistically highly significant (P<0.001). This result is similar with findings of Bures et al. (2008).

Table3.Relation between birth weight (BW) and circumference of wrist (CW)

Traits	circumference of wrist (CW)
birth weight (BW)	r= 0.6684^{+++}

$^{+++}$ P<0.001

The linear model to represent coefficients of determination on birth of weight (BW) with all fixed effects R^2 = 0.467093%. These effects were significant (P<.0001).

Table4. Factors affecting birth weight (BW) in Slovak Spotted calves (S_0)

Sources of variability	DF[1]	Mean Square	CV[2]	F Value	Pr> F	R-Square [3]
						Birth weight (BW)
Years of Birth (YB)	4	861.158082	12.37	35.80	<.0001	0.287604
Month of Birth (MB)	12	50.7737145	14.41	1.55	0.1125	0.062176
Sex of calves (SC)	2	525.7658896	14.17	16.66	<.0001	0.058531
Sire (F)	26	138.419980	12.0	6.12	<.0001	0.385238

[1]grades of freedom, [2]coefficient of variation,[3] coefficient of determination (R^2), $^{+++}$ P<0.001 or - P>0.05

In showed the analyses of the effects on birth of weight (BW) revealed higher effect of sire R^2 = 0.385238% than effect of years R^2 = 0.287604% (Table 4). These effects were significant P<.0001.These results are similar with results of Krupa et al. (2005); Ozluturk et al. (2007); Shahzad et al. (2010); Hozáková et al. (2019).

Conclusions

Based on the available data, the coefficients determination (R^2) for calf birth weight for all fixed effects was calculated. It can be concluded that the birth weight of the calves was most affected by the sire and the year of birth. The correlation between birth weight of calves and wrist circumference was highly significant.

Acknowledgments

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TICK FAUNA OF SHEEP IN EAST SERBIA

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Abstract

The present study was conducted in 52 sheep flocks from the territory of east Serbia in the period of March to November 2017, using into consideration the sesonal occurance and prevalence of ticks. Ticks were from sheep by means lightly sprung forceps. The tick species and sex/gender were identified by morphometric characteristics. In total, we examined 216 sheep and tick infestation was detected in 39.24% of examined animals. The most abundant species was *Ixodes ricinus* found in 41.91%, followed by *Dermacentor marginatus* (27.91%), *Rhipicephalus bursa* (10.92%), *R.sanguineus* (9.92%), *D.recticulatus* (5.51%) and *Haemaphysalis punctata* (3.97%). Of the total number of collected ticks, 57.15% were females and 42.85% were males. The population maximum for *Dermacentor marginatus*, *D.recticulatus* and *Haemaphysalis punctata* occurred in April. In May was population peak for *I.ricinus* and it was noted that this species started to decrease in abundance in June. *Rhipicephalus sanguineus* and *R.bursa* reached maxima decreasing until August, and disappearing completely in September and October. The autumn population peak in September occurred for the *I.ricinus* and R*hipicephalus sanguineus* and in October for the *I.ricinus* and *R.bursa.*

Keywords: sheep, ticks, biodiversity, season distribution, east Serbia.

Introduction

Eastern Serbia is a mountain-basin region that stretches from the Danube and the border with Romania and Bulgaria. This part of Serbia is well known for large number of pastures suitable for growing small and larger ruminants. Breeding of small ruminants in the hilly and mountainous areas of Serbia has a long tradition and is well developed. Today, small flocks of sheep and goats play an important role in providing animal protein for diet, especially for those people who live there. Both, sheep and goats are milked and they produce the bulk milk supply, together with a large proportion of the meat that is consumed [Petrović et al.2021, Pavlovic and Ivanovic,2022].

Ticks represent one of the indispensable elements of that specific biotope. A diverse tick fauna present in this region mainly influences the health status of grazing sheep. Ticks are known as vectors for a number of diseases [Papadopoulos et al., 1996, Dimitrić,1999, Jongejan and Uilenberg, 2004, Nuttall and Labuda 2008, Sevinc et al., 2013, Nieder et al.2013, Pavlović et al.2016b]. However, in the semi-intensive breeding system, which is the most often practice in this region, is very difficult to avoid infections with different types of ticks that are contaminants of the grasslands and pastures [Becskei et al.,2015, Pavlović et al. 2018; 2019]. Tick infestations are common, especially during late spring and autumn months of the year [Harlan and Foster, 1990, Fourie and Horak, 1991; Milutinović, 1992].

The aim of the study was to examine biodiversity, relative abundance, sex ratio, and the effects of environmental factors (temperature, relative air humidity and precipitation) on tick populations in small ruminants in east part of Serbia.

Material and methods

Eastern Serbia is a mountain-basin region that stretches from the Danube and the border with Romania in the north to the Zaplanjsko-Lužnica basin and the Ruj mountain in the south, and from the border with Bulgaria in the east to the Velika valley and part of the South Morava river in the west. Eastern Serbia is characterized by a mosaic of rocks of all types and geological formations and a "chess relief" with alternating mountains and valleys. The relief is dominated by ridged mountains, separated by numerous river valleys and basins. Đerdap is the largest tunnel in Serbia and Europe (96 km). It connects the Pannonian and Wallachia-Pontic basins. It consists of a system of valleys and gorges. Eastern Serbia has a diverse climate: parochial in the valleys, moderately continental in the river valleys and mountainous on the mountains. Summers are moderately warm; winters are quite cold and windy. Kosava is the most common wind. The amount of precipitation ranges from 500 to 700 mm, and in the mountains up to 1,000 mm [Pavlovic et al.2022b). Geographical conditions in examined area favor for breeding of small ruminants because there was a large number of pastures suitable for grazing.

The present study was conducted in 52 sheep flocks from the territory of east Serbia in the period of March to November 2017, using into consideration the sesonal occurance and prevalence of ticks. Ticks were collected by removing them from sheep with tweezers and were placed in tube with 70% ethanol. The tick species and sex/gender were identified by morphometric characteristics. The main attribute of identification of tick family is a plain dorsal sclerotised scutum or shield, which is often ornate with patterns in white or gold against a brown or grey background and which distinguishes these ticks from other families. This sclerotised plate covers the entire dorsal surface of the male, but only one third of the female's dorsal surface. Second one was the capitulum of hard ticks which just as the mouthparts and is visible from a dorsal view. The peritreme or groove is big and clearly visibly around the stigma plate. Grooves are deep, linear depressions in the body cuticle, usually on the ventral surface. Hard ticks can be easily differentiated by the shape of the basis capitulum and by the form of anal grooves [Kapustin,1955, Kolonin, 2009].

Result and discusion

In total, we examined 216 sheep and tick infestation was detected in 39.24% of examined animals. The most abundant species was *Ixodes ricinus* found in 41.91%, followed by *Dermacentor marginatus* (27.91%), *Rhipicephalus bursa* (10.92%), *R.sanguineus* (9.92%), *D.recticulatus* (5.51%) and *Haemaphysalis punctata* (3.97%).

The found species of ticks are most common in sheep in the regions of the Western Balkans including Romania, Macedonia, Mediterranean and Central Europe [Omeragić, 2011; Dumitrache et al., 2012; Mihalca et al., 2012; Dumitrache et al.,2012, Pavlović et al.,2014, 2020; 2016a,c].

In temperate habitats, feeding and generation cycles of hard ticks are closely synchronized with periods of suitable temperature and humidity conditions (Anderson and Magnarelli, 2008). The considerable interchange between spring and autumn tick populations can be attributed mainly to environmental conditions. In general, the climate in examined area is continental, with cold, relatively dry winters and warm, humid summers. Summer temperatures in the mountainous areas are notably cooler, averaging about (17°C). For these

reasons, some species occur later than in the lowland and hilly parts of Serbia. The population maximum for *Dermacentor marginatus, D.recticulatus* and *Haemaphysalis punctata* occurred in April. In May was population peak for *I.ricinus* and it was noted that this species started to decrease in abundance in June. *Rhipicephalus sanguineus* and *R.bursa* reached maxima decreasing until August, and disappearing completely in Septembar and October. The autumn population peak in Septembar occurred for the *I.ricinus* and R*hipicephalus sanguineus* and in October for the *I.ricinus* and *R.bursa.*

Of the total number of collected ticks, 57.15% were females and 42.85% were males. The sex ratio of detected tick species showed a higher number of females in four species (*Ixodes ricinus, Haemaphysalis punctata, Rhipicephalus sanguineus* and *Dermacentor marginatus),* while higher number of males was detected in *Rhipicephalus bursa,* and an equal number of ticks of the *D.pictus.* This is in agreement with the research of the tick sex ratio that have been made around the world [Milutinović, 1992, Milutinović et al., 1997a, Anderson and Magnarelli, 2008]. The female abundance of established tick species has been in correlation with previously established population dynamics. The females of *Ixodes ricinus* species were present from March to October, with a peak population in May and June. Females of two species of the genus *Rhipicephalus* (*sanguineus* and *bursa*) have been found most often in the summer months - June and July. Findings of the females of species *Dermacentor marginatus* and *Haemaphysalis punctata* were most common in April and May, while sporadic finding of females of *Dermacentor pictus* species was attached to the spring months. This population dynamics of female ticks is characteristic for this microclimate [Belozerow,1982, Černy et al.,1982, Hornok 2009].

Our results confirmed the results of the similarly studies carried out in northeast and south-eastern part Serbia [Milutinović et al., 1987; 1996; 1998; Miščević et al.,1990]. This data is in correlation with the results of other examinations of the seasonal dynamics of ticks in Europe including West Balkans [Daniel,1978, L'Hostis et al., 1995, Omeragić, 2011, Mihalca et al.2012, Sevinc et al., 2013, Pavlović et al., 2014;2016a,c,2020b].

Conclusion

Based on the obtained results, it can be seen that ticks represent a significant problem of small ruminant production in east Serbia. Biodiversity of ticks of sheep holding allows permanent infections with these parasites and the climatic conditions favor their development and maintenance on grazing surfaces. Climate conditions have a great influence on the population dynamics of ticks which had two picks-at late spring and early autumn. Fauna, seasonal dynamics and sex ratio of ticks found do not differ much from the results obtained in other regions of Serbia. Likewise, the presence of ticks points to the ever-present possibility of infections zoonotic character that the ticks carry.

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PHENOTYPE EVALUATION OF GATACKO CATTLE FROM THE TERITORY OF GACKO MUNICIPALITY IN BOSNIA AND HERZEGOVINA

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Abstract

Phenotype evaluation of Gatacko cattle has been determined on a total of 48 female necks (age between 4 and 8 years), at the end of 2022, on the territory of the municipality of Gacko, at a total of eight locations (Republic of Srpska, Bosnia and Herzegovina). The territory of the municipality of Gacko was chosen because of its distinctiveness when it comes to breeding and distribution, i.e. the representation of the local (autochthonous) breed of cattle (Gatacko cattle). The phenotype was evaluated on the following parameters: height at withers, back height, loin height, body lenght, chest width, chest depth, chest girth, hip width, pin bones width, front shin girth, back shin girth, head lenght, forehead width, horn lenght, horn girth. For the purpose of data analysis, the aim was to determine the variability of the average values of the examined parameters, correlation of investigated parameters and significance of calculated correlation coefficients. Based on the results of our research on Gatacko cattle in the Gacko municipality, Republic of Srpska (BIH), an increase in most of their body dimensions is noticeable. The contribution of these researches lies in the continuation of previous and future researches on phenotypic parameters of autochthonous cattle breed (Gatacko cattle), given the inestimable value of autochthonousness.

Key words*: Gatacko cattle, morphometric parameters, phenotypic correlation.*

Introduction

Although there is a dominance in the use of commercial cattle breeds, which dominate livestock production, they cannot fully meet the expectations and demands of producers, nor consumers. There are several reasons that confirm the above, and the most important one is the reduced resistance and impossibility to express the maximum of genetic potential. Unlike commercial, i.e., introduced breeds, domestic (autochthonous) breeds (more precisely: Gatacko cattle) give their best in the conditions that are characteristic for this geographical area. Domestic breeds are suitable for the use and maintenance of rural areas (pastures, prevention of biotope devastation), but also for generating additional income for the population of rural areas. However, the trend of displacement of rural areas is still present and increasing, so as a result we have the neglect of livestock production, which has also led to a decrease in the number of domestic breeds of cattle. In the Republic of Srpska and BH, there has been a general decrease in the cattle population, and thus the autochthonous breed that is the subject of this paper – Gatacko cattle (*Law on livestock, Official Gazette of the RS, 44/15, Article 40, paragraph 2)*. The Gatacko cattle was created by breeding Busha with Viptal and

Oberintal cattle. This breed belongs to the group of short-legged cattle. It is short-headed with a wide and uneven forehead and it has a dark pigmented muzzle with a light edge. The horns are thin, pointed forward. The color of the hair is grey, it can also be brown, with dark shading on certain parts of the body. Females weigh about 400 kg and males about 750 kg. Gatacko cattle is most valued for its milk production, which goes up to 2,500 litres (Katica et al., 2004).

The aim of this research was to analyze morphometric parameters of Gatacko cattle in the region of Gacko Municipality (8 locations), Republic of Srpska (BIH), on a total of 48 female necks, ages between 4 and 8. For the purpose of data analysis, the aim was to determine the variability of average values of the examined parameters (height at withers, back height, loin height, body lenght, chest width, chest depth, chest girth, hip width, pin bones width, front shin girth, back shin girth, head lenght, forehead width, horn lenght, horn girth), correlation of the investigated parameters and to determine the significance of the calculated correlation coefficients.

Material and Methods

Phenotype evaluation of Gatacko cattle has been determined on total of 48 female necks (ages between 4 and 8 years) by the end of 2022, in the Gacko Municipality (Entity of Republic of Srpska, Bosnia and Herzegovina - BiH), on total of 8 locations. The territory of the municipality of Gacko was chosen because of its distinctiveness when it comes to breeding and distribution, i.e. the representation of the local (autochthonous) breed of cattle (Gatacko cattle).

The phenotype was evaluated on the following parameters: height at withers, back height, loin height, body lenght, chest width, chest depth, chest girth, hip width, pin bones width, front shin girth, back shin girth, head lenght, forehead width, horn lenght, horn girth. Based on the registry (ear tag), and the statement of the owners, the age of each cow was defined. Measuring of body dimensions (morphometric, phenotypic) were performed using zootechnical aids. Zootechnical aids that were needed to measure this group of parameters are the cattle tape and Lydtin's rod (*Lalovic* and *Zdralic*, 2018). The rule states that body measurements (morphometric) must be performed in the way where the animals/necks must stand on a flat and firm surface. During the measurement, it is best that the animal stands in a natural position, i.e., that it rests evenly on all four legs. Also, as a rule, measures are taken first from the left side, then from the right side of the animal, after which the average value for the measured parameter is calculated (*Krajinovic et al*., 2000). Lydtin's rod measured linear body dimensions: height, length, depth and width, while the following body dimensions were measured with a cattle tape: head length, forehead width, horn length, horn girth, chest girth and shin.

In the following text, we will explain in more detail the method of taking measurements for each parameter, which is included in this paper. Height at withers is measured from the base, behind the front hoof, to the highest point on the withers. It is the area between the second and fifth dorsal vertebrae. Back height is measured from the base to the highest point of the last thoracic vertebra. Loin height is measured from the base to the point where the line connecting the hips intersects the spinal column. Body length is measured from the front edge of the shoulder blade joint to the rear point of the pin bone. Chest width is measured behind the shoulder blades, at the narrowest part of the chest. Chest depth is measured from the lower edge of the sternum to the highest point at the withers. Chest girth represent the torso girth behind the shoulder blades. Hip width is measured from the outer point of one hip to the outer point of the other hip. The pin bones width is measured from the outer point of one to the outer point of the other pin bone. Front and end shin girth is measured at the thinnest point.

Head length is measured from the top of the frontal bone to the top of the nose. Forehead width is measured from the outer edge of one to the outer edge of the other orbital arch. Horn length is measured from the base to the top of the horn. Horn girth the girth of the horn at the base (*Lalovic* and *Zdralic*, 2018).

In order to process the collected data for each parameter, a descriptive analysis was performed (mean, mean error, standard deviation, coefficient of variation, minimum, maximum) by using Microsoft Office Excel 2010. R Core Team package (2015) was used to calculate the phenotypic correlation between all morphometric parameters.

For the purpose of data analysis, the aim was to determine the variability of the average values of the examined parameters, correlation of investigated parameters and significance of calculated correlation coefficients. The method of descriptive statistics was in use (program package Microsoft Office Excel 2010).

Results and Discussion

Phenotype evaluation of Gatacko cattle (a total of 48 female necks) was determined on following parameters: height at withers, back height, loin height, body lenght, chest width, chest depth, chest girth, hip width, pin bones width, front shin girth, back shin girth, head lenght, forehead width, horn lenght, horn girth.

The average values and variability of morphometric parameters of Gatacko cattle are shown in Table 1.

Table 1. Average variability of morphometric parameters in Gatacko cattle

Parameters (cm)	$\overline{X}$	$S\overline{x}$	Sd	CV (%)	Min.	Max.
Height at withers	131.31	0.37	2.56	1.95	121	136
Back height	131.08	0.42	2.88	2.20	120	135
Loin height	131.77	0.29	1.99	1.51	124	136
Body length	153.10	0.61	4.26	2.78	134	160
Chest width	57.71	0.78	5.38	9.32	46	68
Chest depth	74.06	0.18	7.46	10.07	45	80
Chest girth	179.25	0.47	3.23	1.80	170	186
Hip width	60.38	0.35	2.45	4.06	50	64
Pin bones width	41.96	0.49	3.37	8.03	25	49
Front shin girth	13.96	0.17	1.15	8.24	12	17
Back shin girth	13.60	0.16	1.14	8.38	12	16
Head lenght	46.31	0.26	1.82	3.93	40	49
Forehead width	24.23	0.26	1.82	7.51	14	27
Horn lenght	13.42	0.52	3.60	26.83	7	23
Horn girth	12.56	0.25	1.76	14.01	9	16

The determined height at withers in this research (131.31 ± 0.37 cm), is close to the values determined in the research by *Nikitovic et al*. (2022), which amount to 133.3 cm, with the variation width for this parameter, ranging from 121 to 136 cm (15 cm), in this research. The difference in the research of the mentioned authors amounted 33 cm, which is approximately in line with the values obtained in the research by *Varatanović* (2018), *Nikitović et al.* (2022), *Nikitović et al.* (2021). In the older literature data, we find much lower values for the average height at withers in the Gatacko cattle. In the research by *Ilancic* (1952), the average value amounted 112.56 cm, while in the research by *Gutic et al.* (2003) in which 125 necks were analyzed, the average value for height at withers amounted 122.71 cm. Undoubtedly, the

format of Gatacko cattle has been upgraded due to numerous paragenetic factors. When it comes to back height, the value obtained in the research is 131.08±0.42 cm, which is in accordance with the results obtained in the research by *Nikitovic et al.* (2022), *Nikitovic et al.* (2021), while in the research by *Varatanovic* (2018) obtained values were lower and ranged from 128.83 to 134.00 cm.When it comes to loin height, the average value in the research is 131.6±0.77 cm, with a variation of 124 to 136 cm, and is in accordance with the results obtained in the researches of authors *Varatanovic* (2018*), Nikitovic et al.* (2022), *Nikitovic et al.* (2021). Following the obtained loin height results from the previous researches, we can hint at the potential conclusion, that there was over 10 cm increase in this morphometric parameter (*Ilancic, 1952; Gutic et al., 2003*). The average value for body lenght in the research is 153.10±0.61 cm, which is slightly lower than the values obtained in the research by *Gutic et al.* (2003), *Nikitovic et al*. (2022), *Nikitovic et al.* (2021). The average value of chest width is 57.71±0.78 cm, the average value for chest depth in this research is 74.06±0.18 cm. In the research by *Nikitovic et al.* (2021) higher average values were obtained for chest width and depth (64.3 cm and 78.5 cm), while the obtained values, on average, for chest width and depth in research by *Nikitovic et al.* (2022) are in accordance with the values obtained in this research and amount to 59.3 cm, i.e. 75.5 cm. The average value for chest girth in this research is 179.25±0.47 cm. We can notice a far higher average value in research by *Nikitovic et al.* (2021) which amounts 183.6 cm, while in the research by *Pajanovic* (1961) and *Popovic et al.* (1979) much lower values were recorded for this parameter (for the first group of authors: 151.41 cm, 147.25 cm and 148.07 cm - three locations; for the second group of authors: 166.21 cm), which again points to an increase in the format of the Gatacko cattle over time. The average value for hip width in the work is 60.38±0.35 and it is in accordance with the result (61.4 cm) obtained by *Nikitovic et al.* (2021), while the significantly higher average value (70.76 cm) was obtained in the research by *Gutic et al.* (2003). Pin bones width in this research is 41.96±0.49 cm and that is a slightly lower value compared to recent research by *Nikitovic et al.* (2022) which amounted 43.6 cm, i.e. *Nikitovic et al*. (2021) which amounted 42.9 cm. When it comes to the average values for the shin girth, both front (13.96±0.17 cm) and back (13.60±0.16 cm), the obtained values are in accordance with the obtained values in the research by *Nikitovic et al.* (2021) amounting on average 13.7 cm i.e., 13.1 cm. The average value for the head lenght is 46.31±0.26 cm, for the forehead width 24.23±0.26 cm, for the horn length 13.42±0.52 cm and for thehorn girth 12, 56±0.2250 cm. Obtained values are in accordance with results obtained in the research by *Nikitovic at al.* (2021) on the other hand, slightly higher results were obtained in the research by *Nikitovic at al.* (2022) (47,4 cm; 25,0 cm; 14,7 cm; 13,0 cm). Also, it should be added that the coefficient of variability for the horn length and horn girth is extremely high in this research and for the horn lenght it is 26.83%, while for the horn girth it is 14.01%. Certainly, the high coefficients of variation for the horn length and horn girth can be explained as the result of smaller movements of the animals during the measurement.

Correlational relationships of average values of examined body dimensions as well as statistical significance of correlation coefficients are shown in Table 2.

Table 2. Phenotypic correlations of morphological characteristics in Gatacko cattle

	BH	LH	BL	CW	CD	CG	HW	PBW	FSG	BSG	HL	FW	HNL	HNG
WH	.425***	.536***	.515***	.193^{ns}	.069^{ns}	.209^{ns}	.361**	-.238*	.091^{ns}	.057^{ns}	.324*	.021^{ns}	.159^{ns}	.007^{ns}
BH		.673***	.651***	-.096^{ns}	.538***	-.091^{ns}	.499***	-.008^{ns}	.046^{ns}	.178^{ns}	.383**	.279*	.166^{ns}	.171^{ns}
LH			.471***	.166^{ns}	.364**	.204^{ns}	.402***	.040^{ns}	.061^{ns}	.015^{ns}	.184^{ns}	.155^{ns}	.337**	.244*
BL				-.03	.365**	.060^{ns}	.553***	-.104^{ns}	.140^{ns}	.192^{ns}	.600***	.382***	.176^{ns}	.258*

				2 ns										
CW					**-.045 ns**	**.184 ns**	**-.059 ns**	**.004 ns**	**.039 ns**	**.067 ns**	**.025 ns**	**.009 ns**	**.171 ns**	**.022 ns**
CD						**.195 ns**	**.327****	**.348****	**-.069 ns**	**.070 ns**	**.061 ns**	**.455*****	**.091 ns**	**.285***
CG							**-.061 ns**	**.200 ns**	**.289****	**.200 ns**	**-.017 ns**	**.156 ns**	**-.157 ns**	**.038 ns**
HW								**-.045 ns**	**.157 ns**	**.312****	**.412*****	**.538*****	**.103 ns**	**.182 ns**
PBW									**-.121 ns**	**.001 ns**	**-.171 ns**	**.133 ns**	**-.220 ns**	**.212***
FSG										**.862*****	**.047 ns**	**-.117 ns**	**.113 ns**	**.033 ns**
BSG											**.040 ns**	**.024 ns**	**-.001 ns**	**-.056 ns**
HL												**.470*****	**.297****	**.302****
FW													**.063 ns**	**.237***
HNL														**.076 ns**

WH – height at withers; BH – back height; LH – loin height; BL – body lenght; CW – chest width; CD – chest depth; CG – chest girth; HW – hip width; PBW – pin bones width; FSG – front shin girth; BSG – back shin girth; HL – head lenght; FW – forehead width; HNL – horn lenght; HNG – horn girth

nsP>0.05; *P<0.05; **P<0.01; ***P<0.001

Height at withers is positively correlated with all examined parameters, except for pin bones width parameter (r_p = -0,238) and is statistically confirmed at the level of P < 0.001 for back height, loin height and body length parameters; P<0,01 for hip width parameter; P<0,05 for pin bones width and head lenght parameters. Back height is in a highly positive correlation (P < 0.001) with loin height, body length, chest depth and hip width, i.e., height (P<0,01) with head lenght and statistically confirmed at the level P<0,05 for forehead width. Loin height is in a highly positive correlation (P < 0.001) with body length and hip width, i.e., height (P<0,01) with chest depth and horn lenght and statistically confirmed at the level P<0,05 for horn girth. Correlational interrelations between body length, hip width, head length and forehead width are positive and by significance confirmed at the level P<0,001. Connection of body lenght and chest depth is confirmed at the level P<0,01, i.e., connection of horn girth at the level P<0,05. Correlation between chest width and other parameters are not statistically confirmed (P>0,05). The same can be concluded for the interrelation of the back shin girth and horn length to the parameters examined in this research. Correlation between chest depth and forehead width is confirmed at the level P<0,001, i.e., at the level P<0,01 for hip width and pin bones width. Chest girth is in a positive correlation, statistically significant at the level P<0,01 (front shin girth). Hip width is in a correlation statistically significant at the level P<0,001 for forehead width and head lenght, while statistical significance between pin bones width and horn girth is confirmed at the level P<0,01. Front shin girth is in a high correlation (P<0,001) with back shin girth. Correlation coefficient between head lenght and forehead width is confirmed at the level P<0,001, i.e., at the level P<0,01 for horn lenght and horn girth. Forehead width and horn girth are in a correlation confirmed at the level P<0,05.

Conclusions

The purpose of this research is to determine morphometric parameters of autochthonous breed Gatacko cattle, on a total of 48 necks (age 4 to 8 years) in the region of Gacko municipality (8 locations), Republic of Srpska (BIH). For the purpose of data analysis, the variability of average values of the examined parameters (height at withers, back height, loin height, body lenght, chest width, chest depth, chest girth, hip width, pin bones width, front shin girth, back

shin girth, head lenght, forehead width, horn lenght, horn girth), correlation of the investigated parameters and the significance of the calculated correlation coefficients are determined.

Based on the results of research, the increase in most body dimensions is noticeable, as well as a large number of statistically confirmed correlation coefficients between the examined morphometric parameters. The most likely reasons are the criteria for cattle breeding, which is commonly based on phenotype of the animal, the appearance of genetically impure material etc.

At the end, an unavoidable procedure for phenotypic characterization of a race is morphometric characterization. Knowledge on racial characteristics is necessary while making decisions on race development and breeding programs. Furthermore, we can say that the contribution of these researches lies in the continuation of previous researches on morphometric parameters in autochthonous cattle breed (Gatacko cattle) which further provides material for future researches.

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THE EFFECT OF POTASSIUM HUMATE ON THE PRODUCTIVITY, HAEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF DAIRY COWS

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Abstract

In present market economy, the most important task of the agricultural sector is to achieve a significant and rapid increase in livestock production and to fully meet the needs of the population of the country. The organization of a comprehensive diet based on knowledge of different nutrients and bioactive substances promotes the successful resolution of the problem. The objective of the study was to investigate the effect of potassium humate, a new biologically active feed ingredient from Latvian black peat, on productivity and haematological and biochemical parameters in the blood of dairy cows. The study was conducted at "Dukāti" farm of Vītiņi Rural Territory, Auce Municipality. The study was conducted between October 2020 and August 2021. Two analogous groups (in terms of milk yield, lactation and lactation phase) of Holstein Friesian cows were created for the trial, 15 animals per each group. The study included 2nd lactation cows with an average milk yield of 40.3 kg day^{-1}, fat content of 3.47%, protein content of 3.44%, and average live weight of 700 kg. The cows selected for the study were housed in common untethered housing and received partially mixed ration (PMR) as feed. In the cows of the trial group the average milk yield at the end of the trial was 5.19 kg per day higher, compared to the control group. In the analysis of the overall trends in haematological blood results over time, no significant changes that could have a major impact on the health of the cows were detected. Feeding potassium humate as a feed ingredient did not demonstrate any direct effect on the biochemical parameters of blood serum during the study.

Keywords: *dairy cows, potassium humate, productivity, blood parameters.*

Introduction

In animal nutrition, in addition to deficiencies in protein, energy and other key nutrients, there is a deficiency of biologically active substances, which leads to metabolic disorder and can have significant effects on animal health, productivity and milk quality. The problem has become acute due to the use of various feed additives, including synthetic ones, in animal nutrition Biologically active substances – humic substances – can play an important role in solving this problem. Humates are composed of substances such as carbohydrates, amino acids and phenols, which are formed by decomposition and decomposition of plant and animal residues in the soil. Humates include humic, fulvic, ulmic acid and some microminerals originating from humus, and humates are organic substances capable of chelating with some metal ions and making electron transfer. Histopathological and histochemical studies have shown that humates are harmless on blood, cardiovascular system, endocrine system, and other important organ systems. Studies have also shown that humates have a protective effect against diseases, they can be used safely even in pregnant animals, and they have no embryotoxic effect (Islam et al., 2005; Yüca & Gul, 2021). Studies by

scientists from different countries have shown that humic substances work at both the cellular and extracellular level in the animal's body. They enter the cell and take part in the metabolic process, optimising the metabolism and facilitating the passage of inorganic ions into the intestinal wall. This results in a stimulatory effect of humic substances on individual organ systems and the whole organism (Tomassen and Faust, 2018; Potůčková and Kouřimská, 2017; Kouřimská et al., 2014; Wang et al., 2007). Yüca and Gul (2021) reported that humates addition increased dairy cow milk yield and ratio of milk fat to milk protein. Little information is available on the influence of humates supplementation to dairy cows on the milk production and blood parameters. The objective of the study was to investigate the effect of potassium humate, on the productivity and haematological and biochemical blood parameters of dairy cows.

Materials and Methods

Trials were carried out on the farm "Dukati", Vitinu Parish, Auce Municipality in Latvia. For the research, Holstein-Friesian Black-and-White cows were selected and assigned by the analogy principle (according to yield, lactation phase and live weight) to two treatment groups: trial and control, with 15 dairy cows in each group. The average live weight of cows in both groups was 700 kg, the mean age was 2.0 lactations. Cows with an average yield of 40.29 kg per day, fat content of 3.47% and protein content of 3.44%, lactation days 121–250 were included in the research. The trial was carried out from 1 October 2020 to 31 August 2021. The research design is presented in Table 1.

Table 1. Scheme of the trial.

Group of cows	Number of cows	Basic feed ration
Trial	15	Partial mixed ration (PMR) + 7 g potassium humate per cow per day
Control	15	Partial mixed ration (PMR)

The dairy cows were kept in freestall barns. The cows had a deep bedding area in the barn, where straw was used for bedding. Animals were allowed *ad libitum* access to water and fed six times a day (at 5:00, 8:00, 13:00, 17:00, 20:00 and 22:30) with PMR (Figure 1 and Table 2) prepared according to the Nutrient Requirements of Dairy Cattle (NRC, 2001).

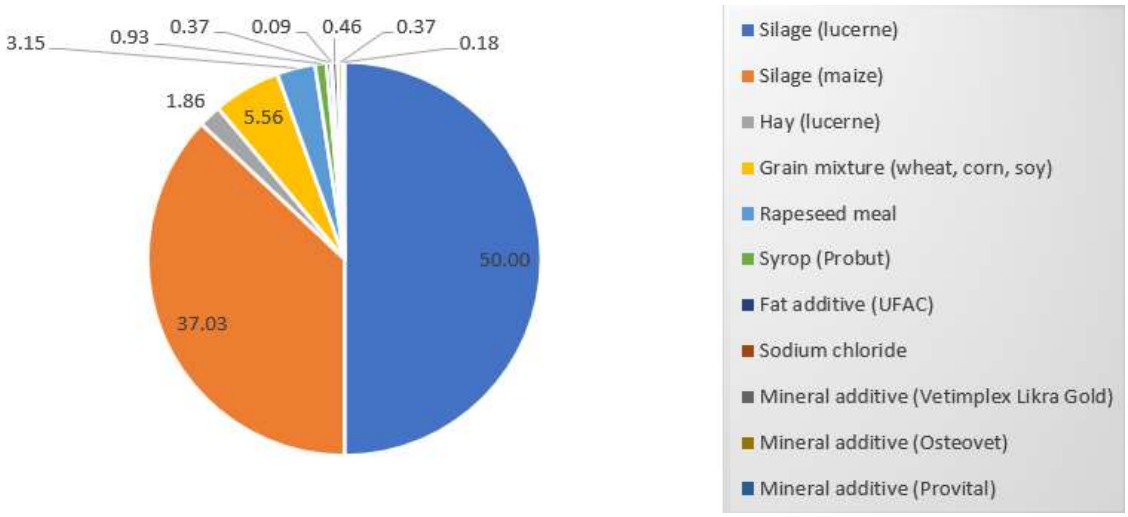

Figure 1. Composition of PMR.

The difference between the feeding of one group and the other was that the cows in the experimental group received potassium humate at a rate of 7 g per cow per day added to the supplementary feed consisting of maize, barley, soybean meal and molasses (liquid) from the company "Baltic Agro", which the cows received in the milking robot depending on the level of productivity.

Table 2. Chemical composition of PMR.

Component	Content
Dry matter, %	40.7
Crude protein (CP), % DM	15.4
Degradable intake protein (DIP), % CP	48.0
Undegradable intake protein (UIP), % CP	32.9
Crude fibre, % DM	17.2
Net energy for lactation (NEL), MJ kg^{-1} DM	7.1
Crude fat, % DM	4.1
Neutral detergent fibre (NDF), % DM	32.4
Acid detergent fibre (ADF), % DM	19.8
Calcium (Ca), % DM	0.91
Phosphorus (P), % DM	0.44

The feeding ration varied according to each cow's milk yield and physiological state, and was corrected monthly depending on the results of the control milk yield, dry period and state of health. In order to compare and evaluate the results between the groups, the amount of energy-corrected milk (ECM) per kg per day^{-1} was calculated using the following formula (Garcia *et al.,* 2006):

$$ECM = \text{Milk yield x } \frac{0.383 \text{ x Milk Fat,\%} + 0.242 \text{ x Milk Protein,\%} + 0.7832}{3.14} \quad (1)$$

where: *ECM* – energy-corrected milk

To monitor the health status of dairy cows during potassium humate feeding, blood samples were taken from cows in the experimental and the control group. Blood samples were taken from the milk vein before and six months after the study using a vacutainer and a needle. 3 ml EDTA tubes were used for haematology, 6 ml tubes containing serum stabiliser were used for biochemistry, and 6 ml tubes containing heparin were used for glutathione peroxidase determination. During one sampling occasion, other samples were collected on the same day from each cow participating in the study, through a single puncture.

During the blood sampling process, a break was taken every ten cows in order to immediately determine the concentration of glucose and ketone substances in the blood using the express test device "Freestyle optimum". After collection, the blood samples were immediately placed in a cold bag (temperature +6 $^{\circ}$C), cooled and delivered to the laboratory for further processing within a maximum of 2 hours.

After sample collection, the laboratory performed haematological examination of the blood using an automated counter to determine the total leukocyte count (WBC), red blood cell count (RBC), haematocrit (HCT), haemoglobin content (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and to perform white blood cell (WBC) counting analysis in blood smear (segmented nucleus leukocyte count (Sg), eosinophil count (Eo), monocyte count (Mo), lymphocyte count (Ly). Serum biochemistry samples were centrifuged and serum separated after arrival at the laboratory. The following serum biochemical

parameters were determined: aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), urea (UREA), creatinine (CREAT), total protein (TP), albumin (Alb), calcium (CA), phosphorus (P), cholesterol (CHOL) and triglycerides (TRIG). Full value nutrition analysis was conducted by the accredited biotechnology scientific laboratory of Latvia University of Life Sciences and Technologies following the accredited ISO standard methods and "J.S Hamilton Baltic" according to the generally accepted methodology. Determination of serum parameters was performed at the veterinary clinic of the Latvian University of Life Sciences and Technologies. MS Excel and SPSS data processing computer programs were used for calculations and analysis. Differences between group indicators were determined using t-tests and Mann-Whitney and Wilcoxon tests. The results were considered statistically significant at $p<0.05$.

Results and Discussion

The chemical composition of potassium humate is shown in Table 3.
As can be seen from Table 3, the chemical composition of potassium humate (dark brown natural organic powder) contains a wide variety of biologically active components – minerals, vitamins and other biologically active substances that can significantly increase the biologically functional value of the feed ingredient.

Table 3. Chemical composition of potassium humate.

Determined component and its unit of measurement	Test result
Dry matter, %	94.66
Crude protein, %	3.76
Crude fibre, %	38.87
NEL, MJ/kg	3.03
Crude ash, %	31.08
Calcium, %	1.68
Magnesium, %	0.22
Potassium, %	5.92
Sodium, %	0.11
Zinc, mg/kg	7.67
Copper, mg/kg	2.00
Manganese, mg/kg	59.45
Iron, mg/kg	2970.89
Beta-carotene, µg/100 g	<5
Vitamin A, µg/100 g	<21
Vitamin D3, µg/100 g	<0.25
Vitamin E, mg/100 g	5.79
Vitamin C, mg/100 g	<0.5
Vitamin B1, mg/100 g	<0.015
Vitamin B2, mg/100 g	<0.01
Vitamin B9, µ/100 g	<5
Vitamin B12<, µg/100 g	<0.25
Humic acid, %	4.17
Fulvic acid, %	2.0

Humic acid is an important component of the bioactive substances in potassium humate. Due to its specific chemical composition, humic acid reacts with biologically active substances such as choline, thiamine, riboflavin, nicotinamide, pantothenic acid and is able to absorb

various harmful substances – toxic heavy metals, pesticides, radionuclides, etc., ensuring their elimination from the body, thus acting as a bio-regulator. The use of biologically active substances, including humic substances, activates digestive and metabolic processes in the animal's organism, promoting the transformation of feed nutrients in available forms, raising daily milk production and milk quality (Bezuglova and Zinchenko, 2016; Kucukersan et al., 2005). Cow productivity during the trial is shown in Figure 2. Productivity is one of the most important indicators of the effect of one or another dietary factor on the cow's production performance (Islam et al., 2005; Batchelder, 2000). The analysis of productivity indicators shows that when cows were fed with potassium humate, the obtained milk production was also different. The average milk yield was 5.19 kg/day higher in the experimental group at the end of the study, compared to the control group. The difference in milk yields demonstrated an increasing trend in favour of the experimental group, but there were no statistically significant differences between the average daily milk yields between the two groups ($p<0.05$).

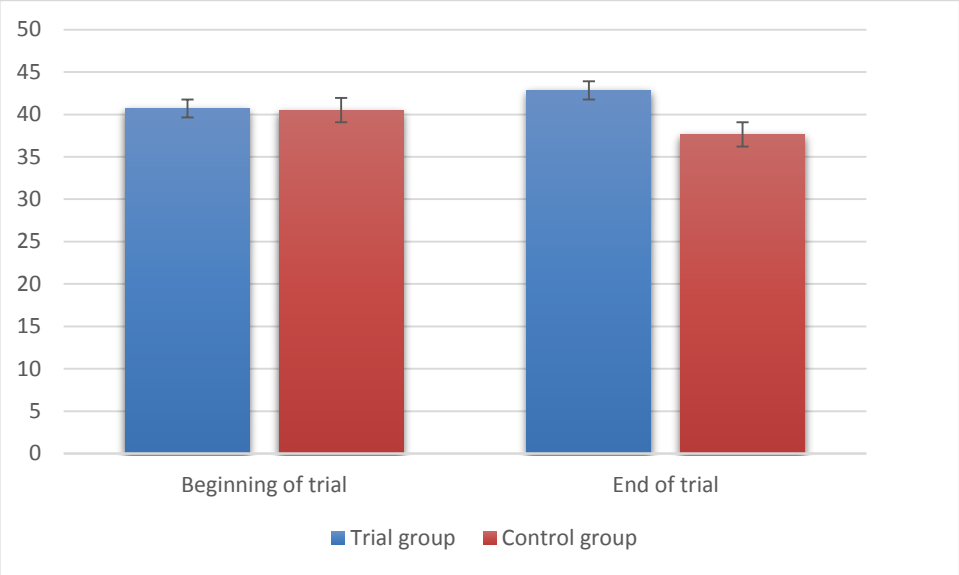

Figure 2. Cows' productivity during the trial, on average, kg.

The serum haematological parameters obtained during the study are shown in Table 4. All automatically determined haematological parameters were not statistically different between groups or between the time period of blood sampling ($p>0.05$). The mean values of the automatically determined haematological parameters, highlighting the significant WBC, RBC HTC, HGB, were within the normal range in all cases. When total leucocyte counts and other parameters were analysed for individual cows, some parameters were above the normal range on one sampling occasion but within the normal range on the next occasion (Kerr, 2002). In the leukocyte formula, the mean values of Sg and Eo were slightly increased, while Ly was decreased in both groups of study. These results can be explained by the fact that the changes in the mentioned indicators were based on the changes in the indicators of individual animals in both groups, which affected the average indicators. In the analysis of the overall trends in haematological blood results over time, no significant changes that could have a major impact on the health of the cows were detected.

Table 4. Serum haematological parameters before and 6 months after potassium humate feeding.

Parameter	Group of cows			
	Trial		Control	
	At the beginning of the trial	6 months after the trial	At the beginning of the trial	6 months after the trial
WBC 10^9/l	7.41±1.15	7.03±0.92	8.15±2.32	7. 97±1.27
RBC 10^2 /l	5.81±0.59	5.93±0.57	5.76±0.66	6.12±0.72
HTC %	26.76±2.37	28.29±3.35	26.90±3.66	30.66±4.63
HGB g/dl	9.9±0.89	10.61±1.09	10.01±1.21	11.28±1.53
MCV fl	46.19±2.51	47.68±2.25	46.59±1.81	49.98±2.39
MCH pg	17.28±0.95	17.92±0.63	17.39±0.58	18.43±0.69
MCHC g/dl	37.42±0.69	37.62±0.79	37.36±0.72	36.98±0.75
PLT 10^9/l	236.70±63.10	295.89±26.37	271.90±81.24	262.2±52.71
Sg	4.69±1.28	3.89±0.56	5.04±2.66	4.39±1.00
Eo	0.72±0.28	0.34±0.20	0.96±0.70	0.48±0.25
Mo	0.17±0.16	0.26±0.08	0.18±0.07	0.36±0.13
Ly	1.81±0.49	2.54±1.12	1.96±0.63	2.71±0.64

The mean values of serum biochemical parameters are shown in Table 5. Aspartate aminotransferase (ASAT) is an enzyme associated with parenchymal liver cells, but it's also found in the kidney, brain, heart muscle and skeletal muscle. This is a non-specific indicator and can increase during inflammatory, degenerative processes. Analysing the results of ASAT, the indicator did not change clinically significantly during the study.

Table 5. Blood serum biochemical parameters before and 6 months after potassium humate feeding.

Parameter	Group of cows			
	Trial		Control	
	At the beginning of the trial	6 months after the trial	At the beginning of the trial	6 months after the trial
AST U/L	111.6±37.75	87.77±17.12	116.2±54.97	73.5±21.40
GGT U/L	40.5±8.71	39.11±11.08	163±199.88*	34.4±10.30
LDH U/L	1165.1±159.73	1010.75±49.06	1424.3±534.39	945.4±171.64
Urea mmol/L	3.92±0.34	5.38±0.78	4.39±0.63*	5.57±1.03
CREAT umol/L	69.17±4.19	62.48±6.08	65.62±8.75	68.09±10.26
TP g/L	74.2±7.01	78.55±5.5	75.77±6.03	73.7±7.04
Alb g/L	32.9±3.62	33.5±1.41	32.77±2.62	32.77±1.87
CA mmol/L	2.37±0.12	2.31±0.08	2.28±0.08	2.23±0.12
P mmol/L	1.81±0.24	1.77±0.16	1.89±0.21	1.91±0.42
CHOL mmol/L	5.51±1.32	6.29±1.10	5.32±1.21	4.78±1.76
TRIG mmol/L	0.182±0.04	0.19±0.08	0.15±0.03	0.201±0.08

* significant difference between a and b ($p < 0.05$)

This suggests that the potassium humate component did not affect ASAT blood levels during the study (Cozzi et al., 2011; Kerr, 2002). Gamma-glutamyltransferase, or GGT, transfers amino acids across the cell membrane. Serum GGT levels are mainly elevated in diseases of

the liver, biliary tract, pancreas and kidneys. The mean GGT measurements in the study were within the normal range, except for the control group, which had an elevated mean GGT level at the first examination. In the other tests, GGT values were within the optimal range in both groups. Lactate dehydrogenase (LDH) is an enzyme that helps to turn lactate into pyruvate, so it is not tissue-specific and is found in many organs such as the liver, heart and skeletal muscle. The mean LDH levels in both study groups were elevated above the normal range at the time of sampling, which was attributed to the stress of fixation of the animal at the time of sampling. Literature also indicates higher LDH levels in dairy cows – up to 2146 U/L (Cozzi et al., 2011). Changes in serum urea levels can also indicate a disturbance in nitrogen metabolism, a biomarker for kidney disease and liver function (McMurphy et al., 2009). Serum urea levels were within normal range in this study. When serum urea and creatinine levels were analysed together to judge potential renal failure, the results indicate that potassium humate supplementation had no adverse effect on renal function, as both parameters were within the normal range in both groups throughout the study or the changes were clinically insignificant. Total protein (TP) and albumin (Alb) levels in both groups were within the normal range throughout the study and no significant differences were observed between groups ($p>0.05$). Similarly, when CA and P levels were analysed, no significant differences were observed between groups ($p>0.05$). The results for serum phosphorus and calcium during the study are attributable to the different lactation phases and on average are acceptable for lactating dairy cows. Changes in cholesterol levels can be a sign of a lipid metabolism disorder. Overall, CHOL levels were within the optimal range in both groups. Serum triglyceride levels were within normal range and no differences were observed between groups.

Conclusions

The feed ingredient potassium humate promoted physiologically important processes in the bodies of dairy cows and contributed to an increase in milk yield by 5.19 kg per day, compared to the control group and better maintained milk yields during lactation ($p<0.05$). Potassium humate feeding to cows during the study had no effect on haematological parameters and no direct effect on serum biochemical parameters. Further research would be needed to draw deeper conclusions on the effects of potassium humate on productivity and health parameters in dairy cows.

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THE IMPACT OF BREEDING HONEYBEES IN DIFFERENT REGIONS OF SERBIA AND BOSNIA AND HERZEGOVINA ON THEIR MORPHOMETRIC PARAMETERS

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Abstract

In order to facilitate better comparative analysis, a sample of bees originating from Slovenia was also analyzed. A comparison was made between the values of the morphological parameters of the front wings of bees from the following locations: Svojnovo (I), Ražanj (II), Prokuplje (III), Zlatar (IV), Negotin (V), Čajniče (VI), Tuzla (VII), Višegrad (VIII), Bijeljina (IX) and Slovenia (X). The aim of the study was to determine the existence of differences between bees from various sites. The standard methodology included the measurement and analysis of 11 angles on the front wing (A4, B4, D7, E9, J10, J16, K19, L13, N23, G18 and O26), wing length and width, and cubital index on samples of 15 bees each. Univariate statistical analysis was performed, and comparisons were made between locations using Duncan's Studentized Multiple Range Test. Based on the results of the statistical analysis, a highly significant difference was found between location I and locations II, IV, VII, VIII, and IX for angles A4 and B4. When comparing the value of angle E9, a statistically significant difference ($p<0.01$) was found between location IV and all other locations. In addition to the established differences between bees from individual localities, there are similarities that indicate the maintenance of a stable population of *A. m. carnica* in the region.

Key words: *Honey bee, Morphometry, Angle, Cubital index, Serbia.*

Introduction

In Serbia, due to the significant expansion of beekeeping in recent decades, the honeybee is increasingly influenced by human activities. The increasing number of beekeepers, beehives in apiaries, breeding centers, and modern beekeeping techniques (including queen trading, artificial selection, and migratory beekeeping) have resulted in genetic diversity in cultivated and wild (feral) populations of this ecologically and economically important species in breeding areas (Patenković et al., 2022).

Morphological characteristics play a role in the classification and characterization of honeybees into specific subspecies (Abou-Shaara et al., 2013). Furthermore, they have an essential role in their foraging activity for nectar collection and pollination (Ajao et al., 2014). As Rahimi and Mirmoayedi (2013) point out, weather conditions, the presence of parasites, and bee colony migration have an impact on the morphological characteristics of bees.

Measuring the morphological characteristics of honeybees (Apis mellifera) at different time periods is significant for assessing the purity of the breed and predicting colony productivity, as well as understanding the impact of the environment on honeybees (Abou-Shaara and Ahmed, 2015).

The fundamental cause of variability in the beekeeping process is the use of local ecotypes that are well adapted to the agricultural and ecological environment of a specific region

(Jevtić et al., 2007). Specific features of the terrain, landscape exposure, and eco-climatic conditions in Serbia contribute to the heterogeneity and variability of honeybee traits.
Abou-Shaara (2013) emphasizes that a representative number of bees per sample, along with the application of an appropriate standard morphometric analysis, can be sufficient for clear discrimination and identification of honey bee races.
The aim of the research was to determine the differences between bees from different locations. The standard methodology involved measuring and analyzing 11 angles on the forewing (A4, B4, D7, E9, J10, J16, K19, L13, N23, G18, and O26), as well as measuring wing length and width and calculating the cubital index on samples of 15 bees each.

Materials and Methods

The determination of morphometric traits was conducted on ten samples of bees, with nine samples originating from the territory of the Republic of Serbia and the territory of Bosnia and Herzegovina (BiH). The study included five bee samples from Serbia and four samples from BiH, collected from different locations. To facilitate better comparative analysis, a sample of bees originating from Slovenia was also included. Within the analyzed samples, a comparison was made between the obtained values of the parameters of the forewing of bees from the following locations: Svojnovo (I), Ražanj (II), Prokuplje (III), Zlatar (IV), Negotin (V), Čajniče (VI), Tuzla (VII), Višegrad (VIII), Bijeljina (IX), and Slovenia (X). The processing of collected samples for the research was conducted in 2022.
From each location, 100 bees were sampled, preserved in 96% alcohol, and then dissected. Fifteen bees were randomly selected from each sample, and the right forewing of each bee was dissected. The wings were then arranged on slides in two rows. After the wing preparation process was completed, they were scanned using an Epson Perfection V600 Photo scanner. The scanning resolution for the wing slides was set at 4800 pixels. After scanning and image formation, individual measurements of characteristics on the forewings of bees were performed using the ImageJ software. The measurements included a total of 14 parameters, with 11 angles on the forewing and additional parameters of forewing length (FWL), forewing width (FWW), and cubital index (CI).
Univariate statistical analyses were conducted for the 14 morphological traits. Descriptive statistical analysis was performed, and comparisons between locations were made using Duncan's Studentized Multiple Range Test. All statistical analyses followed standard procedures, and the results of these analyses were interpreted.

Results and Discussion

Descriptive statistical values of the morphological characteristics of bees from different locations in the territory of the Republic of Serbia and the area of Bosnia and Herzegovina are presented in Table 1. The measurement results of the sampled worker bees exhibited a wide variation in mean values and standard deviations for the investigated forewing characteristics.
The average value for angle A4 ranged from 27.27° to 30.19° across the different locations. The most pronounced separation in this angle was observed between the Svojnovo and Prokuplje locations, which were highly statistically significantly different from the other locations, except for two locations (V and VI). The average value for angle A4 is in line with the results reported by Mladenović and Simeonova (2010), where the average angle A4 for the Lešak location was 30.30°.

Table 1. Comparison of the significance of differences in mean values

Characteristic	Group										Average
	I	II	III	IV	V	VI	VII	VIII	IX	X	
A4	27,27	29,83	27,47	29,20	28,46	28,59	29,38	29,72	30,19	29,90	29
	ac	bd	ac	b	ad	ad	b	b	b	bd	
B4	115,23	109,05	114,02	108,46	110,66	112,31	106,95	109,20	108,76	110,27	110,49
	a	bc	ac	b	ac	ac	b	bc	b	ac	
D7	97,85	99,13	99,70	99,13	100,80	100,57	95,29	98,01	97,84	98,75	98,71
	ab	a	a	a	a	a	b	ab	ab	a	
E9	23,30	22,99	22,15	19,80	22,22	23,23	22,36	22,87	23,44	22,74	22,51
	b	b	b	a	b	b	b	b	b	b	
J10	53,65	54,78	57,21	55,12	55,42	56,92	53,99	52,84	56,13	53,83	54,99
	ab	ab	b	ab	ab	b	ab	a	ab	ab	
J16	98,75	97,92	95,95	95,45	96,32	96,07	95,12	93,13	94,74	95,62	95,91
	a	ad	ac	bcd	ac	ac	bcd	bc	bcd	ac	
K19	76,77	74,75	77,90	79,17	76,42	77,96	77,86	78,60	76,63	74,67	77,07
	ab	b	ac	ad	b	ae	af	ag	ab	acb	
L13	12,89	14,45	14,38	13,33	12,01	13,88	13,30	12,77	13,37	12,95	13,33
	abf	c	cd	bce	f	ac	ac	aef	ac	aef	
N23	100,16	98,67	100,15	100,41	98,66	99,40	99,07	98,12	99,48	99,49	99,36
	a	a	a	a	a	a	a	a	a	a	
G18	89,89	92,67	90,95	90,22	89,86	88,75	92,24	89,86	90,91	89,87	90,52
	ab	a	ab	ab	ab	b	ac	ab	ab	ab	
O26	35,42	37,48	37,60	37,41	39,08	36,79	38,00	35,47	37,92	41,14	37,63
	db	ab	ab	ab	ac	ab	ac	f	ac	c	
FWL	9,05	9,11	9,20	9,11	9,28	9,40	9,01	9,34	9,23	9,36	9,21
	a	ac	cd	ac	bd	b	a	bd	cd	b	
FWW	3,10	3,07	3,16	3,08	3,19	3,26	3,07	3,27	3,23	3,17	3,16
	a	ab	ac	abd	ce	af	abd	f	cef	ce	
CI	2,72	2,35	2,49	2,38	2,28	2,28	3,11	2,82	2,56	2,17	2,52
	abc	d	bcd	cd	d	d	a	ab	bcd	d	

The angles are given in degrees (°), while the length and width of the forewings are given in millimeters (mm)

Statistically significant differences were also found for angle B4 among the mentioned locations, with an average value of 110.49°, which did not significantly differ from the value reported by Nedić et al. (2011), where the average value for the same angle was 107.89°.

A distinct difference between the locations in Bosnia and Herzegovina and the territory of Serbia was observed for angle D7, where the Tuzla location significantly differed from the locations in Serbia. Additionally, a statistically significant difference was found compared to the Slovenia location, while the samples from Bosnia and Herzegovina did not significantly differ from each other. The average value for angle D7 across all locations was 98.71°, which is consistent with the results highlighted by Nedić (2009), with an average angle D7 of 98.33°.

Considering that the bees sampled from the Zlatar location originate from the local ecotype of that area, the average value for angle E9 of these bees significantly differs from all the analyzed locations. The average value for angle E9 in the Zlatar location was 19.80°, differing from the values reported by Rinderer et al. (1993) for commercial (21.50°) and feral (21.10°) European honeybee populations. Güler et al. (2010) emphasize that the average value of the E9 angle in Apis mellifera carnica in the Thrace region of Turkey was 22.58, which

significantly differs from location IV, which exhibited significant separation compared to other locations in the study.

Regarding the average values for angle J10, a significant difference was observed between the Višegrad location (52.84°) and the Prokuplje (57.21°) and Čajniče (56.92°) locations. The highlighted values for the Višegrad location do not significantly differ from those reported by Mladenović and Simeonova (2010), where the average value for angle J10 across the analyzed locations was 51.87°.

Bees bred in the Pomoravlje region (location I) exhibited significant differences in terms of the average value for angle J16 (98.75°) compared to the bees from locations II, III, V, and VI. The average value for angle J16 in the I location was lower compared to the value (100.75°) reported by Bouga et al. (2011) for Apis mellifera carnica colonies from Portugal.

When comparing the values for angle K19 between the Ražanj and Negotin locations (74.75° and 76.42°) in Serbia and the Bijeljina location (76.63°), which are characteristic for being different from the other locations, the results differ from those of Kauhausen-Keller and Keller (1994), where the average values for Apis mellifera carnica for the same angle were 79°. However, the average value for the autochthonous ecotype of the IV location for angle K19 is 79.17°. Considering the time difference between the obtained research results and the results used for comparison, the focus should be on the existence of originality and the preservation of the genotype, which is reflected in the absence of significant differences in terms of average values for certain morphometric characteristics.

In contrast to angle N23, whose average values did not significantly differ among locations, angle L13 illustrates diversity in terms of breeding conditions between the Negotin location in the Bor District and the locations in central Serbia and Bosnia and Herzegovina. A significant difference was observed for angle G18 between location VI and locations II and VII.

The average values for the analyzed locations for angle O26 ranged from 35.42° (Svojnovo location) to 41.14° (Slovenia location). The average for all locations was 37.63°, which differs from the values reported by Abou-Shaara and Al-Ghamdi (2012), where the average angle size for the Carniolan honeybee was 40.57°.

The average length of the forewing of the bees was 9.21 mm across all locations, which differs from the values reported by Mladenović et al. (2011), who found an average length of 8.96 mm for bees from six locations in southern Serbia. The average wing length in the research falls within the range reported by Georgijeva (2014), where the wing length of selected lines of Apis mellifera carnica Poll. ranged from 9.19 to 9.55 mm (with an average of 9.40 mm) across all lines.

According to Mladenović and Pešev (2011), the average wing width per location ranged from 3.11 mm to 3.14 mm, with the obtained average value in the research being almost the same (3.16 mm).

The values of the cubital index ranged from 2.17 to 3.17 on average per location, with an overall average of 2.52, which does not differ from the value reported by Mladenović et al. (2011), where the cubital index value for yellow bees from Vojvodina was 2.40. Kauhausen and Keller (1994) reported a cubital index size of 2.70 for the reference sample of A. m. carnica bees. In the study conducted by Kulici (2021) on Albanian bee populations, the values of the cubital index ranged from 2.33 to 2.94 within the examined regions.

Conclusion

Based on the research results, significant variability was found among bees from different locations in the territory of the Republic of Serbia and Bosnia and Herzegovina. The analysis of variance revealed high statistical significance in the differences of the average values for angles: A4, B4, D7, E9, J10, J16, K19, L13, G18, and O26. Despite the differences observed

among various sampled locations based on multiple examined wing morphometric parameters, a clear discrimination can be noticed in the bee sample originating from the original ecotype at the Zlatar location based on the E9 angle. Additionally, statistically highly significant differences were observed in the values of forewing length, forewing width, and cubital index between bee samples from different locations. Based on the analysis of average wing width values, the Čajniče locality from the territory of Bosnia and Herzegovina showed a highly statistically significant difference compared to localities from the territory of Serbia, where a clear distinction in terms of the examined wing dimensions of honeybees was observed among the analyzed regions. In terms of the average cubital index values, the Tuzla locality exhibited a highly statistically significant difference compared to all other examined localities. However, there were no statistically significant differences in the average values of angle N23 among the analyzed locations.

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POPULATION STRUCTURE OF THE ENDEMIC MAKAL DACE (SQUALIUS MICROLEPIS HECKEL, 1843) FROM THE MATICA RIVER

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Abstract

The freshwater ichthyofauna of Adriatic basin is characterized by a large number of endemic species, which are distributed on a restricted area. The makal dace (*Squalius microlepis*) is an endemic species living only in the Neretva River basin and mainly in the sinkhole river Matica in Bosnia and Herzegovina (BiH) and Croatia, Prološko Lake and accumulation of Ričica near Imotski in Croatia. Population structure was studied on 51 individuals caught by gill nets at location on the Matica River near Drinovci in BiH. The aim of this study was to determine the population structure of makal dace, according to length and weight, and length-weight ratio between total length (cm) and weight (g). The results are presented in the form of frequency histograms, linear and weight structure, and length-weight ratio between total length (TL) and weight (W). Individuals with total body length of 19-20 cm are dominating, with maximum total length of 28 cm. The maximum recorded weight was 269.30 g. The frequency histogram of body weight show that weight class 60-90 g dominates too. Positive allometric growth with high b-value of 3.47 has been determined. This research provided new results on structure of population of the endemic makal dace.

Keywords: *population structure, endemic makal dace, length-weight relationship, Matica River.*

Introduction

Bosnia and Herzegovina and the Republic of Croatia are known for presence of numerous endemic species and subspecies of fish, associated with two major river basins, the Danube and the Adriatic. Rivers of the Adriatic basin are significantly short and isolated, compared to the rivers of the Danube basin. Thus, a certain number of insufficiently described endemic species exist in the Lower Neretva area, where a small number of researchers studied their ecology, biology, taxonomy and correlations between them. A large number of endemic species of freshwater ichthyofauna of the Adriatic basin are distributed on a restricted area (Vuković and Ivanović, 1971; Economidis and Banarescu, 1991; Mrakovčić *at al*., 1995, 2006; Kottelat i Freyhof, 2007). For these species, there is a small number of published scientific papers, referring to their systematic position, distribution, problem of endangerment and biological-ecological character (Crivelli and Maitland, 1995a, b; Crivelli, 1996; Kottelat and Freyhof, 2007). A type of fish that, in this context, has not yet been sufficiently researched is the makal dace (*Squalius microlepis*). This species, as well as other endemic fish species, is characterized by a limited area of distribution, as well as a low ability of the adaptations to changes in external factors (Mrakovčić *at al.*, 2006). Makal dace is mentioned in the Red Book of Freshwater Fishes of Croatia (Mrakovčić *at al.*, 2006) as a critically endangered species. In the book Croatian endemic fish species (Ćaleta *et al.*, 2015), the makal dace is mentioned as an endangered species on the IUCN list, and for the territory of the Republic of Croatia it is listed as endemic for the Adriatic basin and a critically endangered species. The introduction of non-native species, increased anthropogenic pressure on their habitats, and lack of water due to climate change contribute the most to their endangerment

(Mrakovčić *at al*., 2006; Kottelat i Freyhof, 2007). Adopting more efficient protection measures for each endemic species is limited by the small amount of knowledge about their biology and ecology, despite their threat and value. There is little published scientific data for the makal dace, mostly related to basic biological-ecological features, distribution, and problems of endangerment (Crivelli i Maitland, 1995; Kottelat i Freyhof, 2007, Ivanković *at al*., 2017). Morphometrics and meristics are the most frequently applied morphological methods for distinguishing fish populations. Therefore, the use of meristic and morphometric traits proved to be useful in differentiating carp populations (Treer *at al*., 2000). The aim of the present work was to provide data about length and mass structure of the makal dace population, and relation between total length (cm) and weight (g). The results are presented in the form of frequency histograms, linear and weight structure, and length-weight ratio between total length (TL) and weight (W).

Material and Methods

The sampling was done in the area of Matica River near Drinovci in BiH. Fishes were caught by anchored gill nets, mesh diameter 28-72 mm and 30 meters long, while net height varied from 1 to 3 meters. Nets were set up at night and checked the next morning. Fish population that has been processed was total 51 specimens of makal dace and they weren't analysed for sex determination. Determination of species was done by system key for determination of fish species presented by Vuković i Ivanović (1971) and Kottelat and Freyhof (2007). Total length (TL; cm) was measured by Ichthyometer from the beginning of the head to the end of the tail fin, while the body weight (TW; g) was analysed by analytical scale. The formula according to Ricker, 1975 was used to determine the length-mass ratio (Ricker, 1975) where: Length-to-weight relationships (LWR) were calculated from log W = log a + b log L, W is the weight of the fish (g), L is the total length of the fish, a is the intercept, and b is the slope. Parameters a and b of the LWR were estimated by linear regression analysis on log-transformed data. The obtained coefficients were analysed with ANOVA. The obtained research data were processed with the statistical program SPSS 20.

Results and Discussion

The maximum registered total body length of makal dace was 30.6 cm and the minimum 13.6 cm, while the average length was 20.25 cm, which is in accordance with the data from Mrakovčić *at al.* (2006), who state that the makal dace is a spindle-shaped fish that can grow up to 30 cm with an average length of between 20 and 25 cm. Also, Jelić *at al.* (2008) state that the makal dace is endemic to the Adriatic basin with a body length of up to 30 cm. According to Bogutskaya *at al.* (2019) the maximum standard body length (SL) in makal dace (phenotypes 1) was 22.34 cm, while the minimum was 6.11 cm. In makal dace (phenotypes 2), the maximum standard body length was 25.57 cm, while the minimum was 7.20 cm. In the histogram frequency of total body length makal dace (Figure 1), it's noticeable that the majority of specimens were in the third class with total body length between 19 and 20 cm.

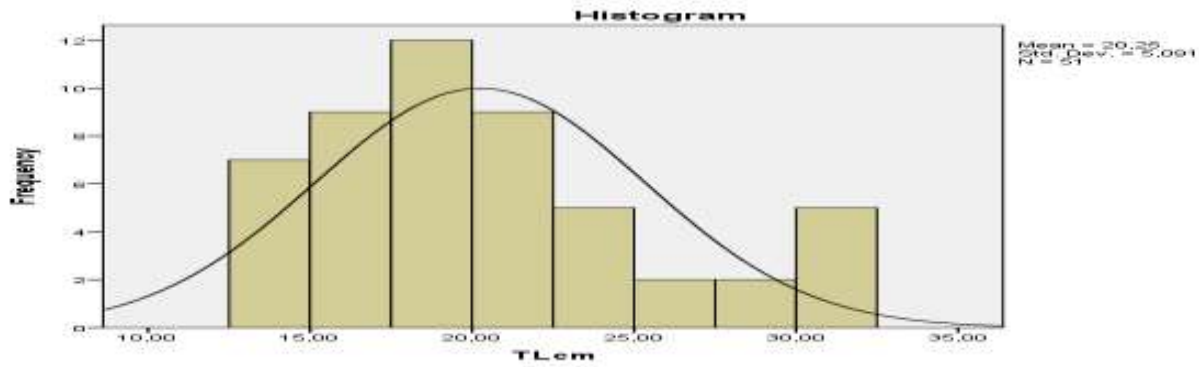

Figure 1. Frequency of makal dace total body length

The maximum measured body weight of makal dace was 313.8 g., while the minimum was 24.9 g. The average body mass of 51 measured specimens was 98.48 g. The frequency histogram of makal dace body weight showed that it is classified in ten classes according to body weight, where the second and third class with a body weight of 60 to 90 grams dominated, while first with body weight of 20 to 40 g and the seventh i eight weight classes, with body weight of 150 to 200 g had a minimum value (Figure 2).

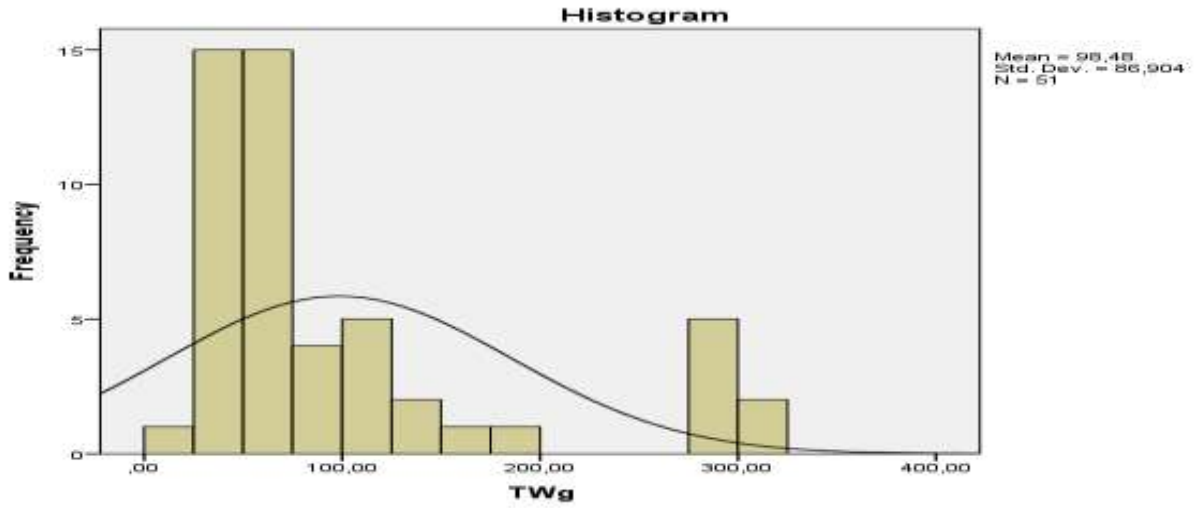

Figure 2. Frequency of makal dace body weight

The function that best describes length-weight relationship specimens of makal dace is: $W = 0.0072\ L^{3.10}$ with the determination coefficient $R^2 = 0.98$. According to the obtained b-constant value of 3.10, demonstrated positive allometric, i.e. almost isometric growth for whole sample of makal dace specimens (Figure 3). Acording to Dulčić *at al*. (2009) b-constan value of 3.22 in a related species Neretva chub in the waters of Hutovo blato indicates its positive allometric growth, which is also confirmed by Ivanković *at al*. (2017) with b-constan value of 3.47.

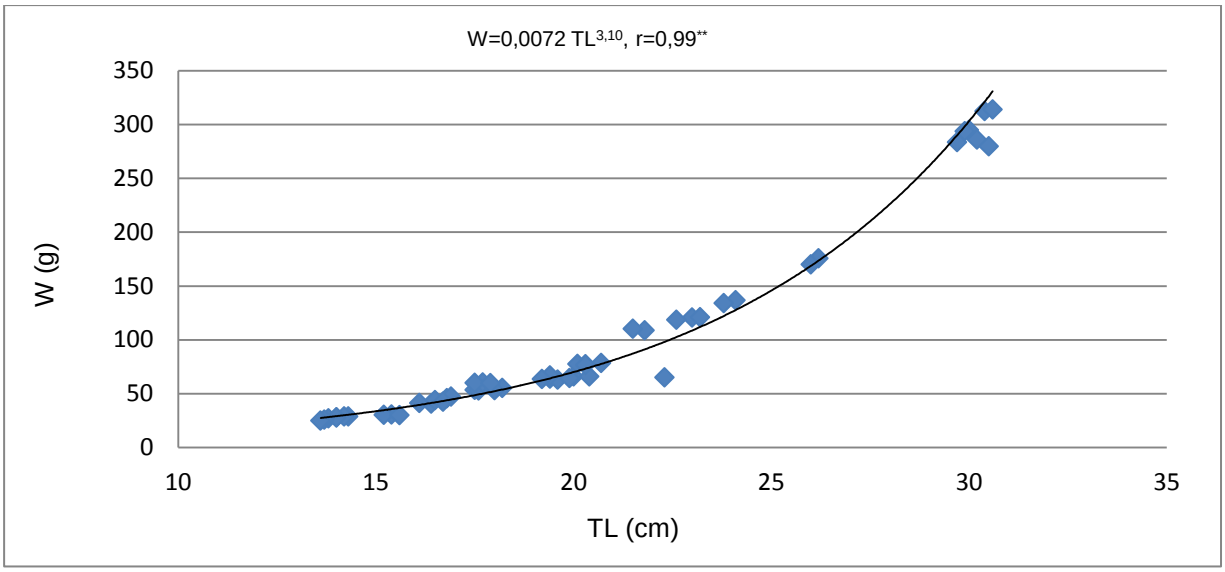

Figure 3. The length-weight relation between total length (cm) and body weight (g) of makal dace from the Matica River

According to Ivanković *at al*. (2017), the potential growth of makal dace, expressed through the maximum length on the von Bertalanffy growth curve, was: length $L\infty$ = 49.838 cm and weight $W\infty$ = 1256.17 g. This is in accordance with the data of local fishermen's that the largest specimen of macal was over 40 cm long and 1 kg heavy (IMO art, 2016). The makal dace grows slightly faster compared to Neretva chub, from the same area (Ivanković *at al*., 2010). However, the growth of both of these species is significantly faster compared to the chub *S. cephalus* from the north Croatia (Treer *at al*., 1997). These differences can be attributed to the characteristics of different fish species, but also to the habitat, which is located in a very fertile area with a mild Mediterranean climate (in Croatia called "Croatian California").

Conclusions

One of the main characteristic of rivers in Bosnia and Herzegovina is the population of many endemic species and subspecies of fish. Here, the lower basin of the Neretva River, which belongs to the Adriatic basin, can be stands out with a large number of endemic fish. One of them is the makal dace with quite limited living space. Only a small number of articles study its ecology, biology and taxonomy. Makal dace is a species that is listed as an endangered species according to the IUCN Red List, while it is listed as a critically endangered species in the Red Book of Freshwater Fishes of Croatia. There is no data of its endangered status in the area Bosnia and Herzegovina. The largest registered total body length of the makal dace from the Matica River was 30.6 cm, and the largest body weight was 313.8 g. From the shown frequency histogram for the body length, it can be concluded that the majority of specimens were in the classes with total body length between 19 and 20 cm, while in the frequency histogram for the body weight dominated specimens of makal dace between 60 and 90 g. The obtained b-constant with a value of 3.10 shows that the makal dace has a positive allometric, i.e. almost isometric, growth.

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STATUS OF THE MIGRATORY BEEKEEPING IN LATVIA

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Abstract

Migratory beekeeping is an approach to potentially increase the honey production, by moving bee colonies from one geographical location to another, based on the flowering times of the specific crops to exploit seasonal nectar flows. As well, migratory approach can be applied for the pollination of the critical agricultural crops by providing the pollination service for the farmers and it is becoming as a vital component of modern apiculture. This study investigates migratory beekeeping in the unique ecological and climatic context of Latvia, a Baltic country renowned for its diverse floral resources and rich beekeeping tradition. Within this study authors are making the social study to analyze the Latvian beekeepers' awareness about the migratory beekeeping and its popularity in Latvia. Online survey was conducted and 237 respondents shared their experience and participated in the questionnaire. This research helped to identify key factors and benefits of migratory beekeeping and shed a light on beekeepers' doubts about this important topic. In addition to elucidating migratory patterns, this study examines the implications of migratory beekeeping in Latvia. The research highlights the positive effects of hive movements on honey production and pollination services, providing a substantial economic benefit to the beekeeping industry and agricultural sector. Furthermore, the study discusses potential challenges faced by migratory beekeepers, including transportation logistics, exposure to environmental stressors, and the risk of disease transmission among colonies.

Keywords: *migratory beekeeping, beekeepers survey, beekeeping in Latvia.*

Introduction

Honeybee is the most dominant pollination specie globally in agricultural (Allen-Perkins et al., 2022) and natural ecosystems (Hung et al., 2018). As well, honeybees are the main pollinators of the most important agricultural crops (Garbach and Morgan, 2017). Beekeeping is considered as activity that have positive impact on the environment, social and economic aspects (Guiné et al., 2021). Sometimes to meet the demand for pollination of agricultural crops, honey bee colonies can be transported to remote locations and in case colonies are transported frequently more than once per season, this is called migratory beekeeping. Travel distances can vary, depending on the area of the country and the target fields. For example, in the USA bee colonies are transported at a continental scale to pollinate such crops as cranberries, almonds, blueberries etc. (VanEngelsdorp et al., 2013). In the USA, there are an estimated 2.7 million commercial honey bee colonies, which are migratory ones. In other countries, for instance in Saudi Arabia 93% of beekeepers reported moving their hives 2–9 times a year (Adgaba et al., 2014). In India also migratory beekeeping is very common and popular, as it can increase the economic income (Kishan et al., 2017). In China it is evaluated that 80% of *A.mellifera* beekeepers migrate across the country (Zheng et al., 2018). Migratory beekeeping is applied as a strategy to harvest multiseasonal honey also in Ethiopia (Kumsa et

al., 2020). Also, in some European countries migratory beekeeping is a common practice. For instance, in Spain about 80% of colonies are moved in an annual cycle (Jara et al., 2020), as well in Turkey there are up to 75% of bee colonies which are exposed to migratory beekeeping (Albayrak et al., 2021), In Norway most migratory beekeepers move their colonies 200-400 km in late July to areas with blooming heather *Calluna vulgaris* (Dahle, 2010). Traditionally bee colonies are migrated to maximise honey production according to the availability of floral resources in the specific regions and based on a different flowering periods of crops. However, in recent decades, migratory beekeeping has been promoted as a pollination service for economically important crops such as almond or apple orchards (Martínez-López et al., 2022).

But there are also some challenges of migratory beekeeping. Migratory beekeeping may affect the health of both managed and wild bees, but the impact of migratory beekeeping on the health of wild bees is still unknown (Martínez-López et al., 2022).

In relation to Latvia, previously there was no investigation of this topic and it is generally assumed that migratory beekeeping is not common for this country. Based on the information, provided by the Latvian Beekeeping Association, there are 3499 registered association members, but real number of beekeepers in Latvia is potentially higher and there are 101 568 bee colonies (based on Latvian data centre). Bees are producing approx. 2 152 t of honey per year (https://www.lsm.lv/raksts/zinas/ekonomika/latvijas-medum-gruti-konkuret-ar-dienvidnieku-zemajam-cenam.a383305/). There are up to 68% of apiaries, where the number of bee colonies is from 1 to 30 bee colonies. But still there are some professional beekeepers with more than 100 colonies. Beekeeping in Latvia is a traditional branch of agriculture and its development continues. Several subspecies of honeybee are used in Latvian beekeeping like *Apis mellifera carnica, Apis mellifera ligustica, Apis mellifera mellifera and Buckfast strain.* The average honey yield in Latvia is about 20 kg per colony (Liepniece, 2015), but it is fluctuating year to year and for instance in 2021 was about 35-40 kg per colony (https://www.saimnieks.lv/raksts/biskopibas-biedriba-medus-raza-sogad-no-vienas-bisu-saimes-palielinajusies-par-videji-15-kilogramiem). Potentially honey yield can be increased using the migratory beekeeping approach.

Sometimes, discussing with the beekeepers about migratory beekeeping approach they do not really correctly understand this term and think that taking their colonies to one remote field and then bringing back near to the living place is considered as migratory. But as defined earlier one transportation is not corresponding to the migratory approach, but only in case of several location changes migratory approach can be established.

Aim of this research was to identify the behavior of the Latvian beekeepers in relation to the transportation of the hives and get the information about the popularity of the migratory beekeeping in the country.

Materials and Methods

Survey was conducted to study the behaviour of the Latvian beekeepers in relation to the bee colony transportation and migratory beekeeping approach. Survey was implemented as an online questionnaire in the local Latvian language using Google Forms. The questions mainly focused on multiple choice answers and open-ended responses. Survey consisted of the general questions to describe a respondent's profile: gender, age, experience in beekeeping etc. Then based on an answer to the question about migratory beekeeping and its applicability, the user is guided to a separate section for detailed description of using a migratory approach or not.

Survey was conducted in December, 2022 as this time corresponds to a more or less quiet period in beekeeper's life and they are not so busy with daily beekeeping activities. The

questionnaire in its original form was disseminated using the direct email contacts and also information was posted in a homepage of the Latvian Beekeeping Association.

Results and discussion

In total, 237 respondents completed the survey and below we will present the main findings and results of the questionnaire, analysing the beekeeper's motivation towards migratory beekeeping approach.

Beekeepers profile

There were 79 women (33%) and 158 men (67%) participating in an online survey in the age group from 18 to 88 (Figure 1).

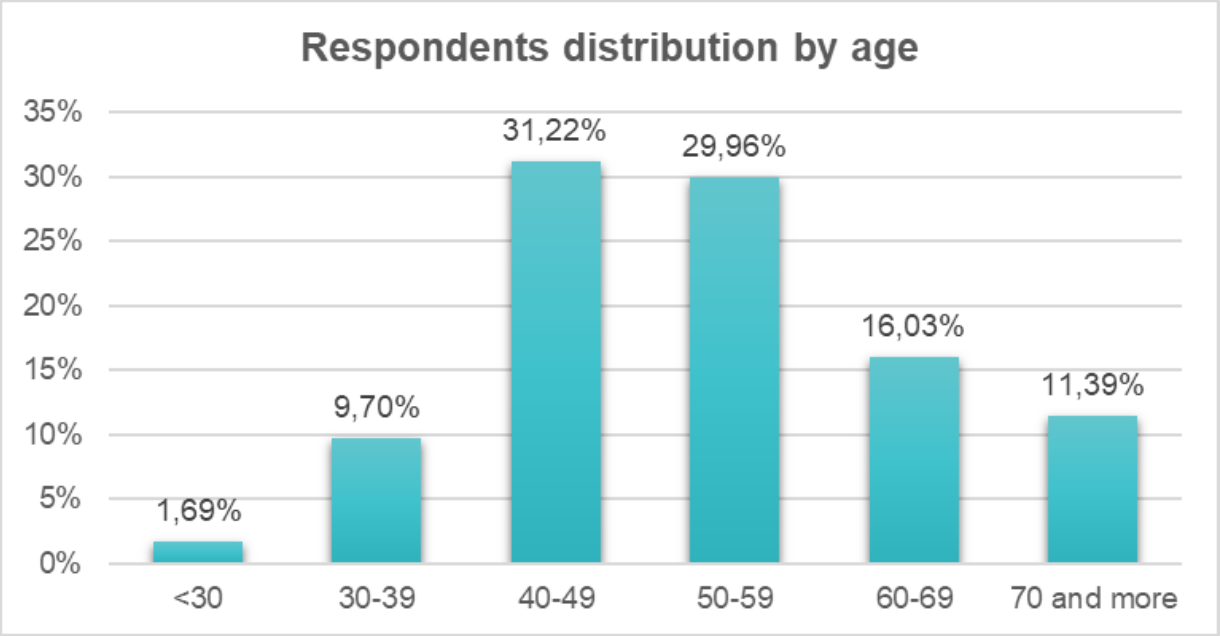

Figure 1. Age groups of participants

Looking at the bee colony count for the beekeepers it can be concluded that mainly there were small beekeepers, for 221 participant bee colony count was less than 100, and only for some it was more than 100, for one beekeeper it was 500. For 30% of respondents number of bee colonies was from 1 to 10 colonies (Figure 2).

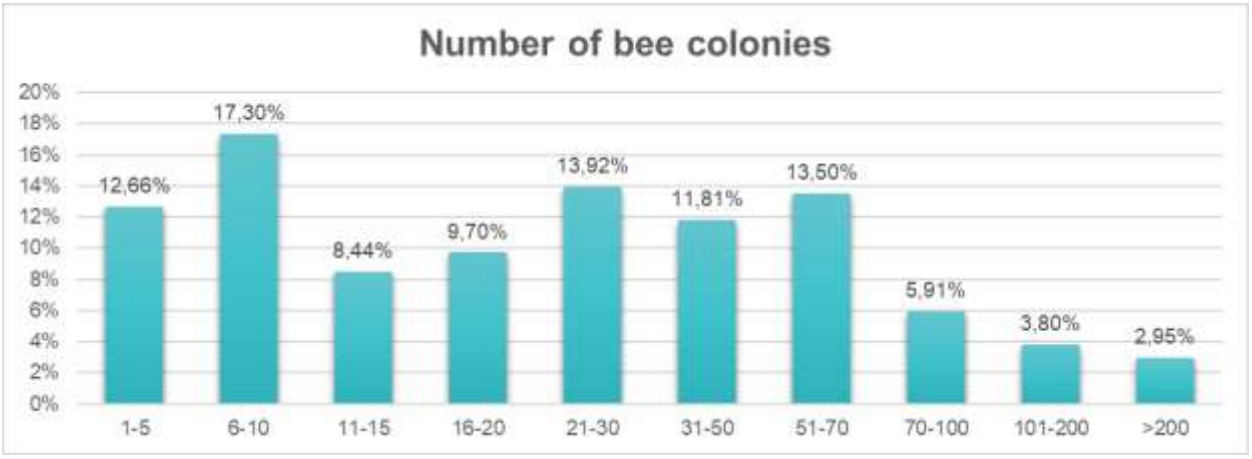

Figure 2. Number of bee colonies per beekeeper

Analysing the geographical region of the participants, it was concluded that most of the respondents were from Vidzeme (97) 41% and less from the capital city Riga (27) 11%, 39 (17%) were from Zemgale region where authors' university is located (Figure 3). For the reference on planning regions of Latvia please see https://en.wikipedia.org/wiki/Planning_regions_of_Latvia.

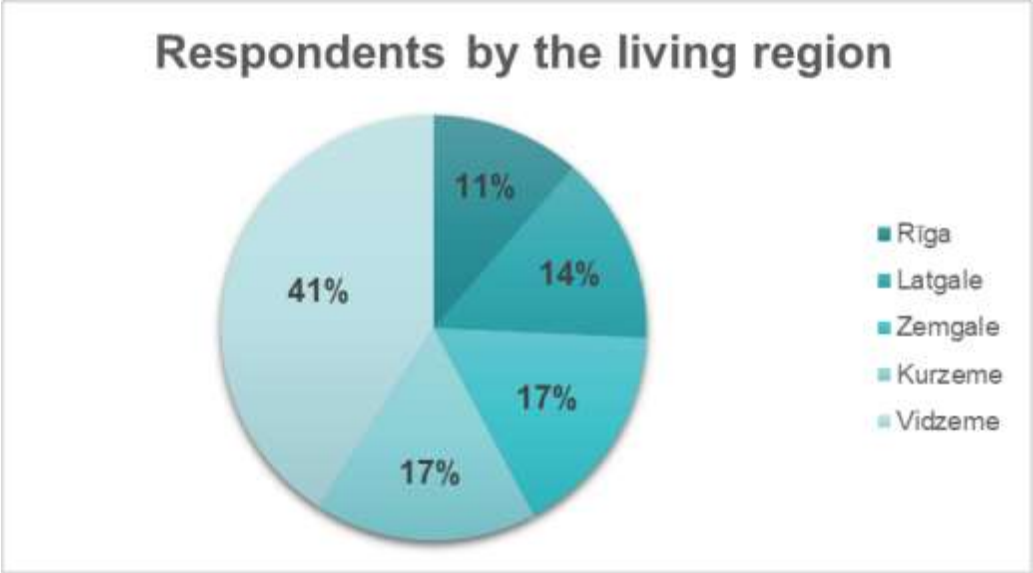

Figure 3. Respondents distribution by the living region

Experience level of the beekeepers also differs starting from 1 year and going even to 68 years of active beekeeping (Figure 4).

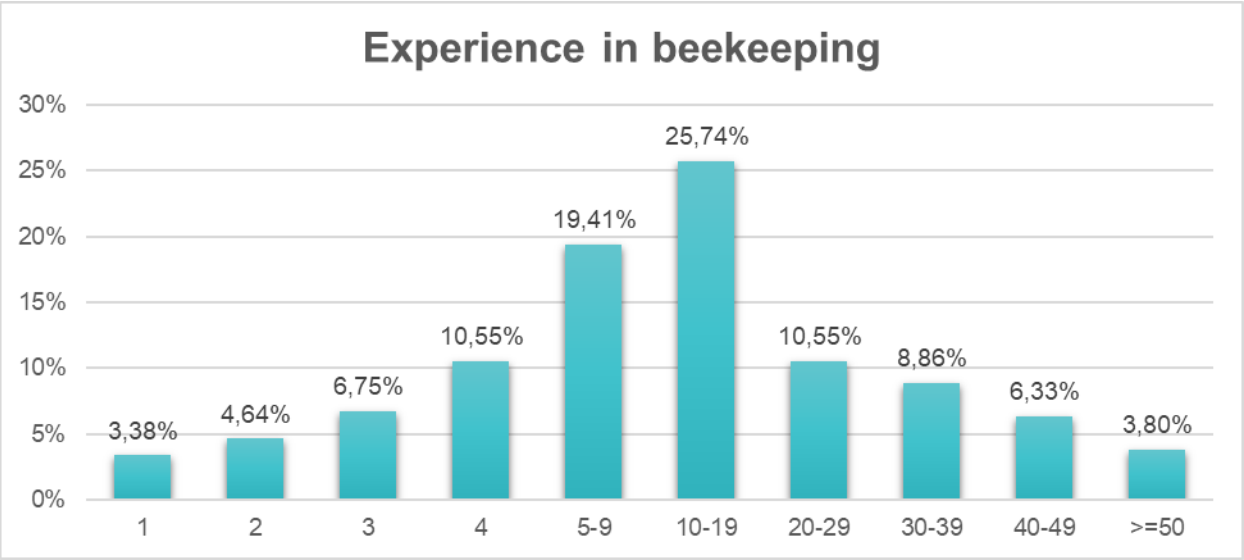

Figure 4. Respondents distribution by the experience in beekeeping

It is worth mentioning that beekeeping as the main economic activity is only for 49 (21%) of respondents, but for others it is only as an additional activity and they are not spending full-time doing the beekeeping activities.

Does the Latvian beekeepers use the migratory approach?

Most of the beekeepers (count: 147 (62%)) place the apiary stationary and bee colonies are not transported at all (Figure 5).

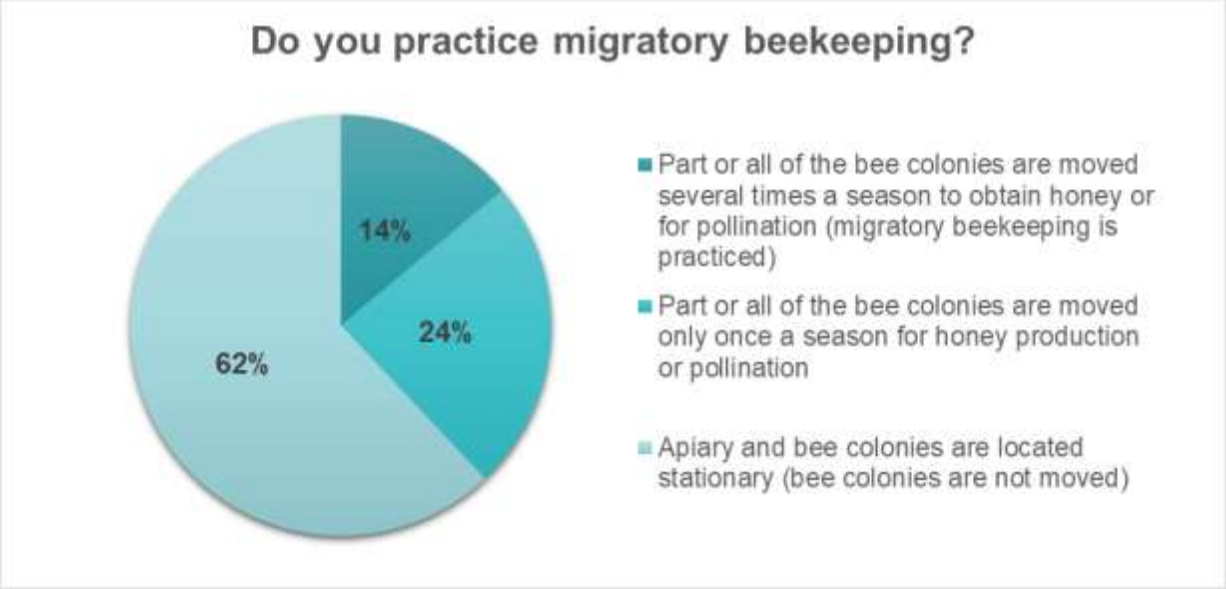

Figure 5. Respondents distribution by the application of migratory approach

These beekeepers are not practicing migratory beekeeping as there is enough foraging resources for the bee colonies in the apiary location (65,3%). But 75 persons are mentioning that special transport is required to transport the apiary to a remote location (51%). As it is

identified, for many beekeepers beekeeping is only as an additional activity, doing migratory beekeeping is very labor intensive and they do not have time to do this (52,4%). Some of the beekeepers (count: 30 (20,4%)) think that transporting the colonies can cause an additional stress for the bees and even doing migratory beekeeping can increase the bee colony risk of disease. But looking at migratory beekeeping from the economic perspective, only 9 beekeepers (6,1 %) assume that this activity is not economically feasible (Figure 6). At the end 43 % of the stationary beekeepers are willing to try the migratory approach in the future.

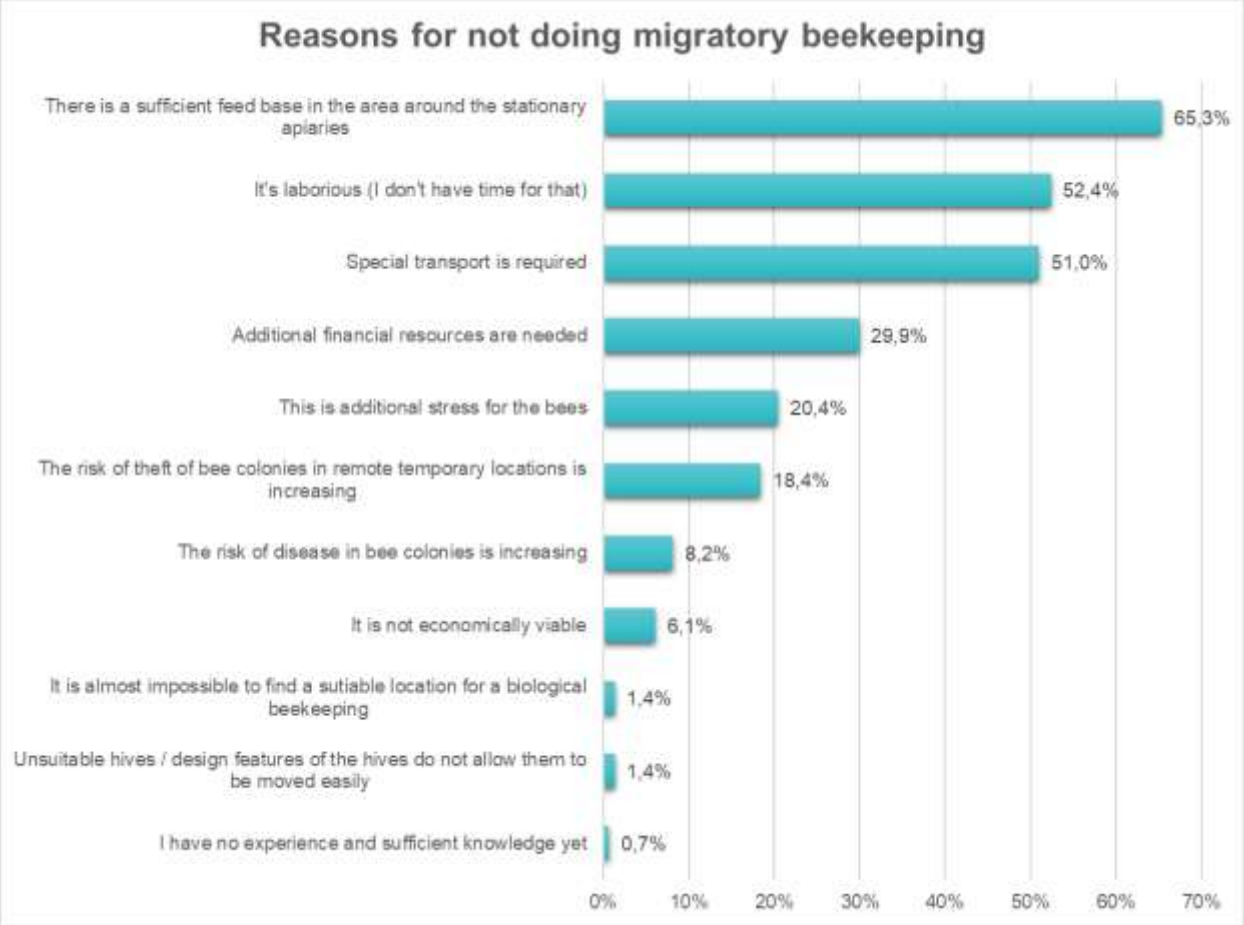

Figure 6. Reasons for not doing migratory approach, which are mentioned by the beekeepers

Next section of beekeepers (count: 57 (24 %)) one time per season are transporting whole or part of the apiary to one remote location for the pollination service or for the honey yield increase. Majority (84 %) transport only part of the bee colonies, thus leaving one part in a stationary location usually near own living place. Looking at the target crops, where colonies are transported the list is very diverse. Some of the target crops or locations are: heather (22.8 %), buckwheat (17.5 %), forest (15.8 %), linden trees (12.3 %), phacelia (10.5 %), etc. (Figure 7). By analysing the transportation distance, it can be concluded that most of the beekeepers (Count: 39) transport the colonies to a close remote location up to 30 km, but 12 persons transport from 30 - 60 km. And then there are some individuals, who transports apiary to more distant locations up to 120 km. Some of the beekeepers (Count: 11 (19%)) are providing the pollination service to farmers and this is the main reason for them to transport the bee colonies.

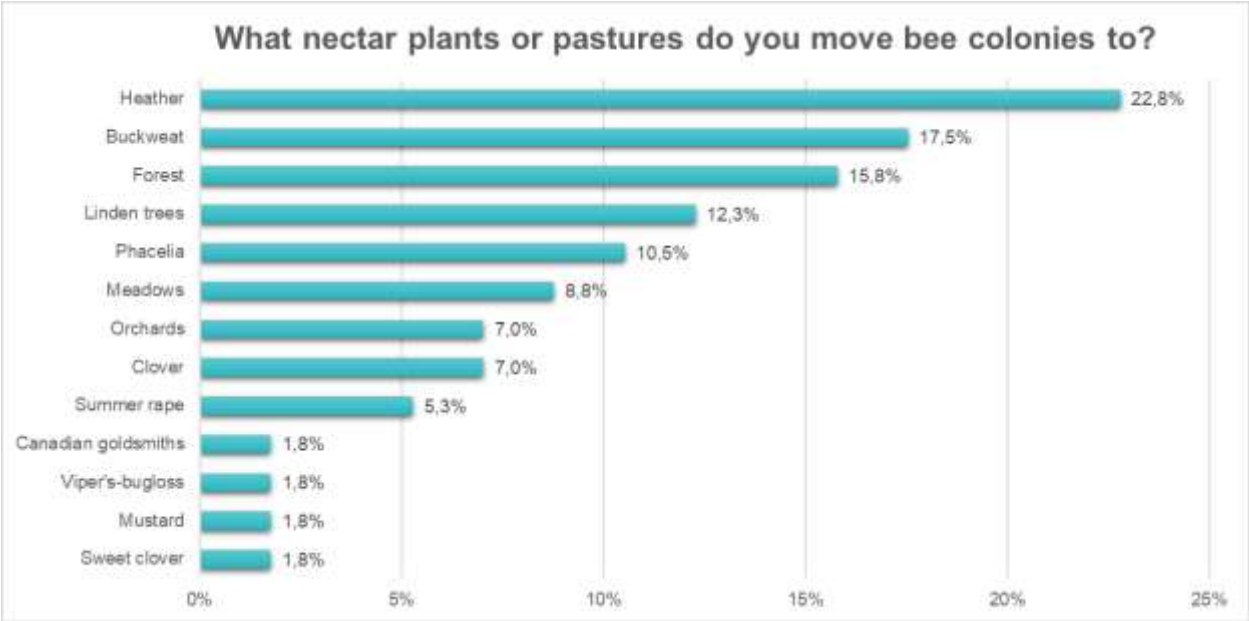

Figure 7. Most important plants and pastures where bee colonies are moved to

The smallest part of the beekeepers (count: 33 (14 %)) several times per season migrate the apiary from one location to another, thus practicing the migratory beekeeping approach. And similar to the previous group of beekeepers only small part 30.3 % migrate the whole apiary. Analysing the target crops or locations the most visited are: heather (75.8 %), linden trees (63.6 %), winter rape (57.6 %), buckwheat (54.5 %), forest (30.3 %), orchards (18.2 %) (Figure 8). But the traveled distance for the migratory beekeepers are higher than for the previous group of the beekeepers. 19 of migratory beekeepers travel less than 100 km, but others more, and there is even some individuals traveling 400 km and more. The main part (count: 16) of the migratory beekeepers change the location 2 times per season, then 11 migratory beekeepers change 3 times and there are some individuals which change the locations more than 3 times per season. Almost half of the migratory beekeepers (count: 16 (48%)) are providing the pollination service to farmers.

For the beekeepers the main motivation (count: 29 (88%)) for practicing the migratory beekeeping is to diversify the assortment of honey. Other important reasons are: to provide a food base for bee colonies throughout the season (count: 21 (64%)), to get a higher honey yield (count: 21 (64%)). It is surprising that only 9 beekeepers selected an option, that it is economically feasible to do a migratory beekeeping.

It is clear that practicing the migratory beekeeping asks for additional workload and effort and there should be some challenges of doing this. Some of the main challenges mentioned by the migratory beekeepers are: there is no information about nearby nectar plants, transportation and logistics, finding a suitable remote location, lifting of the bee hives.

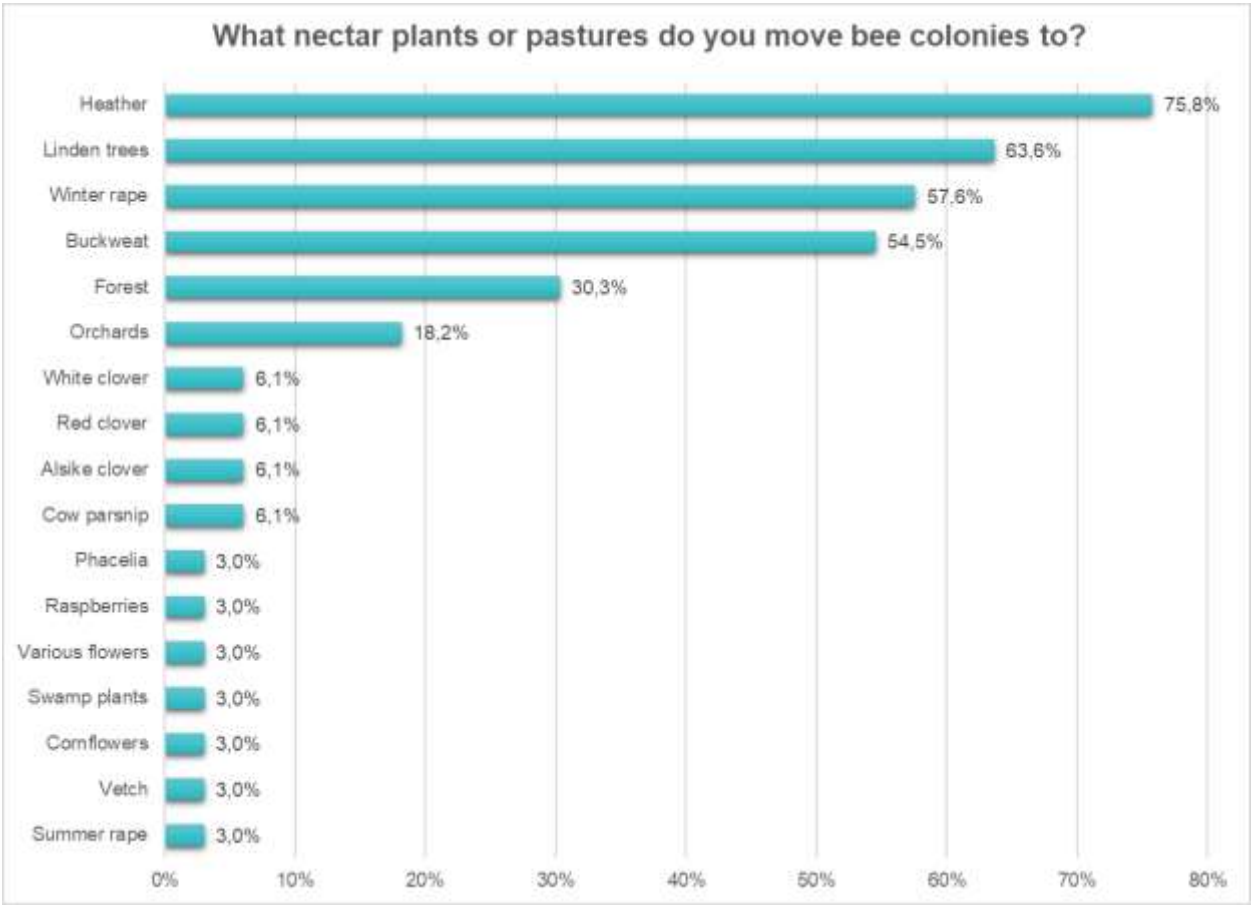

Figure 8. Most important plants and pastures where bee colonies are moved by the migratory beekeepers

As it can be concluded from the completed survey migratory beekeeping is not popular in Latvia, as only 14% of surveyed beekeepers practice this approach. Authors can explain it by several factors. Latvia is a small country, and beekeeping is common almost in whole territory, meaning that it is difficult to find free remote locations to place the apiary. As well in Latvia there are small number of non-hobbyist beekeepers with a number of bee colonies, that exceeds 100 colonies. In 2022 there were only 3.6 % of such beekeepers. And for hobbyist beekeepers, as mentioned in the survey there is enough foraging resources around the stationary located apiaries, thus there is no need to migrate the apiary to different remote locations. Migratory beekeeping in Latvia is more related to the transportation of bee colonies to specific yields. For example, the density of bee colonies in heather pastures is significantly higher.

Migratory beekeeping is also connected with the popularity and establishment of the pollination service in the countries. As it is seen in USA, where pollination for instance of almonds is very common and almond growers are seeking for the beekeepers for pollination purposes. Another example, which can be mentioned is Denmark, where portal https://bestoeverportalen.dk/

is established to ensure better contact between beekeepers and farmers who need pollination of their crops. In case of Latvia there is no pollination system developed and farmers are not forced to search for the beekeepers, rather the opposite situation is observed, when beekeepers are searching for fields where to place their apiaries.

Within the completed survey some beekeepers indicated as a reason for not doing migratory beekeeping the limited opportunities for finding pesticide free fields for the biological beekeeping. This emphasises the necessity to open the data about biological agricultural fields and provide this information for biological beekeepers to ease the process of finding of suitable apiary locations. In Latvia there is a GIS system available https://karte.lad.gov.lv/ where it is possible to search for biological fields, but still, there are some limitations for beekeepers doing biological beekeeping, like required distance from other fields, where intensive agriculture methods are applied.

Conclusions

In Latvia migratory approach is not very common and only small number of beekeepers are practicing it. The main reasons for this: enough foraging resources for the bee colonies in the apiary location and additional effort and time resources are required.
Main reason to use the migratory approach for the Latvian beekeepers is to diversify the assortment of honey.
In other countries migratory approach is used by the beekeepers to provide a pollination service for the farmers, but in Latvia this approach also is not very common, as farmers are not very actively requesting the pollination service for their crops and there is no separate system to easily connect farmers with the beekeepers.
Still in Latvia nectar crops are flowering in different periods of the active summer period and there is a possibility to increase the honey yield of the honeybee colonies by changing the foraging places, when nectar flow is finished in one location it potentially could be started in another location.

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ASSESSMENT OF THE MICROBIOLOGICAL STATUS OF DRINKING WATER ON ANIMAL FARMS

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Abstract

The experiment used drinking water originating from animal farms from Republic of Srpska (Bosnia and Herzegovina) sampled in 2022. A total of 251 samples were examined, out of which 23.11% originated from the water supply system, and 76.89% from wells. The aim of study is to determine the microbiological status of drinking water from animal farms. For microbiological testing used methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1. The research determined 54.18% satisfactory samples and 45.82% unsatisfactory samples. Analyzing the results of satisfactory samples in relation to the origin, 87.93% of the satisfactory samples were from the water supply system, and 44.04% were from wells. In relation to the tested parameters, 26.69% of samples were due to an increased number of total count of bacteria cultivated at 22°C and 31.87% of samples were due to an increased number of total count of bacteria cultivated at 37°C. When it comes to pathogens, 26.29% of the samples were due to the presence of intestinal enterococci, 27.49% due to the presence of *Escherichia coli* and 28.29% due to the presence of coliform. The microbiological status of water in animal farms is of concern, as almost half of the samples are unsatisfactory. This is particularly significant considering the fact that over three quarters of farms are supplied with water from wells, which are not under constant sanitary supervision. High microbiological risk is of intestinal enterococci, coliforms and *Escherichia coli*, which were found in more than one quarter of the samples.

Keywords: *drinking water, animal, farms, microbiology.*

Introduction

Water is essential for life, and a satisfactory (adequate, safe and accessible) supply must be available to all. Improving access to safe drinking-water can result in tangible benefits to health. Therefore, every effort should be made to achieve a drinking-water quality that is as high as possible (WHO, 2008). Water plays a crucial role in the livelihood and well-being of rural communities. In rural areas, in addition to basic human needs, water is also used for other livelihood productive activities such as livestock drinking, among others (Makoni *et al*., 2004).

The microbiological quality of drinking water for livestock is often neglected (Beede, 2012). The suitability of water for human or livestock consumption depends on the microbiological and/or physicochemical properties of water (Schlink *et al*., 2010; WHO, 2011; Beede, 2012). Low microbiological quality of water is associated with contamination of water sources with human or animal excreta (WHO, 2011). The concern of low quality water is due to its negative health impacts or the reduction in the palatability of water for humans or livestock consumption (Willms *et al*., 2002; WHO, 2011).

Some studies, however, revealed water as an important vehicle for the transmission of various clinically important pathogens such as *Salmonella, Cryptosporidium* and *Eimeria*, urging to

ensure good microbiological quality of water offered to farm animals (Bolton *et al*., 2012; Mitchell *et al*., 2012). Specifically, waterborne pathogens bear problems for young animals (Mitchell *et al*., 2012).

Another possible adverse health effect of contaminated water by faecal matter for animals is associated with algal overgrowth in water bodies (Beede, 2005). In the presence of faecal contamination and high organic matter, toxic blue-green algae can grow specifically in stagnant water.

The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard (EU, 2020). *E. coli*, intestinal enterococci, coliform bacteria and colony count on 22°C shall be monitored in accordance with the monitoring frequencies. *E. coli* and intestinal enterococci are considered "core parameters".

In Republic of Srpska (Bosnia and Herzegovina) the limit value for total count on 22°C (TC 22°C) is 100 CFU/ml and for total count on 37°C (TC 37°C) the limit is 20 CFU/ml. Also, coliform bacteria (CB), *Escherichia coli (E. coli)* and intestinal enterococci (EC) must not be detectable in 100 ml sample of water (Official Gazette, 2017).

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites capable of surviving for different periods, and raise the health risk for many people throughout the world. Monitoring of water sources involves the determination of important microbiological and physico-chemical parameters which indicate first of all potential organic pollution, particularly pollution originating from animal excrement, storage of waste, natural and artificial fertilisers, and others (Sasakova *et al*., 2013; Fridrich *et al*., 2014).

E. coli is a coliform bacteria and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. *E. coli* occurs in the faeces of all mammals, often in high numbers (up to 10^9 per gram of faeces) (Edberg, 2000; WHO, 2008). Enterococci include a number of species that occur in the faeces of humans and warmblooded animals (WHO, 2008).

The health benefits associated with improvements in water supply depend on the quality of water at the source and the point-of-consumption (Oswald *et al*., 2007). Children, women, immunocompromised individuals, and rural residents are considered to be at the highest risk of contracting waterborne pathogenic microorganisms (Obi *et al*., 2006). People can become infected by waterborne pathogenic agents, if they either consume contaminated water directly or indirectly through its use in food production, processing, or preparation (Kirby *et al*., 2003). Hruskova *et al.* (2016) concluded that weather (precipitation) most likely affected the quality of water on farms and was associated with some risk to animals consuming this water, as indicated by the presence of total coliform bacteria in examined samples.

In a study that included microbiological analysis of water on farms in the Republic of Srpska, Kalaba *et al*. (2015) found that 62.66% of water samples from animal farms were unsatisfactory and that the most common cause of water malfunction was enterococci, *E. coli* and total count. Kalaba *et al*. (2020) found that 26.20% of water samples from animal farms were unsatisfactory in the Republic of Srpska for the period 2015-2017. Also, they found that there were 63.40% of unsatisfactory samples due to the increased total count on 22°C and 54.90% due to the increased total count on 37°C, 58.80% due to the presence of intestinal enterococci, 31.40% due to coliform and 19% because *E. coli*. The lowest risk of the presence of coliforms and *E. coli* is in water supply system, and it is significantly higher in well water system.

In Croatia, two studies were conducted in which drinking water was analyzed from different farms (chicken broiler and laying hen farms, cattle and swine farms), where 40% of unsatisfactory samples was found (Denžić Lugomer *et al*., 2019) i.e. 20% (Kiš *et al*., 2017).

The aim of study is to determine the microbiological status of drinking water from animal farms.

Material and methods

The experiment used drinking water originating from animal farms from Republic of Srpska (Bosnia and Herzegovina) sampled in 2022. A total of 251 samples were examined, out of which 23.11% originated from the water supply system, and 76.89% from wells.
Microbiological examination was carried out according to the Official Gazette (2017). This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C and 37°C according to BAS EN ISO 6222 (ISBIH, 2003a), intestinal enterococci according to BAS EN ISO 7899-2 (ISBIH, 2003b), coliform bacteria and *E. coli* according to BAS EN ISO 9308-1 (ISBIH, 2018).

Results and discussion

The results of the study of LeJeune *et al*. (2001) demonstrate that drinking water offered to cattle is often of poor microbiological quality. Water troughs are a major source of exposure of cattle to enteric bacteria, including a number of foodborne pathogens, and this degree of bacterial contamination appeared to be associated with potentially controllable factors.
When it comes to the representation of samples in relation to the category, 23.11% of samples was from water supply system and 76.89% from wells.
Figure 1. shows the regional representation of samples in % for the observed period, and Figure 2. shows the regional representation of samples in % in relation to the category.

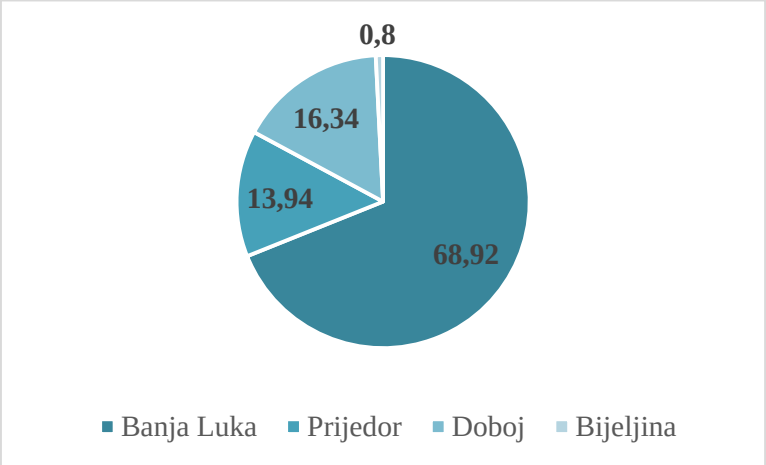

Figure 1. Regional representation of samples in % for the observed period

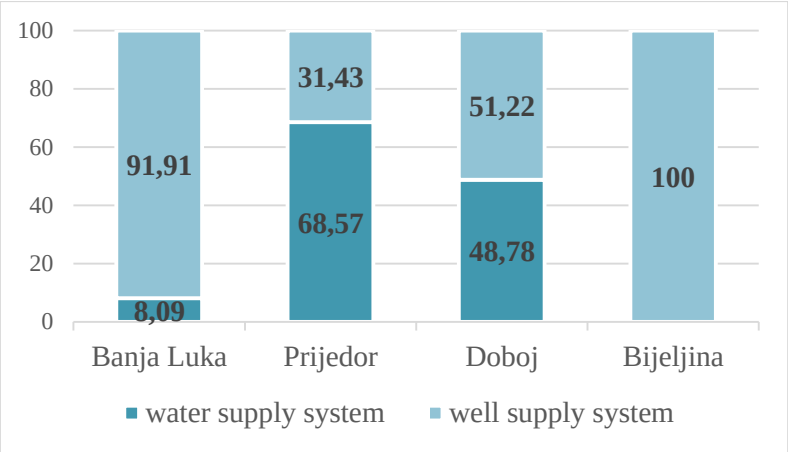

Figure 2. Regional representation of samples in % in relation to the category.

The territorial and economic organization of the Republic of Srpska is conditionally created at the level of six regions: Banja Luka, Prijedor, Doboj, Bijeljina, East Sarajevo and Trebinje (MSPCE, 2015). The distribution of water is such that it is not enough where it is most needed (in the northern, most developed part of Republic of Srpska), and flows are most scarce during periods of the year when needs are greatest and when water quality protection problems are most serious. Analysis of the testing samples taking into account the region and category indicates that most samples come from three regions (Banja Luka, Prijedor, Doboj). The analyzed number of samples originating from the region of Bijeljina is negligibly small, so it was left out of the discussion, while none of the samples analyzed from the region of East Sarajevo and Trebinje.

Of the total number of tested samples, 54.18% were satisfactory, and 45.82% were unsatisfactory.

There are large differences between water and quality control systems for water destined for human or animal consumption (Van Eenige *et al*., 2013). For example, testing for microorganisms occurs less frequently in cattle systems; substrates for bacteria are often present in cattle systems; few cattle systems are screened for faecal contamination, even though this is a major source of contaminants; in many cattle systems water can flow in more than one direction, which is not the case for human water systems; the existence and implementation of cleaning and disinfection protocols are poor in cattle systems and biofilms are more often present in the pipelines of cattle systems. The latter phenomenon often leads to the presence of many different bacteria in cattle drinking systems.

Table 1. shows the test results by the category.

Table 1. Test results by category in %

Year	Satisfactory	Unsatisfactory
Water supply system	87.93	12.07
Well supply system	44.04	55.96

The obtained results for all samples and for water supply system differ significantly and are better than results of Golić *et al*. (2021b) and Kalaba *et al.* (2020), but they are less favorable by well supply system. The obtained results also are better than results Kalaba *et al*. (2015) for water supply system, who state that 30.50% of samples originating from water supply system are unsatisfactory, and for well supply system where is 76.72% of samples are unsatisfactory.

Table 2. shows the test results by region in %, and Table 3. shows the results by region in % in relation to categories.

Table 2. Test results by region in %

Region	Satisfactory	Unsatisfactory
Banja Luka	45.09	54.91
Prijedor	74.29	25.71
Doboj	75.61	24.39
Bijeljina	50.00	50.00

The obtained results are in accordance with the results of Kalaba *et al*. (2015) and Golić *et al*. (2021b), who found that the largest number of unsatisfactory samples were from the region Prijedor and Banja Luka.

Table 3. Test results by region in % in relation to categories

Region	Water supply system		Well supply system	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
Banja Luka	85.71	14.29	44.51	58.49
Prijedor	87.50	12.50	45.44	54.55
Doboj	90.00	10.00	61.90	38.10
Bijeljina	-	-	50.00	50.00

The obtained results are in accordance with the results of similar studies (Denžić Lugomer *et al*., 2019; Kiš *et al*., 2017; Kalaba *et al*., 2015; Golić *et al*., 2021b). All types of water sources may be subjected to contamination by agricultural activity. Freerange animals may excrete faeces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of faecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The potential source of coliform bacteria in water supplies result from sub-optimal operation of water treatment processes or ingress of contamination from breaches in the integrity of the distribution system. Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognised sources of these organisms.
Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well water supplies one or fewer farms, is not under constant control but very rarely, most often once a year as an official control, or in the event of an animal health incident. Also, well waters are not flowing but stagnant, so the microbiological status of this water is greatly influenced by the number of animals drinking from the well, i.e. the speed and amount of water consumption from the well.
Table 4. show unsatisfactory results in % according to test parameter.

Table 4. Unsatisfactory test results in % according to test parameter

Test parameter				
TC 22°C /ml	TC 37°C /ml	EC /100ml	*E. coli* /100ml	CB /100ml
29.69	31.87	26.29	27.49	28.29

Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies (WHO, 2008; EA, 1998). The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. The heterotrophic plate count includes all of the microorganisms that are capable of growing in or on a nutrient-rich solid agar. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources. The test results indicate that the causes of microbiological water malfunction mostly come from animals, having in mind that the largest number of unsatisfactory samples is due to the increased total count at 37°C, but we should not ignore the fact of the possibility of contamination from the environment due to the increased total count at 22°C. Contamination from animals is very possible due to inadequate drainage of waste and fecal water and consequent contamination of groundwater, because wells are usually not planned and are mostly located near farms.

The obtained results are in accordance with the results Kalaba *et al*. (2015), Kalaba *et al*. (2020) and Golić *et al*. (2021a) and indicate significant fecal contamination of water, especially with *E. coli* and coliforms. In relation to the results of Golić *et al*. (2021a) and Golić *et al*. (2021b) for the period 2018-2020, there was a significant deterioration in terms of *E. coli* and coliforms. The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Official Gazette, 2017). A possible explanation for this is dilapidation and damage to water supply installations in farms leading to water contamination. This is in line with observations Interact (2006), which indicate that it is the microbiological quality of drinking water for cattle diminishes once the water has entered the farm. A field survey in the Netherlands has pointed out that at the entry point (130 dairy farms; 285 samples) 98% of water was of suitable microbiological quality, but at the end point (199 samples) this percentage had dropped to 60%, so that 40% of the water samples were unsuitable as drinking water for cattle.

Compared to the period 2015-2017 (Kalaba *et al*., 2015; Kalaba *et al*., 2020), the level of pathogenic bacteria in drinking water is reduced, especially for intestinal enterococci and coliforms, as well as the total number of microorganisms at 22°C and 37°C. The obtained results indicate a significantly improved microbiological status of drinking water compared to the results Jaki *et al*. (2010) and Denžić *et. al.* (2012), and they are in accordance with the results Denžić *et al*. (2013).

Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well water supplies one or fewer farms, is not under constant control but very rarely, most often once a year as an official control, or in the event of an animal health incident. Also, well waters are not flowing but stagnant, so the microbiological status of this water is greatly influenced by the number of animals drinking from the well, i.e. the speed and amount of water consumption from the well.

Conclusion

The microbiological status of water in animal farms is of concern, as almost half of the samples are unsatisfactory. This is particularly significant considering the fact that over three quarters of farms are supplied with water from wells, which are not under constant sanitary supervision. High microbiological risk is of intestinal enterococci, coliforms and *Escherichia coli*, which were found in more than one quarter of the samples.

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NUTRITIONAL VALUES OF DOMINANT FESTUCA SPECIES IN SANDY GRASSLANDS ALONG THE DANUBE BASED ON A SYSTEMATIC SAMPLING

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Abstract

Extensive animal husbandry has a long tradition in Hungary. The most valuable feed for livestock is provided by plants on grassland. Supplementing the feed rations with fibre improves the digestive saturation, making the animals calmer and enhancing animal welfare. Grasses are especially important because they contain a lot of digestible fibre. Therefore, in the present study, our objective was to analyse the cut samples of six dominant *Festuca* species (*Festuca vagianta, Festuca pseudovaginata, Festuca wagneri, Festuca tomanii, Festuca pseudovina, Festuca rupicola*) from Pannonian sand grasslands along the Danube which were collected every two weeks (15 April - 30 May) to find out their nutritional values during the period. Based on the results of this analysis, we could conclude what type of grazing animal, what period of the year and what vegetation type in the sample area are the most suitable for grazing. Various nutritional elements were analysed for example content of different mineral materials, crude protein, crude fat, crude fibre, sugar and selenium. As a reference, we considered the ideal nutrient input of 600 kg non-pregnant beef cow, 500 kg horse and 50 kg non-pregnant ewe. The results were compared with the optimal nutritional requirements of these animals. Based on the comparative statistical results, it was observed that mainly the *Festuca pseudovina* specimens that were collected at the end of April had the closest rank values to the theoretical optimum, considering the requirements of all three animals.

Keywords: *animal husbandry, fescue, sandy grassland, grazing, grasses.*

Introduction

Nowadays, conservation of biodiversity of the preserved semi-natural habitats has become a very important global issue (Jones *et al*., 2018). Across Europe, species diversity in pastures and grasslands is declining, according to some observations, because intensive agricultural technology is transforming natural habitats (Walker *et al*., 2009). The decline in biodiversity is caused mainly by anthropogenic impacts, or it can result from improper grassland management and abandonment of grassland management (Fischer and Stöcklin, 1997; Bischoff *et al*., 2005). Grazing pressure is important for pastures, but overgrazing or complete abandonment can also lead to decreased biodiversity (Guo 2007; Antal and Juhász, 2008; Cornwell and Grubb, 2003; Gillman and Wright, 2006; Mittelbach *et al*., 2001). The European Union has also set a target to improve degraded ecosystem condition (Palmer *et al*., 2016), and pastures provide important ecosystem services in terms of regulatory, cultural and supply functions (Millennium Ecosystem Assessment, 2005). In addition, pastures have an outstanding role in agriculture as well as in the conservation of wild flora and fauna. In order

to ensure the proper quality of animals for food processing, optimal feeding is essential resulting in an increasing role for extensive grazing (Török *et al*., 2018).
According to McNaughton (1979), moderate grazing can double the production of grasslands, although intensive grazing already causes strong selection effects on the species composition of the vegetation. These types of low disturbances are often necessary in grasslands because they have a positive influence on species diversity. However, if they are not implemented, scrub encroachment can occur (Morris, 2000). To sustain grasslands, regular removal of biomass is important, which can be carried out by mowing or grazing. Grazing is practised in many regions with different animals, such as sheep, goats, cattle or buffaloes (Fenu *et al*., 2022; Póti *et al*., 2007; Yayota *et al*., 2020; Fűrész *et al*., 2023; Catorci *et al*., 2017; Centeri *et al*., 2009; Czóbel *et al*., 2012).
The most characteristic species of grasslands are *Festuca* species, which are often dominant in plant associations within the Pannonian biogeographical region (Borhidi *et al*., 2012). In addition to their role as competitors, their ability to survive in habitats that are already too extreme for most plant species is a significant feature (Penksza *et al*., 2021a, 2021b). Fescues with thin leaves occur in grasslands with poor productivity, but despite this, their relevance is an important aspect of their utility in dry habitats in the context of climate change. Fescues can be a suitable supplement, because they contain a lot of digestible fibre (Fűrész *et al*., 2022).
Crude fibre is a chemical composition of residues that are generated after cooking in dilute acid and alkali. Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and the group of non-starch polysaccharides (NSP), which have an important role, and their component polymers can be defined (Halász *et al*., 2022). Introducing fibre into the feed rations enhances the saturation of the digestive system, which makes the animals calmer and thus increases animal welfare.
In the current work, our aim was to analyse samples of different dominant *Festuca* species from Pannonian sand grasslands collected every two weeks in order to find out their nutritional value during the period.

Materials and methods

Sample collection
In the present study, authors collected six native *Festuca* species systematically. The studied species were collected from the following sample areas: *Festuca vagianta* (Vácrátót, Tece legelő), *Festuca pseudovaginata* (Vácrátót, Tece pasture), *Festuca wagneri* (Kunpeszér), *Festuca tomanii* (Újpest Homoktövis Természetvédelmi Terület/Újpest Sea-buckthorn Conservation Area), *Festuca pseudovina* (MATE Botanical Garden of Gödöllő), *Festuca rupicola* (Vácrátót).

Nutritional values analysis
The collected specimens were analysed for different nutritional parameters. The analysis of the cut samples was carried out at the Hungarian University of Agriculture and Life Sciences Laboratory Centre of the University according to different criteria and standards.
The studied parameters the applied criteria: crude protein content (MSZ EN ISO 5983-2:2009 (Kjeldahl-method), crude fat content (MSZ 6830-19:1979 (withdrawn standard), crude fibre content (152/2009/EK III/I), crude ash content (MSZ ISO 5984:1992 (withdrawn standard), calcium content (MSZ EN ISO 6869:2001), phosphorus content (MSZ EN ISO 6869:2001), magnesium content (MSZ EN ISO 6869:2001), kalium content (MSZ EN ISO 6869:2001), sodium content (MSZ EN ISO 6869:2001), manganese content (MSZ EN ISO 6869:2001), copper content (MSZ EN ISO 6869:2001), zinc content (MSZ EN ISO 6869:2001), iron

content (MSZ EN ISO 6869:2001), selenium content (MSZ 21470-50:2006), NDF content (MTK-1990.II.8.2), ADF content (MTK-1990.II.8.2.), ADL content (MTK-1990.II.8.2.), cellulose content (MTK-1990.II.8.2.), hemicellulose content (MTK-1990.II.8.2.), total sugars content (MSZ 6830-26:1987).

Statistical analysis

Preparation and database evaluation were carried out using the Microsoft Office Excel 2007 XL-STAT statistical software package (Addinsoft XLSTAT, 2016), using the rank sum of differences method (Héberger and Kollár-Hunek, 2011) for comparative multicriteria analyses. The method was based on the closeness of the evaluated data to the reference SRD value. In this study, the theoretical best type values of a 600 kg non-pregnant beef cattle, a 500 kg horse and a 50 kg non-pregnant ewe (Schmidt 1993; Schmidt, 2000) were the optimal reference requirements for the comparison of the nutritional values of *Festuca* species (Table 1).

Table 1: Studied parameters with theorical optimal values

Standard	**Parameters**	**Beef cattle (600 kg, not pregnant)**	**Horse (500 kg)**	**Ewe (50 kg, not pregnant)**	**Unit**
MSZ EN ISO 5983-2:2009 (Kjeldahl-method)	Crude protein	698	414	99	g/kg
MSZ 6830-19:1979 (withdrawn standard)	Crude fat	20	20	20	g/kg
152/2009/EK III/I	Crude fibre	200	90	200	g/kg
MSZ ISO 5984:1992 (withdrawn standard)	Crude ash	90	90	90	g/kg
MSZ EN ISO 6869:2001	Calcium	3,7	3,1	2,2	g/kg
MSZ EN ISO 6491:2001	Phosphorus	2,6	1,9	1,7	g/kg
MSZ EN ISO 6869:2001	Magnesium	1,6	1	1	g/kg
MSZ EN ISO 6869:2001	Kalium	8	4	8	g/kg
MSZ EN ISO 6869:2001	Sodium	1	1,6	1,2	g/kg
MSZ EN ISO 6869:2001	Manganese	0,06	0,04	0,05	g/kg
MSZ EN ISO 6869:2001	Copper	0,008	0,008	0,008	g/kg
MSZ EN ISO 6869:2001	Zinc	0,04	0,04	0,03	g/kg
MSZ EN ISO 6869:2001	Iron	0,05	0,04	0,05	g/kg
MSZ 21470-50:2006	Selenium	0,0	0,0001	0,0001	g/kg
MTK-1990.II.8.2.	NDF	270	270	270	g/kg

MTK-1990.II.8.2.	ADF	200	100	200	g/kg
MTK-1990.II.8.2.	ADL	30	10	30	g/kg
MTK-1990.II.8.2.	Cellulose	70	170	70	g/kg
MTK-1990.II.8.2.	Hemicellulose	170	90	170	g/kg
MSZ 6830-26:1987	Total sugars	100	100	100	g/kg

Results and discussion

In the Figure 1, it can be seen the results of the nutritional values of *Festuca* that were compared to the theoretical optimal requirements of a 50 kg non-pregnant ewe. Based on the results, the most ideal for sheep from the collected samples was *Festuca pseudovaginata* which was collected on 15 April. In addition, *F. wagneri* and *F. pseudovina* also had outstanding values. Moreover, it can be seen that the specimens of the collected species in April would be the preferred ones for the animal.

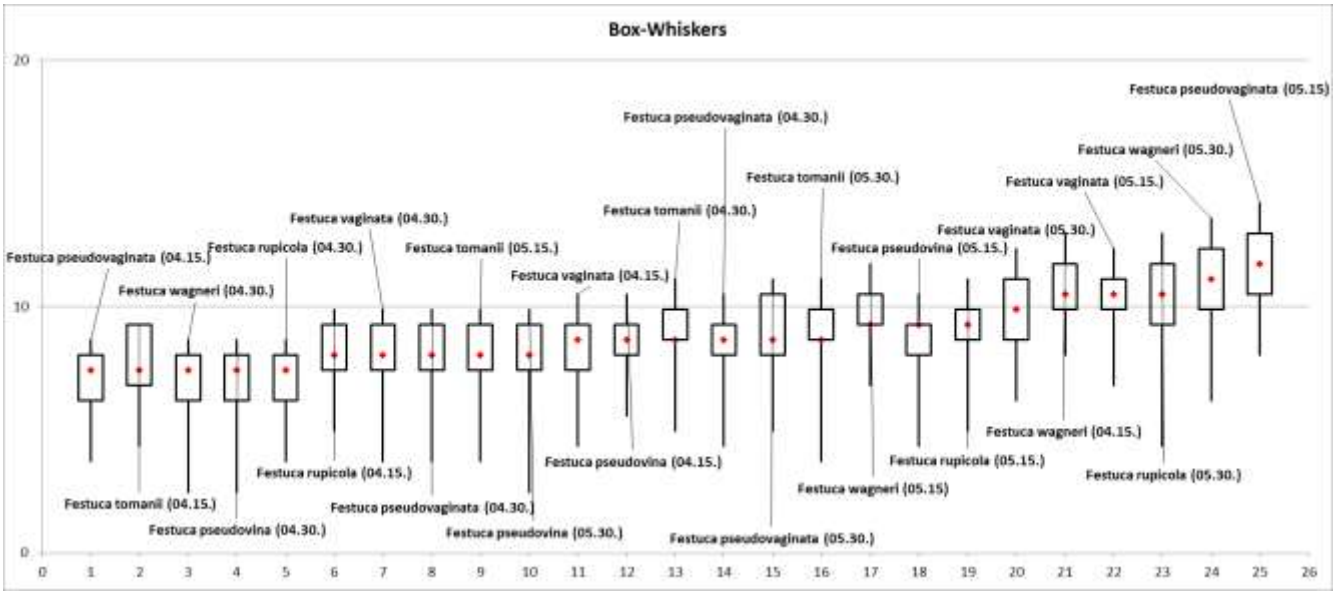

Figure 1. Results of nutritional values of *Festuca* compared to the theoretical optimal requirements of a 50 kg non-pregnant ewe

In the Figure 2, the results of the nutritional values of *Festuca* compared to the theoretical optimal requirements of a 500 kg horse are presented. According to the results, *Festuca pseudovina* which was collected on 30 April was the most ideal for the horse from the collected samples. Furthermore, the specimens of *F. tomanii* and *F. vaginata* also demonstrated high values. In addition, it can be seen that specimens collected in April would be ideal, although in this case several specimens collected in May were ranked higher in comparison to the requirements of the sheep.

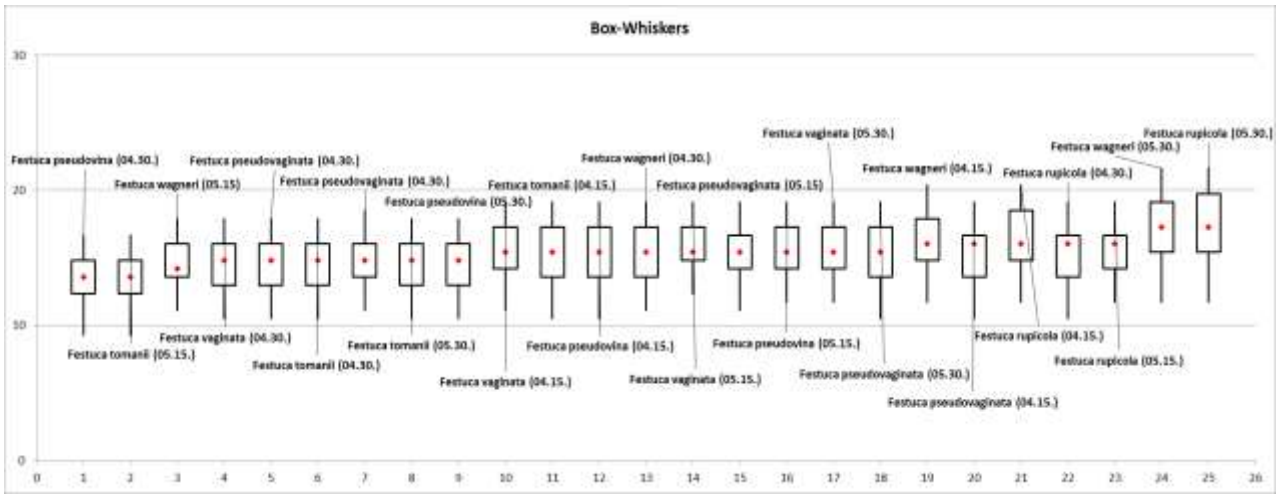

Figure 2. Results of nutritional values of *Festuca* compared to the theoretical optimal requirements of a 500 kg horse

In the Figure 3, it can be seen the results of the nutritional values of *Festuca* that were compared to the theoretical optimal requirements of a 600 kg beef cattle. Based on the results, the most ideal for beef cattle from the collected samples was *Festuca pseudovina* which was collected on 30 April. In addition, *F. pseudovaginata* and *F. vaginata* also had outstanding values. Moreover, it can be seen that the specimens of the collected species in April would be the preferred ones for the animal, similarly to the requirements of the sheep.

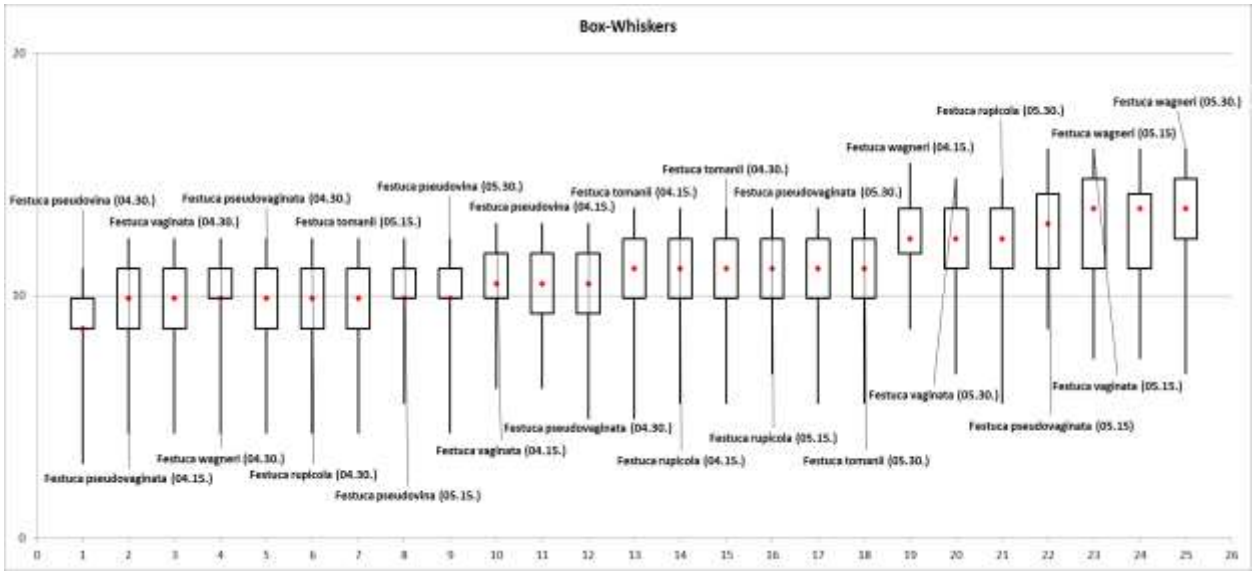

Figure 3. Results of nutritional values of *Festuca* compared to the theoretical optimal requirements of a 600 kg beef cattle

Conclusion

Based on the results, the effect was mainly seasonal rather than species-specific. Consequently, according to the analysed data, the second half of April might be the most optimal period for grazing on grasslands dominated by *Festuca* for beef cattle, sheep and horses. Overall, the presented results of the statistical analyses suggest that *Festuca*

pseudovina may be the most optimal for all three grazing animals in terms of the nutritional values.

However, the obtained results are not applicable in practice yet, because there was only one recorded study season. Moreover, the obtained values were compared to theoretical optimal values, which may require further review to be able to be confident that the optimal values are representative. In addition, it might give a more relevant picture of the optimal species and time period if nutritional parameters were weighted during statistical evaluations.

Considering the results of this research, it may be useful to carry on the work by widening the criteria to other types of grazing livestock. Finally, it may be worth examining the nutritional values over a broader period of time, which would be monitored over several years, to get a more representative picture of the changes in the nutritional values.

Acknowledgments

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THE INFLUENCE OF AGE ON REPRODUCTIVE CHARACTERISTIC IN SIMMENTAL COWS

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Abstract

The objective of this study is to investigate how the age of Simmental breed heifers affects their reproductive characteristics. Research was conducted on a farm of dairy cows TC "Zona" Podromanija Sokolac, Bosnia and Herzegovina. The main objective was to determine age of heifers (cows) at first, second and third insemination (gestation), length of gestation, lenght of service period, length of calving interval, weight of calves at birth. Special emphasis was placed on determining the strength and significance of the correlation relationship by calculating the correlation coefficient between the age the heifers (cows) at the first, second and third insemination, the length of the service period, the inter-calving interval, the weight of calves and the length of gestation. Based on the presented results and discussions, along with the findings of various individual authors in their respective research, it can be concluded that cows on the farm TC "Zona" Podromanija, Sokolac, Bosnia and Herzegovina were bred with a selection plan aimed at enhancing both milk production and herd reproduction, with partial success.

Key words: *heifer, gestation, inter-calving interval, milk production*

Introduction

In intensive beef cattle production, a characteristic feature is that it is built around the animals' life cycle. What it primarily relies on is increasing the birth rate index, the number of calves at one birth and during the productive life. Cow reproduction is one of the most crucial elements in beef cattle production. In this phase, head of livestock are being secured to replace the herd, herd expansion, milk production and fattening, as well as meat production. In order to achieve the best possible reproductive results, it is necessary for all stages of reproduction to be well-organized. When involved in the process of organizing reproduction, it is essential to highlight the importance of employing right heifers breeding methods. Under this, special focus should be directed towards the stage of growth, namely, the age of heifers at first mating and calving interval, respectively, period between two calvings in order to get one calf per cow per year. In this manner, effective reproductive outcomes are attainted, which facilitate longer productive life of milk-production cows and a higher number of calvings (calves), and, consequently, higher income through the sale of milk and calves, or meat in case of fattening specific number of head of livestock. The objective of the study is to investigate how the age of heifers affects their reproductive characteristics of heifers (cows), Simmental breed. Research was conducted on a farm of dairy cows TC „Zona" Podromanija Sokolac, Bosnia and Herzegovina. The main objective was to determine age of heifers (cows) at first, second and third insemination (gestation), length of gestation, length of service period, duration of calving interval, weight of calves at birth. Special emphasis was placed on determining the strength and significance of the correlation relationship (r_p) by calculating the correlation coefficient between the age the heifers (cows) at the first, second and third

insemination, the lenght of the service period, the intercalving interval, the weight of calves and the length of gestation.This way, the cow reproductive capability in first three lactations, on a farm TC “Zona” Podromanija, Sokolac, will be more realistically observed, highlighting potential improvements in the production process.

Materials and methods

Examinations of Simmental breed cow’s reproductive characteristics were carried out within the confines of the farm TC “Zona” Podromanija, Republic of Srpska, Bosnia and Herzegovina. The farm TC “Zona” is located in the municipitaly of Sokolac, specifically within Glasinačko polje (eng. Glasinac field). The Simmental breed exhibits longevity and exceptional adaptability to varying climates, ground, and levels of utilization. Fully grown Simmental breed cows, which have been around 5 years, can weigh around 600 kg and 700 kg. Its color ranges from yellow to red embellished with white patterns, legs and tail are white with pigment fields. The fur is soft, skin is moderately thick and partially pigmented. In accordance with weather conditions, categories and productivity, cultivation practices involve the application of either tethered holding or open holding system. The planned technology for accommodation, healthcare, and nutrition of different categories of cattle (youth, heifers, gestation, dairy and milk cows, as well as fattening of calves) depends on the mentioned conditions. Within the confines of the farm, there are suitable additional structures located next to the facilities designed for housing specific categories of cattle, as well as appropriate machinery. On the farm, meticulous records and documentation for all head of livestock are maintained. The heifers (cows) are marked and identified. Each head of livestock posseses its own pedigree, complete with registration number, date of birth, date of each insemination, date of each calving, length of gestation. On a farm TC “Zona”, Podromanija, Sokolac, the insemination of cows is conducted through artificial methods. In the light of the fact that detailed, consistent and regular documentation regarding the reproductive attributes was accessible for 78 cows, these particular specimens were chosen as the subject for the research. Each cow calved on three separate occasions. Based on the data from inventory and production records, the age of heifer (A.H.) has been determined, namely the age of cows (A.C.) during the first, second and third insemination, the length of the first, second and third gestation (L.G.). The descriptive statistical parameters that have been established include: the average value, the standard error of the mean, the standard deviation (Sd), and the coefficient of variation (C.V.%), (Latinović, 1996). During data processing and significance testing of difference between the investigate indicators standard descriptive statistical methods were employed. The t-test was employed to assess sample means with equal variances. Additionally, the calculation of the coefficient of phenotypic correlation between the established parameters was conducted according to the formula suitable for the corresponding sample (Latinović, 1996). Established correlation coefficients are defined according to the Roemer-Orphal classification for the measure of the association of traits (Tavčar,1948, Latinović,1996).

Results and Discussion

Table 1. presents the average valuables and variabilty of age of the heifers (cows) during the insemination.

Table 1. Average valuables and variability of the age of heifers (cows) during the insemination

Insemination	N	$\bar{x}$	S$\bar{x}$	Sd	C.V.%	Min	Max
First	78	16,54	0,16	1,40	8,46	15,01	20,04
Second	78	29,57	0,27	2,39	8,08	27,23	33,90
Third	78	43,12	0,28	2,45	5,68	41,04	46,88

The average age of heifers during their first insemination was 16,54 months. Derived from the dataset, it is evident tha the average age of the cows during their second insemination was 29,57 months, while during their third insemination, it was 43,12 months (Table 1). Accoridng to *Petrović*'s (2000) research findings, heifers underwent their first inseminations sightly sooner, occuring at approximetaly 17 months, as well as in researches of *Petrović et al.* (2005; 2008). On the other hand, *Đurđević*'s (2001) investigation discovered that the avrage age of head of livestock during their first calving was 27,73 months. Table 2. displays the average values and variations concering the length of the first, second and third gestation, incorporating the gender of the calf.

Table 2. Average values and variability of gestation duration (days)/ gender of the calf

Gestation	Gender	n	$\bar{x}$	S$\bar{x}$	Sd	C.V.%	Min	Max
First	♂ ♀	37 41	286,65 286,49	0,40 0,45	2,45 2,91	0,85 1,01	280 280	295 296
	Both genders	78	286,56	0,31	2,69	0,93	280	295
Second	♂ ♀	43 35	286,58 285,60	0,34 0,45	2,25 2,69	0,78 0,94	280 285	290 291
	Both genders	78	286,17	0,28	2,49	0,87	279	291
Third	♂ ♀	35 43	287,34 287,81	0,27 0,34	1,64 2,25	0,57 0,78	285 285	291 297
	Both genders	78	287,60	0,23	2,00	0,69	285	297

Considering that the length of cow gestation remains biologically constant, the length of gestation during the first three calving events displayed significant similarity. However, there was a slight increase observed in the gestations of cows giving birth to male calves compared to those with female offspring, although the distinction was minimal. Regardless of the gender of the calf, the gestation was the longest in the third calving (287.81 days), and the shortest in the second calving (285.60 days). The calculated variations in the length of gestation between the L.G.1-L.G.3 and L.G.2-L.G.3 occurrences demonstrated a remarkably strong level of significance ($P<0,001$), while the difference between the L.G.1-L.G.2 was not statistically confirmed ($P>0,05$), (Table 6). Among Simmental breed of cows, *Petrović et al.* (2007) discovered that gestation persisted for a length of 284,14 days, whereas *Pantelić et al.* (2005) ascertained a length of 285,51 days for the same, which is several days shorter than in this research. Table 3. showcases the average values and variability of the service period between the first calving and the second insemination, the second calving and the third insemination, and the third calving and the third insemination.

Table 3. Average values and variability of the length of service period, (days)

Service period	N	$\bar{x}$	S$\bar{x}$	Sd	C.V.%	Min	Max
First	78	116,53	1,62	14,29	12,26	90	139
Second	78	120,79	1,24	10,98	9,09	98	143
Third	78	111,49	1,21	10,72	9,61	90	132

The second service period (120,79 days) had the longest duration, while the third service period (111,49 days), was of the shortest span (Table 3). *Caput et al.* (1989) indicate that the duration was roughly equivalent to that in this study, with an average of 125 days. The identified differences among the first (-4,26), second (5,07), and third (9,30) periods displayed a significantly high level of statistical importance (P<0,001), (Table 6). Table 4. displays the mean values and variations of the inter-calving intervals between the first and second, as well as between the second and third calvings. The third inter-calving interval is omitted from the presentation due to the fact that the analyzed cows have not undergone their fourth calving yet.

Table 4. Average values and variability of the inter-calving interval, (days)

Inter-calving interval	N	$\bar{x}$	S$\bar{x}$	Sd	C.V.%	Min	Max
First	78	402,78	1,63	14,46	3,59	375	445
Second	78	408,39	1,30	11,52	2,82	387	430

First inter-calving period lasted, on average, for 402,78 days, whereas the second interval was 5,62 days longer (Table 4). According to *Petrujkić and coauthors* (2011), they propose that the inter-calving interval should ideally span around 380 days. If it falls short of 12 months, it serves as an indicator that either lactation has been shortened or the dry period for cows has been reduced. These factors have a negative impact on the herd's reproductive and productive characteristics. The calculated deviation in terms of the inter-calving period's length (-5.61) received statistical confirmation at a significance level of P<0,001, (Table 6). Table 5. shows the average values and variability of body mass at birth.

Table 5. Average values and variability of body mass at birth, (kg)

Calving	Gender	N	$\bar{x}$	S$\bar{x}$	Sd	C.V.%	Min	Max
First	♂	37	38,07	0,12	0,75	0,32	36,9	39,5
	♀	41	37,11	0,11	0,71	1,92	36	38,6
	Both	78	37,75	0,09	0,86	2,28	36,45	39,05
Second	♂	42	37,90	0,10	0,69	1,82	36,4	39,20
	♀	36	37,31	0,11	0,66	1,76	36,2	39,5
	Both	78	37,83	0,08	0,74	1,98	36,3	39,3
Third	♂	35	37,99	0,13	0,81	2,13	37,2	39,7
	♀	43	37,60	0,08	0,54	1,43	36,4	38,4
	Both	78	37,92	0,07	0,70	1,86	36,8	39,05

From the data shown in the Table 5, it's evident that calves of both genders weigh the least during the first calving (37,75 kg), while slightly heavier calves are born during the second (37,83 kg) and third (37,92 kg) calvings. Furthermore, in the first, second, and third calvings, female calves had a lower birth weight compared to male calves. The calculated variances in

birth weight differences were not deemed substantial (P>0,05), (Table 6). Table 6. illustrates the significance of variations in the reproductive characteristics of cows on the farm.

Table 6. Significance of differences in reproductive characteristics

Indicators	N	Average values	Differences	t-test	Significance
$L.G._1$- $L.G._2$	78 -78	286,56-286,17	0,39	1,050	ns
$L.G._1$- $L.G._3$	78 -78	286,56-287,60	-1,04	-2,977	***
$L.G._2$-$L.G._3$	78 -78	286,17-287,60	-1,43	-3,972	***
$L.S.P._1$- $L.S.P._2$	78 -78	116,53-120,79	-4,26	-5,731	***
$L.S.P._1$- $L.S.P._3$	78 -78	116,53-111,49	5,04	4,249	***
$L.S.P._2$- $L.S.P._3$	78 -78	120,79-111,49	9,30	10,035	***
$I.C.I._1$ – $I.C.I._2$	78 -78	402,78-408,39	-5,61	-7,000	***
$W.C._1$ -$W.C._2$	78 -78	37,57 - 37,63	-0,06	-.474	ns
$W.C._1$ - $W.C._3$	78 -78	37,57 - 37,72	-0,15	-.614	ns
$W.C._2$ -$W.C._3$	78 -78	37,63 - 37,72	-0,09	-1.270	ns

***P<0,001; **P<0,01; ns P>0,05. L.G. 1, 2, 3- length of the first, second and third gestation, days; L.S.P. 1, 2, 3- length of the first, second and third service period; I.C.I. 1, 2 – first and second inter-calving interval; W.C. 1, 2, 3 – weight of calves, first, second and third calving;

Tables 7, 8 and 9 present the coefficients of phenotypic correlation and the measure of association of reproductive characteristics concerning the age of heifers at the first, second, and third insemination.

Table 7. Coefficients of phenotypic correlation and measure of association of reproductive characteristics (in relation to the age of heifers at the first insemination)

Charactheristics	r_{xy}	$t_{exp.}$	Measure of association
A.H.F.I - $L.S.P._1$	$0,109^{***}$	1,04	complete
A.H.F.I - $L.S.P._2$	$0,144^{***}$	1,41	complete
A.H.F.I -$I.C.I._1$	$0,072^{**}$	0,64	complete
A.H.F.I - $I.C.I._2$	$0,136^{***}$	1,24	complete
A.H.F.I - $W.C._1$	$-0,012^{ns}$	0,10	absence
A.H.F.I - $W.C._2$	$-0,162^{***}$	1,48	complete
A.H.F.I - $W.C._3$	$0,014^{ns}$	0,12	absence
A.H.F.I - $L.G._1$	$-0,138^{***}$	1,26	complete
A.H.F.I - $L.G._2$	$-0,215^{***}$	1,97	complete
A.H.F.I - $L.G._3$	$-0,005^{ns}$	0,04	absence

***P<0,001; **P<0,01; ns P>0,05. A.H.F.I - age of heifers at the first insemination; L.S.P. 1, 2 - length of the first and second service period; I.C.I. 1, 2 – first and second inter-calving interval; W.C. 1, 2, 3 – weight of calves, first, second and third calving; L.G.1, 2, 3- length of the first, second and third gestation;

Based on the presented data from the table, we can observe that the calculated coefficients of phenotypic correlation between age at first fertilization, duration of the first and second service periods, the second inter-calving period, calf weight at the second calving, and the length of the first and second gestations showed very high significance (P<0,001). According to the Roemer-Orphal scale, the measure of the association for these factors was complete. It should be added that the calculated correlation coefficients between age at first insemination,

calf weight at the first and second calvings, and the length of the first, second, and third gestation was negative in direction. A high correlation (P<0,01) was observed in the calculated correlation coefficient between the age at first insemination and the first inter-calving interval (r_{xy}=0,072**). However, the calculated correlation coefficient involving age at first insemination, the first inter-calving period, the third inter-calving interval, and the duration of the third gestation did not show statistical significance (P>0,05).

Table 8**.** Phenotypic correlation coefficients and measure of associations of reproductive characteristics (with respect to the age of cows at second insemination)

Performance	r_{xy}	$t_{exp.}$	Measure of association
A.C.S.I - L.S.P.$_2$	0,262**	0,62	strong
A.C.S.I -L.S.P.$_3$	0,153***	1,40	complete
A.C.S.I. -I.C.I.$_2$	0,260***	2,38	copmlete
A.C.S.I -W.C.$_2$	-0,149***	1,36	complete
A.C.S.I -W.C.$_3$	-0,184***	1,68	complete
A.C.S.I - L.G.$_2$	-0,130***	1,19	complete
A.C.S.I - L.G.$_3$	0,061**	0,53	strong

^{ns}

***P<0,001; **P<0,01; P>0,05. A.H.S.I - age of cows at the second insemination; L.S.P. 2, 3 - length of the second and third service period; I.C.I. 2 – second inter-calving interval; W.C. 2, 3 – weight of calves second and third calving; L.G. 2, 3- length of the second and third gestation;

Based on the data presented in the table, it is clear that the calculated phenotypic correlation coefficients between the age of cows at their second insemination, the length of the third service period, the second inter-calving period, the weight of calves during the second and third calvings, and the length of the second gestation, have demonstrated a remarkably high level of significance (P<0,001). Specifically, there is a high level of significance (P<0,01) between the age of cows at their second insemination and the length of the second service period, as well as the third gestation. According to the Roemer-Orphal scale, the measure of association in these instances was classified as both strong and complete.

Table 9**.** Coefficients of phenotypic correlation and measure of association of reproductive traits (with respect to the age of cows at the third insemination)

Characteristics	r_{xy}	$t_{exp.}$	Measure of association
A.C.T.I – L.S.P.$_3$	0,221***	2,02	complete
A.C.T.I – W.C..$_3$	-0,200***	1,83	complete
A.C.T.I – L.G.$_3$	0,081**	0,71	strong

^{ns}

***P<0,001; **P<0,01; P>0,05 A.H.T.I - age of heifers at the third insemination; ; L.S.P. 3 - length of the third service period; W.C.3 – weight of calves third calving; L.G.3 – lenght of the third gestation

Based on the presented table data, it's evident that the calculated coefficients of phenotypic correlation between the age of cows at their third insemination and the length of the third service period, as well as the weight of calves during the third calving, exhibited very high significance (P < 0.001) and high significance (P<0.01) for the correlation between the age of cows at their third insemination and the length of the third gestation. According to the Roemer-Orphal scale, the measure of association in these cases was both complete and strong.

Conclusion

Based on the analyzed reproductive characteristics, it can be concluded that the average age of heifer at the first insemination was 16,54 months, age of cow at second insemination was 29,57 months, while the age of the cows at the third insemination stood at 43,12 months. The average length of gestation during the first, second, and third calving is 286 days. On average, the first service period lasts for 116,53 days, the second service period lasts for 120,79 days and third 11,49 days. The first inter-calving period spanned an average of 402,78 days, with the subsequent period extending by an additional 5,61 days. Heifers of both genders manifest their minimal weight during the first calving (37,75 kg), while relatively heavier calves are observed during the second (37,83 kg) and third (37,92 kg) calvings. Based on the information provided in this study, it is determined that the age of heifers at first insemination is statistically significantly associated with the service period, the second inter-calving interval, length of the first and second gestations, as well as the weight of calves at the second calving. These relationships are confirmed by the calculated coefficients of phenotypic correlation among specific reproductive characteristics. Based on the presented data, it can be observed that the calculated coefficients of phenotypic correlation between the age at second insemination, duration of the second and third service periods, the second inter-calving period, weight of calves at the second and third calving, and the duration of the second and third gestation have shown very high significance levels ($P<0.001$). According to the Roemer-Orphal scale, the measure of association for the mentioned variables was complete and strong. Based on data provides, one can notice that the calculated coefficients of phenotypic correlation between the age at the third insemination, duration of the third service period, weight of calves at the third calving, and the length of the third gestation have shown very high significance levels ($P<0.001$). According to the Roemer-Orphal scale, the measure of association for these variables was complete and strong. Overall, the farm TC "Zona" Sokolac has achieved acceptable results in terms of the studied reproductive characteristics during the analyzed timeframe. Considering its relatively short existence in this location, we believe that it will successfully meet its planned objectives in the future and enhance reproductive efforts to their fullest capacity.

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THE INFLUENCE OF ALTITUDE ON THE QUALITY OF HONEY IN CENTRAL SERBIA

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Abstract

The paper analyzed acacia, meadow and forest honey (honeydew) from Central Serbia. The parameters of honey quality were determined according to the methods of the Rulebook on the quality of honey and other bee products (Official Gazette of RS, 2015). Total nitrogen was determined by the micro-Kjeldahl method, and potassium and trace elements were determined by the AAS-method. In the case of acacia honey, the acidity and amount of mineral substances (ash) increase with the increase in altitude, while the amount of sugar (total, reducing and saccharose) and water decreases. The increase in altitude also had an impact on the quality parameters of meadow honey. With the increase in altitude, the amount of mineral substances and water in this honey increases, while the total sugars and sucrose decrease. The amount of analyzed macro and micro elements in meadow honey increased with altitude, with the exception of zinc (Zn). Honeydew from the hill area had the most ash and the lowest values for other parameters. In this honey, all minerals reach their maximum in the hilly area, and decrease in the plain and mountainous area. The exception is zinc (Zn), which is most abundant in honey from the plains. All analyzed honeys met the conditions prescribed by the Rulebook on the quality of honey (Serbia) and the Directives on the quality of honey in the European Union.

Key words: *honey, altitude, Central Serbia, quality, macro and microelements.*

Introduction

According to the definition (Sl. Glasnik RS 101/15 2015) honey is a natural, sweet substance produced by honey bees (*Apis mellifera*) by processing the nectar of plants, or from juices from living parts of plants, or by collecting the excrement of insects that feed by sucking juices from living parts plants, which bees collect, process and add their own specific substances, dehydrate and deposit in the cells of the comb until maturation.

Honey is a very important high-energy food in the human diet. In addition to its direct use in food, honey is also used as an ingredient in many nutritious foods, primarily based on cereals, where it increases their sweetness, color and taste (Rodriguez *et al*., 2004).

Honey is an excellent source of energy, and its composition contains about 80% carbohydrates (approximately 35% glucose, about 40% fructose and 5-10% sucrose) and 16-20% water. Honey contains over 180 compounds, including amino acids, vitamins, minerals, enzymes, organic acids, phenolic compounds and others (Kahraman *et al*., 2010). The acidity of honey (pH) is about 4.0 (Ouchemoukh *et al*., 2007), while its composition depends a lot on the type of honey plants from which the bees collected nectar and the climatic and pedological characteristics of the area where it was collected. Physico-chemical characteristics of different honeys from a large number of geographical regions were the subject of many scientific works (Golob and Plestenjak (1999); Persano Oddo and Piro (2004); Devillers *et al*. (2004);

Šarić *et al*. (2008); Jevtić *et al*. (2011), Lazarević *et al*. (2012) and many others). Honey quality control is very important because of improving the way of production, processing and use of honey, but primarily because of the protection of consumers and their interests in getting healthy food. It is very important for consumers that information about the quality and origin of the honey on the market is available and that it can be easily verified through analysis. Recently, it has been established that the altitude affects most parameters of honey quality (Bouhala *et al*., 2020; Turk and Sen, 2021).

The aim of this work was to determine how the increase in altitude affects the quality and quantity of macro and micro elements in honeydew, acacia and meadow honey. Bearing in mind the fact that lately there is an increasing demand for honey from Serbia, we wanted to determine whether and to what extent it meets the qualitative criteria set by us as well as the standards of the European Union.

Materials and methods

The paper analyzed acacia, meadow and forest honey from the territory of Central Serbia. Depending on the altitude of the apiary from which the honey was taken for analysis, the honeys were divided into three groups. In the first group were honeys whose apiary altitude was up to 300m (plain area). In the second, there were honeys from the hilly area (300-500 m above sea level), and in the third, honey from the mountain area (above 500 m above sea level). The honey was obtained from the area bordered on the south by the Mojsinje Mountains, on the east by Jastrebac, and on the north and west by the Kopaonik and Goč mountains. The lowest altitude was the apiary in Duboka near Pojate (150 m), and the highest in Kriva Reka on Kopaonik (1191 m). The largest geographical width was the apiary in Stari Bračin near Ražanj ($43^0 46' 08''$ N), and the smallest in Kriva Reka on Kopaonik ($43^0 22' 30''$ N). A total of 46 samples were analyzed and each sample was made in 3 replicates.

The amount of water in honey was determined using a refractometer at a temperature of 21°C. Acidity was determined by titrating honey with 0.1 mol/l sodium hydroxide, in the presence of phenolphthalein solution, until a light pink color appeared. Diastase activity was determined based on the hydrolysis of a 1% starch solution by an enzyme from 1 g of honey during one hour at a temperature of 40°C. The reading was performed with a spectrophotometer at 660 nm. Reducing sugars were determined by titration, based on the reduction of Fehling's solution, with a solution of reduced honey sugars using methylene blue as an indicator. Total nitrogen was determined by the micro-Kjeldahl method, and other macro and micro elements were determined by the AAS-method (AAS-Perkin Elmer 1100 B USA). Each sample was run in triplicate.

Results and discussion

Chemical indicators of honey quality

The amount of water and acids in all three tested types of honey was the lowest in honey from the hilly area (300-500 m above sea level). It was also observed that the amount of (reducing) sugars decreased with increasing altitude in all analyzed honeys (Table 1). In contrast, diastase activity (diastase number) and electrical conductivity increased with increasing altitude in all three types of honey. Bouhala *et al*. (2020) analyzed polyfloral honeys in Algeria and found that honeys from the coastal region of Jijel (altitude 44 m) have higher electrical conductivity, pH, ash and HMF content, while mountain polyfloral honeys from the same area have higher overall acidity.

In acacia honey, the amount of sucrose decreased with increasing altitude, while the amount of substances insoluble in water increased. All quality parameters of acacia honey were within

the standard limits and coincided with the results of most authors (Popa *et al*., 2009; Marghitas *et al*., 2010 and others).

The increase in altitude had a very similar effect on the quality parameters of meadow honey. With increasing altitude, the amount of water and water-insoluble matter increased, while the amount of total sugars and sucrose decreased. Contrary to the above, Turk and Sen (2021) claim that with an increase in altitude, the amount of water in honey decreased, while acidity and diastase activity increased. The total acidity of meadow honey in the hill area was slightly higher compared to domestic standards (40 mmol/l), but it met EU standards (50 mmol/l). Lazarević *et al*. (2012) stated that honeys from these areas can have higher acidity than the domestic standard and reported that the amount of acids in honey ranged from 7.80-42.70 mmol/kg.

Forest honey from the hilly area had the least amount of water, acids and reducing sugars and very little sucrose. Devillers *et al*. (2004) also determined the low content of sucrose in the tested honeys. Mladenović *et al*. (2012) determined a significantly lower content (0.2%) of this sugar in honey. Increased acid content and higher electrical conductivity in honeys from higher altitudes (Zlatibor) were determined in their research by Lazarević *et al*. (2014).

Table 1. Quality of different types of honey form Central Serbia depending on the altitude

Type of honey	Altitude (m)	Water (%)	Free acids (meq/kg)	Reducing sugars (%)	Sucrose (%)	Substances insoluble in water (%)	Diastase activity	Electrical conductivity (mikroS/cm)
Acacia	below 300	17,40	12,29	71,71	5,49	0,02	12,92	164
	300-500	16,67	10,19	68,93	5,25	0,02	12,82	223
	above 500	17,80	14,09	68,06	3,14	0,03	13,12	285
	Average	17,29	12,19	69,57	4,63	0,023	12,95	224
	SD	0,68	4,17	3,11	1,98	0,10	0,87	25,83
Meadow	below 300	18,60	22,79	68,11	3,56	0,03	19,84	358
	300-500	18,23	19,53	67,95	3,51	0,04	20,16	429
	above 500	18,73	23,46	67,57	4,04	0,04	21,99	586
	Prosek	18,52	21,93	67,87	3,70	0,037	20,66	457,7
	SD	1,33	5,31	2,73	1,73	0,25	1,59	27,29
Honeydew	below 300	17,07	41,62	66,17	1,19	0,02	42,50	886
	300-500	16,43	36,46	63,72	3,52	0,02	42,77	1086
	above 500	18,40	39,29	64,44	2,89	0,02	45,45	1218
	Prosek	17,30	39,12	64,77	2,53	0,02	43,57	1063,3
	SD	1,73	10,29	2,31	1,62	0,0	1,82	31,56
Total average		17,70	24,65	67,40	3,55	0,027	25,73	581,67

Macro and micro elements in honey

Acacia honey from the plain area had the highest amount of Na and Ca, from the hilly area the highest amount of P, K, Mg, Fe, and Mn, and from the mountain area the highest amount of Zn (Table 2). With the increase in altitude, the amount of zinc (Zn) increased, while the amount of sodium (Na) decreased. In the research of Gulfraz *et al*. (2010) in acacia honey it was established: 688.0 ± 16.70 mg kg^{-1} K, 412.6 ± 3.54 mg kg^{-1} Na and 586.7 ± 5.53 mg kg^{-1} Ca. Conti *et al*. (2007) found large differences in the amount of K in acacia, flower honey and honeydew. Bodó *et al*. (2021) found a lower content of Ca, Mg, Na, Fe, Zn and Mn in acacia honey compared to our research. The potassium content was similar, and the phosphorus (P) content was significantly higher.

The amount of all analyzed macro and micro elements in meadow honey increased with the increase in altitude. The exception is the amount of zinc (Zn), which was the lowest in honey from the mountain area, and the highest in honey from the hilly area (4.09 mg kg^{-1}). Such a high zinc content in honey (3.58 mg kg^{-1}) was determined in the research of Roman *et al*. (2011), and even higher in Roman and Popiela-Prebran (2011). In the meadow honey from the hilly area, next to Zn, amount of N was the highest, and of Ca and Mg the lowest. The highest amount of P, K, Na, Ca, Mg, Fe and Mn was found in the meadow honey from the mountain area.

In forest honey, all minerals reach their maximum in the hilly area, and then decrease both with increasing and decreasing altitude. There is an exception for the amount of zinc (Zn), which is the lowest in hilly honey, and the highest in honey from the plains. Amount of nitrogen (N) is the highest in honey from the plains, and the lowest in honey from the mountain area. Bodó *et al*. (2021) claim that honeydew contains a very high content of micro and macro elements. The amount of Na, Fe, Zn and Mn had similar values as in our samples. The amount of K, Ca and P exceeds the values determined in our research many times over.

Bogdanov *et al*. (2007) determined that mixed flower honey contains 5 times more iron and zinc, and twice as much manganese and copper compared to acacia honey. Stahovska *et al*. (2008) determined a similar amount of Mg and a slightly higher amount of Zn, Mn, and Na in honey from Macedonia. In their research, a significantly higher amount of K and Ca and a twice lower amount of Fe were found compared to our research. Chakir *et al*. (2011) found higher amounts of K, Na, Ca, Mg, Fe and Zn in honey from Morocco compared to our research.

Table 2. Macro and micro elements in honey from Central Serbia depending on the altitude

Type of honey	Altitude (m)	N mg kg^{-1}	P mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	Fe mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}
Acacia	below 300	262,5	0,06	152,32	53,92	42,20	7,16	3,01	1,86	0,35
	300-500	348,0	0,08	284,91	38,54	25,82	11,51	6,71	1,99	0,77
	above 500	300,0	0,06	265,67	34,37	32,17	6,63	2,47	2,52	0,44
	Average	303,5	0,05	213,72	43,37	34,97	8,76	4,69	2,25	0,62
	SD	34,99	1,54	172,95	16,99	14,54	13,01	5,29	1,03	0,95
Meadow	below 300	346,7	0,04	336,64	13,61	17,30	11,16	1,72	1,49	0,50
	300-500	643,3	0,04	369,27	19,16	12,42	7,40	2,38	4,09	1,13
	above 500	577,5	0,06	458,12	24,83	19,85	19,36	3,84	1,19	1,47
	Average	522,5	0,06	388,01	19,20	16,52	12,64	2,76	2,15	1,08
	SD	127,18	1,13	81,97	9,36	9,98	13,70	1,63	1,96	0,77
Honeydew	below 300	1110,0	0,05	748,94	14,43	21,84	32,49	2,53	1,16	1,36
	300-500	1046,7	0,11	1187,3	21,39	28,15	44,53	3,52	0,96	5,21
	above 500	656,7	0,05	638,10	21,27	20,18	20,68	2,91	1,68	2,37
	Prosek	937,8	0,07	858,11	19,03	23,39	32,57	2,99	1,27	2,98
	SD	200,44	2,03	272,96	5,23	14,11	18,14	0,84	0,42	2,53
Total average		587,93	0,06	486,61	27,20	24,96	17,99	3,48	1,89	1,56

Conclusion

Based on the analysis of the quality of honeydew, acacia and meadow honey produced in Central Serbia in apiaries that differed according to altitude, the following conclusions can be drawn:

- in acacia honey, the amount of sugars (reducing sugars and sucrose) decreases with increasing altitude, while the amount of substances insoluble in water, total acidity and electrical conductivity increases.

- in the case of meadow honey, with the increase in altitude the quality parameters behave very similarly as in the acacia honey, except for the amount of water, which is almost equal at all altitudes.
- honeydew from the hilly area (300-500 m) had the lowest amount of water, the lowest acidity and the lowest amount of reducing sugars, and the highest amount of sucrose.
- the altitude of the area from which the honey was obtained had the greatest influence on the electrical conductivity. The electrical conductivity of all three types of honey increased with increasing altitude.
- considering the amount of macro and micro elements, the altitude had a very big influence on the total amount of almost all observed elements
- observed as a whole, greater differences in the quality of honey and the amount of macro and micro elements were obtained between different types of honey (acacia, meadow, honeydew) than the differences obtained within the same type of honey from different altitudes.
- all analyzed honeys met the conditions prescribed by the Rulebook on the quality of honey in Serbia and the Directives on honey of the European Union. There is a deviation in one sample of meadow and one sample of forest honey, which have a slightly higher acidity than the prescribed domestic standard.

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EFFECTS OF BREEDER AGE ON EGG QUALITY TRAITS IN JAPANESE QUAILS

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Abstract

Many changes in yield and quality characteristics occur with age in poultry. The aim of this study is to compare some egg quality characteristics of eggs collected at 10, 14, 18, and 22 weeks of age from a breeder flock and to determine their changes over time. For this purpose, egg, yolk, and albumen weight, shell weight and thickness, yolk and albumen index, and Haugh unit characteristics were measured in 258 eggs. The egg weight increased from 11.91 g at 10 weeks of age to 12.94 g at 22 weeks of age, accompanied by increases in yolk, albumen, and shell weights (P0.05 for each). It was determined that the eggshell thickness, which was 0.218 mm at the time of the first measurement, decreased to 0.212 mm at the end of the experiment (P<0.05). It was observed that the mean of the shape index, yolk index, and albumen index of eggs also decreased with the age (P<0.05 for all). It was determined that the Haugh unit, which was 92.59 at 10 weeks of age, decreased to 90.84 at 22 weeks of age (P<0.05). As a result, it is possible to say that there are changes in almost all egg quality characteristics with increasing breeder age in Japanese quails.

Keywords: *Egg quality, Breeder age, Japanese quail, Haugh unit, Egg weight*

Introduction

In order to obtain high quality and large numbers of chicks, in addition to providing suitable incubation conditions, hatching eggs must also have the desired quality characteristics. In order for the chicks to be used in production to be of high quality, it is not sufficient to apply the incubation characteristics (temperature, humidity, turning, air circulation) alone, however, genotype, age and live weights of breeders, and external egg quality characteristics (egg weight, shell quality, thickness, shape index, etc.) and internal quality characteristics (Haugh unit, albumen and yolk index, etc.) are also known to be effective (Elibol et al., 2000; Nacar and Uluocak, 1995; Wilson, 1991). The performance traits and egg internal-external quality characteristics may decrease with increasing breeder age. While egg production decreases as the bird ages, the live weight increases. Although the different results of some studies on feed consumption and feed efficiency, the increase in feed consumption and worsening in feed efficiency are noteworthy with increasing age. There is a positive and linear relationship between bird age and egg weight. The accepted view is that hatchability is better in medium-weight eggs (Wilson, 1991; Narushin and Ramanov, 2002;). It has been reported that the hatchability obtained in chicken eggs with egg weights in lower and higher grades is 8-10.5% lower than those with optimum egg weight grade. An increase of 10 g in egg weight reduces hatchability by 10.7%, while a decrease of 10 g in egg weight results in a decrease of 3.9% in laying hens. A similar relationship has been reported in Japanese quail eggs (Narushin and Ramanov, 2002). The rate of abnormally shaped eggs increases in aging birds. For a successful hatching, the shape index of the eggs should be between the optimum limits. It has been reported that while the hatchability is 87.2% in normal shaped eggs, it is 48.9% in non-optimal shape index eggs, 68.1% in eggs with air cell defects and 70.25% in cleaned eggs (Kamanlı et al, 2010; Elibol, 2009; Narushin and Ramanov, 2002). The aim of this study is to

compare some egg quality characteristics of eggs collected at 10, 14, 18, and 22 weeks of age from a breeder Japanese quail flock and to determine their changes over time.

Material and Methods

The experiment was carried out in an environmentally controlled quail coop in the Animal Production Research Unit of Akdeniz University Faculty of Agriculture. The quails used in the study were obtained from a randomly mated flock without any genetic improvement studies. A total of 45 female quails were used in the study. The quails were assigned wing numbers at hatch and were housed in rearing cages with 96 x 43 x 21 cm compartments on each floor for the first three weeks. During the rearing period, quails were given mixed feed with 24% crude protein and 2900 kcal/kg metabolic energy content. Quails were placed in individual chambers measuring 14 x 20 x 21 cm from the third week. During the rearing period, 23 hours of daily lighting was applied to the quails. Randomly selected 108 quails were placed in groups in five chambers measuring 96 x 42 x 20 cm. Quails were placed in these cages using stocking densities of 120, 140, 160, 180, 200 cm^2/quail. Randomly selected 110 quails were placed in quail cages with individual compartments at a stocking density of 300 and 360 cm^2/quail.

In order to examine the growth in quails, the following form of the three-parameter Gompertz nonlinear regression model, which has been demonstrated in similar studies, was used (Akbaş and Oğuz, 1998):

$Y= \beta_0.\exp(-\beta_1.\exp(-\beta_2.t))$

The meanings of the terms used in the Gompertz model are as follows:

t : time

β_0 : mature (asymptotic) weight

β_1 : growth rate (integration constant)

β_2 : growth rate

Using the model parameters, the inflection point weight (IPW) and the inflection point age (IPT) were calculated as:

IPW: β_0/e

IPT: $\ln(\beta_1)/\beta_2$ Model parameters estimated in SAS 9.3 NLIN procedure with Levenberg-Marquardt iteration method (SAS 2003).

When the quails were six weeks old, immobility durations (TI; tonic immobility) were measured in order to determine the fear level of all quails. During the application of this test, the operator taking the test laid the quail to be tested on a special device on its back with its head hanging down and placed one hand on the quail's chest without applying any pressure and waited for the bird to become immobilized (calm) for 10 seconds. At the end of 10 seconds, the hand was slowly withdrawn, and the chronometer was started. In the measurement of inactivity time, the highest value was accepted as 5 minutes (Campo and Davilla, 2002).

In order to measure the effects of housing type and stocking density on tonic immobility duration, sixth week body weight, and growth curve parameters, variance analysis was performed according to nested-design, and the following statistical model was used:

$Y_{ijk} = \mu + b_i + ys_{j(i)} + e_{k(ij)}$

Yijk; Trait, μ; mean, b_i; effect of i^{th} housing type, $ys_{(i)j}$, the effect of the j^{th} stocking density in the i^{th} housing type, $e_{(ij)k}$; error term.

All statistical analyzes were performed using SAS 9.3 statistical software.

Results and Discussion

The tonic immobility durations, body weights, and parameters of Gompertz growth model measured in Japanese quails housed in group cages and individual cages according to different stocking densities are presented in Table 1. The tonic immobility durations determined in the study are consistent with the results of many studies performed in Japanese quail (Benoff and Siegel, 1976; Mignon-Gresteau and Minvielle, 2003). According to the short and long tonic immobility duration in Japanese quails, at the end of long-term (43 generations) mass selection, TI durations were found to be 113 seconds in the randomly mated control group, 399 seconds in the line selected according to the long TI duration, and 22.3 seconds in the line selected according to the short TI duration (Calandreau et al. .2011). The TI durations found in this study are consistent with the TI duration reported by Calandreau et al (2011) for quails mated by chance in control group.

Tonic immobility durations were found to be 68.47 seconds for quails housed in group cages and 83.55seconds for quails housed in individual cages. The difference observed in terms of tonic immobility times between housing types was found to be statistically significant ($P<0.05$). Salzen (1963) and Jones (1996) reported that tonic immobility duration measured in group housed animals were higher than in individually housed birds. In addition, in a study conducted by Kujiyat et al. (1983) with 1, 5, 15 and 17 laying hens in the cages, it was reported that the TI durations of the hens housed individually were higher ($P<0.01$). Salzen (1963) suggested that the level of fear of poultry isolated from their social environment increases, which will lead to stress and lead to loss of productivity. However, in many studies investigating the relationship between TI time and yield traits in poultry, it was reported that TI time did not have a significant effect on yield traits (Minvielle et al., 2002; Mignon-Gresteau and Minvielle, 2003; Skinner-Noble et al., 2003; Buijs et al., 2009).

Table 1. The mean values and statistical analysis results for tonic immobility duration, body weight, and parameters of Gompertz growth model according to stocking density and rearing type

Rearing Type	Stocking Density (cm^2)	TI (sec)	Body Weight (g)	β_0	β_1	β_2	IPW	IPT
Colony	160	72.18^{b}	204.42	256.49	3.45	0.068	94.36	18.21
	180	74.49^{b}	201.78	248.69	3.51	0.073	91.49	17.20
	200	75.21^{b}	199.86	252.81	3.55	0.066	93.00	19.20
	220	63.99^{d}	208.42	253.27	3.48	0.071	93.17	17.56
	240	56.49^{e}	207.54	245.89	3.62	0.069	90.46	18.64
Mean		68.47^{B}	204.40^{B}	251.43	3.52	0.069	85.48	18.24
SE		3.21	4.56	3.55	0.18	0.001	1.56	0.29
Individual	280	82.43^{a}	221.48	259.29	3.53	0.073	95.39	17.28
	360	84.67^{a}	218.56	255.57	3.54	0.073	94.02	17.32
Mean		83.55^{A}	220.02^{A}	257.43	3.54	0.073	85.26	17.32
SE		4.23	3.78	3.76	0.13	0.001	1.24	0.27
Variation Sources				P Value				
Rearing Type		0.001*	0.458	0.741	0.332	0.886	0.741	0.546
Stocking Density		0.001*	0.034*	0.484	0.128	0.749	0.484	0.548

*P<0.05. Lower case letters in the same column denote the difference between stocking density groups, and upper-case letters denote the difference between rearing types.

Japanese quails housed in individual cages have higher body weight averages at 6 weeks of age than those reared in colony cages ($P<0.05$). Cage type and stocking density applications did not affect the Gompertz growth curve parameters.

Conclusions

In conditions where fear factors are effective, irreparable economic losses occur both in terms of poultry and breeders. For these reasons, the importance of fear for poultry should be well investigated. For this purpose, some developed fear tests were tested on birds. Although fear tests alone do not provide sufficient information about bird welfare, it is necessary to know to what extent these tests can detect fear. It would be useful to investigate the contribution of this factor to general stress, without evaluating fear as a single factor on productivity characteristics.

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HOLSTEIN BREED LIVESTOCK IN GEORGIA

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Abstract

The presented article aims to discuss the possibility of Holstein breed cattle's introduction in the Kakheti intensive agricultural zone of Georgia and the phenotypical, internal, and external peculiarities of the dairy cattle researched by the authors. In addition, the authors analyzed the scientific research regarding the important factors contributing to the successful breeding of Holstein livestock in local natural and climate conditions. The study of the acclimatization process of the Holstein breed demonstrated that the heat resistance index in newly born cows was 2.2-3.3 units lower than in non-parturient cows. Adhering to the recommended amount and quality of nutriment ration ensured normal health conditions in cattle and maintained the appropriate level of reproductive ability. The locally produced cows from the introduced Holstein cattle lagged behind the European standard of the Holstein breed by only 2-3 % in terms of live weight and height. Given the conditions of Georgia, it should be mentioned that the received data can be considered acceptable. As for the task of bringing arterial pulse and breathing rate to the norm – it became possible after installing recirculating fans in cow stalls. Thus, the clinical analysis as well as live weight growth and development dynamic of the tested Holstein-type heifers demonstrated that the tested indices were within the physiological norms. Based on the results obtained from the research and the conclusions drawn, practical suggestions and recommendations were developed for the correct management of fodder nutrition for cows during heat stress. This is a valuable material for farmers which will enable them to effectively conduct targeted activities.

Key words: *Holstein, adaptation, heat resistance, productivity, nutriment ration, growth and development.*

Introduction

Natural milk production has been the most urgent problem in Georgia for a long time which in the conditions of existing extensive systems in stock breeding requires immediate intensification, implementation of various necessarry activities in line with breeding and adaptation of intensive breeds of livestock.

Today in most of the countries where the natural milk production problem is solved there is Holstein stock bred. It is not occasional that the Holstein breed cattle is in the center of attention, it is the most popular breed in milk production sector which is also distinguished for its live weight, distinct milking forms, with the special form and location of udders which despite the capacity is located higher and spreads in width when filled up. Besides the above, the Holstein breed of cattle has peculiar quality of its intensive growth and development as well as big capacity of milking which is achieved through healthy and balanced food (Gotsiridze et al., 1986; Miglior et al., 2005).

In the USA more than 80% of livestock is Holstein breed and the milking capacity of each is more than 9000 kg. The Holstein Association has registered 19 million of cattle in the

country (*Campbell* et al., *2016)*. In Germany the Holstein share in the population is 60% in 28500 livestock famrs; more than 1,7 million of cattle of high capacity. In Europe the improvement of the breed is implemented according to the plan and the process is managed by EAAP (European Federation of Animal Science) (Benechis, 2002). If we review East and, in particular, Israeli experience today there is approximately one hundred thousand cattle which produces around 1 billion kg of milk annually which fully meets the needs of local population on milk and milk products and a part of the products is exported (Benechis, 2002).
Over the last years farmers import European breed cattle in Georgia. Due to the peculiarities of homeostatic capacities the set of physiological disorders causing negative results is revealed. It is identified that apart from other breeds, the Holstein breed more easily adopts to environmental conditions and to achieve the maximum of its genetic capacity it is necessary to create conditions which best correspond to its genotype. Consequent from the above breeding of Holstein cattle in untypical natural environment is very important. In general the genetically predetermined norm of reaction of animals defines its environment adaptation capacity limits. It is noteworthy that in some cases the ignorance of adaptation peculiarities impeded the adjustment to extreme environmental conditions (Larry*, 2006; Thomas, 2009;* Ansell, 1974; Tumanian, 2003; Tunikov, 2011).
Today there are thousands of cows (industrial breeds) imported in our country. Out of this number is Hosltein breed and the most of them are in Kakheti region. It should be mentioned that the breeding of Hosltein cattle in Kakheti intensive agrarian zone has not been scientifically studied and there are no corresponding recommendations developed. It is urgent to study the peculiarities of Holstein cow productivity potential realization as well as to study the economic and biological peculiarities of it in the new environmental conditions which is of significant national economic importance (development of intensive farming and increase of raw milk production), while on the other hand, in order to reveal the high genetic qualities of this breed, it is necessary to create such conditions which fully correspond to its genotype.
The goal of our research was scientific justification of Holstein cattle breeding in the conditions of Kakheti region in Georgia.

Methodology of the Research

Consequent from the mentioned goal the following results were obtained in the period of the research:

- Description of the clinical status indicator of the cattle in different periods of procreation:
    - Arterial pulse measurement - by the method of palpation of the subcaudal artery;
    - Breathing frequency - observing the movement of the chest and abdominal cavity during inhalation and exhalation;
    - Body temperature - with a veterinary thermometer, rectally;
    - We studied the resistance of the heifers to high temperatures by calculating the heat resistance index, in accordance with A. Rauschenbach.
- Register of the received nutriments - Performed twice a month, it was calculated as the difference between the amount of feed given before the meal and the amount left after the meal.
- Study of the nutritiousness of the nutriments - Zoological analysis of the feed was carried out;
- Dynamics and calculation of average daily weight gain of heifers – We used methods of monthly weighing (once a month), in the morning, before feeding. Growth

parameters and growth rate were calculated using generally accepted methods. The average daily gain (D) was calculated according to the formula:

$$D = \frac{W_1 - W_\diamond}{t}$$

where:

W_1 - live weight of the animals at the end of the period, kg;

$W_\diamond$ - live weight of the animals at the beginning of the period, kg;

t - days.

Results and discussion

Development of peculiarities of an organism depends on heredity (genotype) and environmental conditions. When we are talking about the adaptation of animals to these or those extreme conditions we agree to take into consideration the fact that any dislocation of animals is accompanied by negative results. Environment is the basic limiting factor of revealing genetic capacities of an organism. In the condition of extremely high air temperature influence European milk cow breeds are characterized by limited homeostatic capacity which results in the whole set of physiological disorders causing decrease in reproductivity, growth intensity, milking productivity, period of agricultural exploitation and other undesirable results. It is obvious that considering the existing natural and economic conditions and biological peculiarities of cattle predefines the selection of relevant technological methods in cattle farming (Gogoli, 1999; Gogol et al., 2017; Gugushvili et al., 2016; Mikadze, 2014; Roche et al., 2006).

On the first stage of cattle farming of the nulliparous and primaparous cows the whole set of problems related to feeding and breeding arose. Though the revealed mistakes were eliminated and the technological process was improved.

It is particularly important to define clinical indicators (changes in the indicators according to various factors) of cattle in the process of adaptation to new environmental conditions which enables to define its health condition. Therewithal the important criteria which characterize the quality of adaptation and its maintenance in the given environment are growth and development, productivity and reproductivity (Tillard et al., 2008; Trevisi et al., 2012).

Visual observation of the animals demonstrated that the imported animals are less adapted to the summer heat. We studied the resistence to the high temperatures by nonporous speices by calculating the heat-resistence index according to I.A.Rauschenbach (Gogoli, 1999). The first test was done in the morning (8-9 o'clock) when air temperature was 16-20^{o}C; the second test was done during the hot daytime period at 15-16 o'clock when the temperature was 28-32^{o}C. The heat resistance index of nonporous cows was lower than of prim parous cows. The body temperature of nonporous cows in the morning under the condition of 20^{0}C air temperature was 38.8^{0}C, while the body temperature of prim parous cows varied from 38.2 to 38.4^{0}C; under the condition of 30^{0}C air temperature the body temperature of nonporous cows increased by 0.8^{0}C and in prim parous cows it increased by 0.2 -0.3^{0}C. Correspondingly the index of heat-resistance of prim parous cows was 2.2-3.3 units less than that in nonporous cows.

In line with it we revealed the number of visually observed problems such as saliva foam formation during the chewing process, inflammation of joints and cloven hoofs, liquid stool and in some cases loss of the cattle The conducted zoological analysis of the feed and its ingredients revealed:

- The protein content is low;
- The portion of nutritiousness in the feed is 30-40%;

- The concentrated feed content in corn silage is 60-70% consequent from its nutritiousness though it should not exceed 38-40%;
- The ration contains big number of carbohydrates (sugar+starch) while according to the norm the ration of a milking cow must contain 62-108 gr. of sugar per 1 FU (290 gr. DM. per kilo) and 93-100 gr. of starch;
- The use of 0.7 kg of soda as a buffer reduces the energy content in the ration which in turn causes the reduction of the milking;
- The corn grains in silage are not powdered which hinders its digestion;
- The nutrient mixture is over-grinded.

Unbalanced feed of cows, the use of concentrates including carbohydrates in big amount demonstrated negative effect on PH of first stomach on generation of evaporating fatty acids and their correlation. As a result of it the ethanoic acid decreases to 40%, propionic acid is increased by 40%. The decrease of ethanoic acid in its turn causes the following: decrease of milk and fat formation, toughening of active champing, decrease in saliva formation. Insufficiency of buffer substance (neutralizer) results in increase of acidity which, in its turn, is a precondition of decrease of cellulose digestion (Chkuaseli, et al., 2011). As it was already mentioned in case that such process lasts it may cause fat reduction in milk, inflammation of joints and cloven hoofs, liquid stool. All the above caused disproportion of sugar-protein correlation (the norm is 0.8=1.0 which means that 100 gr. m/protein must contain 80 gr of sugar). It is known that the quality and volume of the feed component substances as well as their correlation defines the effectiveness of the feed which ensures the productivity, health and reproductive capacity of animals (Gogoli et al., 2010; DeLaval, 2008).

In parallel we observed the breeding of the calves. We selected 12 units of the same age based on the analogy. It is known that the correct breeding of the calves defines the health and reproductivity of the mature cows at the same time the live weight of an animal at certain age and its exterior represents the indicator of the growth quality.

We used the breed standard for comparison (Chkuaseli et al., 2011). Based on the capacity of the farm we tried to use the rational system of breeding which supports the normal growth and development, and ensures formation of high capacity and strong constitution. The feed ration was composed of the available feed components with the consideration of feeding norms which gave us the guarantee that we receive such desirable weight of the animals which will meet the requirements of the first class and higher standards during the first mating. All the above ensures that we get desirable live weight of cows in future.

Table 1. Feed consumption from birth to the age of 15 months per one animal

Feed	kg	Dry substance. kg	mgj	Feed unit	Digestable protein, kg
milk	410	32.0	1.11	123	13.5
concentrate	650	578.5	7.67	650	72.15
hay	720	597.6	5.04	324	36.7
Oat straw	430	356.9	2.45	94.6	5.59
Silage	2070	517.5	4.76	476.1	28.98
Green mass	3400	697.0	88.4	680	88.4
Total	7750	2779.5	109.4	2347.7	245.32

Table 2. Growth and development dynamic of heifers, n = 12

Age/months	Standard/mass/kg	Result in correlation with the standard, kg	Standard/height, cm	Result in correlation with the standard, cm
2	75	71	83	82
8	235-240 (237)	237	112	110
15	390-400 (395)	386	128	125
22	550-560 (555)	520	137	134

Holsteiner breed standard considers: live mass of 75 kg in 2 months of age, by the end of milk feed period (Ots, 2017). The indicators of the tested heifers was 5% less than the standard. At the age of 8 months the standard is 237 kg (Ots , 2017), which is 3% more than the tested heifer mass; and at the age of 15 months the standard is 395 kg while the locally bred heifers achieved 386 kg. This indicator is considered a good result in Georgian reality but it lagged behind compared to the European standard. The tested heifer also showed retardation by 3-4% in height growth compared to standard. The listed disadvantages need to be taken into consideration when planning the growth of heifers though usually these are ignored.

The observation over the dunamic of the growth and development of the animals demonstrated 2-3% of retardation from the breed standard – live mass and whithers height. Despite the above these indicators may be considered applicable in local conditions.

Breathing is an unintegrated sign of life. The volume of oxygen in the organism of cattle is limited thus it requires to get oxygen from the environment. The dynamic of respiratory movements demonstrated that the respiration frequency is directly related to the age. It is similar to puls frequency variability – the highest indicator is registered at birth and it dicreases with the age which varies within the norm.

Table 3. Clinical Status of the Heifers

Age, month	Temperature ^{0}C	Pulse (minute)	Respiration frequency
At birth	39.02	81.0	34.20
6 months	38.40	70.40	31.20
12 months	38.14	59.7	20.60
18 months	38.6	57.50	18.40
24 months	30.17	54.8	18.30

According to the literature data the body temperature of animals is almost permanent and does not suffer significant changes (except sick animals). Apart to the temperature the puls of animals and especially the breathing frequency significantly changes.

Table 4. Clinical Status of Animals

Test period	Frequency of Arterial Pulse	Breathing Frequency
Pregnancy 5-6 months.	66	26.0
Pregnancy 8-9 months.	79	29.0
First parturition	85	33

The puls frequency of the tested six to nine months pregnant nulliparous cows showed the puls frequency increase by 13 beats (11.9%). The puls increase to 85 beats per minute during the first parturation. Hence the pulse frequency was several times less in the first half of the pregnancy period than in the second half and especially compared to the parturition. The results of the respiration frequency data analysis are similar which can be justified by the hot

summer days.

The summer heat became injurious for the newly imported animals. Due to the bad ventilation of the building the first symptoms of thermal stress were revealed (frequent breathing, concetration of animals at water places, saliva formation, foam at the mouth and etc.). In instant response to the mentioned the recirculation fans were montaged in the right and left sides of the farm in 12-14 m distance from the feed table.

The body temperature of an animal is the complex indicators of its thermal condition which gives clear picture of its organism condition. In hot weather the evening temperature of the cattle is 0.5-0.6 C higher compared to the norm which must not be considered a pathology (Kurashvili et al., 2012). 1-2 degrees increase of the body temperature is the sign of pathology. The body temperature during the parturition is particularly worthy attention. High temperature may be caused by the influence of thte environment on the body of a newborn calf, later the temperature may slightly change though it varies within the physiological norm limits. As for the heart contraction frequency, i.e. puls – it is periodic rythmal blood vessels widening which is connected to the dynamic of vessels filling during the one cycle of the heart. Particularly high puls was detected in claves during the parturition which later was decreased and in the age of six months it reaches 70, 20 beats a minute. Later it decreases more to 20 beats and changes significantly until the copulation. At the same time all variations of the indicators were within the physiological limits of the breed.

Conclusions

- Index of heat-resistence of primaparous cows was 2.2-3.3 units less than that in nonporous cows.
- The quality and volume as well as the correlation of nutriments in the animal feed daily ration defined the stabilization of the feed consumption ration for animals to maintain production and normal health and reproduction capacity.
- 2-3% of retardation from the breed standard in live mass and whithers height can be considered an applicable dynamic in Georgian conditions.
- The clinical analysis and the live weight growth and development dynamic of the tested Holstein breed hifers demonstrated that the studied indicators are within the physiological norms which justifies good adaptation capacity of the animals in the hot climate conditions of Kakheti region.

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AN OVERVIEW OF THE CURRENT STATE OF GRASSLANDS IN THE PODRINJE REGION AS A SOURCE OF HEALTHY ANIMAL FEED

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Abstract

Grasslands are widespread terrestrial ecosystems with the primary agronomic importance of providing quality fodder for domestic animals. Recent studies have revealed that grasslands in Serbia have an unfavorable floristic composition and low yield, and that they are an insufficient and frequently improperly used resource. The goal of this study was to assess the condition of the grasslands in the Podrinje region using a sample of 49 farms' actively used grasslands. Each of the 49 grasslands was subjected to soil and biomass analyses. Soil laboratory analyses determined the pH value, mineral element content (N, P, K), and carbonate content, whereas biomass analyses determined crude protein content, fiber content (ADF and NDF), and fat content. According to Braun-Blanquet, phytocenological releve were taken on a portion of the grasslands. The results show that the majority of grasslands grow on soils with a low pH value (38), and that the 60% of biomass sampls had a low protein content (>8%), indicating a low nutritional value. Both the low amount of legumes in grasslands - 36 samples had less than 10% of legumes in biomass - and late mowing during the flowering and seed-ripening stages have been attributed for this condition. All farmers received instructions to take steps to improve the floristic composition of grasslands and, as a result, the quality of produced fodder, which will have a positive economic impact in the near future.

Keywords: *Lawns, Healthy animal feed, Husbandry*.

Introduction

Grasslands are widespread terrestrial ecosystems, covering a significant portion of the world's agricultural land (Huber et al., 2022). Grasslands provide a variety of ecosystem functions according to TEEB study (2009): 1) provisioning services (crops, fresh water and plant-derived medicines); 2) regulating services (e.g., filtration of pollutants by wetlands, climate regulation through carbon storage and water cycling, pollination and protection from disasters); 3) cultural services (e.g., recreation, spiritual and aesthetic values, education). Grasslands, despite their importance, are endangered ecosystems especially due to ecosistem disruption (Liu et al., 2008).

The following global factors have a negative impact on these communities: the spread of forest species, the conversion of grasslands into arable land and urban-industrial zones, climate changes manifesting themselves through modifications to precipitation distribution throughout the year, and the use of fire (Ravi et al., 2010; Blair et al., 2014).

Grasslands make up 29% of agricultural land in the Republic of Serbia, however, a substantial percentage of the area under meadows and pastures is not used due to inaccessibility, weediness, or economic unprofitability (Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2014-2024). Over the last decade, researchers from the Institute for forage crops Kruševac conducted research across Serbia, including hundreds of

farms where the condition of the grasslands was carefully analyzed. Previous research has shown that natural grasslands in Serbia have a high percentage of weeds and other unfavorable species and a low percentage of legumes and quality grasses, that they are misused, and that they are an insufficient and frequently improperly utilized resource (Babić et al., 2019).

The abandonment of grasslands is linked to a decline in population, a phenomenon that is evident in eastern and southeastern Serbia. Western Serbia is still inhabited, and the population is agriculturally active. However, the population's attitude toward grasslands as a resource at their disposal, is questionable.

The objective of this research was to examine the current state of grasslands in the Podrinje region as a source of high-quality animal feed and recommend suitable measures to increase the efficiency of feed production.

Material and methodes

Research area

The studied area is located in the Podrinje region (western Serbia) and includes smaller domains entitled Jadar, Rađevina, and Azbukovica. We included 49 farms set in the villages: Donje Nedeljice, Uzaonica, Korenita, Cvetulja, Donja Badanja, Trbosilje, Bela Crkva, Zavlaka, Cerova, Vrhpolje, Drlače, Dvorska, Donja Borina, Gornja Badanja, Donja Sipulja, Brezovica, Bela Crkva, Likodra, Jadranska Lešnica, Tekeriš, Gunjaci, Dragodol, Lopatanj, Komirić, Belotić, Ostružanj and Bastav. Villages extend from the Drina's banks (Ljubovija municipality) all the way to Osečina municipality in the east. With such a large geographical range, it was possible to get a better picture of the state of the grasslands throughout the region.

Sampling

Each of the 49 grasslands was subjected to soil and biomass analyses. Soil was sampled at depths ranging from 0 to 25 cm using a sampling tool. Each plot was sampled in multiple locations, and the number of individual samples was increased on non-homogeneous patches. Individual samples from the same surface were combined and mixed before being separated into one average sample. Each of 49 samples was labeled and transferred to the Institutes labs where was analyzed.

The aboveground was sampled with a hand tool, and 1 kg of biomass was collected coming from all plant species growing on the grassland. Each of 49 samples was labeled and transferred to the Institutes labs where was analyzed. Vegetation studies are conducted using the Braun-Blanquet method and his cover-abundance scale. Species diversity was calculated based on collected data using the Shannon index, which is an indicator of the richness and uniformity of the observed community (Lakićević, 2023).

Analyses

The following methods were used to examine the soil samples: the soil reaction (pH value) using potentiometric method, total nitrogen by semimicro-kjeldahl method by Bremner modification, available phosphorus and potassium by the AL method by Egner-Riehm, humus by Tjurin in the modification of Simaks. The biomass samples have been split into three groups: *Poaceae* species, *Fabaceae* species, and all other plant species. Following separation, the mass of each of the three groups was measured and their proportion in the sample was calculated. The sample was then carefully mixed again, cut into tiny pieces, and subjected to an AgriNIR analyzer to determine its crude protein, fiber (ADF and NDF), ash, and fat content.

Results and discussion

We analyzed soil pH as well as the content of available macronutrients - potassium, phosphorus, and nitrogen - to compare soil samples. According to the pH values, all 49 samples have been separated into four groups (Fig. 1a). The majority of the samples (38) had pH values less than 5,5 and were categorized as strongly and moderately acidic soils. Four samples alone represent neutral soils that are suitable for the majority of plant species used in agriculture.

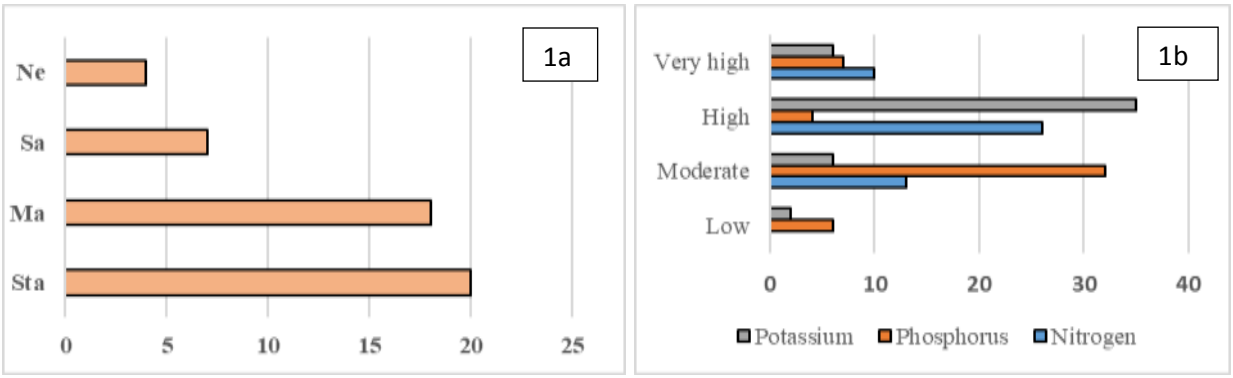

Fig.1a: Soil acidity, soil samples are classified in four categories according to pH values: Sta –Strongly acid (<4,5), Ma – Moderately acid (4,51-5,50), Sa – Slightly acid (5,51-6,5) and Ne – Neutral (6,51-7,2)
Fig.1b: Available macronutrients; soil samples are classified in four categories according to availability of potassium, phosphorus and nitrogen.

Macronutrients (nitrogen, phosphorus and potassium) are essential for plant growth (Sinha and Tandon, 2020). These primary nutrients were the object of laboratory analyses, the findings of which are shown in Fig. 1b. The samples are divided into four groups based on their nutrient content. The nutrient content is expressed as a percentage of total N in soil (<0,03 low - > 0,3 very high) and as content in mg of available P and K per 100g of soil (<8 till >35 mg). The overall conclusion is that the majority of the analyzed soils have high potassium and nitrogen content, with a moderate phosphorus content.
The aboveground part of grassland is the target area for farmers as a source of animal feed. The findings of crude protein content assays as well as legume % in biomass samples are shown in Fig. 2. A large number of the samples (60%) included less than 8% proteins and less than 10% legumes. Less than 10% of legumes were present in 36 samples, and no legume species were present in 7. Only 7 biomass samples had sufficient crude protein level, which was greater than 14%. These findings are strongly related, and the deficiency of protein is caused by a lack of high-quality legumes as well as delay in catting.
About 40% of grasslands were analysed using Braun-Blanquet visual assessment technique and the results were utilized to calculate Shannon diversity index. For 55% of grasslands, the calculated index indicates intermediet species diversity and the conclusion is limited to this collection. In comparison to results from other regions of the country, where the average number of plant species per grassland is 50 or more, the plant diversity of the studied collection is rather low, ranging between 14 and 30 species per grassland while overall number of species at all grasslans was 125. Only 50% of the samples that were analyzed contained high-quality legumes such *Trifolium pratense, Trifolium repens, Lotus corniculatus, Trifolium arvense, Medicago falcata, and Medicago minima* or high quality grassis such as: *Dactylis glomerata, Poa pratensis, Festuca pratensis, Phleum pratense.* These grasslands are under the influence of ruderal and invasive species and one of the biggest problem of this area as well as whole country is strong ocurence of *Ambrosia*

artemisiifolia L. This annual weed greatly outnumbers natural flora and creates "carpets" of plants in some areas. This is a major issue for both plant production and animal meal composition, as well as for human health. Establishing grass-clover mixtures is one strategy for battling and preventing the spread of ambrosia.

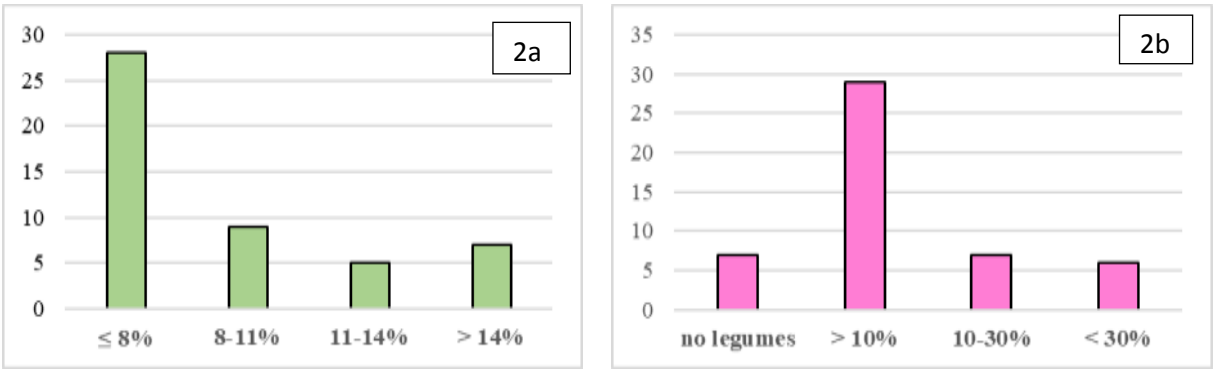

Fig. 2a: Crude protein content; biomass samples are classified in four groups depending of crude protein content.
Fig. 2b: Legumes share; biomass samples are grouped according to the legume share in each sample.

One of the most significant aspects of this work was the establishment of demonstrative trials in a region of the studied area as well as the recommendation of appropriate actions to each farmer. After 6 to 7 months, the results were visible, and all farmers were very pleased. (Fig. 3b)

Fig. 3a: Natural grassland in village Dvorska near Krupanj; Fig. 3b: A specific grass-legume mixture for acid soils, village Lopatanj near Osečina.

The outcomes of an inappropriate attitude toward grassland as a resource are shown in Fig. 3a. Most of the plants have a yellow color, and grasses and *Asteraceae* species including *Hipericum*, *Artemisia*, and *Crepis* are common. There are empty areas near the ground, and short species are uncommon. In the area, this picture is not rare. Natural grasslands are frequently mowed throughout the plant ripening phase. That has an adverse effect on both feed quality and botanical composition.

Conclusion

On the farms that were studied, grassland made up a relatively small portion of the total land area. The majority of the land is cultivated and planted with field crops. A variety of issues have arisen on the existing semi-natural grasslands as a result of inadequate application of agrotechnical methods. Soil acidity is a common issue, and the use of calcareous materials, manures, and fertilizers with higher phosphorus content is advised. As a conclusion, it may be

claimed that the proportion of grassland in the sowing structure is insufficient and that ongoing efforts must be made to change farmers' perspectives on their property.

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IMPROVING POSSIBILITIES OF INDIGENOUS HAIR GOAT BREEDING IN BİNGÖL PROVINCE IN TÜRKIYE

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Abstract

The purpose of this survey is to put forward the current situation and the potential of hair goat husbandry and breeding opportunities in Bingöl province of Eastern Anatolia in Türkiye. Small ruminant activities in Bingöl province are an important source of income for the local people. The Hair goat breed is one of the most widely raised breeds among small ruminants. Especially hair goat husbandry is indispensable for indigenous people in Bingöl province. Although sheep husbandry is still very high in Bingöl province, the demand for sheep decreases in the process, and goat husbandry is gaining importance with the increasing demand. These goats have evolved naturally through adaptation to socio-economic and ecological conditions of region. However, Hair goat is not a prolific breed. The sheep and the goats have been raised together in the region. These goats are generally black, rarely brown and grey. In Bingöl province, the number of goats has a share of 28.8%, which is above the Türkiye average. Hair goats are preferred for supplying the meat for household for long time periods. Although goat meat production is mostly produced from small family businesses, investments in the production of animal products have been increasing in recent years. With the increase in awareness of increasing economic gain, the way of cultivation carried out with traditional methods in recent years has left its place to the facilities where production is made in modern enterprises. As a result, preservation and development of Hair goats as a gene source for meat production is very important.

Key words: *Goat, Hair, Meat, Traditional breeding system.*

Introduction

The most critical sub-sector for entry into the European Union is livestock. Many reasons such as the development of the industry based on animal-derived raw materials, the creation of employment in rural areas, the development of priority regions for development, the increase of productivity in agriculture, and the establishment of foreign trade balances are of strategic value for Türkiye.

In Bingöl province, herds of small ruminants consist of about 5 goats per 100 sheep. The hair goats were preferred for supplying milk and meat for household for a long time period, to make leadership in small ruminant flock and to enhance the movement capability of flock in pastures and plateaus. The goats' hair is used for make clothes, felt, saddlebag, hair tent and suitable socks and shawl for winter. The goat milk is consumed by household or processed into local cheese. Purebred goats are still found in and around Bingöl province. Hair goats are the mainstay for the landless poor families of this region and the they need to be conserved in its breeding region. The sheep-goat breeders of this region had to migrate from the home tract to the cities along without their flocks and herds, due to economic and social problems of the region. During the course of migration, the number of sheep and goat in the region decreased

dramatically. Currently they are returning back to their villages systematically (Aygün, 2021b).

The Hair goat has the potential to meat and milk the nutritional as well as economic needs of the farmers living in this region. Nowadays, social and economic problems, involuntary migrations to the cities are of major concern to the sheep-goat breeders of the mountain region. The role of the Government and policy towards animal genetic resources need to be critically evaluated for the conservation of this type of goat. It can be concluded that conservation of the Hair goat is of paramount importance and efforts to conserve the breed in an integrated manner should be taken up. In addition, genetic improvement offers solutions for satisfaction of needs in small ruminant husbandry (TOB, 2023).

Small ruminant husbandry in Eastern Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications. Results in survey studies have determined that yield of goat is low, the number of animals raised are small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). The first practices that should be done to increase the profitability in livestock enterprises should be directed to environmental regulations. As the environmental factors improve, the genotype needs to be improved accordingly (Öztürk, 2009).

Low investment cost, preference of milk by consumers and ice cream production, better utilization of scrub and rocky areas distinguish goats from other farm animals (TOB, 2023). Livestock activities in Eastern Anatolia are an important source of income for the local people. In the province of Bingöl, small ruminant breeding takes place a very high important in the animal production sector in this region of country (Esen, 2017). In addition, the preservation and the development of Hair goat breed as a genetic source is very important in Bingöl province. Bingöl province is one of the most prominent cities of this area in which its public sustain themselves by animal production. For this reason, it is very important to define the condition, potential, and problems of animal breeding sector especially with respect to the goat stock in this province. The aim of this survey is to put forward the current situation and the potential of goat husbandry and breeding opportunities in Bingöl province of Eastern Anatolia in Türkiye.

Demographic Structure of Bingöl Province

Bingöl province is located in the Upper Euphrates section of the Eastern Anatolia Region. It is surrounded by Muş in the east, Erzurum and Erzincan in the north, Tunceli and Elazığ in the west, and Diyarbakır in the south. Bingöl Province is located between 41º - 20 and 39º - 56º east longitudes and 39º - 31 and 36º - 28º north latitudes. Its area is 8.125 km^2 (Anonymous, 2023). Economy of Bingöl province is an economy mainly based on agriculture and animal husbandry, and aquaculture. The livestock sector in Bingöl province has been developing and gaining importance day by day.

There are 7 districts of the province, namely Adaklı, Genç, Karlıova, Kiğı, Solhan, Yayladere, and Yedisu. The city center is located at an altitude of 1151 meters from the sea, in the northwest corner of the Capakçur plain, on a plain overlooking a branch of the Göynük stream, which meets the Murat water around Genç District.

Bingöl, which has an ideal structure for forestry in terms of climate and land structure, is one of the provinces with the richest forest area in the Eastern Anatolia Region. However, the use

of forests to meet the need for fuel for a long time and to be used in animal husbandry has resulted in it becoming a degraded coppice (Anonymous, 2023). Different plant species, distributed in meadow and pasture areas in Bingöl province, are seen as the main food source for nutrition of cattle, sheep, and goat.

Bingöl city is sixty-second the most crowded city in Türkiye. As of the begin of 2023, its population is 282.556 people. The educational status of the animal breeders and families of Bingöl province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country (Anonymous, 2023).

The Importance of Goat Husbandry in Bingöl Province

Hair goats are the most common breed of goats in Turkey. It is also known as the "Black Goat" among the people. Although they are widespread in all regions, they are densely found in forested and bush areas close to the sea. Due to the fact that they prefer leaves and branches as a feed source in their diet, hair goats are referred to as animals that harm the forest by many people and institutions. For this reason, various studies have been carried out for the removal and breeding of Hair goats from the forest (Özder, 2006; Ata, 2007).

The fact that the province has a large pasture area creates an important potential for small ruminant breeding. Livestock enterprises established in the province in recent years will be able to meet the raw material needs of cattle and small ruminant for integrated meat facilities. Despite widespread economic activity in Bingöl province, the animal production sector is not at the desired level. However, goat breeding is one of the most important livestock sectors for the province of Bingöl. Because are;

-the interest in goat meat is high.
-the feeding of goats is easier than other species.
-the majority of livestock enterprises are small family businesses,
-animal shelters are suitable for rearing and the productivity of pasture areas is wide,
-plateaus and pastures are suitable for small ruminant breeding,
-goat products are easily marketed, and
-forage crops are sufficient level.

It is known that the cultivation made by traditional methods needs some improvements on the basis of the enterprise. By the modern goat breeding training to be given to the breeders, the breeding can be more beneficial. With the breeding to be carried out under the roof of the organization such as cooperatives and unions, the increase in milk and meat yield of goat can be increased to the desired level, and the evaluation and marketing of products will be made more effective.

Although goat husbandry used to be very common in this province, its population has gradually been declined in the last 20 years. Because, roughage and concentrate feed costs have gradually increased. However, goat breeders in Bingöl still consider the state's incentives insufficient. The sheep and the goats have been raised together in the region. Small ruminant husbandry in the study area is carried out with traditional methods. Goat production is an animal sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Goat production is indispensable and an important source of income for farmers in Bingöl province. Actually, this province is suitable for both the small ruminant breeding and the cattle and the water buffalo husbandry in terms of large pasture areas, water resources, and climate characteristics (Esen, 2017).

The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient internal space for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001).
It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. However, small ruminant husbandry is a major industrial sector in the Eastern Anatolia of Türkiye (İnan and Aygün, 2019).

Goat Presence and Future in Bingöl Province

The Eastern Anatolia Region is one of the regions where animal husbandry is intensively made in Turkey. Small ruminant breeding in Bingöl province has been performed by traditional methods and production per goat is low in comparison with intensive goat production systems. Therefore, it is important to carry out the projects on Hair goats for both conserving as a genetic resource and increasing production characteristics.
The most common of goat breeds in Türkiye is the Hair goat breed. It is very important to define the condition, potential and problems of breeding sector especially with respect to the small ruminants in this province. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. The most important of these problems are the pasture and feed problem. Hair goat is regarded as an important genetic and cultural source in Bingöl province.
Inventory studies constitute the basis of all kinds of studies that are planned to be carried out at national or regional level. Therefore, important suggestions for the Bingöl region will be presented with this study. Number of goat in Bingöl province is presented in Table 1.

Table 1. Number of goat in Bingöl province (head) (TÜİK, 2023).

Year	Goat
2019	168486
2020	191553
2021	188771
2022	170043

As can be seen in the table, there was no significant change in the number of goats. The number of domestic hair goats is low and there is a need for the conservation of domestic pure breeds and their spread to other suitable areas.
Sheep and goat breeding is mostly based on pasture. In this region, 80-90% of the forage needs of animals are met from natural grazing areas such as meadows, pastures and plateaus. Breeding is generally carried out with low yielding native breeds that are resistant to diseases. Although it is suitable for the organic livestock model, this potential is not utilized sufficiently (Ata, 2007).
Regions that are not polluted due to intensive agriculture and industry, especially in the Eastern Anatolia Region, are of great importance in terms of ecological livestock breeding. However, there are problems in the export of animal products due to some animal diseases, and the lack of demand due to the low purchasing power and awareness of the consumer in the domestic market negatively affects the development of ecological livestock. However, research and production studies on ecological livestock should be supported and increased,

not only in terms of exports, but also in order to protect the environment and ecology, and to feed our country's people with healthier animal foods. If the potential of ecological livestock is evaluated well, the disadvantage in animal production can be turned into an advantage with ecological livestock (Ata, 2007; Esen, 2017).

Hair goat breeding has developed in the forested areas of the province. However, due to both hair goats and unconscious use, it has caused the forests to be destroyed and turned into corrupt coppice. Therefore, uncontrolled and unconscious grazing should be prevented. However, degraded forest areas in recent years have been rejuvenated (Kılıç, 2018).

One of the issues that negatively affect production costs in goats is the losses due to diseases. It is inevitable for breeders to take preventive measures in this regard. Considering the education level of the breeders, it is seen that they are mostly primary school graduates. Most of them have not received training on animal husbandry, and the training of those trained is insufficient. It would be beneficial to give training modules on animal husbandry to breeders.

Yield level should be increased in animals. For this, it is necessary to improve the maintenance and feeding conditions. The incentives and supports that the government has been implementing in recent years will contribute significantly to the increase in the existence of sheep and goats. In addition, improving the maintenance and feeding conditions is as important as improving the breeds in terms of increasing productivity. Breeders should also pay attention to product quality and hygiene issues (Ata, 2007).

As a suggestion, it is possible to say the following. One of the most important elements in livestock activities is marketing. Marketing is the most important and final stage of production. When it comes to animal marketing, the livestock market comes to mind. It is believed that the livestock market place is not used effectively.

Goat Products in Bingöl Province

Unfortunately, in most of the enterprises studied in the region, there are no suitable environments for animal husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to the country's economy. Meat and milk products from goat in Türkiye are presented in Table 2 and hair product from goats in Bingöl and Türkiye are presented in Table 3.

Table 2. Meat and raw milk products from goats in Türkiye (ton) (TÜİK, 2023).

Product	Year of 2022
Meat	94555
Raw milk	577208

Table 3. Hair product from goats in Bingöl and Türkiye (ton) (TÜİK, 2023).

Year	Bingöl	Türkiye
2019	92,865	6161,505
2020	94,294	6401,075
2021	90,416	6699,848
2022	87,416	6393,276

Demand for goat products was higher than the other animal products in Bingöl province. In a market-oriented definition, sustainability of production relies on maintenance of marketing and producer satisfaction accordingly. Producer satisfaction is interrelated with consumer

satisfaction, raising awareness and development of the market with a marketing point of view. Motivating consumer satisfaction is also related to raising producer satisfaction and improvement of marketing chains and structures in other terms. Lack of market demand and weak market structures also result in negative producer reflections and non-progressive stance in breeding.

The milk productivity of the indigenous Hair goat in the region is very low and it is more obvious especially on small farms. Improvement in productive and reproductive performance is the key for increase the milk production.

There are generally small businesses related to animal husbandry in the province. Considering the animal production situation in Bingöl Province, a significant part of which economy is based on animal husbandry, it is not at a sufficient level. Especially the meat production in Bingol province does not show the animal husbandry potential of the province. The main reason for this situation is that the number of slaughtered animals and most of the meat production are not carried out by official institutions or are not reflected in the statistics of Bingöl due to the slaughtering in other provinces (Esen, 2017; Kılıç, 2018). Thus, milk yield should be increased by improvements in animal production. In this way, milk yield could reach a desired level. There is a need to increase the milk production in relatively low performing dairy states to achieve future milk demand and make dairy farming sustainable for farmers (Işık and Gül, 2016). The number of sheep and goats, which create a heavy burden on the pastures, has decreased significantly. Although unconscious and untimely grazing continues as an important problem, there is a decrease in the problems caused by the number of animals grazing on the pastures. Sheep in pastures in Eastern, Southeastern and Central Anatolian regions, cattle breeding in other regions and plains and plains comes to the fore. Goat, on the other hand, can only survive on rough terrain. Again, due to the migration from the village to the city during this period, it is seen that the pressure on the pastures has decreased and the pasture vegetation has formed in the derelict areas.

The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the husbandry. Then, solution suggestions should be presented to remove or minimize these risks. Taking precautions for occupational health and safety are very difficult, costly and time consuming (Aygün, 2021a).

To producers related to livestock and plant production supports; subsidies have been implemented by the state in the fields of full-grown cattle and buffalo breeding, artificial insemination calves, breeding sheep and goats, combating animal diseases, scheduled vaccinations, breeding male cattle, and protection of animal genetic resources (TOB, 2023).

Bingöl province has an important potential in terms of ecological animal husbandry. Small ruminant husbandry is mostly done extensively. For this reason, very low input is used in sheep and goat breeding. The breeders experience marketing problems because the product they obtain is not sufficient. In order to overcome the marketing problem, breeders need to be organized. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the husbandry. Then, solution suggestions should be presented to remove or minimize these risks (Aygün, 2021b).

Conclusion

All scientific researches carried out so far have shown that small ruminant breeding in this region is done with traditional production methods. The most common of goat breeds in Bingöl province is the Hair Goat breed. The milk from this goat breed is either consumed by the household as raw milk or in making cream and yoghurt. As a result, preservation and development of native Hair goat breed as a genetic source is very important. If the current potential especially in the Eastern Anatolian Region and in Türkiye is evaluated, this breed

can become important in region. It can be said that the presence of goats has an important place in terms of the characteristics and animal production of the province of Bingöl. Goat breeding, which will contribute to the economy of Bingöl province, should be strengthened with planning and projects. In addition, breeders should be re-encouraged to obtain high yields from goats.

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BREEDING TECHNOLOGIES FOR HIGH LAMB CARCASS QUALITY

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Abstract

The aim of this work was to investigate the effects of different breeding technologies, including sex (male *vs.* female), breed (Improved Jezersko-Solčava *vs.* their crosses with Texel) and weaning age (early *vs.* late) of lambs on growth performance and carcass quality of lambs in Slovenia. Lamb production in Slovenia remains low, inconsistent and not uniform in carcass quality. Sex influenced growth and carcass traits. Females had a lower daily gain from birth to slaughter and a higher dressing percentage and carcass fatness than males. Texel crosses were found to have a higher daily gain from birth to slaughter than purebred lambs. The carcasses of Texel crosses had a better carcass conformation and were wider in leg and shoulder. However, there were no significant differences in carcass fatness between the breeds. Lambs weaned at 60 days of age grew faster (252 g/day) than lambs weaned at 120 days of age (223 g/day) and had a higher internal fatness. No differences were found in subcutaneous fatness between lambs weaned at 60 and 120 days of age. Based on the project results, finishing of weaned lambs and commercial crossing with Texel is recommended. Early weaning of lambs is recommended to take advantage of rapid growth ability. Daily gains of growing lambs from birth to slaughter should be above 270 g/day to achieve a slaughter weight of at least 37 kg at 120 days of age at slaughter. It is recommended to slaughter female lambs with a lower live weight than male lambs.

Key words: rearing technology, lamb meat quality, carcass traits.

Introduction

The European Innovation Partnership for agricultural productivity and sustainability (EIP-AGRI) was launched to contribute to the "Innovative European Strategy" for smarter, more productive and sustainable agriculture. The EIP-AGRI is a new European policy concept and brings together innovation actors (farmers, advisors, researchers, businesses, NGOs) to jointly develop solutions and opportunities for practical implementation. In Slovenia, such an agricultural policy concept is promoted and funded under the Rural Development Programme. In 2019 – 2021, the EIP project »Rearing various small ruminant breeds for high quality meat and meat products« took place in Slovenia. The partnership of the project linked operational groups of seven sheep breeders, agricultural advisory service, research institutions, a slaughterhouse and a public relations company, 12 partners in total.

In Slovenia, two main sheep production systems are characteristic, with a variety of breeding technologies depending on the breed of sheep, the time of weaning and the slaughter weight. The first is the production of light suckling lambs and the second is the production of heavier weaned lambs. Early weaning is characteristic of dairy sheep breeds (Bovec breed and Istrian Pramenka). These lambs are traditionally sold as suckling lambs for slaughter (Cividini et al, 2001), mainly to Italy. In the case of meat sheep breeds (Jezersko-Solčava and Improved Jezersko- Solčava breed), lambs traditionally stay with their mothers on pasture or in stable until they are slaughtered, when they reach a final weight of 25, 30 or 35 kg live weight. More

commonly, lambs of meat breeds are weaned and fed concentrates or cereals in the stable or pasture until they reach a slaughter weight of between 35 and 45 kg (Cividini et al., 2004).
The market for lamb in Slovenia is not yet well developed. In Slovenia, lamb production is still low and the slaughter weight and age of lambs are not uniform, mainly because of the small flocks and inconstancy of lamb production. The Slovenian market mainly offers whole carcasses. The offer of carcass cuts is rarely found on the market. The organisation of the purchase of lambs for slaughter is under development. The number of lambs slaughtered in slaughterhouses is still very low, but higher on the black market. The meat industry prefers consistent carcass quality and the retail sector needs meat products all year round.
Therefore, the EIP project conducted numerous studies investigating the effects of different breeding technologies on carcass traits, meat quality, meat sensory characteristics and fatty acid composition of intramuscular fat of lambs. For example, the effects of different slaughter and weaning weights/ages, industrial crosses with some terminal breeds, nutrition, fattening, breed and sex on carcass traits and meat quality of lambs were investigated. The aim of the EIP project was to recommend sustainable breeding technology that can improve carcass traits and meat quality of lambs. In addition, this breeding technology must also be applicable nationwide to ensure a larger number of lambs of the same quality.
This paper presents some improved breeding technologies that have been investigated in the project. The effects of the breeding technologies on growth and carcass performance are reported and discussed.

Materials and methods

Animals and experimental design
During the first six months of the project, the first study was conducted. Data from 58 lambs (27 male and 31 female) of two breeds (28 lambs of Improved Jezersko-Solčava (JSR) and 30 crossbreds (JSRT) with Texel) in two different farms/breeding technologies (60 *vs* .120 days of age of lambs at weaning) were analysed. All lambs had free access to water and from 10 days of age to concentrates. Lambs of both breeds and both sexes were presented on both farms and the study was conducted in the same year but under two different conditions regarding weaning system. Lambs were born in winter and suckled until 60 and 120 days of age, respectively, when they were weaned and fed with hay and concentrates for 50 days. Data were analysed using a GLM of the SAS statistical package (SAS Inst. Inc., 2014), with weaning system, breed and sex as fixed effects. Statistical significance between groups for the traits studied was reported at $P < 0.05$.
Later in the project, some studies focused on industrial crossbreeding with meat breeds such as Texel. One of these studies included a total of 27 winter-born lambs (15 male, 12 female) of two genotypes born and raised on the same farm. The lambs had free access to commercial concentrates from the age of 10 days. All lambs were weaned at an average age of 67.34 ± 12.2 days, when they reached an average live weight of 21.03 ±4.2 kg. On the day of weaning, the lambs were devided into two groups according to their breed. One group consisted of 13 (7 male, 6 female) Improved Jezersko-Solčava lambs (JSR) and the other 14 Texel crosses (JSRT). They were fed *ad libitum* with hay and commercial concentrates. The concentrate contained 18% crude protein, 8.4% crude fibre, 4.5% crude fat, and 7.2% crude ash. To monitor daily gains, the lambs were weighed at 10-day intervals. Lambs were slaughtered at an average weight of 39.2 ± 3.9 kg. Data were analysed using a GLM of the SAS statistical package (SAS Inst. Inc., 2014), whit breed and sex as fixed effects and slaughter weight as a linear regression. Statistical significance between groups for the characteristics studied was reported at $P < 0.05$.

Slaughter and carcass measurements

Slaughter and carcass measurements were carried out in the same way in all experiments. On the day of slaughter, the lambs were weighted just before transport to the commercial slaughterhouse. Daily gain from birth to slaughter was calculated using body weight and age at slaughter. All lambs were electrically stunned and slaughtered according to the standard commercial procedure. After slaughter, the hot carcass weight (HCW) was recorded and a dressing percentage of carcass was calculated. HCW was defined as the weight of the whole carcass after bleeding, removal of viscera, head, pelt, forefeet, hindfeet, tail and udder. The kidney with kidney fat and the knob channel fat belongs to the carcass.

The carcasses were stored at 17°C for 2 hours, and then refrigerated at 4°C for the next 24 hours. After the cold storage the weight, length, leg width and shoulder width of carcasses were measured. Carcass length was measured from the cranial edge of the pelvic symphysis to the cranial edge of the first rib. Leg and shoulder width were defined as the greatest width of the leg or shoulder measured in a horizontal plane on the hanging carcass. Carcass conformation was subjectively scored on a scale of 15 for E+ (excellent) to 1 for P- (poor) of the EUROP classification system (E - excellent, U - very good, R - good, O - moderate and P - poor), while carcass fatness degree was scored on a scale of 1 (1-, very low) to 15 (5+, very high) of the classification system (1 - low, 2 - slight, 3 - average), 4 - high and 5 - very high). Two different types of carcass fatness were assessed, subcutaneous and internal. The areas in the cross-section of the *Longissimus dorsi* (LD) muscles between the 12^{th} and 13^{th} thoracic vertebrae (at the loin cuts) were measured with a polar planimeter. The dressing percentage was calculated as the quotient between the HCW and the slaughter weight of the animal. The pH of the LD on the right side of the carcasses was recorded 45 minutes (pH 45) and 24 hours (pH 24) *post-mortem* behind the last rib using a pH-meter equipped with a penetrating electrode.

Results and discussion

The results of the studies are presented in Tables 1 and 2, where the least square means (LSM±SE) of growth performance and carcass traits for the observed effects are reported. Table 1 presents the data from the first study comparing rearing technology for lambs on two different farms. Lambs of both breeds and both sexes were presented in both farms and the experiment was conducted in the same year, but the two locations/farms differ in weaning system.

Table 1: Growth and carcass traits of male and female lambs of Improved Jezersko-Solčava (JSR) and their crossbreds with Texel (JSRT) from two farms (LSM±SE) from the first study

Growth and carcass traits	*Sex (S)*		*Breed (B)*		*Weaning system (W)**		*Sig.*		
	male	*female*	*JSR*	*JSRT*	*60 days of age*	*120 days of age*	*(S)*	*(B)*	*(W)*
DG, g/day	*245±4.9*	*230±4.6*	*232±5.3*	*243±5.3*	*252±6.0*	*223±5.8*	*	*ns*	**
EUROP	*8.69±0.26*	*8.61±0.24*	*8.06±0.28*	*9.23±0.28*	*9.06±0.31*	*8.23±0.31*	*ns*	**	*ns*
DP, %	*46.44±0.5*	*48.23±0.5*	*46.80±0.5*	*47.88±0.5*	*47.55±0.6*	*47.13±0.6*	**	*ns*	*ns*
SFat, 1-15	*9.10±0.3*	*10.57±0.3*	*10.29±0.3*	*9.38±0.3*	*10.18±0.4*	*9.49±0.4*	**	*ns*	*ns*
IFat, 1-15	*9.79±0.3*	*11.03±0.3*	*10.42±0.3*	*10.39±0.3*	*11.01±0.3*	*9.80±0.3*	**	*ns*	*
KPFat, %	*0.30±0.02*	*0.46±0.02*	*0.36±0.02*	*0.40±0.02*	*0.47±0.03*	*0.29±0.03*	***	*ns*	***
pH45	*6.55±0.03*	*6.45±0.03*	*6.55±0.03*	*6.45±0.03*	*6.51±0.04*	*6.48±0.04*	*	*ns*	*ns*

pH24	*5.62±0.02*	*5.58±0.02*	*5.61±0.02*	*5.59±0.02*	*5.58±0.02*	*5.62±0.02*	*ns*	*ns*	*ns*
Fat, %	*2.47±0.16*	*2.94±0.15*	*2.48±0.17*	*2.94±0.17*	*2.93±0.19*	*2.48±0.19*	*	*ns*	*ns*
LW, cm	*19.79±0.2*	*19.95±0.2*	*19.48±0.2*	*20.25±0.2*	*20.22±0.2*	*19.52±0.2*	*ns*	*	*ns*
SW, cm	*18.83±0.2*	*19.46±0.2*	*18.72±0.3*	*19.57±0.3*	*19.35±0.3*	*18.94±0.3*	*ns*	*	*ns*

*60 days of age: Lambs were weaned at 60 days of age; 120 days of age: Lambs were weaned at 120 days of age; EUROP=body conformation; DP=dressing percentage; SFat=Subcutaneous carcass fatness; Ifat=Internal carcass fatness; KPFat= Kidney-pelvic fat; pH45=pH of the muscle after 45 min after bleeding; pH24=pH of the muscle after 24 h after bleeding; Fat=Total fat; DG= Daily gain from birth to slaughter; LW=Leg width; SW=Shoulder width

Sig.=statistical significance; ns=not significant; *P<0.5; **P<0.01; ***P<0.001

The daily gains from birth to slaughter and some characteristics of the carcasses and meat quality of the slaughtered lambs were determined. Lambs weaned at 60 days of age grew faster (252 g/day) than lambs weaned at 120 days of age (223 g/day). Daily gain from birth to slaughter was influenced by sex and breed, being higher in males than females and higher in crossbreds than purebreds. In addition, lambs weaned at 60 days of age reached a higher slaughter weight (39.4 kg) and were younger at slaughter (126 days) than lambs weaned at 120 days of age (35.3 kg and 168 days, respectively). Commercial crossbreeding improves EUROP conformation and carcass width. Crosses with Texel (JSRT) had a better carcass conformation and were wider in shoulder and leg. However, there were no significant differences in carcass fatness between breeds. Sex influenced many carcass traits. Females had a higher dressing percentage but also had a higher carcass fatness score than males. Consequently, it is recommended that female lambs are slaughtered at a lower live weight than male lambs.

The results of the second experiment are shown in Table 2.

Table 2: Growth and carcass traits of male and female lambs of Improved Jezersko-Solčava (JSR) and their crossbreds with Texel (JSRT) (LSM±SE) from the second study

	Sex (S)		*Breed (B)*		*Sig.*	
Growth and carcass traits	*male*	*female*	*JSR*	*JSRT*	*(S)*	*(B)*
Daily gain from birth to slaughter, g/day	*285.1±6.6*	*268.5±7.8*	*271.6±7.3*	*282.0±7.3*	*ns*	*ns*
Dressing percentage, %	*46.8±0.4*	*49.0±0.4*	*47.3±0.5*	*48.5±0.5*	**	*ns*
Hot carcass weight, kg	*18.3±0.2*	*19.2±0.2*	*18.5±0.2*	*19.0±0.2*	**	*ns*
Cold carcass weight, kg	*18.0±0.1*	*18.8±0.1*	*18.2±0.1*	*18.7±0.1*	**	*
EUROP conformation	*7.9±0.4*	*8.6±0.4*	*7.4±0.4*	*9.0±0.4*	*ns*	*
Internal fatness, 1-15	*9.4±0.3*	*10.9±0.3*	*10.6±0.4*	*9.7±0.4*	*	*ns*
Subcutaneous fatness, 1-15	*8.6±0.3*	*10.9±0.3*	*10.0±0.4*	*9.5±0.4*	**	*ns*
Kidney-pelvic fat, %	*1.8±0.1*	*2.6±0.2*	*2.5±0.2*	*2.0±0.2*	*	*ns*
Carcass length, cm	*59.5±0.4*	*59.4±0.4*	*59.9±0.3*	*58.9±0.3*	*ns*	*ns*
Shoulder width, cm	*19.2±0.1*	*20.3±0.1*	*19.5±0.2*	*20.1±0.2*	***	*
Leg width, cm	*20.1±0.2*	*20.3±0.1*	*19.8±0.2*	*20.5±0.2*	*ns*	*
Rib-eye (Longissimus dorsi) muscle, cm^2	*14.3±0.4*	*14.2±0.4*	*13.3±0.4*	*15.3±0.4*	*ns*	**

Sig.=statistical significance; ns=not significant; *P<0.5; **P<0.01; ***P<0.001

In the second experiment, the effect of breed on some carcass traits was confirmed. Texel crosses had a better carcass conformation (9.0) compared to purebred Improved Jezersko-Solčava lambs (7.4). The best crossbred carcasses were classified in class U and the best of the JSR in class R. This agreed with the results of Scales et al. (2010), where the Merino x Texel carcasses were classified in class E, while the purebred carcasses were classified in the lower class (U). In the second study, the carcasses of crossbreds were wider at the shoulder

and leg (P<0.5) than the carcasses of pure breed JSR lambs. The surface area of the rib-eye muscle (*Longissimus dorsi)* was larger in JSRT than in JSR carcasses for 2.0 cm^2 (P<0.01). Similar results for muscle surface area are also found by Scales at al. (2010), where Merino x Texel crosses had a larger rib-eye muscle surface area than purebred Merino lambs. However, the daily gain of the crosses was higher in JSRT (282 g/day) than in JSR (271.6 g/day), but the difference was not statistically significant. JSRT lambs had a higher cold carcass weight than JSR lambs. In agreement with the results of the first and second study, Nunes et al. (2019) found that Texel crosses had significantly higher carcass conformation scores than purebred lambs, while no difference was found in the fatness of carcasses between the genotypes considered. Nunes et al. (2019) found no difference in rib-eye muscle area or leg width, while our study found that Texel crosses had significantly larger rib-eye muscles and wider legs than purebred JSR lambs.Sex influenced daily gain and carcass traits in both studies. Male lambs had a significantly higher daily gain from birth to slaughter (245 g/day in the first and 285 g/day in the second study) than female lambs (230 g/day in the first and 268 g/day in the second study). However, the differences were statistically significant only in the first study. Perez et al. (2007), Bizjak and Cividini (ASD, 2020) also reported faster growth in male than in female lambs. The carcasses of male lambs had a lower dressing percentage as well as a lower carcass fatness score. In addition, the carcasses of male lambs had a lower value for external and internal fatness. These results were confirmed by many studies (Pouliot et al., 2009; Facciolongo et al., 2018; Nunes et al., 2019; Scales at al., 2010), in which the carcasses of female lambs of different breeds had a higher fatness.

The results of our studies, showed daily gains between 223 g/day and 285 g/day when using the rearing technology of early weaning (at about 60 days of age) and commercial crossing with Texel. Finishing the lambs after weaning resulted in higher weights at slaughter, better carcass conformation with little or even no negative effect on the carcass traits of the lambs. The breeds mentioned, such as the Improved Jezersko-Solčava and its crosses with Texel, can be fattened up to a live weight at slaughter even above 40 kg as reported in previous studies (Žgur et al., 2003; Cividini et al., 2004) and some other studies from the EIP project (not yet published).Hot dressing percentage ranged from 46.44% to 49.00%, with final body weights at slaughter of 35.3 kg and 39.4 kg. Many authors reported a higher dressing percentage of lambs slaughtered at higher body weights compared to lambs at lower body weights. The results of previous studies for Slovenian breeds confirmed the lower dressing percentage (45.82 %) for lambs slaughtered at about 30 kg (Cividini, 2021).

Carcasses were classified in EUROP classes U and R and for subcutaneous fatness in classes 2 and 3. For both traits, carcass conformation and carcass fatness there was only one class difference.

Conclusions

The efficiency of lamb production on the Slovenian market can be improved by increasing the number of lambs per ewe using the proper breeding technology, with earlier weaning and controlled crossbreeding programmes. In addition, finishing weaned lambs can improve growth and carcass traits and allow marketing of higher weight lambs for confectionery purposes. However, the results of the studies clearly indicate that the use of Texel rams for commercial crossing with JSR ewes improves carcass traits and growth performance of the lambs. Early weaning of the lambs enable earlier mating of the ewes and exploitation of the rapid growth period of the lambs in the first months of their life.

The number of lambs born per ewe per year is the key to economic farming. The higher it is, the higher the coverage rate of the farm (results of the calculation of the profitability of livestock farming during the EIP project-not yet published). The meat of lambs bred in this

way should in any case fetch a reasonable price, so that the recommended technology could be economically viable for farmers.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

DIVERSIFICATION OF AGRICULTURAL VALUE CHAINS AND RURAL DEVELOPMENT IN MALI: CASE OF KITA CERCLE

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Abstract

This paper aims to examine the added value of agricultural value chain diversification in rural areas development in Mali, as illustrated by the case of Kita Circle. To do so, qualitative and quantitative data were collected in May 2023 among 97 surveyed persons through a questionnaire. Descriptive and comparative methods were used. Focus groups were also organized in order to evaluate the benefits and challenges of value chain diversification. The results demonstrated the role of value chain diversification in rural development. While diversifying their production systems, farmers are able to increase their income by expanding production and accessing new markets. Diversification so contributed to poverty reduction and improved living standards in rural areas: farmers have spread their risks and reduced their dependence on one product and one market. Diversification also contributed to food security, malnutrition reduction and health improvement. In addition, it also promoted environmental sustainability by reducing pressure on natural resources, such as land and water. Despite such positive aspects, challenges are still to be addressed. Access to credit is uneasy for farmers who would like to invest in new crops and technologies. Mali is also vulnerable to climate change effects and farmers have a limited access to markets for high-value products like fruits and vegetables, which limits the prices they can get and the diversification possibilities.

Keywords: *diversification, rural development, Mali.*

Introduction

Agriculture forms the backbone of Mali's economy, with the sector playing a vital role in driving economic growth, ensuring food self-sufficiency, and providing livelihoods to a significant portion of the population. Mali heavily depends on the cultivation of a single or limited range of crops to ensure national food security, a trend commonly observed in many African countries. The agricultural sector accounts for approximately 36% of Mali's gross domestic product (GDP) and engages nearly 80% of the population (Maiga *et al.*, 2019). While this reliance on agriculture signifies its crucial role in the country's economy and livelihoods, it also exposes Mali to significant risks associated with climate change, market fluctuations, and pests or diseases affecting specific crops. Thus, diversifying agricultural activities becomes essential to mitigate these risks and promote sustainable economic development. Diversification refers to the strategy of cultivating multiple crops and engaging in livestock production simultaneously, aiming to improve income and livelihoods. This practice offers farmers a more reliable and varied source of income (Dembele *et al.*, 2018).
Rural development refers to improving the economic, social, and cultural conditions of rural areas while integrating them with society as a whole (Guinjoan *et al.*, 2016). The contemporary understanding of rural development entails a comprehensive and multidimensional approach to promote sustainable progress in non-urban areas, recognizing

the need for holistic development in response to challenges like depopulation, aging population, landscape stagnation, and socio-economic disparities, emphasizing the crucial role of diversifying the rural economy as a prerequisite for achieving demographic stability and overall sustainability (Croatian Rural Development Network, 2023). Upon reviewing the provided definition, we can conclude that Rural Development refers to improving economic, social, and cultural conditions in rural areas while integrating them with society. It involves diversifying the rural economy to address challenges like depopulation and the aging population, ensuring sustainable development for the overall well-being of rural communities.
Despite the recognized importance of agricultural diversification, developing countries like Mali face challenges that hinder their progress. Limited availability and accessibility of essential agricultural resources, as well as institutional constraints such as inadequate support services and weak market infrastructure, pose significant barriers to diversification (Kasem & Thapa, 2011; Nguyen, 2017). Insufficient diversification in agricultural value chains negatively affects the production of essential commodities like cash crops, food crops, and livestock products. This lack of diversification leads to decreased income, heightened food insecurity, and increased poverty among smallholder farmers (Ellis and Freeman, 2004; Okoboi and Barungi, 2012; Makate *et al.*, 2016). Therefore, promoting agricultural value chain diversification is crucial to enhance income generation, food security, and poverty reduction among smallholder farmers. In this context, this study aims to explore the unique situation of the Kita Circle in Mali, evaluating the economic, social, and environmental dimensions of value chain diversification. By analyzing qualitative and quantitative data and considering the experiences and viewpoints of farmers in the region, the research seeks to uncover the benefits and limitations of value chain diversification. The findings of this study aim to provide valuable insights for policymakers and practitioners to address the identified problems and fully realize the potential of agricultural value chain diversification for rural development in Mali.
The research aims to explore the potential of agricultural value chain diversification in the Kita Circle of Mali for promoting rural development. It seeks to achieve two main objectives: 1) Explore the advantages of agricultural value chain diversification for rural development; 2) Identify and analyze the challenges hindering diversification in the Kita Circle.

Materials and Methods

The study was carried out in May 2023 in the southern-west parts of Mali, with a particular focus on the Kayes region in the Kita Circle. The selection of these locations and agricultural cooperatives was based on the targeted areas of intervention by Caritas Suisse, with the objective of including a significant number of cooperatives and farmers engaged in diverse agricultural value chains. Caritas Suisse provided assistance to cooperatives situated in six communes within the Kita district, namely Bendougouba, Benkadi Founia, Bougaribaya, Djidian, Sébékoro, and Sirakoro. The project aimed to benefit a total of 600 households, consisting of approximately 3,950 individuals, who formed the population of study for this research. The questionnaires included open-ended questions and closed questions face-to-face with respondents. Qualitative data were collected using a five-point Likert scale enabling respondents to indicate their level of agreement or disagreement with statements on a scale ranging from "Absolutely Disagree" to "Completely Agree." The scale assumes equal intervals between each point, providing a standardized rating system. Secondary data sourced from the project report was utilized to provide insights into the impact of agricultural diversification on income levels. This secondary data allowed for an examination of the extent to which income increased as a result of engaging in diversified agricultural practices. By incorporating both primary and secondary data, a comprehensive understanding of the

relationship between agricultural value chain diversification and income generation could be achieved, thereby contributing valuable insights to the research findings.
In order to ensure a precise representation of the communes, a two-stage stratified sampling technique was employed in this study. During the initial stage, a sample was selected from the six communes benefiting from the project. In the subsequent stage, the sample was further refined by specifically targeting cooperative members who fulfilled certain criteria. These criteria included being active members of Caritas Suisse-supported cooperatives for a minimum of three years and actively participating in the diversification of the value chain through various crops such as maize, sesame, groundnuts, and vegetables. This two-stage approach was adopted to ensure that the collected data accurately reflected both the beneficiary communes and cooperative members actively engaged in value chain diversification. The table below provides a complete overview of the sampling data received from six communes.

Table 1. Sampling by commune and value chain diversification

Communes	Number of Respondents Selected
Bendougouba	13
Benkadi Founia	22
Bougaribaya	12
Djidian	15
Sébékoro	14
Sirakoro	21
Total respondents	**97**

*Source: Author's field survey elaboration, May 2023

Limitations

Data collection in rural Kita posed challenges linked to deficient infrastructure, like roads and electricity. In certain villages with inaccessible roads, we had to arrange transportation for respondents from nearby centers, incurring additional costs. Additionally, the presence of low literacy rates necessitated more time for clarifying questionnaires and recording responses. Furthermore, security concerns in specific Kita locations led to delays in our research, prompting the implementation of precautionary measures to ensure effective data collection.

Results and Discussion

Diversification of Value Chain Participation by Commune

While the research primarily focuses on agriculture within the three value chains of maize, vegetables, and peanuts, it is important to recognize that not all participants are engaged in all three value chains. There is variation among respondents, with some involved in all three, others in two, and some in just one value chain, often complemented by additional value chains outside of the aforementioned three. This is why categorizing the respondents based on their value chains and geographical areas is crucial. This categorization enables a comprehensive understanding of the unique dynamics and specific needs of each value chain, leading to more effective and targeted efforts in agricultural development. The table 2. below provides an overview of the distribution of value chain participation by communes.

Table 2. distribution of respondents by commune and value chain

Communes	Maize, vegetables, peanuts	Maize and vegetables	Maize and peanuts	Vegetables and peanuts	Others
Bendougouba	12%	3%	11%	11%	0%
Benkadi Founia	3%	0%	0%	0%	0%
Bougaribaya	5%	2%	0%	6%	0%
Djidian	10%	6%	10%	9%	0%
Sébékoro	0%	0%	1%	7%	0%
Sirakoro	0%	1%	0%	3%	1%

*Source: Author's field survey elaboration, May 2023

Based on the data, it is evident that each commune has variations in value chain participation. To effectively support agricultural development, it is recommended to tailor interventions and resources according to the specific needs and dynamics of each value chain and commune. By focusing on the predominant value chains in each area and considering the unique requirements of participants, targeted efforts can be made to enhance productivity, increase market access, and address challenges within each value chain. Additionally, further research and data collection could help identify opportunities for diversification or expansion into other value chains that may have untapped potential in certain communes.

Assessing the Respondents' Comprehension of Agricultural Diversification

While delving into the benefits and drawbacks of agricultural value chain diversification, the researcher aimed to assess respondents' understanding of the concept of agricultural value chain verification below responses were collected: Agricultural value chain diversification is the process of expanding and diversifying the range of products and activities within the agricultural value chain. It includes introducing multiple crops, developing their by-products, and organizing various links in the value chain to enhance networking and increase income. The main objectives are to promote sector development, improve the resilience and sustainability of agricultural systems, and create more economic opportunities for farmers and stakeholders. Diversifying crops helps mitigate risks associated with mono-cropping, reducing vulnerability to pests, diseases, and adverse weather conditions. By cultivating different crops on the same farmland or rotating crops across seasons, farmers can spread risks and potentially achieve higher overall yields. Additionally, diversification involves organizing various actors in the value chain, such as farmers, input suppliers, processors, wholesalers, retailers, and consumers. Strengthening linkages and networks among these actors leads to a more efficient, transparent, and market-responsive value chain. It also enables knowledge sharing, technology transfer, and collective decision-making, ultimately resulting in improved productivity and income generation. Based on the information presented, we can infer from the respondents' feedback that: *Agricultural value chain diversification involves the expansion and diversification of products and activities within the agricultural value chain. The main objective is to enhance sector growth, market structure, and income generation. This entails introducing various crops and their by-products and establishing and reinforcing the relationships among various stakeholders involved.*

Active Actors Driving Agricultural Diversification in the Kita Circle

In the Kita Circle, several organizations and initiatives are actively promoting agricultural diversification to support farmers and encourage sustainable practices. Caritas Suisse's Kita III project focuses on training and infrastructure development, particularly for vegetable

gardens, to enhance the region's agricultural potential and resilience. They aim to reduce the use of mineral fertilizers for land restoration and income improvement. Stop Sahel, an NGO, supports farmers with activities like vegetable growing, tree planting, and providing groundnut seeds. Other stakeholders like AGRECO, PDRIK, Caritas Mali, MPDL, CMDT, and JMI are also involved in diversifying value chains and promoting income-generating activities, encouraging the cultivation of various crops alongside traditional cotton production. These efforts collectively aim to facilitate a shift towards more diverse and sustainable agricultural practices in the region.

Benefits of Agriculture Value Chain Diversification

The findings from participants who have experienced the benefits of value chain diversification in agriculture and its impacts on rural development have been summarised in the table below:

Table 3. Advantages of agriculture diversification in Kita

Advantages	**Description**
Risk Management and Improved Nutrition	Reduces negative environmental impacts of mono-cropping; Minimizes the effects of pests and unexpected occurrences; Improves food security and nutrition by offering a wider variety of crops
Income Opportunities and Poverty Reduction	Creates income opportunities for farmers; Allows exploration of new crops or products; Enters more profitable markets; Increases farmers' incomes and improves food security
Market Access, Value Addition, and Processing	Enables farmers to cater to diverse consumer demands; Expands customer base; Captures a larger share of the value chain through processing and transformation activities; Reduces rural-urban migration
Resilience to Market Fluctuations and Adaptability	Spreads risks by diversifying production and marketing strategies; Enhances resilience to market fluctuations
Environmental Benefits and Sustainability	Promotes sustainable agricultural practices; Mitigates soil degradation; Preserves biodiversity
Rural Economic Growth and Job Creation	Generates employment opportunities in rural areas; Stimulates economic growth

*Source: Author's field survey elaboration, May 2023

Additional information has been collected from both focus group discussions and secondary data from the project report. Respondents from FGD test that diversification has played a significant role in empowering farmers, enhancing food security, and improving the overall well-being of farming households: "*In my opinion, the cultivation of maize, groundnuts, and vegetables by farmers receiving support from Caritas Switzerland's intervention in Kita is a highly effective approach to combat food and nutritional insecurity. By diversifying their agricultural activities and cultivating these crops, farmers ensure a diverse and nutritious diet for their families. Moreover, the promotion of crop processing, such as maize meal and flour, paste, and groundnut cocoa, has proven beneficial for farmers in Kita, providing additional income and contributing to food security. The success of COPROSEM cooperative in Kita, which initially focused on onions and expanded to include crops like gombo and tomatoes, serves as a compelling example of how diversification positively impacts rural*

development. COPROSEM's seeds being sold by major agricultural input suppliers in Mali further underscores the recognition and acceptance of their products in the market" (Sadia DIARRA, Sébékoro commune/Kita Circle).

Furthermore, data were gathered from project reports in March 2023. The data revealed that more than half of the farmers who received support experienced a boost in their income. Findings from a survey conducted by the project team and analyzed in KOBO software between January and May 2022 indicate that 229 maize-producing households in the project's four intervention communes (Bendougouba, Benkadi Founia, Djidian, and Sébékoro) observed an approximate 15% increase in their earnings since the project's initiation. This figure represents 54.52% of the final target of 420 households. The initial feedback on the satisfaction level related to the implementation of marketing plans in 2022 suggests a significant increase in households, confirming the growth of their income in 2022.

Effect of Diversification on Rural Development

Diversification in agriculture has a profound impact on rural development. Firstly, it strengthens farmers' ability to cope with unexpected events and challenges by reducing their vulnerability to pests, diseases, and climate variations. This leads to more stable incomes and livelihoods, making farmers resilient and adaptable to changes in their environment. By enhancing their capacity to withstand shocks, diversification contributes to the long-term sustainability of agricultural systems. Secondly, crop diversification promotes sustainable farming practices and environmental conservation. Through techniques such as crop rotation, agroforestry, and reduced pesticide use, farmers prioritize the health of land, water resources, and biodiversity. By acting as stewards of the environment, farmers contribute to the preservation of ecosystems and soil health, mitigating environmental degradation. This sustainable approach ensures the long-term viability of rural communities and safeguards natural resources for future generations. Furthermore, crop diversification drives economic growth, poverty reduction, and improved living conditions in rural areas. It leads to increased incomes for farmers, creates employment opportunities, and stimulates entrepreneurship and economic diversification in rural communities. By reducing income disparities and addressing rural-urban migration, diversification promotes balanced regional development. Moreover, diversification enhances farmers' access to local and international markets, improving the economic viability and competitiveness of the agricultural sector. This, in turn, uplifts livelihoods, raises the standard of living, and reduces unemployment rates, fostering self-sufficiency and resilience within the rural economy.

Challenges in Diversification

Despite the numerous advantages of diversification in agriculture, there are several challenges which hinder effective diversification and have a negative impact on rural development. The table 4 is the summary of feedback collected from respondents.

Table 4. Challenges and their impacts on diversification and rural development

Challenges	Impact on Diversification	Impact on Rural Development
Limited financing and credit	Inhibits investment and restricts innovation.	Hampers economic growth and job creation.
Limited availability of inputs	Hinders adoption of modern practices and crop diversification.	Reduces productivity and limits food security.
Inadequate infrastructure	Impacts transportation, storage, and market access.	Reduces efficiency and hinders market expansion.
Gender inequalities in land ownership	Limits women's participation and underutilizes their potential.	Reinforces gender disparities and hampers inclusive development.
Insufficient technical knowledge and skills	Constrains productivity and competitiveness.	Hinders innovation and technological advancements.
Lack of suitable seed varieties	Restricts crop diversification and resilience to market demands.	Reduces adaptability and income opportunities.
Disease management	Impacts crop productivity and agricultural sustainability.	Reduces incomes and threatens food security.
Limited market access	Hinders sales of diversified products and market expansion.	Reduces income opportunities and market integration.
Climate-related challenges	Reduces crop yields and increases vulnerability to shocks.	Impacts productivity, food availability, and requires adaptive practices.

*Source: Author's field survey elaboration, May 2023

Conclusions

The research findings indicate that diversifying agricultural value chains in the Kita Circle has significant advantages in fostering rural development. It positively impacts food security, income generation for farmers, risk management, poverty reduction, market access, value addition, job creation, resilience to market fluctuations, and environmental sustainability. However, there are several challenges and barriers that impede the process of agricultural value chain diversification and its impact on rural development. These challenges include limited access to financial resources, inadequate availability of inputs, insufficient infrastructure, gender disparities in land ownership, lack of technical expertise, limited market reach, climate change-related difficulties, and disease management. It is crucial to address these challenges through appropriate interventions to fully harness the potential benefits of agricultural value chain diversification and ensure sustainable rural development in the Kita Circle. To address challenges in agricultural value chain diversification in Mali, several recommendations have been proposed for different stakeholders:

- *Collaboration and Coordination:* Reinforce the presence of support services and research institutions which can contribute to the success of diversification efforts. State and private entities, including research organizations operating in Mali such as Institut d'Economie Rurale (IER), Institut International de Recherche sur les Cultures dans les Zones Tropicales Semi-arides (ICRISAT), and NGOs like Caritas Suisse, should provide valuable guidance, training, and knowledge to farmers.

- *Gender Equality:* Address gender inequalities in land ownership and resource access through legal reforms, awareness campaigns, and capacity-building initiatives to empower women farmers.
- *Climate Change Adaptation:* Prioritize climate change adaptation strategies (conservation agriculture, agroforestry, water management) and support farmers in accessing climate information, and insurance schemes,
- *Financing:* Collaboration among governments, financial institutions, and development organizations to offer affordable and accessible financing options such as agricultural loans and microfinance for diversification. Implementing these recommendations will enhance collaboration, empower women, build climate resilience, and provide financial support for agricultural value chain diversification

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THE ECONOMIC IMPORTANCE OF AGRICULTURE AND TOURISM FOR THE ECONOMIC DEVELOPMENT OF BOSNIA AND HERZEGOVINA

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Abstract

In the last decade of the 20th century, economic stability in Bosnia and Herzegovina was based on the assumption of transition to market conditions. The aim of the paper is to analyze the role of agriculture and tourism in the economic reform of Bosnia and Herzegovina. In the study, it was established that the changes in the economy developed in the area of transformation of ownership rights in the environment of liberalization of trade chains and investments, instability of the currency exchange rate, increase in costs, reduction of the social product, occurrence of inflation. All these factors have influenced material and human resources and the transformation of agriculture and tourism in Bosnia and Herzegovina. At the time of the transition, unemployment of people, changes in the ownership structure, changes in activities, introduction of new technological processes, and dismissal of employees were expressed. Agriculture and tourism had capacity for the development of entrepreneurship and new investments in innovative programs of agricultural production and tourist services. In the studies, it was established that the role of agriculture in the economic development of Bosnia and Herzegovina is different depending on the level of economic growth itself. It was also established that tourism has a multiple impact on economic development, due to the demand for agricultural and food products, accommodation capacities, recreational and cultural facilities, traffic communications.

Keywords: *economic development, agriculture, tourism, transition, reforms*

Introduction

Economic development in an country based on changes traditional technology to modern technology, on intensive industrial development and high-income service economy which contributes to improving Gross Domestic Product (GDP). However, agriculture have share in economic development in an country through ensuring food security and improving nutrition and accelerate growth in GDP per capita (Loizou et al., 2019). For progress in agriculture was very important to make modern technologies available in both public and private sector, as well important role of government investing in agricultural research and development. Modernization in industry contributes to raising incomes and productivity of poor farmers, lowering food prices, and modernization in agriculture can improve better nourishing and avoiding of malnutrition the population.

The natural resource base on which agriculture depends is poor and deteriorating. Productivity growth is more difficult to achieve. The integration of rural and urban areas affected the change in the population structure, by reducing the number of young and healthy people in the countryside, where old people, sick and with reduced ability to work remained. During the

global technology change and increasingly integrated markets, prices fall faster than yields rise. So, rural incomes fall despite increased productivity if they are net producers. Liberalization in the 1990s and greater openness to trade led to a reduction in the economic potential of the rural sector, which was greatly influenced by the import of cheap Chinese plastic products, which are cheaper and competitive with local pottery, wood and metal products. Agriculture have role in Economic growth based on agricultural resources which are involved in function of industrial sector and urban centers, service sector and other economy branches as well as construction tourism, mainly for providing food, and u has a large share in the increase in employment in food production, to provide food supply for increasing population, for feeding workers in other economy branches, to provide surplus for increasing exports. Raising supply of foods by agriculture sector has ,therefore, great importance for economic growth of a country (Anderson, 1987; Anderson and Ponnusamy, 2019).

Agriculture has large share in growth gross domestic product (GDP) primary before industrial development. As the process of industrial development accelerates , the share of nonagricultural sector in GDP tends to increase steadily. In the same time the relative share of agriculture decline and increased share of manufacturing and service sectors. This does not imply that the agricultural production does not increase. It only implies that the growth in production of industrial and service sectors is faster than the growth in agriculture sector. Also, as the economy strengthens, the share of agriculture in GDP and employment tends to decline, as a result of lower demand for food compared to other goods and services and rising incomes, and because of the faster development and introduction of new technologies for agriculture than for other sectors (market, traders), leading to expansion higher productivity and food supply per hectare and per worker. In addition, the relative reduction of the share of agriculture in GDP was influenced by the rapid growth of modern economies in the use of intermediate (chemicals, machines) investments purchased from other sectors which over the time become more important than labor, land and capital which contribute decreasing of their share in gross value of agricultural and in employment (Anderson, 2023). The results of this is that producers receive a lower price and in return for which their households spend less time marketing.

Tourism is strongly associated with agriculture and with a wide range of activities contributes to increasing economic and social development in country. The development of tourism on local and international level increased by development of globalization process of integration of numerous activity, economy, science, culture, sport etc. Tourism can generate development of national economy and accelerate investing in infrastructure, accommodation, services, natural and social heritages, innovation of standardized products with favorable prices (Herman et al., 2017). Tourism classified as invisible export, which means domestic tourism can be assessed as an export from the domestic region to another local region. International tourism includes immigration procedures and money exchanges that are recorded, which facilitates monitoring of the effects on the national economy (Bianchi, 2018; Cooper et al., 2008). Tourism activity have effect on other economic activity, but influence on increasing food prices and cost of living (Gavranovic, 2018). The effects of domestic tourism are much more difficult to measure than the effects of international tourism, what is very important for economic policy system for investment in development and improving insufficient activity in sector of tourism (Demirović and Radosavac, 2018).

The aim of the paper is to analyze the role of agriculture and tourism in the economic development of Bosnia and Herzegovina and role of government for improvement and modernization of tourism and agricultural cooperation.in function of economic development.

Agriculture and economic development

The disintegration of the SFRYU influenced the appearance of war in Bosnia and Herzegovina, which, together with the bombing of the territory of Bosnia and Herzegovina by NATO forces, influenced the destruction of natural resources, agricultural areas, tourist sites and facilities, settlements. Also, during the war period (1992-1995) disrupted work of existing economic entities and infrastructure. After this period foreign financial donation focused on rural infrastructure and housing, agric. machinery, seeds, fertilizers, revitalization of crop production and population in rural area. This is very important considering that in Bosnia and Herzegovina have 2.1 million hectares of agricultural land, of which 46.5% is arable and as much as approximately 50% of arable land remains unused. That is great potential for agricultural production. After the period of war (1992-1995) over 50% population lives in rural area in B&H. Agriculture was primary economic activity, and international investments focused on rural infrastructure and housing, agricultural machinery, seeds, fertilizers, revitalization of crop production and population in rural area. The share of 61% rural population in 1995 year decline to ~48% in 2023 year in B&H (Figure 1). During this period international financial resources are mostly invested on production (38%), banking sector (21%), service sector (5%), trade (9%), transport (0.3%) and tourism (1.0%) Figure 2 (World Bank, 2022).

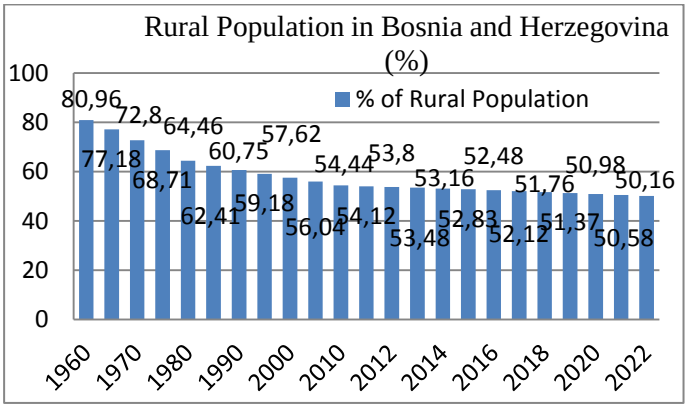

Figure 1. Dynamics of rural population in B&H

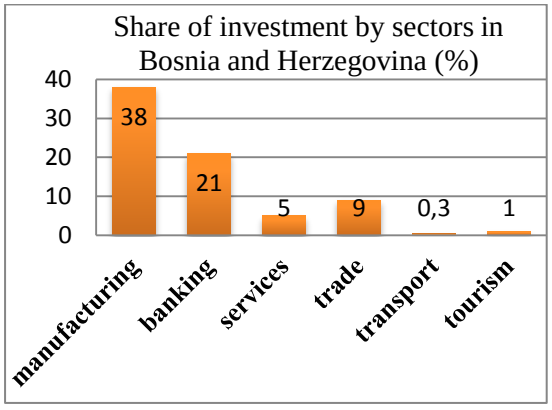

Figure 2. Share of international investment by sector in B&H

Rural areas characterized a lack of physical infrastructure, culture and social infrastructure as well as job opportunities. In Bosnia and Herzegovina. Agriculture is mostly privately owned with domination of small farms with insufficient competiveness, with low potential of quantity production and could not provide enough food, which is why B&H was a food importer. Farming and related activities make base for development of rural life, contributing significantly to developing rural regions in terms of employment and business opportunities, infrastructure and quality of the environment, what is important source of livelihood (Figure 3).

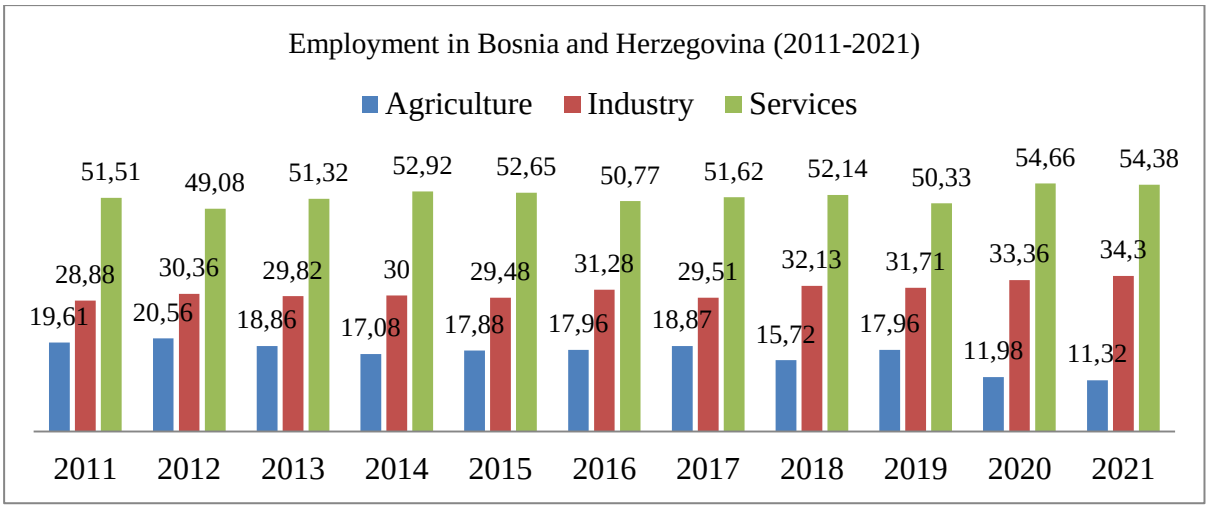

Figure 3. Comparison of employment in agricultural sector, industry and services

The share of farming in rural economy indicate level of intensity of agriculture and its, economic contribution to rural development in B&H, what linked with political and economic system. In B&H the general characteristic of agriculture is low size of farms ~4-5 ha, which are formed from small parcels, often dislocated from each other, which makes the production costlier and less income. Some 250.000 farms (50%) are less than 2 hectares, and 400.000 or 80% are less than 5 hectares, while only 4% have areas over 10 hectares (EC, 2006). This structure of agricultural farms is a reason why is low cooperation food processing industry on long term contract with farmers (Ministry of Foreign Trade and Economic Relations (MoFTER 2012). Also food industry is not able to take role for convergent connectivity with smallholders, as well as smallholders are not jointly organized to offer contract quantity and quality of products, and on the other hand have no power offer to foreign market because farmers are not able adopt require standards. Domestic producers have narrow assortment of product, and rest of market left to import companies what is reason that processing industry have choice on domestic market (Federal Ministry of Agriculture Water Management and Forestry, B&H, 2019).

The main problem of the agricultural sector is low productivity per unit of production, and low productivity per farm. The low productivity caused by lack of extreme dependence on weather conditions producer specialization for crop production, low technology levels of farms and low knowledge of marketing. The rural population as a whole has around 25% less years of education than the urban population. Women have around 15% less education than men – in rural and in urban area. In the 21st century rural girls receive around 4% less education than boys, and children in rural areas average as many or more years of education as those in urban areas (UNDP, 2013). Particularly important is adoption of modern technologies in production process and knowhow which helps farmers for including in market infrastructure to access these inputs and sell their agricultural. The government need invest in forming human capital to ensure a skilled labor force to master new technology, handle logistics from production to trade. Economic development is a process of structural transformation, in which agriculture plays a driving role in the accelerated initiation of processes through cross-sector linkages through which agricultural growth supports overall economic growth as a rule, agricultural production is a activity of the rural economy and generates significant economic value for the country B&H.

The growth of Gross Domestic Product (GDP) after period (1992-1995) was high rate up to 1999 (~29%) due to an extremely low starting base, than growth of GDP reduced from 2000-2002 and from 2003 growth of GDP showed partial stagnation up to 2007, and negative value in 2008 year. The high foreign trade imbalance and high trade deficits, are the main

causes of the negative current account balance Ministry of Foreign Trade and Economic Relations (MoFTER 2012).

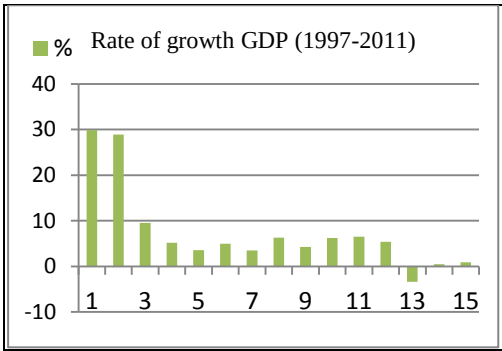

Figure 4. Rate of growth GDP in B&H

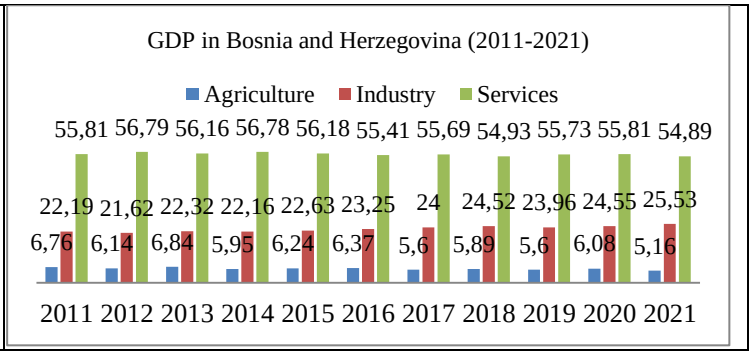

Figure 5. Share of GDP by sector in B&H

Source: World Bank, 2010; 2022

The high negative trade balance is significantly affected by import of agri-food products. The primary imported food products include: grains and grain products, beverages (alcoholic and non-alcoholic), and meat and dairy products. The share of agriculture in GDP is constantly decreasing (10% in 2000 to 6% in 2018), employing 16% of workforce (Figure,3. 4. 5) (World Bank, 2010; 2022). In 2021, agri-food import was $20.6 billion, while export was valued at $552.6 million. Share of agricultural products in export is ~6%, while share in total import is 15%.

The complex constitution structure of Bosnia and Herzegovina with two entity Republic of Srpska, Federation of Bosnia and Herzegovina (consist 11 cantons) plus district of Brcko (BD) has influence on govrement management competence and capacity of agriculture, involving different regulationsat different levels, limited capacities and communication channels, different vision and failure to implement necessary reforms

Tourism and Economy in Bosnia and Herzegovina

In Bosnia and Herzegovina there are numerous natural and social resources of tourisms'. Some of them are destroyed, neglected and require financial investments for the needs of tourist activity. In the period after 1995, tourism resources were renewed individually, which generated the development of other economic activities. In addition, tourism in Bosnia and Herzegovina represents one of the important economic activities that contributes to growth gross domestic product GDP through job creation and export. Tourism contributed to the increase in employment labor and increasing the procurement of local food products, what generate establishing small and medium enterprises (SME) in or near major tourism centers. The development of tourism included joint activities associated with cultural, mountain and winter; eco and rural; outdoor and adventure; sport; religious; and health and spa tourism. These activities have provided consistent employment opportunities (12% of total employment in the country) and contributed to economic growth ~10.5% of GDP (WEF, WTTC, 2019) Figure 6.

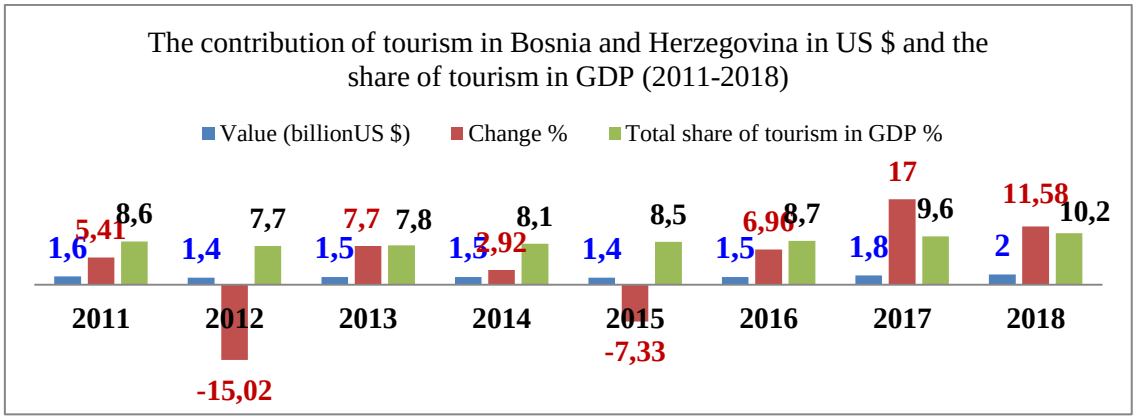

Figure 6. Share of tourism in GDP in Bosnia and Herzegovina.
Source: Agency for statistics Bosnia and Herzegovina, 2021

The achieved success in tourism sector's is largely the result of individual efforts in the private sector, what mean that tourism is not planned and strategically supported. This statements require clear strategy to promote tourism and tourism opportunity in B&H. Tourism in B&H directly generates about 2.7% of GDP (Mrkaić Ateljević and Popović, 2020), which have contribution to accomodation services, catering, retail-trade, hospitality, transportation, sports, entertainment and recreation, national parks or museum. An increase in tourist arrivals as well as in overnight stays as well as in overnight stays was achieved in Bosnia and Herzegovina. Over the last ten years, from 2010 to 2020, the number of tourist arrivals increased by 250% and the number of overnight stays by 87%, indicating an increased demand in tourism (Figure, 7 and 8).

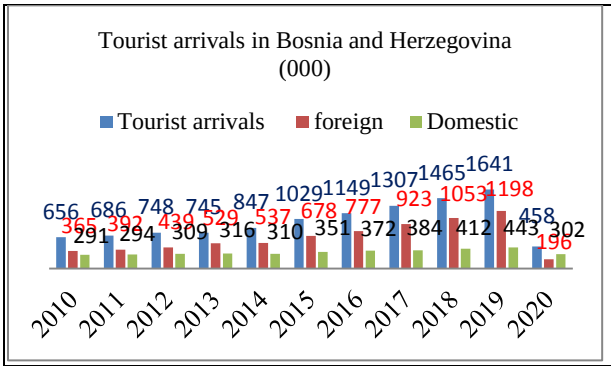

Figure 7. Tourist arrivals in B&H

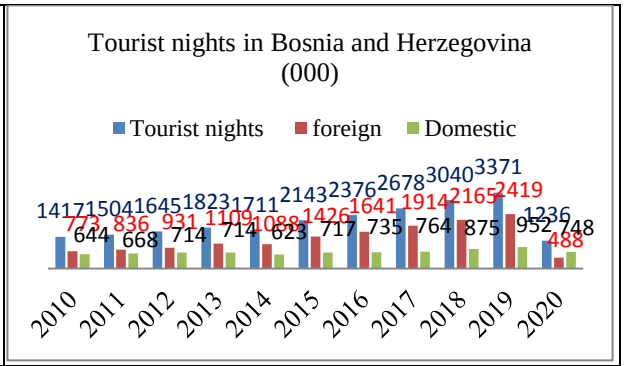

Figure 8. Tourist nights in B&H

Source: Agency for statistics Bosnia and Herzegovina, 2021

Over the last ten years, from 2010 to 2020, the international tourism receipts rose by 34% in B&H, what is in line with the growth of international tourism arrivals and overnights in B&H recorded in this period.

For faster growth and development of tourism in B&H the important role have investment in tourism as well as foreign direct investment (FDI) which will provide the resources required to adjust tourism supra-structure and infrastructure, as well as enhancing human resources and technologies (UNCTAD, 2007). However, in FDI should be improve cooperation on long term period and type of interest and share in management and income (Cró & Martins, 2020).

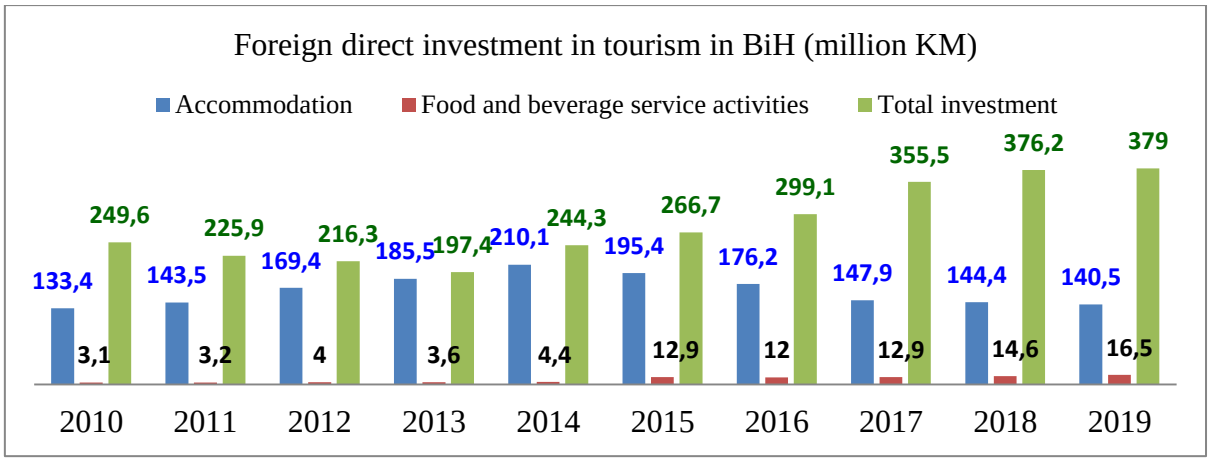

Figure 8. Foreign direct investment in tourism in Bosnia and Herzegovina (Source: WEF, WTTC, 2019)

In sector tourism for accommodation and food and beverage service activities in B&H over period from 2010 to 2019 foreign direct investment rise in range 249.6 -376.2 million Bosnian-Herzegovinian convertible marks (BAM), what mean that that the average growth rate of investments in tourism was 3.7% (WEF, WTTC, 2019) Figure 8.

The positive influence of tourism on other service industries underlines its strategic importance. Tourist inflows foster services such as in the food industry, transport, health and well-being, culture, sports, and adventure activities that are of interest to tourists. Tourisms' direct impacts are related to accommodation or tourism transport services, indirect contributions are related to the expenditure on food and beverage suppliers to tourism enterprises, whilst induced impacts are related to the expenses of people working in the tourism sector (or benefitting from the sector) within the local economy. The employment tended to increase in B&H and was 85700 persons in 2019. The highest annual growth rates of tourism jobs were achieved in 2011 (21.5%) and 2016 (11.4%) Pasovic and Efendic, (2018). For sustainability of tourism in Bosnia and Herzegovina should be promote elements such as a favorable natural and cultural resources, safety and security human resources, and labor market and business environment.

The development of tourism, especially rural tourism, creates the conditions for the rural population to stay at their hearths, which will acquire additional funds by selling traditional agricultural and food products, organic agricultural products, souvenirs and organizing courses in the preparation of traditional food products, picking medicinal herbs and forest fruits and other

Half of rural households have little or no involvement with agriculture, at most keeping a vegetable garden. About 36% rural householders produce food for own requirements, achieve very little cash income. Around 13% of rural households may be considered as full-time or part-time farms, producing significant quantities for sale, yet even these gain more than three-quarters of their income from outside agriculture. Less than 1% of households can classified as "commercial farms". All categories have chance for linkage with tourist sector.

Conclusion

Agriculture have significant role for economic development to meet the demand for food, to meet the growing demand for employment and to raise more income of rural people. In order to maintain and increase the number of rural agricultural people, it is necessary to develop new models of small agricultural enterprises, farms that will growing employment of rural labor force and reduce poverty. Should be developing intensive agricultural production – producing vegetables uses five times as much labor as cereals; livestock production.

Development of rural non-farm enterprises - which can eventually become an engine of growing out of expanded agricultural production. Investment in agricultural research & extension - improved agricultural productivity, and aplying smart technology in agricultural production. Developing rural institutions for education, credit, governance,
Investments in rural infrastructure, especially to give access to both input and output markets. Improve road transport in B&H in function of developing agric., economy and tourism.
Improve capacity of airports: Banja Luka, Mostar, Sarajevo and Tuzla, for the development of trade transfer of agric., products, tourist trip, business. Investing in: tourism in places of winter ski resorts, rural tourism, river tourism, summer tourism on the coast of the Adriatic Sea, spa tourism, congress tourism.

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AGRICROWDFUNDING AS AN INNOVATIVE AGRI-FINANCING MODEL

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Abstract

The constant growth of the world's population requires a considerable increase in the production of agricultural products. Therefore, it implies more significant financial investments in agricultural production. However, farmers, especially in developing countries, face a chronic lack of financial resources and limited access to traditional financial intermediaries. The rapid development of financial technologies (FinTech) offers an innovative financial solution to this problem, known as AgriCrowdfunding. AgriCrowdfunding is an increasingly popular type of crowdfunding that allows a number of interested individual and/or institutional investors to invest money in agricultural projects. This agri-financing model is implemented through online platforms that connect investors with farmers and other agricultural producers, providing them access to missing financial resources. It means AgriCrowdfunding platforms, or AgriCrowdfunding FinTechs, seek to improve farmers' access to finance. In addition to the model connecting all parties (land owners, farmers, investors and consumers of agricultural products) in a platform that promotes transparency in the collection of financial resources, the economic empowerment of farmers and the engagement of the public in agriculture, it also contributes to the financial inclusion of the most vulnerable categories of the population (poor, rural population, women, etc.), all with minimal transaction costs. The primary goal of this paper is to point out the potential of AgriCrowdfunding as an innovative financial solution that can help farmers and other agricultural producers meet their financial needs. Seen from the perspective of the investment public, this model is an efficient and affordable way to invest in the agricultural sector, and potential investors can expect high rates of return on invested funds. Due to the above, the paper will also point out the multiple benefits of integrating this innovative financial solution into the broader agriculture ecosystem.

Keywords: *AgriCrowdfunding, Financial Technology (FinTech), agriculture, alternative finance, financial inclusion*.

Introduction

AgriCrowdfunding is part of a broader universe of financial innovations made possible by technological progress – FinTech (European Commission, 2016), i.e. it is part of a FinTech subcategory known as alternative finance (AltFi).

This agriculture financing (agri-financing) model offers new financial products and services, lower transaction fees, and new intermediaries such as FinTech platforms. It is a valuable option for financing those agricultural enterprises, ventures and farmers who would be denied financing if they relied upon traditional financial intermediaries. On the other hand, it is also acceptable to investors, which precisely reflects the potency and popularity of this innovative and alternative form of financing.

AgriCrowdfunding's rise in popularity is the result of three catalysts. The first was the great economic crisis of 2008, followed by a credit crisis that affected the sector of agricultural enterprises and the population engaged in agriculture. It questioned the financing of numerous

high-quality agricultural projects and business ventures because, suddenly, the traditional financial intermediaries were no longer able or keen to provide financing under pre-crisis conditions. The second is that many consumers are increasingly interested in how their food (agricultural products) was grown, its quality, nutritional value (due to changes in the eating habits of a significant part of the population), where it comes from (geographical origin) and the like, and this innovative agri-financing model provides opportunities for individual investors and consumers (backers) to be more directly involved in the early stages of the food production cycle (Bifani, n.d.). The third is undoubtedly the COVID-19 pandemic, which has changed us forever, forcing many people to switch to a digital way of trading and communicating in search of food products and connecting on FinTech platforms. The disruptions and complete interruptions of the supply chain during the health crisis caused by the COVID-19 pandemic led to FinTech platforms also serving as a digital market for agricultural products where it is possible to get cheap, high-quality agricultural products from different geographical locations (Anshari et al., 2019).
This paper aims to point to AgriCrowdfunding as a new funding trend that has the power to significantly expand the base of the investment pyramid of the agricultural sector. The paper is organized as follows: the first part elaborates on the stages of AgriCrowdfunding. We then look at the different AgriCrowdfunding models and their advantages for both initiators and potential backers. Although fundraising is the essential advantage of using the AgriCrowdfunding model, this model has numerous other non-financial – marketing advantages, discussed below.

AgriCrowdfunding process

AgriCrowdfunding is the practice or the process of funding any agricultural project or venture by often collecting modest financial contributions from considerable individuals without the participation of traditional financial intermediaries. In this model, specialized FinTech platforms that offer farmers practical ways to ensure a source of financing performs the role of an intermediary. Using the financial services they provide, FinTech platforms can help the agricultural sector compete in the global economy through AgriCrowdfunding (Anshari et al., 2019).
Basically, the AgriCrowdfunding model has five fundamental stages (see related numbers in Figure 1):

- Stage one: Agricultural companies and farmers with innovative projects and business ideas that they deem attractive to the public – crowd and do not have the financial means for their realization, nor can they get them from traditional sources, decide for an alternative through AgriCrowdfunding. This model can benefit them in various ways because this is an opportunity to collect the missing funds and to test their business ideas and projects for free, using online (a FinTech platform placed on the Internet) communication with potential investors. In this phase, agricultural companies and farmers present their projects and ventures in the best light, using FinTech platforms and numerous social networks as intermediaries, sending a direct call to collect the missing financial resources. In short, fundraising begins with the presentation of a project and/or venture and a request for a contribution, i.e. investment, highlighting data on the amount of necessary financial resources and what is offered in exchange – a perk (Ahlers, Cumming, Günther, & Schweizer, 2013).
- Stage two: Crowdfunders (backers) usually do not have the knowledge and experience expected of professional investors, but on the other hand, they like a challenge and are constantly looking for attractive and innovative agricultural companies and projects, and they recognize the opportunity to invest through AgriCrowdfunding. Through the

FinTech platform, they get information about tempting ideas, farming enterprises, projects and ventures. If they become interested in some, they are willing to expand their knowledge in that area, take on an advisory role to improve a specific idea and exchange opinions and experiences with already established participants in AgriCrowdfunding. In the end, they make their contribution – they invest. Their motives are different. Most frequently, they partake in AgriCrowdfunding because of a specific return, i.e. reward, identification with the project, personal – internal needs and motivation to contribute and participate in creating something new.

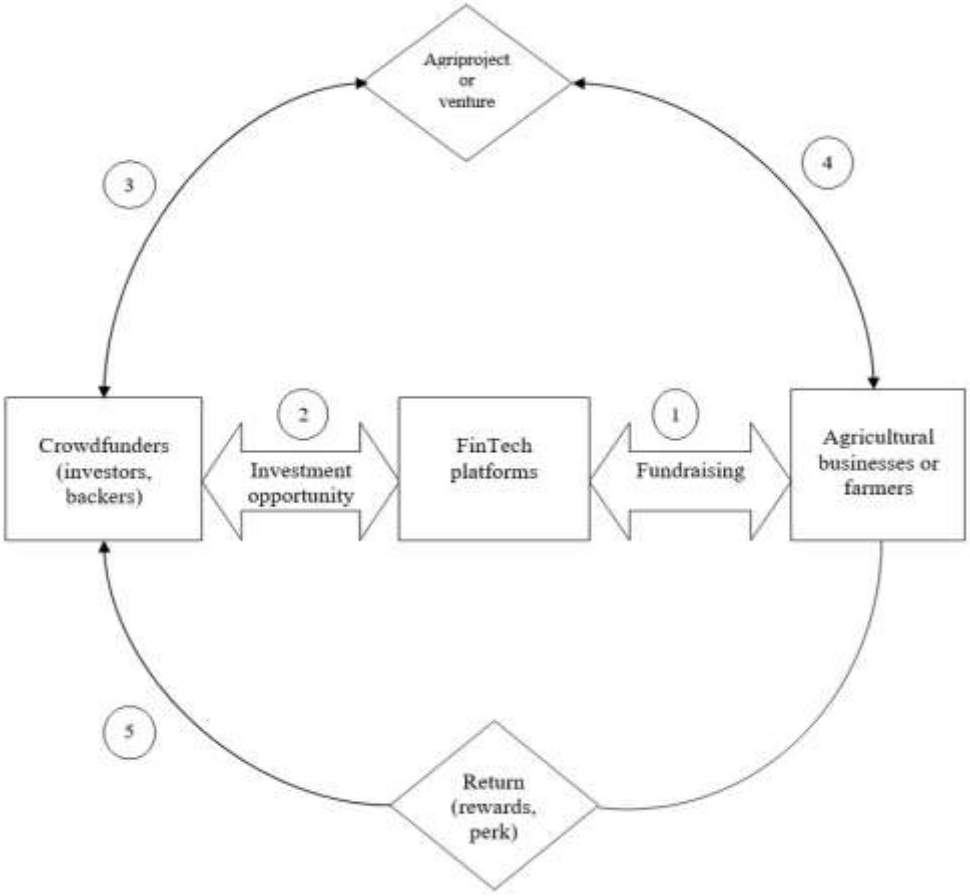

Figure 1. The stages of AgriCrowdfunding model (adapted from: Golić, 2016)

- Stage three: In this phase, crowdfunders – backers decide on a specific agricultural project or venture and invest in it. Some evidence presented in the paper of Gerber, Hui and Kuo (2012) indicates that individuals decide to invest after the positive experiences of other investors.
- Stage four: When the originators of the project or venture collect the necessary amount of financial resources through the AgriCrowdfunding initiative, they start implementing ideas. This phase ends after the concrete product sees the light of day and is accepted in the market, followed by the final stage of AgriCrowdfunding – return.
- Stage five: Return in the Agri Crowdfunding model implies certain returns, i.e. perks that can be material and immaterial in the case of AgriCrowdfunding donations.

Types of AgriCrowdfunding

There are four main types of AgriCrowdfunding: reward-based, equity-based, P2P (peer-to-peer) lending and donation-based (Fig. 2). A different degree of complexity and risk

characterize each of the four basic types of AgriCrowdfunding, with AgriCrowdfunding based on shares being the most complex form, both from an organizational and legal perspective, given the information asymmetry between initiators and investors (Marzban, Asutay, & Boseli, 2014). In this part of the paper, we will explain each individual AgriCrowdfunding model in the order in which they are presented in Figure 2.

Figure 2. Main types of AgriCrowdfunding (adapted from: Xsero, n.d.)

● Rewards AgriCrowdfunding. It is the most popular type of crowdfunding with the most extensive number of participants, both on the side of the initiator and the side of contributors - investors, with both preferring this form because of their funds' safety. In reward-based crowdfunding, the target amount of funds must be determined first. Then the company or individual decides the rewards, i.e. perks proportional to the amount of monetary resources invested in the project. One of the main advantages of reward-based crowdfunding is that it has little risk for the business or individual behind the fundraising campaign. Once the company reaches its financial goal, i.e. accumulates the required amount of funds for the project, it will be able to avoid dealing with debts, credit applications and implications, and interest on loans or government grants. Hence, this crowdfunding method allows companies to avoid the mentioned problems entirely and initiate their business venture. The reward-based crowdfunding procedure also lets investors be personally involved with the project they have invested in and/or the people they fund. Given the nature of their deal or the incentives of a particular project, investors get the chance to meet the initiators and/or owners of the projects. However, it is first necessary to research other projects and the rewards they have offered to people to ensure that investors feel interested enough to decide whether to invest their funds in the project. The reward is very significant for the project because the main reason people are interested in investing is the attractiveness aspect of the project.

At the same time, this crowdfunding model is the most popular and most often used in financing agricultural companies and/or projects, including farmers, for various reasons, of which we will mention only the most noteworthy. It is common for agricultural producers to use rewards-based AgriCrowdfunding as a mechanism for market research because every investor is also a potential consumer of farming products. The abundance of interested investors guarantees the product's success on the market. Most often, the award itself implies the delivery of an agricultural product (which means we could treat this model as purchasing the product in advance) within the deadline indicated in the project proposal. It means that the model has gradually evolved into commercial reward-based AgriCrowdfunding, whereby FinTech platforms can be used as virtual sales channels: invested financial resources are "rewarded" with specific agricultural product/products that are delivered on demand (Reffell, 2021) and most often according to the "field to the front door" model.

The recent COVID-19 pandemic that forced many people to switch to a digital way of trading and communicating in search of agricultural products contributed to the reward-based

AgriCrowdfunding commercialization. In addition, disruptions and even complete interruptions of the supply chain of agricultural products during the health crisis caused by the COVID-19 pandemic often required the delivery of farming products "to the door", and all this was possible thanks to the reward-based AgriCrowdfunding model.

● Equity AgriCrowdfunding. It is typical of this AgriCrowdfunding model that the investor, in proportion to the invested funds, receives a share in the ownership or profit of the agricultural company that collects financial resources. Investors get the opportunity to invest under far more favorable conditions than market ones because the owners of agricultural enterprises and/or projects must make their proposal and project attractive to potential investors through competitive prices and future growth potential. Through equity AgriCrowdfunding, investors can achieve a high return on investment (ROI) because when farmers have enough capital to allocate for all agricultural costs, they have a great chance to make each project more successful, i.e. they can produce quality agricultural products, which can ensure a qualified and potential market where they can expect good sales, and therefore high sales revenues (Fundraising Script, 2022).

Thanks to the FinTech platform, equity AgriCrowdfunding makes it possible to reach an agricultural company for potential investment. The essential role of the platform is to ensure (virtual) meetings between farmers and investors. In addition, FinTech platforms allow people with similar businesses and ideas to communicate with each other and exchange advice on best practices and best ways to improve business. It means that platforms are not only a place, i.e. a space that is only useful for amassing financial resources, but also ideas and plans about the best ways to improve business, given that there are many other members of the group – the crowd – with the same participation or similar ideas and business ventures (the so-called wisdom of the crowd – one of the fundamental non-financial advantages of using group financing, which will be discussed in more detail in the next part of this paper). It can also lead to the merger and consolidation of two or more different agricultural businesses or projects as equity AgriCrowdfunding targets those farming businesses and business people interested in raising their venture capital.

Investors, however, bear the risk of loss if the agricultural enterprise or project in which they have invested financial resources does not perform well.

● P2P lending or simply lending AgriCrowdfunding. According to this model, the borrowers themselves set the amount they need, i.e. they want to borrow, and the contributors, i.e. financiers interested in a specific agricultural enterprise/project, provide financial resources to the borrower in advance with the promise that the exact amount will be returned at a future date. FinTech platform has the role of guarantor and/or intermediary between the borrower and the lender in the lending AgriCrowdfunding. More precisely, the platform connects borrowers and lenders and performs the role of guarantor and/or intermediary between these parties to ensure that the lender gets the invested financial resources back on the specified date. It facilitates the pooling of funds from a multitude of lenders to collect the necessary funds for the borrower. For providers of financial resources, i.e. contributions, this is the safest model of group financing because if an agricultural company, its owner or a farmer does not pay the given loans, the FinTech platform is responsible for paying the loans to the providers of financial resources (Tomczak, & Brem, 2013).

Borrowers consider this type of AgriCrowdfunding to be quite an appealing method of financing their companies or projects because they can get funds under exceptionally favourable conditions. Namely, in this model, the borrowers determine the interest rate in such a way that the determined interest rate is acceptable and guarantees the return of the money within the stipulated period (Mollick, 2014). On the other hand, lenders themselves must evaluate the agricultural enterprise/project based on its risks or benefits and potential and determine whether they are willing to lend financial resources at the interest rate set by

the borrower. Based on the above, it is clear that this arrangement is most similar to a bank loan. The main differences are that the borrower determines the interest rates, unlike a bank loan where the borrower must agree to the conditions set by the bank or the authorities (Althoff & Leskovec, 2015), and the fact that in this model, the borrower (the debtor) does not have to provide any form of collateral to the lender.

● Donations AgriCrowdfunding. In contrast to the previous three types of AgriCrowdfunding we considered, there are no material rewards (for this reason, the dotted line in Figure 2) in this type. Backers (crowd) donate financial resources without expecting anything tangible in return (Reffell, 2021). They are motivated primarily by altruistic motives, and their reward is a sense of personal satisfaction for financially supporting a specific project and/or venture, an idea with which they sympathize (Golić, 2014; Reffell, 2021). Donations AgriCrowdfunding is primarily used as financial assistance to poor farmers to provide equipment, machinery, seeds and other things necessary for agricultural production (Fundraising Script, 2022). Thus, the financially excluded and most vulnerable population categories (the poor, rural population, women, and national minorities) can obtain innovative and high-quality agricultural tools and quality seeds. It, again, will have broader socio-economic implications: quality seeds mean an increase in the yield of crops in the future, and they will not only use the reapings for their own needs but also make them one of the permanent sources of income in the future (Fundraising Script, 2022). Therefore, AgriCrowdfunding can contribute to financial inclusion and poverty reduction in a broader sense.

Non-financial – marketing and other advantages of using AgriCrowdfunding

Fundraising is an essential, but not the only advantage of using the AgriCrowdfunding model because AgriCrowdfunding also benefits agricultural companies/farmers for market research, and it is also a powerful marketing tool for promoting new farming products and cultures (De Buysere, Gajda, Kleverlaan, & Marom, 2012). Nevertheless, we consider that the advantages of using the AgriCrowdfunding model in financing agricultural projects and/or ventures are far more numerous. Therefore, in addition to the primary benefits, i.e. collection of financial resources, we also point out the following:

- Product promotion. Contributors (investors, backers) are, at the same time, potential buyers of agricultural products and also ambassadors of the project, i.e. ventures promoted through their social networks (Golić, 2014). Given that they usually invest in projects that they are personally interested in and identify with in any way (Gajda & Walton, 2013), agricultural businesses and/or farmers use AgriCrowdfunding as a powerful marketing tool to draw attention to their products, agricultural projects in the early stages of their development, which enables them to provide certain competitive advantages even before the products are produced, i.e. the projects are realized (Mollick, 2014).
- Market research. Considering the previously stated, if agricultural companies, farmers, i.e. the initiators of the campaign notice that their product or project is drawing a lot of attention from the audience/crowd (backers) and that a considerable number of people decide to invest, this can be a reliable signal that the market will react satisfactorily on the agricultural product/s and that the demand will be high because the assumption is that the contributors (backers, investors) are also potential buyers of the product (Golić, 2014). In short, the AgriCrowdfunding model can give an agricultural company or farmer a reliable indication of the market potential of his product or project (Schwienbacher & Larralde, 2010).
- Savings in expenses for the development of products of particular nutritional values. By actively suggesting product characteristics (e.g. the nutritional value of certain

crops and/or products) and with an advisory role in the broadest sense, contributors (backers) contribute to creating value for the agricultural company, i.e. the farmer. On the other hand, it makes it possible to significantly shorten the time required for developing a product with the desired characteristics and obtain a product that potential customers will accept nicely. Therefore, the outcome is significant cost savings (Schwienbacher & Larralde, 2010).

- Facilitated flow of information to potential investors (backers). By centralizing access to data on available investment opportunities (on a FinTech platform), AgriCrowdfunding facilitates the flow of information to potential investors and makes it faster than ever before. Traditional financing models very often require companies to hold dozens or hundreds of meetings to seek the remaining funding, which means that these meetings are both labour and capital-intensive and very ineffective in contrast to FinTech platforms.
- Removing geographic impediments to investment. In their paper, Agrawal, Catalini and Golfarb (2011) suggest that the average distance between investors and companies is about 5,000 kilometres. Thus, in the crowdfunding model, the geographical distance between the initiator of the fundraising campaign and the investor is not an obstacle to investment.

  AgriCrowdfunding enables the crowd (backers) to back farming projects and ventures in their local community, thus contributing to its local and sustainable economic development. It is a fact that many agricultural companies opt for projects that they will implement within their geographical area and will also collect the missing financial resources outside of it (Valanciene & Jegeleviciute, 2013). It implies that the AgriCrowdfunding model offers the opportunity for local projects to attract global investors and the diaspora (Golić, 2014; Golić, 2020).
- The wisdom and power of the crowd argument. On the one hand, the fact that a group, i.e. a crowd, can very often be more effective and more powerful in solving the issues and dilemmas faced by agricultural companies and farmers (campaign initiators) than a few lone investors explains the argument of the wisdom and power of the crowd. On the other hand, the risks taken by the contributors (backers, investors) may be lower not only because of investing less money but because they are potential consumers and are motivated to promote the product. In addition, Ellman and Hurkens's (2016) paper studies how the interaction of campaign initiators – project owners and potential investors from the crowd who participate as project collaborators – determines the consumer, producer and overall well-being. The authors highlight that one of the main advantages of this financing model is the opportunity for project owners to adjust production according to information, i.e. the needs of the crowd.
- Retention of management control over the enterprise. By collecting the missing capital through AgriCrowdfunding, business owners do not lose the right to make business decisions (Golić, 2014). Funding enterprises through venture capital funds and business angels gives investors considerable control over the company's decisions. However, depending on the modality, AgriCrowdfunding may or may not grant investors such rights (Valanciene & Jegeleviciute, 2013).

Conclusion

An adequate response to the challenge of feeding the growing world population (which will reach over 9 billion people by 2050) when facing climate change, burdened food production resources, and accelerated urbanization trends (Bifani, n.d.) is increased capital investment in agriculture. As an innovative financing model, AgriCrowdfunding has the potential to

significantly expand the base of the investment pyramid of the agricultural sector, and we can expect numerous benefits from its integration into the broader agriculture ecosystem.
By providing better access to funds and financial assets, AgriCrowdfunding can potentially contribute to financial inclusion efforts.
Very often, agricultural projects may require considerable financial investments that individual investors cannot afford. However, using the AgriCrowdfunding model, small investors (even with modest contributions) have the opportunity to participate in promising agricultural ventures (smaller or larger) in different parts of the world (without geographical barriers to investment).
Limited access to equity and debt financing is a central challenge for agricultural companies that need more considerable sums of risk capital at their disposal to accelerate innovation in new foodstuffs and agritech and to be able to take proper risks that would enable them to offer new agricultural solutions and significantly build enterprise value (Bifani, n.d.).
As all investors in the agricultural sector are also food consumers, the AgriCrowdfunding model provides them with a financial return on investment and also the delivery of farming products. It leads to producers (farmers) and consumers (investors), who are on opposite sides of the food chain, becoming partners because backers can become more directly involved in the earlier stages of the production cycle of agricultural products through FinTech platforms.

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REGIONAL SOCIO-ECONOMIC IMBALANCES IN BULGARIA

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Abstract

After the EU integration of Bulgaria in 2007 and the accompanying socio-economic challenges, the approaches to regional politics changed. A number of support measures and interventions were included with the aim of harmonizing regional interventions with the EU. However, regional differences are still present, often contrasting the overall "picture". The aim of this study is to measure and evaluate the regional socio-economic differences of rural areas in Bulgaria, to help in the preparation of appropriate regional policies and interventions in the development of the New CAP 2023-2027. For the analysis of socio-economic differences in rural areas in Bulgaria, an adapted general methodology for Shift-Share analysis is applied. According to the results, among most rural regions in Bulgaria for the period 2008-2020, reduction in unemployment is ahead of the changing trend compared to non-rural regions and the average for the country. Only the South-Eastern and North-Eastern regions lag behind the trend of change based on the reference regions for comparison. With regard to the unemployed by educational attainment in rural areas, there is a lag in change compared to the reference areas among those unemployed with tertiary and secondary education. There is a lag in employment in rural areas in the North Central region and, with some exceptions, in the South East, compared to the change in employment in non-rural and average areas of the country. The coefficient of economic activity by statistical region in rural areas is ahead of the rate of change of the reference ones for comparison.

Key words: *regional sustainability, rural areas, socio-economic imbalances, eemployments, unemployment*

Introduction

Due to interregional and intraregional imbalances, issues related to regional policies are constantly on the agenda among stakeholders and politicians. The reason for this is that regional disparities are increasingly deepening and growing, which provokes researchers to focus research on the adequacy of applied strategies (Gezici, F. and Hewings, G., 2004; Monastiriotis, V., 2008; Totev, S., 2011; Timiryanova, V. et al., 2021). After the integration of Bulgaria into the EU in 2007 and the accompanying socio-economic problems, approaches to regional politics have changed. A number of measures and support schemes were included in order to harmonize regional interventions with those of the EU. However, there are still regional differences (Doitchinova and Stoyanova, 2020; Petrov, K., 2020; Marinov and Mutafov, 2022) that often contrast with the overall "picture".
The aim of the present work is to measure and evaluate the regional socio-economic differences of rural areas in Bulgaria, to help in the preparation of appropriate regional policies and interventions in the development of the New CAP 2023-2027.

Material and Methods

In the present study, an adapted general methodology of Shift-Share analysis is applied. It is one of the well-known traditional tools for measuring and evaluating the differences in economic results from a regional aspect.

A Shift-Share analysis is a method of comparing regional differences against a selected reference level. Socio-economic differences in rural areas in Bulgaria will be compared with the non-rural and, respectively, the average indicators for the country for the period 2008-2020. The approach is suitable for identifying regional structural changes and imbalances in the regions of the country. The economic activity rate represents the ratio between economically active persons (the labor force) and the population. The employment rate is the ratio between the number of employed persons and the population. Unemployment rate (unemployment rate) - the ratio between the number of unemployed and the number of economically active persons" (National Statistical Institute - NSI, 2021)

The purpose of labor force inclusion is to focus on the basic characteristics of employment and unemployment in Bulgaria. NSI defines as unemployed people between 15 - 74 years of age who do not have a job during the reported period, but are actively looking for work. Unemployed are also persons who are not actively looking for work, but have found, waiting to start work up to 3 months after the observed period. The choice of employment and unemployment indicators is justified by the fact that they are leading socio-economic indicators. They show the level of economic development and the potential of each country. The following components were selected for analysis:

- Unemployment rate by statistical region in rural/non-rural regions and the average for the country;
- Unemployment rate by degrees of education by statistical regions in rural/non-rural regions and average for the country;
- Employment rate by statistical region in rural/non-rural regions and the average for the country;
- Coefficient of economic activity by statistical regions in rural/non-rural regions and average for the country.

A Regional Shift is that part of the shift that is due to the regional advantage or lag of the selected component. The Regional Shift (RS) component measures the differential displacement due to differences in the rates of change in the region. They can be the result of a number of factors, such as the creation of a favorable economic environment, appropriate sector policies, availability of labor resources or other comparative advantages/disadvantages, entrepreneurial activities, and of course regional policy (Stimson et al., 2006). Regional Shift-Share could help to identify the strengths of the respective region and accordingly the comparative advantage of a particular region over another. The wide application of Shift-Share analysis is explained by its simplicity, and minimal data requirements, and naturally, the results are relatively easy to evaluate and interpret (Marquez et al., 2009).

The following calculation formula is used (Knudsen, D., 2000):

$$RS_j = r_{i;t} * (((r_{i;t} - r_{t-1})/r_{t-1}) - ((R_{i;t} - R_{t-1})/R_{t-1})) \quad (1)$$

Where:
t = current time period
t-1 = previous year
i = component
r = region
R = reference region

The resulting RS coefficient should be interpreted as follows:

- At RS coefficient > 1.0: The region has a larger share of the component in the relevant indicator compared to the reference region. The higher the RS, the greater the variation of the regional component in that sector;
- When the ratio RS = 1.0: The share of the regional component in the industry is equal to that of the reference region. It is assumed that the region fully corresponds to the reference region change in this industry;
- When the coefficient RS < 1.0: If the component of the region in a given industry has a smaller share than the component of the reference region - the region falls below the level of change and must continue to develop to reach the reference region in a given industry. specific industrial sector.

The positive side of Shift-Share analysis is its easy application and the clarity of the results since the calculation of the selected components does not take much time. An important condition is to choose an appropriate time frame in which the method will be applied in order to avoid distortion of the results.

NUTS-2 statistical regions in Bulgaria are regions designated with the main purpose of statistical reporting of territorial units, according to the requirements of Eurostat. According to NUTS-2, six planning regions are distinguished in Bulgaria: South-West Region, South-Central Region, South-East Region, North-East Region, North-Central Region, North-West Region **(**Images 1).

Images 1. *Statistical regions for planning in Bulgaria*
Source: *https://en.wikipedia.org/wiki/NUTS_statistical_regions_of_Bulgaria*

Data from the National Statistical Institute in Bulgaria (2022) for the period 2008-2020 were used.

Results and Discussion

- ***Regional Shift-Share Component: Unemployment Rate by Statistical regions 2008-2020***

The regional Shift-Share component: Unemployment rate by statistical region for 2008-2020 focuses on the change in the ratio of rural/non-rural regions (Fig. 1). Only the North West planning region most closely followed the rate of change in non-rural unemployment. The North-Central and South-West regions have an RS coefficient > 1.0. These two regions have a larger share of the component compared to the reference region, which means that the change in the component outpaces the change in non-rural regions. Three regions, respectively South Central, South East and North East show a lag in the rate of change of unemployment compared to the change in non-rural regions - the coefficient RS < 1.0.

When comparing the rate of change in rural unemployment compared to the average change for the country, South-West and North-Central stand out. They are anticipating change trends with a coefficient RS > 1.0. The remaining four planning regions North-East, South-East, North-West and South-Central lag behind the change on average for the country (Fig.2).

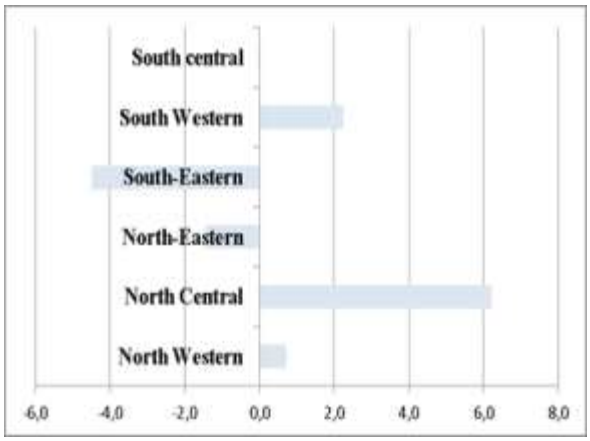

Figure 1. Regional Shift-Share Component: Rural Unemployment Rate by Statistical regions vs. Non-Rural Unemployment Rate, (2008-2020)

Source: own calculations based on National Statistical Institute data. Classification by Statistical Area - Labor Force Survey, (2022)

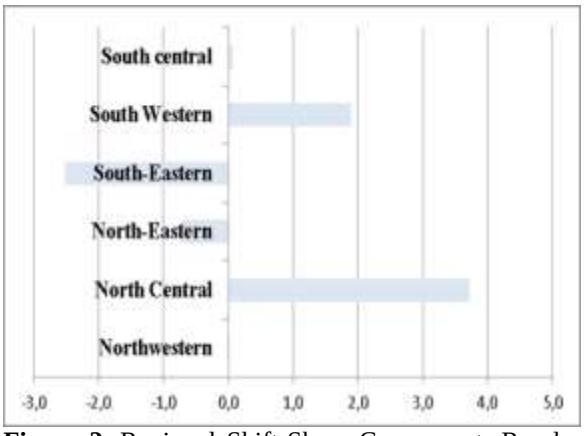

Figure 2. Regional Shift-Share Component: Rural Unemployment Rate by Statistical regions vs. National Average Unemployment Rate, (2008-2020)

Source: own calculations based on National Statistical Institute data. Classification by Statistical Area - Labor Force Survey, (2022)

- ***Regional Shift-Share Component: Unemployment Rates by Level of Education 2008-2020***

When the coefficient RS > 1.0, the compared region occupies a larger share of the component compared to the reference region – in this case, the non-rural region. The most sensitive is the change in the direction of increasing unemployment among the unemployed with primary and lower education, followed by primary and secondary. (Fig.3). The higher the RS coefficient, the more noticeable the change of the regional component in the corresponding sector. The unemployed with average general and acquired professional qualifications most closely approximate the change in non-rural areas with an RS coefficient closer to 1.0.

The unemployed with higher education in rural areas are below the level of change relative to those outside rural areas. The ratio RS <1.0, which means that the component of the analyzed region has a smaller proportion than the component of the reference region. The same trend is observed among the unemployed with secondary and secondary general education. In other

words, the change in the unemployed with higher education in rural areas deviates to the greatest extent from the change in this component in non-rural areas of the country.
In the regional Shift-Share component: The unemployment rate in rural areas by educational level compared to the average unemployment rate for the country (Fig. 4), some differences stand out. The coefficient RS < 1.0, which means that the analyzed component for the region occupies a smaller share than the component of the reference region - on average for the country. It can be concluded that the rate of change of unemployed persons in rural areas at all levels of education is slowing down compared to the change of this component on average for the country (2008-2020).

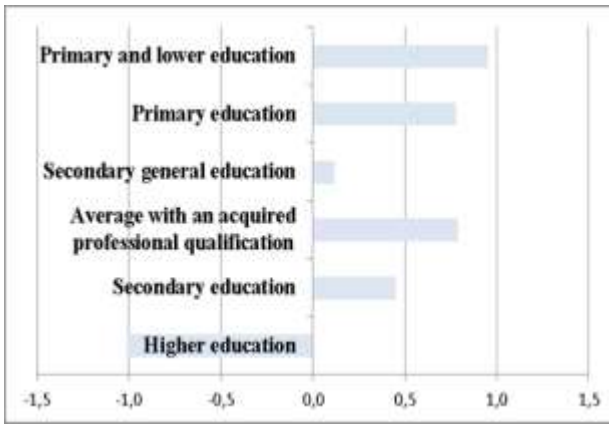

Figure 3. Regional Shift-Share Component: Rural Unemployment Rate by Level of Education vs. Non-Rural Unemployment Rate, (2008-2020)

Source: own database calculations National Statistical Institute. Classification of Educational Attainment - Labor Force Survey, (2022)

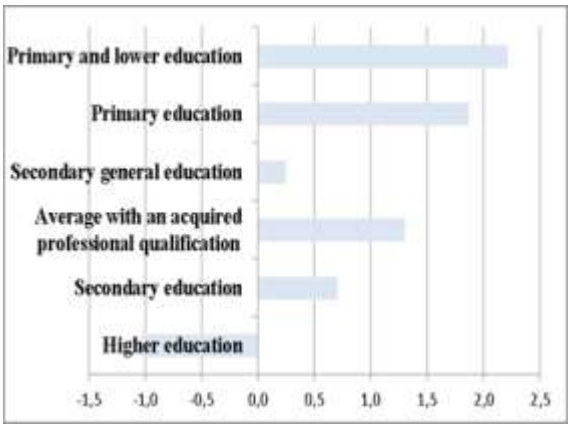

Figure 4. Regional Shift-Share Component: Rural Unemployment Rate by Level of Education vs. National Average Unemployment Rate (2008-2020)

Source: own database calculations National Statistical Institute. Classification of Degrees Completed - For Workforce Monitoring, (2022)

- ***Regional Shift-Share Component: Employment Rate by Statistical regions 2008-2020***

The RS coefficient of employment in the Southeast region in rural areas compared to the employment coefficient in non-rural regions (2008-2020) is equal to 1.0 (Fig. 5). This region fully corresponds to the modification of the reference region. With the exception of the North Central region, the remaining four planning regions in the country (North-West, North-East, South-West and South-Central) have an RS coefficient > 1.0. In them, the change in the component outpaced the rate of change in non-rural areas. In the South-East and North-Central region, the coefficient RS < 1.0 (Fig. 6). This means that the employment for the mentioned two regions occupies a smaller share of the component of the reference region - on average for the country. The pace of employment change in rural areas is slowing down compared to the national average employment change. The North-West, North-East, South-West and South-Central regions have a coefficient RS > 1.0, which means that the change in the component is ahead of the rate of change in employment on average for the country.

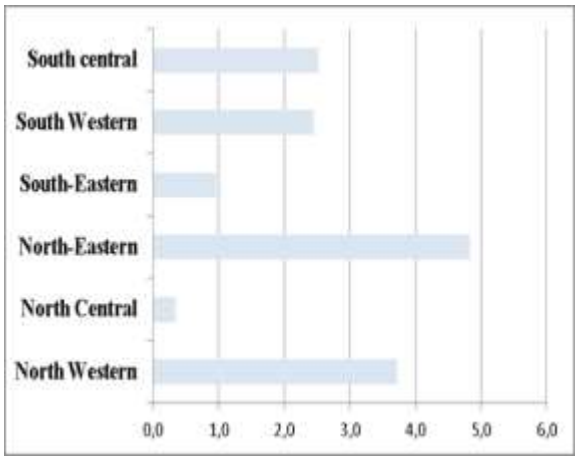

Figure 5. Regional Shift-Share Component: Rural Employment Rate by Statistical regions vs. Non-Rural Employment Rate (2008-2020)

Source: own calculations on National Statistical Institute database. Employment rates by place of residence and statistical areas, (2022)

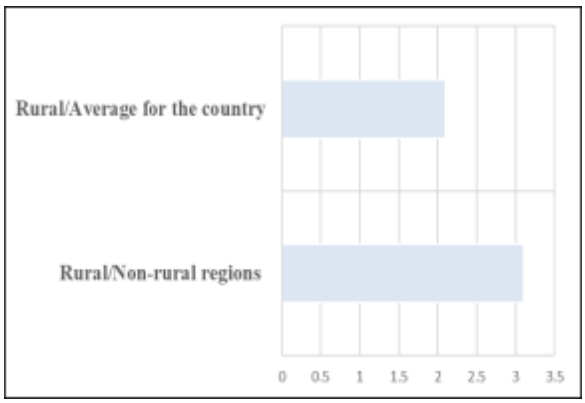

Figure 7. Coefficient of economic activity by statistical regions in rural/non-rural regions and average for the country (2008-2020)
Source: own calculations on a database from the National Statistical Institute. Employment rates by residence and statistical area, (2022)

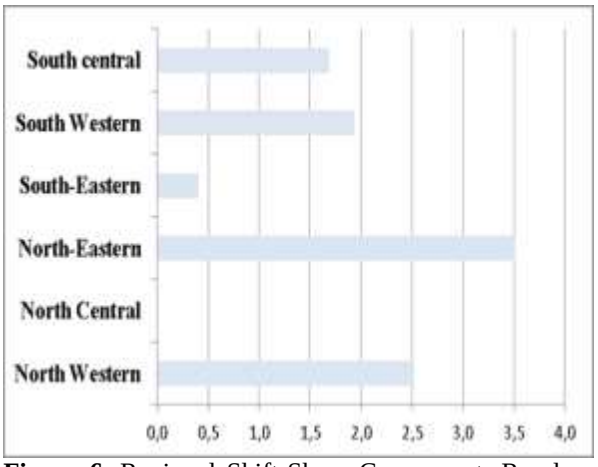

Figure 6. Regional Shift-Share Component: Rural Employment Rate by Statistical regions vs National Average Employment Rates (2008-2020)

Source: own calculations on a database from the National Statistical Institute. Employment rates by residence and statistical area, (2022)

Regarding the Regional Shift-Share component: „Economic activity rate by place of residence and statistical regions for the period 2008-2020"(Fig. 7), it can be concluded that rural areas occupy a larger share of the component compared to the reference regions - non-rural and on average for the country. The coefficient RS > 1.0, which means that the variation of the component is significantly larger compared to the non-rural and national averages.

Conclusions

In the Shift-Share analysis based on the selected rural/non-rural and country average components, some interesting results were achieved.

According to the results, among most rural regions in Bulgaria during the considered period 2010-2020, changes in unemployment are ahead of the changing trend compared to non-rural regions and the average for the country. Only the South-Eastern and North-Eastern regions lag behind the trend of change based on the reference regions for comparison. With regard to the unemployed by educational attainment in rural areas, there is a lag in change compared to the reference areas among those unemployed with tertiary and secondary education. There is a lag in employment in rural areas in the North Central region and, with some exceptions, in the South East, compared to the change in employment in non-rural and average areas of the country. The coefficient of economic activity by statistical region in rural areas is ahead of the rate of change of the reference ones for comparison.

Viewed in this way, it would not be possible to group more closely the potential instruments for policy interventions in the regions. In order to increase their economic results, incentives should be provided for attracting fast-growing industries to the regions and sectoral specialization. This seems to be a difficult task, considering the strong processes of regional divergence.

Acknowledgements

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EFFECT OF THE TRANSITION TOWARDS CIRCULAR ECONOMY

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Abstract

The trend for sustainable development imposes the concept of the circular economy, which is one of the main priorities of the European Union (EU). In a circular economy, the resource chain is closed, so that the vast volume of exhaustible resources is put back into production and reused. The application of this logic in the economy means a profound change in the basic structures of industrial systems. A key argument in favour of the circular economy approach is that achieving a sustainable world does not require changes in the quality of life of consumers, nor does it require loss of profit or additional costs for producers and other economic agents. The transition towards a circular economy is based on three pillars: benefits for the environment, cost savings from reduced demands on natural resources, and creating new markets providing additional economic benefits from circular practices. In theory, the circular economy promises significant environmental and economic benefits and should therefore quickly replace the linear economy, but in practice the old model still dominates. To achieve economically and environmentally sustainable models, circular economy focuses on areas such as design thinking, systems thinking, product life extension and recycling. According to The
The main objective of this paper is to present the basic steps of development of the circular economy concept and the international efforts aimed at introducing more resource-efficient production practices in the context of evaluating the measures taken to stimulate the transition towards a circular economy.

Keywords: *circular economy, sustainable development, climate neutrality, EU.*

Introduction

The current socio-economic system is based on a linear economy (Michelinia et al., 2017), in which companies produce products and consumers use and dispose of them. This linear model of production leads to unnecessary waste of resources in several ways related to production chains, amount of waste disposed of, excessive use of energy and erosion of ecosystems. To achieve economically and environmentally sustainable models, the circular economy focuses on areas such as design thinking, systems thinking, product life extension and recycling.
The need to achieve sustainable development deeply relates to the concept of a circular economy, which is one of the main priorities of the EU (EC, 2020). Concerns about resource scarcity in economic thought (Gregson et al., 2015) can be traced back to Malthus and Ricardo, but the concept of the circular economy began to develop in the 1970s as an alternative economic model, questioning the traditional linear economy. The European Green Deal (EC, 2019) is a response to the challenges related to climate change and the circular economy is a fundamental element of the Green Economy as it represents a regenerative system in which resource inputs and waste, emissions and energy loss are minimized by slowing, closing and narrowing energy and material connections. This is achieved through sustainable design, maintenance, repair, reuse, rework, repair, and recycling, and contrasts with the linear economy, whose production model follows the "take, make, throw away" line.

Although the sustainable models of production and consumption are partially integrated into many official documents and strategies, the first document to bring the circular economy to the fore is the Circular Economy Action Plan adopted in December 2015 (EC, 2015). It underlines that the circular economy covers the whole life cycle of products as in every phase there is an impact on resource use, waste generation, etc. The focus is on those sectors and activities which have specificities of their products or value chains, their environmental footprint or dependency on imported materials - plastics, food waste, critical raw materials, construction and demolition, biomass and bio-based products.

The concept of a circular economy is set as a goal (Gregson et al., 2015) in the context of rising resource prices and climate change. In scientific circles, it is gaining followers in many areas, including sustainability and the environment, and has a major development in terms of measures to limit carbon emissions. The concept attempts to decouple economic growth from increasing resource scarcity and promotes the minimization of waste and its transformation into resources through recycling and reuse.

The circular economy is substantial to reducing the overall environmental footprint, to reaching the climate goals of the Paris Agreement and it paves the way for the EU and European companies to remain competitive in a global market (EP, 2020).

The concept of circular economy (Kirchherr et al., 2017) is of great interest both in academic circles and in practice, because it is considered as the basis for achieving sustainable development. Significant concepts offering the operationalization of sustainable development for business are those of green economy and green growth, where the circular economy concept is argued to be one of the main driving forces nowadays (Ellen MacArthur Foundation, 2014).

Kirchherr et al. (2017) presented a study that analyses 114 definitions of the circular economy. The existence of over 100 definitions for circular economy and the lack of a commonly accepted one, may result in offering the stakeholders the opportunity of adjusting it to their needs. Such a concept, which can be interpreted in different ways, can collapse or remain stagnant due to constant rivalry between different views and the permanent conceptual contention. This requires further theoretical development of the concept and transparency regarding the current understanding of it.

The definition of a circular economy given by the European Parliament (EPRS, 2016) which will be adopted herein is "an economic model based inter alia on sharing, leasing, reuse, repair, refurbishment and recycling, in an (almost) closed loop, which aims to retain the highest utility and value of products, components and materials at all times."

The main objective of this paper is to present the basic steps of development of the circular economy concept and the international efforts aimed at introducing more resource-efficient production practices in the context of evaluating the measures taken to stimulate the transition towards a circular economy.

Materials and methods

This paper is based on a detailed review of reports, analysis and official documents as action plans, declarations, programs and data related to the circular economy, its implementation and the policies aimed at supporting the transition towards a circular economy at the EU level in the context of the efforts for achieving sustainable development.

The transition towards a more circular economy (EC, 2014) is central to the delivery of the resource efficiency agenda set out in the Europe 2020 Strategy for Smart, Sustainable and Inclusive Growth. Higher and sustainable improvements in resource efficiency are achievable and can lead to large economic benefits. The basis of the circular economy is the replacement of raw materials with biological ones, extending the life of the product, utilizing resources

from waste, which simultaneously leads to a reduction in the amount of waste as well as in the need for raw materials. The transition towards a circular economy is based on three pillars: benefits for the environment, cost savings from reduced demands on natural resources, and creating new markets providing additional economic benefits from circular practices, for example in terms of job creation or welfare improvement.
The transition towards a circular economy is one of the main priorities of the EU's policies, aiming to help Europe achieve its obligations in achieving the UN's Sustainable Development Goals. The aim of the EU in the context of the Circular economy and quality of life sub-programme, part of the LIFE programme (EU, 2021) is facilitating the transition toward a sustainable, circular, toxic-free, energy-efficient and climate-resilient economy and at protecting, restoring and improving the quality of the environment, either through direct interventions or by supporting the integration of those objectives in other policies.

Results and discussion

Half of total greenhouse gas emissions and more than 90% of biodiversity loss and water stress are a result of resource extraction and processing (EC, 2020). At the same time, the global consumption of materials is expected to double in the next forty years, and the annual waste generation is projected to increase by 70% by 2050 (Ellen MacArthur Foundation, 2021). In this context, the EU has the ambition to achieve climate neutrality by 2050 and differentiate economic growth from resource use. To fulfill this ambition, the EU needs to accelerate the transition towards a regenerative growth model that is aimed at reducing the consumption footprint and doubling the circular material use rate in the coming decade. Economic losses (EC, 2021) from climate-related extreme events in the EU are already over €12 billion per year. If global temperature reaches a level of 3°C above pre-industrial levels it would result in an annual loss of at least €170 billion (1.36% of EU GDP) and will endanger life, especially in the coastal areas which produce about 40% of the EU GDP and are home to around 40% of its population.
According to the Impact Assessment Report of the EU Strategy on Adaptation to Climate Change (EU, 2021) losses will increase over time and that effect will be multiplied with increasing global temperature. So, it is estimated that a more ambitious approach to change is needed, which includes additional and more comprehensive measures. With their implementation, it is evaluated that by 2050 the economic, social, environmental and cross-cutting impacts on the long term could be significantly mitigated. For example, the circular economy can reduce global CO_2 emissions (Ellen MacArthur Foundation, 2021) from cement, steel, plastic, and aluminium production by 40% or 3.7 billion tonnes in 2050 and so to achieve almost half of their net-zero emissions target.
International efforts to tackle the climate crisis (EC, 2020; Ellen MacArthur Foundation, 2021) have focused on a transition to renewable energy and increasing energy efficiency. But these measures can only address 55% of CO_2 emissions. The remaining 45% come from the production of materials used on a daily basis so they also should be taken into account. The circular economy can contribute to completing the picture of emissions reduction by transforming the way products are made and used. For transforming the economy into a circular one (EC, 2020) an approach based on appropriate assessments for creating science-based policymaking is needed as well as the circularity and sustainability principles to be embedded in all stages of the value chain.The new Circular Economy Action Plan (EC, 2020) provides a future-oriented agenda for achieving a cleaner and more competitive Europe by integrating it in the regulatory framework, close cooperation with all interested parties and building on circular economy actions implemented since 2015, including sustainable product

design, waste prevention, creating a market for secondary raw materials, limiting the use of toxic substances and transition to circular production and consumption models. For the 2021-2027 programming period, EU will provide over €550 billion or 30% of its budget for climate action (EU, 2023) and additional funds from the Social Climate Fund for the 2026-2032 period. The EU main tools, mechanisms and programmes supporting measures and activities related to mitigating and adapting to climate change and achieving climate neutrality are represented in Table 1.

Table 1. EU financial support for transition towards climate neutrality.

Tool	Financial support, billion €	Period	Supported measures/activities
Social Climate Fund	Up to 65	2026-2032	People and businesses most impacted by the introduction of the new (ETS)
Modernisation Fund	48	2021-2030	• Renewable energy • Energy efficiency • Energy storage • Energy networks • Just transition in carbon-dependent regions
Innovation Fund	38	2020-2030	Development of innovative low-carbon technologies
Just Transition Mechanism	55	2021-2027	Measures for addressing the social and economic effects of the green transition
Horizon Europe	95.5 total; about 33.4 (35%) for climate-related projects	2021-2027	• Tackling climate change • Achieving the UN's SDGs • Boosting the EU's competitiveness and growth
LIFE	5.5	2021-2027	• Nature and biodiversity • Circular economy and quality of life • Climate change mitigation and adaptation • Clean energy transition
Next Generation EU (NGEU)	672.5 total; about 248.8 (37%) on climate-related objectives	2021-2027	

Source: European Union, official website of the Council of the EU and the European Council, 2023.

Social Climate Fund, Modernisation Fund and Innovation Fund will be financed from the revenues from the sale of the allowances under the new emissions trading system (ETS) and in this context, the exact amount of the financial support depends on carbon price. So far, for the 2021-2023, €4.11 billion from the Modernisation Fund have been disbursed for 61 projects in 8 countries. Also, the EU and its member states, as the largest providers of public climate finance in the world, have allocated €23.04 billion in 2021 (EU, 2023) to help developing countries reduce their emissions and build resilience against the effects of climate change.

Almost €1.35 billion from the budget of the LIFE Programme (EU, 2021) is intended for the Circular economy and quality of life sub-programme, which offers support for the transition

toward a sustainable, circular, toxic-free, energy-efficient and climate-resilient economy and at protecting, restoring and improving the quality of the environment, through direct interventions or by supporting the integration of those objectives in other policies.
Still, Europe's economy remains very resource dependent. A report of Ellen MacArthur Foundation (2015) puts the focus on three human needs that together account for 60% of EU household expenses and 80% of resource use - mobility, food, and housing. Shifting to the circular model could contribute significantly to achieving Europe's growth, employment and environmental objectives. The study shows that in the circular development scenario by 2050 the average household income and European GDP will increase respectively by 44% and 27% compared to a growth of 24% of income and 15% of GDP in the current development scenario. Shifting towards a circular model would deliver annual benefits for the European economy of up to €1.8 trillion by 2030.

Conclusion

The circular economy is considered as the basis for achieving sustainable development as it promises significant environmental and economic benefits. Therefore, it should have yet replaced the linear model, but in practice still, the linear one dominates. This could be partially related to the existence of more than 100 definitions for the circular economy. The lack of a commonly accepted definition allows the stakeholders to interpret it in different ways and to adjust it to their needs. So, it is of great importance for the concept to be further developed in order for the current understanding of it to become much more clear.
So far, when addressing the need to decrease the levels of CO_2 emissions, the focus is mainly on the transition to renewable energy and increasing energy efficiency. These measures can address 55% of CO_2 emissions so it is vital that the remaining 45% comes from the production of materials used on a daily basis to be addressed too. The circular economy can contribute to emissions reduction by transforming the way products are made and used by taking into account the circularity and sustainability principles in all stages of the value chain.
For the 2021-2027 programming period, the EU will provide over €550 billion or 30% of its budget for climate action. The implementation of all measures taken into account will lead to benefits for the European economy of up to €1.8 trillion by 2030.
Although the efforts yet put forward for shifting towards a more circular model of production and consumption, the EU economy is still very resource-dependent and economic losses from climate-related extreme events in the EU are over €12 billion per year. This imposes an approach that will lead to more ambitious changes in the existing economic model with a focus on areas such as design thinking, systems thinking, product life extension and recycling.
To achieve climate neutrality by 2050 and differentiate economic growth from resource use, the EU should accelerate the transition towards a regenerative growth model which will contribute to the reduction of the consumption footprint and to a significant increase in the circular material use rate.

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YOUNG CONSUMERS AND ETHICAL MEAT CONSUMPTION

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Abstract

Animal production and meat consumption is a topic that contains ethically and environmentally questionable aspects. This paper presents qualitative research based on some principles of grounded theory and uses semi-structured interviews with young people who live in a large city and are interested in the origin of the meat they eat. The research questions were: How do young people who live in a big city and are interested in the origin of the meat they consume deal with the meat consumption? What factors influence the consumption of meat with a clear origin? How do these young consumers deal with the ethical and environmental demands of meat consumption? What relationship do these consumers have with the farmer? The research results show that meat consumption is influenced by minimalism, i.e. by buying a smaller amount of meat that is sufficient for consumers and at the same time less environmentally demanding. Consumer decision-making is formed by criticism of overconsumption and conventional agriculture. Consumers are aware of the ethical issues of meat consumption, but do not attach much importance to it and prefer quality over quantity, which is reflected in their willingness to support smaller farmers, from whom they look for quality.

Keywords: *meat consumption, qualitative research, Czech Republic, consumer behaviour, animal ethics.*

Introduction

Agriculture has undergone considerable change over the past two centuries transformation. It moved away from traditional agriculture based primarily on human labour, solar energy and the work of farm animals to a form of industrial using advanced technology, and especially energy-intensive inputs, which are often obtained from fossil fuels resources. This transformation was mainly driven by the idea of feeding the rapidly growing population, which but it was not fulfilled. The intensification of agriculture did not lead to solving the problem of the hungry people, but rather to create socially and environmentally demanding food production. (Fraňková and Cattaneo, 2018). Livestock production itself also becomes ethically and environmentally problematic and specifically, meat production is a problem within the global sphere. Intensive farm breeding animals are often criticized in the world, for example, because of their high carbon and water footprints, land occupation for the cultivation of fodder, deforestation and the associated loss of biodiversity of the territory, creation a large amount of waste and, last but not least, depersonalization when working with animals, soil or other sources. Here, the animal often becomes a mere tool to satisfy human needs (McDermott, 2017).

It is not only production that is a problem on the market.

According to Krautová and Librová (2009), it is not possible to researching the impact of human activity on nature also neglects the consumer side of the whole process. It is consumers who influence the behaviour of companies with their purchasing preferences. The inseparability of production and consumption can be seen in the example given by Røpke (2003), who talks about two concepts of understanding consumption, i.e. resource consumption and final consumption. Resource consumption it is not created only with the

help of technologies of production, subsequent redistribute in and distribution, but in a way, it is also affected by the size of the final consumption, which is multiplied by the size population. Final consumption is understood as the volume of goods and services intended for satisfaction needs and is made up of 70% household consumption, the remaining percentage is final consumption government institutions and private non-profit institutions. (Krautová and Librová, 2009).

With increasing consumerism, an anti-consumer society is also developing. Iyer and Muncy (2009) describe a society that is responding to the uncontrolled growth of consumerism in the world. The authors divide anti-consumers into four groups. Groups differ in whether people limit overall consumption or focus only on certain products or brands. And at the same time, the groups are divided according to whether people consume less with regard to social problems, such as environmental problems, or whether they are solving a more personal side of things, such as simplifying their lives. *Global Impact Consumers* who are interested in reducing the total waste on the planet. The reasons for the reduction are primarily environmental, at the same time they point to inequality between people, which is closely related to consumption. They criticize the inequality between the poor and the rich, where the overconsumption of the rich contributes to the poverty of the lower classes. The second group is the *Simplifiers*, who believe that maximizing their consumption, the kind that is common in society, has undesirable consequences such as stress or detachment from the pursuit of higher goals. Spiritual elements can be found in them - they say that it is morally wrong to spend so much energy on self-centered consumption. They experience important decisions about whether to act on a sensitive choice of ethical alternatives or when to reduce consumption to a more sustainable level through voluntary simplicity. *Market Activists* boycott buying a particular brand of goods because they believe that particular brand raises specific social issues – for example, a product that causes environmental degradation or a brand that promotes negative social behaviour. The fourth group of *Anti-Loyal Consumers* represents the opposite of "Brand Loyalty" - this is the commitment to buy a certain brand, to be loyal to it because of real or often imagined superiority over other brands. The Anti-Loyal Consumers group represents the opposite – not buying a certain brand because of its possible negative impact on society or the environment.

The goal of the research was to characterize young consumers of meat with a clear origin and factors influencing their consumer behaviour.

Materials and Methods

The research questions were - The main research question: How do young people living in Brno and interested in the origin of the meat they consume deal with its consumption? Side questions - What factors influence the consumption of meat with a clear origin? How do these young consumers deal with the ethical and environmental demands of meat consumption? What relationship do these consumers have with the farmer?

The paper is based on a review of secondary data and primary data collected by a qualitative research - in-depth semi-structured interviews. Face-to-face interviews were conducted in 2020 with eight respondents (age 18-30) living in the city of Brno (appx. 400 000 inhabitants) in Czech Republic. Respondents were obtained by a snow-ball method and an equal number of women and men was randomly selected so that the data would not be distorted by the predominance of one gender. These were mainly university-educated people, in two cases the respondents had a secondary education. In order to obtain various monthly income levels students, full-employed and a mother on maternity leave were included. The coding method was used in the analysis of the data from the in-depth interviews. After all texts were coded,

all codes were assigned categories they could fall into. The codes in the categories may intermingle in different ways or one code may fall into several categories at the same time.

Results and discussion

The paradigmatic model according to Strauss and Corbin (1999) was used during axial coding: Causal conditions - Phenomenon - Context - Intervening conditions - Action and interaction strategies - Consequences. Based on the coding the following main categories were identified: a) Criticism of conventional agriculture, Criticism of overconsumption – b) Looking for meat of known origin – c) Meat consumption – d) Environmental reflection, Ethical side of meat consumption – e) Conscious consumer behaviour – f) Procurement of meat with a story.

Criticism of conventional agriculture, Criticism of overconsumption - All of the respondents see the beginning of the problems in overconsumption, which humanity builds up through its consumption. This is not only a criticism of overconsumption of meat, but of overconsumption in general. Respondents sympathize with the concept of zero waste and try to reuse all the goods they buy. They plan their shopping and think about the meals they will cook: *“I just care about not throwing food away. So that there wouldn't be any loss of food because of me. And as with the meat, it's probably the highest form for me.*" In a certain way, the classic form of transgression is disturbed here, as stated by Librová (1994). The respondent does not experience the expected good feeling from accumulating property. She thinks carefully about what she buys to produce as little waste as possible.

Respondents also see a problem in overconsumption of meat, people should indulge in better quality meat but in smaller quantities. The increase in the amount of goods produced also makes it impossible for people to appreciate the given product. One respondent recalls how, as a child, the fact that they had meat meant that they were well off. For her, meat was a sign of wealth. Today, however, this is not the case for some people, because they have the option of buying kilos of packaged meat in supermarkets, but that is very impersonal. One respondent even described buying meat in a supermarket as "disgusting". On the other hand, she does not find it disgusting to work with animal entrails at a domestic slaughterhouse. Disgust here does not mean an aversion to meat, but an aversion to depersonalization and anonymity, an aversion to the loss of the ritual experience that killing an animal was in earlier forms of society.

The respondents agree that the form of conventional agriculture is unsuitable both for themselves and for the animals and natural resources that are used in it: *"So I realize that there is an awful lot of input. Well, I'm talking about the work put in as a human, but also those resources, such as water, energy, soil, everything. Actually, it is the most demanding production of the meat. As in the entire menu, it is the most demanding"*. And according to the respondents, a large part of society is not aware of this fact. Another respondent sees a problem mainly in the way livestock are raised: *"I see conventional farming as a problem when it is done in a cafo (concentrated animal feeding operation). And those are the ones where the animal is one on top of the other. Of course, soya, I don't know what else, grain is grown for them.*" And this is precisely one of the big problems of intensive agriculture that the respondents are aware of. By finding information about where the animal they eat lived, they pressure farmers to change routine patterns of farm animal husbandry. When analysing the behaviour of the producers, the respondents also came to a criticism of the unnatural life of the animal to which it is exposed in today's agriculture. "*You see the farm, in my opinion, meat should not be kept in halls like this. The animal must see, be in the air.*” This is a cycle in which the animal is dissatisfied, and thus
a disgruntled consumer who eats an animal.

Looking for meat of known origin - For some respondents, the need to know the source of the consumed meat stems from the situation of their family, which owned farm animals. The family has become a source of information for them, which in their own way consumption respondents could later use. This is also where the feeling of naturalness that they have from eating meat originates. The places of origin where the respondents buy their meat vary, and it is not always a direct purchase. We can talk about acquiring rather than buying because if you they get meat from their family, usually they get it as a gift. Smaller farms in the vicinity of Brno are used by about half of the respondents. They also use the options of small butchers in the centre of Brno or online shopping, in which case it is most often a box delivery scheme service.

Finding out the source of the meat is definitely important for the respondents, but they do not always succeed. The amount of meat consumed varies, but the emphasis is always on lower consumption than the general population. So, the quality of the meat prevails over the quantity here, just as it is in the consumption of other products. The respondents do not directly feel the need to consume meat from local sources in the vicinity of Brno. Since most of them still have two “homes” (in Brno and with their family), local is a broader term for them than just the surroundings of their only permanent place of living. Respondents do not seek entirely certified organic label as a clear guarantee of quality: “"*But then again, it could be that they're feeding it organic grain or organic soy, which the cow isn't quite made for. So, it doesn't quite work for her and then it doesn't quite work for me. But I was just saying that organic is not as important to me as "grass fed". I don't really care if there is an organic sign. But if it's pastured, that's what makes the meat healthy or better.".* In a different form, they talk about organic, criticizing the high price of organic meat and preferring to get meat from their family. One respondent does not buy organic meat mainly because of the price: *"I almost don't buy organic meat. Unless it is on sale because it's terrible expensive.”*

Meat consumption - The amount of meat consumed from a clear source varies. The research criterion was meat consumption at least once per month and that meat of known origin prevailed. Too little consumption could indicate vegetarianism. The scale of the frequency of eating meat is varied among the respondents. The lowest frequency of meat consumption was once or twice a month. Another respondent says: "*At the moment I try to keep it at a certain level, that is 500 g per week. And I've never even come close to going over it. Such an imaginary border that I really never want to get there.*" The amount of meat consumed also varies in its type. Another reason for eating meat or other parts of animal bodies is health: "*I probably also eat a lot of organs. Mainly the liver. Like mainly, but I try to eat it, I set myself the ideal of having the liver once a week. But I'll take it once every 14 days, when I'm coming home to my family, for example.*"

Environmental reflection, Ethical side of meat consumption - All respondents are aware of the impact of animal production on the planet, as well as the ethical issues that accompany meat consumption. Each of them decided to consciously consume meat for a different reason. Some emphasize more environmental awareness, soma was the trigger rather the ethical side. Others, on the other hand, see awareness in taking care of their health. We can say that the respondents may have stopped at the so-called "meat paradox", when they are aware of the impact of animal production on the planet, but do not want to give up meat consumption. They realize that even their style of consumption is demanding for the planet. However, the animal is definitely no longer viewed as a thing, as stated by Frey and Pirscher (2016). But at the same time, we see that the animal is judged according to the purpose it fulfils for consumers. At the same time, animals are evaluated by the respondent as better creatures than people. But that doesn't stop her from identifying animals as food for humans. But everyone feels respect for the animal in a certain sense. Respect for the animal itself and the fact that it will be their food - *"And I also realize that the animal had a life. I respect it so much. I*

respect it as if it were my friend and I care that it is well. I want them to have good conditions if they are to serve me in some way."

Conscious consumer behaviour - Respondents believe that by purchasing meat from a smaller farm, the ethical problem is not solved, but at least mitigated. Criticism of local meat purchase by Stanescu (2019) is commented by the respondent as saying that death awaits everyone. Buying meat from a smaller farm will contribute to at least a partially better life for the animal. In general, killing an animal for one's own sustenance is not seen by the respondents as problematic, some would even be able to kill some animals themselves -*"I can kill a hen, but I don't know a goat. I find that so cute. But I guess if I had to stand up to it, I would. I don't know why, somehow it would bother me more to kill the goat than the hen. The chick looks like she doesn't know anything. When you hold her upside down, by those legs, she looks like she doesn't know anything. She looks so out of it. But that goat roars quite loudly.'* Meat is not perceived by the majority of respondents as a necessary component of food, but at the same time, none of them see veganism as the right path. Respondents also sometimes get carried away by their taste and consume meat of which they are not 100% sure of its source.

Procurement of meat with a story - So what led the respondents to conscious consumption? This decision was mainly influenced by the values that the respondents attribute to individual things. The change in values came especially during their vegan periods. Respondents decided to trade their own comfort for the comfort of animals or the planet. But after a certain time, the value scale changed and their health or taste satisfaction became more important than, for example, the animal's welfare. At the same time, they did not want to continue mainstream meat consumption and took something away from veganism, a need inform yourself about the food consumed - *"Somehow I noticed in myself that veganism is not quite the path for me. But I wanted to preserve some of the values I had acquired during that veganism. Some environmental values. And I try them at least to preserve it by not consuming the meat as much as before and I try to choose a quality source of the meat when possible*". Respondents evaluate the period of hunters and gatherers, as described by Bulliet (2005), as inspiring, for example, in consuming only the amount that is needed. According to the respondents, the death of an animal is a ritual that belongs to their life. There is also an interesting idea that compares the lives of animals and plants. Many authors such as Singer (1975) or Regan (1983) would certainly disagree with this picture. Conscious consumption is important to the respondents, but sometimes comes second to other issues that are more important to them. An interesting counterpoint is presented by Stanescu (2019). In his article he opens an interesting opinion on the choice of local meat by the consumer. He criticizes the idea that free-range livestock is more environmentally friendly to the planet than indoor farming. He also sees the problem in the fundamental approach in breeding, where farm animals are kept free, but still imprisoned behind a fence or not allowed to cross a certain border. According to the author, the emphasis on "returning to nature" is pointless when it comes to local production. Even with local production, the animal is bought, sold, inspected and killed. According to him, this process does not even come close to "natural" (which we expect when returning to nature). Based on the results the most prevailing approach would be the Simplyfiers one, as formulated by Iyer and Muncy (2009). Thus, the ordinary consumer becomes a conscious consumer who is interested in what he buys. He takes responsibility for the consequences of satisfying his needs. (Ulčák, 1997)

Conclusion

The research results contribute to understanding why young consumers decided to limit meat consumption and what conscious consumption means to them. It can be seen that the impetus for this type of consumption came mainly from the criticism of overconsumption in today's

society and the reluctance to support classical conventional agriculture. These factors could be called external. The internal factor is that they want healthy and tasty meat for themselves, which they can get in this way. One part of the respondents went through a vegan life, they found in it a change in values, which they have preserved in a certain form to this day.

None of the respondents is significantly involved in activism related to meat consumption, they are aware of their consumer responsibility, but at the same time they blame the "system" that affects their decision-making in a certain way, but they do not want to influence it more actively. Each of the respondents is aware of the ethical and environmental issues of meat consumption. Some of them even decided on conscious consumption precisely because of the overconsumption of meat, which they criticize as environmentally demanding. Even if they feel respect for the animal that they will consume, ethical issues come second when deciding on consumption, behind their own health or environmental benefits. They deal with meat consumption by minimalism, i.e. by buying a smaller amount of meat that is enough for themselves. Attitude of these consumers to farmers is different, but they agree that they want to get as much information as possible about the farm before buying meat, which will satisfy them as demanding consumers. One part gets meat from their family, the other bets on smaller farms.

The study of young conscious meat consumers is seen as an important insight into today's society intensively debating the pollution of the planet, which is partly caused by animal production. But this production is still for a large part of people a source of tasty food, and therefore also a pleasure that they do not want to give up. Therefore, it is important to know how the meat consumption of these young people takes place, if they consume meat but still want to maintain some values that they attribute to their health, the health of the planet or the health of the animal.

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VITALITY OF EDUCATION ORGANIZATIONS IN QALIUBIYA RURAL AREAS (CASE STUDY IN SHEBIN DISTRICT IN EGYPT)

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Abstract

The present study aimed to study the vitality of education organizations in Qaliubiya governorate as one of the high ranking governorates according to the Human Development Index (2008). The study aimed first to identify the vitality of rural education organizations Q. Secondly it aimed to identify the impact of personal variables and administrative on vitality degree for education organizations, thirdly to estimate the size of the gap between the current vital degree and optimal vital degree of educational organizations. The sample frame of Shibin el-Qanater district included 92 primary schools, .50 junior high schools, represented by 18 elementary schools, 12 junior high total of 30 schools. The study results indicate that vitality degree of education organizations was 58%, while the vitality gap was 42%, and the variables of internal environment, efficiency of the school class, and the effectiveness of parents boards had top score on the scale and ranked first among 11 variable, followed by the importance of efficiency of the human element variable, and the success rate, and the efficiency of the external environment, while the variable of school activities, horizontal communication, and vertical communication represents the minimum degree in their contribution to the construction of the scale. The results also indicate that the age variables, and practical experience, management experience are all negatively associated within dynamic scale, while the variables associated with training, labor relations, and the effectiveness of parents boards have positive vital degree.

Key words: *vitality, vitality gap, basic education organizations.*

Introduction

Education is considered the most powerful tool for achieving economic and social growth and for reducing poverty and improving the standard of living. Countries have realized the importance of education in accumulating human capital, which in turn affects productive capacity. Recent studies have indicated that productivity is linked to the form of educational wealth, as well. Other studies showed the effective impact of education on longevity (Human Development Report 1999).

Due to technological change and globalization, education has become extremely important from two angles, the first: providing society with its needs of a labor force capable of acquiring new skills, and second: supporting the expansion and spread of knowledge, which contributes to the development of human resources.

In recent years, the Arab Republic of Egypt has pursued several strategies, all of which serve the educational process, especially basic education as a social organization, to raise its efficiency and effectiveness to maintain the quality of its enrollment. Therefore, it becomes important to study its institutions to determine the extent of their vitality and ability to continue in what serves their purpose. As a base for supplying active and influential elements in human development that represent the locomotive of economic and social development, which reflects the problem of the study and its importance at the same time.

Material and method

The study used the research methodology of social evaluation and the mechanism of building social measures (Barakat 2000).

Study goals:

1. Identify the vitality of rural education organizations.
2. identify the impact of personal variables and administrative on vitality degree for education organizations
3. estimate the size of the gap between the current vital degree and optimal vital degree of educational organizations

The sampling framework Shebin Al-Qanater district includes 92 primary schools, 50 preparatory schools, with a total of 142 schools at the basic education level. Draw a regular random sample with a fraction20%. (the third item in the order), so the study sample was represented by the basic education organizations in the Shebin Al-Qanater district by about 18 primary schools and 10 preparatory schools, which were raised to 12 schools with a total of 30 schools, representing about 20.8% of their total, in order to ensure the quality of the statistical analysis of the data and the statement of these schools.

The questionnaire forms were prepared and tests to ensure their validity, as they were collected from the sample schools with the help of the educational administration at the Shebin Al-Qanater district. experience - training - number of years of management - work relations - effectiveness of the parents' council), and a questionnaire for members of the parents' councils that included 6 areas to measure the effectiveness of schools in serving their local communities from their point of view (health, education, religious, social, moral and agricultural),

In addition to a form for schools (in the quality unit of the educational administration), which answered many of the quantitative questionnaire questions (number of schools, number of teachers, number of classes, number of students, number of administrative workers, percentages of completed success, and finally the degree of effectiveness of council meetings parents) and data were collected during August and September

Operational concepts and numerical measurement of variables:

1. The dependent variable: the degree of vitality of the organization

   The study used 11 axes to measure vitality according to the procedural definition adopted by the study, and these axes are: the external environment - the internal environment - the availability of educational tools - the available capabilities (school rooms) - the human element - organizational activities - horizontal communication - vertical communication - organizational coordination - The organization community effectiveness from the perspective of the parents' councils - the actual achievement from the perspective of the goals (end of academic year success %).

Table 1. Characterize the digital measurement of the axes of the vital scale.

Variables	Theoretical range		Actual range		Arithmetic mean	standard deviation
	Lower limit	Upper limit	Lower limit	Upper limit		
external environment	6	18	6	18	10.4	3.9
internal environment	30	180	124	172	144.6	14.8
educational tools	23	66	24	62	35.6	10.7
school rooms	-	296	132	282	218.5	45.3
human element	10	30	13	28	20.8	3.9
Organization activities	12	300	72	114	95.4	12.8
horizontal communication	10	40	12	26	18	4.1
vertical communication	3	15	3	12	7.3	2.5
Organization coordination	10	40	10	32	19.8	7.7
organizational community effectiveness	6	30	12	28	21.1	5.8
academic year success %	1	10	5	9	6.3	1.1
Tota;	111	1025	450	771	597.8	95.6

Source: study sample

Independent variables and The numerical measurement of the study

Within the framework of the reference review of previous studies, six independent variables were considers important in influencing the degree of vitality of basic education schools, (The current age of the school principal, the number of years of his practical experience, the number of training courses he attended, administrative experience, work relations within the school, the effectiveness of the parents' council, and Table No. (2) Presents a description of these variables.

Table 2. Description of the independent variables

variables	Theoretical/actual range		Arithmetic mean	standard deviation
	Lower limit	Upper limit		
age	42.00	57.00	49.0667	4.7989
practical experience	20.00	34.00	25.2000	3.8363
training	2.00	9.00	4.6000	2.5811
administrative experience	5.00	15.00	9.3667	2.8465
work relationship	1.00	5.00	3.2333	1.5013
parents' council effectiveness	1.00	5.00	3.5000	1.1055

Source: study sample

Results and discussion

Identify the vitality of rural education organizations

To build the vitality scale, 11 sub-axis of measurement were used, and by using the correlation matrix for the eleven axes Where the scores of the eleven indicators were collected as a measure of the degree of vitality.

The scale's validity coefficient was *0.98, and its stability coefficient was 0681. This theoretical range of the scale ranged between 108 and 1025 degrees, with an arithmetic mean of 597.8, and a standard deviation of 95.6 degrees. By dividing the theoretical range into three

categories of equal length and progressively ascending, it can be said that the majority of the schools fell into the medium-vital category, with a rate of 90% of the total sample schools.

Table 3. Distribution of basic education organizations on the scale of vitality:

Data	Low vitality 111-415.6	Medium vitality 415.7-720.3	Height vitality 720.4-1025
Number	-	27	3
%	-	90	10

Source: data from field study.

The stepwise regression method was used to show the most important axes in the formation of the scale.

Table 4. The relative importance of the axes involved in constructing the vitality scale:

Variables	R^2	R^2 adjusted	%explained variance	F test
human element	**0.879**	**0.875**	**87.5**	18
horizontal communication	**0.948**	**0.945**	**7.0**	172
external environment	**0.969**	**0.965**	**2.0**	62
school rooms	**0.979**	**0.976**	**1.1**	282
vertical communication	**0.986**	**0.983**	**0.7**	28
internal environment	**0.993**	**0.992**	**0.9**	114
Organization activities	**0.994**	**0.993**	**0.1**	26
educational tools	**.0996**	**0.995**	**0.2**	12
academic year success %	**0.997**	**0.997**	**0.2**	32
organizational community effectiveness	**0.999**	**0.998**	**0.1**	28
Organization coordination	**1.000**	**1.000**	**0.2**	9

Source: sampling data significant :0.001

Identify the impact of personal variables and administrative on vitality degree for education organizations.

Table 5. Simple correlation coefficients for the relationship of the vitality scale of basic education organizations with the degree of their vitality

Independent variables	R
age	-0.821
practical experience	-0.544
training	0.860
administrative experience	0.910
work relationship	0.916
parents' council effectiveness	0.895

Source: field study data.

In order to show the most important factors affecting the degree of vitality of the basic education organizations, the study used the stepwise regression method, which indicated that the most important of these factors are administrative experience, practical experience,

training, and finally work relations, as these variables explain about 91.7% of the variation in The degree of vitality of these organizations, as indicated by the data of Table No. (6).

Table 6. the combined effect of the independent variables on the dependent variable

Independent variables	R^2	R^2 adjusted	% explained variance	F test
administrative experience	0.829	0.822	82.2	125.769
practical experience	0.895	0.886	6.4	106.292
training	0.915	0.904	1.8	86.164
work relationship	0.930	0.917	1.3	75.938

Source: data of field study.

Estimate the size of the gap between the current vital degree and optimal vital degree of educational organizations.

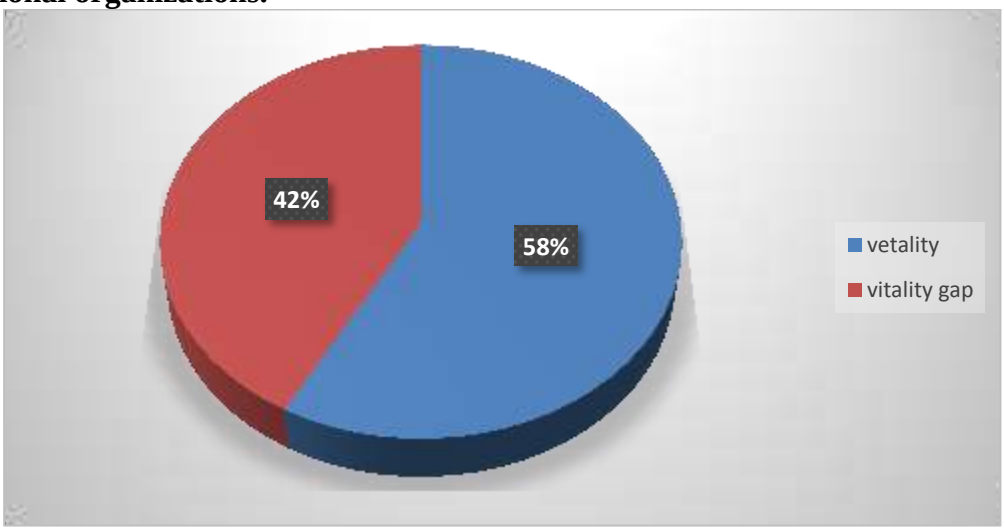
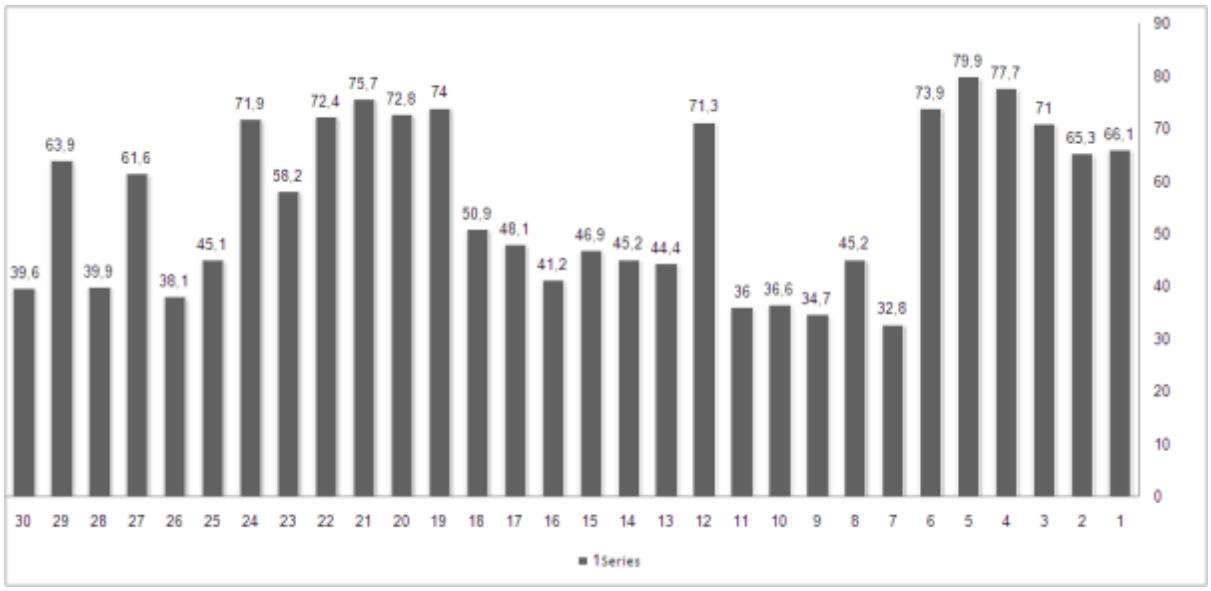


Diagram 1. Gap of educational organization vitality

That was done on three levels:

a. total schools included in the study sample
the average degree of vitality for the total basic education organizations was 597.8 degrees Out of the total score of 1025 degrees, which means that the percentage of vitality of basic education organizations in the sample was 58%, and the gap percentage of the vitality of these organizations is 42%.

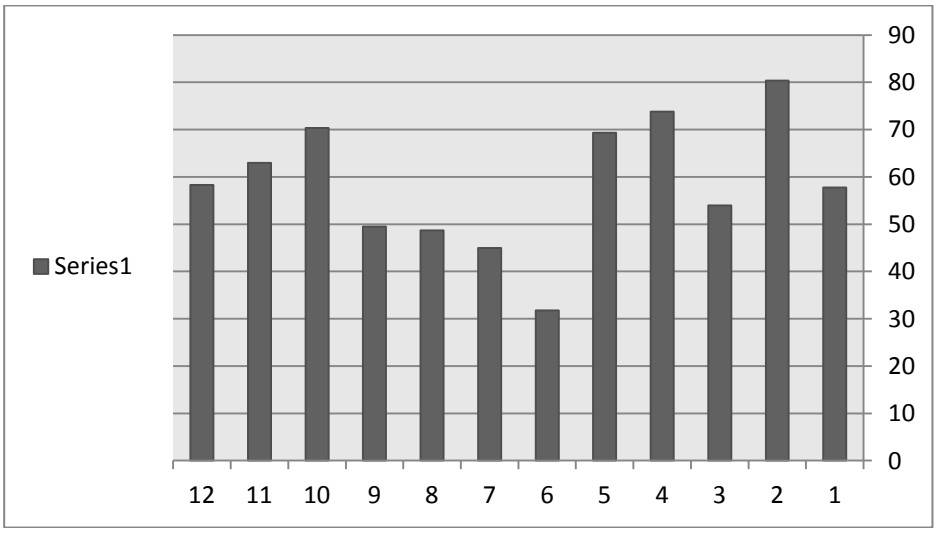
b. Measuring the degree of vitality gap for Every school from the study sample.
At the school level, the following graphic display was used to indicate the degree of vitality and gap for each educational organization, It is clear that there are 5 schools with a degree of vitality of 70%.
Diagram 2. Graphic representation of the degree of vitality of basic education schools in the study sample



c. Measuring the vitality gap for each variable used in the study

To show the vitality gap for each of the studied axes, the study used the percentage of the arithmetic mean divided by the upper limit of its measurement.

Diagram 3. Vitality gap for the study variables.



Source: data of the field study

1-External environment, 2- internal environment. 3-educational tools, 4 school rooms, 5-human element, 6- organization activities, 7-vertical communication.8- horizontal communication, 9-organization coordination, 10-organizational community effectiveness, 11- academic year success %, 12-total

Conclusion

The vitality gap of the basic education organizations is 41.7%, which is a satisfactory result of the quality unit in the educational administration from the perspective of social evaluation it is considered alarge gap that needs to more efforts to upgrade schools with low vitality

There are only 5 schools whose vitality is more than 70% of the total score of the measurement. The vitality scores of the majority of the 17 schools in the sample were between (50% - 70%), while the vitality of 8 schools were less than from 50%.

The variable of the internal environment, the efficiency of school rooms, and the effectiveness of parents' councils are the highest degree among the eleven axes that entered the index.

The absence of school activities is considered a missing variable of vitality, despite being an important educational input for developing students' creative skills and hobbies and helping them to taste aspects of arts and literature, which reflects the failure of higher administrative levels to support the development of these creative aspects of students .

The lack of horizontal communication reflects the isolation of basic education organizations from their social reality, and also means that social organizations operate in isolated islands despite being in one geographical area!

Vertical communication with higher administrative levels is weak

According to The weakness of the horizontal and vertical connections, it is natural that the degree of organizational coordination of basic education organizations decreases with social organizations working in the same geographical scope.

Parents' councils play a major role in the vitality of basic education organizations, , which is unexpected in light of what is known about the formality of these councils! However, the Quality Unit explained that the emergence of this variable is a logical result of its efforts to involve the local community in facing some of the problems facing the school during its educational activity.

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APPLYING CATEGORICAL REGRESSION FOR EXPLORING VETERINARY STUDENTS' ATTITUDES TOWARDS CAREER PERSPECTIVES

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Abstract

This study focuses on exploring attitudes of veterinary students towards issues reflecting career perspectives, in relation to basic classification criteria. A survey was carried out using a specially designed questionnaire on a sample of 105 veterinary students, who were asked to indicate their "level of agreement" on certain individual issues of multithematic variables concerning their job prospects. Based on the data collected, the relationships between the variables of interest and independent classification criteria of students, such as age, family's residence, parents' occupation, etc., were estimated using the categorical regression. The analysis revealed that mother's occupation and family's residence play a decisive role in students' attitudes to issues related to career perspectives. In particular, students with a private/civil servant mother, a mother employed in the veterinary field or from families located in urban and/or rural areas consider the perspectives for the veterinary profession (companion and productive animals, food hygiene and technology, veterinary medicinal products) to be highly positive, contrary to those with a mother who is engaged in farming or from semi-urban areas, who consider them unpromising. Age, parents' profession and place of residence largely determine the respondents' attitudes regarding problems they are expected to face as young professionals. Younger students, and those who origin from the metropolitan area of Thessaloniki or smaller urban areas, appear to be more concerned about these issues. In contrast, older students, or those with a civil servant/entrepreneur/freelance professional father, or with a mother who is a veterinarian, are less cautious. Overall, the findings of the study could contribute to the debate regarding the entrepreneurial prospects of veterinary students but also to the improvement of their training in issues related to the modern business market.

Keywords: *Veterinary students' attitudes, categorical regression, career perspectives.*

Introduction

Veterinary medicine, being a primarily professional science, orients the veterinarian to professional roles which highlight a variety of duties and responsibilities, both in protecting the animal health and providing medical care to animals, but also ensuring the public health. Therefore, the choice of a professional and/or business career by veterinary students is an important process which concerns them throughout their studies and especially in the pre-degree stage, since it largely determines their subsequent professional and social future.

In Greek labour market, the veterinary profession is expanding and the prospects for veterinarian employment are estimated to be rather favourable due to the increasing demand for animal health and medical care services (Kirmizoglou, 2020). At the same time, there is a clear shift of veterinarians towards the private professions, with an increase in the number of those employed as freelancers and private employees and a decrease in those employed in the public and broader public sector or even in local authorities (GEOTEE, 2022).
Undoubtedly, the career choices of veterinary students will have an impact on the future distribution of the veterinary workforce in the labour market when they graduate and seek employment as young veterinarians. Since employment is a two-way and continuously feedback-led process of choice between supply and demand, it is considered to be important and timely to profile the attitudes of current veterinary students on issues related to their career perspectives. In particular, for the educational bodies involved, it is especially important to be aware of the professional needs expressed by veterinary students in order to initiate actions to develop the appropriate skills on relative fields, before students face the increasingly competitive labour market (Zhang *et al.*, 2020).
The aim of the study is to outline the attitudes of veterinary students on issues of multithematic variables concerning their job perspectives, in regard to basic classification criteria, considering that the resulting findings could be useful for the educational bodies involved.

Material and Methods

A survey (Heller & Rosemann, 1974) was conducted on a sample of 105 veterinary students studying in Thessaloniki who were in their last semesters of studies and asked to indicate their "level of agreement" on certain individual issues of multithematic variables concerning their job prospects. The completeness of the specially designed questionnaire used in terms of structure and clarity of questions was initially checked through preliminary completion by a small number of students, and in December of 2022 it was finally administered for completion.
Students declared the "level of agreement" on individual issues of multithematic variables related to career perspectives-(Q21**):** *["a. Positive prospects for the veterinary profession in the companion animal sector", "b. Positive prospects for the veterinary profession in the field of productive animals", "c. Positive prospects for the veterinary profession in the sector of food hygiene and technology", "d. Positive prospects for the veterinary profession in the public sector", "e. Positive prospects for the veterinary profession in the field of veterinary medicinal products/feedingstuffs/supplies" and "f. Scientifically ready to start your professional career"],* as well as to the challenges/problems they are expected to face as young veterinary professionals-(Q22): *["a. Lack of knowledge/orientation for a comprehensive planning of his/her future business", "b. Lack of knowledge as to what the labour market requires and what its trends are", "c. Financing (lack of sufficient funds, difficult access to sources of finance, lack of a financial plan)", "d. Insufficient staffing with appropriate personnel", "e. Insufficient technical support from secondary level partners (e.g. accountants, suppliers, etc.)"].*
All students' responses are in the form of a Likert-type scale with four levels of "agreement", where 4 corresponds to the highest value and 1 corresponds to the lowest value of the scale. In particular, for the statements of the level of agreement, the values used were: "Highly agree"=4, "Fairly agree"=3, "Slightly agree"=2 and "Not agree at all"=1.
The collected data were statistically analyzed with multivariate methods, with respect to independent classification variables/criteria of students such as gender, age, work experience, etc. More specifically, the categorical regression was applied to investigate the relationships

between the multithematic variables "Viewpoints on career perspectives-Q21" and "Challenges/problems the veterinarians are expected to face as young entrepreneurs/professionals-Q22", and the independent classification criteria of students (Kooij and Meulman, 1997; Siardos, 2000; Moutopoulos *et al.*, 2007; Batzios, 2021; Batzios *et al*., 2022). The method was applied using the sums of the scores of all individual issues of the multithematic variables of interest as the dependent variables in the respective regression models. "Age" was used as an independent ordinal variable, while "gender", "method of enrolling the Veterinary Medicine", "work experience", "father's profession", "mother's profession" and "family's place of residence" of the respondent were used as independent nominal variables.

Categorical regression is a multivariate method of analysis that quantifies data of categorical variables by assigning numerical values to their categories, resulting in an optimal linear regression model of the modified variables. The F value of the ANOVA table determines whether removing an independent categorical variable from the initial model significantly reduces the forecasting ability of the model. The multicollinearity between the variables in the model is assessed by the tolerance test (T), with a T value of an independent variable close to 1 denoting lack of multicollinearity (Moutopoulos *et al*., 2007).

Moreover, for the efficient handling of the multithematic variables of interest, a reliability analysis (Reliability test: Cronbach, 1951) of the issues they include was applied (Siardos, 2000). Cronbach's alpha test (Cronbach's alpha coefficient) was used as a validation criterion, where a value greater than 0.80 is considered completely satisfactory, while lower values are considered as acceptable, with a threshold around to 0.60 (Malhotra, 1996; Siardos, 2000).

Results and Discussion

The reliability analysis of the multithematic variables reflecting on career perspectives of veterinary students resulted in generally satisfactory values of Cronbach's coefficient α. In particular, regarding the multithematic variables "Viewpoints on career perspectives -Q21", and "Challenges/problems the veterinarians are expected to face as young veterinary professionals -Q22", the internal consistency of the responses is assessed as moderate, since Cronbach's coefficients α take values close to the acceptable threshold of 0.60 (i.e., α=0.590 for Q21 and α=0.588 for Q22, respectively). However, considering the number of issues of the multithematic variables, the research's stage and the objectives, the estimated Cronbach's α values can be considered as acceptable (Spector, 1992; Norusis, 1999; Karathanassi, 1995; Siardos, 2005; Malhotra, 1996).

Moreover, the evaluations of the statistical indicators and overall assessment of the estimated categorical regression models resulted in relatively high values for the multiple coefficients of determination R^2, based on both the nature of the research and relevant literature (Batzios *et al.*, 2005; WCD, 2000; ADB, 1999). Respectively, statistically significant F values of ANOVA ($P \leq 0.05$) were estimated, indicating that the models fit the data well. For all independent variables remained in the models, the tolerance (T) values were estimated relatively high, confirming lack of multicollinearity (Moutopoulos *et al.*, 2007).

The categorical regression model for "Viewpoints on career perspectives-Q21" of veterinary students revealed that the highest significant values of the regression coefficients b were estimated for the independent variables "mother's occupation" and "family's place of residence", with almost similar relative importance in the model (Table 1).

Table 1. Categorical regression model for the key question "Viewpoints on career perspectives" [Q21]

Independent	Standardized coefficients	F values	Sig.	Importance	Tolerance

	Beta	Std. Error				
Mother's profession	0.335	0.100	11.205	0.000	0.485	0.994
Family's place of residence	0.346	0.098	12.379	0.000	0.516	0.994
Model Summary: R Square=0.249, F value of ANOVA test =2.81 (Sig =0.003, df: ν_1=11, ν_2=93)						

From the quantified values of the independent variables and the corresponding transformation diagrams (Table 1/ Figure 1), it is evident that respondents whose mother works as a civil/private servant or as an employee in the veterinary field tend to be highly positive about the perspectives of the veterinary profession in the general fields of employment (companion and productive animals, food hygiene and technology, veterinary medicinal products, etc.). On the contrary, those whose mother is engaged in farming or is engaged in household duties tend to consider the perspectives of the veterinary profession as unpromising. In parallel, those from urban or rural areas tend to be highly positive about the perspectives for the veterinary profession, attitude that is probably driven by their general views on the veterinary profession (e.g., companion animal veterinary practices). Conversely, those from semi-urban areas are less or not positive at all about career prospects, perhaps due to the sluggishness of the relevant market in these areas, in terms of size and dynamic (e.g., provision of veterinary services).

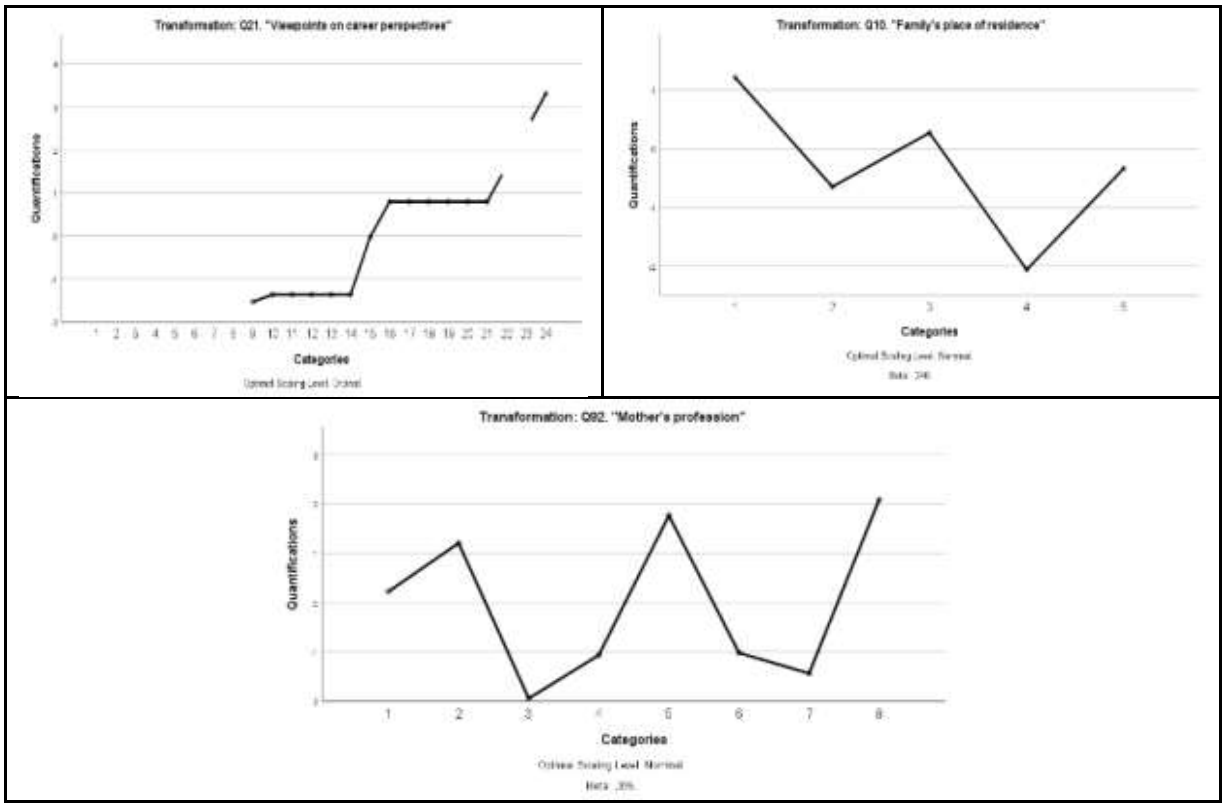


Fig. 1. Quantification plots of the key question "Viewpoints on career perspectives" [Q21], against independent categorical variables of socio-economic status of the respondents

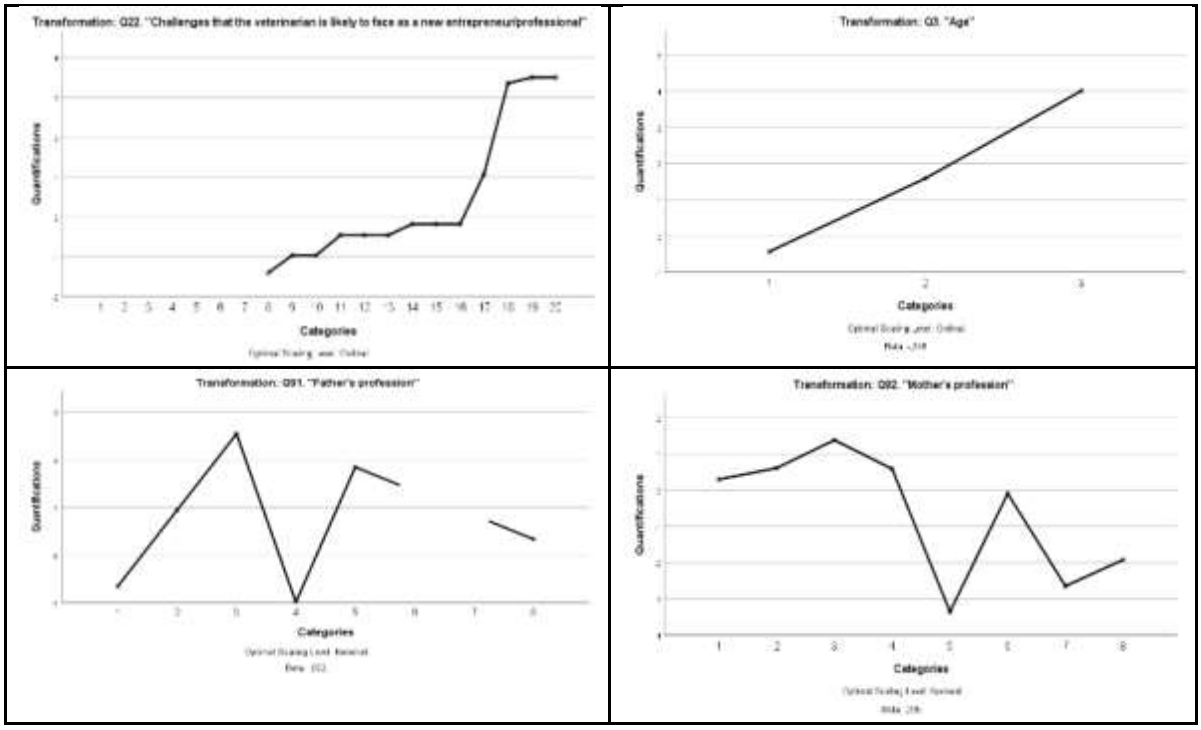
The categorical regression model for "Challenges/problems the veterinarians are expected to face as young entrepreneurs/professionals -Q22" demonstrated that the highest significant values of the regression coefficients b were estimated for the independent variables "age", "father's profession" "mother's profession", "family's place of residence" and "method of

enrollment in Veterinary Medicine", with the dominant relative importance (59.9%) in the model being the "father's profession" (Table 2).

Table 2. Categorical regression model of the key question "Challenges/problems the veterinarians are expected to face as young entrepreneurs/professionals" [Q22]

Independent variables	Standardized coefficients		F values	Sig.	Importance	Tolerance
	Beta	Std. Error				
Age	-0.348	0.175	3.949	0.023	0.019	0.593
Father's profession	0.552	0.179	9.544	0.000	0.599	0.892
Mother's profession	0.335	0.142	5.573	0.000	0.123	0.907
Method of enrollment in Veterinary Medicine	0.326	0.191	2.893	0.093	0.127	0.598
Family's place of residence	0.237	0.127	3.459	0.011	0.132	0.933
Model Summary: R Square=0.389, F value of ANOVA test =2.847 (Sig =0.001, df: ν_1=19, ν_2=85)						

From the quantified values of the independent variables, combined with the signs of the standardized regression coefficients and the corresponding transformation diagrams (Table 2/ Figure 2), it can be seen that younger students appear to be more concerned about the problems they are expected to face as young veterinary professionals, in contrast to older students.



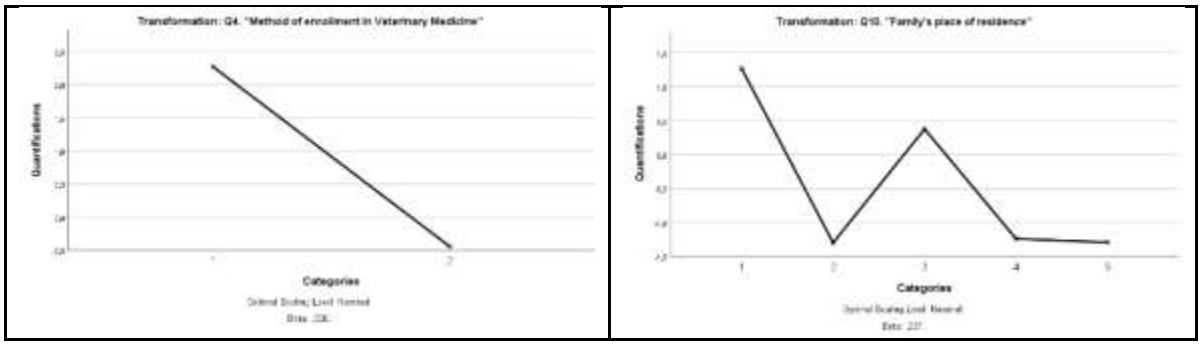


Fig. 2. Quantification plots of the key question "Challenges/problems the veterinarians are expected to face as young entrepreneurs/professionals" [Q22], against independent categorical variables of socio-economic status of the respondents

In addition, respondents with a civil servant or an entrepreneur/freelance professional father, or those with a mother employed in the veterinary field, are less cautious, contrary to those whose father is engaged in farming who assess the problems they are expected to face as young veterinary professionals as very serious. Furthermore, those who have enrolled in Veterinary Medicine after qualifying exams appear to be more aware and assess these problems as very serious. Similar attitudes are expressed by those from families living in the metropolitan area of Thessaloniki or smaller urban areas.

Conclusions

From the estimates of the categorical regression models, it is concluded that gender and work experience do not significantly affect the students' attitudes towards their career perspectives, in contrast to age, method of enrolling in Veterinary Medicine, parents' profession and their family's place of residence.

The place of residence of students' families plays a key role in their attitudes towards career prospects. Both students from urban areas and those from rural areas have an optimistic view of the veterinary profession, presumably indicating that the first ones intend to work in the companion animals or in the sector of food hygiene and technology which mainly thrive in urban centers and the second ones in the livestock sector which mainly involves activities in rural areas. In contrast, those from semi-urban areas express doubts about career prospects in the veterinary field, perhaps due to the limited potential of the relevant market in these areas (e.g., provision of veterinary services). At the same time, the employment of student's mother as a civil/private servant or as a veterinarian also gives optimism about the professional prospects of a veterinary student. Students with a civil servant or entrepreneur/freelance father or a veterinarian mother are optimistically placed towards possible challenges/problems they will encounter as new veterinary professionals/entrepreneurs, in contrast to those whose father is engaged in farming or younger students or those who have enrolled in Veterinary Medicine after qualifying exams, who seem to be more aware and cautious.

Overall, the results of the study are expected to provide valuable information to educational institutions in order to adapt their curricula to better meet the students' needs and expectations about their professional future.

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A SOCIAL NETWORK ANALYSIS ON THE COLLABORATIVE CONSERVATION OF RANGELANDS IN LOCAL COMMUNITIES OF IRAN

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Abstract

Social networks play an important role in facilitating effective and sustainable communication between people responsible for regional conservation plans and those responsible for local conservation measures in rural areas. However, few studies have used social network analysis in conservation planning projects. The purpose of this study was to use the Social Network Analysis (SNA) approach in order to design and facilitate strategic networks to strengthen inter-local and regional links and provide policies that develop collective communication to solve various issues including fire conservation, change of land use will pay. According to this issue, this study used social network analysis, while modeling the network relationships of various actors present in Jiroft rangelands of Kerman province, Iran, to identify structural gaps that lead to the weakness of collaborative conservation of pastures. Quantitative and qualitative information necessary for the study were collected through interviews and questionnaires of network analysis. Results indicated the existence of relatively suitable of collaboration in between different active communities in the study area. Evaluation showed that the density index in network of collaboration was weak, indicating poor social cohesion and reduced resilience of livestock beneficiaries when facing with environmental stresses. The analysis of the index of reciprocity index indicated high interaction that has led to the connection of the entire network. However, the most important problem is the lack of diversity among effective actors in cooperative conservation so that diverse powers can act more successfully in the realization of a multi-centered governance system.

Key Words: *Stakeholders Analysis, Social capital, Rangeland Management, Policy.*

Introduction

To conserve natural resources, stakeholder group cooperation is very important. In the past few decades, attention has been paid to the influence of stakeholders in environmental decision-making (Michael et al., 2022). Environmental policy and decision-making are characterized by complex interactions between different actors and sectors (Lienert et al., 2013).

Pastures are one of the most important natural resources of a country, which have a place in regulating the earth's ecosystem, preserving the genetic resources of plants, providing fodder for livestock, food security, economic development, and people's well-being (Mousavi et al., 2020). In recent years, these valuables resources have been exposed to severe destruction for various reasons. Severe destruction of pastures creates significant social, economic and environmental problems. Dealing with the destructive factors of pastures and efforts to protect and revive them are considered essential measures for sustainable management of pastures.

The most effective management strategy, applying the ecosystem approach and protection at the level of natural areas. Participatory behavior is a set of conscious actions of people in the society towards the fields of natural resources, which includes a wide range of emotions, special inclinations and preparations for favorable behavior towards natural resources. Local people have the best analysis and solutions of the state of natural resources, and because they have a sense of ownership towards those areas, if they have enough knowledge and skills, they can get the best results as environmental protectors. In a research, Turner et al. (2020) introduced the weak connection between diverse resource users and more complex governance networks as a challenge in environmental governance.

One of the tools used to understand stakeholder relationships and strengthen collaborative conservation is social network analysis (Blacketer et al., 2022). Stakeholder analysis is defined as a holistic procedural approach to understanding a system by identifying key actors or stakeholders and evaluating their respective interests in the system. The analysis of social networks, which is applied to the analysis and implementation of policies, focuses on structural patterns between actors. Contrary to the state-oriented views in policy-making, the network approach considers informal decision-making arrangements and the participation of non-state actors as vital (Lienert et al., 2013). The more individuals, communities or organizations are involved in strategic conservation activities, the greater the potential for conservation impact (Maynard et al., 2022). The main core of social network analysis is the systematic and quantitative analysis of relationships between actors. Network analysis makes it possible to identify the structures of the participation policy process in detail and reveal the central role of certain actors. After identifying the important players in the first stage, analyzing the social networks, the relationship patterns of the actors and the overall structure of the process are analyzed. The multi-level divisions of the network of actors in the network analysis make it possible to determine their importance and evaluation. The purpose of this study is to use the Social Network Analysis (SNA) approach in order to design and facilitate strategic networks to strengthen inter-local and regional links and provide policies to develop collective communication such as the ability to respond adaptively to complex environmental changes and manage the flexibility of social ecological systems.This accountability, in the majority of a network communication system, leads to the development of common values and the development of innovative solutions for rangeland problems, including fire protection, change of use, construction and mining projects, grazing management, direct protection of fields, handovers, etc. to be the structural model of actors' collaborative links strongly affects the actors' behavior and, as a result, the effective results. According to this issue, this study uses social network analysis (SNA), while modeling the network relationships of various actors, identifying the network role of stakeholders in conservation cooperation, and identifying the structural gaps that lead to wrong flows in the studied area.

Materials and methods

Jiroft city in Kerman province in the southwest of Iran was the socio-ecological border of this study. Pastures play a significant role in the livelihood of the local people in the native areas of Iran. The fires that occurred in the pastures have played the biggest role in the damage to the pastures and the shrines of the native people of these areas. Therefore, the study of rangeland users as people who play a role in cooperative conservation has been done. Organizational forces such as natural resource departments that are in charge of rangeland protection have also been studied. In recent years, due to the lack of manpower and facilities, these organizations are not able to protect 830 thousand hectares of national lands in Jiroft city alone, and due to the wide range of pastures and forests, these areas require cooperative

protection, which has caused all institutions and individuals to Their role in this study should be considered.

Determining the effective key actors in the cooperative protection of Jiroft pastures in Kerman. At this stage, active and hidden local actors are analyzed based on the method of social network analysis (Social Network Analysis) to identify and structure the social relations of trust and their participation. In this research, according to the socio-ecological boundary of the studied area and based on the identification of actors at different central or peripheral levels, various indicators of network analysis will be evaluated (Islami, 2021). A social network, built through some operating systems, can be a special type of database from which data can be obtained by structured queries, a social network is a special type of graph. which can be analyzed as different network data structures and also a social network can be a special type of knowledge that has formal semantics and reasoning (Ehrlinger and Wöß, 2016). In summary, the main advantage of a social network that includes highly enriched ontologies is that it allows us to extract hidden relationships and patterns (Patel et al., 2018; Wang et al., 2020). SNA is an innovative tool in collaborative relationship discovery that can help the development and dissemination of knowledge in a social network system (Wang et al., 2020) and also show who knows what and visually It can show the importance of stakeholders over time and looks for stakeholders who have the most intelligence about a particular problem or activity (Nissen and Levitt, 2004).

The indicators of density, concentration, size, ambivalence are the most important indicators of social network analysis that have been investigated. Since the defined goals and questions of this research are focused on identifying the key actors, their relationships in the cooperative protection of Jiroft pastures in Kerman province, therefore, emphasis on macro-level indicators is a priority.

In this research, by using questionnaire tools and interviews with experts, partnership relationships were completed in the form of relationship matrix. In order to know the network of actors effective in protecting and receiving communication data, two methods were used. In the first method, by preparing a list of effective activists using the information of local people and public and private organizations, and in the second method, using the snowball method to introduce new people who probably did not have a place in the initial list. These two methods are used in a complementary way with the aim of obtaining the most information. To collect information from a set of survey methods including observations and field visits, Questionnaires and interviews with experts will be used (Tang et al., 2013). The questionnaires in the relationship identification section are network analysis questionnaires, and in the challenge evaluation section, they are construction researcher questionnaires, the validity of which is confirmed by experts, and the reliability of which is tested through Cronbach's alpha test.

The unit of measurement of social relations analysis and the investigated network is the complete network and in the form of a survey at the local level of the pastures of the study area, which includes groups of individuals, non-governmental organizations, and effective activists. After field studies, preparation of data bank in SPSS environment and analysis of information and graph drawing of relationship network are done using Ucinet and Netdraw 6.0 software.

Results and discussion

Figure 1 shows all the communication and behavioral trends among the actors present in the cooperative protection network, according to these graphs, the entire cooperative protection network consisting of 197 actors in Jiroft city was evaluated and in all types of organizational stakeholders, the organization Non-governmental organizations, villagers and independent

rural people. The actors in these graphs were separated in terms of size, which shows the level of communication between the actors and the importance of that actor.

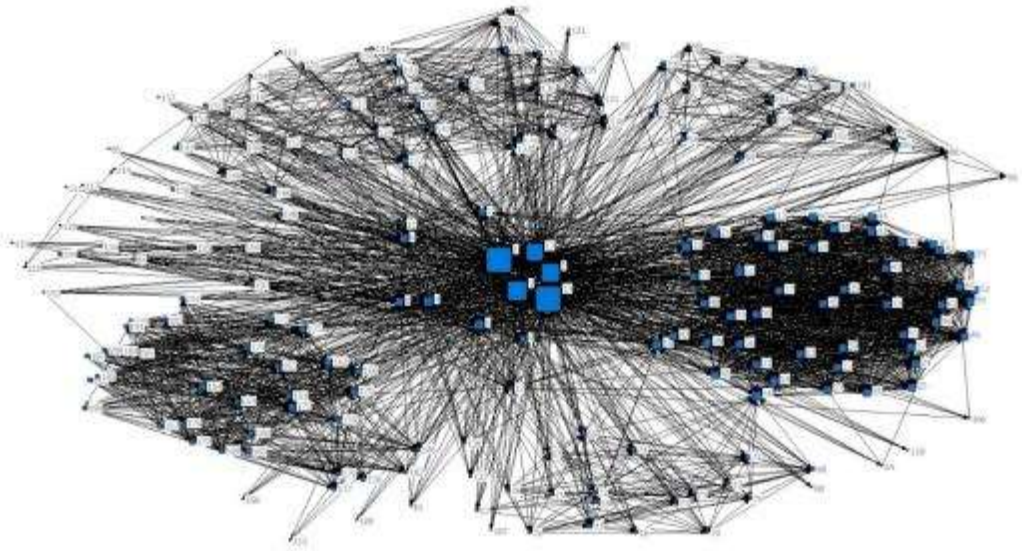

Figure 1. Cooperation network of actors in cooperative protection of local communities of Iran.

In order to evaluate the coherence of the whole collaborative protection network, macro-level indicators of social network analysis (compression, density, concentration, average geodesic distance, interactive relationships, transferability and clustering coefficient) were used.
The results of evaluating the indicators for this collaborative protection network are given in Tables one and two. Actors of this network have good communication continuity according to the compression index (0.570). On the other hand, due to the low density (0.139), the network has low cohesion, and due to the low level of concentration, the distribution of actors within the cooperative protection network is suitable, and the communication in the network is not managed only by a few specific actors.

Table 1. Evaluation of compression indices

Index	**Compactness**	**Centralization (Percent)**	**Density**
Model output	0.57	23.77	0.139

The average geodesic distance in this network indicates the length of the path between two actors in the shortest possible time and indicates the speed of information circulation slightly more than optimal in critical times. According to the obtained information, the average geodesic distance (1.861) outside the optimal value (1-1.5) and high clustering coefficient (6.896) of the network of actors present in cooperative protection indicates that this network is a small world network. Is. The average value of the transitivity index (0.553) shows the stability and relative strength of the network and the balance and resilience of the medium to quickly deal with problems. In terms of mutual communication between the actors in the network (Reciprocity Index= 0.75), the situation is also good. It shows the stability of the cooperative protection cooperation network between the different stakeholders of Jiroft pastures.

Table 2. Evaluation of average geodesic distance, reciprocity, transitivity in collaborative conservation

Index	***transitivity***	***Reciprocity***	***clustering coefficient***	***average geodesic distance***
Model output	*0.553*	*0.750*	*6.896*	*1.861*

Conclusions

The results show a non-dense network but with good connectivity. The greatest weakness can be seen as the need for greater variety of communication actors along with greater expansion in the entire network space. This causes concentration in the entire network to be lowered and more appropriate management, which leads to better network governance. Participatory protection requires extensive group cooperation with the presence of all people, institutions and activists, and if the weak points are removed by managers and policy makers, we will witness the least problems in facing accidents, and on the other hand, the speed of encountering will be reduced to the minimum possible.

The suggestion of this study is the development of private and public institutions alongside local operators. The capacity of local institutions was measured in this study. The results showed that it should be possible to use the local capacity more than before. The ability of the local community to spontaneously deal with destructive social or ecological phenomena requires planning based on a community-oriented management perspective.

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MODELING THE TIME SERIES OF RASPBERRY YIELDS IN THE REPUBLIC OF SERBIA USING THE ARIMA MODEL

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Abstract

In terms of intensity and productivity, fruit harvesting is a very important branch of agricultural production. Namely, the harvesting of fruit produces 10 to 20 times higher value of production per hectare than the harvesting of wheat and corn. Raspberry is the most profitable export product of Serbian agriculture. More than 90% of the produced raspberries are intended for export, while the rest is sold fresh or processed into other products. Approximately 25% of the world's raspberry production originates from Serbia, which indicates the exceptional importance of this fruit type, which positively reflects on our country balance of payments. The aim of this work is to analyze the time series of raspberry yield in the period from 1990 to 2022, as well as to predict the tendency of the volume of raspberry yield in the Republic of Serbia for the period from 2023 to 2025 using the ARIMA model, based on official data from the Statistical Office of the Republic of Serbia. The time series is modeled with the Box-Jenkins modeling strategy. The results of this research show that the raspberry yield, in the observed period, is a trend-stationary time series that can be modeled with the ARIMA (1,0,0) model. The mentioned model predicts that in a defined three-year period, there will be an increase in the total yield of raspberries.

Keywords: *raspberry yield, time series analysis, ARIMA models, Republic of Serbia.*

Introduction

The Republic of Serbia has great agricultural potential. Our country is characterized by favorable natural and climatic conditions, which positively influence the development of agriculture. "Fruit harvesting is one of the most productive branches of agriculture" (Milić et al., 2013). Fruit production accounts for about 11% of the value of total agricultural production in Serbia (Government of the Republic of Serbia, 2014). Raspberry is the most profitable export product of Serbian agriculture. More than 90% of raspberries produced are intended for export, while the rest are sold fresh or processed into other products. About 25% of the world raspberry production comes from Serbia (Kljajić, 2014). This fact indicates the extraordinary importance of this fruit, which has a positive impact on the balance of payments of our country. The regional distribution of raspberry production in the Republic of Serbia includes not only traditionally recognized areas for the cultivation of this fruit, but also some new areas where plantations of larger area are established. Recognized areas for raspberry production are Arilje, Ivanjica and Valjevo, where small farms with a high percentage of human labor dominate. The Mačva region, on the other hand, is characterized by large-scale plantations where mechanical picking is predominant and lower unit production costs are achieved (Cecić et al., 2007). The aim of this paper is to analyze the time series of raspberry yield in the period 1990-2022, as well as to predict the tendency of raspberry yield volume in the Republic of Serbia for the period from 2023 to 2025 using the ARIMA model. The literature, which serves as a starting point for defining the topic and the objective, is systematized into two groups. The first group includes literature that presents the basic concepts of time series analysis, ARIMA modeling, and their advantages and disadvantages.

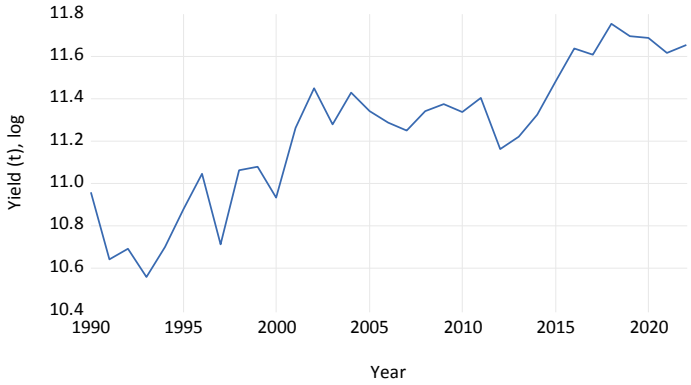
The second group includes literature presenting the practical application of quantitative methods in different areas of agriculture. Dabetić (2016) studied the application of mathematical and statistical methods in the research of meat supply and demand. Ilić et al. (2016) modeled the time series using the ARIMA model, which enables prediction of corn production for the upcoming period. Novković et al. (2010) predicted the behavior of the three most common vegetable crops in Vojvodina (potatoes, beans and tomatoes) using the appropriate ARIMA models. Mutavdžić et al. (2016) focus their research on the most common arable crops in Serbia, i.e., their prices and the correlation between them. Mutavdžić and Novković (2016) analyzed the production parameters of cabbage and used adequate ARIMA models for prediction. Petrović et al. (2022) studied the trends in tomato prices on the market of the Republic of Serbia using the ARIMA model.

Material and methods

The time series was modeled using the Box-Jenkins modeling strategy based on official data from the Statistical Office of the Republic of Serbia. Box and Jenkins (1976) developed a methodology that makes it possible to determine whether the model is an AR model, a MA model, an ARMA model or an ARIMA model based on the determination of the parameters p, d and q. The application of the Box-Jenkins methodology includes several stages. First, it is necessary to identify the model parameters-p, d and q. At this stage, it is desirable to plot the time series to gain insight into whether the series is stationary or not, whether there is a seasonal component, a trend component, etc. The second step is to determine the optimal model of the time series - forming an ARIMA model based on the parameters p, d and q determined in the previous step. After that, it is important to evaluate the quality of the model. The optimal model is additionally analyzed to confirm that the prediction made on the basis of the given model will be as accurate as possible. The last step of the methodology is the prediction. The prediction is based on the defined model and the previous values of the time series. In this study, we analyze the volume of raspberry yield in the Republic of Serbia from 1990 to 2022 using the ARIMA method. The following subsections describe the data used and the result of ARIMA modeling. The analysis was performed using Eviews 12 student version Lite.

Results and discussion

In our analysis, we considered logarithmic data because such transformed data stabilise the variance over a time series and reduce the potential distortions due to outliers. Graph 1 shows that raspberry yield was quite volatile over the period 1990-2000, but with a positive trend. The data also show that since 2000 there was a noticeable rise in log raspberry yield, which continued until 2002. In the following years, the yield stabilised. After a slight decrease in 2012, the raspberry yield recovered and reached its maximum in 2018. The presented data show that there is a trend in raspberry yield.



Graph 1: Total yield of raspberries in the Republic of Serbia, 1990-2022, logarithmic data
Source: Author's data analysis

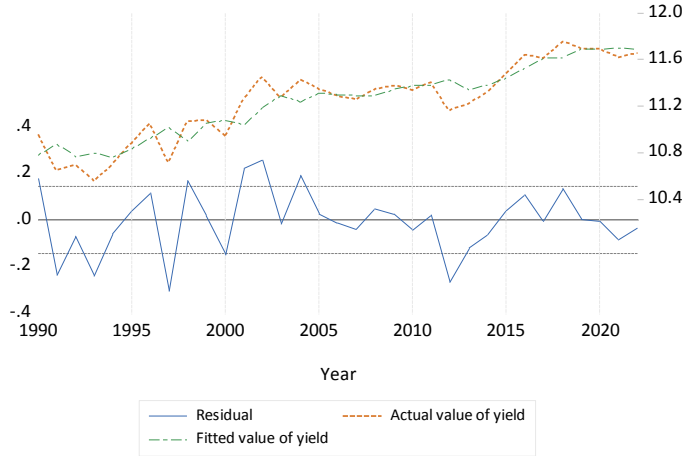
Among the different ARIMA models tested, we chose as the best model the ARIMA (1,0,0) model, which includes the constant and trend component. All three model components are statistically significant, with a p-value of less than 0.05. The detailed model assessment is shown in Table 1. The measured trend component is 0.0298. Since the trend component is positive, it indicates an increasing upward trend. Also, since the trend component is close to zero, it can be interpreted as a low-intense trend. The AR (1) implies that the log value of raspberry yield is linearly related to its immediately preceding value. The AR (1) coefficient value can be interpreted as follows: The expected change in raspberry yield for a one-unit increase in previous years, with other factors held constant, results in a0.4228 t (logarithmic data) increase. The R-squared of the model is 0.8329. Since the obtained R-squared is close to 1, it can be concluded that the model is of good quality. The value of F-statistic is 48.1713, with a p-value of 0.000, indicating the statistical significance of the calculated coefficient (R-squared). In addition, we can consider the adjusted R-squared, which is the R-squared penalised by the number of model parameters. The adjusted R-squared is 0.8156, and since the difference between the two coefficients is minimal, we can conclude that the quality of the model is satisfactory. The EViews software provides three measures of time series model quality: Akaike Information Criterion (AIC), Schwarz Criterion (also known as the Bayesian Information Criterion (BIC)) and Hannan-Quinn Criterion. The measured AIC value is -0.9236, indicating that the ARIMA (1,0,0) model has a much better fit than any other model created and provides a better trade-off between goodness of fit and model complexity. The other two information criteria can be interpreted in the same way. To test for the presence of autocorrelation in the model, the Durbin-Watson test was used. The value of the statistic is 2.0185, which is close to 2 and indicates that there is no autocorrelation in the model.

Table 1: Estimated parameters of the selected model

Dependent Variable: Raspberry yield, logarithmic data, 1990-2022.				
Method: ARMA Maximum Likelihood				
Variable	Coefficient	Standard Error	t-Statistics	p-value
Constant	10.7646	0.0654	164.5616	0.0000
Trend Component	0.0298	0.0047	6.3538	0.0000
AR(1) Component	0.4228	0.1755	2.4089	0.0226
R-squared	0.8329	Akaike info criterion		-0.9236
Adjusted R-squared	0.8156	Schwarz criterion		-0.7422
F-statistic	48.1713	Hannan-Quinn criterion		-0.8625
Prob (F-statistic)	0.000	Durbin-Watson statistic		2.0185

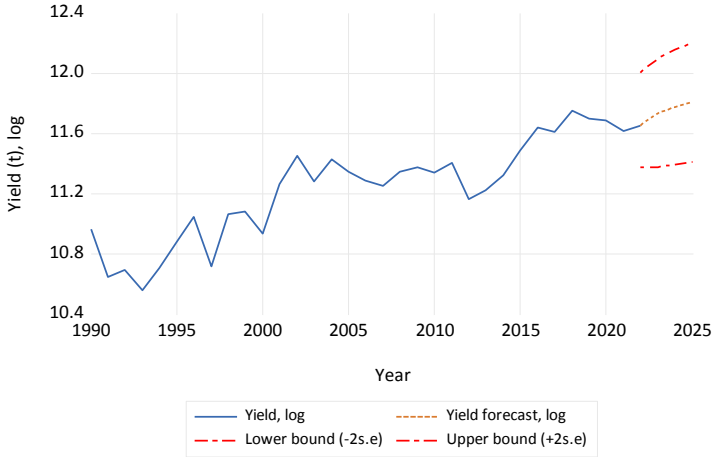
Source: Author's data analysis

After checking the model, it can be concluded that the quality of the model is good and that the model can be used for predictions. Graph 2 shows the actual logarithmic values of raspberry yield (orange line), the fitted values (green line), and the residuals (blue line). The fitted values closely follow the actual values, while no pattern can be seen in the residuals.



Graph 2: Actual and estimated values (by the model) of raspberry yield and residuals of the selected model, logarithmic data
Source: Author's data analysis

The final step is to consider the predicted values of raspberry yield according to the time series model. The graph below shows the log yield predictions and the confidence interval of the prediction. As can be seen, the log yield value will increase in the years 2023-2025.



Graph 3: Actual (1990-2022) and forecast values (2023-2025) of raspberry yields, logarithmic data
Source: Author's data analysis

The logarithmic values were transformed and the forecasted values of raspberry yields for the period 2023-2025 are presented in Table 2. According to the model forecast, raspberry yield will continue to increase from 122,955.77 tons in 2023 to 133,455.05 tons in 2025.

Table 2: Forecast values for raspberry yield in the period 2023-2025.

Year	Predicted values (t)
2023	122,955.77
2024	128,680.77
2025	133,455.05

Source: Author's data analysis

Conclusion

Raspberry, as the most profitable export product of Serbian agriculture, represents an extremely important factor for the balance of payments of the Republic of Serbia and, as such, can be characterized as a unique brand of our country in the field of agricultural and food products. It is precisely for the above reasons that the prediction of future trends in raspberry yields would be of great strategic importance. The ARIMA model (1,0,0) with the trend component was selected for forecasting raspberry yields in the Republic of Serbia for a three-year period using the Box-Jenkins methodology. The conducted analysis of the time series of raspberry yield in the Republic of Serbia indicates that the volume of raspberry production will increase in the three-year period (2023-2025). It should be noted that the presented predictions cannot be made with certainty, as the annual yield of a given variety is influenced by a large number of natural and economic factors that are not captured by the model.

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IMPORTANT PARTNERS OF SERBIA IN THE FOREIGN TRADE EXCHANGE OF FOOD

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Abstract

The focus of this research is the analysis of Serbia's foreign trade in agricultural and food products between 2012 and 2022. Serbia had a positive average export balance with all countries for products from sections 0-Food, 1-Beverages and tobacco, and 4-Animal and vegetable oils and fats, according to the SITC international classification. Serbia achieved the highest average balance in the Food section at USD 1.08 billion. Average food exports amounted to USD 2.57 billion, with a coefficient of variation (CV) of 21.82% and a positive rate of change of 5.61%. The most represented products in the average export were 04 Cereals and related products, accounting for 24.2%, as well as products 05 Fruits and vegetables at 26.2%, although this group of products had a large share in the average import at 21.9%.
Serbia's largest food exports are to EU countries with a 53.9% share, followed by CEFTA countries with a 28.6% share. The most important partner countries in food trade from Serbia within CEFTA are BIH, which holds 44.9% in this group. Germany is an important trade partner in the EU, accounting for 14% of Serbia's average exports.
The main exports to Germany are frozen raspberries and other frozen fruits (primarily blackberries and sour cherries), soybeans and soybean oil, and rapeseed. Serbia mainly imports food products for nutrition, cheese and related products, frozen pork, tobacco, cigarettes, and inputs for agricultural production from Germany. The most exported products to BIH are malt beer, corn, sunflower seeds, sweet biscuits and waffles, products for animal nutrition, and other food products. Serbia mainly imports frozen raspberries, milk and sour cream, meat, sweet biscuits, salt, trout, and poultry eggs from BIH.

Keywords: *food and products, export, import, balances*

Introduction

Economically developed countries are both large exporters and importers of food and implement their foreign trade policy through customs and duty-free barriers and affecting the world food market. The offer of agroindustrial products on the food market depends on the development of agriculture and the food industry, as well as the productivity and economy of production of the participating countries. Multinational companies also influence changes in supply and demand, realizing their interests, to make as much profit as possible in international exchange. On the world food market, the turnover of processed products is increasing, and re-export plays an increasingly important role and is recognized by many countries as an export opportunity. The Republic of Serbia has a rather modest participation in the international exchange of agro-industrial products in the structure of world exports, it participates with 0.10%, while to European participation is 0.20%. Due to its small importance in international trade, it adapts to the conditions of the international market, that is, it can have very little influence on the flows and characteristics of exchange (Vlahović and Radojević, 2023). For many years, Serbia's foreign trade has been characterized by the export of products with a low degree of processing of raw materials and semi-finished products,

while at the same time, final products of a higher value are imported, as stated in the research of Veljković et al. (2010) and Veljković et al. (2018). Important partners of Serbia in foreign trade exchange are the EU countries Italy, Germany, Romania and others. and the CEFTA groups traditionally Bosnia and Herzegovina, Montenegro and Macedonia. Foreign trade liberalization by the EU, as well as the implementation of the CEFTA agreement, had positive effects on Serbia's export and bilateral food exchange (Dragutinović and Popović 2013). The goal of the work is to, based on the analysis of foreign trade exchange in agroindustrial products for the period 2012-2022 point to the countries and products in which Serbia achieves the largest balance.

Material and Methods

The statistical data base of foreign trade for agro-industrial products of the Statistical Office of the Republic of Serbia was used in the research. The data were analyzed according to the SITC Standard International Classification, in which products participating in commodity exchange are classified into sectors marked from 0-9. Sectors are divided into sections and divisions marked with two digits and further divided into groups and subgroups of products. The research covered sectors 0, 1 and 4, as well as sections 00-09, 11-12, and 41-43, in the period 2012-2022 years. The groups of products ranked highest in exports and imports from Serbia were also analyzed.
The data were calculated by the authors and the results are shown by tables and graphs. The intensity of changes was quantified by calculating the annual rates of change, and the stability of exports and imports was given through the calculated coefficients of variation. Also, the average export-import balance by SITC4 commodity groups for products is given.

Results and Discussion

Balances in the foreign trade exchange of Serbia in the period 2012-2022 were positive for all three examined sectors, and Serbia has been achieving a positive balance in the foreign trade exchange of agro-industrial products since 2005. In sector 0 - Food and live animals, an average balance of 1.08 billion USD was achieved, in sector 1 - Beverages and Tobacco 229.5 million USD, and in sector 4 - Animal and vegetable oils, fats and waxes 142.7 million USD in relation to the total foreign trade according to all countries (Table 1 and Chart 1). The achieved average balances are mostly positive, except for the balance in Sector 1-Beverages and tobacco, which is negative: with the EU -56 million USD and Germany -6.6 million USD, more of these products were imported than exported.

Table 1. Foreign trade balance by sector and partner countries in the period 2012-2022.

0 Food and live animals	Export	Import	Balance in USD 000
All countries	2576929	1495121	1081808
EU	1388108	965578.8	422529.2
Germany	194880.3	133352.9	61527.4
CEFTA	735698.4	159513.3	576185.1
BIH	330397.4	72767.56	257629.84

1 Beverages and tobacco	Export	Import	Balance in USD 000
All countries	543511.9	313989.2	229522.7
EU	123209.2	179236	***-56026.8***
Germany	11316.85	17916.95	***-6600.10***
CEFTA	186615.9	37972.47	148643.43
BIH	86432.54	6354.2	80078.4

4 Animal and vegetable oils, fats and waxes	Export	Import	Balance in 000 USD
All countries	209818	67025.76	142792.24
EU	128263.6	45381.62	82881.98
Germany	7188.26	5628.29	1559.97
CEFTA	76526.61	2951.31	73575,3
BIH	39453.32	778.05	38675.27

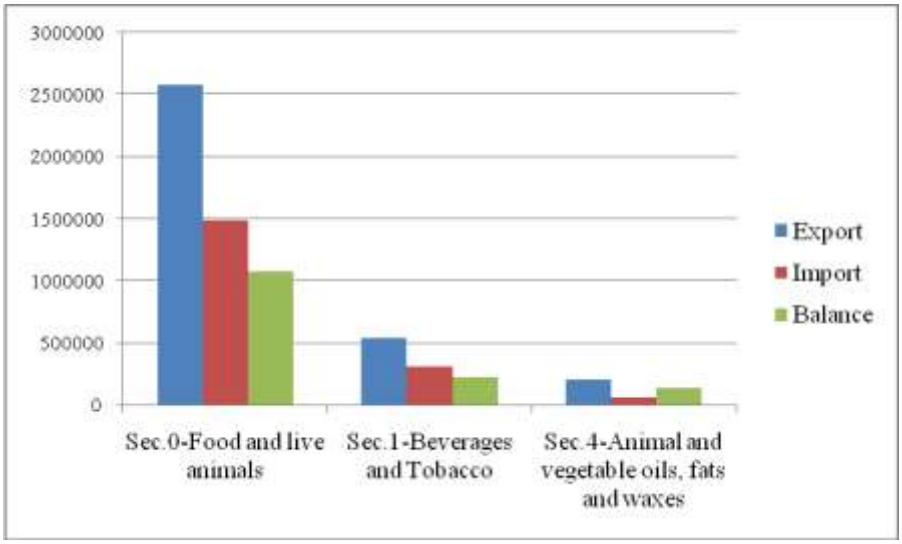

Figure 1. Serbia's foreign trade balance by sectors for all countries in 2012-2022.

In the observed period, the largest export was realized in the sector 0-Food and live animals, USD 2.57 billion, of which the EU was USD 1.38 billion, which is 53.9%, and with CEFTA USD 735 million, which is 28.5%. Products from the other two sectors are mostly exported to the EU and CEFTA 4-Animal and vegetable oils and fats 97.6% and 1-Beverages and tobacco 57%. In all sectors and partner countries, export rates of change are positive, and the highest values are in sector 1-Beverages and tobacco in relation to all countries, the EU, Germany and Bosnia and Herzegovina (Table 2). By analyzing data in 2012-2022 in the ranking of exports, Germany is in first place within the EU, with a share in Serbia's exports of 14%. Within the framework of CEFTA, BiH is with a participation of 44.9%, so these countries are also the most important export partners of Serbia. In terms of the export of agro-industrial products from Serbia to the EU and CEFTA, the 0-Food sector is the most prominent, as depicted in Figures 2 and 3. Serbia maintains a positive foreign trade balance with the European Union and Germany. During the analyzed period, the annual rates of change indicate a higher growth in imports. For all countries, the rate of change in food exports from Serbia is 5.6%, while the rate of change for imports is 7.7%. Among EU countries, the export rate of change is 5.7%, and the import rate is 9.7%. Import growth in the exchange with Germany outpaced exports, with an annual rate of change of 14.5% for imports compared to 5.3% for exports (Figure 2).

Table 2. Serbia's average export by sector and partner countries in 2012-2022.

All countries by sectors SITC	Average in USD 000	Variation interval		CV%	Annual rate %	Share in % by SITC
		Min.	Max			
0 Food and live animals	2576929	2085996.5	3614209.3	21.82	5.61	77.4
1 Beverages and tobacco	543511.9	286127.7	888046.7	34.52	10.97	16.3
4 Animal and vegetable oils,	209818	150953.6	315846.6	26.24	5.65	6.3

Evropska Unija by sectors SITC	Average in USD 000	Variation interval		CV%	Annual rate %	Share in % all countries
		Min.	Max			
0 Food and live animals	1388108	1080685.2	2100165.2	25.49	5.68	53.9
1 Beverages and tobacco	123209.2	67266	226887.1	38.49	10.67	22.7
4 Animal and vegetable oils,	128263.6	83615.2	220909.2	37.73	6.54	61.13

Germany by sectors SITC	Average in USD 000	Variation interval		CV%	Annual rate %	Share in %	
		Min.	Max			∑	In EU
0 Food and live animals	194880.3	140366.7	279736.6	22.63	5.34	7.56	14
1 Beverages and tobacco	11316.9	1174.9	55839.5	150.24	12.12	2.1	9.2
4 Animal and vegetable oils,	7188.3	2238.7	11151.2	43.92	11.14	3.43	5.6

CEFTA by sectors SITC	Average in USD 000	Variation interval		CV%	Annual rate %	Share in % all counties
		Min.	Max			
0 Food and live animals	735698.4	636724.9	935876.1	12.58	2.96	28.55
1 Beverages and tobacco	186615.9	160216.5	245830.2	13.49	2.19	34.3
4 Animal and vegetable oils,	76526.61	57175.1	99729.1	16.57	2.67	36.5

BIH by sectors SITC	Average in USD 000	Variation interval		CV%	Annual rate %	Share in %	
		Min.	Max			Total ∑	In CEFTA
0 Food and live animals	330397.4	277169.5	400617.6	10.36	2.31	12.8	44.9
1 Beverages and tobacco	86432.54	74619	108255.3	12.79	9.93	15.9	46.3
4 Animal and vegetable oils,	39453.32	22043.6	64674.2	32.60	0.92	18.8	51.5

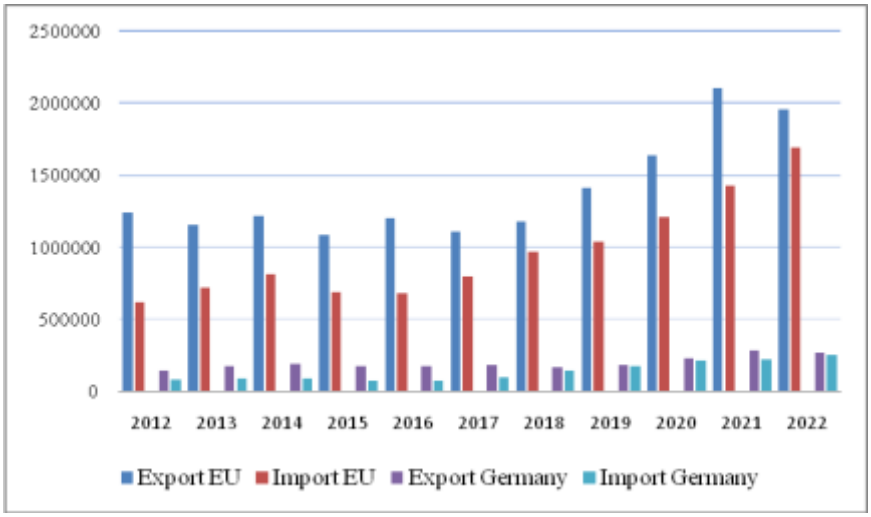

Figure 2. The foreign trade balance of the 0-Food sector with the EU and Germany in USD (000)

The CBI results show that Serbia is in the group of countries, in addition to China, Turkey and Egypt that supply the EU market with frozen vegetables, given that it has developed processing capacities for freezing vegetables. Besides Poland, Serbia also supplies the EU market with frozen berries, primarily raspberries and blackberries. Major exporters are Sirogojno, Zadrugar, Elixir, Agropartner and others, as well as over 150 small processors. The structural analysis of the export of products to Germany includes frozen raspberries, blackberries, cherries and other fruits and products, soybeans, soybean oil, rapeseed, cigarettes, etc. Serbia has negative foreign trade balances with the EU and Germany by product groups: meat and processed foods, milk and dairy products, various ready-made food products, tobacco, fish, coffee, tea and spices. Food products, cheese, dairy products, frozen

pork, tobacco, cigarettes, as well as inputs for agricultural production are imported from Germany. Import of meat and processed products from EU countries is at an annual rate of 12.8%, and of milk and products is 10.1%.
Among the CEFTA countries, Serbia achieves higher values of food exports compared to imports, as shown by the foreign trade balances (Figure 3). Food foreign trade balances are also positive in relation to Bosnia and Herzegovina, but the annual rate of change in exports is 2.3%, and imports are 5.1%.

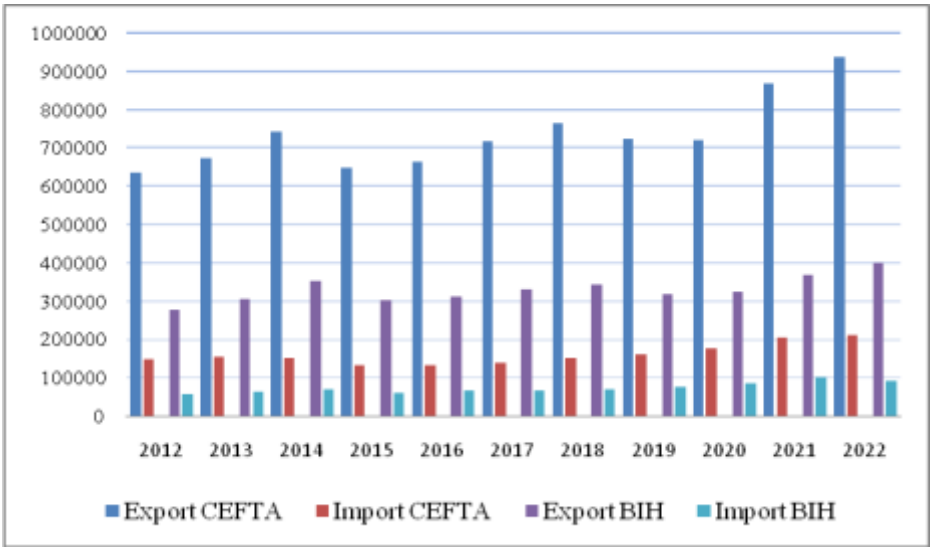

Figure 3. The foreign trade balance of the 0-Food sector with the CEFTA and BIH in USD (000)

According to the structural analysis, the products that are most represented in exports from Serbia are section 04 Cereals and products, with an average share of 24.2% according to all countries and 31.9% from the EU, and section 05 Fruits and vegetables represented with 26.2% and 31.7%, respectively. In the structure of imports 05 Fruits and vegetables are represented by 21.9%, and a lot is imported from the CEFTA group 33.7% (Table 3).
Analyzing the data by product groups, Serbia mostly exports corn, frozen raspberries, wheat and sugar, and the export structure has been the same for a long time, as confirmed by Mitrović et al. (2017) and Vlahović and Radojević (2023).

Table 3. The structure of Serbia's foreign trade exchange by product groups in 2012-2022.

SITC by sections and divisions, Share in %	Export ALL	Export EU	Export CEFTA		Import ALL	Import EU	Import CEFTA
00 - Live animals other than animals of divis. 03	1.5	0.0	4.6		1.7	2.5	0.7
01 - Meat and meat preparations	3.2	0.9	6.5		7.8	10.0	12.2
02 - Dairy products and birds' eggs	3.6	1.7	6.1		5.4	7.0	8.5
03 - Fish crustaceans molluscs and preparations	0.4	0.5	0.5		5.3	3.8	2.7
04 - Cereals and cereal preparations	24.2	31.9	25.9		6.8	8.4	9.9
05 - Vegetables and fruit	26.2	31.7	6.9		21.9	16.3	33.7
06 - Sugars, sugar preparations and honey	3.9	6.0	2.8		2.7	3.2	1.4
07 - Coffee, tea, cocoa spices, and manufactures	3.1	2.0	6.3		13.5	10.1	4.7
08 - Feeding stuff for animals	5.9	6.0	6.3		5.1	6.5	3.6
09 - Miscellaneous edible products, preparations	5.4	4.1	7.7		9.6	13.3	2.1
11 - Beverages	7.4	4.7	14.9		6.0	6.4	15.0
12 - Tobacco and tobacco manufactures	8.9	2.8	3.7		10.7	8.7	4.0
41 - Animal oils and fats	0.1	0.2	0.1		0.6	0.8	0.1
42 - Fixed vegetable fats and oils, crude, refined	6.0	7.4	7.4		2.7	2.8	1.3
43 - Animal or vegetable fats and oils, processed	0.2	0.3	0.2		0.2	0.2	0.0

Various agro-industrial products are exported to the CEFTA countries, mainly corn, wheat and various flours, sunflower oil, beer, and non-alcoholic beverages as well as other products. Most of the exports to BIH are malt beer, corn, sunflower seeds, sweet biscuits and waffles, products for feeding animals and other food products. Frozen raspberries, milk and cream, meat, sweet biscuits, salt, trout, poultry eggs, etc. are imported. The import of milk from Bosnia and Herzegovina gradually increased at a positive annual rate of 7.7% in the period 2012-2022 year. These tendencies were pointed out by Veljković et al. (2010). It should be noted that Serbia imports about 90% of milk from Bosnia and Herzegovina compared to other CEFTA countries, and in 2020 and 2022, more milk and products by section 02 were imported than exported, and Serbia had a negative balance. In addition to food products, Serbia exports electricity and other energy products to CEFTA countries.

Conclusion

The global agro-industrial products market is undergoing a shift from a global nature to a regional focus, as individual countries increasingly dominate trade within their respective economic groupings. Serbia has entered into Trade Agreements with several countries; however, it has not fully capitalized on the benefits provided by these agreements. To enhance Serbia's export potential in terms of both volume and quality, it is imperative to restructure domestic agricultural production in line with the capacities of the agro-industry and the requirements for successful market penetration.

Acknowledgments

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RZS Republički zavod za statistiku Srbije, https://www.stat.gov.rs/sr-Latn/ (Accessed May 2023)

FORECAST OF AND TRENDS IN THE SERBIAN RASPBERRY PRODUCTION

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Abstract

As a highly valued early-maturity fruit crop, raspberries are the predominant berry fruit in Serbia according to the production volume. They are characterized by early cropping (within the first season after planting), regular and high yields, and increased adaptability to various cultivation technologies. Raspberries are also considered a fruit crop of diverse utility value, which are mostly frozen or processed into rolend raspberries, raspberry groats and block raspberries. In the period 1960–2022, the productive raspberry area in Serbia averaged 10,110 ha, indicating a growing trend (at a rate of change of 3.16%). Compared to 1960, the productive raspberry area in Serbia increased by 17,467 ha in 2022 (an index of 881). In the period under consideration, the total raspberry production in Serbia averaged 50,771 t, increasing at an average annual rate of change of 4.91%. Observed over shorter periods of time, the Serbian raspberry production showed considerable variability. Compared to 1960, the volume of raspberry production in Serbia increased by 110,353 t in the final year of the period under consideration (an index of 2,023). The forecast values obtained indicate that the increase in the Serbian raspberry production in the period under consideration will continue throughout the entire forecast period (2024–2028). At the end of the forecast period, the expected raspberry production in Serbia will approximate to 120,000 t.

Keywords: *Raspberry, Productive area, Production, Trends, Forecast.*

Introduction

Raspberries are considered the most important berry fruit crop in Serbia. The following advantages distinguish them from other fruits: extreme adaptability to a variety of natural habitats (allowing for their successful cultivation in hilly and mountainous areas up to an attitude of 1,000 m) and soil types (including less-endowed soils with poor texture), straightforward and undemanding cultivation requirements, early cropping (in year 2), an exploitation period of 7–10 years, and a rapid return on investment in raspberry plantations. Conversely, raspberry production is plagued with high fruit sensitivity, limited fruit longevity, poor fruit transportability, and high labour requirements (especially for harvest purposes).

Raspberry fruits are an important raw material in the fruit processing industry. A share exceeding 90% of the total raspberry production in Serbia is frozen in cold storages or processed into semi-processed products (namely rolend raspberries, raspberry groats and block raspberries). A 7–8% share of mostly second-class raspberries is processed in hot processing facilities, whereas only 2–3% of raspberries produced are used fresh or processed for both domestic and industrial purposes (such as flavouring sweets and chocolates in the hospitality and confectionery industries, etc.) (Petrović and Milošević, 2002*)*.

The raspberry cultivar assortment in Serbia has been established according to the market demands (Nikolić, 2012). 'Willamette' claims the largest share of approximately 95% in the structure of Serbian raspberry cultivar assortment, followed by 'Miker' (3–4%) and all the remaining raspberry cultivars (1–2%), which include the summer-bearing 'Tulameen' and 'Glen Ample' cultivars and ever-bearing cultivars such as 'Polana', 'Polka', 'Heritage', 'Autumn Bliss', etc.

Under market conditions, a successful production depends on analysing and forecasting both the results and most important factors. Forecasting the future is a principal component of planning (Novković, 2003). Different quantitative models of forecasting were developed in agriculture: for trends in fruit production parameters with regard to apples (Sharma *et al.*, 2014) strawberry (Lukač-Bulatović and Đurić, 2022) mangoes, avocadoes and guavas (Hamjah, 2014), for trends in vegetable production parameters (Novković *et al*., 2010) with regard to wheat (Falak and Eatzaz, 2008) and rice (Awal and Siddique, 2011; Suleman and Sarpong, 2012).
The purpose of this paper is to assess the forecast of and development trends in raspberry production. The research examines changes in the distribution of productive raspberry area and the total raspberry production in Serbia in the period 1960-2022. A performance analysis of the Serbian raspberry production was based on descriptive statistics, whereas ARIMA models were employed for forecast purposes.

Materials and methods

The time series of the production parameters observed were completely excerpted from the website of the Statistical Office of the Republic of Serbia. For the purpose of defining some basic features of the parameters observed, the following descriptive statistical indicators were calculated: the mean value (x), coefficient of variation (V) and basic index.
Provided a time series analysis is used for forecasting, a time series model is developed and assessed on the basis of the available historical data, and subsequently used for estimating the future values. Furthermore, a series of statistical tests and criteria are simultaneously utilised in verifying the goodness of fit of the model assessed.
For the analysing and forecasting in this research, the autoregressive–moving-average (ARMA(p,q)) model was employed. The ARMA model is a combination of the autoregressive (AR) part, which involves regressing the variable on its own legged (past) values, and the moving average (MA) part, which entails modelling the error term as a linear combination of error terms occurring at the same and different times in the past.
Provided a time series is not stationary, the autoregressive–moving-average model for an integrated time series (ARIMA(p,d,q)) is used with an integrated (differential) time series $(1-L)^d = \Delta^d$, where d is the smallest number of differentiations required for reaching stationarity.

Results and Discussion

In the period 1960–2022, the (productive) raspberry area in Serbia averaged 10,110 ha, with annual variations ranging from 2,236 ha in 1960 to 24,028 ha in 2020, i.e. an absolute variation interval of 21,792 ha (Table 1). The productive raspberry area in Serbia and Autonomous Province of Vojvodina (hereinafter: Vojvodina) increased at average annual rates of change of 3.16% and 5.03%, respectively. The share of Vojvodina in the total productive raspberry area in Serbia averaged 3.39% in the period under consideration.
The total raspberry production in Serbia averaged 50,771 t, with annual variations ranging from 5,050 t in 1963 to 127,010 t in 2018 (an absolute variation interval of 121.960 t). The rates of change computed indicate a growing trend in the actual Serbian raspberry production in the period under consideration at average annual rates of change of 4.91% and 7.40% in Serbia and Vojvodina, respectively.
The coefficient of variation computed suggests significant variations in the Serbian raspberry production (CV = 72.25%). The coefficient of variation computed for the raspberry production in Vojvodina amounts even up to 125%, with the share of Vojvodina in the total Serbian raspberry production averaging 2.54%.

Table 1. Trends in the productive raspberry area and raspberry production in Serbia in the period 1960-2022

Parameters	Serbia		Vojvodina	
	Productive area (ha)	Production (t)	Productive area (ha)	Production (t)
Average 1960-2022	10,110	50,771	342.35	1,289
Absolute interval of variation	21,792	121,960	1,424	8,640.00
minimum	2,236	5,050	32	40.00
maximum	24,028	127,010	1,456	8,680.00
Annual Rate of Change (%)	3.16	4.91	5.03	7.40
Coefficient of Variation (%)	56.31	72.25	97.18	152.03
Share of Vojvodina (%)	*100.00*	*100.00*	*3.39*	*2.54*

Source: Author's calculation based on *https://www.data.stat.gov.rs/*

With an actual production of 116,093 t in 2022, raspberries accounted for 7.73% of the total Serbian fruit production considered (Table 2). Compared to the base year of 1960, the raspberry production in Serbia increased by 110,353 t (or 2,223 index points) in the final year of the period under consideration. Moreover, the share of raspberries in the Serbian fruit production structure increased fourfold (or 391by index points) in 2022 over that recorded in 1960.

Table 2. Raspberry production in Serbia in the years considered

Region	1960		2022			
	Production (t)	Share (%)	Production (t)	*Production Index 1960=100*	Share (%)	*Share Index 1960=100*
Serbia						
Raspberries	5,740	1.98	116,093	*2,022.53*	7.73	*390.67*
Other fruit species*	284,390	98.02	1,385,943	*487.34*	92.27	*94.13*
Total	290,130	100	1,502,036	*517.71*	100	/
Vojvodina						
Raspberries	60	0.09	4,260	*7,100.00*	1.56	*1,733.37*
Other fruit species	66,630	99.91	268,907	*403.58*	98.44	*98.53*
Total	66,690	100	273,167	*409.61*	100	/

Source: Author's calculation based on https://www.data.stat.gov.rs/

*All the fruit species included have official records kept by the Statistical Office of the Republic of Serbia from 1960.

Raspberries claimed a 1.56% share in the Vojvodinian fruit production structure in 2022 (Table 2). The raspberry production in Vojvodina increased by 4,200 t in 2022, or by 7,100 index points, over that recorded in the base year of 1960. The share of raspberries in the Vojvodinian fruit production structure increased seventeen-fold in 2022 over that recorded in 1960 (an index of 1.733).

In 2021, Serbia claimed an 18.82% share in the total (productive) raspberry area structure in the world (ranking second in the world), whereas the Serbian raspberry production accounted for 12.47% of the total raspberry production in the world (ranking third in the world) -Table 3.

Table 3. The share of Serbia in the total productive raspberry area and raspberry production in the world in 2021

Production parameters	World	Serbia	Share of Serbia (%)	Rank of share
Productive area (ha)	110,567	20,807	18.82	2
Production (t)	886,539	110,589	12.47	3
Yield (t/ha) - average	8.0	5.3	/	/

Source: Author's calculation based on https://www.fao.org/statistics/

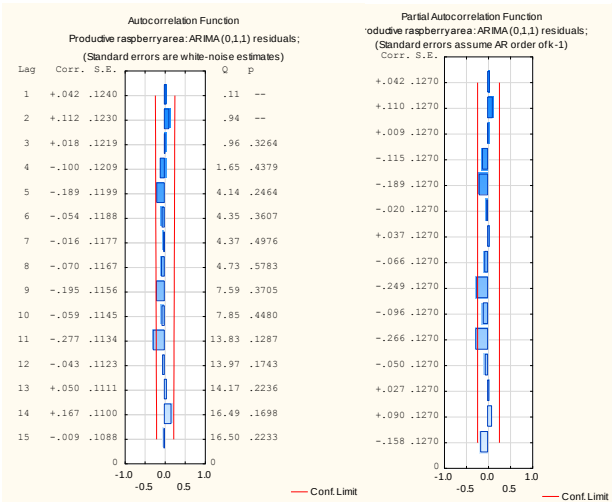
Forecast of raspberry production in Serbia - using the Statistica 14 program, an adequate model for forecasting trends in the Serbian raspberry production was determined. The model parameter estimates are represented in tabular form. Although parameter estimates are not statistically significant with the autoregressive part (AR), the exclusion of the AR part from the model is not an option in the Statistica 14 program. Upon analysing the residual and partial autocorrelation function plots of the model assessed, the model was found adequate. Furthermore, it was also used for computing the parameter estimates and 95% confidence interval. As the model assessed features a stochastic trend, standard forecast errors are relatively great.

Using the Statistica 14 program, the ARIMA(0,1,1) model was selected for forecasting trends in the productive raspberry area in Serbia, whereas the ARIMA(1,1,1) model was selected for forecasting trends in the volume of raspberry production in Serbia. Table 4 shows the parameter estimates of the ARIMA models employed.

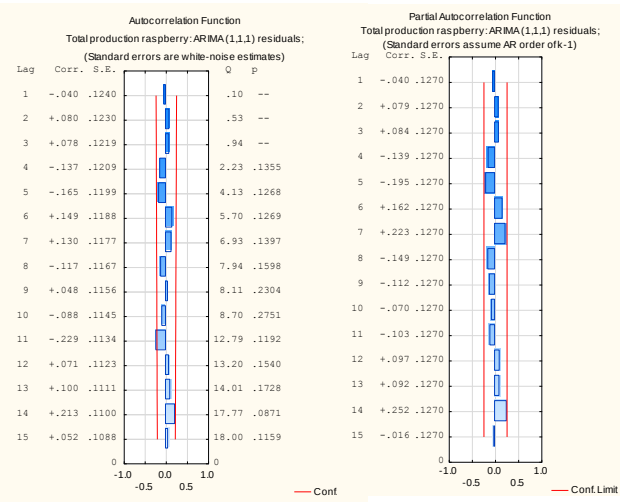
Table 4. Models for forecasting the distribution of productive raspberry area and the volume of raspberry production in Serbia

ARIMA Model Parameters		Raspberries			
		Productive area		Production	
Model		(0,1,1)		(1,1,1)	
MS Residual		9621E2		6689E4	
Parameters		Constant	q(1)	Constant	p(1)
Param.		281.6163	-0.4229	1797.560	0.679
SE		177.6122	0.1127	323.6481	0.1953
t		1.58557	-3.75262	5.554058	3.474574
p		0.118094	0.000397	0.000001	0.000966
Confidence interval (95%)	Lower	-73.6610	-0.6484	1149.942	0.288
	Upper	636.8936	-0.1975	2445.178	1.040

Productive raspberry area in Serbia - Residuals: ACF PACF



Total volume of raspberry production - Residuals: ACF PACF



The model employed for analyzing and forecasting trends in the Serbian raspberry production indicates that the current year's parameter values are greatly affected by the previous year's parameter values and incidental occurrences.

The forecast trend values of the distribution of productive raspberry area in Serbia suggest an annual increase throughout the entire forecast period (2024-2028). At the end of the forecast period, the productive raspberry area in Serbia will approximate to 21,000 ha (Table 5).

Table 5. Forecast of trends in the total raspberry production and the distribution of productive raspberry area in Serbia (2024-2028)

Raspberries	Forecast				
	2024	2025	2026	2027	2028
Production areas (ha)	20,059.96	20,341.57	20,623.19	20,904.80	21,186.42
LCL	18,097.93	16,929.24	16,214.29	15,686.29	15,268.03
UCL	22,021.98	23,753.90	25,032.09	26,123.32	27,104.81
SE	980.86	1,705.90	2,204.12	2,608.86	2,958.75
Production (t)	116,925.94	118,068.76	119,421.90	120,917.82	122,510.63
LCL	10,0561.12	97,464.51	96,558.87	96,649.95	97,271.47
UCL	133,290.76	138,673.00	38,457.58	142,284.94	147,749.79
SE	8,178.34	10,297.00	11,425.83	12,127.90	12,613.30

The forecast trend values of the raspberry production in Serbia indicate an annual increase throughout the entire forecast period (Table 5). By the end of 2028, the expected raspberry production in Serbia will approximate to 120,000 tones.

Lukač Bulatović *et al.* (2019) have used Box-Jenkins ARIMA model to forecast productive trees and production of apple in Serbia. From their study, it was found that the adequate selected ARIMA model for apple productive trees is ARIMA(1,1,0) and for apple production is ARIMA(0,1,1). Sharma *at al.* (2014) has used Box-Jenkins ARIMA model to forecast area and production of apple in Himachal Pradesh. Hamjah (2014) made a paper with the title „Forecasting Major Fruit Crops Productions in Bangladesh using Box-Jenkins ARIMA Model". The analysis is revealed that the best model is ARIMA(2,1,3) for forecasting banana production, ARIMA(2,1,3) for forecasting mango production and ARIMA(1,1,2) for guava production. Novković *et al.* (2022) have used models to forecast productive areas and production of plum in Serbia.

Conclusion

Over the past decades, raspberries have assumed considerable industrial importance in Serbia as the country has emerged as one of leading raspberry producers in the world. Compared to other global raspberry producers, Serbia boasts favourable agro-ecological conditions conducive to high-quality raspberry yields. Although Western Serbia has always been renowned for raspberry production, the raspberry production in other Serbian regions has been gaining prominence (especially in Vojvodina).

In the period 1960–2022, the (productive) raspberry area in Serbia averaged 10,110 ha. The productive raspberry area in Serbia and Vojvodina increased at average annual rates of change of 3.16% and 5.03%, respectively. The share of Vojvodina in the total productive raspberry area in Serbia averaged 3.39% in the period under consideration.

In the period 1960–2022, the actual raspberry production in Serbia averaged 50,771 t, indicating a growing trend (at a rate of change of 4.91%). With an actual production of 1,289 t, Vojvodina accounted for 2.54% of the total Serbian raspberry production. The share of Vojvodina in the total raspberry production in Serbia increased from 1.1% in 1960 to 3.7% in the final year of the period under consideration.

The forecast values obtained suggest that the increase in the total raspberry area and production in Serbia in the period under consideration (1960–2022) will continue throughout the entire forecast period, i.e. up to 2028. At the end of the forecast period, the productive raspberry area in Serbia will approximate to 21.000 ha and the actual Serbian raspberry production will approximate to 120,000 t.

In order to increase and improve the raspberry production in Serbia, the following recommendations can be made: establishing new intensive plantations with cultivars whose quality, yield and size would meet cost-effective and international market requirements; requisite production insurance and various insurance models in agriculture.

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THE INFLUENCE OF CORN AND FATTENING PIGS PRICE FROM CURRENT YEAR ON THE NUMBER OF FATTENING PIGS IN FOLLOWING YEAR

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Abstract

The subject of this research is the dependence of annual production of fattening pigs on price elements, inputs and products. The aim of the research is to observe the impact of the price of corn, as the most important concentrated feed, and the price of fattening pigs from current year on the number of fattening pigs in the following year in Serbia. The analyzed period is 2006-2021 and basic tools of descriptive statistics are used for data processing. The results of descriptive statistics show moderate variability. The average price of corn is 14.70din/kg, and the price of fattening pigs is 140.30din/kg. Greater variability is confirmed for the corn prices (CV=27.41%). Corn prices (X_1) and fattening pigs prices (X_2) are growing at annual rates of 7.92% and 3.88%, while number of fattening pigs (Y) decrease at annual rate of 1.75%. In order to establish the influence of corn and the fattening pigs prices on the number of fattening pigs a multiple linear regression model: $\hat{Y} = 1358312 - 18928X1 + 2255X2$ is created. The statistical significance of model is tested by ANOVA and the obtained results determine the statistical significance of the model ($p<0,05$). The estimated parameters of the regression model are also significant, and there is negative relationship between the price of corn from current year on the number of fattening pigs in following year, while the relationship between the price for fattening pigs and their number is positive. The model demonstrates a significant impact of both independent variables on the annual production of fattening pigs, but the price of corn has a greater impact than the price of fattening pigs.

Key words: *price, corn, state, tendency, fattening pigs.*

Introduction

The development of pig production is recognized as a very important economic activity. Increasing the number of pigs can contribute to the development of both agriculture and the economy as a whole. That is supported by the results of Mirilović et al., (2012) who claimed that pig production represents the largest and the most important part of Serbian agriculture. Generally, animal husbandry as a whole encourages various economic activities. Zekić et. al., (2007) state that the growth rate of agricultural production exhibits a tendency to grow primarily due to the influence of the shorter production process of animal production, under the influence of a shorter turnover period of invested funds. Although animal production is an economic branch recognized as a driving force for development, individual agriculture production lines do not have the same impact. In European Union, in 2018, pig production took part in total agriculture production by 9%, while in total meat production all categories of pigs took part by 35% (Renaudeau and Dorumad, 2022). However, in the Republic of Serbia, all livestock production lines decreased in annual production rate, while pig production showed growing rate of 0.8% (Statistical yearbook 2021). Considering the importance of pig farming as a whole, it is important to observe and determine the possibilities of raising the efficiency of pig production. One way is to implement cost efficiency. Profitability of pig farms largely depends on the prices of inputs used to make feed

mixtures, and especially the price of corn, which can be a decisive factor in making a decision on the expansion of pig production. Corn represents a significant input in the preparation of food mixtures for feeding pigs, primarily due to the high value of the nutritional properties of corn (Chen et.al, 2019). Moreover, the authors state that apart from quality, the decisive role and imposition of the need to use corn is also played by its availability, that is, its dominant participation in the sowing structure.

The subject of research in this paper is the analysis and change of the annual production of fattening pigs depending on the dynamic of price elements: the price of fattening and the price of corn, as the most important input in pig production.

The goal of the research is to show and quantify the dependence (legality) of the impact of changes in the price of corn and the price of fattening pigs from the current year, on the number of fattening pigs in the following year.

Material and Methods

The research is based on secondary data and the application of standard quantitative methods. The collected data were processed with standard statistical tools:

1. Average value $\overline{X}$
2. Extreme value (min, max)
3. Coefficient of variation (CV)
4. Annual rate of change % (r)

The rate of change is calculated directly from the absolute data of the analyzed series using the following expression:

$$Y = (G - 1); G = (\frac{Y_n}{Y_1})^{\frac{1}{n-1}}$$

Where is: r - annual rate of change, G - constant relative change of the phenomenon, Yn - absolute value of the last member of the series, Y1 - absolute value of the first member of the series and n - total number of members of the series.

For the purposes of revealing the impact of the price of corn and fattening pigs from the current year on the number of fattening animals in the following year, a multiple linear regression model was applied. Regression analysis is a method which is widely used to determine the relationship between the included variables. According to Munćan and Božić (2018) regression analysis is a method used to identify relationships between observed variables. Additional analysis determines the direction and strength of the identified connections. Also, regression analysis can be defined as an assessment of the value of dependent variable based on one or more independent variables (Mutavdžić and Đorić, 2018):

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} + \varepsilon_i$$

Where Yi represents the value of the dependent variable, X_{1i}, X_{2i}, … , X_{pi} represent the values of the independent variables and β_1, β_2, ….. β_p are regression parameters. Parameter β_0, shows the average initial level of the dependent variable, while ε_i is accidental mistake (Novaković, 2019). To check the statistical significance of the defined model as a whole, a regression variance analysis was performed. The data used in the analysis were taken from the website of the Statistical Office of the Republic of Serbia. The data refer to the price of corn, the price of fattening pigs as well as the number of fattening pigs in the Republic of Serbia for

the period from 2006-2021 years. The IBM STATISTICS 21 software package was used for statistical data processing.

Results and discussion

Based on the official statistical data of the Statistical Office of the Republic of Serbia (Table 1), a quantitative analysis of the annual production of fattening pigs, as well as the price of pigs and corn in the period from 2006 to 2021 in the Republic of Serbia, was carried out. Based on the average annual prices of fattening pigs and corn, the average annual parity was calculated.

Table 1. Number and price of fattening pigs and price of corn in the Republic of Serbia, 2006-2021.

Year	Number of fattening pigs (000)	Price of fattening pigs (RSD/kg)	Price of corn (RSD/kg)	Price parity pigs/corn
2006	1.831	84.86	7.47	11.36
2007	1.451	73.06	12.24	5.97
2008	1.329	119.64	9.84	12.16
2009	1.470	138.16	8.92	15.49
2010	1.414	116.26	13.49	8.62
2011	1.305	135.39	17.05	7.94
2012	1.361	172.94	20.82	8.31
2013	1.365	176.89	16.03	11.03
2014	1.533	174.62	14.10	12.38
2015	1.576	148.64	15.18	9.97
2016	1.425	140.65	15.14	9,30
2017	1.301	165.47	16.19	10.22
2018	1.305	144.48	14.57	9.92
2019	1.382	150.98	14.39	10.49
2020	1.469	152.60	16.31	9.36
2021	1.404	150.22	23.43	6.41

Source: Statistical Office of the Republic of Serbia

In the analyzed period in the Republic of Serbia, the average parity was 9.5, which means that one kilogram of fattened pig worth as 9.5 kilograms of corn. Parity varied in the interval from 5.97 kilograms of corn per kilogram of fattening pigs in 2007 to 15.49 kilograms of corn per kilogram of fattening pigs in 2009.
The basic indicators of descriptive statistics for the observed parameters of corn and fattening pigs, i.e. prices, as well as the number of fattening pigs, are shown in Table 2.

Table 2. Descriptive statistics of corn price, price and number of fattening pigs

Product	**Parameter**	**Average**	**Min**	**Max**	**CV (%)**	**Rate of change (%)**
Corn	Price (RSD/kg)	14.70	7.47	23.43	27.41	7.92
Fattening pigs	Price (RSD/kg)	140.30	73.06	176.89	21.22	3.88
	Number (000)	1432.56	1301.00	1831.00	9.30	-1.75

Source: Author's calculation.

The price of corn showed slightly higher variability than the price of fattening pigs. However, the price of corn grew by more than twice (2.04 times) the price of fattening. This caused the number of fattening pigs to show negative tendencies in the observed period. The research

results and conclusions are in accordance with the results obtained by Novković et al., (2019), where the authors predicted the rise in the price of corn in the future using a group of ARIMA models. In the period from 2001-2012 year, according to Mirilović et al. (2015) the number of fattening pigs decreases annually by 4,248 heads, while the price of corn and the price of fattening pigs tends to increase in the territory of the Republic of Serbia, which is in accordance with the indicators obtained for the time interval covered by the research (2006-2021). In the next part of the research, a regression model was formed based on the presented variables, to determine the dependence of the number of fattening pigs in the following year on the selling price of corn and fattening pigs in the current year. Before the calculation, the model was tested as a whole using regression analysis of variance (Table 3). The starting hypothesis for testing the model as a whole is: $H_0: \beta_1 = \beta_2 = \cdots = \beta_k = 0$, if it is accepted, it is considered that the model is not statistically significant.

Table 3. Analysis of variance regression

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	41824.536	2	20912.968	4.533	.034[b]
	Residual	55361.464	12	4613.455		
	Total	97186.000	14			

Source: Author's calculation.

The obtained results of the regression variance analysis for regression model 1 indicate the significance of the model, the null hypothesis is rejected and it is concluded that the formed model is statistically significant ($p<0.05$). The estimated parameters of regression model 1 and the dependence of the price of fattening pigs in the next year in relation to the price of corn in the current year are shown in table 4.

Table 4. Regression model, Dependence of the number of fatteners on the price of corn and the price of fatteners

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig
		b	Std. Error	Beta		
1	Constant	1358,312	88,665		15,320	0,000
	Corn price	-18,928	7,164	-0,773	-2,642	0,021
	Fattening pigs price	2,255	0,794	0,831	2,839	0,015

Source: Author's calculation.

The estimated regression model has the following form:

$$\hat{Y} = 1358312 - 18928X1 + 2255X2$$

The obtained regression coefficient (b) confirms the statistical significance of the regression parameters related to the price of corn and fattening pigs in the current year. The influence of the first independent variable (the price of corn) has a negative impact, while the influence of the second independent variable (the price of fattening pigs) has a positive impact. It is concluded that the number of fattening pigs decreased under the influence of the increase in the price of corn, that is, the number of fattening pigs increased under the influence of the increase in the price of fattening pigs. That is, if the price of the input (maize) increases by one unit (1 din/kg) in the current year, it is expected that the number of fattening pigs will decrease by 18,928 (000) heads in the following year. With the increase in the price of fattening pigs in the current year by one unit (1 RSD/kg), it is expected that the number of fattening animals will increase by 2,255 (000) heads in the next year. Regarding the significance of the impact of corn on the determination and development of pig production,

the research results of Santa and Wantasen (2018), are in accordance with the obtained results in this research, that the price of corn has a significant negative impact on the profitability of pig farms, should not be ignored, because if the price of corn increases by 10%, a drop in profitability by about 3.681% can be expected.

Conclusion

The results for the observed period from 2006-2021. year showed:
- the average number of fattening pigs in the analyzed period is 1,432,560 heads,
- the number of fattening pigs shows moderate variability (CV=9.30%),
- the number of fattening pigs is decreasing at an average annual rate of -1.75%,
- the average price of corn is 14.70 RSD/kg,
- the variability of the corn price in the observed period is high (CV=27.41%)
- the annual average growth in the price of corn amounts to 7.92%,
- the average price of fattening is 140.30 din/kg,
- the price of fattening has high variability (CV=21.22%).
- the average price of fattening pigss is growing at an annual rate of 3.88%
- the average price parity for fattening pigs and corn is 9.5 and varies in the interval from 5.9 to 15.5.

The research results show a statistically significant influence of the price of corn and fattening pigs from the current year on the number of fattening pigs the following year. The impact of the price of corn is negative, and with an increase in the price of corn of 1 RSD/kg in the current year, it is expected that the number of fattened pigs will decrease by 18,928 in the next year, also an increase in the price of corn by 6.8% will affect the decrease of the number of fattened pigs by 1.39%. Opposite to corn, the influence of the price of fattening pigs on the number of fattening pigs is positive. With the increase in the price of fattening pigs by 1 RSD/kg in the current year, it can be expected that in the next year the number of fattening pigs will increase by 2,255 heads. Therefore, a grew rate of fattening pigs price by 0.71% has impact on increasing of fattening pigs number by 0.17%. This kind of research is very important for decision making process not only at the macroeconomic level, but for agricultural producers as well.

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DISTRIBUTION AND EFFICIENCY OF ECONOMIC SUBSIDIES FOR SOIL CONSERVATION ALONG TWO DECADES IN SPAIN

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Abstract

Measures for soil conservation in EU countries have been vertebrated through Rural Development Programmes (RDPs) within the Common Agricultural Policy's framework, providing detailed statistics for investments in this field. Despite data availability, there's a lack of spatial and temporal analysis as well as it's correlation with soil conditions at a regional level in Spain. This paper analyses the spatial distribution of public investments directly or indirectly related with soil conservation during two decades and evaluates if the financing of soil conservation measures (SCMs) was related to the real soil condition or socioeconomic factors.
The methodological approach of this study consisted in (i) data mining and inventory development of agri-environmental and forestry measures aimed at soil conservation published by the Ministry of Agriculture; (ii) a spatial and temporal analysis of the measures' progress, and (iii) a preliminary exploration of the potential relationship between the selected measures and soil conditions, using the National Inventory for Soil Erosion (INES). It was observed that investment decreased over a 3 programme period. Also, distribution of investment was inconsistent, with scarce correlation between investments per hectare and soil degradation processes such as sheet and rill erosion intensity. Whilst it may be true that the environmental perspective gains more relevance with each RDP specific measures aimed at soil protection and conservation become less prevalent in the last programming period. The latest CAP reform incorporated sustainable agriculture goals that translated into broader environmental conditions towards receiving subsidies from a variety of measures. This study intends to shed light upon the efficacy of SCMs in the CAP's second pillar.

Keywords: *soil conservation processes, subsidies, soil erosion, CAP, Rural Development Programmes*

Introduction

The European Green Deal (EUGD) leads the way to achieve a climate neutral Europe. It re-establishes the commitment of the European Commission to address the challenges related to the environment and climate, which aims to transform the European economy and decouple economic growth with the exploitation of resources for 2050 (COM [2019] 640). The EUGD has 3 key land-management strategies: Farm to Fork (F2F), Biodiversity (BS) and Soil (SS), that together with other initiatives as the Long-term Vision for EU Rural Areas have sustainable agriculture, including healthy soil, as a common objective to reach the sustainability goals and progress towards a neutral climate continent (Boix-Fayos and de Vente, 2023). In doing so, it continues a trend initiated during the Common Agricultural Policy's 2003 reform, where policies changed from an agri-territorial perspective towards the incorporation of environmental priorities (van Leeuwen *et al*., 2019). Rural Development Programmes (RDPs) appeared after the 1992's CAP reform. Member states could choose sub-objectives from RDPs key areas that suited their specific economic, environmental, and social conditions for policy implementation (European Commission, 2013a, 2013b). In Spain,

besides a national RDP specifying horizontal measures, 17 RDPs were implemented for each regional administration to adapt to their singularities. Soil Conservation Measures (SCMs) may be specified in the RDPs as a sub-measure, a line of action or a condition towards receiving subsidies. To date, there have been 3 RDPs during the periods 2000-2006, 2007-2013 and 2014-2020.

Intensification of agricultural activities and the loss of natural vegetation cover in Spain, were linked to increments in soil loss by erosion (Sanjuán *et al*., 2013) which, in turn, affected soil fertility by reducing organic matter, porosity and degrading its structure and water retention capacity (Boix-Fayos *et al*., 2005; Martínez-Mena *et al*., 2020; 2021a) accelerating desertification processes. Efforts to monitor erosion processes resulted in the publication of the National Soil Erosion Inventory (INES, 2002), which intended to detect, quantify, and map the main erosion processes in Spain and determine how they evolved in time.

Sheet and rill erosion processes were the main focus of this work, as they widely affect agricultural land, which were the main aim of the RDPs. Soil conditions in Spain vary widely due to differences in geography, geology, and climate across the country. Additionally, various regions receive different budgets for their RDPs. Political decisions can also influence the priorities within each regional RDP. Therefore, it's challenging to determine if funding for soil conservation measures aligns with the diverse soil conditions on a national scale.

Materials and methods

Data on SCMs and their funding was extracted out of the three RDPs executed during years 2000 to 2021 for each regional administration. Measures directly related to soil conservation can be found in the RDPs agri-environmental measures. Afforestation measures were also selected and considered as indirect SCMs (Zethof *et al*., 2018). Data on executed investment (Figure 1, right) was extracted from financial tables in RDPs *expost* evaluations and annual execution reports published by the Ministry of Agriculture, for each regional administration. The latest available common frame was December 2021, as some programmes are still ongoing.

Data on erosion rates was obtained from the National Soil Erosion Inventory (INES), which intended to detect, quantify, and map the main erosion processes in Spain (Figure 1, left) and determine how they evolve in time (INES, 2002-2012). Sheet and rill erosion processes were the main focus of this study, as they widely affect agricultural land (Fernández-Carrillo, 2015), which was the main aim of the RDPs. Tolerable soil loss rate was established at 10 t ha-1 year-1 (INES, 2002) as seen in the INES categorization. Though the INES suggests values change according to the effective depth of the soil it didn't provide such detailed data for each region.

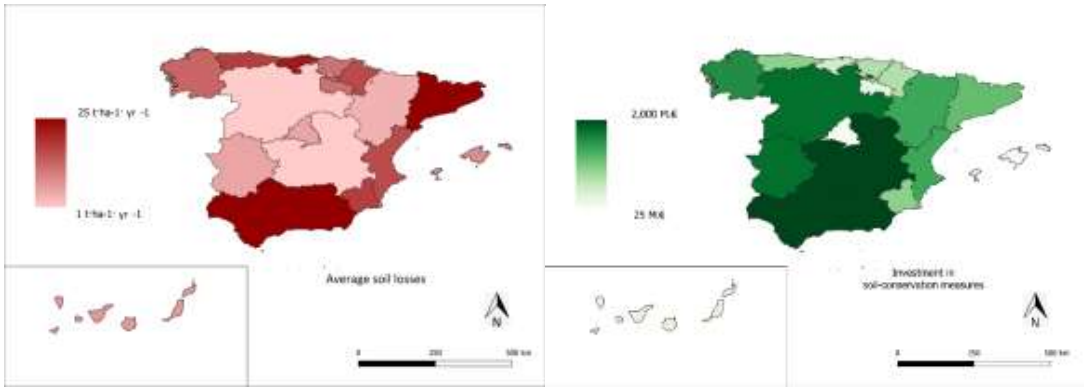

Figure 1. Left: soil loss distribution. Right: investment in SCMs in millions of euros.

Qualitative categorizations "severe" and "very severe" provided in the INES and "degraded" and "degrading" in Sanjuán *et al*. (2013) were also used. Furthermore, other data related to land cover and other socio-economic indicators extracted from the National Statistics Institute (INE, 2000-2022) was used for further statistical analysis (Table 1). Data sources for each indicator can be found in Supplementary Material 1 (SM1).

Table 1. Indicators and sources of data used in analysis. Letters correspond with vectors in PCA (Figure 2).

Indicator	Unit	Description
€measures (a)	€	Investment in SCMs to date (December 2020)
€Ameasure (b)	€	Investment in soil conservation within agri-environmental measures to date
€Fmeasure (c)	€	Investment in forestry measures to date
€/haATL (d)	€/ha	€ per hectare affected by sheet and rill erosion above 10 t ha-1 year -1
€Ameasures/Carea (e)	€/ha	€ invested within the agri-environmental block per cultivated surface area
€Fmeasures/ha (f)	€/ha	€ invested in afforestation from the forestry block per hectare (total surface area of the region)
%RDP (g)	%	Percentage of RDP's budget used for SCMs
S-Rero (h)	ha	Surface area affected by sheet and rill erosion above 10 t ha-1 year -1
SAarea (i)	ha	Surface area affected by severe erosion from INES variable tolerance categorization
LDarea (j)	ha	Surface area of degraded or degrading soils
WASL (k)	$t/ha^{-1}/year^{-1}$	Soil loss intensity
WASL-ATL (l)	$t/ha^{-1}/year^{-1}$	Soil loss intensity above 10 t ha-1 year -1
SL-ATL (m)	t/yr^{-1}	Total soil losses in tonnes per year
WASL-Carea (n)	$t/ha^{-1}/year^{-1}$	Soil loss intensity on cultivated land
WASL-Farea (o)	$t/ha^{-1}/year^{-1}$	Soil loss intensity in forests
G-Rarea (p)	ha	Surface area occupied by ravines and gullies
%G-Rarea (q)	%	% of the region covered by ravines and gullies
Farea (r)	ha	Surface area occupied by forests
Carea (s)	ha	Surface area occupied by crops (woody or herbaceous)
ALarea (t)	ha	Surface area covered by arable land (includes fallow plots)
PParea (u)	ha	Surface area covered by permanent pastures
RP (v)	No.	Number of people living in rural areas with less than 3,000 people.
GDP (x)	€	Average GDP during the 2000-2020 period
GP (w)	1,2,3	Longest governing party during the three programming periods

Weighted variables were created to compare data amongst regions (Table 1). Three types of analyses were conducted using statistical computing program R 4.2. Correlations between variables were tested by (i) Spearman's rank correlation test (p-value <0.05 and <0.01). The correlation test was set on two data frames measuring correlations between grouped variables. (ii) A Principal Component Analysis (PCA) was also conducted. By plotting the vectors, dependency, and variability among pairs of vectors were determined. Individual plot enabled the observation of clustering amongst regions with similar tendencies. QGIS 3.16.13 was used (iii) for spatial analysis of investment in SCMs, erosion, land cover and population indicators, as well as the visual representation of the data on maps.

Results and discussion

Investment in agri-environmental and forestry measures in Spain has decreased after each programming period by 13.36% and 1.60%. This decline can be partly attributed to Spain's regions no longer being classified as "underdeveloped" within the EU, leading to a shift in

financing priorities to other member states (European Commission, 1999; European Commission 2006; European Commission 2015).
Investment across regions is uneven, with larger regions managing higher RDP budgets allowing for better SCMs funding (Figure 1, right). However, when considering investment per hectare or the percentage of regional RDPs dedicated to SCMs, correlations are neither strong or significant (Table 2).

Table 2. Spearman's correlation matrix. p-value <0.05 and p-value <0.01 (*). Descriptions for the indicators may be found in table 1. Indicators with no significant correlation along entire rows or columns were eliminated. For a more detailed matrix including all indicators see tables 2 and 3 in SM1.

	S-Rero	**SAarea**	**WASL**	**SL-ATL**	**WASL-Carea**	**WASL-Farea**	**G-Rarea**	**Farea**	**Carea**	**ALarea**	**PParea**	**RP**
€measures	0,91*	0,88*	-	0,90*	0,89*	0,86*	-	0,80*	0,81*	0,72*	0,79*	0,88*
€Ameasures	0,91*	0,88*	-	0,88*	0,88*	0,90*	0,54	0,78*	0,83*	0,73*	0,70*	0,85*
€Fmeasures	0,87*	0,77*	-	0,78*	0,78*	0,85*	-	0,73*	0,68*	0,66*	0,90*	0,86*
€/haATL	0,49	-	-	0,52	-	-	-	0,52	-	-	0,55	0,52
€Fmeasures/Farea	-	-	-	-	-	-	-	-	-0,52	-	-	-
%RDP	-	-	-0,50	-	-	-	-	-	-	-	-	-

As seen in table 2, results showed distribution of investment is coherent with erosive states such as soil loss due to the double factor: on the one hand, RDP budgets were found to be heavily correlated to size, on the other, a greater surface area will naturally be more inclined to include a higher overall soil loss.
A very strong correlation was found between soil loss intensity in cultivated and forested area and investment in agri-environmental and forestry measures respectively. Nevertheless, when weighted by area (euros per hectare), no correlation was found between investment per cultivated hectare and soil loss intensity.
Rural population highly correlates with investment, as farmers can often benefit from land management subsidies, and are one of the main targets of RDPs.
Finally, the percentage of RDP budget invested in SCMs didn't correlate with the regional erosive states implying regions that make a bigger effort towards soil conservation aren't necessarily those that suffer higher erosion rates.
A PCA was applied to identify regional patterns in the combination of variables (Figure 2). The results were highlighted in two clusters that represent most of the data (red and orange) and two smaller clusters (blue and green), whilst Andalusia and Catalonia were outliers. Clusters express similarities in the regions' behaviour regarding the analysed variables a-x (Table 1). Regions with similar climate, topography, lithology, and land cover have similar erosive states (García Ruiz *et al*., 2013), and thus, should need similar intervention and finance in soil conservation (van Leeuwen *et al*., 2019). This was not found true in some clusters. Clustering seemed to be highly dependent on size above any other factor, as the most

similar-sized regions appeared in proximity. This was due to size dependency of unweighted variables such as gross investment, total soil loss or areas.

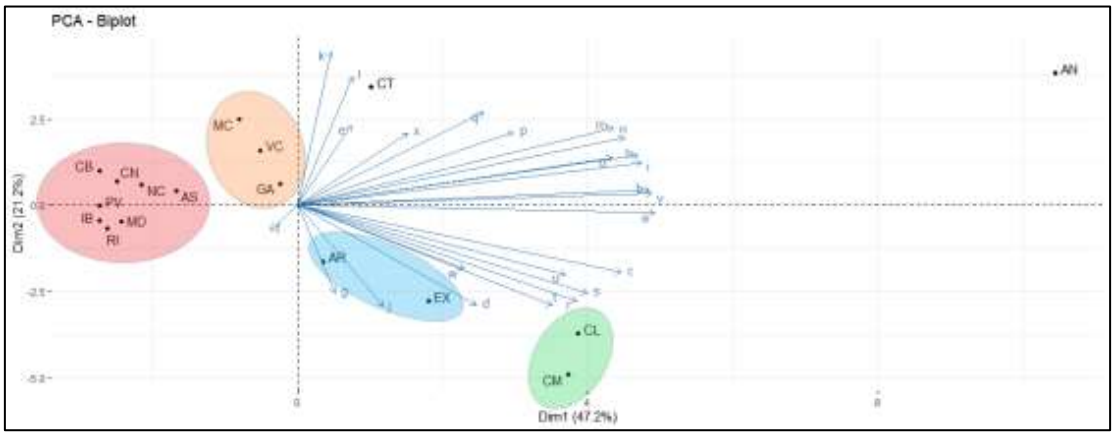

Figure 2. PCA biplot: individuals PCA & PCA graph pf variables a-x. Regions: Andalusia (AN), Aragón (AR), Asturias (AS), Balearic Islands (IB), Canary Islands (CN), Cantabria (CB), Castilla-La Mancha (CM), Castile & León (CL), Catalonia (CT), Extremadura (EX), Galicia (GA), La Rioja (RI), Community of Madrid (MD), Murcia (MC), Navarre (NC), Basque Country (PV), Valencia (VC). Vectors lettering correspond to variables as shown in Table 1.

Although the correlation between investment and size can be considered a positive outcome for soil conservation, heterogeneous climate patterns, topography, lithology, land uses or population cause variability in the erosion rates' distribution as shown in Spain's National Plan to Combat Desertification (PAND, 2008), suggesting subsidies should consider non-size-dependent criteria as well.

Soil conservation provides a multitude of ecosystemic services that are beneficial to the whole community (van Leeuwen *et al*., 2019). Although afforestation has been shown as a good method to combat soil erosion its scope is limited (Zethof *et al*., 2019), as agricultural intensification has continued over the three programming periods jeopardizing soil conservation. Studies confirmed that agricultural intensification, when mismanaged, leaded to intense soil degradation (Martínez- Mena *et al*., 2021b), showing the importance of effective legislation for soil conservation.

Conclusion

The tracking of SCMs in Spain's RDPs had obstacles related mainly to the (i) lack of transparency in financial data during the first programming period, (ii) final data on programming period 2014-2020 is due to be published along 2023 and (iii) decrease of specific SCMs in the last programming period which incorporates soil conservation goals within a multitude of different broader measures. Furthermore, investment was proven to be highly size-dependent as confirmed by correlation matrix and PCA results (Table 2 and Figure 2). Surprisingly higher erosion rates did not correlate to a higher investment per hectare, revealing weaknesses in programme planning and/or implementation. Non-significant correlations of key variables indicated a lack of standardization in soil management strategies at a national level. Finally, this paper shows rural population is strongly associated to soil conservation subsidies, suggesting rural peoples play an important role in the application of soil conservation measures. This highlights the problem of rural exodus and land abandonment which has been linked to higher rates of soil degradation (van Leeuwen *et al*., 2019).

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GREEN BONDS AS A FINANCIAL TOOL TO SUPPORT AGRICULTURAL SECTOR – OPPORTUNITIES AND CHALLENGES

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Abstract

In recent decades, the agricultural sector has faced numerous challenges and obstacles, such as the increase in food demand due to the growing population and their changes in lifestyle and eating habits, the shortage of natural resources and biodiversity, including the increase in crop yields. Discouraging are United Nations predictions, according to which the world population will reach 9.8 billion by 2050, suggesting that this population growth will significantly impact global food demand, which could increase between 59% and 98% in this period. On the other hand, sudden climate changes can reduce crop yields by more than 25%, while the consequences of climate change can be catastrophic for farmers, crops and soil. Given these devastating data, it is evident that the transformation of the agricultural sector and increased productivity will be unattainable until the amount of capital available for sustainable agricultural investments significantly increases. Green bonds are a way to channel significant funds into the agricultural sector and, at the same time, support agricultural practices that positively affect the environment. These "green" financial instruments could become a powerful complementary source of financing for governments, big corporations, and smaller agricultural companies that play a prominent role in the global food chain. The aim of this paper is to point out the importance of green bonds as a unique financial instrument that has the potential to contribute to bridging the gap between the need for increased agricultural production and environmental preservation.

Keywords: *sustainable finance, green finance, agricultural productivity, green bonds, sustainable agriculture initiatives*.

Introduction

Green finance and sustainable finance play a vital role in building a more resilient and sustainable economy. They represent the types of financial activities that support the transition to a low-carbon sustainable economy while at the same time dealing with the global challenges of today (environmental and sustainability risks, climate change, etc.). Green finance includes financing projects and initiatives that positively impact the environment (e.g. sustainable agriculture). Sustainable finance integrates environmental, social, and governance (ESG) factors into investment decisions to promote long-term economic growth, social outcomes, and environmental sustainability (Chartered Banker, 2023). Their shared goal is to initiate positive changes by mobilizing capital that will be directed towards activities that promote sustainability and reduce the negative impact on the environment. Hence, they represent necessary tools for achieving this transition by redirecting investments towards environmentally friendly projects and integrating ESG factors identified by the UN Sustainable Development Goals - SDGs (United Nations [UN], 2015) in making investment decisions.

Modern society is a post-COVID one, but also a society of the current Russian-Ukrainian crisis, which faces tremendous challenges in the agri-food sector, including the increasing importance of food quality and protection, but also disruptions and/or interruptions in supply chains. Apart from the fact that policymakers and economists in the agricultural sector are concerned about the growing significance of sustainability criteria, they also worry about the sources and methods of financing, as trends indicate a rising demand for green investments in the agricultural sector to support its sustainability.

Green bonds are the most important financial instrument of green finance, but what is their role in agriculture financing, and what should it be? This paper aims to clarify this issue. The work is organized as follows: the first part deals with the evolution of financing through the issuance of green bonds while considering their types. The second part of the paper examines the current state of the European green bond market, analysing the sectors to which a considerable portion of the revenue collected from the issuance of green bonds is directed. Even though Europe is the global leader in issuing green bonds, the facts show that a minimum percentage of the capital collected in this way goes to sustainable agricultural initiatives, which is the worst possible scenario. The following part of the paper deals with the possibilities and challenges of financing agriculture by issuing green bonds, after which comes a conclusion.

The (r)evolution of green bonds financing

Green bonds are fixed income securities that raise capital for use in projects or activities with specific climate or environmental sustainability purposes. They are structured the same way and the same characteristics as standard bonds in terms of rating, seniority, execution process, and pricing, but with proceeds dedicated to climate or environmental projects (Rosembuj, & Bottio, 2016).

The European Investment Bank (EIB) issued the first green bond in 2007, as a structured bond with proceeds dedicated to renewable energy and energy efficiency projects, in what was a giant leap towards building a responsible banking industry. The following year the World Bank (WB) issued its first green bond, and already in 2013, the International Finance Corporation (IFC) issued a 1 billion USD benchmarked bond (Pareek, 2023; Rosembuj, & Bottio, 2016). According to Kochetygova and Jauhari (2014), since its inception in 2007, the green bond market has grown rapidly at a 50%+ compound annual growth rate (CAGR), and it continues to evolve into a mainstream subset of the broader fixed-income market. As a result of increased liquidity in the sector, large multinational companies, such as Toyota, Apple and Engie, issued green bonds in developed countries in 2013 (Sovilj, 2020). A few years later, some states emerged as issuers of green bonds (state green bonds). Poland was the first country to issue government green bonds as a debt instrument at the end of 2016. France also decided on this venture at the beginning of the following year (Giuliani, 2018).

The adopting of the Green Bond Principles (GBP) in January 2014 (last updated in June 2021) by the International Capital Market Association (ICMA) contributed to the further secure development of the green bond market. The GBP seek to support issuers in financing environmentally sound and sustainable projects that protect the environment and foster a net-zero emissions economy. GBP-aligned issuance should provide transparent green credentials alongside an investment opportunity. By recommending that issuers report on the use of green bond proceeds, the GBP promote a step change in transparency that facilitates the tracking of funds to environmental projects, while simultaneously aiming to improve insight into their estimated impact (International Capital Market Association [ICMA], n.d.). The GBP emphasise the required transparency, accuracy and integrity of the information that will be

disclosed and reported by issuers to stakeholders through core components and key recommendations. The four core components for alignment with the GBP are (ICMA, n.d.):

1. use of proceeds,
2. process for project evaluation and selection,
3. management of proceeds,
4. reporting.

The key recommendations for heightened transparency are (ICMA, n.d.):

- green bond frameworks,
- external reviews.

Besides GBP, also important are the Climate Bonds Standards (CBS) and Certification Scheme launched in 2012 by the Climate Bonds Initiative (CBI), a not-for-profit organization working to mobilize global capital for climate action (Climate Bonds Initiative [CBI], 2023a). The Climate Bonds Standard and Certification Scheme is a labelling scheme for entities, assets, bonds, loans and other sustainability-linked debt instruments. Rigorous science-based criteria ensure that it is consistent with the goals of the Paris Climate Agreement to limit warming to 1.5 degrees. The Scheme is used globally by bond issuers, governments, investors and the financial markets to prioritise investments which genuinely contribute to addressing climate change (CBI, 2023a; 2023b).

The expansive growth of the green bond market in previous years has led to the emergence of various types of these financial instruments. Today, we distinguish four basic types (Iyer, 2022):

- Standard green bonds (secured by assets). A standard green bond is a bond with an obligation to pay interest and principal following the regulations of green bonds, and with these bonds, a general charge of fixed assets is offered as security to the investor (Iyer, 2022; Weber & Saravade, 2019).
- Green revenue bonds (secured by income-producing projects). Unlike the previous one, this type of green bond does not include the obligation to pay interest and principal. The credit exposure of the bonds is related to cash flows of income, taxes and fees (e.g. excise taxes, toll collection, etc.) (Iyer, 2022).
- Green Project Bonds (secured by a project asset). Kaminker (2015) states that these bonds are issued to finance one or more green projects. The specificity is in the fact that the issuer is a special purpose vehicle (entity) - SPV/project, and all assets and cash flow generated by the entity is ring fenced and available to investors as collateral (Iyer, 2022; Weber, & Saravade , 2019).
- Green Securitized Bonds (secured by an asset pool). The characteristic of this bond is that it is securitized with one or more specific green projects, an advantage of taking such a route is that investors can potentially get diversification benefits by relying on cash flows of several projects (Iyer, 2022; CBI, 2017).

According to the issuer type, green bonds can be corporate – i.e. issued by financial and non-financial corporations, and state and municipal (Golić, 2012) – issued by lower levels of government (see Figure 2).

European green bond market – current state

Even though prevailing market conditions fuelled a decline in debt issuance volumes across all categories of bonds, green bonds maintained 3% of overall issuance volumes (Michetti, Chouhan, Harrison, & MacGeoch, 2023). Since introducing green debt financial instruments, the global green debt market has grown slowly but continuously, reaching impressive growth rates of over 50% in the last five years (CBI, 2022). To date, green bond demand has far exceeded supply, and the trend persisted during 2022. According to the issuers, investors thus represent themselves as green or socially responsible by using green bonds (Michetti et al.,

2023). According to the Climate Bond Initiative (2022) assessment, the issuance of green bonds at the global level will continue to grow in the coming years, reaching 5 trillion USD by 2025 (Figure 1).

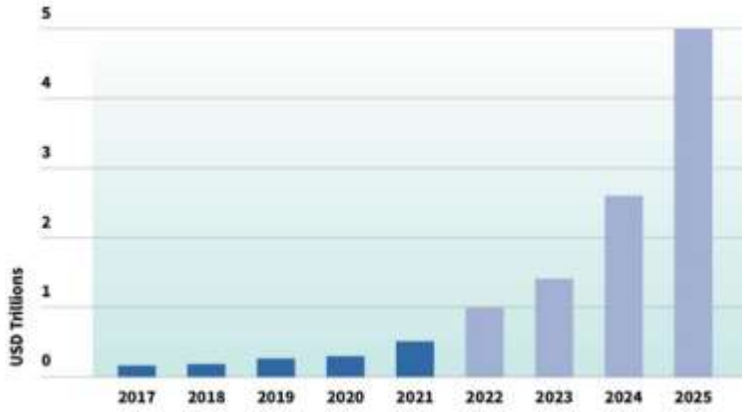

Figure 1. Green bond issuance reaching 5 trillion USD by 2025 (CBI, 2022)

As a financial instrument that encourages sustainability and green trends, green bonds represent a novelty on the capital market that can be of interest to any entity (financial institution, public entity, private company, etc.). At the same time, it allows all participants to assess how concretely financing reflects investments and functions of the real economy (Kadunc & Blažant, 2021). The commitment of the European Union (EU) to achieve climate neutrality by 2050 (European Parliament [EP], 2021; European Commission [EC], 2019; Božić, 2022) has led to modern Europe being the world leader in issuing green bonds. Different issuers from multiple European countries (Germany, France, Italy, Spain, Portugal, Holland, Croatia, Greece, etc.) backed its bond issuing. Figure 2 shows the distribution of green bond issuance by issuer type.

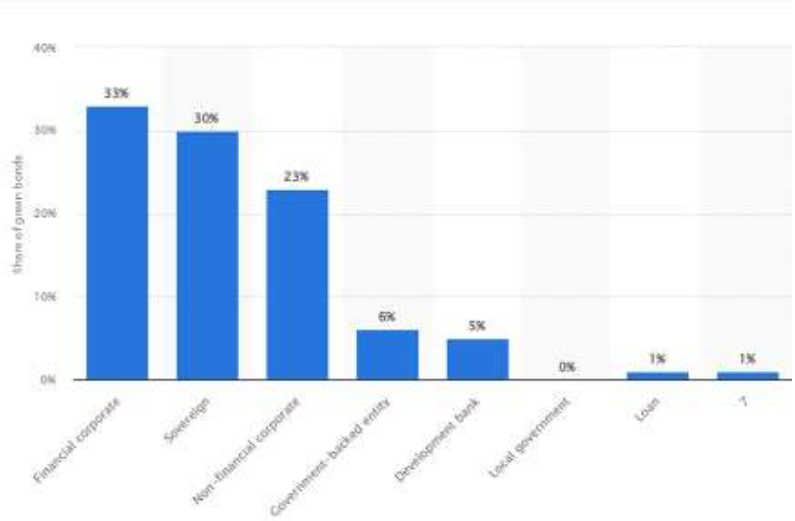

Figure 2. Distribution of green bonds issuance in Europe in 2022, by issuer type (Statista, 2023)

During 2022, financial corporations were the biggest green bonds issuer in Europe with a share of 33%, followed by sovereign issuers (30%) and non-financial corporations (23%), which account for almost 90% of the total issuance of green bonds (Fig. 2) (Statista, 2023). Green bonds imply fixed income securities that finance investments with environmental or climate benefits. These investments can finance new or existing green projects in the domains

of renewable energy, energy efficiency, sustainable waste management, land use and water management, clean transport or adaptation to climate change (Statista, 2023).

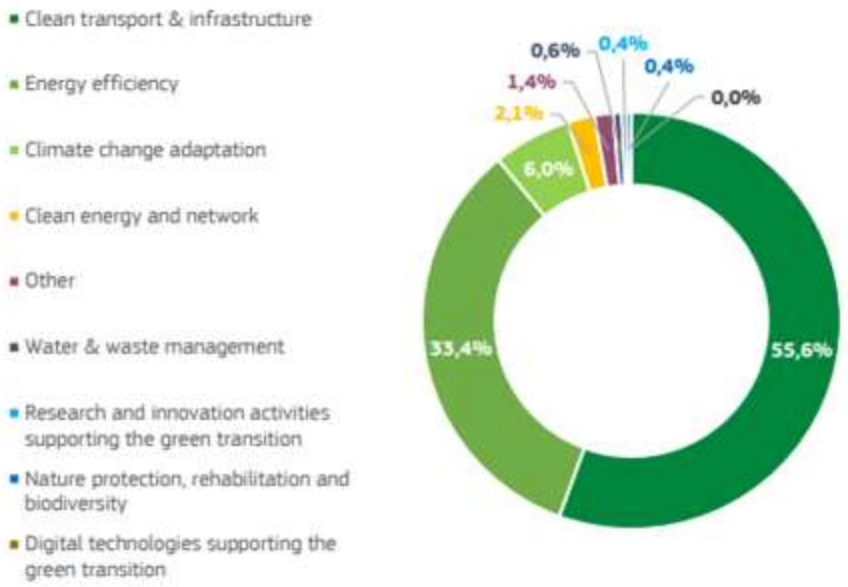

Figure 3. Sector wise Distribution of Green Bond Proceeds in Europe in 2022 (EC, 2022)

According to the European Commission data (2022), a significant amount of the revenue collected through the issue of green bonds in 2022 was invested in the green transport sector - a share of 55.6% of the total revenue allocation, followed by the sectors of energy efficiency (33.4%) and climate adaptation changes (6%). It means that a total of 95% of the income from the green bonds in Europe was invested in these three sectors. All other sectors account for the remaining 5% of the total issue of green bonds income, and sectors such as clean energy and network, water & waste management and others are still fighting for participation in the total allocation of income, as shown in figure 3.

Green bond financing in the agricultural sector – opportunities and challenges

The increase in world population, which will reach over 9 billion inhabitants by 2050 (UN, 2017), will cause global food demand to increase between 59% and 98% over the same period (Valin et al., 2014). In addition, increasingly frequent health (pandemic COVID-19) and geopolitical (Russian-Ukrainian conflict) crises (Glauben et al., 2022) signal that it is crucial to encourage and financially support sustainable agriculture initiatives. In their paper, Gernego, Urvantseva, & Sandulskyi (2022) state that this is achievable in case of attracting additional resources following specific roadmaps to understand available green investment opportunities across the agricultural sector. The same authors claim that green investments, and thus green bonds as the most attractive green financial instrument, aim to encourage progress in European sustainable agriculture (Gernego, Urvantseva, & Sandulskyi, 2022).

It is necessary to comprehend the agricultural sector's unsustainability due to negative impacts on natural resources and the environment to understand the growth and increase in its productivity (Gernego, Urvantseva, & Sandulskyi, 2022). Despite this, the potential for developing the agricultural sector depends on its ability to be competitive (Figure 4).

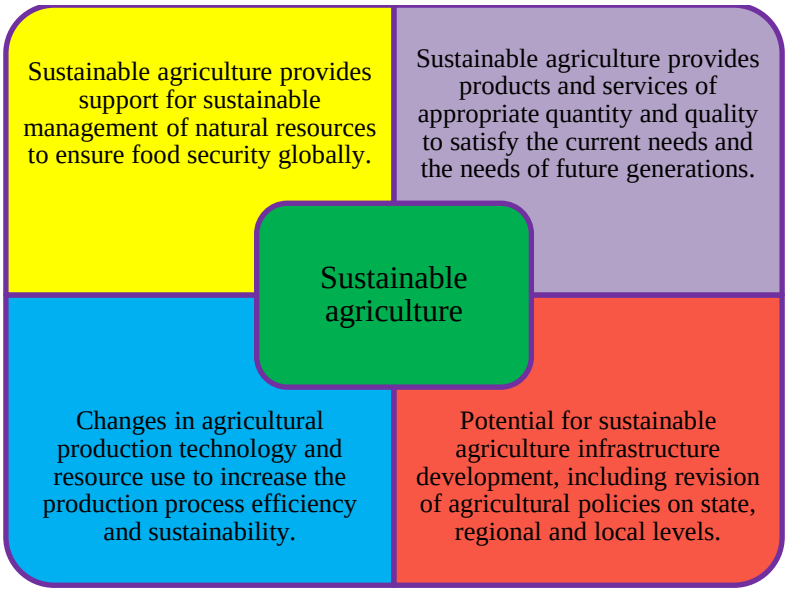

Figure 4. Characteristics of sustainable agriculture (adapted from: Group BPCE, 2021; Gernego, Urvantseva, & Sandulskyi, 2022)

Based on the characteristics presented in Figure 4, we conclude that sustainable agriculture is significant for several reasons. In the first place, it allows us to produce food in a way that does not harm the environment and/or does not deplete natural resources. In addition to environmental benefits, sustainable agricultural initiatives are also economically valuable because farmers who use sustainable farming methods have lower input production costs usually, and there is also an increased demand for environmentally friendly products, which implies higher prices. Furthermore, sustainable agriculture supports local communities by providing jobs and strengthening the local population and economy. Finally, sustainable agriculture is crucial in ensuring sufficient food to feed a growing global population without compromising the planet's health. The fact is that food systems cannot be resilient to crises such as the COVID-19 pandemic if they are not sustainable. It is necessary to redesign our food systems and move to sustainable food ones that include a collective approach at all levels of management, including public authorities, participants from the private sector, non-governmental organizations, social partners in the total food production and supply chain (Farm to Fork, 2020; Rangone, & Ali, 2021).

Although Europe is the world leader in issuing green bonds, the agribusiness sector lags significantly behind other sectors in the green bond market (see Figure 3) because only 1% of the revenue collected from the issue of this financial instrument is invested in sustainable agriculture. Due to the importance of the agribusiness sector, the collective engagement of all main actors is necessary to strengthen green financing (primarily through the issuance of green bonds) in this sector. Thus, there should be a series of measures to bridge this gap in financial support for the agri-food industry so that it can support the entire economic segment and keep up with other sectors (Figure 3) in which the revenues collected through the issue of green bonds are used for financing (Rangone, & Ali, 2021). Thus, in their paper, Rangone and Ali (2021) declare that public-private actors (governments – large corporations; governments – smaller agricultural companies) could play an extremely significant role in demonstrating and implementing green bonds focusing on sustainable agriculture.

Bonds can provide issuers with long-term financing at a lower price, i.e. they can be an alternative to expensive bank loans, which is especially relevant for long-term investments in agricultural practices with positive environmental consequences. Moreover, some research indicates that green bonds provide issuers with lower capital costs than conventional bonds

but also slightly lower yields (on average by 0.02%) than conventional bonds (Tuhkanen, 2020). Investors are indifferent to returns lower by solely two basis points (0.02%) on average if they know that investments do not hurt the environment. However, Tolliver, Keeley, and Managi (2019) indicate a lack of investors' awareness and ability regarding green bond investments due to short-term thinking and lack of sector expertise, issues that arise for any potential green bond investment.

Conclusion

Although the importance of the existence and introduction of green bonds is undeniable, as well as the visible progress in the use of green bonds in financing activities that promote sustainability and reduce negative impacts on the environment, an insufficient amount of capital collected in this way is still directed to the agricultural sector.

The analysis of the current state of the European green bond market presented in this paper indicates the need for issuing more green bonds focusing on the agricultural sector. Additionally, sustainable agriculture in Europe requires more investors interested in developing ecologically and socially acceptable agricultural enterprises. Therefore, it is necessary to work on promoting these investments and raising the awareness of the investment public. The reason investors are interested in adding green bonds to their portfolio can and should be an increase in awareness of the need to act globally and for individuals and companies to be more responsible for the environment.

Even though green bonds are a unique financial instrument that has the potential to contribute to bridging the gap between the need for increased agricultural production and environmental protection, it seems that the following years will be crucial to determine whether this potential will reach its maximum capacity in this sector. For this to happen, issuers must generate success stories and continue to build market momentum for this funding approach. By doing so, they can help create a significant new capital market able to provide financial support to sustainable agricultural initiatives.

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EXPANSION AND DECLINE OF NATURAL RUBBER EXPORTATIONS IN THE BRAZILIAN AMAZON REGION

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Abstract

This study has the following objectives: a - to estimate the instabilities and behavior of the trajectories of prices and exports of Brazilian natural rubber in each of the periods; b - to estimate forecast models of prices and exports of natural rubber from 1827 to 2021; c - to estimate the impacts of price forecast errors on the forecast errors of Brazilian rubber exports in the three periods that classify the trajectory of natural rubber exports. The research used data from the Brazilian Institute of Geography and the Brazilian Ministry of Industry and Trade. ARIMA models are estimated to predict the series of exports and prices of Brazilian natural rubber. From these models the forecast errors are generated and the influence of price forecast errors on the forecast errors of natural rubber exports is evaluated. Growth rates of exports and prices in the three periods studied were estimated, as well as the levels of instability of exports and prices. The results showed that both prices and exports of Brazilian natural rubber were quite unstable in the three periods evaluated and that the forecast errors of prices, as was one of the hypotheses of the research, interfered differently in the forecast errors of natural rubber exports and that exports had positive growth rates before and during the rubber cycle, while prices only had positive growth rates before and during the rubber cycle.

Key words: *Brazilian Amazon; Rubber Cycle; Development of the Amazon; Native rubber trees; Cultivated rubber trees.*

Introduction

Natural rubber is an important raw material in the world and has great relevance, especially since the period of industrial development at the end of the nineteenth century. This development, mainly of the automobile industry, caused the international demand for natural rubber to grow rapidly. According to Weinstein (1993), the result of this confluence of economic forces generated unprecedented commercial and demographic growth in the Amazon region, and made this part of Brazil one of the most promising trade centers in the country. Also according to this author, in the early 1900s, demand continued to grow, and by 1910 the price of the commodity reached its highest level.

The rubber tree (Hevea brasiliensis), from which natural rubber is extracted, is native to the Amazon region in South America, where it is found mainly in its native form. From the end of the second decade of the 19th century, when the first records appeared, and the first decade of the 20th century, natural rubber was a product of great importance for Brazilian international trade. In this period, its exports reached the same importance as those resulting from coffee. Moreover, this activity was an important source of employment and a driver of the Brazilian Amazon Region, in the various stages of preparation of natural rubber: from the bleeding of the trees, through the collection and smoking of latex, to the first processing that occurred within the areas of exploitation. (AKERS, 1912; SANTOS, 1980; LEMOS, 1983; PINTO, 1984; SILVA, 2022).

As labor was scarce in that region, the extraction of latex from rubber trees also served as a work alternative for people devastated by the frequent droughts that occurred in the semi-arid region of the Brazilian Northeast, who were recruited or migrated there spontaneously. This contributed to increase the occupation of the Brazilian Amazon, with Brazilians coming from that region (WEINSTEIN, 1993).

Until the end of the 19th century, Brazil was the only exporter of natural rubber. In this condition of monopoly of the market, the Brazilian exporters practically defined the price that the product would be traded on the world market. With the entry into the market of natural rubber produced in Southeast Asia, at the end of the nineteenth century and the beginning of the twentieth century, and synthetic rubber, also at the beginning of that century, Brazilian exports of natural rubber from native rubber plantations, went into decline. Still, they remained at high levels until the year 1912 and, after that year, went into decline (LEMOS, 1983).

Thus, it is likely that natural rubber extracted from rubber trees in the Amazon, including those in neighboring countries, has followed the economic cycle that Homma (2014) described for extractive products. According to this author, this cycle manifests itself in three stages. In the first stage there is an expansion of production, with an increase in extraction, when natural resources are transformed into economic resources driven by the growth in demand. In the second stage stabilization will occur, because the extractive natural resource will reach the limit of its production capacity. At this stage the domestication process should already take place to also have cultivated species that should replace the extractive ones. In the third stage is the decline, which is characterized by the exhaustion of reserves caused by increased external demand. In this phase the process of domestication and cultivation should already be underway, which did not happen with the rubber produced in the native rubber trees of the Brazilian Amazon. In the case of this extractivism the situation worsened due to competition from the production of countries of southwest Asia (HOMMA, 2014; LEMOS, 1983. WEINSTEIN, 1993).

This research seeks to describe the trajectories of exports and prices of natural rubber by Brazil between the years 1827 (when records begin) and 2021. The research starts from the premise that this long period was not homogeneous with respect to both price and export variations. For this reason the export and price series of natural rubber are subdivided into three periods that follow, in some way, the trajectory described in the work of Homma (2014). The first period develops between the years 1827 until the year 1878, when the rubber produced in the rubber plantations cultivated in countries of Southwest Asia began to enter. The second period begins in 1879 and extends to 1912, a period that became known as the "Rubber Cycle" (GONÇALVES et all, 2021). The last period of the study begins in 2013 and extends to the present day, which is characterized by the collapse of Brazilian natural rubber exports.

Thus, it is believed that the errors in the formation of expectations of natural rubber prices, in each of these stages, motivated by their instability, must have influenced the instability and the formation of expectations of demand for the commodity.

Anchored in these scenarios this study has the following objectives: a - to estimate the instabilities and behavior of the trajectories of prices and exports of Brazilian natural rubber in each of the periods; b - to estimate forecast models of prices and exports of natural rubber from 1827 to 2021; c - to estimate the impacts of price forecast errors on the forecast errors of Brazilian rubber exports in the three periods that classify the trajectory of Brazilian natural rubber exports

Material and Methods

The variables used in the research were: quantities exported and prices of rubber in Brazil, between the years 1827 and 2021. The information for the years 1821 to 1987 were taken from IBGE (1990), while the information for the years 1988 to 2021 were extracted from COMEX STAT (2022). During this period, according to the Central Bank of Brazil (BACEN, 2007), there were nine (9) different types of currencies in Brazil. The prices were updated to 2021 Real, using the general price index, domestic availability (IGP-DI) of the Getúlio Vargas Foundation. Then the average exchange rate of the end of 2021 was taken and the whole price series was converted into 2021 US Dollars.

3.1 Methodologies to reach the first objective.

The instabilities of natural rubber exports and prices were measured by the coefficient of variation (CV), which measures the percentage ratio between the standard deviation and the mean of a random variable. The larger the magnitude of the CV, the more unstable, or more heterogeneous, will be the distribution of the observed values of the random variable around its mean (SANTOS & DIAS, 2021).

To assess how the trajectories of natural rubber exports and prices have happened, we estimate instantaneous geometric rates of growth (GGR). In general, the GGR, or acceleration/deceleration rate of a continuous random variable (Y_t), by definition, is expressed by the following equation:

$Y_t = \rho_0.e^{(\rho 1T + \lambda t)}$

In the equation, "e" is the base of natural logarithms; $d[\log(Y_t)]/dT = \rho_1$ multiplied by 100 is the instantaneous GGR associated with the variable (Y_t); T = 0, 1, 2, ...,n). Its values will be defined in each of the periods in which the trajectories of exports and prices of natural rubber are studied. The random term (λ_t) assumes the assumptions of being white noise (WOOLDRIDGE, 2015).

3.2 Methodology to achieve objective b: estimate forecast models for natural rubber prices and exports from 1827 to 2021

The predicted value (Y_P) of a random variable Y_t, will differ from its observed value due to the occurrence of random factors (ξ_t), which can be caused by exogenous variables. This information can be summarized by Equation (1):

$Y_t - Y_P = \xi_t$; or, similarly, $Y_t = Y_P + \xi_t$ (1)

For this study the random variable (Y_t) can be either the quantity exported annually in tons of natural rubber, or its price in USD per kilogram. It is for these variables that the forecasts will be created in this study, using the Box and Jenkins model. The formulations proposed by Box & Jenkins (1976) - ARIMA (Auto Regressive Integrated Moving Average) - are mathematical structures that aim to capture the behavior of a random variable that has values distributed over time series. It is considered that the time series Y_t can be represented as follows:

$$Y_t = \mu + \sum \psi_{k.} u_{t-k} = \mu + \psi(B).u_t \quad (2)$$

Where the linear filter ψ is defined by:

$\psi(B)=\theta(B)/\phi(B)$ (3)

The terms in equation (3) are defined by the following polynomials:

$$\theta(B) = 1-\theta_1 B - \theta_2 B^2 - \ldots . - \theta_q B^q \text{ and } \phi(B) = 1-\phi_1 B - \phi_2 B_2 - \ldots - \phi_p B^p$$

Defining $\tilde{Y}_t = Y_t - \mu_t$, where $\tilde{Y}_t$ is the expected value of Y_t, we obtain the following transformation:

$$\phi(B)\tilde{Y}_t = \theta(B)u_t \quad (4)$$

In equation (4), u_t is a generally Gaussian white noise. To do so, it must satisfy the following conditions: i) $E(u_t) = 0$; ii) $E(u_t^2) = \sigma(u_t)^2 < \infty$; e iii) $E(u_t, u_{t+k}) = 0$, para k = ±1, ±2, ... (BOX, JENKINS, 1976; WOOLDRIDGE, 2015; CAMELO *et al*., 2018; BOX *et al*., 2015.)
According to Box, Jenkins, (1976), equation (4) is called ARMA(p,q) and can be rewritten as follows:

$$\tilde{Y}_t = \theta(B)\phi^{-1}.(B)u_t \quad (5)$$

The types of Box and Jenkins models are:
(i) - moving average (MA) models: are those in which $\phi(B) = 1$ and are said to be MA(q).
ii) - autoregressive models (AR): are those where $\theta(B) = 1$ and are said to be AR(p). These models are so called because Y_t, at moment t, is a function of the values of this variable at moments prior to t. iii) Autoregressive moving average models (ARMA): are those that have one part (AR) and one part MA and have the notation ARMA (p,q).

Before applying the ARMA model, it is necessary to evaluate whether the series being analyzed are stationary. This is done by looking at the graph of the series and analyzing the autocorrelation and partial autocorrelation functions (LIET *et al*., 2019). Another way to check whether the series is stationary is to estimate the first-order AR model over the original series and test whether the coefficient ϕ is less than 1. A stochastic process $Y_t = \psi(B)ut$ will be stationary if:
$\psi(B) = \Sigma_{k=0}^{\infty}\psi_k(B)^k$ converges to $|B| < 1$.

If the time series under study is not stationary, it must be transformed to become stationary. The autocorrelation function between the residuals is estimated. If the autocorrelation function stabilizes with the first difference, it can be assured that the series has become stationary. Otherwise, the second, third or more differences should be performed to search for stationarity. In general, series need no more than three lags to become stationary. In this case, the model is called an autoregressive moving average integrated model (ARIMA). "I" is the number of lags needed for the series to become stationary (LI *et al*., 2019).

3.2.1 Tests to assess the qualities of the fits.

When choosing statistically appropriate models, one of the criteria was to look for the most parsimonious ones, from the perspective that the smaller the number of estimated parameters, the better the model fit. There are several tools to evaluate the quality of fit and performance of a predictive model, the most relevant measures to make this measurement, and which were used in this research, are: R-squared test (R2); Bayesian Information Criterion (BIC); Mean Absolute Percentage Error (MAPE); Ljung-Box test, and Pearson's correlation coefficient between the observed and the predicted series. All estimates in the paper were performed using Statistical Package for the Social Sciences (SPSS) software, version 27 (BOX & JENKINS, 1976; BOX *et al.*, 2015; WANG *et al*., 2018; WOOLDRIDGE, 2015).

3.3 Methodology to achieve the third objective: to estimate the impacts of price forecast errors on the forecast errors of Brazilian rubber exports in the three periods of the Brazilian natural rubber export trajectory

We define $Є_t$ as the errors generated in the export forecast model and εt as the errors generated from the natural rubber price forecast model. To estimate the impact of the price forecast errors (ε_t) on the natural rubber export forecast errors ($Є_t$) the following exponential equation is used:

$$|Є_t| = \beta_0.|\varepsilon_t|^{\beta 1}e^{\upsilon t} \quad (6)$$

In equation (6), the random terms ($Є_t$; ε_t) are estimated in positive and negative values. The research seeks to assess the magnitudes of the impacts of these deviations on natural rubber exports and prices, regardless of the signs. Therefore, their absolute values are taken. The coefficient β_0 is the log-linear parameter of the equation; the constant "e" constitutes the

base of natural logarithms; υ_t is the random term associated with equation (6); the coefficient β_1 is defined as follows:

$$\beta_1 = (\alpha_0 + \alpha_1 D1 + \alpha_2 D2) \quad (7)$$

In equation (7), the variable D1 = 1 in the years before the natural rubber cycle (1827 to 1878) and D1 = 0 in the other periods. The variable D2 = 1 in the post rubber cycle period, from 1913 to 2021; D2 = 0 in all other years. When D1 = D2 = 0 was the rubber cycle period from 1879 to 1912. Thus, equation (7) can be rewritten as follows, already applying the natural logarithm operator:

$$\ln(|\epsilon_t|) = \ln(\beta_0) + [(\alpha_0 + \alpha_1 D1 + \alpha_2 D2)]\ln(|\varepsilon_t|) + \upsilon_t \quad (7a)$$

In equation (7a), the coefficients α_0, α_1 and α_2, respectively, measure the sensitivities (elasticities) of the forecast errors of Brazilian natural rubber exports in response to the forecast errors of their prices: during, before and after the cycle of Brazilian natural rubber exports.

Results and Discussion

The results found to achieve the first objective show, as expected, that exports and prices of natural rubber were quite unstable in the three periods in which the trajectories of these variables were divided in this research. In the period before the rubber cycle, from 1827 to 1878, the average exports were 2130.27 tons of natural rubber with a CV=100.07%. The prices in this period had an average of USD30.25/kg with a CV = 20.20%, showing the lower instability of prices. This is the period when only Brazil exported the product.

In the period called rubber cycle, the average of Brazilian exports reached 21,140.15 tons at an average price of USD24.72/kg. The exports of this period had a CV=53.32%, high, but the lowest observed in the three periods. The prices had a CV=27.97%.

In the post rubber cycle, from 1913 to 2021, both exports and prices of natural rubber had the highest instability CV=122.76% and CV = 60.68%, respectively. Average exports for this period fell to 8141.52 tons and average prices stood at USD4.78/kg. Estimates of the GGRs for exports and for natural rubber prices in each of the periods studied are shown in Table 1:

Table 1 - Estimates of geometric growth rates (GGR) for exports and prices of Brazilian natural rubber before the cycle, during the cycle, and after the rubber cycle.

	Natural rubber exports (tons)				
	Adj. R^2	Constant		Regression Coef.	
Periods	Value	Value	Sign	Value	Sign
Before the cycle (1827 – 1878)	0,938	4,441	0,000	0,095	0,000
During the cycle (1879 – 1912)	0,914	8,783	0,000	0,061	0,000
After the cycle (1913 – 2021)	0,397	10,498	0,000	-0,059	0,000
	Natural rubber prices (USD/kg)				
	R^2	Constant		Regression Coef.	
Periods	Value	Value	Sign	Value	Sign
Before the cycle (1827 – 1878)	0,031	3,468	0,000	-0,003	0,110
During the cycle (1879 – 1912)	0,350	2,877	0,000	0,018	0,000
After the cycle (1913 – 2021)	0,284	1,907	0,000	-0,009	0,000

Sources of the original data: IBGE, 1990; MIC, 2022.

From resuls shown in Table 1, Brazilian natural rubber exports experience the highest GGR in the years before the rubber cycle, when Brazil was the sole exporter of the product. In those years exports expanded at a GGR of 9.5% annualy and prices were practically stable, since the GGR was not statistically different from zero.
During the rubber cycle, the GGR of Brazilian natural rubber exports was also high (6.1% p.year.). In this period, as already discussed in this paper, Brazilian exports of the commodity experienced competition from natural rubber grown in Southeast Asian countries, which had higher productivity per rubber tree and per worker, but still expanded at a significant rate. During this period, prices grew at an average rate of 1.8% per year, which peaked in 2010. These 32 years were called the rubber cycle, because it was and classified by authors like HOMMA (2003) as "Bele Epoque". There the cities of Belém and Manaus were consolidated and still today keep signs of the progress caused by the "golden age" for exports and prices of Brazilian natural rubber (Table 1).
During the long period from 1913 to 2021, some facts occurred that interfered with the world market for natural rubber, among them: a greater and increasing participation of natural rubber grown in Southeast Asia; two world wars; the emergence of synthetic rubber. Brazilian natural rubber continued to be produced mainly from raw material extracted from native trees in the Amazon region until the early 1970s, with low productivity.
The decadence of Brazilian naural rubber exports manifests itself in an evident and continuous way, which seems irreversible. Over this long period the production and productivity of rubber grown in Asia have made Brazil's position in this market unsustainable. This contributed to the fact that the prices of natural rubber exported by Brazil showed an average annual decline of 0.9%, while exports also had an average annual decline of 5.9% (Table 1).

4.1 The results found to achieve the second objective: estimation of the models for the forecasts of exports and prices

In Table 2 are presented the results found in the creation of the models used for the forecasts of exports and prices of natural rubber between the years 1827 and 2021. These results showed that both the export and price series were not stationary and needed a difference to make them stationary. Both estimated models were ARIMA(0,1,1) at their original values, without the constant terms.

Table 2 - Models fitted to Brazilian export (EX) and natural rubber price (PR) forecasts from 1827 to 2020

Fitted model	ARIMA(0,1,1) (EX in ton)	ARIMA(0,1,1) (PR in USD2020/kg)
Direrence	1,0	1,0
MA lag1	0,189*	0,224*
R^2	0,945	0,923
MAPE	53,740	20,230
Normalized BIC	15,709	2,537
Ljung Box	$36,169^{NS}$	$25,569^{NS}$
$r_{Pearson}$	0,997	0,998

Original data sources: IBGE, 1990; MIC, 2022.*Significant to less than 1% error; NS: Not significant to at least 25% error,

As can be seen in the evidence shown in Table 2, the adjustments found were parsimonious and proved robust from the statistical point of view, which allows the projections to be made

and the analyses proposed in this research to be performed. The adjusted coefficients of determination were 0.945 and 0.923, respectively, for the forecast models of exports and prices of natural rubber produced in Brazil, for the period 1827 to 2021. The other statistics associated with the adjustments such as MAPE, Normalized BIC and the Pearson correlation coefficients estimated to evaluate the adequacy of the predicted values to the observed ones, were also robust, from a statistical point of view (BOX *et al*., 2015; NASCIMENTO CAMELO *et al*, 2018; GUJARATI, PORTER, 2011; PINHEIRO *et al*, 2021; VANDEPUT, 2021; WANG *et al.,* 2018; WOOLDRIDGE, 2015).

4.2 Results found to meet the third objective: to estimate the impacts of price forecast errors on the forecast errors of Brazilian rubber exports in the three periods of the Brazilian natural rubber production/export pathway

The results found to find estimates of elasticities that assess the impacts of price forecast errors on those arising from rubber exports in each of the periods analyzed are shown in Table 3.

Table 3 - Estimates of the impacts of price forecast errors on natural rubber exports in Brazil between 1827 and 2020

Variables	Coefficient	Sign.	Elasticities
Constant	8,208	0,000	-
Period 1827 - 1878	0,979	0,011	0,578
Period 1879 - 1912	-0,401	0,100	-0,401
Period 1913-2021	0,858	0,006	0,457
Adjusted R^2	0,810		

Original data sources: IBGE, 1990; MIC, 2022.

The results shown in Table 3 confirm the assumptions that guided the construction of this research. One of these assumptions is that the forecast errors of prices interfered with the forecast errors of natural rubber exports. The other assumption was that these interferences were different.

This evidence suggests that when Brazil was hegemonic in natural rubber exports in the years before the rubber cycle, price forecast errors had the largest impact on export forecast errors as measured by the elasticity of 0.578 (Table 3).

Throughout the rubber cycle, when prices of the product became competitive with the entry of the product grown in the rubber plantations of Southwest Asia, the elasticity was negative and with the smallest magnitude among the three periods. Because of this competition, prices grew at a lower rate than they did in the period before the cycle (Table 2) and, probably, exporters captured this phenomenon and overestimated exports, motivated by a slower expansion of these prices (Table 3).

In the post-rubber cycle phase, as seen in the discussion, the export capacity of Brazilian natural rubber became unsustainable, due to historical factors that occurred in the period, among them, the most relevant were the emergence of rubber grown in the countries of Southwest Asia and the emergence of synthetic rubber. The conjunction of facts of this period must have contributed to the forecast errors of exports due to the forecast errors of natural rubber prices had a positive magnitude of 0.457.

This evidence also suggests that there has been a kind of accommodation, both of natural rubber producers and policy makers, since for over a century the situation of rubber production in Brazil has only deteriorated.

Conclusions

The production and export of natural rubber extracted from native rubber trees was of great importance for the economic and social development and for the occupation of Brazilians from cities and states in the Amazon Region, especially in the 19th and early 20th centuries.
There are reports that in the 1700s and early 1800s the extraction of natural rubber in that region already occupied people. However, it was only after 1827 that Brazilian exports of this commodity began to be recorded. It generated enough wealth to transform cities like Belém (now the capital of the state of Pará) and Manaus (now the capital of the state of Amazonas), which in the rubber cycle, between 1879 and 2012, were even compared to already exuberant cities in Europe, like Paris.
The production of natural rubber in the Amazon also played an important role in the occupation of the population of the Northeast that was always hit by periodic droughts. Mainly northeastern men were recruited or attracted to work in the Amazon region in all phases of natural rubber production.
One can characterize the Brazilian rubber production in three periods, from the time when the first record of export was obtained: 1 - Before the Rubber Cycle, which occurred between 1827 and 1878. In this period Brazil had a monopoly on the export of the commodity, all of it extracted from rubber trees in the Amazon. 2 - During the Rubber Cycle, which occurred between 1879 and 1912. In this period natural rubber grown in Asian countries entered into the market. This period was also characterized as a phase of sharp growth in commodity prices that peaked in 2010. This phase ended in 2012 when Brazil recorded its highest volume of natural rubber exports. 3 - Post natural rubber cycle phase that began in 2013 and extends to the present days. This long period saw two world wars, the emergence of synthetic rubber, the increasing advance of cultivated rubber in Southwest Asia, and the first attempts to cultivate rubber in Brazil, still in the Amazon. However, the producers were faced with a disease known as "rubber leaf disease" which was unknown to researchers and therefore had no way to control. In this post-rubber cycle phase there was an evident decline in Brazil's participation in this market, so that, in the early 1950s the country became an importer of natural rubber.
The research sought and succeeded in building parsimonious models to capture the trajectories of exports and prices of natural rubber as well as generate the forecast errors of prices and forecast errors of exports of natural rubber during all these years (1827-2021). In this part the research attempted to prove the assumption that price forecast errors differentially affected the three periods in which the research was constructed.
The overall conclusion of the research is that probably the decline in the export of natural rubber produced in the Brazilian Amazon was mainly due to four reasons: 1 - the low production and productivity of the native rubber trees of that region, from which rubber was extracted in Brazil; 2 - the entry into the market of rubber grown in Southeast Asia from the end of the 19th century; 3 - the entry of synthetic rubber, mainly during the first and second world wars; 4 - the negligence with which the producers/exporters, as well as the Brazilian policy makers, observed the evident advance of natural rubber production from Asian plantations.

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THE INFLUENCE OF INTERNATIONAL MARKETS ON BARLEY PRICES IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

The price formation for agricultural and food products in Macedonia is under the intense influence of political processes and preferences of the state and the series of signed agreements on free trade and trade integration. Barley accounts for 27% of the total area dedicated to cereal crop cultivation in Macedonia, playing a vital role as a feed source for livestock production. The objective of this paper is to analyze the influence of international markets on the formation of barley prices in Macedonia. Empirical methods have been employed to analyze and compare the data on the purchase price of barley for a period spanning from December 2009 to December 2021, encompassing a total of 145 months. The statistical data encompasses the purchase price of barley for Macedonia, the EU, Bulgaria, Romania, and Serbia, enabling a comprehensive comparative analysis. The correlation coefficient shows a relatively high relationship and mutual dependence of purchase prices in Macedonia, compared to prices in the EU, Bulgaria, Romania and Serbia (over 55%), with the highest relationship with the movement of prices in Bulgaria (0.75) and the lowest with Serbia (0.56). The number of lags and months of time delay in purchasing prices of barley in Macedonia compared to other countries ranges from no delay (BIC and HQIC-test) to a one-month delay (AIC and FPE-test). The models show that the price of Barley is strongly influenced by external markets and it is necessary to improve its market competitiveness.

Keywords: *barley prices, international markets, correlation coefficient, lag length, forecasting*.

Introduction

The agricultural and food sector is an important sector for the Republic of Macedonia, which has a great contribution both in the formation of the gross domestic product and through the participation in the trade exchange of the state. According to data from the State Statistics Office, agricultural production in 2021 accounts for 7.60% of the total gross domestic product (GDP).

The Republic of Macedonia is an import-dependent country. The coverage of imports with exports is 71.82%. The EU member states (77.33%) and the countries of the Western Balkans (11.81%) have the largest share in exports, and the EU member states (46.24%) in imports (MAFWE, 2021).

The level of market integration and consequences of this connection on the prices of the products are primarily based on one of the key principles of economics is that markets allow prices to be transmitted horizontally (spatially) and vertically (Conforti, 2004). Most of the

scientific research focused on the vertical integration of markets, focus on price and its movement in the food supply chain (Goodwin & Holt, 1999); (Balcombe & Morrison, 2002).
As a result of so-called food crisis in the period 2007-2008, when the prices of agri-food products on international markets begin to vary significantly with the occurrence of large falls and increases in sales prices (European Commission, 2008); (Irwin et al., 2009), the interest for prices of agri-food products and the level of horizontal integration and mutual influence of markets has increased.
The spatial arbitrage is the main rule of market integration and price formation, based on the assumption that the difference in the price of the product between related markets will not exceed the costs of transferring it. Based on this rule, the law of one price is developed (Marshall, 1920); (Fackler & Goodwin, 2001); (Tomek & Robinson, 2003).
Even that researchers and economists in general placed their focus on connection and influence between prices, still other factors, especially the product and its characteristics (Asche et al., 2007), market positioning, competitiveness, market power as role of intermediaries (Dhar & Cotterill, 1998); (Wohlgenant, 1999); (Azzam, 1999), productivity and profitability as vertical integration (McCorriston et al., 2001), complementarity or complementarity of a certain agricultural-food product (Saadi, 2011); (Esposti & Listorti, 2013), the level of trade and product heterogeneity and homogeneity (Armington, 1969), different currencies and exchange rates (Dornbusch, 1987); (Froot & Klemperer, 1989); (Knetter, 1993), are also significant and have great impact on prices movements and formation. Especially in the last two decades, the import and export policy and additional costs for exporters are one of the main driving forces for market integration and price formation (OECD, 2017).
Barley accounts for 27% of the total area dedicated to cereal crop cultivation in Macedonia, playing a vital role as a feed source for livestock production. As the agri-food sector in Macedonia has a modest competitive power, it is expected that international market movements have a strong impact on domestic production and prices.

Material and methods

Monthly local purchase prices of barley for the period from December 2009 to December 2021 (total 145 months times series with data) have been analysed using statistical methods and models and compared with the prices of barley in EU, Bulgaria, Romania, and Serbia.
The monthly price indices data for barley from the State Statistical Office of Macedonia were used and transferred into prices based on the price of purchased products in the baseline year 2015, calculated based on value and quantity of purchase barley (SSO, 2022). Data for the EU countries are taken from the official agricultural data site of the European Commission (EC, Agridata, 2022) and for Serbia from official System for Agriculture Market Information of Serbia (STIPS, 2022).
Exponential smoothing was used for weighting and cleansing of the series seasonal atypical variations. Having in mind that our series has trend with multiplicative seasonal variation, the triple exponential smoothing and the multiplicative Holt-Winters model with multiple degrees was used. In order to compare and determine the correlation and dependence of the purchase prices of barley in the Republic of North Macedonia compared to other countries, the Pearson correlation coefficient was used. ADF test, as the most common statistical tests was used to determine the stationary/ non-stationary aspects of the time series. The Akaike's information criterion (AIC), Bayesian information criterion (BIC), Final prediction error (FPE) and Hannan–Quinn information criterion (HQIC) were used in order to determine number of lags and time reaction of the series. The Granger's test was used as a predicted model to test

causality, dependence and measuring the future predicted value of the time series, using the real prices in the time series.

Results and discussion

As the barley is one of the important products for Macedonia agriculture production, especially the livestock, this paper focus is place on the analyse of the influence of international markets on barley prices in the Republic of North Macedonia.
The graphic analysis of the data for the purchase prices of barley in Macedonia and the EU shows that the prices in Macedonia are higher than the prices in the EU, excluding the mid of 2019 and last months of 2021 (Figure 1).
The missing monthly data for purchase prices of barley, in the process of exponential smoothing were replaced with the calculated mean of the previous months. For Macedonia ($R^2 = 0.86$) and relatively for all others countries, the Goodness of fit statistics (degree of suitability and deviation) has a high level of fitness of the model.

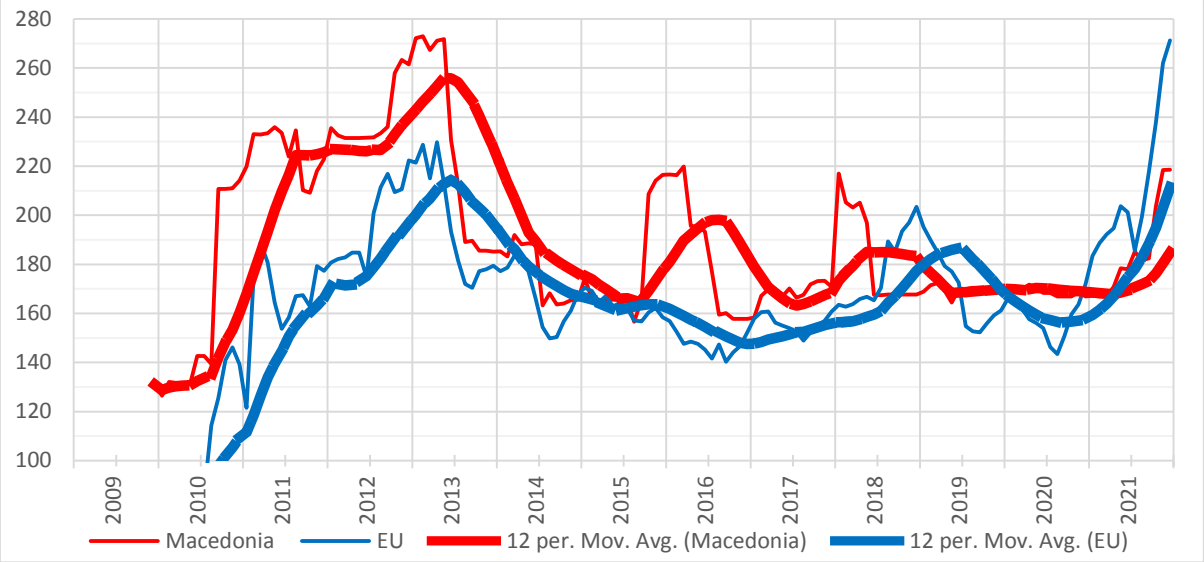

Figure 4. Barley purchase and price trends (12-month moving average) o in Macedonia and the EU, in EUR per 1,000 tons, December 2009 - December 2021

The minimum, maximum and average purchase prices of barley and standard deviation (SD) for Macedonia, EU, Bulgaria, Romania, and Serbia for the period from December 2009 to December 2021 (Table 1). In this period, Macedonia is with highest average monthly purchase price of barley of 188.48 EUR/t or 0.18 EUR/kg of purchased barley, while Bulgaria is with lowest average price of 150.32 EUR/t and standard deviation (29.10). Serbia has high monthly price variation and with 76.05 EUR/t and 274.91 EUR/t is the country with lowest and highest evidenced monthly purchase price and has highest standard deviation (40.44).

Table 1. Purchase prices of barley in EUR per 1,000 tons, December 2009– December 2021

Country	Minimum	Maximum	Average	SD
Macedonia	126.50	272.93	188.48	32.95
EU	86.74	271.26	167.70	30.59
Bulgaria	88.58	227.53	150.32	29.10
Romania	84.90	242.74	155.57	33.32
Serbia	76.05	274.91	167.23	40.44

The purchase barley prices correlation coefficient shows a relatively high relationship and dependence of prices in Macedonia, compared to prices in the EU, Bulgaria, Romania, and Serbia. (over 55%). The highest correlation and mutual dependence of purchase prices of barley in Macedonia has with prices of barley in Bulgaria (0.75) and lowest with Serbia (0.56).

Table 2. Correlation coefficient of barley purchase prices

Country	Macedonia	EU	Bulgaria	Romania	Serbia
Macedonia	**1.00**	**0.58**	**0.75**	**0.66**	**0.56**
EU	**0.58**	**1.00**	**0.86**	**0.90**	**0.35**
Bulgaria	**0.75**	**0.86**	**1.00**	**0.92**	**0.55**
Romania	**0.66**	**0.90**	**0.92**	**1.00**	**0.47**
Serbia	**0.56**	**0.35**	**0.55**	**0.47**	**1.00**

*The values in bold have significant statistical importance with α=0.05 and different from 0

The ADF test and obtained p-value significance level is lower than 0.05 shows that time series is stationary. The number of lags and months of time delay of barley purchase prices in Macedonia are ranging from no delay (BIC and HQIC tests) to a delay of three months (AIC and FPE test) compared with other countries (Table 3).

Table 3. Number of lags and months of time delay of purchase prices of barley in Macedonia

	AIC	BIC	FPE	HQIC
0	23.34	23.45*	1.37E+10	23.38*
1	23.30*	23.99	1.317e+10*	23.58
2	23.44	24.7	1.52E+10	23.95
3	23.59	25.42	1.77E+10	24.33

*Values marked with an asterisk is the lag number or monthly lag

In our case, 2 (two) lags were used in forecasting model and Granger causality test.

Table 4. Real, forecast and deviation of the forecast to real purchase prices of barley in Macedonia, in EUR per 1,000 tons

Month	Actual prices	Forecast prices	Difference forecast-actual	Monthly difference
11/2020	168.57	169.63	1.06	1.06
12/2020	166.84	171.82	4.98	3.92
1/2021	167.12	173.53	6.41	1.43
2/2021	166.84	174.88	8.05	1.63
3/2021	166.84	175.94	9.10	1.06
4/2021	170.80	176.77	5.97	-3.13
5/2021	178.32	177.43	-0.89	-6.86
6/2021	177.95	177.94	-0.01	0.89

7/2021	185.21	178.35	-6.86	-6.85
8/2021	181.66	178.67	-2.99	3.87
9/2021	182.39	178.92	-3.47	-0.48
10/2021	203.65	179.12	-24.53	-21.06
11/2021	218.41	179.28	-39.14	-14.61
12/2021	218.50	179.40	-39.10	0.04
1/2022	218.43	179.50	-38.93	0.17
2/2022	218.41	179.59	-38.82	0.10
3/2022	218.41	179.66	-38.75	0.07
4/2022	237.76	179.72	-58.04	-19.29
5/2022	218.30	179.77	-38.53	19.51
6/2022	225.36	179.82	-45.54	-7.01
Average	**194.49**	**177.49**	**-17.00**	**-2.28**

Until October 2021, the predicted purchase prices of barley through the model show a symbolic deviation compared with the real ones and on average have higher estimation for 1.94 EUR/t or insignificant 0.002 EUR per kilogram of barley. The higher negative deviation can be noticed in October (-21.06) and November (-14.61) 2021, as April 2022 (-19.29), which results with total model forecast cumulative negative deviation of -17.00 EUR/t or -0.02 EUR per kilogram of barley (Table 4).

Conclusion

It is evident that barley prices in Macedonia are relatively strongly connected and under influence of international markets, primary as result of the lower completive advantage of the country, but also as result of low production and yields, huge demand and lower (deficit) supply of barley. The highest correlation and mutual dependence of purchase prices of barley in Macedonia has with prices of barley in Bulgaria (0.75) and lowest with Serbia (0.56).

Based on the analytical and statistical analyzes of monthly purchase prices of barley for the period from December 2009 to December 2021 (total 145 months times series), it can be concluded that Macedonia has highest average monthly purchase price of 188.48 EUR/t compared with EU, Bulgaria, Romania and Serbia.

The ADF test and obtained p-value significance level is lower than 0.05 shows that time series is stationary.

The number of lags and months of time delay of barley purchase prices in Macedonia are ranging from no delay (BIC and HQIC tests) to a delay of three months (AIC and FPE test) compared with other countries and demonstrate that actually the domestic prices are under influence and follows the prices on international markets.

Until October 2021, the predicted purchase prices of barley through the model show a symbolic deviation compared with the real ones and on average have higher estimation for 1.94 EUR/t or insignificant 0.002 EUR per kilogram of barley. As of October 2021, the model clearly shows the impact of food economic crisis and unexpected, immediate rise prices as result of the post Covid-19 and Ukraine war crisis and the higher negative deviation can be noticed in October (-21.06) and November (-14.61) 2021, as April 2022 (-19.29), which results with total model forecast cumulative negative deviation of -17.00 EUR/t or -0.02 EUR per kilogram of barley.

It is evident, that methodology, analyzes and forecasting model used in this research can give detail and significant information of the barley price dependences and trends, can point out the market distortion, which makes them suitable to be used as early markets shock indicators, management and decision-making tools for producers, traders and processors, but also for the policy makers.

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THE LAND CONSOLIDATION INFLUENCE ON DEMOGRAPHY: CASE STUDY ON VOJVODINA IN SERBIA

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Abstract

Land consolidation as agricultural activity predominantly aiming to group fragmented agricultural parcels is utilized for centuries. The main measurable effects of land consolidation are the costs of agricultural production reduction, solving the property relations and the improving efficiency of land cultivation. Bearing in mind the complexity of land consolidation the research of other consequences of its utilization has been started to perform recently. The research in domain of land consolidation was connected with different issues of agriculture including reducing greenhouse gasses emission but also with societal and cultural effects on the area where it was performed. The main aim of this research is to find the effects of land consolidation on demographic issue – case study Vojvodina, Republic of Serbia depending on the level of land consolidation performed in each municipality and other improvements of agricultural production. For this analysis the multidimensional linear regression was utilized.

Key words: multidimensional linear regression, statistics, population, demography.

Introduction

The land consolidation is an issue researched by numerous authors and from different aspects on its influence on agricultural production, area where it was provided, sustainable development and many other questions of importance for agricultural economic issues. The land consolidation covers the wide spectra objectives and procedures depending on the country in question (Vitikainen, 2004). The economic, social and environmental impact of land consolidation is also one issue which was researched (Crecente et al., 2002). The research of influence of land consolidation in China showed that in case when the land fragmentation has not been reduced the positive effects were obtained through improvement of land quality and possibly through the production structure (Wu et al., 2005) The intensified social and economic decline in rural areas of Central and Eastern European countries could be solved by establishing new policies in relation to the fundamentals of land ownership and management (Pašakarnis and Maliene, 2010). The complexity of land consolidation requires complex simulation models in order to evaluate its economic and non-economic effects (Van Huylenbroeck, 1996) because it encompasses numerous stakeholders whose objectives could oppose to each other. The research conducted by Miranda et al. (2006) answered on five questions related to land consolidation influence on area where it was provided in period of a half-century. The result showed that land consolidation improved agricultural land structure by reducing number of plots per holding. Different researches argue that land consolidation leads to the better economic performance (Lerman and Cimpoieş, 2006), rural revitalization (Louwsma, 2017), and sustainable development (Ma, 2020). The spectra of land consolidation influences imply the multiple possibilities for further investigation. In this paper authors

researched the possible influence of land consolidation to the change of population in Vojvodina region, Republic of Serbia. The 45 municipalities were included in research through the available parameters obtained from different official sources like a Statistical Office of the Republic of Serbia and Republic Geodetic Authority of the Republic of Serbia.

Materials and Methods

The materials for this research are data for 45 municipalities belonging to region Vojvodina, the Republic of Serbia.
The data for each municipality (independent variables) are sorted as follows:

- X1: The percent of finished land consolidation in certain municipality
- X2: The number of farmers in municipality
- X3: Average area per plot
- X4: Average number of plots per title deed
- X5: Road network length [km]
- X6: Average cadastral class of agricultural land
- X7: Total area of agricultural land
- X8: Irrigation system coverage
- X9: Average precipitation per year and
- X10: Rural area regularization (divided into three categories-smaller number means better regularization)

Dependent variable (Y) was the change of population in each municipality during period of 40 years (from the year 1971 to the year 2011). This choice was made because the most land consolidation projects were realized in that period.
The model for analysis is multidimensional linear regression (Perović, 2012) defined as follows:

$$Y_i = \theta_0 + \sum_{j=1}^{10} \theta_j * X_j + \varepsilon_i \quad (1)$$

where ε_i is stochastic part of the model (1).

The solution system of linear equations (1) was obtained by using least square method. Firstly, all of 45 municipalities were considered and after that the reduced set of municipalities were considered and analysed. The municipalities which were extremely increased population were excluded from analysis because it was obvious that influence of industrialization prevailed.

Results and discussion

The first analysis included all municipalities nevertheless of the fact that some of them were under faster urban and industrial development related to the others. The diagram 1 illustrates the population change in all municipalities in Vojvodina region. The obtained results of parameters' values are given in table 1. The level of explanation ($R^2 = 0.33$) indicate that included model is not adequate and does not explain the considered dependent variable well. From that reason the three municipalities (Novi Sad, Stara Pazova and Pančevo) were excluded from analysis and obtained results were given in next part of the paper. The diagram 2 shows reduced set of municipalities and table 2 shows the obtained results.

Diagram 1. Population change for all municipalities in Vojvodina region

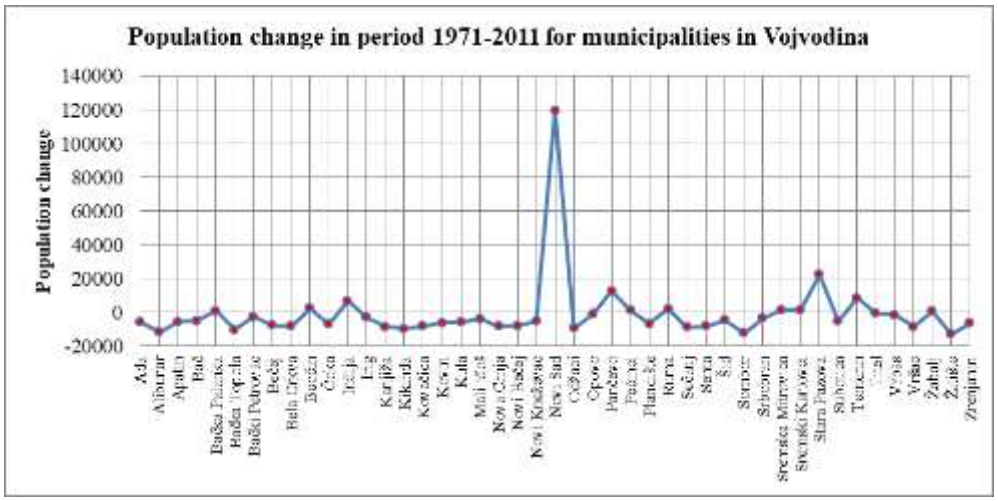

Table 1. Parameters and their values for all 45 municipalities

Parameter	Value
□□□□□	81.91
□□□□□	-5.55
□□□□□	-23655.36
□□□□□	-5096.25
□□□□□	152.39
□□□□□	-5504.72
□□□□□	-0.30
□□□□□	11.65
□□□□□	0.18
□□□□□□	-6322.53

Diagram 2. Population change for reduced set of municipalities in Vojvodina region

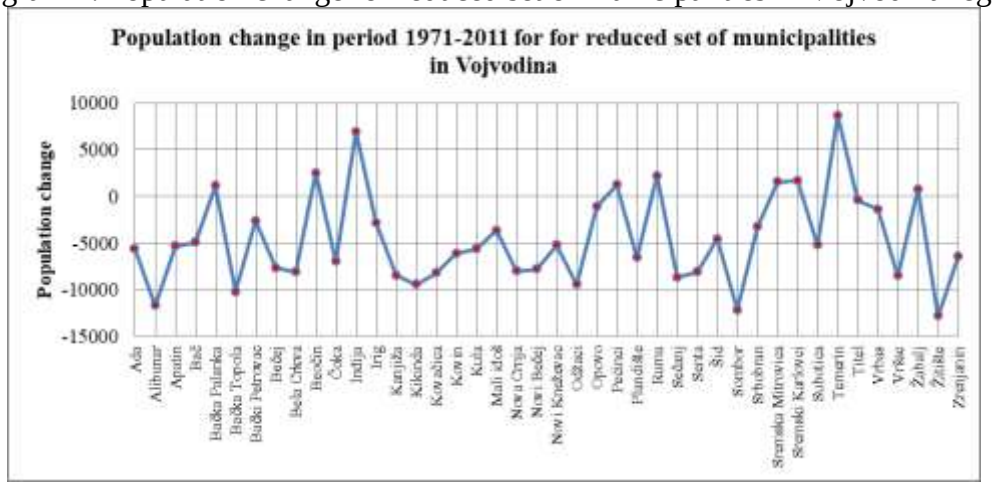

Table 2. Parameters and their values for reduced set of municipalities

Parameter	Value
□□□□□	-13.05
□□□□□	0.14
□□□□□	959.32
□□□□□	-1101.17
□□□□□	45.24
□□□□□	303.04
□□□□□	-0.29
□□□□□	193.88
□□□□□	-10.14
□□□□□□	-148.88

The level of explanation ($R^2 = 0.49$) indicate that model with reduced set of municipalities better explains the considered dependent variable than in case when all municipalities were included but it still remains the significant level of independent variables which are not included in model. The positive values of parameters indicate that increment of one parameter increase while negative value decrease population in certain municipality.
The meaning of each parameter requires further analysis and should not be simplified without deep analysis. For example, it sounds paradoxically that increment of land consolidation decrease population. But it could mean that value of the land increase and could be sold and obtained money could provide movement of the owner to the other municipality.

Conclusion

The main idea of this research was to explain the population changes in region of Vojvodina, the Republic of Serbia. This research showed that land consolidation could not explain the population changes in considered period at the high level of significance. The attempt with reduction the set of municipalities improved the significance of explanation dependent variable but still more than 51% of the population change caused by other influences remained unexplained. The model of multi-dimensional linear regression needs to be harmonized with the wider and more complex reality. The fact of industrialization rate and urbanization rate combined with the technological improvement of agricultural production shall be included in further research. The complex societal and technological development needs the holistic approach which should be modelled and supported by adequate data in order improve the model.

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FORESTRY AND AGRO-FORESTRY

STRUCTURAL AND FUNCTIONAL RESPONSES OF *ALNUS GLUTINOSA* [L.] GAERTN. TREES ON DIFFERENT SITE TYPES IN CENTRAL BOSNIA

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Abstract

The black alder (*Alnus glutinosa* [L.] Gaertn.) is recognisable as tree species that occupies headwaters, lowland and follow riversides and streams in central Bosnia. It appears in a form of canopy closed stands as well as small group of trees or in line formations on three site types: waterlogged, plateau and riverside site type. This paper examined the research hypothesis that black alder selected structural and functional variables differ in relation with site type. In addition, importance of the selected environmental variables is evaluated. Selected structural variables: diameter at breast height (DBH), tree height and crown radius of three dominant trees were measured and height crown ratio (HDR) and crown projection area (CPA) were determined for 23 temporary plots distributed in upper Bosnia river basin. Two functional variables were determined: mean annual tree leaf chlorophyll content (CCI) and fractional ground cover percentage (FGCC). Selected environmental variables were: altitude, slope, annual mean temperature (Tmean) and annual maximal precipitation (Pmax). Multivariate analysis of variance (MANOVA) was conducted to test whether site types have same multivariate means and then pair-wise comparisons were applied. MANOVA reviled a statistically significant differences between site types on the combined structural, functional and environmental variables, $F(2,20)=2.17$, $p=0.036$). Post-hoc tests reviled significant differences in mean tree height and CCI between site types ($p<0.001$). We found statistically higher CCI values on plateau and riverside site types, but lower tree mean height on riverside type. The group consisted of plateau and riverside sites indicate trees exposed to environmental stress. Obtained results could be used to address a need for strategy and action to improve conditions on plateau and riverside sites contributing to habitat protection, conservation and biodiversity. Further research should examine other influential factors as soil, water regimes, air conditions, management and human impact on black alder site types in central Bosnia.

Keywords: *black alder, waterlogged, plateau, riverside, tree height, tree leaf chlorophyll content.*

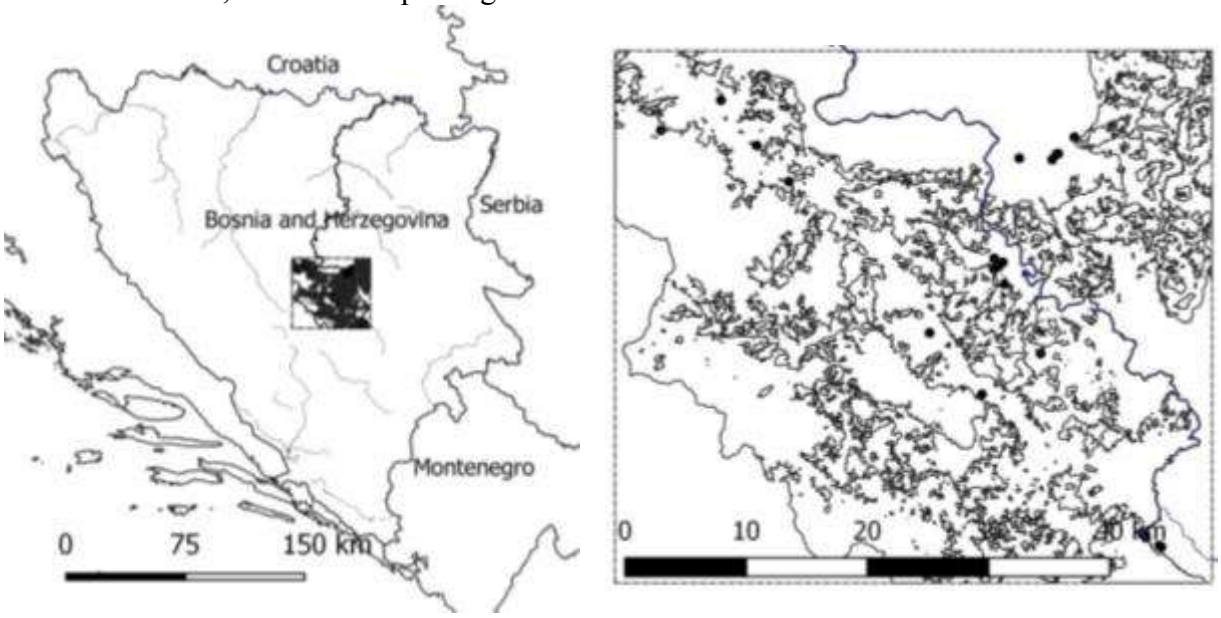
Introduction

In Bosnia and Herzegovina (B&H) all forests and wooded land occupy about 42.7% of area with about 2.170.000 ha productive forests (USAID – FIRMA 2012). Black alder forests occupy less than 1% area of productive forests with about 22,600 ha. About 70% of the area are coppice forests, mainly in private ownership (87%) (Dukić *et al*., 2012). Beside high productive native mixed high beech, spruce and fir forests that dominate on B&H forested area, black alder remained neglected without detailed information about ecology, structure, function and taxonomy. A few studies examined and reported about productivity of black alder stands in the north Bosnia (Dukić *et al*., 2012) and taxonomical classification in Sutjeska National Park in the south-east B&H (Milanović and Stupar, 2017).

Black alder is situated in Bosnia river basins and small watersheds within in central Bosnia. Here black alder exists in different stages from canopy closed forest stands to degraded sparse groups of trees without forest stand character. We focused on central Bosnia where black alder is situated on Bosnia headwaters in protected nature monument, along the Bosnia river and streams in line formations and as small groups of trees on lowland near settlements in the upper river basin. Along the Bosnia river black alder sites are exposed to different environmental conditions and anthropogenic impact. Obtained communities are situated on different site types that correspond with Claessens *et al*. (2003, 2010) description of site types in relation to the type of water supply. Claessens *et al*. (2003, 2010) differentiated (1) sites that have waterlogged subsoil thought the year, which constitute the *Alnetum* community, (2) plateau site with deep loamy soils with a temporary water-table (*Carpinion* community) and (3) riverside sites where the rooting zone is well aerated during the growing season (*Alno-Padion* community). There are no sufficient findings about structural, functional and environmental characteristics of black alder trees and stands in central Bosnia. Recent growing interest in riparian forests, climate changes (floods and extreme temperatures) and rapid urbanization lighted the importance of black alder for protection and conservation. In this study, stated research hypothesis was that structural and functional characteristics of black alder trees differ in relation with site type in central Bosnia. The aims were (1) to determine and compare selected structural and functional tree variables and (2) to evaluate effect of selected environmental variables (altitude, slope, Tmean and Pmax) on black alder trees on different site types. Selected structural variables are: tree height, diameter at breast height (DBH), height diameter ratio (HDR) and crown projection area (CPA). Functional variables are assigned: crown leaf chlorophyll content (CCI) and ground cover percentage (FGCC).

Material and Methods

The study area is located between 43° 81´ and 44° 14´ N and between 17 °77 and 18 °29 E in central Bosnia, (B&H) (Fig. 1a). Black alder sites in this study are spread between 385 and 500 m in altitude, while the slope ranged from 0 to 12.3°.



(a) (b)

Figure 1. Study area: (a) Bosnia and Herzegovina, (b) Central Bosnia – upper Bosna river basin (black dot - sample plot)

The mean annual temperature and maximal daily precipitation of the study area are 14° and 57.73 mm respectively. Climate data are based on estimates of B&H Federal Hydro-meteorological Institute. The soil types in the study area stretching from the spring of the Bosna River downstream are humic fluvisols, eutric and humic cambisols (Ćesir, 2022). According to national classification, beech forests and oak forests dominate in the study area (Stefanović et al., 1983).

Plots varying in size from 60 to 300 m^2 with an average area of 190 m^2 were selected within black alder dominant site types. The plots are situated along a longitudinal gradient and are exposed to various environmental conditions (Fig. 1b). In each plot, dominant and two neighboring trees above of 7.5 cm diameter at breast height at least (DBH, height at 1.30 m above the ground) were identified, and their diameter at breast height, height, crown diameters were recorded. The caliper, Vertex and diameter tape were used to measure tree DBH, height and crown diameters, respectively. *In-situ* measurements related to chlorophyll content in leaves and the coverage area of ground vegetation were conducted in the vegetation period in 2022 and mean values were calculated. The chlorophyll content in leaves was determined using a handheld chlorophyll content meter (CCM-200) and standard measurement protocol. The values were registered as CCI between May and October 2022. The coverage area of ground vegetation was considered as functional variable assuming tree crown impact on area of ground vegetation and their relationship. The FGCC was recorded using Canopeo, digital image-based software for canopy crop measurement. This application is available for Android (http://www. Canopeoapp.com). Canopeo estimates green cover based on an automatic color threshold differentiated green cover as white, and bare land area as black pixels. It produces an image where the green cover is white-colored and expressed in percentage (Wang *et al*., 2018).

Typical site types situated in the study area are presented in Figure 2.

(a) (b) (c)

Figure 2. The black alder on Plateau (a), Riverside (b), Waterlogged (c)

Data analysis started with explanatory analysis checking normality of distribution and homogeneity of variance for all (structural, functional and environmental) data. Then, all data were log transformed and multivariate analysis of variance (MANOVA) was performed. To determine the effect of site types on structural and functional variables with significant differences a univariate analysis of variance (ANOVA) was performed. The pair-wise comparisons were conducted to identify variables with significant differences of means between site types. All statistical analyses were performed using "psych" packages (R Statistical Software version 4.2.2; R Core Team 2022).

Results and Discussions

Structural and functional variables are measured and registered on 23 sample plots with varying size (from 60 to 375 m2) on different site types. Table 1 shows the results of descriptive statistics of structural and functional variables of black alder trees on three different site types and total.

Table 1. Summary statistics for environmental, structural and functional variables on selected site types.

Site type	Plateau		Riverside		Waterlogged		Total	
Descriptive statistics	Mean	St.dev.*	Mean	St.dev.*	Mean	St.dev.*	Mean	St.dev.*
Structural variables								
Number of trees	24		21		24		69	
Height (m)	18.2	4.4	15.0	3.6	21.7	2.6	18.4	4.4
Diameter at Breast Height (cm)	39.6	10.4	39.4	17.2	37.9	8.8	38.9	11.8
Height Diameter Ratio (%)	47.9	15.4	42.1	14.5	59.4	11.2	50.2	15.0
Crown Projection Area (m^2)	66.4	28.0	46.6	44.6	45.8	27.8	53.2	33.7
Functional variables								
Number of trees	8		7		8		23	
Chlorophil Content Index	34.9	4.6	30.3	4.5	20.4	4.3	28.4	7.6
Ground Cover Percentage (%)	24.1	10.6	26.1	11.3	23.2	10.9	24.4	10.5

* Standard deviation

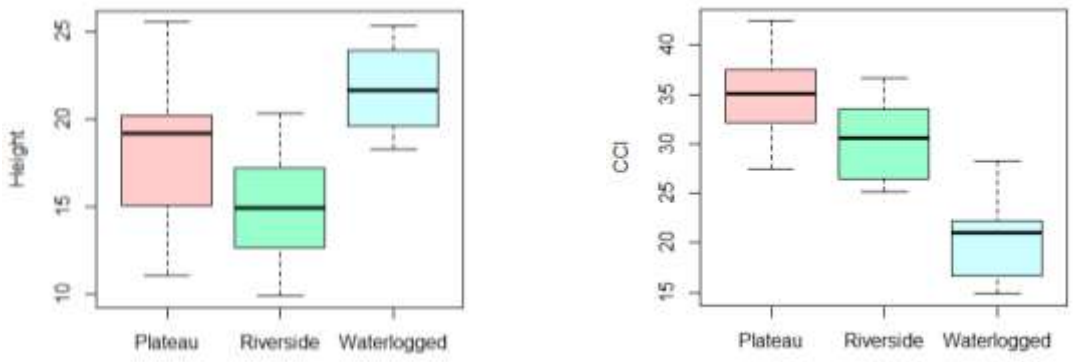
The mean height of all dominant trees was 18.4 m. The mean DBH of all dominant trees was 38.9 cm. The mean HDR of all dominant trees was 50.2%. The mean CPA of all dominant trees was 53.2 m^2. Related to functional variables, the mean CCI of all dominant trees was 28.4 and the mean FGCC of all dominant trees was 24.4%. MANOVA revealed statistically significant differences between site types on the combined structural and functional variables, $F(2,20)=2.17$, $p=0.036$). Then, to determine the effect of site types on structural and functional variables ANOVA was performed. Results showed that there was a statistically significant difference in tree height ($F(2,20)=6.09$, $P<0.01$) and CCI ($F(2,20)=22.78$, $P<0.001$) between site types while other differences were non-significant (Table 2).

Table 2. Univariate ANOVA results.

Variable	F value	Pr(>F)
Structural variables		
Height	6.086	0.009**
Diameter at Breast Height	0.063	0.939ns
Height Diameter Ratio	3.274	0.059ns
Crown Projection Area	1.771	0.196ns
Functional variables		
Chlorophyll Content Index	22.777	0.000***
Ground Cover Percentage	0.138	0.872ns

, * Significant at the .05 and 0.1 significant level; ns not significant

Pair-wise comparisons between mean heights on site types revealed that height mean of 21.7 m on waterlogged is significantly higher ($p=0.009$) than on plateau and riverside sites with mean values of 18.2 m and 15.0 m respectively (Figure 3). Trees growing on waterlogged sites had height that was higher than trees on plateau and riverside sites by 3.5 m and 6.7 m respectively.



(a) (b)

Figure 3. Box-plots of tree height (F=6.09, p<.01) (a) and chlorophyll content index (F=22.8, p<.001) (b) for three site types.

Our findings are consistent with other studies (Claessens, 1999; Thibaut et al., 2004; Laganis, 2007; Rodríguez-González *et al*., 2010). Thibaut *et al*. (2004) reported that site types can affect top height growth patterns of black alder (marshes, riparian forests, spring areas, etc.). For the same age of tree, the highest heights are expected on riverbanks with permanently good moisture and aeration conditions. In our case higher mean height on waterlogged sites could be a manifestation of the most suitable site type and where trees appear in high density and the competition for light is strong. On plateaus and riversides trees are more exposed to environmental stress what could affect height growth.

The mean differences of other tree structural variables (DBH, HDR and CPA) did not differ significantly on different site types.

Related to functional variables, dominant trees growing on plateau and riverside sites reached significantly higher CCI means with differences of 14.5 and 9.9 units then trees on waterlogged site ($p<0.001$). Many studies reported about forest trees' response to water stress by increasing chlorophyll production (Hemery *et al*., 2009; Rodríguez-González *et al*., 2009; Arend *et al*., 2016; Croft *et al*., 2017.; Sever *et al*., 2018; Anadol-Rosel *et al*., 2022). Sever *et al*. (2018) related increase in tree photosynthetic activity with insufficient water supply and plant efforts to prevent tissue damage. In our study, higher CCI values could be related to environmental pressures present on plateau site type. Further research is needed to differentiate main drivers of unstable CCI dynamics of black alder trees on plateaus.

Conclusions

In this research, structural and functional responses of dominant black alder trees in relation to environmental variables on different site types (waterlogged, plateau and riverside site types) in central Bosnia were examined. Structural and functional characteristics of black alder revealed different responses on the plateau and riverside compared with waterlogged sites. The average tree heights were lower and average CCI values were higher on the plateau and riverside sites indicating that site conditions affected black alder structural potential and functional stability. Considering structural and functional characteristics, we found that the plateau and riverside sites need attention and strategy to improve their conditions contributing to black alder structural and functional potentials.

Further research is needed to examine other relevant factors (soil, water, air, human impact) affecting structural and functional variables as well as biodiversity, protection and conservation capacities of black alder sites in central Bosnia.

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SEASONAL CHANGES OF FUNCTIONAL RESPONSES ON *ALNUS GLUTINOSA* (L.) GAERTN. SITES

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Abstract

Black alder sites are exposed to multiple pressures like climate changes with consecutive effects and anthropogenic pressures (vicinity of settlements and agriculture lands, land use change, insufficient environmental management). The aims of this paper are to analyse seasonal variations of over-story (tree) and under-story (ground vegetation) functional responses that could address environmental pressures on five sites in central Bosnia in one vegetation season. Overstorey response was related to chlorophyll content in black alder leaves and expressed as chlorophyll content index (CCI). The understorey component was related to fractional ground canopy coverage (FGCC) (herbaceous coverage) expressed in percentage. The CCI measurements were conducted on leaves of 23 dominant trees situated on five sites, monthly in the period from May to the end of October in the vegetation season 2022. The FGCC was registered under a canopy of dominant trees using Canopeo app on an android in the same period. Descriptive statistics, normality test and analysis of variance (ANOVA) were used to examine differences between tree dimensions, orography variables and functional responses between sites. Time-series of CCI and FGCC values were analysed using repeated measures ANOVA (RM-ANOVA). The ANOVA resulted in non-significant differences between all variables except altitude ($p<.01$). The RM-ANOVA revealed statistically significant differences in CCI and FGCC during the vegetation season as well as in interaction month x site with 95% confidence level. Although sites were relatively similar in average CCI and FGCC values, their interactions with changeable seasonal conditions affected differences in responses during the season. It seems that seasonal changes affected sites differently: some sites reacted with an increase in CCI (indication of water stress) and some with an increase in FGCC (indication of invasive plant expansion). Obtained results could be used to differentiate black alder sites for distinctive treatments. Seasonal effects relate to edaphic and uncontrollable climate changes while site conditions could be improved by adequate measures and strategies in the frame of environmental management.

Keywords: *Dominant trees, Chlorophyll content index, Fractional ground canopy coverage, Central Bosnia.*

Introduction

The black alder is described as one of the most tolerant species resistant to stress and environmental conditions (Zhu *et al.*, 2023). Also, it has particular importance for ground vegetation biodiversity due to specific habitats exposed to changeable water levels and human impact (vicinity of settlements, land use change, wood overuse). The black alder sites are characterized by smaller stands, groups of trees or riverside line formations where over-story and under-story vegetation respond both to environmental impacts and human pressures in central Bosnia. To examine the seasonal dynamics of vegetation on black alder sites in central Bosnia we addressed two indicators related to over-story and under-story vegetation

responses. Seasonal changes in over-story are related to chlorophyll content in leaves of dominant trees.
Recently, leaf chlorophyll content was addressed as the index (CCI) for tree condition that reflects the effects of environmental stress, changes in temperature and humidity, soil and air pollutants as well as human activities (Talebzadeh *et al.*, 2022). Previous studies of seasonal variation of leaf chlorophyll content of broadleaved species described a spring increase in chlorophyll, a stabile period in midsummer and a final decrease before senescence (Demarez *et al.*, 1999; Kodani *et al.*, 2002; Zhang *et al.*, 2007; Croft *et al.*, 2017; Atar *et al.*, 2020). Many studies found out that quantities of chlorophyll content could detect environmental stress even before visible signs (Hendry *et al*., 1987; Sauceda *et al.*, 2007; Percival *et al*., 2008; Terzi *et al*., 2010; Zhang *et al*., 2011; Gholamin and Khayatnezhad, 2011).
As an indicator of environmental impact to under-storey vegetation we selected herbaceous coverage expressed as fractional ground canopy coverage (FGCC). Changes in FGCC reflect changes in plant phenology as well as other impacts (climate change, water storage, drought, invasive plants, surrounding landscape) (Havrdova *et al*., 2023).
The main objectives of this paper were to analyze seasonal variations of chlorophyll content in black alder leaves and fractional ground canopy coverage below tree canopy aiming to address environmental pressures on five sites in central Bosnia in one vegetation season.

Material and methods

The study area is situated on area of 1,512 km^2 (41.2 km by 36.7 km) from 43 °81 to 44 °14 N and 17 °77 to 18 °29 E in central Bosnia and Herzegovina. The subsection of the Bosna River basin examined in this study stretches from Sarajevo and flows north toward the city of Zenica where the Lašva River joins it (Figure 1).

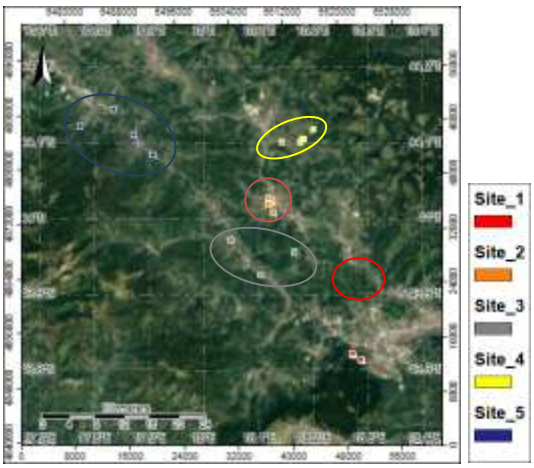


Figure 1. Study area in Central Bosnia

The climate for this region is humid continental tempered by the Adriatic Sea, with an average annual temperature of 10°C, while minimal average temperatures occur in January (-0.5 to -4.3°C) in Sarajevo and Zenica, respectively, and maximum temperatures in July/August (20 to 26°C). Precipitation differs significantly between Sarajevo and Zenica, where in Sarajevo it ranges from around 64 mm in February to 91 mm in September, while in Zenica it ranges from 34 mm in September to 81 mm in May (Federal Hydro-meteorological Institute). The mean, maximal and minimal temperatures and precipitation were recorded monthly at two metrological stations: at the headwaters and at the station near the Bosnia river 100 km far away in the north.

Five sites with similar environmental conditions were identified and a variable number of temporary sample plots were determined. Site 1 is located at the Bosna headwaters, below Igman mountain with a shaded position and colder climate (Ilidža). Sites 2 and 3 occupy sunny exposed planes near fertile agricultural fields around Bosna river (Visoko) and near the industrial area (Kakanj). Sites 4 and 5 are situated on uphill positions, but Site 4 is closer to river (Kiseljak) while Site 5 occupies drier positions (Busovača). The dominant trees were selected at the beginning of spring and structural characteristics were measured (tree height, diameter at breast height and crown projection area determined.
The sites were visited during the first week of each month for nine months with ten visits in total from March up to the end of October 2022. *In-situ* measurements related to chlorophyll content in the leaves of dominant trees and the herbaceous coverage were conducted. The chlorophyll content in leaves was determined using a handheld chlorophyll content meter (CCM-200) and standard measurement protocol. The values were registered as CCI between May and October 2022. The coverage of ground herbs was recorded using Canopeo app (Wang et al., 2018).

(a) (b) (c)

Figure 2. Diameter at breast height measurement (a), Chlorophyll content index measurement (b), Fractional ground canopy coverage recording (c)

Exploratory data analysis is performed and descriptive statistics is calculated. All statistical analyses were performed using standard R packages (R Core Team, 2021).
Descriptive statistics for site conditions and tree characteristics in this study area are presented in Table 1.

Table 1. Descriptive statistics (n=23).

Descriptive statistics	Mean	Standard deviation	Minimum	Maximum
Altitude (m a.s.l)	449.96	35.85	384.40	500.00
Slope (^{o})	1.95	1.70	0.50	5.64
Tree height (m)	18.43	4.42	9.90	25.50
Diameter at breast height (cm)	38.93	11.84	20.00	72.20
Crown projection area (m^2)	53.21	33.69	5.11	142.08

Differences between means of the site, tree variables and functional responses are analysed using analysis of variance (ANOVA). To analyse the seasonal variability of selected functional responses between sites we used repeated measures analysis of variance (RM-ANOVA).
The month and site are assigned as main factors with an assumption of their random characters. Then, further analysis was based on graphical presentations.

Results and discussion

Black alder is distributed in a wide European area fulfilling various ecology services (productive, protective, conservation biodiversity, social and others) in different environmental conditions. In our study, black alder sites' average altitudes differ significantly at the 0.01 significance level and trees differ in mean tree height and CCI (Table 2).

Table 2. Univariate ANOVA results.

Variables	F-Ratio	Pr(>F)
Orography variables		
Altitude (m a.s.l)		11.3 0.000 ***
Slope (^{o})		1.73 0.188 ns
Tree variables		
Height (m)	6.086	0.009 **
Diameter at Breast Height (cm)	0.063	0.939 ns
Crown Projection Area (m^2)	1.771	0.196 ns
Functional traits		
Chlorophyll Content Index	22.777	0.000 ***
Fractional Ground Canopy Coverage	0.138	0.872 ns

, * Significant at the .05 and 0.1 significant level; ns not significant

Obtained differences pointed out environmental impacts that affect black alder sites. Mean values of CCI and FGCC for vegetation season on five different sites are presented in Table 3.

Table 3. Descriptive statistics of chlorophyll content index and fractional ground canopy coverage per site.

Sites	Site 1	Site 2	Site 3	Site 4	Site 5
Number of plots/trees	5	6	3	5	4
Chlorophyll content index	23.4±7.7	34.3±8.5	24.1±8.4	28.3±10.2	24.2±5.1
Fractional ground canopy coverage	17.5±10.4	31.7±12.7	28.7±10.9	29.1±18.4	31.0±13.1

Chlorophyll content index. Talebzadeh and Valeo (2022) stated that chlorophyll content is a species-specific feature that could be upgraded with the assumption that any change in

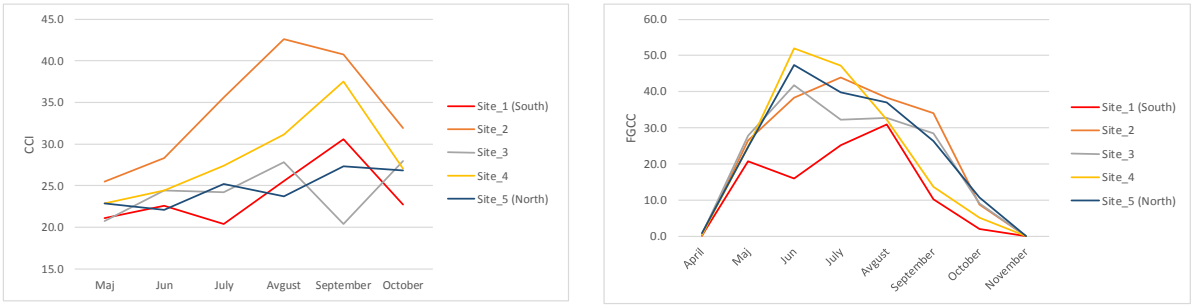
chlorophyll content in a plant reflects weather or environmental impacts on specific sites. In our research the highest CCI mean value appeared on site 2 (Table 3). Values of CCI ranged from 9.7 to 59.9 with mean of 27.5 units for the whole vegetation season. Our CCI results are consistent with other authors' findings related to long and stabile black alder leaves functionality (Taulavuori, 2006; Laganis, 2007).

Table 4. Repeated-measures analysis of variance (RM-ANOVA) results of chlorophyll content and index and fractional ground canopy coverage.

Source	Chlorophyll content index		Fractional ground canopy coverage	
	F-Ratio	P-Value	F-Ratio	P-Value
Site	2.71	0.06^{ns}	1.65	0.21^{ns}
Error(Tree)	10.30	0.00**	7.45	0.00**
Month	12.45	0.00**	33.93	0.00**
Month x Site	1.94	0.02*	2.01	0.01*

*, **Significant at the .05 and 0.1 significant level; ^{ns} not significant

According to RM-ANOVA, variability between trees and months has statistically significant effect on CCI at the 99% confidence level while interaction impacts of month and site have statistically significant effect at the 95% confidence level (Table 4). The larger parts of total variation are related to season and trees with 45% and 38% of total variance, respectively. Our results are in accordance with a study about seasonal trends of native trees and shrubs (Sauceda *et al*., 2007).



(a) (b)

Figure 3. Variations in the chlorophyll content index (a) and in the fractional ground canopy coverage (b) across a growing season and between sites.

Effects of interactive impact month x site on CCI are visible in Figure 3. Sites 2 and 4 exhibited the highest CCI values in August and September while other sites had a similar increasing trend till the end of the vegetation season (Figure 3).

Fractional ground canopy coverage. Usually, herbaceous coverage is measured and registered in floristic and taxonomic research but without inference about its seasonal dynamics (Sciandrello *et al*., 2023). We stated that its dynamics reflecting seasonal impacts could clarify environmental pressures. Values of FGCC ranged from 2.1% to 51.9% with a mean of 27.6% for the whole vegetation season. According to RM-ANOVA, variability between trees and months has statistically significant effect on FGCC at the 99% confidence level while interaction impacts of month and site have statistically significant effect at the 95% confidence level (Table 4). The larger parts of the total variation are related to season and trees with 75% and 17% of the total variance, respectively. Two different dynamics can be noticed in Figure 3. The first type of seasonal variation pointed out the earlier appearance and later disappearance of herbaceous coverage with a lower mean percentage stable during

the season. The second type of dynamics represented low coverage at the start of the season, a sharp increase and high coverage during the late spring and early summer season sharply declining to the end of the vegetation season.
We analysed differences between sites and identified that the first type of annual variation presents sites situated in the closed stand while the second type is situated in carr woodland along the Bosnia river. During the *in-situ* observations, we noticed tall sedges and high grasses on carr woodland near Bosnia river which is represented with a timely bounded period and the highest FGCC values. Mandžukovski *et al.* (2022) addressed the possibility of the rare occurrence of tall sedges and high grasses in the Mediterranean region. Our results could be related to Douda *et al.* (2009, 2016) description of different herb layers on drier hummocks (stand conditions) and waterlogged hollows (carrs conditions) not only in composition but in herbaceous coverage seasonal dynamics.

Conclusions

In conclusion, significant seasonal variation of overstory (tree) and understory (ground vegetation) functional responses on different black alder sites indicate differences related to the interaction of seasonality and site condition. Some sites responded with sharp increases in CCI in the second part while on some sites FGCC values increased in the first part of the vegetation season. The highest component of CCI and FGCC variations relates to climate changes but also differences rely on controllable site conditions. The presented methodology could contribute to differentiating black alder sites for suitable environmental management to stabilize functional responses and maintain site conditions for optimal ecological services (protection and biodiversity conservation).

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POSSIBILITIES AND DIFFICULTIES OF MEASURING CARBON SEQUESTRATION IN AGROFORESTRY SYSTEMS IN HUNGARY

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Abstract

In 2022, the report of the Intergovernmental Panel on Climate Change (IPCC) classified agroforestry as one of the three most important mitigation alternatives within the land use sector. The methods used to estimate carbon sequestration are very diverse. We have global models based on extrapolation of field measurements, which are used in forestry for carbon sequestration estimates. They are likely to result in severe under- or over-estimation of the total carbon stock in agroforestry systems. Using cloud-based systems (eg. Google Earth Engine or Global Forest Watch) we can create maps and graphs displaying biomass, soil carbon content, carbon absorption and greenhouse gas emissions. Their advantage is large spatial coverage, but their disadvantage is medium spatial resolution, so systems with small areas cannot be examined by them. In the case of shelterbelt systems, we can perform a more accurate carbon stock calculation based on the data of the National Forest Stock Database, which is based on field measurements and estimated data. In case of field measurements, we examine the amount of carbon stored in the above- and below-ground biomass, as well as in the soil. The traditional method for measuring above-ground biomass is to cut down individual trees and plants and measure their carbon content in the laboratory. The carbon content of the soil sample is determined according to a methodology, and the root-to-shoot ratio is used to express the belowground biomass. In our research work, we want to assess the mitigation potential of Hungarian agroforestry systems, comparing the accuracy of the mentioned methods.

Keywords: *agroforestry, mitigation, carbon, biomass.*

Introduction

Along with many other sectors, agriculture is one of the most vulnerable in the face of climate change (Verchot et et al., 2007). In recent decades, agricultural research has been driven by the ambition to increase the productivity and resilience of agricultural systems (Kandji et al., 2006). They can also have an indirect effect on carbon sequestration by helping to reduce the pressure on natural forests, which are the largest sinks of the terrestrial carbon (Montagnini & Nair, 2004). The agroforestry management practice has traditions in Hungary, but only a small amount of information is available about its prevalence. In recent decades, only a rough survey of woody shelterbelt systems and silvo-pastoral lands has been carried out. Almost 95% of domestic agroforestry areas are silvo-pastoral lands. The remaining area (cca. 2,000 ha) consists intercropping systems for the production of quality timber.

Carbon sequestration potential

Agroforestry can play a significant role in mitigating the atmospheric accumulation of greenhouse gases and sequestering carbon (Verchot et et al., 2007). In terms of mitigating climate change, agroforestry may be more favorable than other agricultural practices, as its long-term perspective ensures the permanence of sequestered organic carbon, and significant emissions are avoided due to generally high productivity and multifunctionality. Combined cropping systems with several above-ground and below-ground layers result in higher efficiency in terms of biomass production and carbon sequestration than monoculture systems (Aertsens et et al., 2012). It can be said that agroforestry is one of the most promising agricultural technologies with negative emissions and appropriate carbon dioxide management measure that can be rewarded with a CO_2 certificate (Mayer et al., 2022).

Measurement of carbon sequestration in agroforestry systems

Estimating carbon sequestration in agroforestry systems is a serious challenge. The used methods are very diverse, so it is difficult to find identical, consistently used techniques and easily comparable results in the different refences. This problem probably results a serious under- or overestimation of the total carbon stock (Nair, 2011).

The oldest, but most time-consuming, method for measuring *above-ground biomass* is the cutting and harvesting of individual trees and intermediate plants, then measuring their carbon content in the laboratory. To simplify this method, the researchers developed various *allometric equations* and *models* for different forest types. These equations vary greatly depending on climate zone, tree species, age, tree density and other factors, and can lead to large inaccuracies. Furthermore, there are no two agroforestry plots alike: each can be unique in terms of plant composition, planting arrangement and stock density (Mosquera-Losada et et al., 2011).

The amount of carbon sequestered in the *soil* is based on soil analysis, when the carbon content of a soil sample is determined and expressed per hectare. Soil organic carbon (SOC) is often measured on the whole soil. Several studies measure organic carbon by quantifying the amount of CO_2 produced by heating. Other studies measure the change in sample weight after heating (Nair, 2011).

The *below-ground biomass* contains a significant amount of carbon, but its measurement is very difficult. The root-to-shoot ratio is usually used to estimate the living biomass below-ground. The proportions vary considerably between different tree species and ecological regions. In the absence of measured values, many researchers assume that the underground biomass constitutes a specific part of the above-ground biomass and the assumed values range between 25% and 40% depending on factors, such as root system and ecological conditions. It can lead to significant underestimations (Nair, 2011).

Remote sensing technology is considered as an important factor because the data from the satellite has evolved rapidly. Satellite remote sensing can provide spatially explicit information on land cover, stand-age class, and harvesting. Carbon-cycle process models coupled lake regional climate databases can provide information on potential rates of production and related rates of decomposition. The integration of remote sensing and modeling thus produce spatially explicit information you carbon storage and flux (Turner et et al., 2004). The limiting factor of its applicability is that our domestic agroforestry systems are currently only located in small areas, so accurate calculations are difficult to perform.

Materials and Methods

Experimental areas

Földes

The investigated shelterbelt system was established in a 5.1 hectare organic agricultural field nearby Földes (47°17′56″N, 21°23′35″E). The field characteristics are shallow site, meadow solonetz soil turning into steppe formation, with some periodic water affected areas. The trees were planted in 1999, in 8 rows, interrow spacing was 3 m and in-row spacing was 1 m, making up to 20 m each stripe, covering altogether 3 ha. Organic farming is conducted in the enclosed area with the production of pumpkins, zucchini, spelled wheat, and organic corn.

The species used in the shelterbelt were originally chosen according to their significance to apiculture. There were determined by their blooming period to continuously provide pollen and nectar for the bees, and to fulfil windbreak characteristics.

Figure 1: Shelterbelt system in Földes (source: Google Earth)

Fertőd (Sarród)

The cca. 1 hectare experimental area was established on the field of The Hungarian University of Agriculture and Life Sciences, Department of Fruit Growing at Fertőd Research Station (47°37′59″N, 16°52′58″E) in 2016. Among the rows of hybrid poplar clone trees SV-890, there are berries (raspberry, currant, strawberry, blackberry) in intermediate cultivation.

Soil sampling

The experiment started in September 2022 when soil samples were taken at several points in both areas, from 4 depths (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm), in duplicate with a 100 cm^3 sampling cylinder. The samples were transported to laboratory to carbon content test, where other elements were also tested.

During the summer of 2023, the measurements will be repeated, as well as the above-mentioned above-ground biomass sampling. The aggregated results will be presented at the AGROSYM 2023 conference in October 2023.

Calculated carbon sequestration data based on the National Forest Database

The shelterbelt system in Földes is under forest planning obligations in Hungary, so its data can be found in the National Forest Database (NFD), from which carbon content and sequestration calculations can be made.

Data of the yearly statistical states of the years 2011-2021 was used. The standing volume of the sub-compartment is described in four tree species rows, these are: pedunculate oak, silver maple, Siberian elm, and hybrid poplar. The standing volume of other associate tree species is described in the above-mentioned tree species rows.
The estimation of the carbon stock and carbon stock change is based on the methodology of the IPCC (2006, 2019) and it is also consistent with the Hungarian Greenhouse Gas Inventory (GHGI; NIR 2023). The basis of the carbon stock calculation is the annual standing volume data of the NFD. Density and carbon fraction values were taken from the Hungarian GHGI.
In order to calculate the carbon content of the above-ground biomass the 0.25 root-to-shoot ratio was used (NIR, 2023).

Results and Discussion

The collected soil samples were tested in a soil laboratory, from which we obtained the results of humus and total carbon (TC%), total organic carbon (TOC%), total inorganic carbon content (TIC%). Overall, as expected, the humus content of the soil samples collected from under trees and rows of trees was the highest. The following graphs (*Figure 2, Figure 3*) show the average TC% of soil samples from the two study areas. The results match the expectations and will be compared with the results measured in 2023.

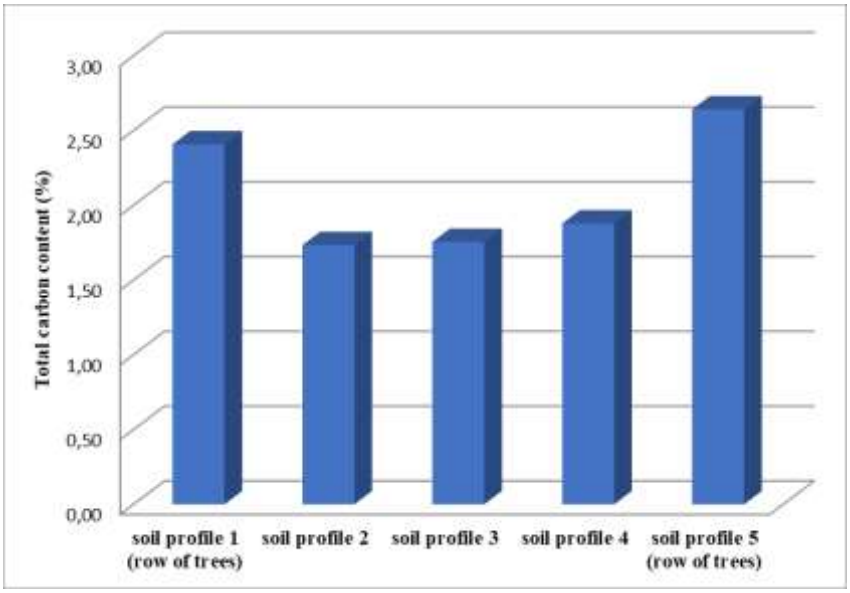

Figure 2: The average TC% of the soil samples in Földes field experimental area

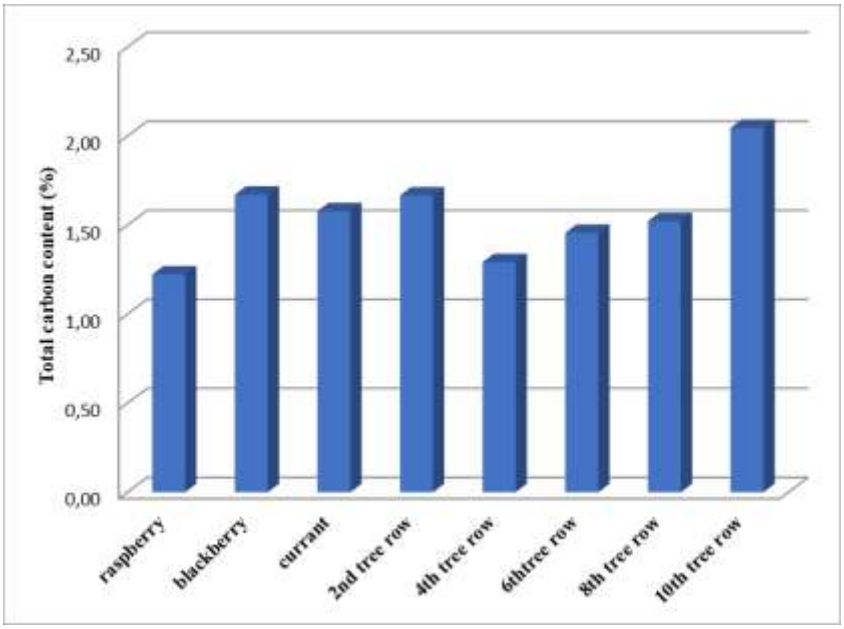

Figure 3: *The average TC% of the soil samples in Fertőd (Sarród) field experimental area*

The standing volume of the stand according to the NFD data is shown in *Figure 4*.

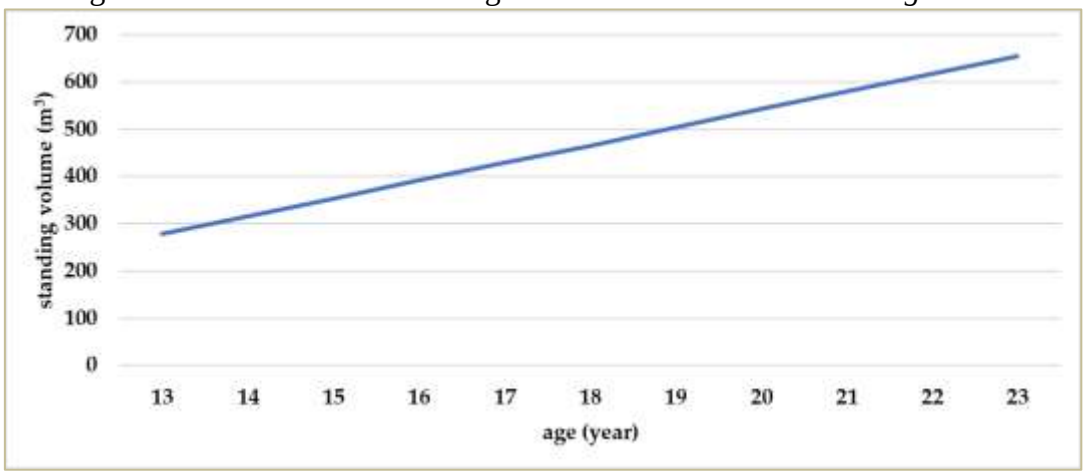

Figure 4: Standing volume of the Földes 68 A forest sub-compartment according to NFD data

According to calculations, *Figure 5* clearly shows that the total organic carbon stock is constantly increasing over the years, from 100 to 200 tons in 10 years. The 12 tons/year average annual carbon sequestration can be a promising result in the face of international literature (*Mayrinck et al., 2019).*

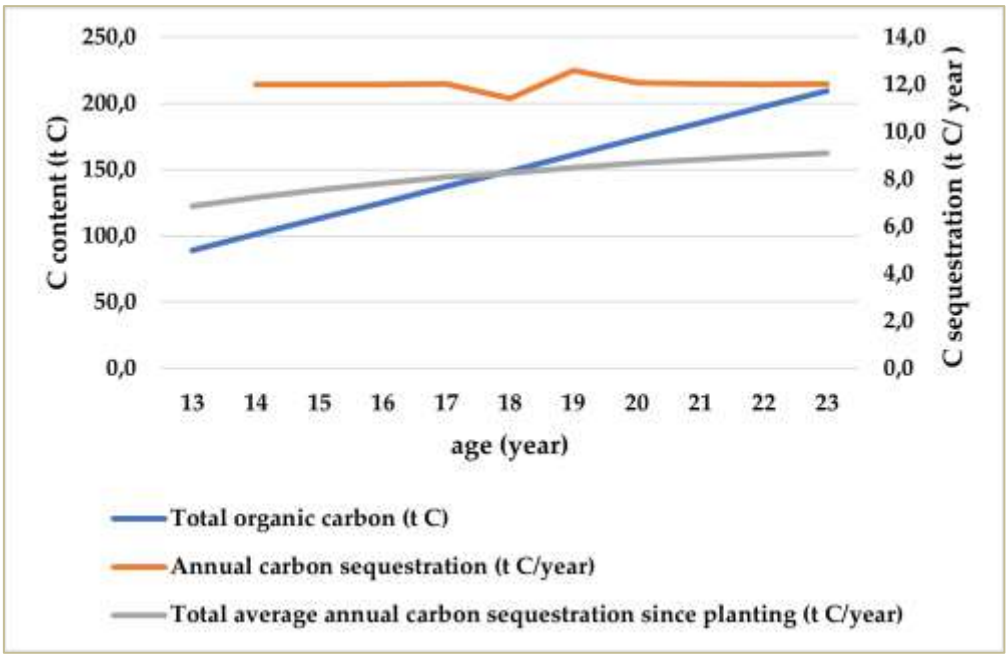

Figure 5: Carbon stock and carbon stock change of the Földes 68 A forest sub-compartment.

Conclusion

The literature concludes that there is potential in the ability of agroforestry systems to sequester carbon, which represents an important value for society in general and the agricultural sector. The high variability of the agroforestry systems complicates the measurement of their mitigation potential, as well as the comparability of the results. Looking at the results of the first year of our research, our goal is to define a methodology for temperate agroforestry systems, which we will carry out the necessary measurements to determine the carbon sequestration and carbon storage capacity. In the future with these results, we are confident to create a model that can be easily applied, thus presenting the mitigation opportunities provided by the systems to both farmers and decision-makers.

Acknowledgement

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MACHINE LEARNING METHODS FOR LAND COVER AND TREE SPECIES CLASSIFICATION USING GOOGLE EARTH ENGINE

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Abstract

The satellite-based forest health monitoring system created in Hungary was further developed and extended using Machine Learning methods of Artificial Intelligence applied in Google Earth Engine cloud platform. Our goal was to apply multiple Machine Learning to Sentinel-2 space imagery for land cover classification, which helps to accurately delineate forests, which further improves the effectiveness of forest monitoring. Random Forest, Minimum Distance Estimation, Support-Vector Machine and Gradient Boost Regression algorithms were used to generate land cover and tree species maps in Hungarian study sites (Farkas-erdő of Sárvár and Nagyerdő of Debrecen). The aim was to delineate the forests as accurately as possible, thus improving the accuracy of forest monitoring, and analysing the variation of land cover classes and spatial distribution of tree species. Compared with the remote sensing-based WorldCover land cover map each method's accuracy was between 70.3% and 80.6%, where Random Forest showed the best agreement with the reference map at 80.6%. Land cover classes were the following ones: forest, shrubland, grassland, cropland, built-up, barren land, and permanent water bodies. Tree species were classified in the study sites using the same Random Forest method. Accuracy assessment was made with confusion matrices where field-surveyed data was compared to satellite data demonstrating 81.8% accuracy for dominant tree species: pedunculate oak (*Quercus robur*), black locust (*Robinia pseudoacacia*), Scots pine (*Pinus sylvestris*), red oak (*Quercus rubra*) and clearcuts. The system could be extended to the entire county based on the applied methods, the promising results and the flexibility of Google Earth Engine.

Keywords: *satellite imagery, machine learning, land cover, tree species classification.*

Introduction

Forestry research is successfully supported by the modern technology of remote sensing. Sensors installed on satellites continuously scan forests and provide useful information. The great advantage of this method is the growing database of objective, free and high-resolution images are available for large areas, which provides an excellent basis for forestry research.
In the present research, we have further developed a cloud-based forest monitoring system for the Farkas-erdő in Sárvár, Hungary, which uses the European Space Agency's (ESA) Sentinel-2 space imagery with high temporal, spatial and spectral resolution, and partially automated methods for continuous monitoring and analysis of forest health (Molnár - Király 2022). The monitoring runs in the Google Earth Engine (GEE) cloud system, where data storage and access, analysis and visualisation are done online (Gorelick et al., 2017).
The precise delimitation of forest areas is a key point of the effectiveness of monitoring. This is where Machine Learning, a branch of artificial intelligence, can help. The Machine Learning (ML) methods built into GEE can identify land cover classes, thereby separating forested and non-forested areas (Basheer et al., 2022). To do this, we show the program where

and what type of land cover category is present and through Machine Learning, it automatically finds all similar ones based on spectral similarity.
This paper aims to apply multiple ML methods of Artificial Intelligence to Sentinel-2 space imagery for land cover classification, which helps to accurately delineate forests, which further improves the effectiveness of forest monitoring.

Material and method

Study sites

The first study area is the inner city of Sárvár (47° 15′ 15″ N, 16° 56′ 08″ E), which covers an area of about 780 hectares. The nine-hectare botanical garden of Sárvár was established in the city centre, and the area around the city is mainly covered by hornbeam-oak (*Querco robori-Carpinetum*) and Scots pine (*Pinus sylvestris*) forests.
According to the categories of the European Space Agency's WorldCover land cover map (Zanaga et al. 2022), produced by remote sensing in 2021, in 10x10 m spatial resolution. From the total area of the study site, 21% is forested, 23% is shrubland, 10% is grassland, 39% is cropland, 5% is built-up, 1% is bare and <1% is covered by permanent water bodies.
The second study site, the Nagyerdő (Great Forest) of Debrecen is situated north of the city of Debrecen (21.63° N, 47.57° E) in Eastern Hungary. It covers a 1092 ha area where the typical forest communities are oak with lily of the valley (*Convallario - Quercetum roboris*), oak-hornbeam (*Querco robori - Carpinetum*) and open oak forest on sand (*Festuco rupicolae - Quercetum roboris*).

Satellite imagery

For image classification, authors used ESA Sentinel-2 satellite imagery (ESA, 2023). Authors produced composites for the growing season of 2021 from the 10x10m spatial resolution images, taken every 2-5 days, to which we applied ML methods. (Molnár et al. 2019).
The production of cloud-free satellite image composites for ML classification involved several steps (Google, 2023). The process started with the surface reflectance data query from the Sentinel-2 satellite image database, which went through three filters: spatial (downtown of Sárvár), temporal (April-October 2021), and cloud cover (<=5%). This method was used to produce a near-cloud-free composite of the growing season of the study area.
Secondly, the WordCover map was classified with values of 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, and 100, corresponding to the values of the land cover categories (Zanaga et al. 2022). These were tree cover, shrubland, grassland, cropland, and. They were built-up, bare/sparse vegetation, snow and ice, permanent water bodies, herbaceous wetland, mangrove, and finally moss and lichen.
In the third step, random sample points were created, where 1000 teaching points were assigned within the previously defined WorldCover classes. The points were used as teaching and validation data with a ratio of 80-20%. In the fourth step, the four types of ML methods (MD, GB, RF, SVM) were trained, with 100 decision trees for GB and RF methods, Mahalanobis distance for MDE, and C_SVC kernel for SVM.
Fifthly, we performed an accuracy test using a confusion matrix, where we compared the classified values of the ML methods to the WorldCover values at the pixel level. In step six, after setting the display parameters (map centre, approximation, colour scale), the map was displayed. Finally, land cover images were exported, allowing further analysis with desktop GIS programs.

Machine learning

The classification of land cover and within this the delimitation of forested and non-forested areas is an important part of the monitoring, which was carried out using ML. The aim is to

separate built-up areas, forests, meadows, ploughland and water bodies from each other in Sárvár, the first study site. Using each 10x10 m pixel of the sample area, a Machine learning-assisted classification was created, which the program uses to teach itself, thus increasing the accuracy of the results. In the present research, pixel-based supervised classification algorithms were tested, where pixels with unknown affiliations were classified into one of the predefined classes. The algorithms tested were Random Forest (RF), Minimum Distance Estimation (MDE), Support-Vector Machine (SVM) and Gradient Boost Regression (GBR) (Zhang et al. 2019).

In our previous study (Molnár et al. 2021), we used a ML method called Random Forest (Ho 1995), which is based on training data (in our case, points) and decision trees. We assigned manually selected sample points (100 pieces), which were then used to generate seven outputs from the initial 100 decision trees by the program running in the GEE cloud based on their similarity to the given samples. Here, however, the program randomly selected teaching areas within the categories of the WorldCover land cover map for the city of Sárvár in the framework of the new method. Here it was possible to increase the sample number by one order of magnitude, i.e. using 1000 points. The distribution of sample points was random within the WorldCover classes. All thirteen satellite spectral channels were used (B1-B12).

The seven output categories correspond to the ESA WorldCover land cover map categories. The classification was based on the classes of forest, scrub, grassland, cropland, built-up area, barren land and permanent water bodies. For the accuracy of the results, the program compared the classes classified by ML to the WorldCover classes at pixel level.

On the second study site tree species classification was made with the ee.Classifier.smileRandomForest classifier of GEE. A median true colour composite of 2021 was used to apply the RF method for determining dominant tree species: pedunculate oak (*Quercus robur*), black locust (*Robinia pseudoacacia*), Scots pine (*Pinus sylvestris*), and red oak (*Quercus rubra*) and clearcuts. We manually selected 123 training points on the RGB image and used them as training input for the classifier. Every S-2 band was used and 100 decision trees were applied.

Results and discussion

The ML methods of the monitoring system have been used to produce the land cover maps for the city of Sárvár. All four methods (MD, GB, RF, SVM) were successfully used in the sample area, Figure 1 shows the RF-based classified map of Sárvár.

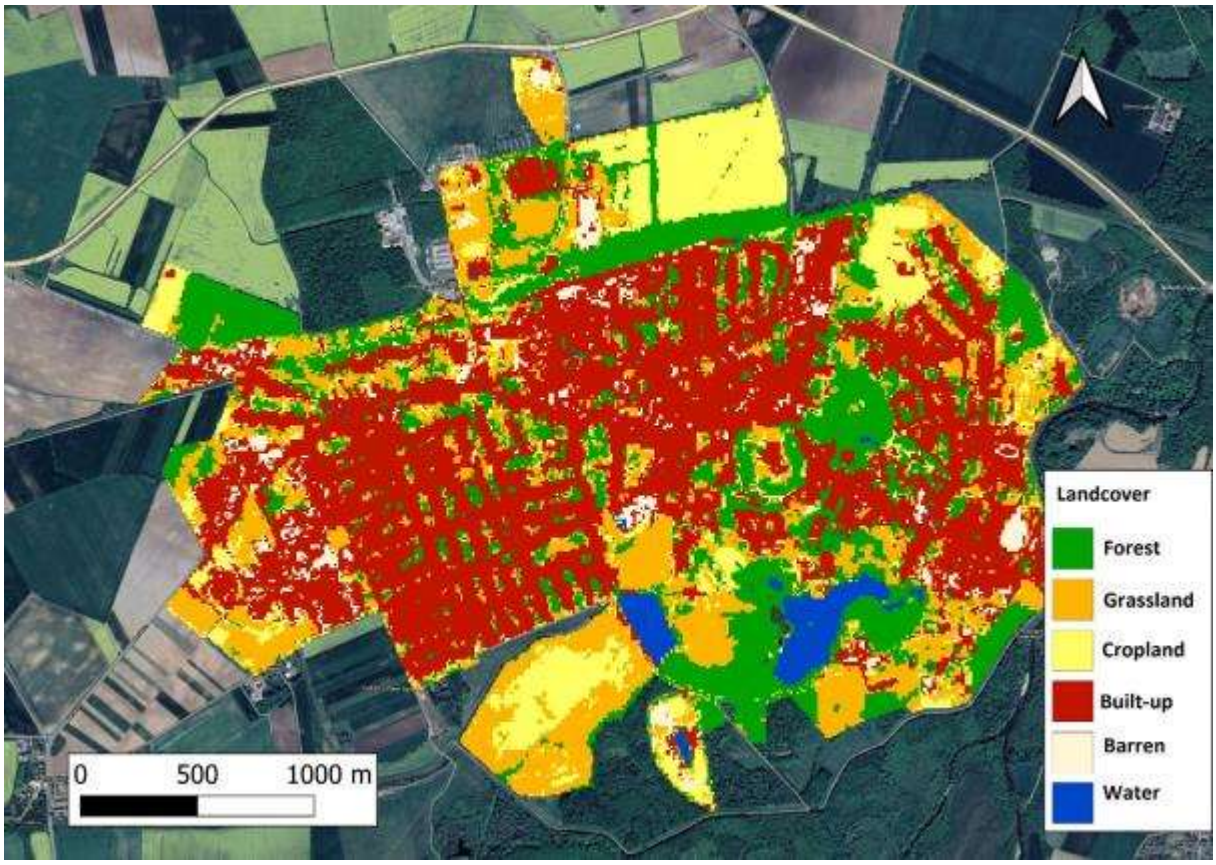

Figure 1. RF-based classified land cover map with six categories for the city of Sárvár.

The land cover classification gave different results with different methods. The result of the classification was a set of colour-coded land cover maps with seven classes (forest, field, grassland, field, or built-up area, barren land, water), from which we calculated statistics for the area percentage of each class in hectares and percentages.

If we take WorldCover as the field truth, i.e. 100% accuracy, then compared to it, RF achieved 80.6%, GB 80%, MD 79.75% and SVM 70.32% accuracy. This can be considered acceptable accuracy because WorldCover itself showed a 76.7% match when compared to the reference, Copernicus Global Cover map.

There were significant differences between the algorithms for different classes of land cover (Figure 2). For us, forest was the most important, in this category almost all algorithms underestimated by ~2-3% compared to the WC data, except MD, which overestimated, but still provided the most accurate data. The built-up area also varied by ~10-15% versus 5% for some algorithms, while cropland varied by up to -10%. The smallest differences were in the water, barren and forest categories. It can be concluded that the method was successfully applied for both land cover and forest classification.

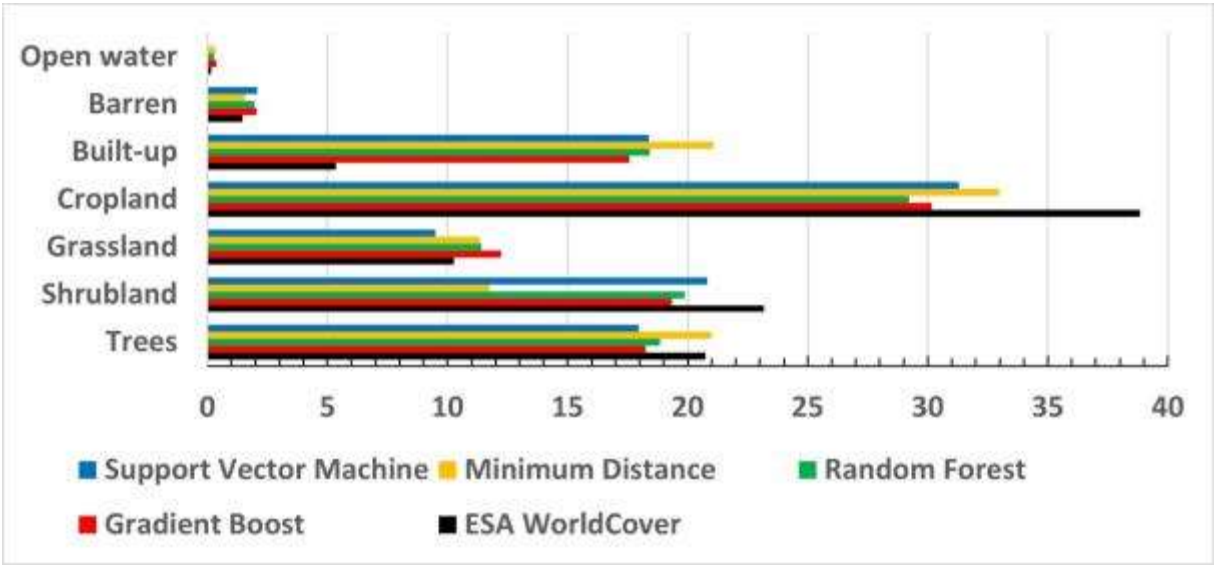

Figure 2: Land cover classes by four Machine Learning algorithms (SVM, MD, RF, GB) for Sárvár.

Regarding the second study site Nagyerdő of Debrecen, RF successfully showed the distribution of dominant tree species within the forest. The majority-based tree species comparison on forest compartment level indicated 76.1% accuracy with five classes, the four dominant tree species plus the clearcut (Figure 3). In 258 compartments we found agreement on the species from the 339 total. It is worth mentioning that we aimed to classify only the dominant tree species and there is an ongoing tree species change in several compartments which resulted in subcompartment-sized clearcuts and lower classification accuracy. Removing the clearcut class resulted in improved, 81.8 % accuracy.

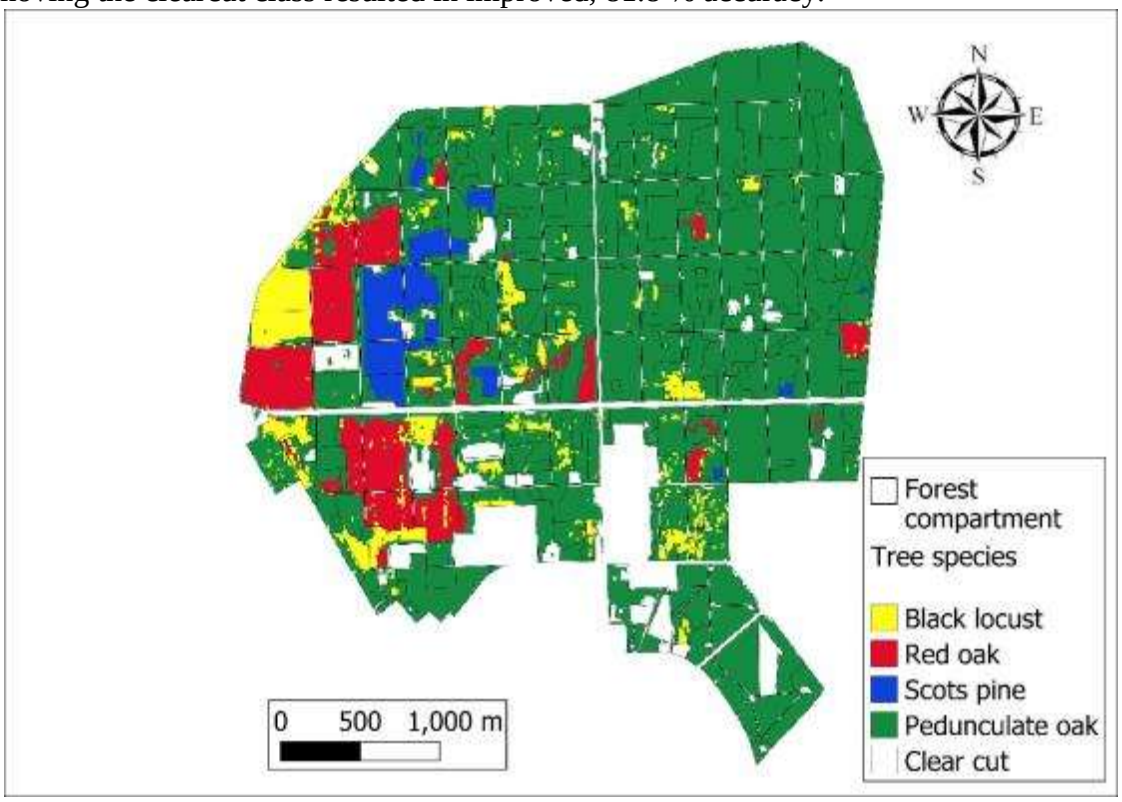

Figure 3. Dominant tree species of Nagyerdő made with Random Forest classifier for the vegetation season composite of Sentinel-2 images in 2021.

It is worth mentioning that due to the computational capacity limitations of GEE, AI support is limited in the number of teaching points and pixels. Furthermore, in the future, it would be advisable to develop the methodology, which would increase the accuracy either by manually selecting the teaching areas or by increasing the resolution of the satellite images used (e.g. Planet 3-4 x 3-4 m).

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TAXONOMY AND PHYTOGEOGRAPHY ANALYSIS OF MEDICINAL PLANTS AT THE AREA OF NATURE MONUMENT "ŠUMA KOŠUTNJAK"

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Abstract

The study was carried out during two consecutive growing seasons (2020-2021) with the aim to record medicinal plants at the area of protected nature monument "Šuma Košutnjak". Based on field research, together with numerous references, there was established presence of total of 147 medicinal plants at the whole researched locality. After the plants were determined, taxonomy and phytogeography analyses of collected material were performed. As for taxonomy, the most distributed families are *Rosaceae* (10.88 %) and *Lamiaceae* (9.52 %), while *Fabaceae* (5.44%) and *Asteraceae* (5.44%) are a bit less present. In the phytogeography analysis participation of different life forms and floral elements was determined. Among life forms the most common are hemicryptophytes (40.14%), then phanerophytes (24.49%) and therophytes (11.56%). The most dominant floral elements are Eurasian (20.41%), submideuropean (17.69%), mideuropean (12.24%) and submediterranean (11.56%). It should be noted that some species, according to destructive human effect, are very endangered due to low rate of their natural regeneration. Therefore it is needed to conduct protective measures in order to conserve gene pool of these species. If we want to obtain reliable information about the number and presence of medicinal plants at this area, it is necessary to perform detailed monitoring which would include several consecutive growing seasons.

Key words: *NM "Šuma Košutnjak", medicinal plants, taxonomy, phytogeography.*

Introduction

The nature monument "Šuma Košutnjak" was declared (Decision on the declaration of the protected area Šuma Košutnjak, decision number 501-2419/14-C-20) as protected due to the significant spatial functions and bioecological value of the complex under the forest vegetation, as well as to preserve the habitat of numerous representatives of mammals, birds, insects and amphibians. This protected area is classified into the third category and two protection regimes have been established within it: the second level protection regime on part of the protected area which includes the pedunculate oak and hornbeam forests near Hajdučka česma with an area of 3.48 ha (1.31%) and the third level protection regime on the remaining part of the protected locality on an area of about 262 ha (98.69%).

Medicinal plants play a crucial role in nutrition and treatment, and the essential oils derived from certain medicinal species are widely utilized in the cosmetic industry (Kišgeci & Adamović, 1994; Tucakov, 2014; Vilotić, 2018; Vilotić, 2021). In terms of the wealth of biodiversity, Serbia is a country that has enormous potential in terms of the number of representatives of flora and fauna, and also applies the international standards contained in the IUCN categorization of protected areas in order to further protect and preserve certain areas (Šijačić-Nikolić & Milovanović , 2010). Floristic research conducted in the area of "Košutnjak Forest" (Šarčević-Todosijević et al., 2018) found out numerous representatives

from the *Rosaceae, Lamiaceae* and *Asteraceae* families that have pronounced medicinal properties. Some earlier research (Ilić et al., 1972) emphasizes the great influence of the anthropogenic factor in this area, which caused a certain succession of vegetation. The above mentioned authors established the presence of a relict shrub species – *Ruscus aculeatus* L., which once grew on the coast of the Pannonian Sea and survived the glacial epoch.
The scope of the paper is, based on taxonomy and phytogeography analysis of the protected area "Šuma Košutnjak", to investigate which families have the most representatives, and to establish the most distributed life forms and floral elements, as well. In addition to this, one of the goals of the research is to draw attention to the state of the gene pool of certain medicinal taxa that are threatened to some extent by anthropogenic influence, as well as to implement adequate conservation measures for their protection.

Material and methods

Floristic research at the whole area of NM "Šuma Košutnjak" was carried out during two consecutive growing seasons (2020-2021). Medicinal plants were determined based on numerous references (Kojić & Vilotić, 2006; Igić et al., 2010; Tucakov, 2014; Vilotić, 2018; Vilotić, 2021; Jokanović, 2021). As for endangered species, they were recorded due to low rate of its' natural regeneration and fragmentary presence just in some parts of the studied area. Life forms classification was conducted due to Raunkier system (1934) which was extended by Mueller-Dombois et. Ellenberg (1974), while Stevanović (1992) did it for taxa in Serbia. Floral elements were determined based on overview of flora species from Serbia with plant-geographic characteristics (Gajić, 1980). Taxonomy features were identified based on many references related to medicinal plants in Serbia (Sarić, 1989; Ranđelović et al., 2002; Tucakov, 2014; Vilotić, 2018).

Results and discussion

An overal monitoring was conducted in the entire area of NM "Suma Kosutnjak", which established the presence of 147 medicinal plants (Table 1). The analysis of proportion of some families by medicinal plants at Kukavica mountain in southeastern Serbia (Jovanović, 2016) showed domination of *Lamiaceae, Asteraceae* and *Rosaceae* families which coincides a lot to our results. Great similarity was found by conducted phytogeography analysis (Jovanović, 2016), as well – the most distributed life form at Kukavica mountain are hemicryptophytes, phanerophytes and therophytes, while among floral elements the greatest proportion was recorded by Eurasian elements which is the same as in our research. Đošić (2016) monitored medicinal plants at Besna Kobila mountain and there is complete similarity with our results in terms of the most distributed families and life forms, however, unlike the area of NM "Šuma Košutnjak", where Eurasian floral element is the most dominant, it is mideuropean floral element on Besna Kobila. Jokanović et al. (2022) investigated medicinal plants at the area of Gornji Srem in the belt of lowland pedunculate oak forests and established that hemicryptophytes are the most represented as life form, while the most numerous floral element is Eurasian, which is completely the same as in our research. As for taxonomy analysis of medicinal plants in the area of Gornji Srem (Jokanović et al., 2022), apart from 4 families which are the most distributed as in our paper, as well (*Fabaceae, Rosaceae, Lamiaceae and Asteraceae*), there is another family (*Poaceae*) with almost 10% proportion.

Table 1: Display of taxonomy and phytogeography plant characteristics (P - phanerophytes; H - hemicryptophytes; G - geophytes; T/H - therophytes/hamephytes; Ch - hamephytes; T - therophytes; S - scandetophytes; H/T - hamephytes/therophytes; DC - woody hamephytes)

Latin name	Family	Life form	Floral element
Acer platanoides L.	Aceraceae	P	Mideuropean
Achillea millefolium L.	Asteraceae	H	Eurasian
Aesculus hippocastanum L.	Sapindaceae	P	Mediterranean-submediterranean
Agrimonia eupatoria L.	Rosaceae	H	Eurasian
Agropyrum repens (L.) Beauv.	Poaceae	G	Cosmopolit
Ajuga reptans L.	Lamiaceae	H	Submideuropean
Allium scorodoprasum L.	Alliaceae	G	Mideuropean
Allium ursinum L.	Alliaceae	G	Boreal
Anemone ranunculoides (L.) Holub	Ranunculaceae	G	Submideuropean
Arctium lappa L.	Asteraceae	T/H	Eurasian
Artemisia vulgaris L.	Asteraceae	H	Circumpolar
Asarum europeum L.	Aristolochiaceae	G	Eurasian
Asperula odorata L.	Rubiaceae	Ch	Subpontic-submeditteranean
Ballota nigra L.	Lamiaceae	H	Subpontic
Bellis perennis L.	Asteraceae	H	Submideuropean
Betula pendula Roth.	Betulaceae	P	Sub-south-siberian
Brassica alba L.	Brassicaceae	T	East-submeditteranean
Brassica nigra L.	Brassicaceae	T	East-submeditteranean
Bromus mollis L.	Poaceae	T	Submeditteranean
Campanula glomerata L.	Campanulaceae	H	Eurasian
Campanula persicifolia L.	Campanulaceae	H	Eurasian
Capsela bursa-pastoris (L.) Med.	Brassicaceae	T	Cosmopolit
Cardamine bulbifera L.	Brassicaceae	T	Subcircumpolar
Carpinus betulus L.	Betulaceae	P	Mideuropean
Chelidonium majus L.	Papaveraceae	H	Eurasian
Cichorium intybus L.	Asteraceae	H	Subeurasian
Cirsium arvense (L.) Scop.	Asteraceae	G	Subeurasian
Clematis vitalba L.	Ranunculaceae	S	Subpontic-submeditteranean
Conium maculatum L.	Apiaceae	H	Mideuropean
Convallaria majalis L.	Asparagaceae	G	Circumpolar
Convolvulus arvensis L.	Convolvulaceae	S	Cosmopolit
Convolvulus cantabrica L.	Convolvulaceae	Ch	Submeditteranean
Cornus mas L.	Cornaceae	P	Pontic-submeditteranean
Cornus sanguinea L.	Cornaceae	P	Submideuropean
Coronilla varia L.	Fabaceae	H	Subpontic
Corylus avellana L.	Betulaceae	P	Submideuropean
Corylus colurna L.	Betulaceae	P	Iranian
Crataegus monogyna Jacq.	Rosaceae	P	Mideuropean
Dactylis glomerata L.	Poaceae	H	Subeurasian
Daucus carota L.	Apiaceae	H/T	Eurasian
Dryopteris filix-mas (L.) Schott.	Dryopteridaceae	H	Cosmopolit
Epilobium parviflorum Schreb.	Oenotheraceae	H	Mideuropean
Euphorbia amygdaloides L.	Euphorbiaceae	T	Subatlantic-submeditteranean
Euphorbia cyparissias L.	Euphorbiaceae	H	Eurasian
Evonymus europaeus L.	Celastraceae	P	Submideuropean
Festuca glauca Vill.	Poaceae	H	Eurasian
Festuca ovina L.	Poaceae	H	Subeurasian
Ficaria verna L.	Ranunculaceae	G	Submideuropean
Fragaria indica L.	Rosaceae	H	Eurasian
Fragaria vesca L.	Rosaceae	H	Eurasian
Fraxinus excelsior L.	Oleaceae	P	Submideuropean
Fraxinus ornus L.	Oleaceae	P	Submeditteranean
Fumaria officinalis L.	Papaveraceae	T	Submideuropean
Galium aparine L.	Rubiaceae	T	Eurasian
Galium sylvaticum L.	Rubiaceae	G	Mideuropean
Galium verum L.	Rubiaceae	H	Eurasian
Geranium dissectum L.	Geraniaceae	T	Eurasian
Geranium robertianum L.	Geraniaceae	T	Subcircumpolar
Geum urbanum L.	Rosaceae	H	Eurasian
Glechoma hirsuta Waldst. et Kit.	Lamiaceae	H	Pontic-east-submeditteranean
Hedera helix L.	Araliaceae	S	Subatlantic-submeditteranean
Helleborus odorus (L.) W.K.	Ranunculaceae	G	Mideuropean
Hordeum murinum L.	Poaceae	T	Submeditteranean
Hypericum hirsutum L.	Hypericaceae	H	Subeurasian
Hypericum perforatum L.	Hypericaceae	H	Mideuropean
Hyssopus officinalis L.	Lamiaceae	H	Submeditteranean
Iris variegata L.	Iridaceae	G	Pontic-submeditteranean
Juglans regia L.	Juglandaceae	P	Mideuropean
Lamium galeobdolon (L.) Crantz	Lamiaceae	Ch	Submideuropean
Lamium maculatum L.	Lamiaceae	H	Submideuropean
Lamium purpureum L.	Lamiaceae	T	Submideuropean
Lapsana communis L.	Asteracaea	T	Submideuropean
Leonorus cardiaca L.	Lamiaceae	H	Eurasian
Ligustrum vulgare L.	Oleaceae	P	Mideuropean

Lolium perene L.	Poaceae	H	Submideuropean
Lonicera caprifolium L.	Caprifoliaceae	P	Mideuropean
Lysimachia vulgaris L.	Primulaceae	H	Eurasian
Malva sylvestris L.	Malvaceae	H	Subeuropean
Medicago sativa L.	Fabaceae	H	Subpontic
Mentha longifolia (L.) Huds.	Lamiaceae	H	Submideuropean
Oxalis acetosella L.	Oxalidaceae	H	Circumpolar
Oxalis europea L.	Oxalidaceae	G	Circumpolar
Paeonia officinalis L.	Paeoniaceae	G	Eurasian
Papaver rhoeas L.	Papaveraceae	T	Mideuropean
Plantago lanceolata L.	Plantaginaceae	H	Eurasian
Plantago major L.	Plantaginaceae	H/T	Eurasian
Plantago media L.	Plantaginaceae	H	Eurasian
Polygonum aviculare L.	Polygonaceae	T	Cosmopolit
Potentilla erecta (L.) Raeusch.	Rosaceae	H	Eurasian
Potentilla micrantha Ramond ex DC	Rosaceae	H	Submeditteranean
Primula vulgaris Huds.	Primulaceae	H	Subeurasian
Prunella vulgaris L.	Lamiaceae	H	Subeurasian
Prunus avium L.	Rosaceae	P	European-westasian
Prunus cerasifera Ehrh.	Rosaceae	P	Sub-southsiberian
Prunus padus L.	Rosaceae	P	Eurasian
Prunus spinosa L.	Rosaceae	P	Sub-south-siberian
Pteridium aquilinum (L.) Kuhn	Pteridaceae	G	Cosmopolit
Pulmonaria officinalis L.	Boraginaceae	H	Submideuropean
Pyrus piraster L.	Rosaceae	P	Mideuropean
Quercus cerris L.	Fagaceae	P	East-submeditteranean
Quercus farnetto Ten.	Fagaceae	P	Submeditteranean
Quercus petraea (Matt.) Liebl	Fagaceae	P	Mideuropean
Quercus pubescens Wild.	Fagaceae	P	Submeditteranean
Quercus robur L.	Fagaceae	P	Submideuropean
Quercus rubra L.	Fagaceae	P	Submeditteranean
Ranunculus repens L.	Ranunculaceae	H	Pontic-submeditteranean
Robinia pseudoacacia L.	Fabaceae	P	Adventive
Rosa arvensis Huds	Rosaceae	P	Submeditteranean
Rosa canina L.	Rosaceae	P	Eurasian
Rubus hirtus L.	Rosaceae	P	Mideuropean
Rumex acetosella L.	Polygonaceae	H	Subcircumpolar
Rumex obtusatum L.	Polygonaceae	H	Submideuropean
Ruscus aculeatus L.	Asparagaceae	G	Eurasian
Ruscus hypoglossum L.	Asparagaceae	G	Submeditteranean
Salvia pratensis L.	Lamiaceae	H	Submideuropean
Salvia viridis L.	Lamiaceae	H	Submeditteranean
Sambucus ebulus L.	Adoxaceae	G	Submeditteranean
Sambucus nigra L.	Adoxaceae	P	Submideuropean
Scila bifolia L.	Asparagaceae	G	Submeditteranean
Sedum album L.	Crassulaceae	Ch	Submideuropean
Silene vulgaris (Moench.) Garcke	Caryophyllaceae	H	Subeurasian
Solanum dulcamara L.	Solanaceae	T	Subeurasian
Sorbus torminalis (L.) Crantz.	Rosaceae	P	Submeditteranean
Stachys sylvatica L.	Lamiaceae	H	Submideuropean
Stellaria holostea (L.) Scop.	Caryophyllaceae	H	Submideuropean
Stellaria media (L.) Scop.	Caryophyllaceae	H	Cosmopolit
Symphytum officinale L.	Boraginaceae	T/H	Submeditteranean-pontic
Tamus communis L.	Dioscoreaceae	S	Meditteranean-submeditteranean
Taraxacum officinale Web.	Asteraceae	H	Eurasian
Teucrium chamaedrys L.	Lamiaceae	Ch	Mideuropean
Tilia platyphyllos Scop.	Tiliaceae	P	Mideuropean
Trifolium alpestre L.	Fabaceae	H	Subpontic
Trifolium campestre Schreber.	Fabaceae	T	Submideuropean
Trifolium pratense L.	Fabaceae	H	Subeurasian
Trifolium repens L.	Fabaceae	H	Subeurasian
Ulmus minor Mill.	Ulmaceae	P	Pontic-european
Urtica dioica L.	Urticaceae	T/H	Eurasian
Verbascum phlomoides L.	Scrophulariaceae	H	Continental-meditteranean
Verbena officinalis L.	Verbenaceae	H	Eurasian
Veronica chamaedrys L.	Scrophulariaceae	H	Submideuropean
Veronica persica Poir.	Scrophulariaceae	Ch	Subboreal-circumpolar
Viburnum lantana L.	Adoxaceae	P	Submeditteranean
Vicia cracca L.	Fabaceae	H	Subeurasian
Viola alba L.	Violaceae	H	Submediterannean
Viola tricolor L.	Violaceae	T/H	Eurasian
Vinca minor L.	Apocynaceae	DC	Submideuropean
Vitis vinifera L.	Vitaceae	P	Subeuxian

Conclusions

Based on the field study during two consecutive growing seasons (2020-2021) at the complete area of NM "Šuma Košutnjak" 147 medicinal plants were found. The taxonomy analysis results showed that families *Rosaceae* (10.88%) and *Lamiaceae* (9.52%) are most presented, then *Fabaceae* and *Asteraceae* should be mentioned with the same distribution (5.44%), and finally *Poaceae* (4.76%) and *Fagaceae* (4.08%) that also have a bit more significant participation. As for life forms, the most dominant are hemicryptophytes (40.14%), then phanerophytes (24.49%), therophytes (11.56%) and geophytes (8.16%). Among floral elements the most distributed are Eurasian (20.41%) and submideuropean (17.69%), then mideuropean (12.24%) and submediterranean (11.56%). Almost 80% from all recorded medicinal plants are herbaceous, while the other belong to shrubs and trees. Some individuals such as *Campanula persicifolia* L., *Hypericum hirsutum* L., *Paeonia officinalis* L. and *Ruscus hypoglossum* L. are due to anthropogenic factor very endangered, because the possibility of their natural regeneration is pretty reduced and, as a result, they are located on very small areas. For that reason is very important, not only to conduct conservation measures and protection of mentioned taxa, but also to explain visitors how they should behave in the urban forest ecosystem like this.

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PREVIEW OF MEDICINAL PLANTS WITHIN LOWLAND PEDUNCULATE OAK FORESTS AND THEIR USEFUL PROPERTIES

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Abstract

The study related to medicinal plants was carried out at the area of Gornji Srem within three management units: MU „Blata-Malovanci" (forest type *Carpino-Fraxino-Quercetum roboris caricetosum remotae*), MU „Raškovica-Smogvica" (forest type *Fraxino-Quercetum roboris aceretosum*) and MU „Vinična-Žeravinac Puk" (forest type *Carpino-Fraxino-Quercetum roboris caricetosum remotae*). At the area of Gornji Srem in the middle of 30's last century protective embankment was built which eliminated flooding influence. As a result, vegetation is supplied with water mainly from groundwater and precipitation. There were recorded 139 medicinal plants that originate from 43 families. The aim of the paper is to determine useful properties of medicinal plants. The analysis of mentioned features of medicinal plants was conducted according to generally accepted methodology (Ranđelović et al., 2002) that all medicinal herbal drugs classified into 14 groups. Based on obtained results, the largest number of medicinal plants act on the digestive organs and cardiovascular system, and then follow drugs that treat problems with the skin, excretory and respiratory organs. Only one herb belongs to the category of antihistamines, while phytoncide herbs, galactose and drugs for improving the flavour and taste of various medicines have two representatives each. It should be noted that certain medicinal taxa have a wide range of useful properties and simultaneously treat several different health problems.

Key words: *Gornji Srem, useful properties, lowland pedunculate oak forests, phytotherapy.*

Introduction

Medicinal plants have always been considered an extremely important natural resource and humans have used them extensively, both to satisfy their needs for food and to treat numerous health problems (Jokanović et al., 2020 a, b; Jokanović et al., 2021; Jokanović, 2021). Based on the Law on Nature Protection, the Rulebook on the proclamation and protection of strictly protected and protected wild species of plants, animals and mushrooms (Official Bulletin of the Republic of Serbia, No 5/2010) was adopted, which recognizes two basic categories of protection of wild species of plants, animals and mushrooms: the first category (strictly protected) and the second category (protected wild species). Species that belong to the first category are either those that have completely disappeared from the territory of the Republic of Serbia or are extremely endangered, relict, local endemic and very significant for preservation of biodiversity of the Republic of Serbia (Igić et al., 2010).
Protected wild species are currently not in danger of disappearing or becoming critically endangered, but they are of particular importance from an ecological, ecosystem, biogeographical, scientific, health and economic aspect (Igić et al., 2010). Serbia is characterized by extremely rich biodiversity which, among other things, includes 3.562 plant

species and subspecies, of which 606 belong to strictly protected and 535 to protected taxa, which is a total of 1.141 of about 1/3 of the total number of vascular flora taxa (Vilotić, 2018; Vilotić, 2021).

It should be noted that forest ecosystems at the area of Ravni Srem are characterized by a large number and diversity of vascular flora, which also includes many medicinal species (Jokanović et al., 2017; Jokanović et al., 2018; Jokanović et al., 2022). Based on a detailed taxonomic and phytogeographical analysis of medicinal species in the area of lowland pedunculate oak forests in Gornji Srem (Jokanović et al., 2022) the presence of 139 medicinal plants which belong to 43 families, and include 6 life forms and 16 areal types was determined. In general, medicinal plants also have significant phytoremediation properties (Stanković et al., 2015 a; Stanković et al., 2015 b; Stanković & Jokanović, 2017) in terms of soil, water and air cleaning, and this is another important reason why the gene pool of wild species should be preserved.

The scope of the paper is to establish useful properties of recorded medicinal plants at the area of Gornji Srem in order to establish what health problems these plants can treat. One of specific goals is also to emphasize the importance of phytotherapy as a preventive treatment method.

Material and methods

Medicinal taxa within lowland pedunculate oak forests at protected area of Gornji Srem were determined based both on numerous references (Kojić & Vilotić, 2006; Vilotić, 2018; Vilotić, 2021) and detailed field study (Jokanović et al., 2022). People's greatest interest when it comes to medicinal plants is the way they are used in practice. Systematic classification of medicinal species from lowland pedunculate oak forests in Gornji Srem was carried out on the basis of their effects on human health. Based on the modern classification principle according to medicinal plants effects on humans (Ranđelović et al., 2002), medicinal herbal drugs are divided into 14 groups:

1. drugs that affect the cardiovascular system;
2. drugs that affect the digestive organs;
3. drugs that affect the respiratory organs;
4. drugs for the treatment of the central and peripheral nervous system;
5. drugs that act on the autonomic nervous system;
6. drugs for the treatment of the urogenital tract;
7. drugs for the treatment of skin deseases;
8. drugs for regulating the circulation of substances;
9. antidiabetics;
10. antihistamines;
11. phytoncidal plants;
12. galactagogues;
13. tonics;
14. drugs for repairing the smell and taste of various medicines

Results with discussion

At the area of lowland pedunculate oak forests in Gornji Srem (Jokanović et al., 2022) 139 medicinal plants were recorded: *Achillea millefolium* L., *Adonis vernalis* L., *Agrimonia eupatoria* L., *Agropyrum repens* L., *Agrostis alba* L., *Ajuga reptans* L., *Alliaria officinalis* M.Bieb., *Althea officinalis* L., *Anchusa officinalis* L., *Angelica archangelica* L., *Aristolochia clematitis* L., *Artemisia vulgaris* L., *Arum maculatum* L., *Asclepias syriaca* L., *Ballota nigra* L., *Bellis perennis* L., *Betula pendula* Roth., *Brassica nigra* L., *Bromus commutatus* Schrad., *Bromus inermis* Leyss., *Bromus molis* L., *Bromus racemosus* L., *Bromus sterilis*, *Calamintha officinalis* L., *Capsela bursa-pastoris* L., *Cardamine pratensis* L., *Centaurea jacea* L., *Centaurium umbellatum* Gilib., *Chelidonium majus* L., *Cichorium intybus* L., *Colutea arborescens* L., *Colchicum autumnale* L., *Conium maculatum* L., *Convallaria majalis* L., *Convolvulus arvensis* L., *Cornus mas* L., *Cornus sanguinea* L., *Coronilla varia* L., *Corylus avellana* L., *Crataegus monogyna* Jacq., *Crataegus oxyacantha* (Poir.) DC., *Dactylis glomerata* L., *Datura stramonium* L., *Daucus carota* L., *Eupatorium cannabinum* L., *Euphorbia cyparissias* L., *Festuca pratensis* Huds., *Filipendula hexapetala* Moench., *Fragaria vesca* L., *Fumaria officinalis* L., *Galanthus nivalis* L., *Galega officinalis* L., *Geranium robertianum* L., *Geum urbanum* L., *Hedera helix* L., *Helleborus odorus* Waldst & Kit., *Hordeum murinum* L., *Humulus lupulus* L., *Hyoscyamus niger* L., *Hypericum perforatum* L., *Hypericum hirsutum* L., *Inula helenium* L., *Juglans regia* L., *Lamium purpureum* L., *Lathyrus tuberosus* L., *Leonorus cardiaca* L., *Lithospermum purpurocaeruleum* L., *Lolium perene* L., *Lonicera caprifolium* L., *Loranthus europaeus* Jacq., *Lotus corniculatus* L., *Malva sylvestris* L., *Marrubium vulgare* L., *Melissa officinalis* L., *Mentha longifolia* L., *Matricaria chamomilla* L., *Medicago lupulina* L., *Medicago sativa* L., *Morus alba* L., *Morus nigra* L., *Ononis spinosa* L., *Origanum vulgare* L., *Quercus cerris* L., *Quercus robur* L., *Papaver rhoeas* L., *Plantago lanceolata* L., *Plantago major* L., *Plantago media* L., *Platanthera bifolia* L.C.Rich., *Poa pratensis* L., *Poa trivialis* L., *Polygonum aviculare* L., *Populus alba* L., *Populus tremula* L., *Potentilla palustris* L., *Potentilla reptans* L., *Prunus avium* L., *Primula acaulis* L., *Prunella vulgaris* L., *Prunus spinosa* L., *Pulmonaria officinalis* L., *Pulsatilla vulgaris* Mill.,. *Ranunculus repens* L., *Rosa canina* L., *Rubus hirtus* L., *Rumex acetosella* L., *Ruscus aculeatus* L., *Sambucus nigra* L., *Sanguisorba minor* Scop., *Stachys sylvatica* L., *Stellaria nemorum* L., *Salix alba* L., *Saponaria officinalis* L., *Solanum dulcamara* L., *Symphytum officinale* L., *Tamus communis* L., *Tanacetum vulgare* L., *Taraxacum officinale* Web., *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Tilia tomentosa* L., *Trifolium alpestre* L., *Trifolium arvense* L., *Trifolium pratense* L., *Trifolium repens* L., *Tussilago farfara* L., *Valeriana officinalis* L., *Vicia cracca* L., *Vicia dumetorum* L., *Vicia pannonica* Crantz., *Vicia sativa* L., *Veronica officinalis* L., *Viola alba* Besser, *Viola tricolor* L., *Urtica dioica* L., *Viburnum opulus* L., *Vinca minor* L., *Verbascum phlomoides* L., *Verbena officinalis* L.

I : Drugs that affect the cardiovascular system:

Helleborus odorus, Urtica dioica, Crataegus monogyna, Crataegus oxyacantha, Leonorus cardiaca, Convalaria majalis, Hypericum perforatum, Hypericum hirsutum, Sambucus nigra, Capsela bursa-pastoris, Rosa canina, Prunella vulgaris, Daucus carota, Achillea millefolium, Fragaria vesca, Rumex acetosella, Malva sylvestris, Potentilla palustris, Potentilla reptans, Geum urbanum, Rubus hirtus, Chelidonium majus, Artemisia vulgaris, Taraxacum officinale, Tilia cordata, Tilia platyphyllos, Tilia tomentosa, Symphytum officinale, Vinca minor, Juglans regia, Prunus avium.

II Drugs that affect the digestive organs:
Sambucus nigra, Artemisia vulgaris, Taraxacum officinale, Geum urbanum, Potentilla palustris, Potentilla reptans, Ajuga reptans, Achillea millefolium, Agropyrum repens, Verbascum phlomoides, Rosa canina, Capsela bursa-pastoris, Prunella vulgaris, Quercus cerris, Cornus mas, Cornus sanguinea, Chelidonium majus, Daucus carota, Agrimonia eupatoria, Brassica nigra, Centaurium ubellatum, Cichorium intybus, Filipendula hexapetala, Fragaria vesca, Matricaria chamomilla, Melissa officinalis, Origanum vulgare, Rumex acetosella, Symphytum officinale, Urtica dioica, Corylus avellana, Juglans regia, Quercus robur, Rubus hirtus, Salix alba, Centaurea jacea.
III Drugs that affect the respiratory organs:
Hypericum perforatum, Malva sylvestris, Quercus cerris, Corylus avellana, Sambucus nigra, Verbascum phlomoides, Viola tricolor, Viola alba, Plantago major, Plantago lanceolata, Plantago media, Crataegus monogyna, Crataegus oxyacantha, Papaver rhoeas, Bellis perennis, Primula acaulis, Althea officinalis, Matricaria chamomilla, Origanum vulgare, Polygonum aviculare, Tussilago farfara, Populus tremula, Populus alba, Brassica nigra, Pulmonaria officinalis, Cardamine pratensis, Prunus spinosa.
IV Drugs for the treatment of the central and peripheral nervous system:
Chelidonium majus, Hypericum perforatum, Hypericum hirsutum, Salix alba, Veronica officinalis, Solanum dulcamara, Ranunculus repens, Pulsatila vulgaris.
V Drugs that act on the autonomic nervous system:
Chelidonium majus, Melissa officinalis, Hyoscyamus niger.
VI Drugs for the treatment of the urogenital tract:
Daucus carota, Taraxacum officinale, Capsela bursa-pastoris, Prunus avium, Sambucus nigra, Pulmonaria officinalis, Agropyrum repens, Urtica dioica, Artemisia vulgaris, Populus tremula, Populus alba, Betula pendula, Viola tricolor, Viola alba, Vinca minor, Polygonum aviculare, Ononis spinosa, Origanum vulgare, Matricaria chamomilla, Leonorus cardiaca, Fragaria vesca, Filipendula hexapetala, Cichorium intybus, Centaurea jacea, Agrimonia eupatoria, Achillea millefolium, Solanum dulcamara, Ruscus aculeatus, Arum maculatum.
VII Drugs for the treatment of skin deseases:
Corylus avellana, Juglans regia, Salix alba, Filipendula hexapetala, Fragaria vesca, Urtica dioica, Hedera helix, Pulmonaria officinalis, Solanum dulcamara, Veronica officinalis, Origanum vulgare, Malva silvestris, Tussilago farfara, Plantago major, Plantago lanceolata, Plantago media, Achillea millefolium, Sambucus nigra, Helleborus odorus, Morus nigra, Morus alba, Betula pendula, Tilia cordata, Tilia platyphyllos, Tilia tomentosa, Eupatorium cannabinum, Hyoscyamus niger, Rubus hirtus, Ballota nigra, Tamus communis.
VIII Drugs for regulating the circulation of substances:
Arum maculatum, Ranunculus repens, Urtica dioica, Rumex acetosella, Populus alba, Populus tremula, Polygonum aviculare, Sambucus nigra, Colchicum autumnale, Solanum dulcamara, Viola tricolor, Viola alba.
IX Antidiabetics:
Galega officinalis, Daucus carota, Achillea millefolium, Urtica dioica.
X Antihistamines:
Hyoscyamus niger.
XI Phytoncidal plants:
Betula pendula, Colchicum autumnale.

XII Galactagogues:
Daucus carota, Galega officinalis.
XIII Tonics:
Filipendula hexapetala, Rosa canina, Achillea millefolium, Centaurium umbellatum, Urtica dioica, Ononis spinosa, Verbena officinalis, Taraxacum officinale, Geranium robertianum.
XIV Drugs for repairing the smell and taste of various medicines:
Mellisa officinalis, Centaurea jacea.

Based on the obtained results, the largest number belongs to drugs which affect digestive organs, cardiovascular system, skin diseases, urogenital tract and respiratory organs. This completely coincides with results of similar studies (Jovanović, 2016; Đošić, 2016). Analysis of useful properties of medicinal plants from the mountain Kukavica (Jovanović, 2016) shows that drugs which act on skin diseases, urogenital tract, respiratory and digestive organs are the most distributed, while there are also many plants which affect the cardiovascular system, central and peripheral nervous system, and regulated circulation of substances in organisms. Đošić (2016) investigated useful properties of medicinal plants at the Besna Kobila mountain and concluded that the most represented are drugs which affect the cardiovascular system, then these which act on central and peripheral nervous system, while drugs which can be used for problems of urogenital, respiratory and digestive organs are much less are distributed. Similar results were obtained by analysis conducted among medicinal plants in the area of Vlasina (Ranđelović et al., 2002) and within nature monument „Šuma Košutnjak" (Jokanović et al., 2021).

Conclusions

At studied area of lowland pedunculate oak forests in Gornji Srem a total of 139 medicinal plants were found. Based on conducted analysis, according to valid, generally accepted methodology (Ranđelović et al., 2002), we found that the most plants affect digestive organs (36), then cardiovasular system (31), skin diseases (30), urogenital tract (29) and respiratory organs (27). After these five numerous groups, there are also drugs which regulate substances circulation (12) and these which treat central and peripheral nervous system (8) represented with many individuals. Bearing in mind the significance of medicinal plants using in phytotherapy, before all for prevention, this valuable natural resource needs to be planted and protected.

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SEASONAL DYNAMICS OF INSECT COMMUNITIES AND THREE MAJOR PESTS IN FRUŠKA GORA NATIONAL PARK (SERBIA)

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Abstract

Insects are highly responsive to changing environmental conditions, particularly climate, which can significantly impact their species composition and abundance. By studying the seasonal dynamics of insect communities, we can contribute to understanding their population status and ecosystem health, adapting pest management measures, and promoting conservation efforts. This study aimed to investigate the temporal variation in insect community composition, the relationship between insect families and meteorological parameters, and the variation in species abundance of the three most abundant pests; *Oulema melanopus* (L.), (Coleoptera: Chrysomelidae), *Melanotus punctolineatus* (Pelerin), (Coleoptera: Elateridae), and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Insects were collected using Malaise traps, while meteorological parameters were measured by an automated meteorological field station in the forest of the Fruška gora National Park, Serbia. A total of 124 insect species, 114 genera, and 78 families were identified. The highest number of species was recorded in June (35.5%), followed by May (34.7%), and August (31.5%). The lowest number of species was recorded in September (17%), and April (8%). Species richness increased with temperature, with peaks in May and June, followed by July and August. No significant correlation was found between species richness and air humidity. The highest abundance of insect pests was recorded for *Oulema melanopus*, *Melanotus punctolineatus*, and *Harmonia axyridis*, with the most individuals recorded in July. Forests may possibly serve as overwintering sites for common insect pests, providing them with alternative food sources.

Keywords: *Insect community, Insect pests, Seasonal variation, Species richness, Meteorological parameters.*

Introduction

Insect communities, including pest communities, their abundance, and population dynamics are influenced by environmental and biological factors and their interaction (Danks, 1999; Schoonhoven *et al*., 2005). Many authors have confirmed the positive correlation between temperature increase and insect species richness (Régnière *et al*., 2012). In addition, the temperature rise is associated with an increase in the development rate of agricultural pests, increasing also their survival rates and dispersal potential (Dukes *et al*., 2009; Kocmánková *et al*., 2009; Prakash *et al*., 2014). Changes in precipitation frequency (Kobori and Amano, 2003), UV radiation (Villena *et al*., 2018), and air humidity (Jaworski and Hilszczański, 2013) have been shown to influence insect survival rates. It is of great importance to study insect communities under varying environmental conditions. It can improve our knowledge of ecological factors that determine their composition and abundance, which is particularly important in the era of climate change (Körner, 2007; Sundqvist *et al*., 2013; Morris *et al*., 2015). It is certainly difficult to predict the future pace of climate change and its impact on ecosystems and species, yet, it is essential to conduct regular monitoring of as many ecosystem components or ecological guilds as possible, particularly insects as informative

indicators of habitat quality and climate change (Colares *et al*., 2021). This study is part of the regular insect monitoring conducted in Fruška gora National Park in Serbia.

Material and methods

The study was conducted in 2022, from April to September at the long-term monitoring site in Fruška gora in Serbia (45°9'26.48"N, 19°48'36.95"E) in a deciduous forest with linden and beech as the main ediphicators, individual conifer trees, and regularly maintained meadow in the immediate vicinity. Insects were collected by using Malaise traps (ground and canopy dwelling) (Fig.1). Air temperature (average daily maximum), humidity, precipitation, wind speed, illumination, and UV radiation were measured using the AgroSense automatic meteorological weather station, which collects daily meteorological data and transmits them to the server via a GPRS network. The station is placed at a height of 196 cm. Collected insect material was determined down to the species level.

Figure 1. Collecting insects from Malaise traps (A), field-based meteorological station (B)

The number of insect species was plotted against temporal scale and meteorological parameters.

The relationship between insect families and meteorological parameters was examined by RDA analysis performed in Canoco 5 (Lepš and Šmilauer, 2003). Redundancy analysis (RDA) was chosen by first employing Detrended correspondence analysis (DCA) and examining the gradients of compositional change. The gradient length was less than 3, implying that the linear method is suitable for the analysis of our data set. Collinearity between variables was checked by examining the inflation factor (VIF factor). All variables yielded a VIF factor below five, excluding their mutual intercorrelation. Variation in species abundance of the three most abundant pests was represented by a funnel plot showing levels of abundance with progressively decreasing or increasing proportions.

Results and Discussion

A detailed examination of insect material collected from Malaise traps during the six months of this study resulted in the determination of 124 species, 114 genera, 78 families, and 11 orders. A bar chart comparing species richness in different months (Fig. 2) shows the highest number of insect species in June (35.5%), followed by May (34.7%), and August (31.5%).

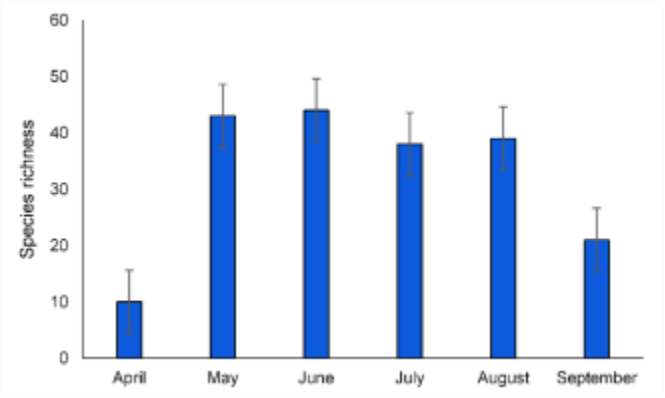

Figure 2. Species richness variation from April to September 2022

Species richness increased as temperatures went higher, with peaks in May and June, followed by July and August (Fig. 3).

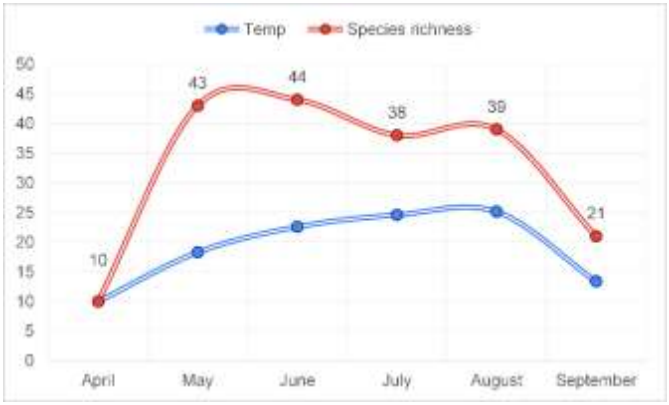

Figure 3. Changes in species richness in relation to air temperature

Temperature is the most important factor affecting primary production, and although there are conflicting results regarding the relationship between species richness and productivity (Waide *et al*., 1999; Dodson *et al.*, 2000; Mittelbach *et al.*, 2003; Brown *et al*., 2004), most authors agree that there is a positive relationship between the two, i.e. higher productivity is associated with high species richness. We did not find a significant relationship between species richness and air humidity, wind speed, and illumination.

Looking into insect pests, the highest numbers of individuals were recorded for three Coleoptera species: *Oulema melanopus* (L.) (Chrysomelidae), *Melanotus punctolineatus* (Pelerin) (Elateridae), and *Harmonia axyridis* (Pallas) (Coccinellidae) (Fig. 4), with most individuals occurring in June and July (Fig. 5).

Figure 4. The three most abundant insect pests: *Melanotus punctolineatus* (A), *Oulema melanopus* (B), and *Harmonia axyridis* (C) collected at Fruška gora forest site in 2022

Considering the linear distance of only 1.7km between our sampling site at Fruška gora and the nearest agricultural site, the forest area possibly acts as an overwintering site for common insect pests, providing them with alternative feeding as well.

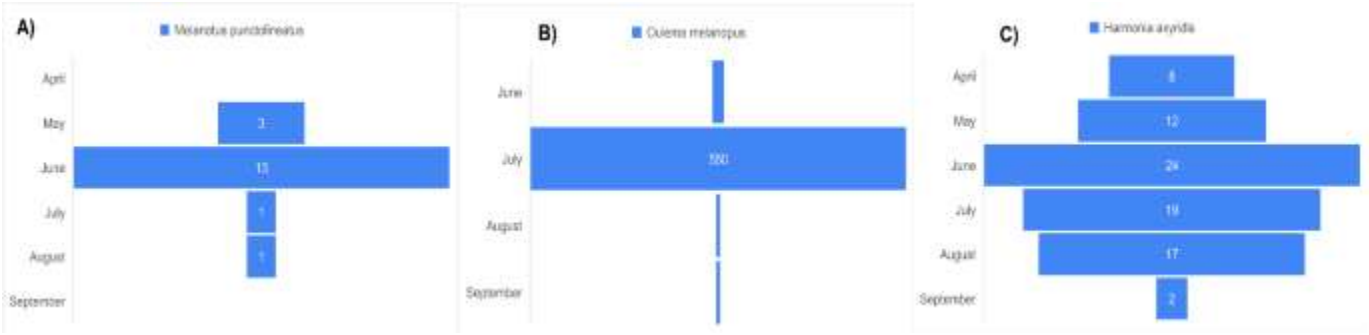

Figure 5. Variation in species abundance of the three most abundant pests *Melanotus punctolineatus* (A), *Oulema melanopus* (B), and *Harmonia axyridis* (C) collected at Fruška gora forest site in 2022

The RDA analysis, performed to examine the relationship between the dataset consisting of insect families and the meteorological parameters, showed a statistically significant correlation (cut-off value <0.05), explaining 75.2% of the variability (Fig. 6). The most influential meteorological variables, affecting the composition and abundance of the recorded insect families are: UV radiation, temperature, and precipitation.

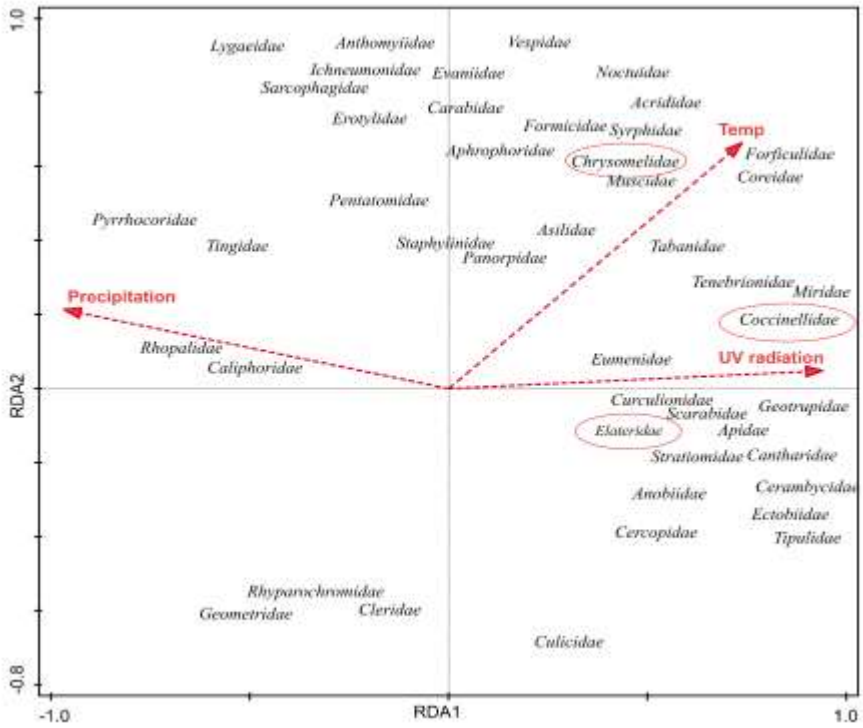

Figure 6. Redundancy analysis (RDA) ordination plot of major meteorological factors and insect families sampled in 2022. The most abundant pest species collected in traps belong to the following families: Elateridae, Coccinelidae, and Chrysomelidae (circled in red). For clarity, a certain number of families was omitted from the graph.

UV radiation and temperature appear to be the most influential meteorological variables affecting the composition and abundance of the families Elateridae, Coccinelidae, and Chrysomelidae, which include the most abundant pest species in our study. UV radiation negatively affects the development time of insect species (Villena *et al*., 2018) and leads to a decline in insect populations (Milanović *et al*., 2023). The contrasting results of our study could be due to the specificity of the research area, i.e., the ability of forests to reduce UV radiation in their immediate environment, particularly under trees and in the shade of the ground (Ryeol Na *et al*., 2014). Our results confirm that the number of insect pests increases with the increase in temperature. Increased precipitation increased the abundance of insects from the families Pyrrhocoridae, Rhopalidae, Caliphoridae, and Tingidae. This could be explained by the impact of precipitation mainly on plant communities, aboveground biomass, and species richness of annual plants (Yan et al., 2015), and the resulting changes in microclimatic conditions (Kamata and Igarashi, 1994), which in turn may have affected the abundance of the above insect families. Continuation of the monitoring program of insect communities, including insect pests, in Fruška gora, is of great importance for deepening the knowledge of insect response to the ever-changing environment and further climate change.

Conclusions

Our study shows temporal variability in the species richness of insect communities, including important insect pests. We found that temperature is one of the most important controlling factors for insect species richness and abundance. High abundance of insect pests: *Oulema melanopus*, *Melanotus punctolineatus*, and *Harmonia axyridis*, at forest sites means that they are forced to leave cultivated areas and seek alternative shelter and overwintering sites, and possibly alternative food sources. Temperature, UV radiation, and precipitation were found to

be the most important factors affecting the distribution and abundance of insect families in the Fruška gora sampling site. Further observations are needed to better assess the response of insect communities to climate change and the changing environment.

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FLORISTIC AND ECOLOGICAL ANALYSIS OF BLACK PINE FORESTS (*Pinus nigra* Arn.) ON WESTERN BALKAN SERPENTINITES

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Abstract

Black pine forests of the Western Balkans constitute a significant complex of azonally and orographically–edaphycally conditioned forests on the ophiolitic massifs in Bosnia and Herzegovina (B&H), Serbia, North Macedonia and Albania. They mainly exist on series of soils on serpentinites - from eutric humus-siliceous to brown soil and pseudogley. In terms of floristic composition, these forests exhibit great similarities, but also significant differences. Forests of black pine on the territory of B&H, which belongs to Illyrian floral province, grow in humid conditions with higher precipitation compared to other countries in the region, which results in larger numbers of mesophillic species. Black pine forests in Serbia belong to transitional Illyrian-Moesian and Moesian province and grow in continental climate with lower precipitation, which tends to further decline towards the East, hence the black pine forests in Central Serbia are more xerophillic with larger presence of sub-Mediterranean species. North Macedonia has even lower precipitation and more prominent influence of Moesian province and Aegean Sea, which results in species from those areas being dominant, while Illyrian species are absent. Black pine forests in Albania belong to the Mediterranean climate, however, in the mountain zone some climatic parameters tend to become more continental. From the ecological point of view, the Albanian black pine communities are in an intermediate position between the typical Mediterranean litoral pines and European or continental ones. Within the natural distribution range, species composition of black pine forests on serpentinite strongly reflects a west-east biogeographical division related to climatic differences.

Key words: *Pinus nigra, serpentinite, floristic composition, Western Balkan.*

Introduction

The Balkan Peninsula used to be one of the most important refugial areas of Europe, because it had a warmer climate due to its geographical position, so glaciation was not fully expressed. This is especially true in the western parts of the peninsula, where during glaciation temperature changes were relatively small (Bennett *et al.,* 1991). The Balkan Peninsula is characterized by a dissected relief (sheltered warm valleys, southern mountain slopes, hills), which made it possible to retain the plant species that have disappeared in other parts of Europe in this area, and due to the unfavorable geographical direction of mountain massifs, the possibility of returning to their original habitats during the interglacial periods was hindered (Janković, 1984; Janković, 1990; Perović, 2013). In this region, considerable areas are occupied by populations of pine trees, primarily black and white pine trees, which represent tertiary relics and include several subspecies and varieties (Soto *et al.*, 2010).
The vegetation cover on serpentinite represents one segment of the flora and vegetation of the Balkan Peninsula (Figure 1), which is extremely rich in plant species, among which there are a lot of endemics. Serpentinites have a high percentage of MgO (32-38%), SiO_2 (35-40%) and heavy metals (Ni, Zn, Mn) (Carmignano *et al.*, 2019). Soils formed on ultramafites, which include serpentinite, may contain several hundred times more nickel (Ni), an element that is

toxic to plants, than other soils (Altinözlü *et al.*, 2012). Soils on serpentinites have an unfavorable chemical composition, shallow, rocky, often formed on steep slopes, so they are unsuitable for most plants and represent a stressful environment for their growth (Kazakou *et al*., 2008).

Figure 1. Distribution of serpentinite on the Balkan Peninsula (Tatić and Veljović, 1992)

On the Balkan Peninsula, significant areas on the serpentine parent rock are occupied by black pine populations. Black pine (*Pinus nigra* Arn.) has scattered but widespread distribution across mountain regions of the Mediterranean basin (Figure 2), ranging from Spain to Turkey, where it has often been planted to restore degraded sites (Nagel and Cerioni, 2023). Black pine forests on the Balkan Peninsula grow on different parent rocks. They make up a significant complex of azonal and orographically-edaphically conditioned forests, especially on the ophiolithic massifs of central and eastern Bosnia, as well as western and to a certain extent central Serbia. Many authors consider these forests to be relict mainly because of the edificator role of black pine (*Pinus nigra* subsp. *gocensis* Vid.), which with the disjunct ranges of certain subspecies is a typical tertiary relict (Tatić and Tomić, 2006). Black pine forests on serpentinite are very dynamic systems, and their development flows in two directions: at lower altitudes towards sessile oak forest and at higher altitudes towards the forest of beech and fir (Jović and Tomić, 1985).

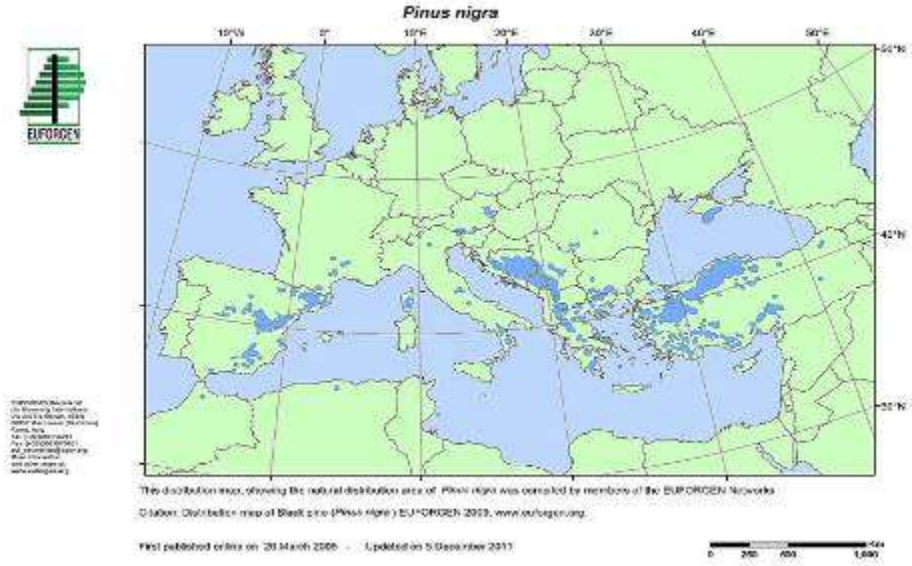

Figure 2. Distribution map of Black pine (*Pinus nigra*) EUFORGEN 2009, www.euforgen.org

In the Balkans, serpentinites are found mainly in the western part of the peninsula (Stevanović et al., 2003): in Albania, Bulgaria, Serbia, B&H, Macedonia, Greece.

Syntaxonomic affiliation of black pine forests

Braun-Blanquet and Oberdorfer (Novaković-Vuković and Eremija, 2020) initially classified all pine forests as *Vaccinio-Piceetea,* which was only logical when acidophilic pine forests were concerned (Zupančič, 2007). On the other hand, if we take a look at the alliance of *Orno-Ericion*, it can be noted that it does not have elements of *Vaccinio-Piceetea* at all, but contains a number of elements of the order *Quercetalia pubescentis*, which, according to Horvat (1959), led Kraft (1955) in his tentative attempt to classify European forests to include the order *Pinetalia* in the *Quercetea pubescentis* class. Considering the ecological and floristic similarity of basiphilous xerothermal pine and oak forests, Tomažić and Horvat originally joined the pine forests of Slovenia and the Velebit sector to the *Quercetea pubescentis* order (Bucalo, 1994). In his later research, Horvat (1959) did not fully accept this syntaxonomy, so he placed all the basiphilous pine forests in the Balkans, which belonged to the *Pineto-Ericion* alliance from the *Vaccinio-Piceetalia* order and the alliance *Orneto-Ericion* of the *Quercetalia pubescentis* order, in the *Erico-Pinetalia* order and the *Erico-Pinetea* class.
However, some authors, including Zupančič (2007), believe that the biggest problem when it comes to the systematics of pine forests is that they do not have their own characteristic species. The author thinks that the class of *Erico-Pinetea* and the *Erico-Pinetalia* order consist of species that often, if not regularly, appear in associations belonging to the *Querco-Fagetea* and *Vaccinio-Piceetea* classes, so that pine forests should be classified, i.e. distributed between these two classes. The author further states that pine forests have relatively characteristic species, that is, species that very frequently occur in pine forests, but are also common in oak, beech, thermophilic broadleaved and other phytocenoses.
The alliance Dinaric forest of black pine with heath (*Orno-Ericion* Ht 1958) was identified within the *Erico-Pinetalia* order Ht. 1959, which encompasses the forests of black and white pine on a wide area from Slovenia to Albania and Macedonia. Based on the parent rock, it is divided into two suballiances*: Orno-Ericenion dolomiticum* on dolomites *and Erico-Pinenion gocensis* (Krause *et* Ludwig 57) Tomić 2004 (Syn. *Orno-Ericenion serpentinicum* Krause *et*

Ludwig (1957), which is widespread on peridotites, serpentinised peridotites and serpentinite. Given the disagreements when it comes to the synsystematic affiliation of pine forests, a new classification is under development, because past classifications of *Pinus nigra* forests lack a formal, broad-scale approach, which hinders a better understanding of these communities (Bonari *et al*., 2022).

Floristic characteristics of black pine forests on serpentinite

The serpentine massif in the territory of B&H is dissected, and with interruptions it stretches from the northwest to the southeast. The largest areas are occupied by basiphilous forests of black pine and sessile oak, as a permanent stage, because due to the edaphic conditions, the development of vegetation towards beech forest communities is hindered. That is why there is diverse vegetation on these surfaces, as a result of the alternation of climatically and edaphically conditioned plant communities. One of the first identified communities is the black pine forest *Seslerio serbicae-Pinetum* Rit. 1970 in the area of the Krivaja watershed (central B&H) and eastern B&H (Varda-Rudo), with a characteristic composition of flora - predominantly serpentinophyte species, which are related to extremely warm black pine habitats, at an altitude of 300 to 800 m (Riter-Studnička, 1963).

The analysis of the floristic composition of black pine forests on serpentinite in central and eastern B&H (Novaković-Vuković *et al.*, 2019), at altitudes between 293 and 590 m, belonging to the Illyrian province, showed a greater share of mesophilous species than in the pine forest in Serbia. Within the B&H releves, species of a wide ecological amplitude were identified: *Pteridium aquilinum, Festuca rupicola, Lotus corniculatus, Fragaria vesca, Melica nutans,* etc., as well as somewhat more mesophilous species: *Symphytum tuberosum, Quercus petraea, Rubus hirtus, Galium rotundifolium, Cardamine glauca,* etc. The presence of mesophilous species and the ones of a wider ecological amplitude in black pine forests in the area of B&H speaks of somewhat more mesophilous conditions within these forests in the Illyrian province. In the B&H area, a typical Illyrian species of *Epimedium alpinum* has been recorded, and its occurrence decreases from west to east. *Calluna vulgaris,* a species belonging to the Atlantic areal type, has been recorded in central B&H, and its presence is expectedly high in western and northern Europe, and reducing when heading to the East. In the territory of former Yugoslavia, it is the most common in Slovenia and Croatia, slightly less common in B&H, while in the territory of Serbia it is recorded in only four locations (Cvjetićanin *et al*., 2014). Stands of black pine can mostly be found on two types of soil: eutric humus siliceous and brown soil on serpentinite, but the stands in which *Calluna vulgaris* occurs have been recorded on pseudogley (Blagojević and Govedar, 2009), which is a rarity for the serpentine parent rock. The Illyrian province is characterized by a significantly higher amount of precipitation, compared to the Moesian province, so it is considered more humid. On the other hand, the climate of the Moesian province is more continental: average differences in temperature between summer and winter are more extreme, yearly amounts of precipitation are lower, and summer heats are followed by the lowest amounts of water residues. Generally speaking, Moesian vegetation, is much more xero-termophilous, compared to the Illyrian vegetation and notably more resistant to high summer temperatures and drought (Jankovic, 1984).

In Serbia, black pine forests on serpentinite belong to the Moesian and transitional Illyrian-Moesian provinces, where they occupy a wide range of altitudes, from 400-1400 m, mostly on shallow and skeletal soils, in a series from rankers to pseudogley on serpentinite, which is very rare. The slopes are also different, and these forests can also occur on plateaus, but they are most commonly found on very steep slopes. They are located in all exposures, but in the territory of Serbia they more often occupy sheltered exposures (N, E, NW, NE). These forests

are less often of primary character, and they are mostly degraded due to the effects of the zooantropogenic factor. Progressive succession flows are present, where pine trees, as a pioneering species, occur in habitats of other species, primarily the Balkan sessile oak and beech-fir sites. Black pine forests on serpentinite in Serbia are dominated by species which are typical of the class *Erico-Pinetea* and the *Erico-Pinetalia* order, and immediately after them the most common are species typical of the order *Quercetalia pubescentis*. In these forests there is a certain number of plants that are closely related to serpentinites, as well as species of a wider ecological amplitude: *Erica carnea, Erythronium dens canis, Pteridium aquilinum, Vaccinium myrtillus, Sesleria serbica, Daphne blagayana, Potentilla heptaphylla, Stachys scardica, Festuca amethystina, Potentilla alba, Crocus veluchensis, Galium lucidum* and many others. In the spectrum of areal types, the dominating one is the Central European areal type (Novaković, 2008; Novaković-Vuković, 2015). The special significance of these forests is reflected in the presence of endemic species, primarily *Potentilla visiani, Euphorbia glabriflora, Stachys recta ssp. baldacii, Sesleria serbica, Hypericum barbatum, Allysum markgrafii, Crocus veluchensis, Thymus jankae* and others.

The floristic composition of pine forests on serpentinite in central Serbia is quite different compared to the western regions. Pine trees live in harsher conditions there. The slopes are generally steeper than in other localities. The soils are more skeletal and shallow, and the exposures are warmer, mostly western and southwestern. In their floristic composition, compared to other sites, they contain the most sub-Mediterranean species, as well as chamaephytes, because these species are able to withstand harsh living conditions. This community has 20% xerophytes (*Dorycnium germanicum, Euphorbia glabriflora, Carex humilis, Calamintha acinos, Euphorbia glabriflora, Carex humilis, Calamintha acinos,* etc.), which corresponds to soil quality class IV of the stand on Kopaonik (Tomanić, 1970). "Moving south from the center of development in western Serbia, pine forests are changing their appearance, composition and structure, they are weaker in vitality and their floristic composition is poorer in terms of the most important species of pine forests. White pine disappears, and black pine dominates" (Pavlović, 1964). Some species that are typical of basiophillous pine forests are disappearing or their presence is significantly reduced, and new ones that have not been recorded in Western Serbia appear. First of all, we should mention heath (*Erica carnea*), which has a much lower number and cover than in other parts of Serbia. Heath has Illyrian geographical distribution, so the climatic conditions of Kopaonik (central Serbia) are not suitable for it. *Sesleria serbica,* one of the typical species of pine forests, has not been recorded on Mt. Kopaonik. A regular companion of these forests is red juniper (*Juniperus oxycedrus*), a subxerophilous species of the sub-Mediterranean areal type, which was not recorded in the research (Novaković-Vuković, 2015) on Tara, Šargan, Pešter and Zlatibor (western Serbia), and is very often common on dolomites, which is another evidence of xerophility of the investigated stands on Mt. Kopaonik. The occurrence of this species is correlated with the climatic impacts of the Mediterranean in certain parts of Mt. Kopaonik. *Epimedium alpinum* was not recorded within the *communities Erico-Pinetum gocensis* and *Pinetum sylvestris-nigrae* on Mt.Kopaonik, while in all other sites this species was recorded, which is another significant difference. Pavlović (1964) listed this species as a permanent companion to the oak and beech forests of the Ibar massif. *Epimedium alpinum* is an Illyrian species, whose presence is increasing in the westward direction, so the subassociation *Erico-Pinetum nigrae epimedietosum alpinae* (Bojadžić, 1969) was recognized in the territory of Bosnia within the black pine forest. In general, the pine forests of central Serbia are poorer in terms of the share of Illyrian species, which is expected, since they are located on the eastern border of the Illyrian floral area.

Although the observed stands in Serbia and B&H are located at different elevations, the distribution of pine forest types is not primarily determined by elevation, but rather by relief, exposure, slope, character of the soil, as well as exposure to winds (Pavlović, 1951).

Pine forests of Macedonia show a lot of specificity compared to other forests of the Western Balkans, because there is black pine in the middle of its southern European areal, where sub-Mediterranean vegetation follows river flows, so these forests have a significant number of sub-Mediterranean species in their floristic composition. Black pine in Macedonia is one of the most important coniferous species, and is found on dolomite limestones, dolomites and serpentinite. In Macedonia sparse stands of black pine on serpentinite are located on the mountain Mariovo-Nidže, (Mandžukovski, 2006), as well as on the massive Kožuf, at the transition from oak to beech belt, at heights of 1000-1200 m, on shallow, rocky brown forest soil of poorly acidic reaction (Em, 1978). *Fraxinus ornus, Sorbus aria, Quercus pubescens, Quercus frainetto, Quercus petraea,* and *Fagus moesiaca* have been recorded in the tree layer. The author states that the ground layer is dominated by the abundance of the species *Sesleria latifolia* and *Moehringia muscosa*, probably as remnants of the pine stage replaced by beech over time. *Lathyrus pannonicus* var. *versicolor, Potentilla rupestris* var. *mollis, Genista carinalis, Tulipa silvestris* are also present in the ground floor. A number of important Illyrian species are absent in the Macedonian pine forests, while species of the Balkan (Moesian) and Aegean regions occur. Climate differences are also significant. Compared to the Dinaric region, the amount of precipitation here in the site of black pine forests is small, and the precipitation regime has a pronounced minimum in summer, which greatly deviates from the precipitation regime within the range of the *Orno-Ericion* alliance (Em, 1978). When of primary character, pine forests of Macedonia, are characterized by low growth and their role is mainly protective. However, when most often after a fire, pine occupies the area of climatogenic forests, it often reaches its maximum growth potentials in the newly formed secondary communities, so its economic importance in this area is high.

In the territory of Albania, black pine forests occupy large areas at altitudes of 300-1850 m, and the optimum is between 800-1500 m. They occur on different parent rocks - dolomite, serpentinite and limestone, and on different soils. They grow mainly in pure stands, but they can also grow in mixed ones, especially with beech and oak. In the oak zone, black pine is mixed with *Quercus robur, Quercus frainetto,* and at the upper border with *Abies alba, Pinus peuce, Pinus heldreichii* (Dida *et al*., 2001). In the territory of Albania, black pine builds communities with *Buxus sempervirens, Erica herbacea, Euphorbia spinosa, Quercus coccifera, Stahaelina unifloscula*... In Albania, the *Pinus nigra-Forsythia europaea* community is also very common, which is of great importance, as *Forsythia europaea* is an endemic species in Albania.

From the phytoclimatic point of view, black pine forests in Albania belong to the mediterranean climate, but in the mountain zone some climatic parameters tend to become more continental (Hoda, 1993). From the ecological point of view the Albanian black pine communities take an intermediate position between the typical mediterranean litoral pines (*Pinus halepensis, Pinus pinea*) and European or continental ones (*Pinus sylvestris, Pinus mugo*).

Black pine forests show significant differences in floristic composition within their range, primarily because they occur in different climatic conditions and on different parent rocks. Within the natural distribution range, species composition strongly reflects a west-east biogeographical division related to climatic differences (Bonari *et al*., 2022).

Conclusions

This paper presents floristic as well as ecological characteristics of black pine forests on serpentinite, which occur mainly in in the Western Balkans area, in B&H, Serbia, Albania and North Macedonia. They have a lot of similarities throughout their range, which are above all conditioned by the specific parent rock. Considering that they grow in different climatic conditions, altitudes, soils, it is certain that these forests can also be distinguished by some peculiarities in different localities.

In the western part of its range, i.e. in the B&H area which belongs to the Illyrian flora-geographical province, black pine forests on serpentinite have an increased number of mesophilous species, due to humid climatic conditions of the Illyrian Province. In Serbia, where they are located in the transitional Illyrian-Moesian and Moesian provinces, the xerophility of these forests increases towards the East, so that they contain a lot of xerophilous species in central Serbia, as well as species of the sub-Mediterranean areal type. In the territory of North Macedonia, the amount of precipitation is significantly lower compared to the above mentioned areas, so these forests have a lot of typical species of the the Moesian and Aegean areas. In the territory of Albania, black pine forests grow under a strong influence of the sub-Mediterranean, but at higher altitudes they have the features of continental pine forests.

Generally speaking, within the natural distribution range of black pine forests the species composition on serpentinite strongly reflects a west-east biogeographical division related to climatic differences.

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SYSTEM MONITORING OF THE CROWN CONDITION ON ICP FORESTS SAMPLE PLOTS LEVEL I IN SERBIA WITH A SPECIAL VIEW OF BIOTIC DAMAGE ON PLOTS WITH OAK AS THE EDIFIER

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Abstract

Monitoring of plant diseases and pests represents the key element of the environmental policy, without which the standards for forests and the environment cannot be applied. Data processing and reporting inevitably follow the uniform methodologies designed in accordance with international standards. The main objective of monitoring the crown condition is provision of a periodic insight into spatial and time variations of the forest condition, relative to anthropogenic and natural stress factors in the European and national systematic networks of wide-range observation. The paper demonstrates that the database in which sample plots Level I data are collected may be used in segments, which enables the search according to tree species, diseases, pests, periods, etc. The data from the base related to specific tree species and population density of major diseases and pests, as well as other types of damage of mechanical and abiotic origin (within certain periods - time series), open broad possibilities in practical application of the research. The paper presents monitoring of biotic damage agents, some of which are quite dangerous and significant, while the most common are harmful insects – 13.7% total on all deciduous trees. Among the oak species the most endangered is Sessile oak (with 31.9% damage). The damages caused by fungi rank second and are most frequently present on coniferous trees (11.0%), whereas among the deciduous species Sessile oak is once again the most endangered (6.9%). Damages caused by anthropogenic and abiotic agents – fire, frost, local pollution, etc., are far less significant.

Key words: *sample plot, forest condition, biotic damage*

Introduction

The condition of forests, ecosystems and biodiversity have a strong mutual connection. The levels of biodiversity largely depend on the health and vitality of forests, while loss of biodiversity leads to reduced productivity and sustainability of forests (Marin et al., 2021).
The system of information on forest health condition is a result of specific activities within the framework of sustainable forest management, which is the basis of numerous international and national policies. Monitoring of plant diseases and pests represents the key element of the environmental policy, without which the standards for forests and the environment cannot be applied. Data processing and reporting inevitably follow the uniform methodologies designed in accordance with international standards. Results of the monitoring must be easily accessible and support the system of ecological indicators. Studies and monitoring are carried out by scientific institutions that prepare the database, assessments, and analyses on the national level.
The initially created database on the condition of forest trees with millions of pieces of information, which due to their subjectivity and poor specificity of international data made it difficult to compare and interpret the data (Horntvedt, 1993; Innes, 1992). Different studies used a range of various indices that are relevant only for specific species only. However, the

development of pertinent indices created an urgent need for improvement of the definitions, training, data collection and reporting (Ferretti, 1998).
The Republic of Serbia has been involved in the ICP program for forests through its National Focal Center for monitoring of the condition of forests (NFC), which in cooperation with the National Expert Group (NEG) conducts data analyses and interpretation of results, assists in scientific management of the Program, and takes part in international expert panels (IEP) and working groups (WG).
The main objective of monitoring the crown condition is provision of a periodic insight into spatial and time variations of the forest condition, relative to anthropogenic and natural stress factors in the European and national systematic networks of wide-range observation. The exposure to stress or constant attacks by insects or diseases makes forest ecosystems more susceptible to outbreaks of epiphytotic diseases (Wulff, 2011), which necessitates monitoring of the occurrence and spread of harmful biotic factors.

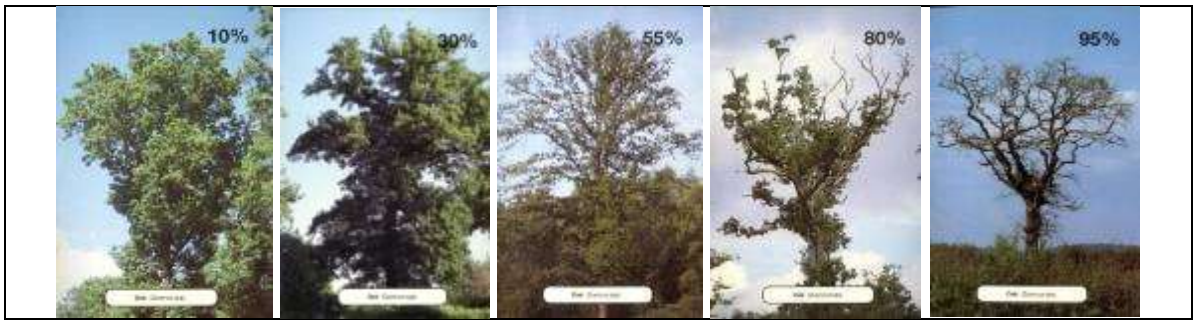
Materials and methods

The Manual *Visual Assessment of Crown Condition* and in the *Submanual on Visual Assessment of Crown Condition on Intensive Monitoring Plots* (Eichhorn et al., 2010) has been redesigned to enable harmonization of the data and a more flexible approach to monitoring of the crown condition, with improved and more transparent quality. All the parameters described in the latest version of the Manual have been tested in multiple countries across Europe or North America, while the values of the parameters are being continuously followed under the control of international expert panels. Any required adjustment will be recommended at the annual meetings of *ICP Forests Task Forces* in the coming years.
All the marked trees on the spot points are numbered clockwise, starting from the geographical north, and each tree is identified taxonomically. Damages observed in the field are noted in 5% intervals (de Lourdes Saavedra-Romero et al., 2021). Given that roughly one-fifth of the defoliation may be attributed to abiotic or biotic damages (Nevalainen et al., 2010), the assessment of chlorosis (decolorization, bleaching, i.e. color change of the leaf mass) and defoliation (branch dieback) is performed on the marked trees every year during the vegetative period. The strength of chlorosis and dieback is marked in percentages ranging from 0 to 100. In addition, injuries according to types and kinds of causal agents are noted in the manuals and marked with codes.
Besides the code, the elements noted for biotic agents of damage include Latin names of the agents of damage, development phase of the harmful agent, description of the attacked part of the plant, age of the attacked needles, etc.

Tree assessment method in practice

- Trees are assessed from multiple directions, if possible (two directions minimum).
- The distance between the assessor and the tree should be at least one height of the assessed tree.
- On a sloped terrain, the assessor should be positioned on or above the tree level.
- To exclude blinding and ensure the most relevant assessment possible, the assessor should avoid looking at the sun while conducting the assessment.

Visual determination of the percentage category of the defoliation is presented in Picture 1.



Picture 1. The visual determination of the categories of the defoliation of trees

Visual assessments of the defoliation of trees are subjective, which frequently brought the consistency of the assessment, which is the most commonly used indicator of the tree condition, into the focus of scientific critique (Haruki et al., 2011). Despite high correlations and discrepancies between the assessors, there is a possibility of occurrence of a systematic error (Eickenscheidt and Wellbrock, 2013). In order to reduce the probability of error to a minimum, efforts are made to insure that the same team visits the same locations each year to conduct the visual determination of the crown condition.

Results and discussion

On the territory of Serbia in the year 2022, the total number of all Level I sample plots amounted to 130, out of which 117 in the central Serbia and 13 in Vojvodina. The total number of points with oak as the dominant species was 69. Out of that number, there were 48 points with Turkey oak (*Quercus cerris* L.), 38 points with Hungarian oak (*Quercus frainetto* Ten.), 17 points with Sessile oak (*Quercus petraea* Matt. Liebl.), 7 points with pedunculate oak (*Quercus robur* L.), and 4 points with pubescent oak (*Quercus pubescens* Willd.).
Among the deciduous species, oak are the most widely present species on sample plots – Sessile oak, Turkey oak and Hungarian oak, all including stands of varied ages. Oak are the main species on the total of 40.9% of points (Turkey oak on 22.0%, Hungarian oak on 11.0%, and Sessile oak on 7.9%). Beech is second with presence on 31.5% of points. In the group of conifers, the most widely present species is spruce (main species on 7.9% of points), followed by ash (3.9%), white pine and fir (3.1% each).
Amongst the insects causing damage to the oak leaves, the early defoliators (Geometriadae) and leafrollers (Torticidae) appear most frequently. On sample plots dominated by Sessile and pedunculate oak, damages from attack ranging from very weak to moderate were detected, mostly by winter moth (*Operophtera brumata* L.) and mottled umber moth *Erannis defoliaria* L., then caterpillar *Alsophila* sp., (Lepidoptera: Geometridae) and lackey moth (picture 2).

Picture 2. *Malacosoma neustria* L., Sample plot Kursumlija

This year, the attack by these pests was noted as mostly weak, and moderate only on sample plot 47. Among the defoliators the Gypsy moth litters is dominant, and most damage is

usually sustained by Turkey oak forests and individual Hungarian oaks. This year it occurred individually, in the egg stage. Litters were found in August on only one sample plot in Negotin area. The majority of litters on older trees were damaged by birds. The trees were found to have sustained a moderate attack by oak leafrollers (Totricidae), with most frequent occurrences of green oak moth (*Tortix viridiana* L.), *Totrticoides alternella* Hbn., early oak leafroller and *Archips xylosteana* L. variegated golden tortrix, and the damage represented by characteristically half-rolled up leaves, most often of sessile oak. Dieback of sessile oak tops and new young shoots is also present as a consequence of the activity of a range of pests – first of early defoliators (Geometridae) and leafrollers (torticidae), followed by a chain action by leaf miners, e.g. representatives of the family (Lepidoptera, Ticheridae) with the species *Ticheria ekedlabella* Bjerk., a very common agent of damage to oak crowns across the country and on a large number of sample plots where it was found in mass, as well as *Profenusa pigmeae* (Klug, 1816) (picture), just as this year skeletal damage caused by *Caliroa annulipes* (Klug, 1816), oak slug sawfly, was frequently seen on oaks on sample plot in Forest Management Office Kraljevo.

Caterpillars of lackey moth *Malacosoma neustria* L. in higher stages of development appeared individually in the area of Forest Management Office Toplica, sample plot in Kursumlija area in early summer 2022. Gall wasps *Cynipidae* did not appear frequently this year, and the registered species included only the common ones: *Cynips quercusfolii* L., *Biorhiza pallida* (Ol.), *Andricus quercustozae* (Bosc), *Andricus caputmedusae* (Htg.). Among the galls, gall midges family Cecidomyiidae were present, but their attack intensity was weak.

Gall midges from the family Cecidomidae, specifically species *Dryomia circinnans* Girauld, frequently appear on the underside of the leaves on sample plots, as does *Janetia cerris* – these galls are similar in appearance, covered in fine hair-like setae, and are equally damaging as they cause physiological weakening of the leaves and stunt the growth of young plants due to general deformity.

The introduced oak lace bug *Corythucha arcuata* Say is present on multiple sample plots with oak, but still mainly in the vicinity of roads.

Altica quercetorum Foudr. is common on top shoots of oak trees, with characteristically deformed and finely formed leaves which are drying out, or have sustained partial or total damage. Visible pitted bite marks are evident as a consequence of the pest's action on the leaf tissue.

Dieback of sessile oak tree tops is also present as a consequence of a range of harmful factors, in addition to those mentioned above, and it may date back to damage accumulated from previous years, with the condition steadily worsening with each passing year. Snout moths *Acrobis tumidella* Zin. (Lepidoptera, Pyralidae) bite the leaves and then paste a number of leaves together on the top of the shoots. These damages were also detected on sample plots dominated by sessile oak in the year 2022.

Synantedon conopiformis Esper. (Lepidoptera, Aegeridae=Sesiidae) creates calloused creases resembling lumps or tumors in root flares of trees, sessile oak in particular, in shoot stands (sample plot Leskovac). Damage by great capricorn beetle *Cerambyx cerdo* was registered on individual felled trees.

Sporocarps of epyxilous fungi such as *Fistulina hepatica*, Schaeff appeared more rarely.

Bacterial tumor-like growths on trunks may reach great scales, but they appear individually and on unnurtured stands. On Hungarian oak trees, the presence of sporocarp of wood-decaying fungi *Coriolus versicolor* (Fr.) Pil., *Fomes fomentarius* was noted, as was the presence of brown central rot, which mostly appears on previously damaged trees. During the monitoring of the crown condition on sample plots I in 2022, several species of pathogenic fungi were found on the leaves of mature oak trees and on the regeneration growth, among

which powdery mildew *Microsphaera alphitoides* Grif.& Maubl was the most widely present (sample plot Ub).
Among other biotic agents of damage, the presence of hemiparasites was registered on individual oak branches – common mistletoe (*Viscum album* L.) and yellow mistletoe (*Loranthus europaeus* Jacq.), which cause physiological degradation of trees and make them susceptible to attack of dangerous pests and wood decay agents (sample plots Petrovac na Mlavi, Zagubica and Vrnjacka Banja).
As for abiotic factors, on sessile oak the most frequent are frost injuries on the tree bark, as well as heat damage of the leaf mass on a smaller scale. Mechanical damage to oak tree trunks, registered on roughly a dozen tested trees, was inflicted through the activity of the anthropogenic factor, i.e. during tree felling and skidding. These injuries are potentially dangerous as they may be the access point for numerous harmful insects and wood decaying fungi.
Table 1 presents the percentage of damage according to causal agents that were determined during field visits to sample plots I on trees and crowns of the marked trees.

Table 1. Damage according to causal agents determined during a field visit to sample plot I on trees and crowns of marked trees in the year 2022

	Damage							
	From insects	From fungi	From abiotic agents	From man	From forest fires	From local pollution	Other	Total
For all species %	13.1	5.0	1.5	0.4	0.0	0.0	0.2	20.2
For broad-leaved species %	13.7	3.2	0.4	0.6	0.0	0.0	0.2	18.1
For conifers %	4.7	11.0	0.0	0.0	0.0	0.0	0.0	15.7
For Turkey oak %	21.4	2.5	0.2	0.0	0.0	0.0	0.2	24.3
For Hungarian oak %	12.9	1.1	0.5	0.0	0.0	0.0	0.0	14.6
For sessile oak %	31.9	6.9	3.1	0.0	0.0	0.0	0.6	42.5

As presented in Table 1, in the year 2022 the highest percent of damage on trees on sample plots I was caused by insects, found on the total of 13.7% of broad-leaved trees. Among the oak species, the most endangered is sessile oak (with 31.9% damage). Damage caused by fungi ranked second and was most frequently present on conifers (11.0%), while among deciduous species sessile oak was once again at the highest risk (6.9%). Damage from anthropogenic and abiotic agents - fire, frost, local pollution, etc., was far less significant.

Conclusions

In the year 2022, on sample plots I the presence of several types of biotic agents of damage was registered, some of which are highly dangerous and significant, with the most common ones being harmful insects and wood-decaying fungi. In addition, there were bacterial tumor-like growths that may reach great scales on trunks, but they appear individually and on unnurtured stands. Among the abiotic factors, on Hungarian oak the frost injuries on the trunk were present on a smaller scale, whereas leaf damage from hail was striking. Mechanical damage to tree trunks of all oak species registered in 2022 was inflicted through the activity of the anthropogenic factor (tree felling and skidding), and represent potential danger and the access point for numerous harmful insects and wood decaying fungi. As presented in the paper, the database in which data from sample plots level I are collected may be used in segments, which enables search according to tree species, diseases, pests, periods, etc. The data from the base related to specific tree species and population density of major diseases

and pests, as well as other types of damage of mechanical and abiotic origin (within certain time periods), open broad possibilities in practical application of the research.

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EDAPHIC CHARACTERISTICS OF THE PARK IN THE ŠARENGRAD AREA IN NOVI SAD IN SERBIA

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Abstract

The paper investigated the soil in the area of the park in Šarengrad in Novi Sad. The park is located in the area of the Novo Naselje district, extends in the southwest-northeast direction, on an area of 1 hectare and contains deciduous and coniferous tree species and various ornamental shrubs. Four pedological profiles were opened in the area of the park, their external and internal morphology was described and the physical and chemical properties of the soil were determined. On the investigated soil, a large anthropogenic influence is visible in the description of the inner morphology of the pedological profiles, i.e. the surface horizon up to 10 cm depth was completely changed by filling in fertile humus soil, while the deeper horizon from 10 to 150 cm depth was also under anthropogenic influence. The analysis of the granulometric composition of the soil shows that the texture classes sandy loam, loam and clayey loam dominate in the surface horizon, while the texture classes in the deeper horizon are sandy loam and sandy clay loam. The chemical properties of the soil studied show an average $CaCO_3$ content of 5.07 to 8.94%, i.e. the soil is carbonate. The active acidity has a pH of 8.23 to 8.44, i.e. it is a medium to strongly alkaline soil. The humus content averages between 1.53 and 2.52% and it is a weak to moderately humic soil. In terms of nutrient content, it is moderately to well supplied with total nitrogen and poorly supplied with readily available phosphorus and potassium. As this is a park area where the vitality of the existing trees must be maintained, it is necessary to improve the physical and chemical properties of the soil to some extent, taking into account the compaction of the deeper horizon, which is a limiting factor for the development of the root system. In the case of the soil studied, it is necessary to improve the water-air balance of the soil through agrotechnical measures, to introduce an irrigation system and to enrich the soil with nutrients by applying mineral fertilisers with a high phosphorus and potassium content. When replanting and replacing withered seedlings, it is necessary to replace the soil at a greater depth to allow unhindered development of the tree roots, i.e. to replace the existing soil with fertile soil with favourable water and air properties.

Key words: *Anthropogenic soil, City park, Land change, Novi Sad.*

Introduction

In the urban area of Novi Sad there are several well-known parks, which are green oases of the city. However, in addition to the larger parks in the older parts of the city, with the urbanisation and expansion of the city in the last two decades, new parks have also been created in the newly built parts of the city. One of these parks is located on the western outskirts of the city in the district of Novo Naselje, i.e. Šarengrad. The main function of building new parks between residential buildings is to provide better living conditions for the population, especially considering the effects of climate change and high temperatures. According to Vratuša (2000), parks fulfil a number of functions, of which he highlights the following: sanitary-hygienic, architectural-urban planning, cultural-historical, educational and

other functions. The new greenery in parks certainly has an aesthetic and decorative function, but also a reduction of temperature extremes. For the construction of new parks to be as successful as possible, it is important to know the edaphic properties of the land intended for the parks. Knowledge of soil properties is also important in newly created parks in order to take additional agrotechnical measures to make the existing greenery as high quality as possible. The area of the studied park in Šarengrad is rich in different types of deciduous and coniferous trees and ornamental shrubs, which were planted in the period from 2008 onwards. The soil of the park was mostly under considerable anthropogenic influence, which will be seen in the work itself. Tešić et al. (2018), describing urban soils, state that the expansion and creation of new urban settlements requires that the existing soil be excavated, moved, often filled with earth materials of different origins, mechanically compacted, covered or various artificial substances introduced into it, various solid or gaseous pollutants. Thus, Škorić et al. (1985) defined human-altered soils as deposol soils. Knežević et al. (2018), who studied the urban park forest in Topčiderski Park (Belgrade), also attribute the altered soil conditions to human activity. Eremija et al. (2019) point out that urban soils - urbisols - have less favourable characteristics than natural soils. As Vukin (2004) states, such anthropogenic ecosystems are of great importance in urban areas and influence temperature extremes and therefore deserve the attention of experts and the entire population. Moreover, this soil study shows the anthropogenic influence on the current condition of the soil in the said park. The aim of the work is to show the characteristics of the soil in the area of the studied park as specifically urbanised soil.

Material and method

In the paper, the soil in the area of the park in Šarengrad in the city of Novi Sad was investigated. The park is located in the district of Novo Naselje (N 45°15'2.03" E 19°47'17.43"). It extends in a southwest-northeast direction over an area of one hectare and contains deciduous and coniferous tree species as well as various ornamental shrubs. Four pedological profiles were established along the longitudinal line of the park. The external and internal morphology of the profile was described, soil samples were collected in disturbed condition and the following physical and chemical soil analyses were carried out. Mechanical composition using the Pipet method prepares the samples for analysis with Na-pyrophosphate according to Thun, and the texture class of the soil is determined according to Tommerup's classification. The content of $CaCO_3$ was determined volumetrically with a "Scheibler" calcimeter. The pH was determined potentiometrically in a soil suspension with water. Humus content according to Tyurin, modified by Simakov. Total nitrogen according to the Kjeldahl method. Easily accessible phosphorus and potassium according to the AL method, Egner-Riehm-Dominigo. Based on the analyses carried out, the characteristics of the soil studied are presented.

Results and discussion

Four soil profiles were worked on the Park site and their physical and chemical properties are listed below. On the investigated soil of the park, a large anthropogenic influence is evident in the description of the internal morphology of the pedological profiles, i.e. the surface horizon (P_1) with a depth of up to 10 cm has been completely altered by filling with fertile humus soil, while the deeper horizon (P_2) with a depth of 10 to 150 cm also shows a considerable anthropogenic influence. As Trowbridge and Bassuk (2004) noted, the problems of urban green functionality arise precisely from the characteristics and quality of the urban soil on which it is planted.

Table 1 Granulometric composition

Profil	Horizon	Depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Total sand (%)	Total clay (%)	Texture class
P1	P_1	0-10	20.58	37.58	25.48	16.36	58.16	41.84	Sandy loam
	P_2	10-150	18.25	55.51	13.64	12.60	73.76	26.24	Sandy loam
	Average	0-150	19.42	46.54	19.56	14.48	65.96	34.04	
P2	P_1	0-10	6.20	32.24	36.60	24.96	38.44	61.56	Loam
	P_2	10-150	9.21	68.95	5.92	15.92	78.16	21.84	Sandy loam
	Average	0-150	7.71	50.59	21.26	20.44	58.30	41.70	
P3	P_1	0-10	7.29	37.47	34.08	21.16	44.76	55.24	Loam
	P_2	10-150	15.50	59.74	14.08	10.68	75.24	24.76	Sandy loam
	Average	0-150	11.40	48.60	24.08	15.92	60.00	40.00	
P4	P_1	0-10	4.50	31.05	36.56	27.88	35.56	64.44	Clay loam
	P_2	10-150	1.16	60.80	17.96	20.08	61.96	38.04	Sand clay loam
	Average	0-150	2.83	45.93	27.26	23.98	48.76	51.24	

Analyzing the granulometric composition of the tested soil (table 1), we can conclude that this soil is dominated by the fraction of fine sand with average values of 45.93 to 50.59%, as well as the fraction of silt with average values of 19.56 to 27.26 %. Slightly lower average values compared to the powder fraction have the share of the clay fraction, i.e. from 14.48 to 23.98%, while the smallest share is the fraction of coarse sand whose mean values are from 2.83 to 19.42%. Looking at the ratio of total sand and total clay, it can be seen (table 1) that at the surface horizon (P_1) the share of total sand decreases, that is, the share of total clay increases from the pedological profile P1 to the profile P4, which results in textural classes: sandy loam, loam and clay loam. Observing the data of the lower horizon (P_2), the ratio of total sand prevails in the first three pedological profiles where the textural classes are determined: sandy loam, while in the fourth profile there is a slightly lower proportion of total sand, so the textural class is determined: sandy clay loam.

Table 2 Chemical properties

Profil	Horizon	Depth (cm)	$CaCO_3$ (%)	pH (in H_2O)	Humus (%)	Total N (%)	P (mg/100g)	K (mg/100g)
P1	P_1	0-10	8.84	8.26	2.27	0.089	9.40	7.55
	P_2	10-150	5.95	8.20	0.78	0.052	6.60	5.22
	Average	0-150	7.39	8.23	1.53	0.07	8.00	6.38
P2	P_1	0-10	7.87	8.23	3.71	0.136	12.93	10.50
	P_2	10-150	8.96	8.36	1.32	0.042	5.81	4.56
	Average	0-150	8.42	8.30	2.52	0.09	9.37	7.53
P3	P_1	0-10	4.82	8.44	3.64	0.121	11.80	9.56
	P_2	10-150	13.07	8.44	0.68	0.049	6.33	5.00
	Average	0-150	8.94	8.44	2.16	0.09	9.07	7.28
P4	P_1	0-10	7.71	8.26	3.41	0.143	13.45	10.93
	P_2	10-150	2.43	8.37	0.14	0.080	8.72	6.98
	Average	0-150	5.07	8.32	1.78	0.11	11.08	8.96

According to the presented chemical properties of the tested soil (table 2), it can be seen that the average $CaCO_3$ content ranged from 5.07 to 8.94%, and we can conclude that according to

the soil classification based on the $CaCO_3$ content, the tested soil is in the carbonate class (Belić et al. 2014). The active acidity of the tested soils shows a pH value ranging from 8.23 to 8.44, which according to the American classification according to soil reaction shows that these soils are classified as medium to highly alkaline (Belić et al. 2014). The humus content of these soils is on average from 1.53 to 2.52%, and according to the Scheffer-Schachtschabel classification, we classify them as weakly to moderately humus soils, while the surface humus horizon can be classified as moderately humus soils. According to the content of nutrients (Predić, 2011) it can be concluded that the tested soils are medium to well supplied with total nitrogen, and poorly supplied with readily available phosphorus and potassium. Analyzing the examined soil, it can be concluded that it has been largely altered and anthropogenized compared to natural soil. According to Tešić (2021), research has shown that the degree of urbanization of the city and the type of soil use change the mechanical composition of the soil. The granulometric composition of the soil indicates an increased content of total clay, especially in the surface humus horizon. The surface humus horizon P_1 with a depth of up to 10 cm was completely changed and was brought from another location for better reception of planting material, and primarily for more successful weeding of the area. According to Sauerwein (2013), removing surface soil and bringing soil from other localities, which have different characteristics from the existing one, spells a change in the ecological characteristics of the soil. The deeper P_2 horizon, from 10 to 150 cm deep, textural class sandy loam to sandy clay loam, is characterized by very high compaction and the presence of small remains of construction material and parts of bricks to a lesser extent, which directly indicates human influence through the presence of heavy machinery and remains of materials during construction surrounding residential complex. According to Tešić et al. (2018) soil compaction affects the reduction of the ease of penetration of the roots of many plants, the reduction of water movement in the soil and affects the reduction of its water-holding capacity, also the compaction affects the gas exchange in the soil, reducing especially the flow of oxygen, which is necessary for the roots to function normally according to Scalenghe and Marsan (2009). The chemical indicators of the examined soil indicate that it is carbonate, medium to highly alkaline, Tešić et al. (2018) states that the decomposition of materials left over from construction objects changes, that is, as a rule, the pH value of existing soils increases, given that lime is one of the ingredients of construction materials. The tested soil is moderately humus in the surface horizon, well supplied with nitrogen but poorly supplied with easily accessible phosphorus and potassium. Bearing in mind that it is a park area where it is necessary to preserve the vitality of woody plants, it is necessary to improve the physical and chemical properties of the soil to a certain extent, and the compaction of the deeper P_2 horizon is a limiting factor for the development of the root system of trees, their weaker reception and growth. Primarily, it is necessary to improve the water-air regime of the soil by applying agrotechnical measures, watering the seedlings by introducing an irrigation system, and increasing nutrients in the soil by applying mineral fertilizers rich in phosphorus and potassium. During the planting of new and replacement of dried seedlings, it is necessary to change the soil at a greater depth, which will enable the unhindered development of tree roots, i.e. replace the existing soil with fertile soil with favorable water and air properties.

Conclusion

The paper shows the physical and chemical properties of the soil in the park in the area of Šarengrad, in the city district of Novo Naselje in Novi Sad. The examined soil consists of two anthropogenic horizons, the surface P_1 and the deeper P_2 horizon. The surface horizon has been completely changed and is characterized by textural classes: sandy loam, loam and clay loam, while the deeper horizon is presented by: sandy loam and sandy clay loam. The

chemical properties of the examined soil indicate that it is carbonate, medium to strongly alkaline, moderately humus in the surface horizon, well supplied with total nitrogen, but poorly supplied with easily accessible phosphorus and potassium. The limiting factor for the development of the root system of trees is the compaction of the deeper P_2 horizon, which affects the vitality of existing trees, as well as the reception and growth of newly planted seedlings. In order to preserve the vitality of the trees in the park, it is necessary to improve the physical and chemical properties of the soil by applying agrotechnical measures, introducing an irrigation system and fertilizing with mineral fertilizers rich in phosphorus and potassium. During the planting of new and replacement of dried seedlings, it is necessary to change the soil at a greater depth.

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DEMOGRAPHIC OF EMPLOYEES IN PUBLIC FOREST ENTERPRISES AS FACTOR FOR IMPROVEMENT OF FOREST PRODUCT AND SERVICES

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Abstract

Public forest enterprises are required to meet public needs for forest products and services. Some forest products and services must be provided free of charge to society as a whole. From a business perspective, the problem is how to motivate employees to improve products and services when some are provided free of charge. Improvement of product and services is a part of entrepreneurial process known as opportunity recognition and relies inter alia on demographic characteristics. We used attitude approach to describe employees' ability to recognize business opportunity based on demographic characteristics. The improvement of forest products and services results from the proper identification of business opportunities. This research begins with research question whether the demographic characteristics of employees in public forestry enterprises have an impact on improving forest products and services. We conducted a survey to collect employees' attitudes from 4 public forest enterprises in Serbia. The results show that male have more positive entrepreneurial attitudes compared to women. Women give more importance to services such as extension service in private forestry than their male counterparts. Younger employees show more positive entrepreneurial attitudes than older ones. Employees with shorter work experience show more positive entrepreneurial attitudes compared to employees with longer work experience. Positive entrepreneurial attitudes are an important momentum for improving forest products and services. Some of products and services did not recognize as business opportunity.

Keywords: Entrepreneurship, Public Forest Enterprise, Innovations, Diversification.

Introduction

In this paper, we analyzed how demographic characteristics of employees in public forest enterprises (PFE) differentiate the possibility for improving forest products and services. Improvement of forest product and services is a specific entrepreneurial process that takes place through business opportunity recognition (Covin, Slevin, 1991; Morris, Sexton, 1996; Shane, Venkataraman, 2000). Business opportunity recognition is a positive entrepreneurial trait of employees that has implications for offering new and improving existing products and services. In general, offering new and improving existing products and services is considered as a result of employees' ability to recognize business opportunity. This entrepreneurial process consists of identification, discovery, evaluation and exploitation of business opportunity and employees who have ability to identify, discover, evaluate and exploit such business opportunity (Shane, Venkataraman, 2000). However, in large and especially public enterprises (PE), the development of new and improvement of existing products and services is considered unstable. Instability occurs due to two forms in the development of business activities. The first is experimenting with new ideas, and the second is using new ideas and turning them into business opportunities, and finally into new or improved products and services (March, 1991).

A fundamental challenge from the entrepreneurial aspect in large companies is managing the conflict between the new and the old (Dess *et al.,* 2003). With PE, given that the state is the

owner, the situation is even more complex because it is the state administration that fully or partially performs the role of entrepreneur (Paunović, 2007). To analyze the possibility for improving products and services in PFE, it is necessary to take into account that the business activities of PE are usually fulfill the public needs (Robinett, 2006). For some of the business activities and related products and services, the private sector has no economically justified reasons to perform them. Sikorski (1993) indicates that "...even though we use public enterprises every day, we are taught to underestimate their importance and value". There are many reasons for such understandings. In Serbia, PE are characterized by "...low efficiency and high costs compared to the quality of services..." (Veselinović, 2014). This situation is not only characteristic for PE in Serbia. It was noted that the efficiency of the PE is not important for the state as the founder (Veselinović, 2014). Although it seems paradoxical, such views are proved by the Armen Alchain's theory of "property rights". Sikorski (1993), quoting Alchain, indicates that the founder of the PE has no motive to develop the company's activities, because there is no possibility to sell a share of his ownership rights. This is especially important for PFE that are engaged in activities of public interest, where it is difficult or impossible to transfer ownership to another owner. Such attitudes rise up questions such as: how and who should discover new business opportunities and how to create new and improve existing products and services (Shane, Venkataraman, 2000).

Following this finding, we started with research question: whether the demographic characteristics of employees in PFE have an impact on improving forest products and services. Improving forest product and service here is understood as ability to recognize business opportunity through introduction of new or improvement of existing product and services. Therefore, it is necessary to analyze the demographic characteristics of PFE employees as a factor in the business opportunity recognition process. As demographic characteristics, the authors use: gender, age, work experience (Hornsby *et al.*, 2002; Autio, 2005; Minniti *et al.*, 2006; Hisrich *et al.*, 2011). Ability to recognize business opportunities is most pronounced for people aged 25 to 45 (Hisrich *et al.*, 2011), and according to global research, this range is somewhat narrower and relates to the age between 24 and 35 years of age. After the age of 35, entrepreneurial motivation and potential decline rapidly (Minniti *et al.*, 2006). Work experience have a negative impact on the readiness to recognize business opportunities. Research results about gender and opportunity recognition indicate that men and women have different approaches and use their specific characteristics to generate new ideas as a part of process of improving product and services (Filser *et al.* 2023). Greater proportions of women in the company creates more favorable conditions for the development of new products and services, but women less often decide to participate in new businesses (Aldrich, Cliff, 2003). In a process of business opportunity recognition, it is noticed that employees hold different beliefs about the value of resources that they manage (Shane, Venkataraman, 2000) what could be important in research of PFE.

Materials and methods

In this research we used demographic approach which assumes that employees' behavior is influenced by demographic characteristics (Robinett, 2006). The survey method was chosen for data collection. The data were collected using the structured questionnaire named Forestry Intrapreneurship Innovativeness Instrument (FIII) (Poduska *et al.*, 2020). The questionnaire consists of 10 groups of questions: 1) demographics (4 items); 2) ability for business opportunity recognition (14 items); 3) professional training (6 items); 4) business environment (8 items); 5) flow of information (7 items); 6) entrepreneurial attitudes and motives (5 items); 7) managerial support (10 items); 8) work autonomy (7 items); 9) rewards system (7 items); 10) work time availability (3 items). For the purposes of this research, we

analyzed respondents' attitudes on two groups of questions: demographics with 4 items (gender, age, work experience and work experience on current position) and attitudes on ability to recognize business opportunity with 14 items representing existing product and services from portfolio of PFE and some new ones. Direct interviews included 137 employees from PFE enterprises in Serbia. The description of the sample is given in the table 1. The survey was conducted from July 2014th to October 2015th. The time required to fill out the questionnaire was an average of 40 minutes, which was assessed as satisfactory, and in this regard, it was concluded that the questionnaire was of appropriate scope.

Table 5. Description of sample.

	N		Mean	Median	Mode	Std. Deviation	Min.	Max.
	Valid	Missing						
Gender	135	2	/	/	/	/	/	/
Age	135	2	44,39	44,00	40	8,445	26	64
Work experience	135	2	16,53	15,00	15	9,062	1	38
Work experience on current position	132	5	9,58	8,00	3	7,215	1	30

source: Authors calculation based on questionnaire survey results

Employees' attitudes were tested by the *Mann-Whitney* U test to compare differences between groups of employees based on variable - demographic characteristics. The following criteria were selected for classifying respondents into independent groups. For the variable "Gender", the division was made into two groups - men and women. For the variable "Age", the division was made by median of the sample (44), into younger (<44 years) and older (>44 years). For the variable "Work experience", the division was made by median of the sample (15), into respondents with more (>15 years) and less (<15 years) work experience. For the variable "Work experience on current position ", the division was made by the median of the sample (8), into respondents with more (>8 years) and less (<8 years) work experience in the current position.

Results and discussion

The results are presented for demographic variables: gender, age, work experiences and work experiences on current position and variable ability to recognize business opportunity. Tests of differences between groups of employees were interpreted and analyzed in text.

The ratio of men to women in the sample is 74% to 26%. This ratio indicates that there are more men in the selected sample, which is expected considering that there are more men than women in the population of employees in PFE. The youngest respondent is 26 years old, and the oldest is 64. The most frequent age of respondents in the sample is 40 years old (7,4%).

The distribution of respondents according to the work experience reveals that the shortest work experience is 1 year, and the longest is 38 years. The most frequent work experience is 15 years (8,9%).

Distribution of employees by work experience on current position reviles that the most frequent is three years of experience in the current position.

Bussines opportunity recognition is analized based on emploeeys' attitudes toward improvement of product and services in FPE. The mean value for 14 potential bussines opportunities are presented in figure 1.

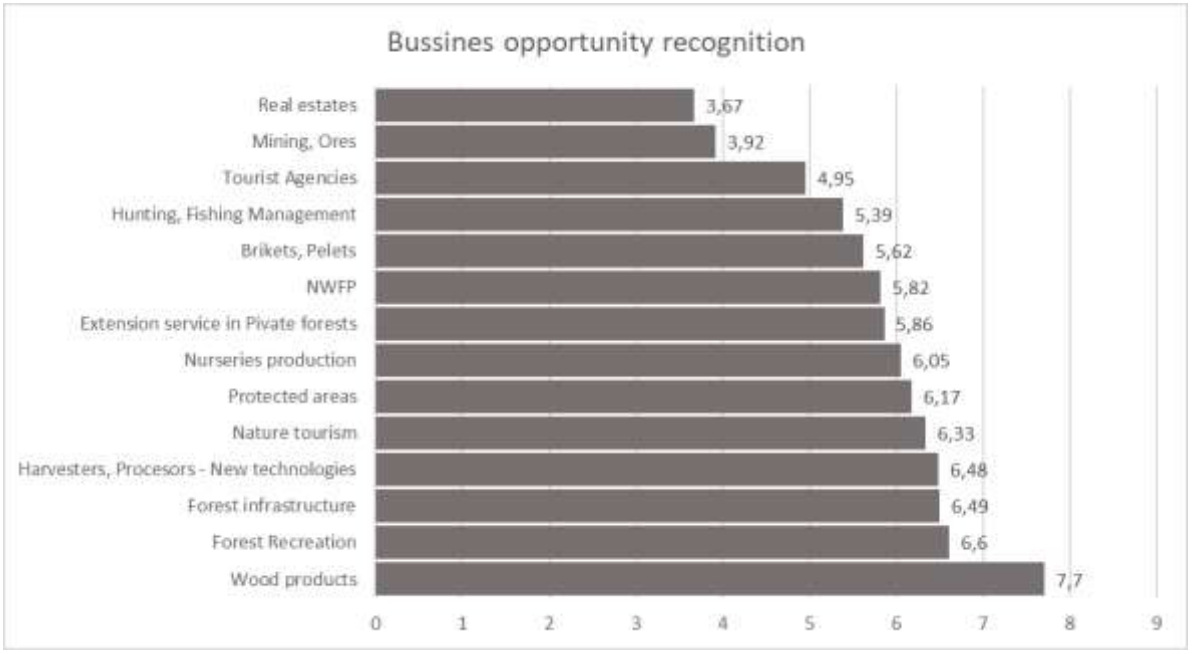

Figure 1. Employees' attitudes on possibility to improve product and services

Results indicate that wood production is the activity recognized as highly ranked (7,7 out of 10) business opportunity. This is expected because it is the main business in PFE. Top five best ranking bussines activity are: forest recreation (6,6), then bussines activities with forest infrastructure (6,49), introduction of new technologies (6,48) and tourism in nature (6,33). This indicate that diversivication of bussines activities need to be in focus on other than wood products. For improvement of forest recreation and tourim in nature should be take into account climate and meteorological factors (Poduška *et al*., 2014). Management of protected areas is business activity dependent from government subsidies while offering variety of ecosistem services (Poduška *et al*., 2013). Impelmentation of new technologies is forest sector is highly ranked (6,48) and it is closly conencted with need for inovation in forestry companies (Poduška *et al*., 2011). Business with pelets and briquets are not highly ranked in spite of variety in potential areas for establishing forest plantations for satisfaction of energy needs (Spasojević, et al., 2019). According to the mean value less that 5,5 the next bussines are not recognised as opportunities for improving product and services: hunting and fishing management (5,39), tourist agencies (4,95), mining and ores (3,92), business with real estates (3,67).

Differences between the groups of employees was tested by Mann-Whitney U test based on attitudes of 4 groups: gender, age, work experience and work experience on current position.

The statistically significant differences[2] between male and female was found for extension services in private forests ($Z=-2,303$) with value of median (Md) for men 5,0 and for women 7,0. Differences about business opportunities between men and women indicate that women are more oriented towards service activities like extension service in private forests, which is fully in line with previous research, that women tend towards service activities, and men towards production (Hisrich *et al*., 2011).

The statistically significant differences between the groups based on age are found for non-wood forest product (Z=-2,454; Md for younger employees is 6,0 and Md for older is 5,0), tourist agency ($Z=-2,652$; Md for younger is 5,0 and Md for older is 4,5); extension service in private forests ($Z=-2,487$; Md for younger old is 6,0; Md for older old is 5,0), recreation in nature ($Z=-2,188$; Md for younger is 7,0 and Md for older is 6,0), nursery production

[2] *p<0,05

(Z=−2,459; Md for younger is 7,0 and Md for older is 5,0); forest infrastructure (Z=−2,327; Md for younger is 7,0 and Md for older is 6,0).
The statistically significant differences between the groups based on work experiences (WE) are found for NWFP (Z=−2,075; Md value for less WE is 7,0 and Md for more WE is 5,0), hunting and fishing (Z=−2,319; Md for less WE is 6,0 and Md for more WE is 4,0), tourist agency (Z=-2,953; Md for less WE is 5,0 and Md for more WE is 4,0), recreation in nature (Z=−2,473; Md for less WE is 8,0 and Md for more WE is 6,0), nursery production (Z=−2,330; Md for less WE is 7,0 and Md for more WE is 5,0).
The statistically significant differences between the groups based on work experiences on current position (WECP) are found for NWFP (Z=−2,284; Md value for less WECP is 7,0 and Md value for more WECP is 5,0) and for forest recreation (Z=−2,140 Md value for less WECP is 8,0 and Md value for more WECP is 6,0).
Values of median less than 5,5 indicate that employees did not recognize business opportunity as possibility for improvement forest product and services. Possible reasons for opposed attitudes regarding NWFP could be found in high impact from climate factors (Ranković, *et al*., 2016; Ranković *et al*., 2017). The similar attitudes associated with median less than 5,5 older employees and employees with more work experience give to NWFP, tourist agencies, extension service to private forest owners, nursery production. Declining entrepreneurial potential with age can be interpreted as a lack of persistence among employees. Persistence is one of the key characteristics for employee entrepreneurship (Hisrich *et al*., 2011). Some of business did not recognize as business opportunity because of possible conflicting activity in management in protected areas (Poduška *et al*., 2018).

Conclusion

Improvement of products and services derived from employees' ability to recognize business opportunity is crucial moment in PFE when public needs should be fulfilled even in situation when consumers and end users do not need to pay for it directly. In our research we found that demographic characteristic can be factor to differentiate employees with positive entrepreneurial attitudes like ability to identify and recognize business opportunity. According to the results of this research we can recommend to management of PFE to use younger teams during the project of improvement business with NWFP, extension service for private forest owners, forest recreation and tourism, nursery production. Women give greater importance to services and should be a core part in business like extension services in private forests. In addition, it was pointed out the activities that cannot improve the business of the company. All of the above indicates that the correct choice of business activities is important for improvement of forest product and services in portfolio of PFE.

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CHARACTERISTICS OF LOWER NON-AGRICULTURAL SOILS IN THE NORTH BANAT REGION, MUNICIPALITY OF KIKINDA AND POSSIBILITIES FOR THEIR AFFORESTATION

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Abstract

This paper presents the results of research in the lower parts of lowland non-agricultural area in the Forest management unit ,,Kikinda" on territory of municipality of Kikinda. According to Development plan of city Kikinda around 90% of territory (70.433 ha) is used as agricultural land. The aim of this research was to analyze soil characteristics of lower non-agricultural land and its possibility for afforestation. In research area, the morphology of four pedological profiles was described together with standard physical and chemical analyzes from each pedologic horizon. Soil type was determined as haplic gleysol. Groundwater was found in two profiles at 60 and 70cm depth. Particle size distribution of the haplic gleysols indicates that total clay has higher share then total sand. Based on obtained results, the most dominant texture class in this type of soil was loam. $CaCO_3$ content in soils varied between profiles while pH values ranged from 7.61 to 9.53. Humus content varied from 1.63 to 4.29% in surface Aa horizon. Based on ecological conditions such as high level of groundwater, together with examined soil properties, this area is convenient for establishment of hardwood forests, primary formed of pedunculate oak (*Quercus robur*) and narrow-leaved ash (*Fraxinus angustifolia*). The most limited characteristic of these soils is low physiologically active profile depth which proves there can not be estabilished highly producive hardwood forests. Considering the current forest percentage of area raising forests on these soils, with protective purpose, have multifunctional value.

Key words: *Lower non-agricultural soil, Haplic gleysol, North Banat, Afforestation.*

Introduction

Banat is an area in the Pannonian Lowland that extends in Hungary (small part), Romania 21,800 km^2 and 8,997 km^2 in Serbia, on a total area of 30,680 km^2. The Serbian part of Banat is the largest of the three regions that make up the Autonomous Province of Vojvodina (Banat, Bačka, Srem). Municipality of Kikinda is administrative center of the North Banat District in Serbia. According to Development plan of city Kikinda around 90% of territory (70.433 ha) is used as agricultural land. Public utility company ,,Kikinda'' rules with forests in the territory of Kikinda and total forested area covered with plan documents is 284 hectares. Total forest amount is around 400 hectares which is around 0,5% of total territory. This data classifies Kikinda as one of the most deforested municipalities in Vojvodina. The genesis of Banat relief is closely related to the dynamics of the plates and micro plates in the base, fragmented and plunged at different depths on lines of major faults and reoriented by local fracture (Oncescu, 1965; Saulea, 1967; Măhăra, 1970; cit. Ianos, 2002). The general aspect of the relief is that of a cuvete with a wide westward opened plain bordered by hills and mountains in the east. Although the low plain relief of Banat is very little varied, the soil layer presents a great diversity accused by the different lithology, by the shapes of micro

relief (plane surfaces, levees, erosion, sagging, deflation depressions, abandoned channel) and by the different position of the phreatic level. The western extremity of Banat plain is influenced by the vast areas of influence still active, from Csongrad-Szeged and Alibunar (Tufescu, 1957; Posea, 1997) that have printed to the soils an accentuated hydromorphism. In these areals made on a basement mode of fluvio-lacustrine deposits there have been evolving mollic gleysols, typical gleyosols, gleyed vertisols together with solonetz (Aranca, Cenei-Ionel-Livezile, Moravita Plains)(Ianos,2002). According to Bukurov (1972) and Nejgebauer (1971) the final forms of the relief of Vojvodina are the result of the periodic blowing of loess, the erosive work of atmospherics and rivers, as well as the accumulation of fluviatile material on the river terraces. The North Banat District mostly lays on alluvial plain and depressions, alltogether with sporadic loess terraces. The area of alluvial plain is characterized by hydromorphic soils that show regularity in distribution in relation to the riverbed (Pekec et. al. 2011). The main running water stream in Kikinda is Great Kikinda channel with embankments and closest river is Tisa 25km western, also with river bents, which means territory of Kikinda is protected from flooding and lower soils are under direct influence from groundwater. Groundwater strongly affects genesis of soils in this area. The most dominant soils in lower non-agricultural areas in Kikinda are gleysols. Although Belic (2011) concludes that every new soil profile has to be considered in particular regarding its diagnostic horizons, properties and materials and cannot be switched from actual national classification to FAO 2006 RSG by default. According to WRB classification (2006) gleysols develop a gley horizon of secondary oxidation and a gley sub horizon of secondary reduction with respect to variation and groundwater level, and the depth of the gley horizon is related to groundwater hydromorphism. The aim of this research was to analyze soil characteristics of lower non-agricultural land in Kikinda municipality, North Banat District and its possibility for afforestation. Based on united results of field and laboratory researches, this paper presents possibilities for afforestation of gleysols.

Material and method

Four pedological profiles were opened in different parts of research area. Profile morphology was described together with standard physical and chemical analyzes from each pedologic horizon. Particle size distribution (%) was determined by international B-pipette method with sample preparation in sodium pyrophosphate. Based on results the texture class of the soil was determined according to the Atteberg's classification. Kopecky's cylinders (volume of 100 cm^3) were used for the determination of soil bulk density (Bošnjak et al., 1997). CaCO3 content was determined volumetrically using a Scheibler calcimeter, while the pH value was measured electrometrically using pH meter apparatus. Humus content according to Tyurin, modified by Simakov. Results based on analyses were presented.

Figure 1. Locations of pedological profiles

Results and discussion

Analysing the data from Table 1 for all four profiles which represent lower lowland gleysols wetted only by groundwater, it is clear that coarse sand has larger share then fine sand. The content of coarse sand ranged from 28.25 to 49.60% while fine sand ranged from 0.65 to 3.20%. The average value of powder for profile P1 Basaid is 28.90% while clay content averaged 42.25% following total clay is 71.15% and classifies P1 Basaid as clay texture class. Profile P2 Banatsko Veliko Selo had larger share of coarse and fine sand then P1 Basaid , together with smaller share of clay 27.4% what classifies it in clay loam texture class for surface horizon and loam texture class for lower gley horizon. P3 Kikinda averaged 43.1% total sand, 36.95% powder and 20.05% of clay which classifies it in Loam texture class. This results are near to the best soils in agronomic aspect when fractions ratio of sand-powder-clay is 35-40% - 35-40% - 20-30% according to Belic et al. (2014). Profile P4 Sajan had larger share of total sand then P3 Kikinda with 51% of total sand, with smaller share of powder that averaged 22.8%, all together with 16.15% average clay. These results classified surface horizon of P4 into sandy clay loam texture class, while gley horizon is loam. Pekeč et al. (2021) exploring the protected part of alluvial plain lists texture classes sandy clay loam to clay loam in surface parts and sand, loam sand, clay loam in lower parts of profile.

Table 1. Granulometric composition of soil

Profile	Horizon	Horizon Depth(cm)	Coarse sand% 2 - 0,2 mm	Fine sand % 0,2-0,02 mm	Silt % 0.02–0.002 mm	Clay % <0.002 mm	Texture class
P1 Basaid	Aa	0-45	33.7	0.6	21.3	44.4	Clay
	Gso	45-80	22.8	0.7	36.5	40.1	Clay
	average		***28.25***	***0.65***	***28.90***	***42.25***	
P2 Banatsko Veliko Selo	Aa	0-40	31.4	3.0	30.8	34.8	Clay loam
	Gso	40-72	48.8	0.8	30.4	20.0	Loam
	average		***40.10***	***1.90***	***30.60***	***27.40***	
P3 Kikinda	Aa	0-30	37.7	2.6	34.5	25.3	Loam
	Gso	30-80	42.1	3.8	39.4	14.8	Loam
	average		***39.90***	***3.20***	***36.95***	***20.05***	
P4 Sajan	Aa	0-34	56.3	0.9	13.8	28.9	Sandy clay loam
	Gso	34-80	42.9	1.9	31.8	23.4	Loam
	average		***49.60***	***1.40***	***22.80***	***26.15***	

Analyzing chemical properties of the soils it was found that all investigated profiles had slightly alkaline to very alkaline reaction. Average pH values ranged from 7.71 to 9.27 while the most alkaline reaction showed in profile P2 horizon G_{so} with value 9.53 (very alkaline). Carbonate content varied from 10.50 to 16.20% but in surface horizon of P2 was 21.05%, then in P3 lower horizon 22.14% and in P4 was 19.56% so we classify them as strongly carbonate soils. Humus content averaged from 1.08 to 2.61%, so these soils are classifed from weak to moderate humus (Scheffer-Schachtschabel classification).

Table 2. Chemical properties of soil

Profile	Horizon	Horizon Depth (cm)	pH	Humus (%)	$CaCO_3$ (%)	Total N (%)	Total K (%)	Total P (%)	Total salts (%)
P1 Basaid	Aa	0-45	7.61	2.66	12.83	0.146	11.1	13.7	0.3
	Gso	45-80	7.80	2.52	8.16	0.172	12.7	15.6	0.19
	average		***7.71***	***2.61***	***10.50***	***0.159***	***11.90***	***14.65***	***0.25***
P2 Banatsko Veliko Selo	Aa	0-40	9.01	2.01	21.05	0.146	11.1	13.6	0.08
	Gso	40-72	9,53	1.05	2.80	0.110	8.8	10.9	0.21
	average		***9.27***	***1.53***	***11.93***	***0.128***	***9.95***	***12.25***	***0.15***
P3 Kikinda	Aa	0-30	7,97	4.29	10.26	0.132	10.2	12.6	<0.03
	Gso	30-80	8,20	0.06	22.14	0.119	9.4	11.6	0.26
	average		***8.09***	***2.18***	***16.20***	***0.126***	***9.8***	***12.1***	***0.14***
P4 Sajan	Aa	0-34	8.08	1.63	4.01	0.092	7.7	9.6	<0.03
	Gso	34-80	8.87	0.52	19.56	0.121	9.6	11.8	<0.03
	average		***8.48***	***1.08***	***11.79***	***0.107***	***8.65***	***10.7***	***<0.03***

Soils in all four pedological profiles, according to Soil classificaton of Yugoslavia (Škorić,1985), belongs to hidromorphic order of soils, class of hypogleyic soils, soil type eugley, subtype hypogley, mineral variety and carbonate – alkalized form. Morphological structure of profiles was A – G_{so}, with A horizon depth from 30 – 45cm. Groundwater was found in P1 Basaid on 60cm depth, and also in P3 Kikinda on 70cm depth. In other two profiles groundwater was not present in 100cm depth. Based on WRB soil classification eugleys in Serbia are classified as gleysols (Knezevic et al. 2011; Mrvic et al. 2016). Area of Kikinda belongs to Panonian province, to the forest-steppe zone, which has no representive climate forests on big areas, so there are xerothermic oak forests *Aceri tatarici – Quercion* where common oak is dominant species. Considering periodical rising of groundwater founded in profiles P1 and P3 gleysols can be convenient for establishment of forests *Fraxinetum angustifoliae* with narrow-leaved ash as main species. Ivanisevic et al. (2006) declared possibility for afforestation eugley with *Salix alba*, what can be considered on parcel where was located P4 Sajan, with total sand of 51%.

Conclusion

This paper presents the results of research in the lower parts of lowland non-agricultural area in the Forest management unit ,,Kikinda" on territory of municipality of Kikinda as a part and administrative center of North Banat District. Total forested area is around 400 hectares which is around 0,5% of total territory which classifies Kikinda as one of the most deforested municipalities in Vojvodina. Four pedological profiles were opened in different parts of research area. Based on obtained results from morphological, physical and chemical analyzes soil types were determined as gleysols in all four profiles. Groundwater was found in two profiles at 60 and 70cm depth. Texture class varied from clay in P1 with 71.15% total clay to sandy clay loam in P4 with average 51% total sand. pH values found alkaline to very alkaline reaction and $CaCO_3$ content classify this soils as strongly carbonate. Altought there is no representive climate forests in this area, this soils can be afforested in first place with hardwood species such as common oak (*Quercus robur)* and narrow-leaved ash (*Fraxinus angustifolia*). In some cases with lighter granulometric composition these soils can be afforested with *Salix alba*. The most limited characteristic of these soils is low physiologically active profile depth which proves there can not be estabilished highly producive hardwood forests. Considering the current forest percentage of area raising forests on these soils, with protective purpose, have multifunctional value.

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BACTERIAL TREATMENT IMPACT ON ONE-YEAR-OLD SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL) SEEDLINGS OF THREE SERBIAN PROVENANCES

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Abstract

Plant growth-promoting bacteria support plant health, development and functioning throughout their life without negative environmental side effects. Their application in the forestry sector is not examined enough. Sessile oak is one of the most valuable forest species in the Serbian forest fond. This research studied the effect of *Viridibacillus arvi* and *Pseudomonas koreensis* on the height and root collar diameter of one-year-old sessile oak seedlings. The results indicate no significant improvements in plant height and root collar diameter as a consequence of bacterial treatment. However, there were notable differences between plants of different provenances. Further research needs to be conducted in order to verify obtained results and get more detailed information on plant-bacteria relations.

Keywords: *bacteria, sessile oak, seedlings, provenances, Serbia.*

Introduction

Demands for good quality seedlings on the global market are increasing. The rise of awareness among the population on environmental pollution and toxicity of different chemical fertilizators and pesticides, used in seedling production, opened space for green solutions, among which biofertilizers and biostimulants take place. In the basis of this concept are different microorganisms and their products that help plant growth. Plant growth-promoting bacteria (PGPB) are a diverse group of bacteria that live on plants or inside their body, making an important part of the plant holobiont (Sanchez-Cañizares et al., 2017). They stimulate plant growth, fructification, resistance to different pathogens and extreme environmental conditions, influence root architecture, and support their development and wellbeing. While helping them to overcome harsh periods of time, they also enhance their adaptivity potential, which is very important in fighting climate change issues (Abdelaal et al., 2021; Fiodor et al., 2021). PGPBs are producers of hormones (auxin, cytokinin, gibberellin), enzymes, acids that dissolve limiting nutrients, siderophores, blocators of ethylene synthesis, and many of them also fix nitrogen (Glick, 2012). Also, through processes of interspecies competence, they indirectly help their plant host (Fira et al., 2018).
PGPB potential for usage in forestry as a "green alternative" for fertilizers and pesticides is not well studied. Serbia has 29.1% forest coverage, according to the latest official data (Banković et al., 2009) and plans to achieve 41 % by 2035, as stated in The Spatial Plan of the Republic of Serbia from 2021 to 2035. Sessile oak (*Quercus petrea* (Matt.) Liebl) is one of the most valuable forest tree species in the Serbian forest fond that has a wide ecological range, and occupies an elevation between 300 and 1300 m. For its wood's excellent technical properties, sessile oak is used in many branches of economy. However, it is equally important in an ecological context, since it is an edificator of numerous forest types that present a significant habitat for the main game species. Compared with other Central European oak species, sessile oak is a more drought-tolerant and different studies predict it will expand its range due toclimate change forecasts (Mette et al., 2013; Stimm et al., 2021). Consequently, it

is one of the most commonly grown species in Serbian nursery production (Popović et al., 2019).

Nowadays, sessile oak forests struggle with intense dieback, due to multiple physiological problems associated with forest age. An additional problem is the low success of natural regeneration, which together emphasizes the need for artificial regeneration measures, which further indicates a greater need for top-quality seedlings. Production of forest seedlings is fundamental for future successful establishment of healthy and high yielding economically profitable forest stands (Binotto et al., 2010). In seedling quality evaluations, stem height and root collar diameter are the main morphological parameters for both bare-root and container-grown seedlings' status (Grossnickle and MacDonald, 2018; Ivetić et al., 2017). Grossnickle and MacDonald (2018) reported these morphological attributes are important markers of future survival because, after replanting, seedlings retain these characteristics for an extended time interval as a result of transplanting shock.

The aim of this study was to investigate the effect of bacterial treatments on the stem height and root collar diameter of one-year-old sessile oak seedlings of three Serbian provenances as a principal seedling quality parameters.

Material and Methods

The one-year-old seedlings used in this paper were produced from acorns collected in the Autumn of 2020 from three Serbian provenances – mountain Rudnik in Central Serbia, mountain Rogozna in the south-west part of Serbia, and Grabova reka in eastern Serbia, from their natural forest stands. Container seedlings filled with peat (Freepeat, Holland) were nurtured in the nursery of the Institute of Forestry, in half-shadow conditions and watered every second day (unless it rained).

Two bacterial species – *Viridibacillus arvi* and *Pseudomonas koreensis*, used in seedling treatment, were isolated from rhizosphere soil samples from sessile oak stands on Mount Rudnik and underwent them laboratory testing for *in vitro* plant growth-promoting traits (unpublished data).

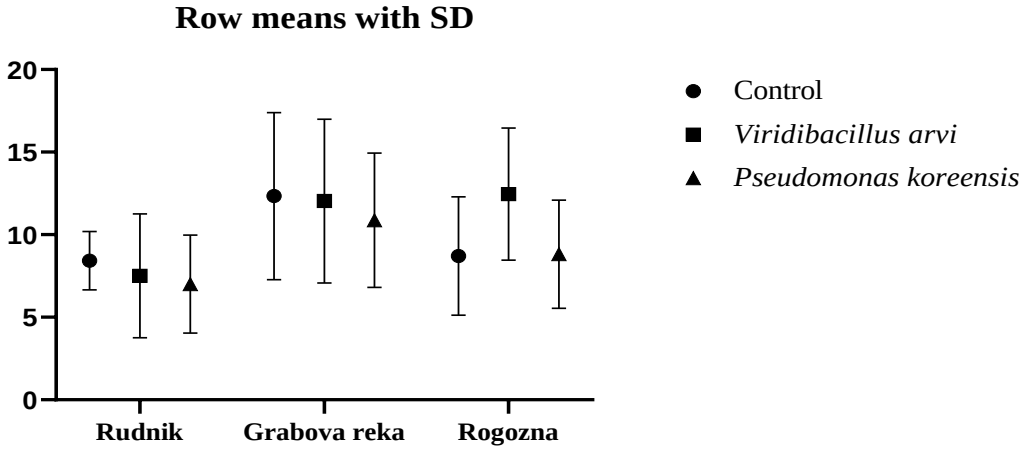
Ten seedlings per treatment were inoculated with 10 ml of bacterial preparations at the end of May, by sterile syringe. The procedure was repeated after 7 days. No additional fertilizers or pesticides were used. Each of the two bacterial treatments contained live bacterial cells of selected bacteria (10^8 cfu/ml) in saline. Tap water was used as a control treatment.

At the end of the growing season, seedling height was measured by a ruler with an accuracy of 0.5 cm. The seedling root collar diameter was estimated by Vernier calliper with an accuracy of 0.1 mm. The morphological characteristics of seedlings were described by descriptive statistics features. A two-way analysis of variance (ANOVA) followed by a Tukey's test were performed to analyse provenance and treatment effect. All statistics were done in GraphPad Prism version 9.0.0. for Windows.

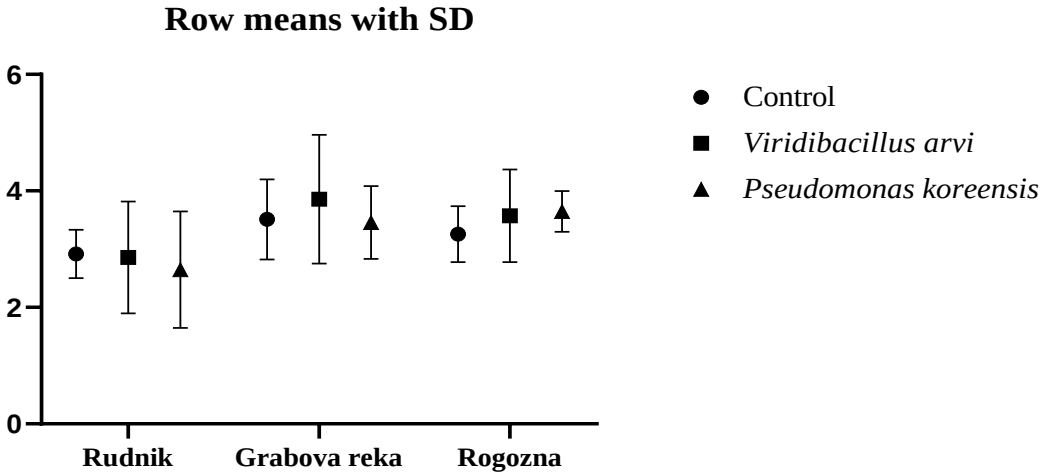
Results and Discussion

Graphs 1 and 2 presents the mean values of seedlings height and root collar diameter regarding provenance and bacterial treatment factors. The maximal mean height seedling value was measured for the group treated with *V. arvi* in the Rogozna population – 12.46 cm, while the lowest was measured for the Rudnik population group treated with *V. arvi* – 6.76 cm. The biggest seedling root collar mean value was detected in a group treated with *V. arvi* in the Rogozna population – 3.57 mm, and the lowest in Rudnik population plants treated with the same bacteria – 2.57 mm. The coefficient of variability value for seedling height ranges from 32.05 % (*V. arvi* treatment in Rogozna population) to 62.94 % (*V. arvi* treatment

in Rudnik population). The coefficient of variability value for seedling root collar diameter ranges from 14.74 % (control treatment in Rogozna population) to 49.7 % (*V. arvi* treatment in Rudnik population. Sessile oak seedlings mean sturdiness quotients with standard deviations and coefficient of variations are presented in Table 1.



Graph 1. Mean height (cm) and standard deviation of sessile oak seedlings



Graph 2. Mean root collar diameter (mm) and standard deviation of sessile oak seedlings

Table 1. The sturdiness quotient (SQ) of sessile oak seedlings

	SQ								
	V. arvi			*P. koreensis*			Control		
Provenances	M	SD	CV	M	SD	CV	M	SD	CV
Rudnik	24.72	7.71	31.20	27.49	8.18	29.76	29.42	7.39	25.13
Grabova reka	30.73	7.65	24.89	31.08	7.59	24.42	34.41	8.74	25.42
Rogozna	36.12	13.54	37.48	24.25	8.62	35.55	26.38	8.37	31.74

M – mean value, SD – standard deviation, CV – coefficient of variation

In order to study provenance and bacterial treatment influence on measured plant growth attributes, two-way ANOVA was performed (Tables 2 and 3).

Table 2. Analysis of variance for seedling height

Parameter	SS Effect	DF Effect	MS Effect	F	P value
Interaction	63.88	4	15.97	F (4, 76) = 1.092	P=0.3664
Provenance	236.2	2	118.1	F (2, 76) = 8.080	P=0.0007
Treatment	44.44	2	22.22	F (2, 76) = 1.520	P=0.2253

Table 3. Analysis of variance for seedling root collar diameter

Parameter	SS Effect	DF Effect	MS Effect	F	P value
Interaction	1.368	4	0.3420	F (4, 76) = 0.5963	P=0.6664
Provenance	10.51	2	5.256	F (2, 76) = 9.163	P=0.0003
Treatment	0.6838	2	0.3419	F (2, 76) = 0.5961	P=0.5535

For Pseudomonas koreensis was shown to produces ammonia, siderophore, protease, amylase and cellulase (Gu et al., 2020). It increased *Arabidopsis* plant fresh weight and lateral root development and antagonized plant pathogenic fungi. Jabborova (2022) reported *P. koreensis* IGPEB 17 to significantly increase plant height, leaf number, length, and width compared to control treatment in *Zingiber officinale*. *Viridibacillus arvi* was first described by Heyrman et al. (2005) as a novel species isolated from soil, and further reclassified by Albert et al. (2007). Both isolate species in our research demonstrated PGP traits (unpublished results). However, in the pot test with sessile oak seedlings, they didn't express a significant impact. A low number of plants might be one reason for this result. Also, concerning the life span of oaks, first year is maybe too early for measuring the effect of bacterial treatment. Some studies reported that PGPB beneficial effect can often be more visible in unfavorable environmental conditions (Aguilera-Torres et al., 2023), which wasn't our case. However, the provenance effect on seedling height and root collar diameter was expressed. Origin impact on seedling attributes is well known and recorded in many studies (Caliskan, 2014). The three presented populations derive from central, eastern and western part of Serbia, regions that differ in site conditions. Different evolution histories affect greatly on seedling's expressed attributes.

Conclusions

The results obtained in this study showed that bacterial treatment of sessile oak seedlings of different provenances didn't significantly improve plant height and root collar diameter. However, there were notable differences between provenances. The obtained results need to be verified, and further tests need to be conducted with a greater plant sample and more plant growth parameters included. Also, plant growth and survival should be examined after transplanting to the field.

Acknowledgments

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AWARENESS AND ADOPTION OF SCIENTIFIC AGRICULTURAL PRACTICES IN AGROFORESTRY HOME GARDENS WITH SPECIAL REFERENCE TO WELIGAMA, SRI LANKA

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Abstract

Agroforestry home gardens offer diverse land use systems, but lack of research exploration raises concerns about the adoption of scientific agricultural practices in Asia. This research attempts scientific practices awareness and adoption in agroforestry home gardens, focusing on Weligama Divisional Secretariat (DS), Sri Lanka. Three (03) Grama Niladari (GN) divisions were selected from the DS based on the availability of the highest number of agroforestry home gardens. The primary data were collected from randomly selected 30 households from each GN division (n = 90) by personal interviews through the use of a structured questionnaire. The adoption level was measured by 5 point Likert scale ranging from 1 to 5 with 1=Never, 5= highly adopted and the awareness was measured by 5 point Likert scale ranging from 1 to 5 with 1=Not at all aware, 5= Highly aware. The level of adoption of various scientific practices was calculated using the adoption quotient. Collected data were analyzed using nonparametric statistics. Results revealed that the majority (38.8%) were aware of scientific agricultural practices. The adoption of scientific agricultural practices in agroforestry home gardens are at moderate adoption level in selection of land (52.2%), land management practices for planting area (51.1%), selection of plant materials (48.9%), management of plant protection (66.7%) and maintenance of plants (58.9%). The overall adoption level of households is moderate (65.6%). A significant correlation ($r = 0.595$, $p < 0.01$) was found between scientific agricultural practices in agroforestry home gardens and awareness (Spearman correlation). Trainings and workshops improve awareness and extension programs for household adoption of scientific agricultural practices.

Keywords: *Agroforestry, Awareness, Scientific Practices, Plant Selection, Adoption.*

Introduction

The interactions between the trees and the other agricultural components of agroforestry should be advantageous from an ecological and economic perspective. Agroforestry can integrate production and conservation goals, which is the exact definition of sustainability, if such interactions are correctly implemented. It must be able to identify and measure the sustainability-related qualities that home gardens are said to possess. Home gardens are agroforestry land-use systems that are maintained by family labor and are situated inside the grounds of individual dwellings, close to seasonal and perennial livestock, and among agricultural crops (Fernandes and Nair 1986).

Food, fruits, vegetables, fodder, fuel, medications, and other items for domestic use are some of the principal objectives of home gardens (Kumar and Nair 2004; Hamilton and Hamilton 2008). Every home garden is different in terms of its composition, structure, utility, and aesthetics since it depends on the local natural ecology, the labor that can be provided by the family, and the interests, abilities, and enthusiasm of the family members. Home garden

cultivation is frequently fairly fluid. The household's needs for consumption and revenue creation dominate the decisions made about the choice of crops, acquisition of inputs, harvesting, management, etc.

The utilization of home gardens as a multifunctional land-use system in Sri Lanka and the benefits they provide during times of food shortage and climate change are not unusual. Similar evidence of various types of agroforestry and its desired influence to mitigate and adapt to climate change and to provide food security is emerging from other parts of the world, as shown in the scientific literature (e.g., Gray *et al*. 2014; Mbow *et al*. 2014b). According to Dilrukshi et al. (2013), the ability to meet changing human demands without harming and, if at all possible, improving the natural resource base on which it depends is how science describes the application of knowledge. A sustainable rural production system is just one component of the broader definition of sustainability, which also covers a number of factors that are not related to agriculture and can be categorized as economic, social, ecological, political, and institutional.

Therefore, this study focuses on accessing the adoption level of scientific practices in agroforestry home garden in Weligama Divisional Secretariat Division, Matara, Sri Lanka.

Materials and methods

Weligama Divisional Secretariat in Matara District was selected as the location based on the availability of comparatively high number of agroforestry home gardens. According to the (Fernandes and Nair., 1986) Agroforestry home gardening definition, many home gardens were identified in Weligama (Weligama Divisional Secretariat 2022). Further, Ministry of Agriculture, Sri Lanka is conducting " Haritha Dayak" Home gardening project in Weligama at present. Kotavila North, Kotavila South and Kotavila West GN divisions were selected from Weligama Divisional Secretariat based on the availability of highest number of agroforestry home gardens (Weligama Divisional Secretariat 2022). These GN Divisions were selected through reconnaissance survey conduct with Agriculture Instructors and Agriculture research and production assistants. In the study region, home gardens with multiple species, several stories, and multiple uses were regarded as agroforestry (De Zoysa 2022). The data were collected through a sample survey method at household level. The random selection of the households from each Grama Niladari (GN) division was based on a list held (N= 130) by the Agricultural extension officers and Agriculture Instructors. Therefore, respondents were selected based on random sampling technique. The primary data were collected from selected 30 households from each GN division (n = 90) by personal interviews through the use of a structured questionnaire. The evolution of home garden agro forestry was collected through interviewing households with open and closed ended questionnaires, and making small group discussion as practiced by Amenu (2017).

Secondary data has already been collected through Journals, website, books, and government records etc…

Table 1. Variables and measurements

Objective	Variable	Measurement
• To analyze households' awareness and adoption level of the scientific home gardening practices	Awareness and adoption of Scientific Agricultural of household practices in Agroforestry home gardens (De Zoysa 2022; Mattsson *et al*. 2017;Jacob & Tomas 2018). **Awareness and adoption to** 1.Selection of land • Topography of land • Availability of Sunlight • Supply of water/Water source • Soil Condition • Existing vegetation 2.Land management practices for planting area • Proper planning and efficient use of space • Integrated soil fertility management • Moisture improvement • Location of Water drainage system • Waste management • Soil conservation management • Soil acidity and soil depth 3.Selection of plant materials • Growing right plant in right place • Amount of Time required to maintain and harvesting • Selecting of quality seeds/plants 4.Management of Plant protection • Selecting cover crops to reduce rain splash erosion and control temperature • Arrangement of plants/trees for wind breaks(air circulation) and shelter breaks • Arrangement of Intercropping and crop rotation systems	5 point likert Scale Awareness 1= Not at all aware 2= Not aware 3= Neutral 4= Aware 5= Highly aware Adoption 1= Never 2= Not Adopted 3=Moderate 4= Adopted 5=Highly Adopted

	• Fencing management 5. Maintenance of plants • Frequency and timing of upgrade soil fertility • Pest and disease control • Biological & organic Pest and disease control methods(Non Chemical) • Integrated pest management (IPM) methods • Weed Management • Use of horticultural techniques like pruning, training and thinning	

Data collected from the interviews were coded, analyzed, interpreted and synthesized. Statistical Package for the Social Sciences (SPSS) was used for the analyses of data descriptively as well as inferentially.

The degree to which scientific farming practices have been adopted has been measured. Each household's adoption level was expressed using a 5 point scale. 1 = Never Adopted, 2 = Not Adopted, 3 = Moderate, 4 = Adopted, and 5 = Highly Adopted were the response categories and weighted values.

The level of adoption of various scientific practices were calculated using the adoption quotient based on formula developed by Sengupta (1976).

$$\boldsymbol{AQ = \frac{\sum_{i=1}^{n} \frac{ei}{pi} \times 100}{N}}$$

Where,

AQ= Adoption quotient

ei = Extent of adoption of each practice (Sum of the adoption score obtained) (Jacob1 *et al*. 2015)

pi = Potentiality of adoption of each practice (Maximum possible adoption score) (Jacob1 *et al*. 2015)

N = Total number of practices selected

After calculating the adoption quotient for the various scientific practices the adopters were categorized into three categories on the basis of mean and standard deviation (S.D.) low adoption = < (Mean – Standard Deviation (SD)), medium adoption = (Mean ± SD) and high adoption => (Mean + SD).Then compare with the standard Rogers curve (Jacob *et al*. 2018).

The other collected data were scored, tabulated and analyzed using different statistical methods like mean, frequency, percentage analysis and Person correlation analysis as proposed by De Zoysa (2022).

Results and discussion

Adoption level of scientific agricultural practices in agroforestry home gardens.

The agroforestry in the traditional home garden system would have been further enhanced by the scientific foundation of household practices. Agroforestry is linked to complexity in the creation of scientific base due to the inherent varied strands of knowledge and practice (Mbow *et al*. 2014). Agroforestry home gardens' multifaceted, multiproduct character necessitates a scientific foundation for their sophisticated management and cropping practices. Science-based agroforestry home gardening methods could be a useful conservation tool for relieving land-use strain and enhancing rural livelihoods.

Table 2. Adoption level of scientific agricultural practices in agroforestry home gardens

Variables	**Level of Adoption and Percentage(%) of households**		
	Low	**Medium**	**High**
1.Selection of land	20.0	52.2	27.8
• Topography of land	8.89	62.22	28.89
• Availability of Sunlight	18.89	76.67	4.44
• Supply of water/Water source	14.44	78.89	6.67
• Soil Condition	18.89	46.67	34.44
2.Land management practices for planting area	22.22	51.11	26.67
• Proper planning and efficient use of space	22.22	72.22	4.44
• Integrated soil fertility management	25.56	37.78	36.67
• Moisture improvement	20	74.44	5.56
• Location of Water drainage system	33.33	31.11	35.56
• Waste management	24.44	65.56	10
• Soil conservation management	16.67	74.44	8.89
• Soil acidity and soil depth	6.67	70	23.33
• Tillage	18.89	77.78	3.33
3.Selection of plant materials	16.67	48.89	34.44
• Growing right plant in right place	16.67	80.00	3.33
• Amount of Time required to maintain and harvesting	21.11	74.44	4.44
• Selecting of quality seeds/plants	21.11	75.56	3.33
4.Management of Plant protection	14.44	66.67	18.89
• Selecting cover crops to reduce rain splash erosion and control temperature	6.67	73.33	20
• Arrangement of plants/trees for wind breaks(air circulation) and shelter breaks	10	71.11	18.89

• Arrangement of Intercropping and crop rotation systems	32.22	30.00	37.78
• Fencing management	13.33	71.11	15.56
5.Maintenance of plants	21.11	58.89	20
• Frequency and timing of upgrade soil fertility	28.89	64.44	6.67
• Pest and disease control (Chemical)	2.22	81.11	16.67
• Biological & organic Pest and disease control methods(Non Chemical)	24.44	61.11	14.44
• Integrated pest management (IPM) methods	0	88.89	11.11
• Weed Management	15.56	78.89	5.56
• Use of horticultural techniques like pruning, training and thinning	20	75.56	4.44

The results shown in Table 2 show that the majority of households were medium adopters of concern about the topography of land (62.22%), availability of sunlight (76.67%), supply of water or water source (78.89%), soil condition (46.67%), and existing vegetation (70%) during land selection. More than 50% of respondents have moderate levels of adoption for the selection of land practices. The mean ± standard deviation of the adoption quotient of land selection is 63.24 ± 15.70. Due to the current water tariff, most farmers have established agricultural wells on their farmland. The households have experience and good knowledge regarding crop rotation and selecting crops according to the availability of sunlight.

In order to ensure a variety of productions and ecosystem services in home gardens, households frequently plant crops and trees, which is one of the key strategies for maintaining biodiversity conservation. According to research by Bardhan *et al*. (2012), agroforestry home gardens, which make up a significant portion of the total land area in human-dominated environments, operate as a bridge for the conservation of biodiversity.

From table 2, it is evident that the majority of the respondents have a moderate adoption level for proper planning and efficient use of space (72.22%), soil improvement (74.44%), waste management (65.56%), soil conservation management (74.44%), soil acidity and soil depth (70%), and tillage (77.78%). The difference between the moderate adoption level of respondents (37.78%) and the high adoption level of respondents(36.67%) of integrated soil fertility management practices is less. 35.56% of respondents have a high adoption level for the location of water drainage systems. The households have experience with the improvement of soil chemical and physical properties by multi-cropping systems, particularly legumes from agroforestry, in their home gardens (De Zoysa 2022).

Due to a lack of scientific information that causes soil erosion during heavy rains and food poverty during droughts, households may occasionally destroy plant diversity in their home gardens (Linger 2014). But according to a study conducted in Sri Lanka, households there engage in conservation measures that considerably improve the soil, water, nutrients, and biodiversity in their gardens at home (Jeyavanan *et al*. 2017).

Growing gliricidia trees (*Gliricidia maculate* syn. G. sepium) on purpose next to fences, as well as different leguminous plants like Kathuru Murunga (*Sesbania grandiflora*), Winged

Bean (*Psophocarpus tetragonolobus*), and others, are common practices that affect the fertility of the home gardens in the study area. They are well aware that in agroforestry home gardens, large levels of on-site nutrient conservation typically establish stable and partially self-generating ecosystems through mixing effects that enhance the fertility of the soil. Indigenous knowledge systems of agroforestry in home gardens demonstrate that families have knowledge of how native legume species increase land productivity more than non-legumes do (Pinho *et al*. 2012).

When considering the adoption level, more than 50% of respondents have moderate level adoption for selection of the right plant in the right place (80%), the amount of time required to maintain and harvest (74.44%), and the selection of quality seeds or plants (75.56%). As mentioned in Table 2, the adoption level for selecting plant materials was medium, and 48.89% responded.

The households prepare the ground in accordance with customary practices and plant crops and trees depending on the availability of preferred and dependable planting materials from the government programs and their neighbors. When competing for nutrients, growing space, light, and soil moisture, trees and associated crops may experience yield losses that go unnoticed by homeowners. There isn't much room for vertical root complementarity even though various and superficial tree root structures are common in agroforestry to replenish water and absorb available nutrients.

The majority of respondents practiced medium adoption. As mentioned in figure 5, selecting cover crops to reduce rain splash erosion and control temperature (73.33%), arranging plants and trees for wind breaks(air circulation) and shelter breaks (71.11%), and fencing management (71.11%) practices were in the category of medium level of adoption. From table 2, it was observed that 37.78% of households practiced intercropping and crop rotation systems in the category of high level of adoption. To prevent pests and diseases, most farmers and households practiced the crop rotation method. This study showed that most of the respondents (66.67%) were moderate adopters of the management of plant protection practices.

In gardens, planting is done both directly and by transplanting from a seedbed. The majority of authors mention intercropping, while Brierley (1985) observed single plantings of clove trees and vegetables. When better planting material is introduced, home gardeners may move from intercropping to single-cropping.

Frequency and timing of upgrading soil fertility (64.44%), pest and disease control (chemical) (81.11%), biological and organic pest and disease control methods (non-chemical (61.11%), integrated pest management (IPM) methods (88.89%), and weed Management (78.89%) and use of horticultural techniques like pruning, training, and thinning(75.56% of practices) were practiced by moderate adopters. Most farmers and households practice organic fertilizers and cultural practices. This study showed that most of the respondents (58.89%) were moderate adopters of plant maintenance practices.

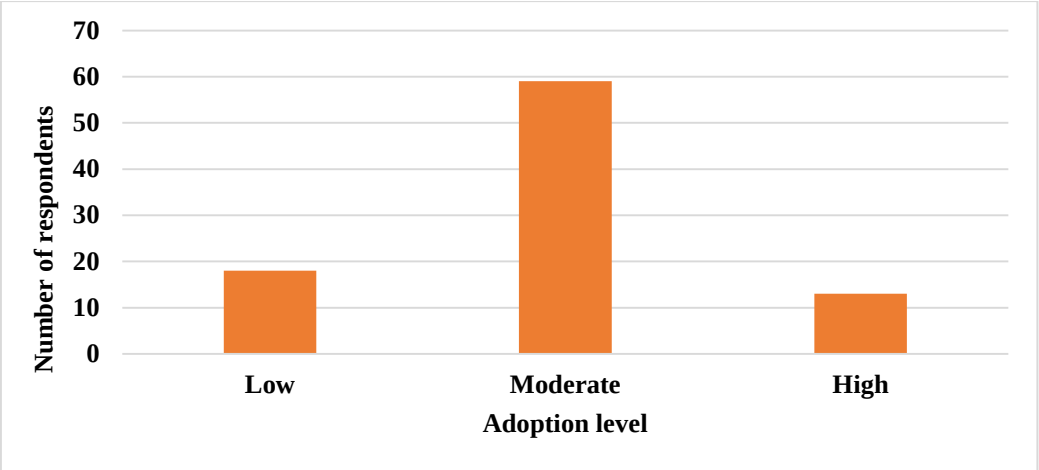

Figure 1: overall adoption level

The mean and standard deviation of the overall adoption quotient are 58.24 and 15.19, respectively, and they range from 34 to 100. The lowest value that can be obtained for the adoption quotient is 0, and the maximum value is 100.
The adoption level is considered as follows:

- If the adoption quotient is greater than 73.43 (58.24 + 15.19), then the level of adoption is high.
- If the adoption quotient is between 43.05 and 73.43, then the level of adoption is moderate.
- If the adoption quotient is lower than 43.05 (58.24–15.19), then the level of adoption is low.

The majority of respondents have a moderate level of adoption, which is 65.6%, as shown in Figure 1. so, the level of adoption of scientific agroforestry home gardening practices is moderate.

Table 3: Households' Awareness on scientific practices of home gardens

	n	%
1. Not at all aware	0	0
2. Not aware	4	4.4
3. Neutral	33	36.6
4. Aware	35	38.8
5. Highly aware	18	20

Table 3 shows that the majority of respondents are aware (35) of scientific applications in agroforestry home gardens, followed by neutral (33) and then highly aware (18). Also, none of them are at all aware of the scientific application of agroforestry in the home garden. Therefore, there is a good level of awareness of scientific application in agroforestry and home gardens.

The awareness and adoption levels of scientific agricultural practices have a statistically significant relationship. The Spearman correlation analysis shows that the correlation coefficient is significant below the 0.05 level since the p-value (0.000) is lower than 0.05. The coefficient is positive, and it is 0.595. So, the strength of the association is moderate. Therefore, adoption levels are significantly increased when increasing awareness about scientific applications in agroforestry and home gardens. Awareness creation is a promising strategy to encourage households to scale up home gardens with appropriate components (Mehari & Abera 2019).

Conclusion

The results of study, identified that households' awareness on scientific practices of agroforestry home gardens was good.

The level of adoption of scientific agriculture practices in agroforestry home garden. Selecting of land, Land management practices for planting area, Selection of plant materials, Management of Plant protection and Maintenance of plants are leveled as medium adoption. Overall adoption level of scientific agriculture practices in agroforestry home garden were moderate.

The adoption level was significantly increased, when increasing the awareness about scientific agricultural practices in agroforestry home gardens. Awareness and the adoption level is positively correlated.

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