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Original scientific paper

Pre-sowing treatments with gibberellic acid in white clover

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Abstract

The aim of this study was to analyze the effect of pre-sowing treatments with different concentrations of gibberellic acid solution, as a growth stimulator, on root and stem growth, seedling weight and nodulation of young white clover plants. The experiment was performed in 2019 in the laboratory for seed seed control at the Faculty of Agriculture in Čačak. Seed of rivendel cultivar were used. Six treatments with gibberellic acid were applied (control, 0.25; 0.5; 0.75; 1.0 and 1.5 mmol L^{-1} gibberellic acid). Root length, stem length and seedling weight were evaluated. Seedlings from the parallel experiment were planted in containers with substrate and cultivate in the greenhouse.Plants were analyzed 45 days after treatment. The obtained results indicate that pre-sowing treatments with gibberellic acid at a concentration of 1 and 1.5 mmol L^{-1} can significantly affect more intensive growth of plants of white clover.

Key words: white clover, gibberellic acid, nodulation, plant growth, seed

Introduction

Gibberellins were discovered in Japan in the 1930s, when a group of agricultural scientists studied rice diseases. The symptoms of the disease are characterized by excessive stem growth, yellowing of infected leaves and lower seed production. Further study has shown that this is a consequence of the action of certain phytopathogenic microorganisms, which, in addition to damaging plant parts, also affect their more intensive growth. It has been determined that the cause of increased growth is the presence of compounds that we now call gibberellins (GA),

which were formed as a product of the secretion of some microorganisms (Camara et al., 2018). Further research has shown that gibberellins are also formed in plants, in tissues that are actively growing, such as the apical parts of the stem, young leaves, roots, immature seeds and fruits. From the site of synthesis, gibberellins are transported in all directions, from the roots by transpiration flow, and from the apical parts through the phloem. Today, about 140 different molecules of gibberellin isolated from plants and microorganisms are known, but only a part of them is biologically active. Those with the highest biological properties are commercially available today (GA3, GA4, GA7).

The most common gibberellin is gibberellic acid (GA3). It is a tetracyclic diterpenoid carboxylic acid, which acts as a growth hormone in plants. There are numerous studies that deal with the possibility of its application in agriculture in order to promote the growth and development of plants. Giberellic acid is used to improve seed germination and plant germination (Chen et al., 2008; Urbanova and Leubner-Metzger, 2016), reduce stress due to adverse environmental conditions (Colebrook et al., 2014), better fruit growth (Li et al., 2011), more intensive stem growth (Wang et al. 2017), has a positive effect on flowering (Muñoz-Fambuena et al., 2012) and other physiological effects that occur in interaction with other phytohormones (Hedden and Sponsel, 2015). In leguminous plants, the optimal level of gibberellic acid in plants affects the increase in nodulation (Liu et al., 2018).

White clover (*Trifolium repens* L.) is one of the most important perennial forage legumes that are sown in a mixture with grasses. Such mixtures, in addition to high yield and quality of forage for feeding domestic animals, are distinguished ability to withstand a wide range of grazing conditions. However, at critical times for livestock feeding, such as following calving or lambing in late winter and early spring, pasture growth rates can be insufficient to meet livestock requirements. Despite low temperatures at this time, it has been shown that the application of exogenous gibberellic acid can stimulate crop growth (Bryant et al., 2016). The aim of this study was to analyze the effect of pre-sowing treatments with different concentrations of gibberellic acid on root and stem growth, seedling weight and nodulation of white clover plants, 10 and 45 days after sowing.

Material and Methods

The experiment was performed in 2019 in the laboratory for seed control at the Faculty of Agriculture in Čačak. Declared white clover seeds of the Rivendel variety (Denmark) were used for the experiment. Six treatments with gibberellic acid were used (control - without

gibberellic acid, 0.25; 0.5; 0.75; 1.0 and 1.5 mmol L^{-1} gibberellic acid). Treatments were applied by soaking the seeds between the filter paper, before placing them on germination, using 4 ml of solution per replication. After 12 hours of treatment, the seeds were washed with distilled water. Germination was performed on filter paper in four replications with 100 seeds each. The seeds were germinated under controlled conditions in an climate chamber at a temperature of 20 °C. After 10 days, as long as the germination lasted, measurements of the length of root and stem of the seedlings were performed with a ruler, and the mass of the seedlings was determined on the analytical scale. Seedlings from a parallel experiment set up in the same way were planted in containers with substrate at this time and its cultivate in a greenhouse. Measurements were performed 45 days after sowing by taking 10 plants for each replication. The substrate from the roots of the plants was removed by rinsing with water. The nodules at the root were then counted and dried on paper at room temperature. After drying, the root was separated from the stem with scissors and the dry matter mass of the root and the dry matter mass of the stem were analyzed.

The obtained results were processed by the method of analysis of variance of one - factorial experiment (ANOVA), using SPSS 4,5 software. The significance of the difference in mean treatment values was tested by the LSD test.

Results and Discussion

Pre-sowing treatments with gibberellic acid had not a significant effect on the average root length of white clover seedlings in the initial stage of growth 10 days after germination (Table 1.) The average root length of white clover in this phase was 5.2 mm. However, treatment with 1.5 mmol L^{-1} of gibberellic acid had a significant positive effect on the length of the seedling stem in relation to the control variant in the mentioned phase. The average length of the seedling stem was 27.5 mm. There were significant differences in seedling weight between pre-sowing treatments with gibberellic acid. Treatment with gibberellic acid at a concentration of 0.5% significantly affected the increase in seedling weight, ten days after germination compared to the control variant. At the same time, a significantly higher mass of seedlings was recorded on the treatment of 0.5 mmol L^{-1}, compared to the treatments of 0.25 and 1 mmol L^{-1}. The average seedling weight was 0.00434 g. These results confirm the results of the other authors. Giberellic acid plays an important role in many essential plant growth and development processes, including seed germination, stem elongation, and leaf growth (Khan and Chaudhry, 2006). It has a positive effect on the activation of embryo growth, mobilization

of nutrition reserves in the seeds and softening of the endosperm (Baskin and Baskin, 2014; Pallaoro et al., 2016). According to Golmohammadzah et al. (2015) gibberalic acid activates the synthesis of proteins and other metabolites required by the embryo for germination.

Pre-sowing treatment with gibberellic acid at a concentration of 1 mmol L^{-1} in this experiment significantly affected the increase in the average root mass of white clover plants in stage 45 days after sowing, when compared to the control variant (Table 2). The same treatment, together with the treatment of 1.5 mmol L^{-1}, significantly increased the mass of the aboveground part of the plants. The results obtained in these studies are in accordance with the results of other authors obtained in other plants. It was noted that pre-sowing treatments of seeds with gibberellic acid affected the improvement of germination and growth parameters of young plants, such as root length, stem length, weight of young plants in *Capparis spinosa* L. (Heydaryan et al., 2014) *Trigonella foenum-graecum* L. (Farahmandfar et al., 2013), *Hibiscus sabdarifa* L. (Ali et al., 2012), *Trifolium repens* L. (Galhaut et al., 2014), *Beta vulgaris* (Dotto and Silva, 2017), Zea *mays* L. (Ghodrat and Rousta, 2012) and *Medicago sativa* L. (Younesi and Moradi, 2014). The positive effect of treatment with gibberellic acid on the initial growth of plants of some species continues in later stages, which leads to an increase in seed yield, as indicated by the research of numerous authors. Pre-sowing seed treatments with gibberellic acid affected the yield of sunflower per head by 123.5% (Ulfat et al., 2017) and the yield of wheat per unit area by 7% (Jafri et al., 2015).

Gibberellins are required for nodule formation at the root of legumes (Liu et al., 2018). However, high concentrations of gibberalic acid can inhibit nodulation (Ferguson et al., 2005). This indicates that nodulation requires an optimal level of gibberellic acid. The number of nodules at the root of young plants in this experiment was significantly higher only on the treatment of 1.5 mmol L^{-1} compared to the control variant. Since this was the highest concentration of gibberellic acid in our experiment, the influence of higher concentrations on nodulation should be analyzed in further research. According to Bryant et al. (2016), the application of gibberellic acid on a mixture of perennial ryegrass and white clover during the growing season had a positive effect on increasing the dry matter content in the shoots, the ratio of leaf and stem proportions and the boron content in perennial ryegrass plants.

Table 1. Effect of pre-sowing treatments with gibberellic acid on root length, stem length and weight of white clover seedlings, 10 days after treatment

Gibberellic acid concentration (mmol L^{-1})	Average root length (mm)	Average stem length (mm)	Average seedling mass (g)
Ø	5,03a	24,02b	0,00358bc
0,25	4,70a	27,82ab	0,00320c
0,5	5,38a	29,00ab	0,00545a
0,75	4,53a	24,60b	0,00502ab
1	7,15a	27,70ab	0,00352bc
1,5	4,70a	31,77a	0,00525ab

Table 2. Effect of pre-sowing treatments with gibberellic acid on root mass, stem mass and number of nodules on white clover root, 45 days after treatment

Gibberellic acid concentration (mmol L^{-1})	Average root mass (g)	Average stem mass (g)	Number of nodules on the roots
Ø	0,524b	1,619b	7,4b
0,25	0,576ab	2,019ab	10,3ab
0,5	0,536b	1,931ab	10,2ab
0,75	0,539b	1,734ab	10,2ab
1	0,663a	2,063a	15,2ab
1,5	0,601ab	2,041a	17,1a

Conclusion

Pre-sowing treatments with gibberellic acid in white clover had a positive effect on stem length and seedling weight in stage 10 days after germination. Also, positive effects of the treatments in terms of root mass and aboveground part were found in plants in stage 45 days after sowing. Pre-sowing treatment with gibberellic acid at a concentration of 1.5 mmol L^{-1} increased the number of nodules at the root of young white clover plants.

The obtained results indicate that pre-sowing treatments with gibberallic acid at a concentration of 1 and 1.5 mmol L^{-1} could significantly affect the more intensive growth of young white clover plants.

Acknowledgement

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Original scientific paper

Analysis of the water regime of chernozem under winter wheat crops in the region of Zemun from 1966/67 to 2019/20

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Abstract

The research covered the period from 1966/67-2019/20 to analyze the water regime of chernozem soil in the area of Zemun under winter wheat crops. The aim was to determine whether the water regime of the rhizosphere layer of soil under winter wheat has improved or deteriorated over the last fifty-four years. The analysis was performed using the FAO CROPWAT 8.0 crop model. Daily minimum and maximum air temperatures as well as the amount of precipitation measured at meteorological station Surcin were used. Reference evapotranspiration (ETo) was calculated by the modified Hargreaves method. Simulations of winter weat potential evapotranspiration (ETc), irrigation requirements and yield reduction were investigated. Analyzes showed that the average ETc was about 350 mm, and winter wheat irrigation requirements were 173 mm. Going from the first (1966/67-1985/86) to the third (2006/07-2019/20) period of the research, an increase in ETc of 7% and irrigation requirements of 10% was registered. A reduction in winter wheat yield was also observed. During the first period (1966/67-1985/86) the yield reduction was 6%, and during the second (1986/87-2005/06) and the third (2006/07-2019/20) period was 10% in relation to genetic crop potential. Winter wheat had the highest water requirements (ETc) during April and May (95mm and 99mm) with a tendency to increase from the first to the third period. The highest irrigation requirements were also in April and May (57mm and 54mm) without regularity in distribution comparing the three examined periods.

The general conclusion is that the water regime of chernozem under winter wheat crops in the area of Zemun had slightly changed in the last 54 years. Simulations show that crop water requirements, irrigation requirements and yield reduction are slightly increasing.

Key words: Winter wheat, CROPWAT, ETc, irrigation requirements, yield reduction

Introduction

In Serbia,winter wheat is grown on an area of about 2600 ha (average for the period 2014-2018) and achieves an average yield of 4.3 t/ha (Statistical Calendar of the Republic of Serbia 2020). From year to year, the variation of yield was expressed as a result of fluctuating weather conditions. The dependence of winter wheat yield on climatic conditions has been established by many authors (Malešević et al., 2008; Jaćimović et al., 2013; Matović et al., 2013). Climatic conditions, in addition to affecting the final yield, are also reflected on the starch quality in grain wheat and flour (Živančev et al., 2017). The greatest limitation of crop growth is caused by temperature stress and water stress (Gibson et al., 2003). Matović et al. 2020 dealt with the analysis of the water regime of chernozem under the maize crop, in the last half century (1966-2019). They presented that there was a decrease in actual evapotranspiration, and an increase in potential evapotranspiration of maize, as well as an increase in water deficit, i.e. the maize irrigation requirements. The increase in the water deficit also led to an increase in the simulated reduction in maize yields.

This paper builds on the previously mentioned research, with the goal of examining the water regime of that same chernozem in the same period, but under the winter wheat crop. This includes the analysis of the growing period, which begins in October and ends in June of the following year, unlike maize, whose growing season lasts from April to September. The goal of this paper is to establish whether there was a change in the water regime of soil under winter wheat crops from 1966/67 to 2019/20. The goal is to examine whether winter wheat potential evapotranspiration, irrigation requirements and yield reduction have increased or decreased over time. Such research is important because it contributes to understanding the problems of previous plant production, and also provides assistance in assessing and planning future plant production and the application of adaptation measures to climate change.

Material and Methods

The research included the analysis of the water regime of chernozem soil under winter wheat crops in the area of Zemun from 1966/67 to 2019/20. The analysis was performed using the FAO CROPWAT 8.0 crop model. During the research, daily data of minimum and maximum air temperature were used, as well as the amount of precipitation from the meteorological station Surcin. Reference evapotranspiration (ETo) was calculated by the modified Hargreaves method (Trajković, 2007). Potential winter wheat evapotranspiration (ETc) was obtained as a product of ETo and crop coefficient. The crop coefficient values are in accordance with data

from FAO publication 56 (Allen et al., 1998). The growing period of winter wheat from sowing on October 16th to harvest on June 22nd was simulated. The soil is chernozem with an amount of total available water 170mm per meter of depth. It was adopted that initial root zone soil moisture was at the level of field capacity. Simulations of ETc, irrigation requirements and yield reduction were investigated. The seasonal and monthly distribution of these parameters were analyzed.

Results and Discussion

The growing period of winter wheat, which begins on October 16th and lasts until June 22nd of the following year, was analyzed. The analyzes were performed by dividing the whole period 1966/67-2019/20 into three periods: the first (1966/67-1985/86), the second (1986/87-2005/06) and the third (2006/07-2019/20).

Mean air temperature in winter wheat growing season (October–June) in the area of Zemun is 9.0 ºC, and average amount of effective precipitation is 341 mm. The air temperature shows an upward trend during the study period, both maximum (Tmax) and minimum (Tmin) values (Figure 1a). The increase is more pronounced at Tmax than at Tmin. In the second period the Tmax is higher by 0.5 ºC, and in the third by as much as 1.8 ºC, compared to the first period.

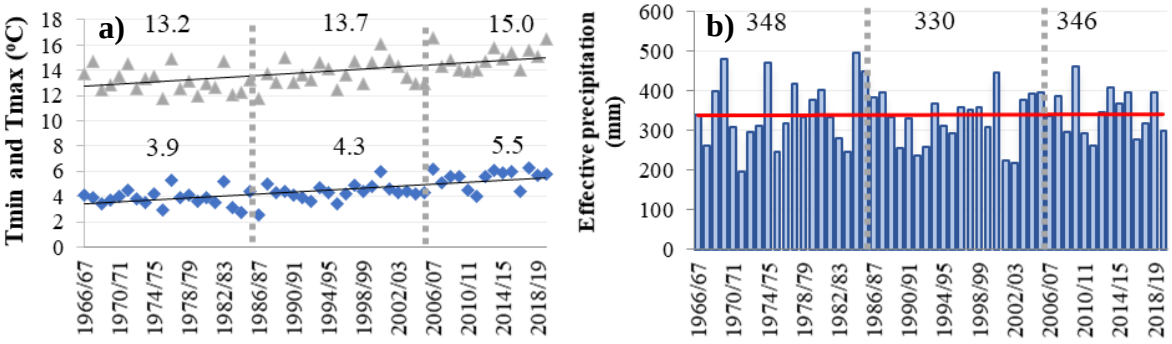


Figure 1. a) Maximum (Tmax) and minimum (Tmin) air temperature and **b)** effective precipitation, during the winter wheat growing season (October 16th to June 22nd) in the area of Zemun from 1966/67 to 2019/20.

During the winter wheat growing season, precipitation ranged from 198mm to 495mm, with an average of 341mm. Throughout the observed period (1966/67-2019/20) no relevant difference in precipitation was found between the three observed periods (Figure 1b). It is interesting to note that the growing season rainfall for spring crops (April-September) in the

same area and in the same period (1966-2019) showed a declining trend of 39% in the third period (2006-2019) compared to the first (1966-1985) (Matović et al., 2020).

The simulated average winter wheat water requirements (ETc) over a 54-year period is 348 mm. From year to year, the values oscillated between 299 and 386 mm, with a slight increase from the first to the third period (Figure 2a). A positive trend of percentage deviation is registered from 1993/94 until the end of the research period (Figure 2b).

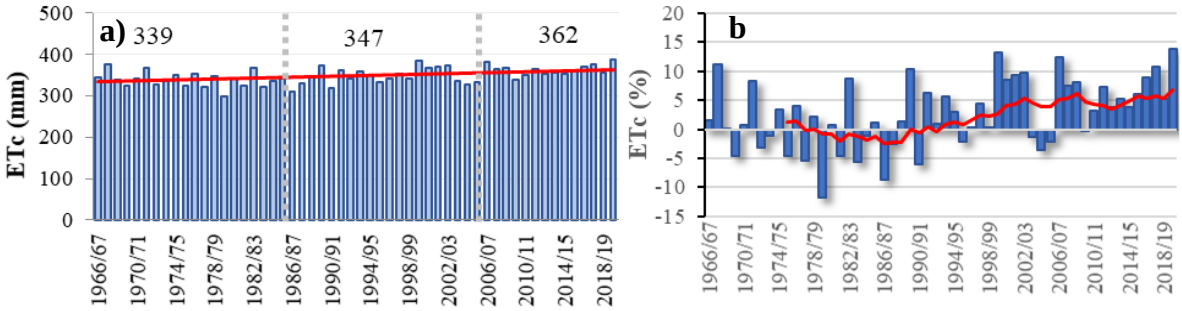


Figure 2. a) Winter wheat water requirements (ETc) during the growing periods from 1966/67 to 2019/20. The printed values represent the average for each of the three selected periods; b) Percentage deviation of ETc during the study period (1966/67-2019/20) relative to the reference period (1966/67-1985/86) mean. The curve shows a ten-year moving average.

Winter wheat irrigation requirements is on average 173 mm, with an amplitude of 95 to 289 mm. During the observed period, there is a slight increase in simulated irrigation requirements (Figure 3a), which in the second period is 9% and in the third 10%, compared to the first period. The percentage deviation of irrigation requirements for winter wheat in relation to the first (reference) period is positive throughout the second half of the study period (Figure 3b).

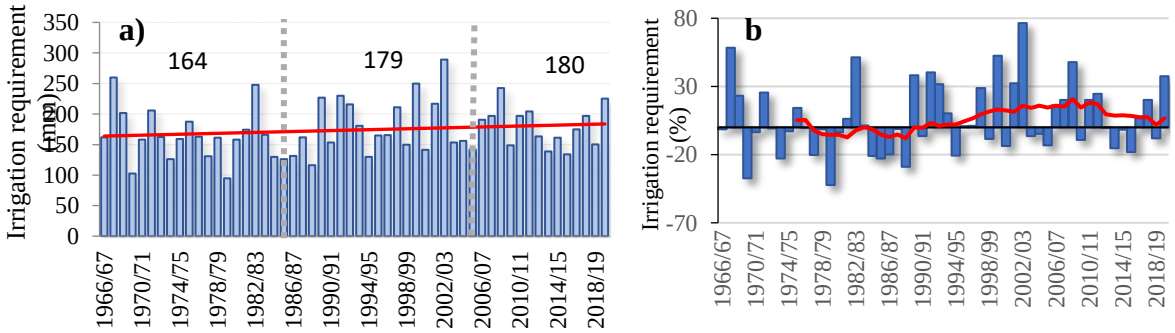


Figure 3. a) Simulated irrigation requirements for winter wheat from 1966/67 to 2019/20. The printed values represent the average for each of the three selected periods; b) Percentage deviation of irrigation requirements during the study period (1966/67-2019/20) relative to the reference period (1966/67-1985/86) mean. The curve shows a ten-year moving average.

In accordance with a slight increase in irrigation requirements, a slight increase in the yield reduction of winter wheat is projected during the second and third research periods compared to the first one (Figure 4). During the first period, the simulated yield reduction in relation to the crop genetic potential was reduced by an average of 6.5%, and during the second and third periods by 9.6%.

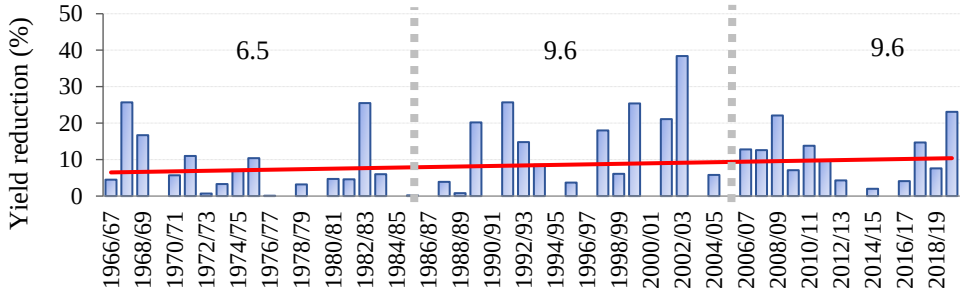
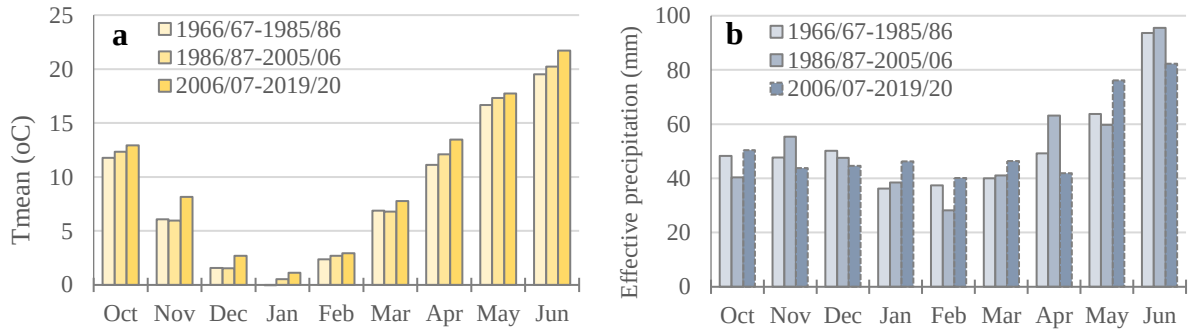


Figure 4. Simulated reduction of winter wheat yield in relation to genetic potential from 1966/67 to 2019/20. The printed values represent the average reduction values (%) for each of the three selected periods.

If we pay attention to the amount of precipitation during the growing period and the potential evapotranspiration of winter wheat (ETc), it is noticed that these values are approximate, i.e. that the total amount of precipitation just provides winter wheat water requirements. However, as shown in Figure 3a, a water deficit (averaging about 170mm) is registered each year. The explanation for this illogicality is in the monthly distribution of parameters on which the crop's water supply depends. The calculation of total precipitation also includes precipitation from the winter period, when the physiological activity, as well as water consumption of winter wheat is reduced to a minimum or not at all.

The monthly distribution of air temperature throughout the three observed periods (1966/67-1985/86, 1986/87-2005/06 and 2006/07-2019/20) shows an increase in Tmean during almost all months. The largest temperature changes were recorded in April, when an increase in Tmean of 1^{o}C (in the second period) and 2.3^{o}C (in the third period) was registered, compared to the first one (Figure 5a). The monthly distribution of precipitation during the three studied periods did not show any regularity (Figure 5b).



igure 5. a) The monthly mean air temperature (Tmean) and b) monthly effective precipitation in the area of Zemun during the three periods: 1966/67-1985/86, 1986/87-2005/06 and 2006/07-2019/20.

Winter wheat had the highest water requirements in April (95mm) and May (99mm) (Figure 6a), when the highest irrigation requirements were registered (in April - 57mm; in May - 54mm) (Figure 6b). During May, winter wheat is in the flowering phase, when it shows the greatest sensitivity to water deficit (Jovanović and Stikić, 2012). The importance of the analysis of the monthly distribution of soil moisture parameters indicated Jaćimović et al., 2013. Their research was carried out on Rimski Šančevi (1965/66 to 2009/10). They showed that the greatest influence on the grain yield of winter wheat had the conditions of humidity/drought in October, December, March and April. During November and April, lower moisture conditions (drought) reduced yields, while in December and March initiated an increase in yields.

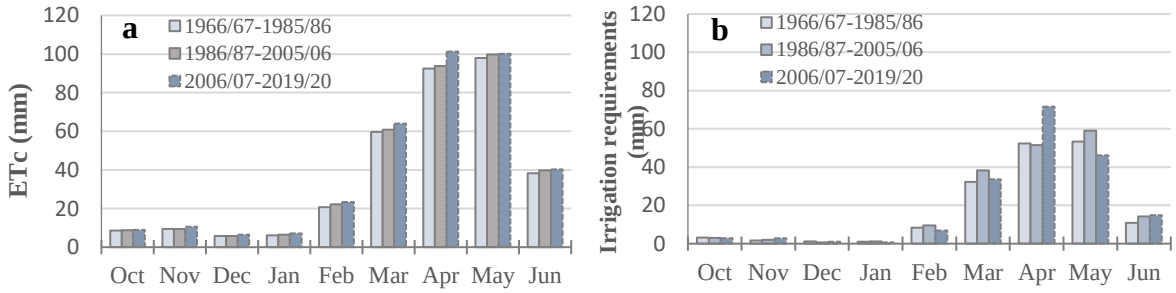


Figure 6. a) The monthly mean winter wheat water requirements (ETc) and b) the monthly mean irrigation requirements in the area of Zemun during the three periods: 1966/67-1985/86, 1986/87-2005/06 and 2006/07-2019/20.

The monthly distribution (Figure 6a), showed a small increase in winter wheat water requirements (ETc) from the first to the third studied period. The largest increase in ETc is observed during April (10% increase in the third period compared to the first). Unlike ETc, the monthly distribution of irrigation requirements does not show any regularity by comparing the

three periods (Figure 6b). This is to be expected, since Irrigation requirements depends on all parameters that affect on water balance in the rhizosphere layer of the soil. Thus, the peak of irrigation requirements in April (in the third period) (Figure 6b) can be explained by specific circumstances that implied a very small amount of precipitation (Figure 5b) and a pronounced increase in air temperature (Figure 5a) which reflected on increase in ETc (Figure 6a).

Conclusion

Based on the analysis of CROPWAT 8.0 simulations of the water regime of Zemun chernozem under winter wheat crops in the period from 1966/67 to 2019/20, the following conclusions were reached:

The average winter wheat water requirements (ETc) is about 350 mm. A slight increase in ETc of 7% was expressed from the first (1966/67-1985/86) to the third (2006/07-2019/20) period.

The average irrigation requirements is 173 mm. There is a slight increase of 10% from the first to the third period.

There is an increase in reductions in winter wheat yield from the first to the third period of research. During the first period the yield reduction was 6%, and during the second and third period 10% in relation to the genetic crop potential.

Analyzing the monthly distribution of simulated parameters, April and May stand out as periods when winter wheat water requirements is highest (over 90mm per month) and when irrigation requirements are over 50mm per month.

Simulations show that there has been a change in the water regime of Zemun chernozem under winter wheat crops in the last 54 years. ETc, irrigation requirements and yield reduction showed a slight increase during this period.

Acknowledgement

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Original scientific paper

Fruit characteristics of *Pyrus elaeagrifolia* Pall. genotypes in Eastern Turkey

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Abstract

More recently, there were increased interest to lesser-known fruits, which found in nature as free of pests and diseases. One of this specie is the oleaster-leafed pear (*Pyrus elaeagrifolia*) and its fruit is highly valued for both processing and human health benefits. In this study, a comparative study on phenological (ripening dates), morphological (fruit weight, fruit length/width ratio, fruit pedicel length, fruit flesh texture, fruit firmness and the number of seeds per fruit) and biochemical (soluble solid content, titratable acidity, total phenolic content, vitamin C and antioxidant activity) characteristics of twenty-six oleaster-leafed pear (*Pyrus elaeagrifolia*) genotypes were determined. Ripening dates, fruit weight, fruit length/width ratio, fruit pedicel length and fruit firmness were in range of 18 October to 07 November; 6.19 g to 21.04 g; 0.75 to 1.03; 7.11 mm to 18.56 mm and 3.84 to 8.22 kg/cm^2, respectively. Soluble Solid Content (SSC), titratable acidity, vitamin C and total phenolic content were found between 11.90-20.35%; 0.42-1.24%; 4.7-7.3 mg/100 g and 57-108 mg gallic acid equivalent per 100 g fresh fruit base, respectively. Oleaster-leafed pear fruit contains edible and non-edible portions, and this study provided the one of the first detailed report on the diversity of a wide range of phenological, morphological and biochemical data in edible parts of the fruit.

Key words: Pyrus elaeagrifolia, diversity, morphology, biochemical content

Introduction

Turkey including three bio-geographical regions namely Euro-Siberian, Mediterranean and Irano-Turanian and their transition zones and climatic and geographical features change within

short intervals of space in the country resulted nine different agro-climatic regions. These regions have diverse ecosystems including forest, mountain, steppe and wetlands and different forms and combinations of these systems. This extraordinary ecosystem and habitat diversity has produced considerable species diversity (Ercisli et al., 2008; Serce et al., 2010).

The country has very rich wild edible fruit diversity. For centuries, wild edible fruits are the main resource, which humans use to maintain their existence on Earth, and they have been used in every aspect of life in Anatolia (Ercisli and Esitken, 2004).

In Anatolia there are hundreds of species of wild edible fruits, which are propagated by seeds for centuries. They are mostly free pests and diseases and eaten raw as a snack food. Many wild edible fruits provide a useful source of nutrients including carbohydrates, lipids, proteins, vitamins, minerals, water etc. In Turkey rural people are familiar with a wide range of different wild edible fruits. There was considerable variation in wild edible fruit abundance and consumption between agro-climatic regions in Turkey and each region characterized by abundance of different wild edible fruits. There were also differences among regions in the amount consumed by different family members; children generally ate the most (Sahin et al., 2002; Ercisli, 2004).

Wild edible fruits in Turkey are still waiting for studies to be conducted on them, especially, have great potential to be beneficial in all dimensions of life: medicine, food, clothing, etc. Hence, through ethnobotanical investigations we can discover new possibilities for our future, arising from our unwritten memory.

Oleaster-leafed pear (*Pyrus elaeagrifolia*) is one of the most popular wild edible fruits in Turkey and native to dry parts in particular central and eastern Anatolia. It is highly resistant against severe climate conditions including cold, heat, calcareous soils etc. Therefore, they easily grow over dry and calcareous sites over which the other *Pyrus* species are not grown. The plant 4-10 m in height with grey-green hairy leaves propagated by seeds, needs very little care, start bearing 7-8 years and lasts for several decades. Young shoots are grey and highly hairy and branches are thorny. The sandy fruits are pseudocarp with 3-4 cm diameter spherical-pear like shape. Non-hairy fruits are initially green and then turn into yellow-brown. The fruits have a short pedicle, acidic taste. Flowering period generally covers spring season between the months April and May. Sandy fruits are collected in fall and flesh mesocarp is eaten fresh or preserved in molasses, jam, pickle, vinegar or syrup and fruits used as tea. Seedlings are used as rootstocks for commercial pear cultivars (Cansaran et al., 2007; Yerliturk et al., 2008). The plant is also native to Albania, Bulgaria, Romania, and Crimea as well.

In eastern Anatolia, oleaster-leafed pears are widespread over northern part of Erzurum where steppe climate with hot and dry summers and cold and snowy winters occurs.

In present study, naturally widespread and locally consumed oleaster-leafed pears grown in Erzurum province located in eastern Anatolia in Turkey were investigated and basic phonological, morphological and biochemical tree and fruit characteristics of different oleaster-leafed pear genotypes were determined.

Materials and Methods

In present study, fruit of 26 wild oleaster-leafed pear trees naturally grown in rural areas of Uzundere and Tortum districts belongs to Erzurum province in eastern Turkey were sampled in 2018 year. For phenological observation fruit-ripening time was determined. For morphological evaluation, fruit weight (g), fruit length/width index, fruit pedicle length (mm), fruit firmness (kg/cm^2), the number of seeds per fruit and fruit flesh texture were determined. For biochemical parameters, Soluble Solid Content (SSC), titratable acidity, vitamin C, total phenolic content and antioxidant activity were analyzed. A total of 50 fruits per genotypes were used for measurements and analysis. Fruit weight (g) was measured with a digital scale sensitive to 0.01 g (Scaltec SPB31). Fruit firmness was determined with non-destructive Acoustic Firmness Sensor (Aweta B.V., The Netherlands) expressed as kg/cm^2. The shape was determined by dividing fruit length by fruit width. A trained panel of five experts evaluated the fruit flesh texture and noted as highly sandy, sandy and slightly sandy for each genotype.

Soluble Solid Content (SSC) were determined by extracting and mixing one drops of juice from each fruit into a digital refractometer (Kyoto Electronics Manufacturing Co. Ltd., Japan, Model RA-250HE) at 22 ^{0}C. Vitamin C (Ascorbic acid) was quantified with the reflectometer set by using RQFlex (Merck Company, Darmstadt, Germany) and expressed as mg/100 g. Fruit extracts were taken in a magnetic stirrer with water. The extract was then filtered and subjected to potentiometric acid-base titration with adjusted NaOH solution. Titration acidity of wild pear samples was expressed in g mallic acid equivalent/100 g sample. Total phenolics of samples were determined by Folin Ciocalteau reactive and reading the absorbance of colorful solution at 765 nm wavelength and expressed in mg gallic acid equivalent/100 g sample in fresh weight base (Singleton and Rossi, 1965). Antioxidant capacity was determined using DPPH method. Fruit juice samples were obtained by pureed and filtered. Samples were homogenized by centrifuge. 950 µl 0.1 N DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was added upon 50 µl supernatant. Then it was read against the blank

at 515 nm wavelength spectrophotometer (Nakajima et al., 2015). Results expressed as μmol of vitamin C equivalent/g fresh weight.

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

Results and Discussion

Phenological and morphological characterization

Studies on morphological and biochemical characteristics of oleaster-leafed pear (*Pyrus elaeagrifolia*) genotypes were very limited in literature. This highlights the importance of present study. Firstly, all genotypes found pest and disease free in their natural habitats.

As indicated in Table 1 and 2, different *Pyrus elaeagrifolia* genotypes varied significantly for phenological, morphological and biochemical characteristics like ripening date, fruit weight, fruit pedicle length, fruit length/width ratio, fruit flesh texture, the number of seeds per fruit, SSC (Soluble Solid Content), titratable acidity, vitamin C, total phenolic content and antioxidant activity.

The ripening dates of oleaster-leafed pear genotypes were found between 18 October (TR-09, TR-20) and 07 November (TR-14 and TR-18), respectively (Table 1). Yilmaz et al. (2015) reported a wide variation on ripening date of *Pyrus elaeagrifolia* genotypes (from 11 October to 10 November) in middle Anatolia in Turkey.

A large number of variations were existed for fruit weight among different *Pyrus elaeagrifolia* genotypes under this study. Fruit weight ranged from 6.19 g to 21.04 g and maximum fruit weight was observed in TR-12 (21.04 g) and TR-18 (20.10 g). Similar high variations on fruit weight were obtained in *Pyrus elaeagrifolia* genotypes by Yilmaz et al. (2015) as 4.71-27.09 g and Gercekcioglu et al. (2016) as 16-22 g, respectively. Kececi (2017) also reported higher fruit weight variation between 18.05-55.50 g among *Pyrus elaeagrifolia* genotypes sampled from Hakkari region in eastern Turkey.

Pedicel length and fruit length/width was found to be between 7.11-18.56 mm and 0.75-1.03, respectively (Table 1). There were statistically significant differences among genotypes for both pedicle length and fruit length/width ratio ($p<0.05$). Yilmaz et al. (2015) and Gercekcioglu et al. (2016) found a great variation on pedicle length that varied from 6.89-24.23 mm and 18.0-25.0 mm, respectively. Same researchers reported fruit length/width ratio between 0.67-

1.09 and 0.77-0.96, respectively. Kececi et al. (2017) also reported high variation on fruit length/width ratio (0.90-1.23) among *Pyrus elaeagrifolia* genotypes sampled from Turkey.

Table 1. Phenological and morphological characteristics of *Pyrus elaeagrifolia* genotypes

Genotypes	Harvest date	Fruit weight (g)	The number of seeds	Pedicel length (mm)	Fruit length/width	Fruit firmness (kg/cm^2)	Fruit flesh texture
TR-1	24 Oct	9.23cd	5.02bc	13.84bc	0.84bc	4.35de	Slightly sandy
TR-2	30 Oct	14.23bc	7.11ab	16.24ab	0.88d	8.22a	Highly sandy
TR-3	01 Nov	15.88b	6.50bc	15.58b	0.80bc	7.55ab	Highly sandy
TR-4	24 Oct	11.02c	5.75bc	9.97cd	0.76c	6.20c	Sandy
TR-5	02 Nov	16.22ab	7.04ab	11.30cd	0.95ab	7.95ab	Highly sandy
TR-6	28 Oct	8.45cd	6.67b	15.22bc	0.75c	6.12cd	Sandy
TR-7	03 Nov	16.65ab	7.80ab	8.02de	0.80bc	6.90bc	Highly sandy
TR-8	30 Oct	13.67bc	7.04ab	18.56a	0.96ab	8.04ab	Highly sandy
TR-9	18 Oct	7.49cd	4.57bc	9.65de	0.90ab	4.18e	Slightly sandy
TR-10	28 Oct	8.41cd	5.67bc	10.80cd	1.01ab	7.20b	Sandy
TR-11	02 Nov	15.23bc	6.36bc	16.13ab	0.95ab	7.76ab	Highly sandy
TR-12	06 Nov	21.04a	8.88ab	17.75ab	0.90ab	5.88cd	Sandy
TR-13	20 Oct	6.19d	4.33bc	8.90de	0.98ab	4.91de	Slightly sandy
TR-14	07 Nov	18.02ab	8.40ab	7.20de	0.84bc	7.89ab	Highly sandy
TR-15	05 Nov	15.10bc	7.10ab	14.45bc	0.96ab	6.78bc	Highly sandy
TR-16	26 Oct	10.65cd	5.40bc	11.33cd	1.03a	5.31d	Slightly sandy
TR-17	28 Oct	12.00bc	6.35bc	17.75ab	0.99ab	7.28ab	Sandy
TR-18	07 Nov	20.10ab	9.11a	8.40de	0.95ab	7.05bc	Highly sandy
TR-19	22 Oct	8.27cd	4.18c	14.87bc	0.77bc	5.55cd	Slightly sandy
TR-20	18 Oct	16.89bc	7.20ab	8.80de	0.79bc	5.40cd	Slightly sandy
TR-21	02 Nov	17.44bc	6.09bc	17.11ab	0.97ab	5.99cd	Sandy
TR-22	28 Oct	13.12bc	7.44ab	12.48c	0.80bc	5.68cd	Sandy
TR-23	30 Oct	12.04bc	7.69ab	13.45bc	0.85bc	7.44ab	Sandy
TR-24	06 Nov	17.79ab	7.46ab	9.70d	0.82bc	6.51bc	Highly sandy
TR-25	28 Oct	14.10bc	8.22ab	7.11e	0.90ab	7.56ab	Sandy
TR-26	29 Oct	13.10bc	6.19bc	10.36cd	0.83bc	5.82cd	Slightly sandy

Same letters in same column indicate statistically significant differences ($p<0.05$) among the genotypes

The number of seeds per fruit, fruit firmness and fruit flesh texture of *Pyrus elaeagrifolia* genotypes are shown in Table 1. There were statistically significant differences ($p<0.05$) among genotypes on the number of seeds per fruit and fruit firmness. The number of seeds per fruit and fruit firmness were in range of 4.18-9.11 and 3.84-8.22 kg/cm^2, respectively. Gercekcioglu et al. (2016) reported a great variation on the number of seeds per fruit and fruit firmness between 4-8 and 4-10 kg/cm^2, respectively. Kececi (2017) reported high variation on the number of seeds per fruit between 6.0-9.0 among *Pyrus elaeagrifolia* genotypes sampled from Hakkari region in eastern Turkey. All above studies supports our findings.

Fruit flesh texture of *Pyrus elaeagnifolia* genotypes are shown in Table 1. Fruit flesh texture varied from highly sandy (8 genotypes), sandy (12 genotypes) to slightly sandy (6 genotypes), respectively. Yilmaz et al. (2015) and reported that among 43 *Pyrus elaeagrifolia* genotypes,

20 genotypes had sandy fruit, 12 genotypes had highly sandy and 11 genotypes had slightly sandy fruits which in accordance with our results.

Biochemical characterization

As indicated before, studies on morphological and biochemical characteristics of oleaster-leafed pear (*Pyrus elaeagrifolia*) genotypes were very limited in literature. This highlights the importance of present study and vitamin C and antioxidant capacity first time determined in this plant in literature.

We found great variation on SSC and titratable acidity content on oleaster-leafed pear (*Pyrus elaeagrifolia*) genotypes. SSC content was the highest in TR-24 genotype (20.35%) and followed by TR-18 (20.20%) and TR-14 (20.05%) whereas it was found minimum in TR-20 genotype as 11.90% (Table 2). The maximum titratable acidity was observed in TR-07 genotype (1.24%) while the minimum titratable acidity was recorded in TR-04 as 0.42% (Table 2). Previously Yilmaz et al. (2015) recorded SSC and titratable acidity between 12.0-20.0% and 0.20-1.40% in fruits of 43 wild grown *Pyrus elaeagrifolia* genotypes. Gercekcioglu et al. (2016) revealed SSC content of 10 *Pyrus elaeagrifolia* genotypes were in range of 13.0-18.0%. Kececi (2017) also found high variation on SSC and titratable acidity content in fruits of *Pyrus elaeagnifolia* genotypes sampled from Hakkari region in eastern Turkey were between 11.1-16.3% and 2.0-4.0%, respectively. Our results of SSC and titratable acidity were comparable to above studies. Plant genotype, environmental conditions, nutrition status etc strongly affect biochemical content in horticultural plants (Vijayan et al., 2008; Marsic et al., 2019). As known, the traditional quality properties in fruits are dry matter, sugars, dietary fiber, organic acids and pigments, whereas soluble solids content (consisting mostly of mono- and disaccharides) and titratable acidity contribute to sweetness and acidity of fruits and their products (Skrede et al., 2011). Fruits of *Pyrus elaeagrifolia* genotypes showed very low vitamin C content varied from 4.7 to 7.8 mg/100 g. Fruits of different *Pyrus* species known that has lower vitamin C content like apple fruits (Galvis-Sanchez et al., 2003; Ozturk et al., 2009).

Total phenolic content and antioxidant activity of *Pyrus elaeagrifolia* genotypes were shown in Table 2 and the results are indicating statistically a great diversity among genotypes for both total phenolic content and antioxidant activity. Total phenolic content and antioxidant activity varied from 57-108 mg GAE/100 g and 66-107 μmol of vitamin C equivalents/g. Galvis-Sanchez et al. (2003) reported that pear flesh have total phenolic content between 123-200 GAE/100 g. Marinova et al. (2005) found that pears include total phenolic content between 91-

125 mg GAE/100 g. Liaudanskas et al. (2017) showed total phenolic content among pear cultivars between 51-111 mg GAE/100 g. They also reported that antioxidant capacity were between 42-60 μmol of trolox equivalents/g dry weight base. Galvis-Sanchez et al. (2003) reported that the antioxidant capacity in pear fruits was correlated with the content of chlorogenic acid (r=0.46), while ascorbic acid made only a small contribution to the total antioxidant capacity of the fruit.

Table 2. Biochemical characteristics of *Pyrus elaeagrifolia* genotypes

Genotypes	SSC (%)	Titratable acidity (%)	Vitamin C (mg/100 g)	Total phenolic (mg GAE/100 g)	AC (μmol of vitamin C equivalents/g)
TR-1	17.35bc	0.58de	5.3^{NS}	66cd	69bc
TR-2	18.30ab	0.95bc	5.0	80bc	104ab
TR-3	18.50ab	0.79cd	6.4	91ab	125ab
TR-4	15.10cd	0.42e	6.0	70c	80bc
TR-5	18.90ab	0.70cd	4.7	85bc	110ab
TR-6	16.40bc	0.95bc	6.3	72bc	77bc
TR-7	19.30ab	1.24a	7.4	89b	114ab
TR-8	17.00bc	1.07ab	6.1	75bc	102b
TR-9	12.30de	0.97bc	7.0	60cd	83bc
TR-10	16.00c	1.04b	6.6	82bc	96bc
TR-11	19.10ab	1.15ab	5.8	77bc	90bc
TR-12	19.60ab	0.85c	7.8	94ab	120ab
TR-13	14.30cd	1.10ab	7.2	57d	78bc
TR-14	20.05ab	0.88bc	5.4	102ab	123ab
TR-15	20.00ab	1.20ab	7.0	108a	137a
TR-16	13.70d	1.06ab	6.1	62cd	66c
TR-17	15.35cd	0.60de	6.6	68cd	74bc
TR-18	20.20ab	0.89bc	4.9	95ab	120ab
TR-19	15.80cd	0.56de	5.8	73bc	88bc
TR-20	11.90e	0.47de	6.3	60cd	80bc
TR-21	19.00ab	1.10ab	5.5	83bc	110ab
TR-22	16.80bc	0.70cd	5.3	67cd	75bc
TR-23	17.50bc	0.66d	4.9	89b	94bc
TR-24	20.35a	1.14ab	5.7	87bc	90bc
TR-25	17.20bc	0.90bc	6.3	82bc	87bc
TR-26	18.25b	0.95bc	5.2	74bc	96bc

Same letters in same column indicate statistically significant differences ($p<0.05$) among the genotypes

Conclusions

A few studies conducted on oleaster-leafed pear (*Pyrus elaeagrifolia*) plants previously thus the results of this study will add more information about this plant on literature. The study points out that oleaster-leafed pear (*Pyrus elaeagrifolia*) genotypes differed each other for phenological, morphological and biochemical characteristics. TR-12, TR-14 and TR-18

genotype were found promising with higher fruit weight, TR-1, TR-9, TR-13, TR-16, TR-19, TR-20 and TR-26 were found promising for slightly sandy fruits, and TR-14, TR-15 and TR-18 were found promising due to higher fruit total phenolic content and antioxidant activity. The values found in this study may be helpful for horticulturists, taxonomists, nutritionists as well as breeders who can promote the cultivation of this species and new cultivars with higher fruit weight, slightly sandy fruits, high total phenolic content and antioxidant activity.

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Original scientific paper

Sugar and organic acids in ungrafted loquat (*Eriobotrya japonica* Lindl.) genotypes in Coruh valley in Turkey

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Abstract

Located northeastern part of Turkey, Coruh valley is accepted one of the 34 plant biodiversity hotspots in the world. The valley is very rich in terms of indigenous fruit species. Present study describes specific sugar and organic acid content in fruits of seven un-grafted loquat genotypes. Specific sugars and organic acid contents of heritage loquat genotypes were studied at harvest. The standard cultivars cv. Sayda was included in the study. HPLC results indicated the presence of four specific sugars such as glucose, fructose, sucrose and maltose and five organic acids such as malic, tartaric, oxalic, citric and succinic acid in loquat fruits. Glucose was the major sugars and malic acid was the predominant organic acid for all genotypes and cv. Sayda. At harvest, the fruit juice content of glucose, fructose, sucrose and maltose ranged from 5.80 to 7.22 mg/100 g, 3.90 to 5.74 mg/100 g, 1.11 to 1.62 and 0.14 to 0.77 mg/100 g, respectively. The malic acid content varied from 407 to 611 mg/100 g, tartaric acid from 104 to 142 mg/100 g, oxalic acid from 22 to 35 mg/100 g, succinic acid from 10 to 24 mg/100 g and citric acid from 7 to 12 mg/100 g, respectively. The distribution pattern of individual sugars and organic acids can be used in the development of commercial, industry and contract industry loquat cultivars that would target specific consumer requirements and consumer health.

Key words: Content, loquat, organic acids, sugars.

Introduction

The loquat (*Eriobotrya japonica* Lindl.) is originated to southeastern China and is widely grown in subtropical regions of the world. The plant introduced from China to Japan first in

very early times and has been moved to western world (France and England) in 1750-1790 years. From France, it was brought to Malta and northern Africa such as Algeria and the Near East (Rodriguez, 1983; Ercisli et al., 2012).

At the end of 1800's year loquat cultivation has been started and spread to India and southeast Asia, the medium altitudes of the East Indies, and Australia, New Zealand and South Africa. Chinese immigrants are assumed to have carried the loquat to all around the world. In the New World, it is cultivated from northern South America, Central America and Mexico to California: also, since 1867, in southern Florida (Lin et al., 1999).

The spreading of loquat was easy in those areas where citrus were grown. However, loquat has stricter environmental requirements than citrus. Flower and fruit development cycle extend during the winter, which implies an average temperature of over 15 ºC. The tree is rustic but the fruits can be damaged by wind and frost; temperatures below -2 ºC damage open flowers and small fruits, recently set fruits can be damaged at a temperature of -1 ºC. For people familiar with the crop, loquat represents a fruit that announces the spring, a nice sweet acid fruit if it has been harvested at the right point of ripeness and if it has been transported with the necessary care (Zhang et al., 2015).

Turkey is one of the most important loquat producers in the world and almost all the plantations are situated in the Southeastern Mediterranean region. The fruit is consumed fresh in the springtime and it ripens early. Small quantities are exported to countries in the Middle East, and Central and Northern Europe. The main problem facing the expansion of this crop in Turkey is the risk of frost and land availability, due to competition with other crops, including citrus, as well as greenhouses for the production of both ornamental and vegetable plants (Polat et al., 2005; Polat, 2007).

Loquat fruit has rich nutritional and commercial importance in terms of its special functional composition and consumer demand. Currently loquat is widely and commercially cultivated in more than 20 countries, including China, Japan, India, Australia, Brazil, Israel, Italy, Spain, Turkey and the US. China is the leading producer of loquat with 420000 tons annual production (FAO, 2020). Loquat is a late spring and summer-harvested small fruit, and regarded as a functional fruit because of its special nutritional content. Consumers highly favor loquat fruit because of its mild, subacid and sweet taste, as well as an attractive flavor (Tian *et al.*, 2007).

Traditional wild fruit is an important foodstuff in the local populations nutrition, and it has been used in folk medicine since ancient times. Increased interest in wild fruit results are primarily from the need for biodiversity preservation and use as a potential source of new

nutraceuticals aimed at prevention of a series of diseases (Ercisli and Sagbas, 2017). In addition, it improves the living conditions of the population in local rural areas, who exclusively engage in collecting and selling wild fruit and medicinal herbs, which are often their only sources of income (Bunea et al., 2011).

Sugars and organic acids are the most common soluble constituents of fruit. They have an important influence on taste, shelf life and nutritive properties, and they are reliable indicators of acceptability by consumers (Kafkas et al., 2006; Tosun et al., 2009). From a technological perspective, they are very important in production of juices and nectars, since they define sweetness index (Lozano, 2006). The representation of individual sugars and organic acids serves also as an indicator of authenticity of fruit products (Evans et al., 1983). In addition, sugars participate in polyphenol biosynthesis; thus, higher sugar content in fruit implies higher polyphenol concentration, which is exceptionally important from the perspective of a nutrient-enriched diet (Milivojevic et al., 2013).

In literature there were studies on organic acid and sugar contents of loquat cultivars. However no study has been done on un-grafted loquat genotypes. Thus the aim of this study is determine individual organic acid and specific sugar contents of seven un-grafted loquat genotypes grown under Coruh valley conditions.

Material and Methods

Plant material

The un-grafted loquat genotypes were found in Coruh valley and the fruits were sampled in 2020. A total of 50 health fruits were harvested from seven genotypes and cv. Sayda was included in the study. Harvested fruits were quickly brought to the Laboratory and juices of the fruits belong to genotypes and cv. Sayda were squeezed with a blender, and the fruit juices were separated from the pulp with the help of cheesecloth.

Specific sugars

Sugar (fructose, sucrose, and glucose) analysis was performed with the methods described by Melgarejo et al. (2000). 1 ml of fruit extracts was centrifuged at 10 000 rev per min for 2 min at 4 °C. Supernatants were passed by SEP-PAK C18 cartridge. HPLC readings were made with μbondapak- NH_2 column using 85% acetonitrile as the liquid phase with a refractive index detector (IR). Fructose and glucose standards were used for the calculations of the sugar contents.

Organic acids

Organic acid composition of the loquat fruit was determined described by Bevilacqua and Califano (1989). Fruit extracts were obtained by crushing the fruits in cheesecloth. 0.009 N H_2SO_4 was then homogenized with shaker for 1 h. The mixture was then centrifuged at 15000 rpm for 15 min and the supernatants were filtered twice through a 0.45 μm membrane filter with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and passed through a SEP-PAK C18 cartridge. Organic acid readings were performed by HPLC using the Aminex column (HPX-87 H, 300 mm x 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths in the Agilent package program (Agilent, USA).

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

Results and Discussion

Specific sugar contents

Figure 1 shows specific sugar contents of seven loquat genotypes and cv. Sayda. The genotypes significantly differed each other in terms of amount of glucose and fructose concentrations ($p< 0.01$) yet sucrose and maltose content were found non-significant among genotypes. The predominant specific sugar in all genotypes and cv. Sayda was glucose. The genotype 5 (G5) had the highest glucose content as 7.22 mg/100 g fresh weight base and followed by G3 with 6.88 mg/100 g glucose content whereas the lowest value was seen in G2 as 5.80 mg/100 g FW (Figure 1). The standard cultivar Sayda had one of the highest sucrose contents as 6.40 mg/ 100 g.

Fructose concentrations of genotypes were found statistically significant (Figure 1). The fructose content of the genotypes and cv. Sayda were in descending order G2 (5.74 mg/100 g)>G7 (5.22 mg/100 g)>G1 (5.10 mg/100 g)>G6 (4.41 mg/100 g)>Sayda (4.30 mg/100 g)>G4 (4.26 mg/100 g)>G5 (4.07 mg/100 g)>G3 (3.90 mg/100 g), respectively (Figure 1).

Sucrose highest glucose content was obtained from G6 as 1.72 mg/g and the lowest values were recorded from G4 as 1.11 mg/g. Genotypes 7 had the highest fructose content with amount of 2.07 mg/g and G2 had the lowest fructose content (1.19 mg/g).

Sucrose and Maltose content of the genotypes were in range of 1.11 mg/100 g (G5)-1.62 mg/100 g (G7) and 0.14 mg/100 g (G5)-0.77 mg/100 g (G2), respectively (Figure 1).

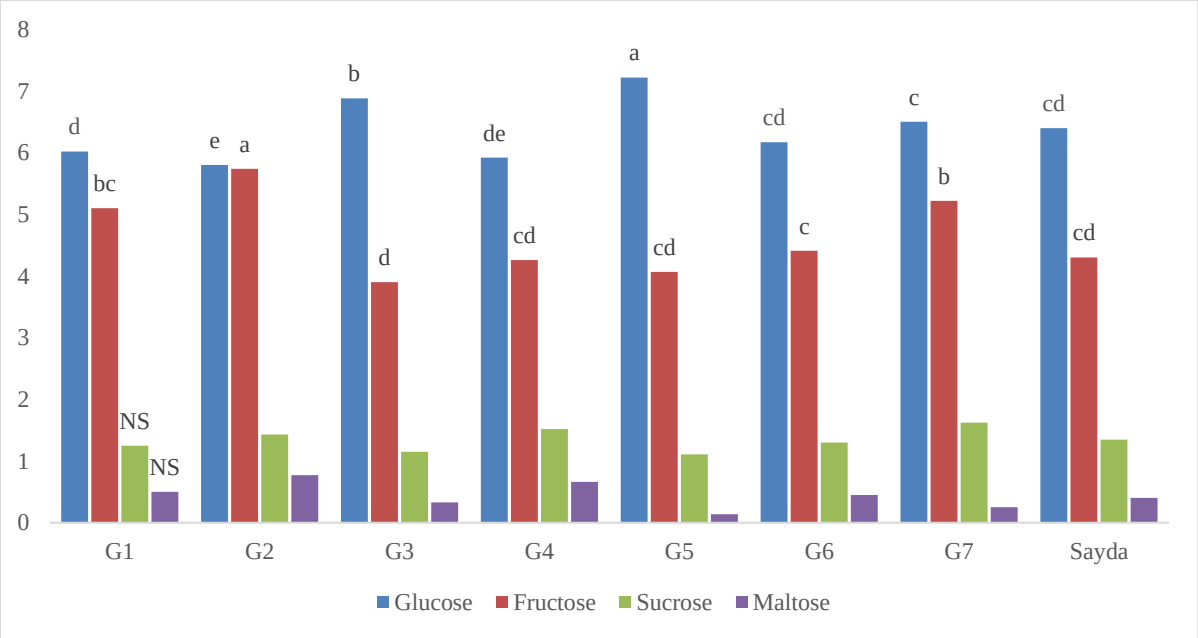

Figure 1. Individual sugar concentrations (mg/100 g) of loquat genotypes and cv. Sayda

Toker et al. (2013) used 15 national and international loquat cultivars to determine biochemical content. They reported that glucose was the main sugar in loquat fruits and Champagne de Grasse had the highest glucose and fructose value as 7.48% and 5.45% and the lowest glucose and fructose content were obtained from cv. Gold Nugget (5.92%) and cv. Taza (3.88%), respectively. They also reported that sucrose and maltose were considerably low in loquat fruits. Topuz (1998) and Xu and Chen (2011) also reported that glucose and fructose main sugars in loquats. Our results related to glucose and fructose amount were similar to that of Topuz (1998) and Toker et al. (2013). However Xu and Chen (2011) reported higher glucose and fructose contents in 12 loquat cultivars than our results. However, Shaw and Wilson (1981) revealed lower glucose content in loquat cultivars that varied from 1.6 to 3.0% while the amount of the fructose ranged from 2.3 to 4.8%. Amount and composition of sugars in loquat generally changed with cultivars, maturation level, cultivation techniques, ecology and also some factors such as amount of fruit per panicle, purple spots, fruit thinning etc. in loquat (Hasegawa et el., 2010; Toker et al., 2013)

Organic acids

As indicated in Figure 2, malic acid and tartaric acid concentrations are greatly varied among genotypes ($p>0.01$). However oxalic, succinic and citric acid concentration of the genotypes were found to be non-significant.

Malic acid was the predominant organic acid in all genotypes and varied from 453 mg/100 g to 611 mg/100 g, respectively. Among the tested genotypes, G6 had the highest malic acid content whereas G7 had the lowest. The cultivar Sayda also had relatively high malic acid content (602 mg/100 g) compared to the local un-grafted genotypes.

Tartaric acid content was found to be between 104 mg/100 g and 142 mg/100 g and cv. Sayda had average 124 mg/100 g tartaric acid. Oxalic, succinic and citric acid content were between 22-35 mg/100 g, 10-24 mg/100 g and 7-12 mg/100 g, respectively (Figure 2).

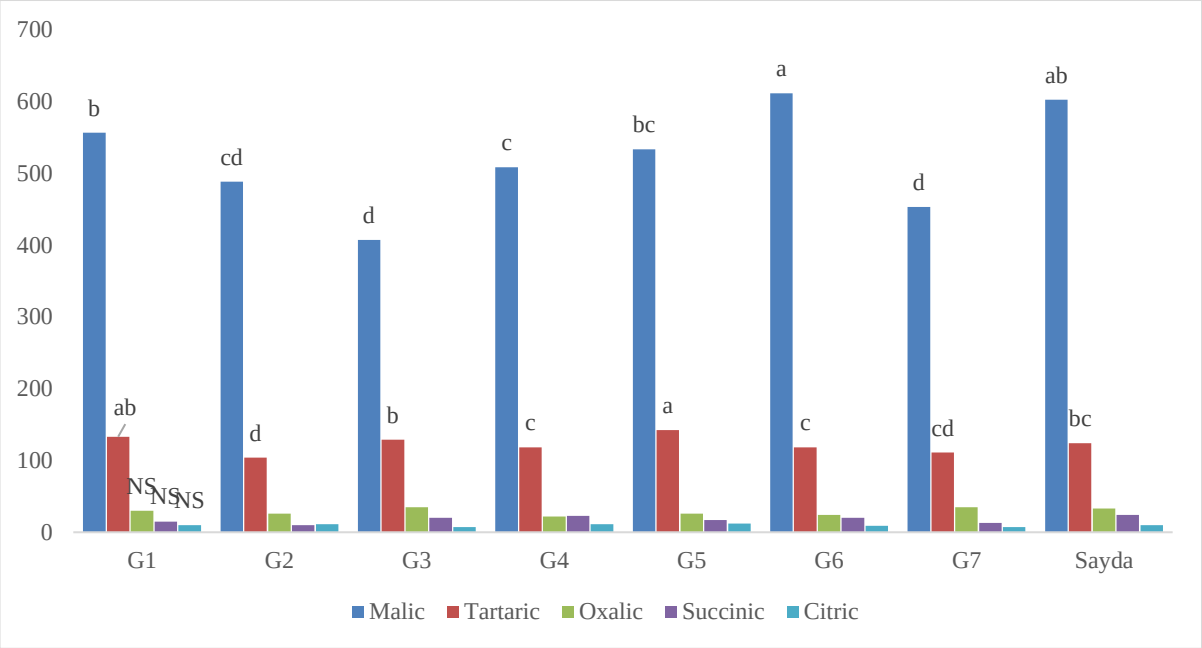

Figure 2. Individual organic acid concentrations (mg/100 g) of loquat genotypes and cv. Sayda

Organic acids are known to particularly affect the fruits' taste formation and many physiological processes. Previous studies are also indicated that malic acid is the predominant organic acid in loquat fruits (Ding et al., 1997; Serrano et al., 2003; Tian et al., 2007; Chen et al., 2008; Hasegawa et al., 2010; Toker et al., 2013), and it was followed by tartaric, succinic, oxalic and citric acids, respectively. Toker et al. (2013) found that organic acid compositions of the loquat cultivars changed significantly and malic acid content were between 368 and 842 mg/100 g. Researchers found various malic acid concentrations in different loquat cultivars

such as 250-850 mg/100 g by Serrano et al (2003), 129-891 mg/100 g by Chen et al (2008), 587-988 mg/100 g and by Hasegawa et al (2010). Besides the genotype, differences in malic acid concentrations could be resulted from ecology, cultivation techniques, harvest date etc.

Conclusions

In literature, very limited studies have assessed on organic acid and sugar content of un-grafted loquats. A comprehensive analysis of the relationship between un-grafted loquats and standard cultivars is highly needed. The un-grafted loquat genotypes studied here presented considerable variation with reference to major sugars and organic acids. A systematic understanding of the variability of un-grafted loquat genotypes by using biochemical traits may enable the development of a reference classification system for commercial purposes.

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Original scientific paper

Biochemical differences between cultivated (*Vitis vinifera* L. ssp. *vinifera*) and wild grapevines (*Vitis vinifera* L. ssp. *sylvestria* (Gmelin) Hegi)

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Abstract

In the present study, berry biochemical composition of 7 wild grapevine and 2 standard grape cultivars grown in Coruh valley in Turkey were examined. Results showed significant differences exist among all genotypes across a number of biochemical properties. The wild grapevine genotypes generally showed smaller berry size, variable berry color and biochemical content. Total phenolic, total anthocyanin and total tannin content were found quite variable among wild and cultivated grapevines ranged from 137 to 441 mg GAE/100 g; 29 to 107 mg/100 g and 33 to 71 mg/100 g, respectively. Radical scavenging capacity of wild genotypes were between 56-81% while it was 61% for cv. Cavus and 76% cv. Manda gozu. Wild genotypes C5 showed some advantages such higher total phenolic content, total tannin and radical scavenging capacity. Biochemical analysis revealed that wild grapevine genotypes present a potential sources for qualitative traits in grapevine breeding programs.

Key words: Grape, wild, biochemistry, differences

Introduction

Among horticultural crops, grapevine (*Vitis vinifera* L) is the most widely cultivated and economically important horticultural crop in the world (FAO, 2020). The specie included both the cultivated form (*V. vinifera* ssp *vinifera*) and the wild form (*V. vinifera* ssp *sylvestris*). The wild form is the putative ancestor of the cultivated form and the morphological differences are considered as key factor between two subspecies. The most conspicuous differential trait is plant sex: wild grapevines are dioecious (male and female plants), while cultivated forms are mostly hermaphrodite plants, with self-fertile hermaphrodite flowers (Carrasco et al., 2019). However, it can be argued that those differences are the result of the domestication process. Although wild grapevines were spread over Southern Europe and Western and Central Asia

during the Neolithic period, archeological and historical evidence suggest that primary domestication events would had occurred in the Near-East (McGovern, 2003).

Anatolia and together with Azerbaijan, Armenia and Georgia within south Caucasus has been considered for a longtime as the birth place for viticulture with the earliest examples of wine-making (McGovern, 2003; Ergul et al., 2011)

Balkan peninsula including Turkey is very rich in terms of wild edible horticultural plants and for centuries those plants have been using in local and traditional cuisine, as well as in traditional and folk medicine (Redzic, 2006; Nedelcheva et al., 2013). Studies from Balkan peninsula highlighted the important beneficial health effects of various wild edible horticultural species that constitute high content in phytochemicals such as phenolic acids, anthocyanins, vitamin C, flavonoids, omega-3 fatty acids, stilbenes and the other secondary metabolites with bioactive and antioxidant properties (Latti et al., 2009; Milivojevic et al., 2011; Veberic et al., 2015).

More recently the increasing demand for functional foods and many recent studies have highlighted the potential of commercial exploitation of wild edible horticultural plants that may diversify modern diets and increase throughout the year the availability of such products (Jurikova et al., 2016; Narwojsz et al., 2019; Kranz et al., 2020). However, domestication of wild edible horticultural species needs several aspects to be considered since various reports highlighted significant changes of bioactive compounds content in domesticated species compared to their wild counterparts, while scarce literature reports exist regarding their agronomic requirements (Milivojevic et al., 2011; Jurikova et al., 2021).

Recent studies indicate the significance of consuming berries including grape due to their high phenolic phytochemical and their contribution to the human nutrition and health (Pezzuto 2008; Serce et al., 2010; Derradj-Benmeziane et al. 2014).

In literature a vast studies has been conducted on cultivated grape cultivars and limited studies conducted wild ones. Thus, the aim of this study was to characterize of wild grape genotypes grown in Coruh valley based on their biochemical composition.

Material and Methods

Plant Material and extraction process

The populations of Eurasian wild grapevine *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) spread from the Yusufeli district to Sarigol village along with Barhal river. Seven wild grapevine samples were obtained from alongside Barhal river (Table 1). The standard cultivars Manda

gozu and Cavus were also included to study. They were harvested in the periods when the berries belonging to genotypes/cultivars reached full maturity. A lot of 1 kg grape berries was carefully collected in the vineyard, cut from the clusters with the pedicel, and transferred quickly to the laboratory. Pedicels were removed and berries were weighted, manually skinned, and the skins were freeze-dried. The freeze-dried tissues were then extracted with 200 mL of 1% HCl in methanol. Extraction was carried out under stirring for 48 h, and repeated in triplicate. Extracts were pooled, and this mixture was used for further procedures after deep-freezing (-70 ºC) for no longer than 3 days (Milivojevic et al., 2011).

Total polyphenol content

To determine the total phenolic content (TPC) by the Folin-Ciocalteu method, 1 mg of each extract or fraction in 1 mL of methanol, was prepared. Briefly, the extract solution (100 µL) was diluted to 3 mL with distilled water and then oxidized with the Folin-Ciocalteu reagent (500 µL); after 3 min, the reaction was neutralized with Na_2CO_3 20% solution (2 mL) and then allowed to react for 60 min in darkness. The absorbance of the resulting blue colored reaction was measured at 650 nm in a spectrophotometer. The calibration curve was performed with gallic acid (concentration range 0.30–9.00 µg/mL) and the results were expressed as mg of gallic acid equivalents per 100 g of fresh berry (Singleton and Rossi, 1965).

Total anthocyanin content

Total anthocyanin content was determined using a previously described method (Connor et al., 2002), in which each grape extract was diluted (5:95, v/v) in 1% HCl in methanol to obtain an absorbance between 0.500 and 1.000 at 530 nm. The values were expressed as mg cyanidin-3-glucoside (c3g) equivalents per 100 g fresh weight using a molar extinction coefficient of 27.900. All determinations were performed in triplicates.

DPPH free radical scavenging activity

The bleaching rate of a stable free radical DPPH was monitored at a characteristic wavelength in the presence of the grape sample. In this, radical form of DPPH absorbs at 517 nm upon reduction by an antioxidant. This activity was measured according the previously described method, briefly as: 100 µM solution of DPPH was prepared in 10 mL of methanol and 2.7 mL of this solution was added to 0.5 mL of grape extract in methanol at the same concentration (0.1 mg/mL). After 10 min, the absorbance was measured at 517 nm. The percentage of remaining DPPH was calculated as, DPPH scavenging effect (%) = [(A Control – A Sample/A

Control) × 100], where A Control is the absorbance of the DPPH reaction and A Sample is the absorbance in the presence of grape extracts. All determinations were performed in triplicate (Nile et al., 2013)

Tannin analysis

Berry tannin concentrations were evaluated using a protein precipitation assay. The method is based on the precipitation of tannins from a solution containing BSA (bovin serum albumin), redisolving the resultant precipitate and then determining the amount of tannin by reaction with ferric chloride, yielding a colored product that can be quantified by its absorbance at 510 nm. Sample preparation and the protein precipitation assay were conducted as described by Harbertson et al. (2002).

Statistical analysis

The study was planned as four replication including 10 bunch and berry per replicate. In the statistical evaluations, Windows SPSS 20 was used and the differences between the means was evaluated by subjecting to ANOVA variance analysis and determined with Duncan multiple comparison test ($p<0.005$).

Results and Discussion

Morphological properties

As shown in Table 1, four genotypes of wild grapes (C1, C3, C4 and C5) had black berry skin color and C2, C6 and C7 had white berry skin color (Table 1). Previous studies are also indicated color differences among grape cultivars throughout the world.

Table 1. Some important characteristics of grape genotypes and cultivars

Cultivars/genotypes	Berry peel color	Sub species	Sampling location
Manda gozu	Purple-black	*Vinifera*	Dereici
Cavus	Green-Yellow	*Vinifera*	Dereici
C1	Black	*Sylvestris*	Bahceli
C2	White	*Sylvestris*	Bahceli
C3	Black	*Sylvestris*	Kupluce
C4	Black	*Sylvestris*	Kupluce
C5	Black	*Sylvestris*	Sarigol
C6	White	*Sylvestris*	Sarigol
C7	White	*Sylvestris*	Balhibar

Total phenolic content

Total phenolic content of the wild grapes and cultivated ones are given in Table 1. We found

statistically differences among genotypes/cultivars at 0.005 level. Total phenolic content was in descending order C5 (441 mg GAE/100 g)>C3 (367 mg GAE/100 g)>Mandagozu (350 mg GAE/100 g)>C4 (334 mg GAE/100 g)>C1 (295 mg GAE/100 g)>C2 (185 mg GAE/100 g)>Cavus (170 mg GAE/100 g)>C7 (156 mg GAE/100 g)>C6 (144 mg GAE/100 g), respectively. These results clearly revealed that black genotypes and black cultivar Mandagozu had higher total phenolic content than white wild genotypes and white cultivar Cavus. The total phenolic content results are also highlighted that wild grape genotypes were very variable for total phenolic amount. In the research, it was determined that there were statistically significant differences among the grape varieties in terms of total phenolic content, total anthocyanin content and total tannin content (Table 2). Yi et al. (1997) revealed total phenolic content between 44 and 184 mg GAE/100 g in table grape varieties and between 57 to 309 mg GAE/100 g in wine grape cultivars. Revilla et al. (2010) found that 21 Spanish-origin grape cultivars had total phenolic content between 92-468 mg GAE/100 g. Kok et al. (2017) reported total phenolic content between 103 mg GAE/100 g fw in Balbal cultivar (white cultivar) and 314 mg GAE 100 g fw in Alphonse Lavallee cultivar (black cultivar). Our results are in agreement with above results. Previous studies are also indicated a great variability among different colored grape cultivars (Soylemezoglu, 2003; Gokturk Baydar, 2006; Cetin et al., 2012; Kok and Bal, 2017). As a biochemical marker, total phenolic contents in grape berries quite variable according to genetic (cultivars) and environmental factors (climate, soil and cultural practices) (Ribereau-Gayon et al., 2000).

Table 2. Some important biochemical characteristics of grape genotypes and cultivars

Cultivars/ genotypes	Total phenolic content (mg GAE/100 g)	Total anthocyanin (mg/100 g)	Tannin (mg/100 g)	DPPH (%)
Manda gozu	350c	101b	65ab	76ab
Cavus	170g	0e	49cd	61c
C1	295e	94c	55c	76ab
C2	185f	0e	33e	56d
C3	367b	86cd	65ab	72b
C4	334d	74d	60b	67bc
C5	441a	107a	71a	81a
C6	144i	0e	45cd	60c
C7	156h	0e	41d	63bc

Different letter in same column indicated statistically significant differences ($p<0.05$)

Total anthocyanins

Total anthocyanin contents of wild and cultivated grape samples were examined in Table 2.

The results show that only black skin-colored wild genotypes (C1, C3, C4 and C5) and cv. Manda gozu had total anthocyanin content with variable level. The white genotypes (C2,C6 and C7) along with cv. Cavus was absent for total anthocyanin. Among black genotypes and cv. Manda gozu, the highest total anthocyanin content was obtained from C5 as 107 mg/100 g, and followed by cv. Manda gozu (101 mg/100 g), C1 (94 mg/100 g), C3 (86 mg/100 g) and C4 (74 mg/100 g), respectively (Table 2). Our findings related to total anthocyanin content were consistent with the values obtained in previous studies. Revilla et al. (2018) used 25 wild grapevines in Spain and reported total anthocyanin content between 27-353 mg/100 g. Kok et al. (2017) used several grape cultivars with variable berry skin color and found that the white cultivar Balbal did not include anthocyanin and the other cultivars included total anthocyanin content between 19-91 mg/100 g. Hallac Turk et al. (2015) found that total anthocyanin content was between 38-179 mg/100 g among 5 grape cultivars. Anthocyanins gave attractive red, blue, purple skin and flesh color to grape cultivars (Ho et al., 2001). Anthocyanins are red pigments accumulated in skins during grape maturation, especially malvidin-3-*O*-glucoside, and their content has been related to several agro-ecological factors (Teixeira et al., 2013), especially light and temperature, light being indispensable for anthocyanin biosynthesis and accumulation in the skins of berries and for phenylalanine ammonia lyase activity. Thus, their concentration is quite variable, even if the same cultivar or the same clone grown in a given location has been examined in several consecutive years (Revilla et al., 2009).

Tannin content

Tannins are a group of polyphenols found in fruits, leaves, trees, etc. in apples, persimmons and grapes. *Vitis vinifera* grapes are commonly grown around the world and are used in winemaking, providing good quality wines with different levels of tannins responsible for the final wine's astringency (Watrelot and Norton, 2020). In this study, the genotypes and cultivars differed each other statistically in terms of tannin amount (Table 2). The highest tannin content was obtained from C5 genotype as 71 mg/100 g and followed by equally Manda gozu cv. and C3 as 65 mg/100 g while the lowest value was observed in C2 genotype as 33 mg/100 g. Kok et al. (2017) reported that tannin content ranged from 11 mg (cv. Balbal) and 62 mg/100 g (cv. Alphonse Lavallee) that indicate good agreement with our present results. Rice et al. (2017) found total tannins between 29-66 mg/100 g among grape cultivars. These variations of tannin composition and concentration in *V. vinifera* grapes have been attributed to variety, climatic conditions, and viticultural practices such as leaf removal post fruit-set, which increases the

temperature of the berry and leads to a higher production of condensed tannins in Merlot grapes (Yu et al., 2016).

Radical scavenging capacity

Radical scavenging capacity of grape genotypes and cultivars are given in Table 2 and statistically significant differences are evident among used materials ($p>0.05$). The highest DPPH activity was found in C5 genotype as 81% and followed by cv. Manda gozu and C1 genotype (76%). The lowest DPPH activity was observed in C6 and cv. Cavus as 60% and 61%, respectively. Nile et al. (2015) used 20 grape cultivars in South Korea and reported the antioxidant activities of grape extracts varied from 32.8% ('Campbell Early') to 87.6% ('Hongiseul') by DPPH. Margaryan et al. (2017) used a large number of grape genotypes in Armenia and they reported that all the extracts of cultivated and wild grape genotypes exhibited appreciable scavenging activity and ranging from 43.3 to 92.2% for aboriginal grape cultivars, from 32.4 to 80.9 % for interspecific and intraspecific hybrids and from 40.61 to 65.48% for wild species, respectively. Grapes and wine contain high amounts of phenolics, flavonoids and anthocyanins and acts as antioxidants (Yildirim et al., 2005). The obtained results demonstrated that scavenging activity was different between the cultivated grapes and wild genotypes, which was due to the difference in their phenolic contents. Presented data indicates the marked antioxidant activity of wild and cultivated grape extracts strongly related with high content of total phenolics, acting as reductones by donating the electrons and reacting with free radicals to convert them into more stable product.

Conclusion

Present study described some important biochemical content of wild and cultivated grapes and obtained results on total phenolic, total anthocyanin, tannin and DPPH radical scavenging in berries will contribute to more comprehensive assessment of their biological activities. The study revealed that a notable difference among the cultivars and wild genotypes in the total phenolic content, total anthocyanin, tannin and DPPH radical scavenging. Obtained results will support the importance of preserving the genetic diversity and favor the reintroduction of grape cultivars and wild genotypes thanks to the present valorization.

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Original scientific paper

Nitrogen rates influence on radicchio yield and yield components

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Abstract

Field experiments were laid out in two consecutive seasons between 2019 and 2020 on the laboratory field of Biotechnical centre in Naklo near Kranj, Slovenia (an altitude: 420 m; φ = 46° 16′ 18″; λ = 14° 18′ 56″). It was conducted with the objective of finding the effect of nitrogen application levels (0 - control, 50, 100, 150 and 200 kg/ha) and 6 cultivars ('Monivip', 'Castel Franco', 'Anivip', 'Foresto', 'Palla rossa' and 'Verona') on yield and yield components of headed chicory. Trial was arranged in spilt plot factorial scheme (main plot – N levels; subplots – cultivars) on randomized complete block design base with four replications for all the seasons. The N fertilizers were applied as KAN (27% calcium amonium nitrate) in two split doses (at transplanting and 40 days after transplanting). The experimental variables measured were fresh weight (g/plant) and yield attributes (dry matter, crop height, leaf number and head firmness).

There was no interaction effect of cultivar and nitrogen application levels of tested parameters. Highest fresh weight (360.2 g/crop) achived when the cultivars received 150 kg N/ha. In contrast, percentage of dry matter content and firmmness of radicchio heads decreased as increased soil N supply. Crops that received 150 kg N/ha recived heighst height (34.5 cm). N levels were not significantly effect on the leaf number of the mature heads.

Key words: radicchio, *Cichorium intybus*, cultivars, nitrogen, yield, yield components

Introduction

The cultivation of radicchio (*Chicorium intybus* L.) found its origin in Italy, and expanded from here to France and later to the other European countries. This perennial cultivated herb is of special interest in Slovenia and always had a leading position in the production of vegetables

in west part of country (Žnidarčič et al., 2004). Radicchio was traditionally produced in the triangle Vipava-Miren-Solkan. The crops are usually grown during autumn and winter season. Traditional production process of radicchio was characterized by a high labour intensiveness, allowing the small farms to obtain a reasonable income with limited capital investments (Al-Snafi, 2016). Much of the recent per square metre-yield increases in radicchio production can be attributed to new cultivars, increased plant populations, improved weed- and pest-management practices, refined fertilizer recommendations, judicious application of irrigation water. Beside these balance applications of sufficient supply of necessary elements play a vital role in the development of radicchio crops (Jančić et al., 2016). Of the various necessary elements, nitrogen (N) is one of greatest importance to radicchio crops (Biczak et al., 1998). Many crops, including chicory, growth depends mainly on N nutrition and the N balance (Kacjan Maršić and Osvald, 2004; Stancheva et al., 2004). In the plant structural function, N is a constituent of amino acids, proteins, nitrogenous bases, enzymes, pigments and secondary products, also having a role in the processes of ionic absorbtion, photosynthesis, respiration, synthesis, cell propagation and differentiation (Cavarianni et al., 2008). It is known that an optimum amount of N fertilizer should be determined in accordance to the cultivars, the cropping seasons and soil conductions (Jablonska-Ceglarek and Rosa, 2003).

The objectives of our study are to evaluate the effect of different N levels on the crop growth and yield of radicchio and to find suitable rate of N fertilizer for the soil and climate conditions which are similar to the present experimental site.

Material and Methods

The trials were carried out in 2019 and 2020 on the laboratory field of Biotechnical centre in Naklo near Kranj, Slovenia (an altitude: 420 m; φ = 46° 16′ 18″; λ = 14° 18′ 56″). Prior to the application of fertilizer soil was sampled 0 to 30 cm and 30 to 60 cm deep with a 2.0 cm-diameter punching tube. The soil test was done in the Centre for Soil and Environmental Science, Ljubljana. The soil is classified as a heavy clay loam with a pH of 6.8. NO_3^- ranged between 4.1 and 8.9 mg/kg, and NH_4^+ between 5.6 and 8.8 mg/kg, with means of 6.5 and 7.2 mg/kg respectively. The average temperature (growing seasons) is 16.8 ^oC with the maximum and minimum being 34.6 and 12.4 ^oC, respectively. The mean relative humidity is of about 70%. In an attempt to deplete the soil residual nitrogen, radish was sown without fertilization as a previous crop, pulled out 90 days later and all the harvest material removed. A split plot design with three replications was used.

Five levels of N, i. e. 0 (control), 50 (inadequate), 100 (semi adequate), 150 (adequate) and 200 (luxurious) kg N/ha, were assigned to the main plots and six commercial cultivars as subtreatment were placed randomize in each main plot. Each sub plot had four parallel rows of 5 m length, with buffer plants at each end. Seeds of cultivars 'Monivip', 'Castel Franco', 'Anivip', 'Foresto', 'Palla rossa' and 'Verona' were densely planted in rows spaced 60 cm apart and thinned to approximately 40.000 plants/ha after emergence.

The N fertilizers were applied as KAN (27% calcium amonium nitrate) into two equal doses of 50%.

Half dose of N along with full doses of phosphorus and potassium were applied at the time of transplanting and the remaining half dose of N was given as top dressing at 40 days after transplanting. All plots were uniformly irrigated after each fertilizer application. All other agronomic practices including eradication of weeds were applied to all treatments uniformly during the course of study. Crops were harvested manually at commercial maturity of each cultivar, i.e. between October 25 and October 31, 2019, and October 30 and November 5, 2020. Plants were cut from the roots as the time when approximately 80% of the raddichio heads had reached relatively compactness. A sample of eight random plants from each sub plot was taken to obtain the following characters: fresh weight, dry matter, plant height, leaf number per plant and head firmness. Head firmness was evaluated by touch on an index from 1 to 10, with 1 (beginning very fluffy) and 10 (a very hard head). All measured and derived data, were analysed by analyses of variance (ANOVA) using Statgraphics Plus for Windows 4.0 computer program. Character means were separated by least significant differences (*LSD, $P<0.05$*) when sources of variation from ANOVAs were significant (*$P<0.05$*).

Results and Discussions

Analysis of variance was conducted for the data set including both years. Results depicted (Table 1) that growth parameters react differently to varying levels of N. Increasing the rate of N had a significant effect on the yield and yield components (except on leaf numbers per plant). N effects on the yield and yield components were also studied by evaluating the effects on the coefficient of variation. The lower the coefficients of variation, the more uniform are the characteristics of plants per treatment. It is clear that the maximum value of fresh weight per plant is the highest in the adequate (150 kg N/ha) treatment and lowest in the control (0 kg N/ha). The fresh weight was 260.5 g/crop in the control plots which were not fertilized, while in the plots treated with 150 kg N/ha N the fresh weight increase was statistically proven (360.2

g/plant). However the further increase of N level resulted in fresh weight decrease. An opposite tendency was observed for the dynamics of dry matter content in the raddichio heads in the various N levels. According to Karić et al. (2005), the dry matter content is the ratio between dry and fresh weight expressed as a percentage. Žnidarčič and Maršić (2008) claims that 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 research the N level had significant effect on this parameter. There were clearly tendencies for the dry matter content to decrease as the N level was increased. The portion of dry matter decreased from 15.24% at 0 kg N/ha to 11.64% at 200 kg N/ha. These results are in agreement with those obtained by Sorensen (1999), Sophea and Preston (2001) and Karić et al. (2005). These researches pointed out that increasing the application rate of N level decreased dry matter content in vegetables. Further, data revealed that application of 150 kg N/ha resulted in the highest crop height (34.5 cm). No significant differences were found for the effect of N levels on leaves number. Relationship between N levels and firmness of raddichio heads show a very similar trend like in a case of dry matter content: at high levels of N, the fertilization gave a decrease in firmness of heads, while at lower levels just the opposite was noticed. Our results are not confirmity with those of Žnidarčič et al. (2004) reported that compact heads by chicory were indicated by the greater head weight.

Table 1: Yield and yield components of raddichio as affected by different levels of nitrogen

N levels (kg/ha)	Fresh weight (g/crop)	Dry matter (%)	Crop height (cm)	Leaf numbers (per plant)	Firmness (1-10)
0	260.5 a	15.24 d	21.1 a	24.4	9.5 d
50	290.8 b	14.02 c	25.6 b	24.8	9.1 cd
100	321.6 c	13.46 bc	27.3 bc	25.6	8.7 c
150	360.2 e	12.81 b	34.5 d	25.3	7.9 b
200	342.1 d	11.64 a	30.7 c	24.8	7.2 a
F-test CV (%)	* 30.8	* 24.5	* 18.4	ns 14.5	* 10.2

ns = non significant; * = significant (*P<0.05*)
Means followed by the same letter(s) are not significantly different at 5% level

Yield and yield components significantly varied among cultivars. The results shown in Table 2 indicated that cv. 'Verona' had a significantly greater production (406.1 g/crop - the highest fresh weight of heads) compared to the other five cultivars, whereas the lowest yield was in cv. 'Palla Rossa' (270.8 g/crop). Among the different yield components observed, no significant

differences were observed on percent of dry matter with different cultivars. Statistically significant differences were found in the mean height of raddichio crops. Cv. 'Foresto' had the highest head (34.6 cm). It was followed by cv. 'Monivip' (29.4 cm). These values were the lowest in the cv. 'Palla Rossa' (22.8 cm) and in cv. 'Verona' (21.8 cm). The highest leaf number was observed in cv. 'Verona' (38.4) and lowest in cv. 'Palla Rossa' (19.2). There were highly significant differences in head firmness among tested cultivars. Cv. 'Palla Rossa' (9.4) and cv. 'Foresto' (9.1) had the best and cv. 'Castel Franco' (6.8) had the lowest grade for head firmness.

Table 2: Yield and yield components of six raddichio cultivars

N levels (kg/ha)	Fresh weight (g/crop)	Dry matter (%)	Crop height (cm)	Leaf numbers (per plant)	Firmness (1-10)
Monivip	348.6 d	12.85	29.4 b	32.1 d	7.5 b
C. Franco	310.4 c	12.04	25.1 ab	27.5 c	6.8 a
Anivip	286.8 b	11.78	21.8 a	23.7 b	8.2 c
Foresto	306.2 c	11.64	34.6 c	26.8 c	9.1 d
P. rossa	270.8 a	11.85	22.8 a	19.2 a	9.4 d
Verona	406.1 1	12.08	24.8 ab	38.4 e	7.6 b
F-test	*	ns	*	*	*
CV (%)	25.6	8.2	12.1	19.4	14.5

ns = non significant; * = significant ($P<0.05$)
Means followed by the same letter(s) are not significantly different at 5% level

Conclusions

N is one of the most important nutrients for producing raddichio. It is also the most difficult element to be managed in fertilization system such that an adequate and not excessive amount of N is available during the whole growing season. An adequate supply of N is essential for vigorous vegetative growth, head formation and optimum yield. An excessive application of N on the other hand is not only uneconomical, but it can prolong growing period and delay crop maturity. The results of the presented research show that the optimum N requirement of the above raddichio when grown in Slovenian ecological conditions is 150 kg/ha. Further work is required on the fate of fertilizer N applied to raddichio, in addition to the development of new tehniques for increasing the efficiency of use of fertilizer N. Emphasis should be placed on the elucidation of the different pathways of N loss from inorganic fertilizers applied applied to raddichio grown under different environmental conditions.

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Original scientific paper

Grapevine water requirements in different regions of Serbia

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Abstract

Grapevine seasonal water requirements and hydromodule of a drip irrigation system were evaluated for different regions of Serbia. Meteorological observations were analyzed at fourteen meteorological stations of the Republic Hydrometeorological Service of Serbia for the last 20 years (2000-2019). The observations were used to calculate referent evapotranspiration, effective precipitation and grapevine evapotranspiration. Water deficit during the vegetation (March-September) were estimated as a difference between the sum of the grapevine evapotranspiration and effective precipitation. The largest water deficit occurs in July, which is the month of peak water consumption. The average seasonal water deficit for the grapevine is about 138 mm. Hydromodule of a drip irrigation system in the month of the greatest water needs (July) is in average 0.45 $l \cdot s^{-1} \cdot ha^{-1}$. Aim of this research is to support producers, based on the grapevine water requirements and available soil and water resources, to select appropriate cultivation system, agro- and ampelo-technical measures that will provide high level yield and grape quality.

Key words: grapevine, water deficit, climate change, irrigation, hydromodule

Introduction

Increased frequency of dry periods during the summer months and altered precipitation distribution in the vegetation period are consequences of the ongoing climate change. Irrigation became a necessary adaptation measure in the agricultural plant production. In order to meet the needs of all water users, as well as the food needs of the increasing world's population, it

is necessary to improve the irrigation strategy with an emphasis on water savings (Fereres & Evans, 2006).

In viticulture, the maximum income is not necessary related to the maximum yield, but rather to finding and maintaining the balance between quality and quantity of the yields (Chaves et al., 2007). Regulated deficit irrigation (RDI) has become widespread as a strategy to save water and improve the quality of red grape varieties (Robi et al., 2004; Santesteban et al., 2011; Basile et al., 2011; Casassa et., 2015). Conesa et al., (2018) pointed out that in the RDI strategy should be implemented carefully, especially in the matter of estimating the value of water deficit and the irrigation timing. Good irrigation management in vineyards can be achieved only by a good knowledge of the grapevine seasonal sensitivity to water stress and by monitoring physiological parameters of the plant, such as the leaf water potential (Girona et al., 2006, 2009). The main concern in the adoption of the RDI strategy in commercial vineyards is the fact that it is very difficult to achieve the uniform water stress level across all parts of a vineyard, due to spatial differences in soil properties and topography, which may greatly influence yield and its quality. The aim of this research is to determine the grapevine seasonal water requirements in different regions of Serbia. It will support producers to adopt the most favorable irrigation regime, depending on the available resources (soil and water).

Materials and Methods

Analysis in this research is done using observations from fourteen meteorological stations of the Republic Hydrometeorological Service of Serbia located in fourteen administrative districts of central, eastern and southern Serbia. In this research, calculated are the grapevine water requirements in the Belgrade Region, Regions of Eastern, Southern and Central Serbia and Šumadija In Table 1 is given a list of regions, administrative districts and corresponding meteorological stations and their location. Daily meteorological observations are analyzed for the period of the last 20 years (2000-2019) and used to calculate referent evapotranspiration, effective precipitation, grapevine evapotranspiration and hydromodule of an irrigation system. Referent evapotranspiration is estimated using the modified Hargreaves method (Trajković, 2007b):

$$ETo = 0.0023 \cdot 0{,}408 \cdot Ra \cdot (Tmax - Tmin)^{0.424} \cdot (Tavg + 17.8)$$

Where: ETo – referent evapotranspiration (mm·day^{-1}); Ra – extraterrestrial radiation (mm·day^{-1}); Tmax is maximum 2 m air temperature (°C); Tmin – minimum 2 m air temperature (°C); Tavg – mean daily 2 m temperature (°C), calculated as a mean of Tmin and Tmax.

Effective precipitation are estimated as 90% of daily precipitation:

$$Pe_{day} = P_{day} \cdot 0.9$$

Where: Pe_{day} – daily effective precipitation (mm); P_{day} – daily observed precipitation (mm)

Grapevine evapotranspiration is calculated as a product of the reference evapotranspiration and the crop coefficient:

$$ETc = ETo \cdot kc$$

Where: ETc – grapevine evapotranspiration ($mm \cdot day^{-1}$); ETo – referent evapotranspiration ($mm \cdot day^{-1}$); kc – crop coefficient. Values of the crop coefficient for the grapevine and the length of the phenophases are adopted from the FAO56 publication (FAO Irrigation and Drainage Paper No. 56).

Grapevine water deficit is calculated as the difference between grapevine evapotranspiration and effective precipitation.

$$In = ETc - Pe$$

Where: In – net water deficit (mm); ETc – grapevine evapotranspiration ($mm \cdot day^{-1}$); Pe – effective precipitation (mm).

Gross water deficit, as a parameter of the hydromodule of the irrigation system calculation, is obtained by increasing the net water deficit for the efficiency of the irrigation system:

$$Ib = \frac{In}{Ep}$$

Where: Ib – gross water deficit (mm); In – net water deficit (mm); Ep – efficiency of the irrigation system, which has a value of 0.8 for the drip systems.

Hydromodule of the system is:

$$q_s = \frac{Ib \cdot 10000 \cdot 24 \cdot 30}{86400 \cdot t \cdot n}$$

Where: q_s- hydromodule of the system ($l \cdot s^{-1} \cdot ha^{-1}$); Ib – gross water deficit (mm); 10000 – number of m^2 in a ha; 24 – number of hours in a day; 30 – number of days in a month; 86400

– number of seconds in a day; t – irrigation system working hours (determined by the designer); n – number of working days of a system in a month (determined by the designer).

Data on the total area of the cultivated agricultural land and percent of grapevine in the plant production for considered regions are adopted from the Statistical Yearbook of the Statistical Office of the Republic of Serbia.

Table 1. Administrative districts and location of meteorological stations that were used in the research.

Region	**District**	**Station**	**Latitude**	**Altitude m a.s.l.**
Belgrade	City of Belgrade	Sopot	44° 31’ N	214
Eastern and Southern Serbia	Podunavski Smederevo	Smederevska Palanka	44° 22’ N	121
	Braničevski	Veliko Gradište	44° 45’ N	80
	Borski	Negotin	44° 14’ N	42
	Zaječarski	Zaječar	43° 53’ N	144
	Nišavski	Niš	43° 20’ N	202
	Pirotski	Dimotrovgrad	43° 01’ N	448
	Toplički	Kuršumlija	43° 08’ N	384
	Jablanički	Leskovac	42° 59’ N	231
	Pčinjski	Vranje	42° 33’ N	433
Šumadija and Central Serbia	Šumadijski	Kragujevac	44° 02’ N	185
	Pomoravski	Ćuprija	43° 56’ N	123

	Raški	Kraljevo	43° 43’ N	219
	Rasinski	Kruševac	43° 34’ N	163

Results and Discussion

Referent evapotraspiration, effective precipitation, grapevine evapotranspiration, net water deficit and hydromodule of an irrigation system were calculated for each year and for each administrative district. Calculated parameters were averaged over the 2000-2019 period and thus obtained values that are used for designing of irrigation systems.

Seasonal water consumption of grapevine for the evapotranspiration processes, effective precipitation, water deficit, and hydromodule of the irrigation system in July are presented in Table 2. Water consumption for the evapotranspiration process ranges from 488 $mm \cdot m^{-2}$ in Raški district to 528 $mm \cdot m^{-2}$ in Jablanički district (511 $mm \cdot m^{-2}$ is a mean value across the administrative districts). Values of effective precipitation vary from 320 mm (Pčinjski district) to 452 mm (Raški district), with an average of 373 mm in all districts. The water deficit is the largest in the Sothern Serbia region (Pčinjski district) with a value of 192 mm, and the lowest in Raški district (35 mm). Hydromodule in July, a month of peak consumption, is the bases of which the future irrigation system would be designed. Its value ranges from 0.36 $l \cdot s^{-1} \cdot ha^{-1}$ (Raški district) to 0.54 $l \cdot s^{-1} \cdot ha^{-1}$ (Pčinjski districts), and the mean across all districts is 0.45 $l \cdot s^{-1} \cdot ha^{-1}$.

Table 2. Seasonal evapotranspiration of grapevine (ΣETc), effective precipitation (ΣPe), seasonal water deficit (ΣIn), water deficit in July (ΣIn July), and hydromodule of the drip irrigation system (q_s July)

Administrative district	ΣETc	ΣPe	ΣIn	qs	ΣIn July
	($mm \cdot m^{-2}$)	($mm \cdot m^{-2}$)	($mm \cdot m^{-2}$)	($l \cdot s^{-1} \cdot ha^{-1}$)	($mm \cdot m^{-2}$)
Beograd	491.6	399.8	91.8	0.46	67.9
Podunavski	510.7	390.8	119.9	0.43	63.5
Braničevski	510.0	387.9	122.1	0.38	56.6
Borski	507.6	338.2	169.4	0.47	69.3
Zaječarski	525.8	335.4	190.5	0.45	70.3*

Nišavski	522.1	337.8	184.3	0.52	77.9
Pirotski	506.1	381.0	125.1	0.45	67.5
Toplički	506.6	377.0	129.6	0.39	58.9*
Jablanički	**527.6**	352.4	175.2	0.53	78.6
Pčinjski	513.3	**320.9**	**192.4**	**0.54**	**80.5**
Šumadijski	502.3	383.2	119.1	0.43	64.0
Pomoravski	524.3	385.6	138.7	0.46	69.2
Raški	**488.1**	**452.7**	**35.4**	**0.36**	**52.8**
Rasinski	512.1	375.2	136.9	0.46	69.1
Average	**510.6**	**372.7**	**137.9**	**0.45**	**67.6**

* The largest deficit in August

Figures 1 i 2 show a spatial representation of the hydromodule values and the grapevine deficit in the studied administrative districts of Serbia.

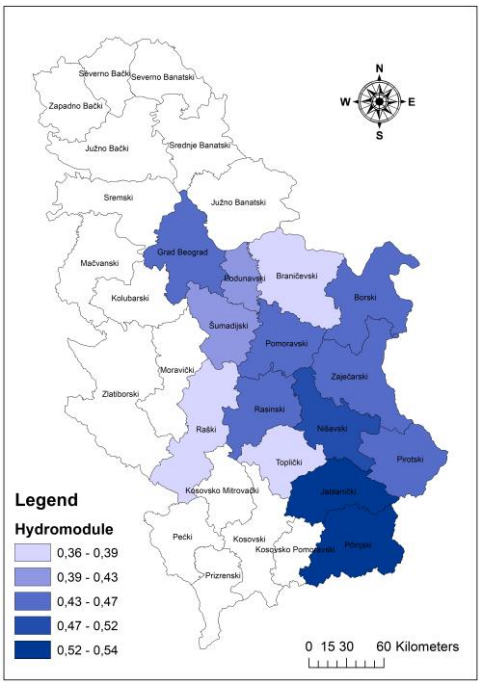

Figure 1. Hidromodule of drip irrigation system for grapewine

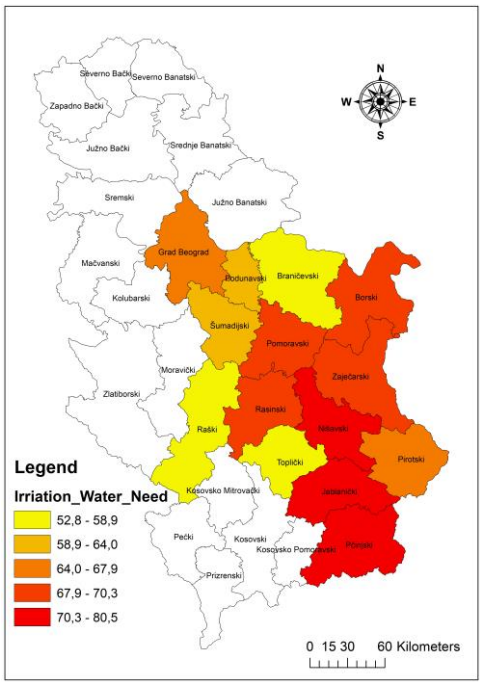

Figure 2. Grapevine water deficit in the period of peak consumption (July)

Conclusions

In viticulture, the maximum income is not necessarily related to the maximum yields, but rather to finding and maintaining the balance between yields and its quality. Irrigation affects the water use efficiency, improves morphological and physiological characteristics of plants and the quality of grapes. However, this is not always achieved by a full irrigation regime, but more often by applying a regime of the regulated deficit irrigation. The application of deficit irrigation has become widespread in viticulture, as an adaptation measure designed to save water and improve yields quality. Still, it is important to estimate the adequate irrigation timing. Proper management of the grapevine irrigation regime can be achieved only by good knowledge of seasonal sensitivity to water stress, as well as by monitoring the physiological properties of the plant. The results of this research present the grapevine water requirements during the vegetation season and could be useful to the producers to achieve the best quality yields by using the good agronomic practice.

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Original scientific paper

The influence of extraction solvents on the antioxidant potential of St. John's wort (*Hypericum perforatum* L.)

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Abstract

Hypericum perforatum L. (St. John's wort) is medicinal plant with high antioxidant, anti-inflammatory, antiviral, antimicrobial and antitumoral activities, used in treatments of many diseases. In this paper content of polyphenols compounds (total phenols, tannins and flavonoids) and antioxidant potential of methanol, ethanol, acetone and aqueous extracts of Hyperici herba were evaluated. The highest concentration of total phenols and total tannins were found in acetone extracts. The highest total flavonoids amount was detected in alcohol extracts. Acetone extracts showed the strongest antioxidant capacity. The results suggested that polyphenols are one of the main compounds responsible for antioxidant activity of *Hipericum perforatum* L. extracts. Due to its chemical composition *Hipericum perforatum* L. is valuable raw material for pharmaceutical and cosmetical industry.

Key words: St. John's wort, antioxidant capacity, polyphenols

Introduction

In recent years, antioxidants from plant sources have gained increasing interest because of its capability to neutralize or scavenge free radicals and protect cells from oxidative damages. Free radical is defined as an atom, molecule, or ion with one or more unpaired electron in its outer shell and normally generated in organisms when cells use oxygen to produce energy. The imbalance between production of free radicals and antioxidant defence system lead to oxidative

stress, which has implications for the progression of many degenerative diseases (cancer, cardiovascular illnesses, Alzheimer's disease, Parkinson's disease and others) (Pham-Huy et al., 2008; Sindhi et al., 2013). Plants are rich in bioactive compounds with high antioxidant activity such as tocopherols, tocotrienols, ascorbic acid, flavonoids, carotenoids and phenolic acids (Zehiroglu and OzturkSarikaya, 2019; Tecucianu and Oanacea, 2020).

Hypericum perforatum L. (St. John's wort) is a rich source of various groups of biologically active compounds e.g. naphthodianthrones (hypericin, pseudohypericin), phloroglucinol (hyperforin, adhyperforin), flavonoids (hyperoside, quercitrin, quercetin, rutin phenolic acid (chlorogenic acid) (Koyu and Haznedaroglu, 2015) and tannins (Maleš et al., 2006). Antioxidant, anti-inflammatory, antiviral, antimicrobial, antioxidant, antitumoral activities of this plant is well documented and related to its complex chemical composition and high concentration of mentioned bioactive compounds, located in buds, blossoms, and tips of twigs. However, the content of these compounds depends on species, growing regions, time of harvesting, extraction process and storage condition (Oliveira el al., 2016; Makarova et al., 2021).

St. John's wort is a member of Hypericaceae family with long traditional use in folk medicine and high distribution all over the world. It is also accepted by conventional medicine due to its positive pharmacological activities and benefits for human health. Dried flowering tops or aerial parts of plants represent the crude drug (Hyperici herba) commercially used in a form of tea, oil extracts, tinctures or sophisticated phytopharmaceutical products (i.e. capsule) (Šavikin et al., 2017) in treatment of internal and external diseases such as gastrointestinal diseases, bronchitis, sore throat infections (Güzel et al., 2019), nerve pain, wounds, skin inflammation, sleep disorders, depression, and haemorrhoids (Müller, 2003; Shrivastava et al., 2015).

The aim of this paper was to investigate the influence of different extraction solvents (70% methanol, 70% ethanol, 70% acetone and distillate water) on polyphenols content (total phenols, total tannins and total flavonoids) and antioxidant capacity of St. John's wort's dry areal parts.

Material and Methods

The wild-growing populations of St. John's wort harvested at full flowering stage were used as plant material in this study. Plants were collected at mountain Kopaonik, Treska, Serbia, during the summer 2020. After harvest, plant material (herba) was air-dried at dark place at room temperature till constant weight and grounded to a fine powder using a blender mill. One

gram of samples was extracted with 10 ml 70% methanol, 70% ethanol, 70% acetone or distillate water during 24 hours. The extracts were centrifuged, filtered and kept in fridge.

Folin-Ciocalteu colorimetric method as described by Nagavani and Raghava Rao (2010) with slight modification was used for determination of total phenolic compounds (TP) and total tannins (TT). Quercetin was used as standard and the amount of total phenolic compounds and total tannins were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW). Total flavonoids content (TF) was estimated spectrophotometrically using aluminium chloride ($AlCl_3$) method, previously described by Saha el al. (2013). The concentration of total flavonoids was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

Ferric-reducing antioxidant power of extracts (FRAP) was assayed following method reported by Valentão et al. (2002), based on reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to blue colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by antioxidants present in samples. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was carried out according to method based on reaction between stable DPPH radical and a substance that can donate a hydrogen atom (Lai and Lim, 2011). ABTS radical cation assay, based on reduction dark blue colored 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$) to colourless ABTS by antioxidants, was estimated by the protocol of Zheleva-Dimitrova et al. with minor modification. The total antioxidant activity (TAA) was carried out by phosphomolybdenum method (Kamath et al., 2015). According to Saha et al. (2013) was estimated the total reduction capacity of extracts (TRC). The results of antioxidant activity estimated by DPPH, FRAP, ABTS, TAA and TRC were expressed as milligrams of trolox equivalents per gram of dry weight (mg Trolox/g DW). The superoxide free radical scavenging activity was estimated using a riboflavin/nitro blue tetrazolium (NBT) method based on ability of superoxide dismutase (SOD) to inhibit photochemical reduction of NBT. The results were expressed as number of International Units (IU) of SOD equivalents per gram of dry weight (IU SOD/g DW) (Kalaskar and Surana, 2014).

The data in triplicate were reported as mean ± standard deviation (Table 1). Statistic evaluation of data was analysed using software STATISTICA ver. 13.2 (StatSoft, Inc., USA). A one-way analysis of variance with the *post hoc* Fisher LSD was used to compare significant difference between the groups at the 5% significance level ($p< 0.05$). The correlation coefficients were done by Pearson.

Results and Discussion

The amounts of polyphenolic compounds (total phenols, tannins and flavonoids) as well as antioxidant activity measured by DPPH, FRAP, ABTS, TRC, TAA and NBT assays are summarized in *Table 1*. The range of total phenols varied between 42.70 and 85.82 mg QE/g DW in different solvent extraction systems. The concentration of total tannins was from 55.75 to 40.47 mg QE/g DW and the content of total flavonoids was from 9.14 to 13.24 mg QE/g DW. In this study, acetone extracts of St. John's wort were showed the highest concentration of TP (85.82 mg QE/g DW) and TT (55.75 mg QE/g DW). The amount of TP in ethanol extracts (63.41mg QE/g DW) was higher than in methanol (57.27 mg QE/g DW) and aqueous extracts (42.70 mg QE/g DW). According to analysis of variance followed by Fisher LSD *post hoc* test, the difference among TP and TT contest in acetone extracts and other tested extracts was statistically significant (p <0.05). Bonoli et al. (2004) were found that extraction of TP from barley flour by mixture of ethanol and acetone was most effective. Our results show that content of TF is not significantly higher in ethanol extracts (13.24 mg QE/g DW) than in methanol (12.30 mg QE/g DW) as well as the significant difference between its content in aqueous (9.14 mg QE/g DW) and acetone extracts (9.69 mg QE/g DW) no exist. In one previously research, Wang and Helliwell (2001) were reported that aqueous ethanol solvent had better performance for extraction of TF from tea, than aqueous methanol and aqueous acetone. Do et al. (2014) were found that 75% acetone extracts of *Limnophila aromatica* had the highest concentration of TP and TF. The differences between TP and TF extraction efficiency in this study and those of other studies may be associated with different chemical properties and polarity of flavonoids and others polyphenol compounds.

Our results demonstrate that *H. perforatum* extracts is a good source of polyphenols compounds and this is in agreement with previous research (Fathi and Ebrahimzadeh 2013; Šavikin et al., 2017). Phenolic compounds (flavonoids, phenolic acid, and polyphenol compounds) are plant secondary metabolites responsible for colour and sensory characteristics of vegetables and fruits as well as they have a significant role in plants` growth, reproduction, protection against pathogen and predators. In human diet phenolic acids, flavonoids, and tannins represent the most abundant antioxidants (Vuolo et al., 2019).

The extraction yields are strongly depending on the solvent polarity and chemical nature of polyphenols, under the same temperature, pH and extraction time (Do et al., 2014). One of widely used solvent for antioxidants and phenols extraction is methanol (Hertog et al., 1993) especially in extraction of lower molecular weight polyphenols while acetone is better

extraction solvent for the higher molecular weight flavanols. However, there is no universal extraction procedure suitable for extraction of all phenolic compounds (Dai and Mumper, 2010).

The results of antioxidant tests suggested that *H. perforatum* extracts have strong antioxidant capacity and this is in agreement with study of Güzel et al. (2019). The acetone extracts showed the highest value of TAA (478.46 mg Trolox/g DW), TRC (310.54 mg Trolox/g DW) as well as NBT (14.03 IU SOD/mg DW), ABTS (311.31 mg Trolox/g DW), DPPH (239.40 mg Trolox/g DW) free radical scavenging activity. The lowest antioxidant activity obtained by FRAP, DPPH, TRC tests was observed in aqueous extracts (119.00, 98.74 and 143.98 mg Trolox/g DW, respectively) while ABTS, TAA, NBT assays showed the lowest antioxidant activity in methanol extracts (206.70, 306.94 mg Trolox/g DW and 10.45 IU SOD/mg DW, respectively). These assays are one of the most frequently used for evaluating antioxidant activity. But different antioxidant tests are based on different chemical reactions and in order to comparing antioxidant properties of selected compounds must be used more than one method (Shalaby and Shanab, 2013; Shahidi and Zhong, 2015).

Table 1. Content of polyphenol compounds and antioxidant activity in St. John`s wort extracted by four different solvents

	Extraction solvent			
	Water	70% Methanol	70% Ethanol	70% Acetone
TP[1] (mg QE/ g DW)	42.70 ± 0.88^{d}	57.27 ± 3.35^{c}	63.41 ± 0.93^{b}	85.82 ± 0.29^{a}
TT[2] (mg QE/ g DW)	40.47 ± 0.94^{c}	52.75 ± 0.32^{b}	53.68 ± 0.69^{b}	55.75 ± 0.98^{a}
TF[3](mg QE/ g DW)	9.14 ± 0.16^{b}	12.30 ± 0.73^{a}	13.24 ± 0.32^{a}	9.69 ± 1.78^{b}
FRAP[4](mg Trolox/g DW)	119.00 ± 2.05^{c}	140.27 ± 20.00^{b}	181.49 ± 4.45^{a}	155.56 ± 0.34^{b}
DPPH[5](mg Trolox/g DW)	98.74 ± 13.96^{c}	131.77 ±27.02^{c}	181.80 ± 9.62^{b}	239.40 ± 27.07^{a}
TRC[6](mg Trolox/g DW)	143.98 ± 2.55^{d}	192.20 ± 7.96^{c}	271.54 ± 0.94^{b}	310.54 ± 18.62^{a}
NBT[7] (IU SOD/mg DW)	12.14 ± 0.25^{c}	10.45 ± 0.57^{d}	12.92 ± 0.40^{b}	14.03 ± 0.37^{a}
ABTS[8] (mg Trolox/g DW)	235.33 ± 1.36^{c}	206.70 ± 4.78^{d}	249.51 ± 1.76^{b}	311.31 ± 6.86^{a}
TAA[9] (mg Trolox/g DW)	372.38 ± 7.02^{b}	306.94 ± 20.87^{c}	379.42 ± 6.26^{b}	478.46 ± 21.81^{a}

Value is a mean of three replicates ± standard deviation (SD)

Value without the same superscript within each row differ significantly at $p<0.05$ (Fisher LSD *post hoc* test)

[1] TP total polyphenol content [2] TT total tannin content [3] TF total flavonoid content [4]FRAP Ferric-reducing antioxidant power [5] DPPH 2,2-diphenyl-1-picrylhydrazyl [6] TRC total reduction capacity [7] NBT Nitrobluetetrazolium[8] ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) [9] TAA Total antioxidant activity

Pearson coefficient (*Table 2*) showed positive correlation among TP and FRAP, DPPH, TRC, NBT, ABTS and TAA assays. FRAP, DPPH and TRC were in strong positive correlation with

TT while TF was in positive correlation with FRAP assay. The correlation analysis in Tusevski et al. (2019) study also indicated that antioxidant activity of *H. perforatum* is related to phenolic compound content.

Table 2. Pearson correlation coefficient between biochemical assays.

	FRAP[2]	DPPH[2]	TRC[2]	NBT[3]	ABTS[2]	TAA[2]
TP[1]	0.5815	0.980*	0.9417	0.6660	0.7443	0.8200
TT[1]	0.7685	0.8138	0.8532	0.2712	0.2892	0.4037
TF[1]	0.694	0.059	0.221	-0.336	-0.503	-0.419

*. Correlation is significant at the 0.05 level (2-tailed).

[1]Expressed as mg Quercetin/ g DW[2]Expressed as mg Trolox/g DW[3] IU SOD/ mg DW

Conclusions

The results of this study showed that the extraction solvent has significant influence on all measured phenolic compounds as well as antioxidant capacity estimated by six different assays. Our results suggested that extraction by using 70% acetone can provide significantly higher yield of total phenolic compounds from St. John's wort's raw material than other tested extraction systems. The strongest antioxidant potential measured by NBT, ABTS, DPPH, TAA, TRC was detected in 70% acetone extracts. Antioxidant activity of *H. perforatum* is directly connected with phenol compounds content in investigated extracts.

Acknowledgement

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Original scientific paper

Effects of thermovinification and carbonic maceration on polyphenols extraction of cv. Cabernet Sauvignon

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Abstract

In this study, the impact of winemaking technique: classical vinification with different maceration time (7 (CC7) and 14 (CT14) days); thermovinification (60°C (T60) and 80°C (T80) and carbonic maceration (CM) on the total phenolic content and phenolic acids in wines was studied. Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard and phenolic acids in wines were performed by UPLC H-Class System. It was conducted that the application of thermovinification (T60°C, 2800mg GAE/l; T80°C, 3130 mg GAE/l) leds to higher total phenolic content then in wines CT14 (1395 mg GAE/l). On the other hand, total phenolic content of CM wines were a lower than those in the wines made by CC7, which lasted 7 days. All phenolic acids have had higher content in samples CT14, except caffeic acid which was the highest for T60 sample (7,5819 mg/l).

Key words: thermovinification, carbonic maceration, total phenolic content and phenolic acids.

Introduction

There are many winemaking techniques (maceration time, heat and cold treatman, yeast and enzyme used, SO_2 dose, fining agents, ageing of red grape processing) used to produce different styles of wines with different content of phenolic compounds. Phenolic compounds depend of the grape variety, ripening stage, cultivation, agrotecnique conditions, location (Ševcech et al., 2015; Borazan & Bozan, 2013). The quantity of phenolic compounds that are transferred from grapes into wine during maceration varies according to the conditions of the process (González-

Arenzana et al., 2020). Thermovinification is a process that can be used for red wines to increase wine color and the contents of some phenolic and volatile compounds during subsequent conventional fermentation. The heat destroys the skins cell membranes, releasing the pigments, tannins and other substances into the wine. Therefore, the yield of extraction is temperature dependent, within the practical range of 60–80°C. Thermovinification can be done either by heating the must after crushing, or by heating the grapes before crushing. For wines that are to be consumed young, and when the colour intensity is a problem, thermovinification may help to enhance the colour (Atanackovic et al., 2012). The use of solid carbon dioxide has an additional advantage: after freezing the grapes it sublimes on the surface of berries protecting them against the subsequent action of oxygen. It is interesting that this method reportedly increases the concentration of tannins while the heat treatment of berries enhances only anthocyanins. Freezing may also disrupt tannin containing cells of the seeds, increasing total polyphenol extractability (Ševcech et al., 2015, Sacchi et al., 2005). Changing the temperature during processing is an effective method that influence extraction because temperature affects the permeability of the cells and membranes in grape berries (Koyama et al., 2007).

Although a large number of studies on the phenolic composition of red wines and a considerable number of studies on the phenolics and their relationship with winemaking technology have been established, there has been a few of published studies concerning the change in the phenolic acids of red wine during the winemaking process. The aim of this study was determination of influence of thermovinification and carbonic maceration on phenolic extraction and their content after storage six months in the bottle.

Materials and Methods

Grapes variety Cabernet Sauvignon were harvested in 2018 at optimal enological maturity, which originated from vineyards belonging to experimental field "RADMILOVAC" of the Faculty of Agriculture in Zemun. Phytosanitary state was 100% health, sugar in must 23% and total acid in must 6.8 g/l as tartaric acid. Sugar content of grape must was analyzed using the Oechsle tester, an apparatus for measuring grape must density, and the titratable acid content was determined by a volumetric method. Grapes for classical vinification were crashed and destemmed, sulfited with 10 g of $K_2S_2O_5$ per 100 kg and inoculated with a pure yeast strain (BDX, Lallemand, Canada). For therovinification crashed grapes were heated on 60°C for one hour (T60) and 80°C for 30 min (T80) and compared with classical vinification without

temperature treatman (CT14). Treated grapes which were cooled to 27°C and inoculated with a pure yeast strain (BDX, Lallemand, Canada) to begin the alcoholic fermentation and maceration 14 days. Contrary to this, grapes for carbonic maceration was prepaired by destemming without crashing. In this sample was added dry ice and covered with a cap. After four days it was inoculated with a pure yeast strain (BDX, Lallemand, Canada) and 7th day must was separated from whole berries. Control sample (CC7) for this experiment was wine produced by classical maceration which lasted also 7 days and inoculated with same yeast. Total phenolic content in all wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard. The analysis of derivates of benzoic acid and derivates of cinnamic acid in wines was performed by using a Waters Acquity UPLC H-Class System (Waters, Milford, MA, USA). Statistical analysis was conducted using SPSS Statistical V20.0 software (IBM, Chicago, IL, USA; 2014) and Origin Pro 8 (OriginLab, Northampton, MA, USA; 2008)

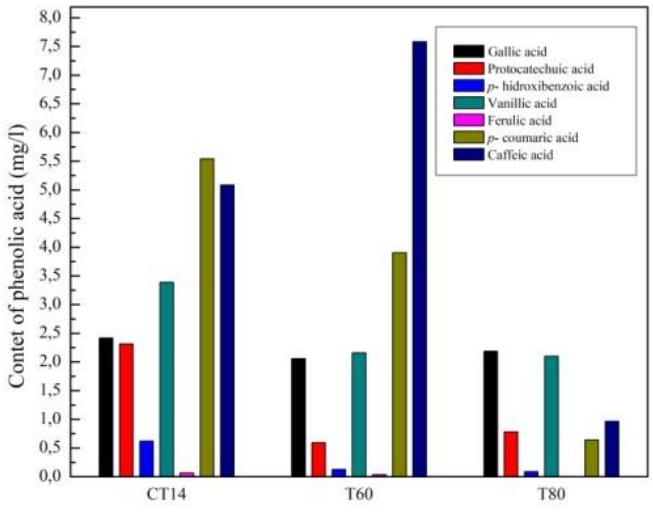
Results and Discussion

Influence on content of phenolic acids by thermovinification and carbonic maceration

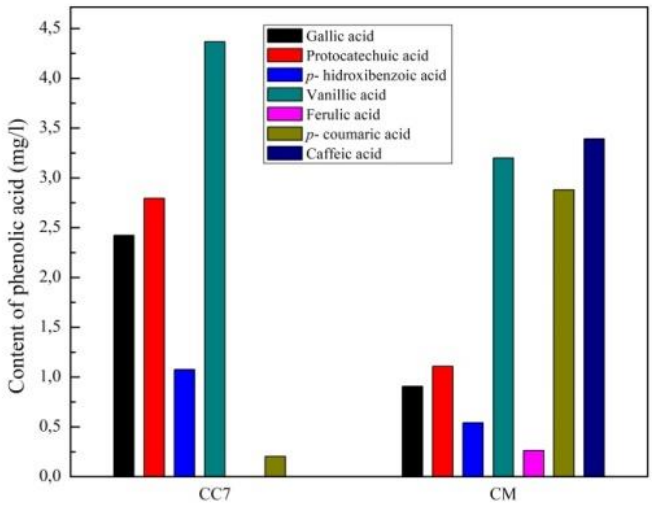
All analysed phenolic acids have had higher content in samples made by classical vinification (CT14), except caffeic acid which was the highest for sample termovinificated on 60°C (7,5819 mg/l). Probably, this was optimal temperature for its extraction because at higher temperature (80°C), content of caffeic acid was notably lower (0,9658 mg/l). It can be concluded that in the must heated to 80 °C the amount of caffeic acid was probably degraded by high temperature or high temperature was inhibited polyphenol oxidase enzyme which hydrolyzes caftaric into caffeic acid in the must before fermentation. (Gómez-Plaza et al., 2001; Wojdyło et al., 2021). These enzymes are only destroyed at temperatures over 60°C, while their activity increases with temperature up to that point, so the must has to be heated very rapidly. Enzymatic activity actually increases at temperatures below 60°C (Ryberau Gayon et al.,2006). Gallic and vanillic acids were the most abundant benzoic acids in wines produced by thermovinification, but the highest content was considered in wines made by classical vinification (CT14). According to Wojdylo et al. (2021), compared with traditional maceration, thermo-maceration pretreatment improved extraction of phenolic acids that is opposite to our results. Liberation of phenolic acids during thermo-maceration could result from releasing them from acylated anthocyanins, notably *p*-coumaric acid, whose the highest content reached wine CT14 in amount 5,5402 mg/l. Ferulic acid was found at the lowest level in all of the wines (T60, T80 not detected, CT14)

which is in accordance with research by Borazan & Bozan, (2013). In our research it was noticed a lower content of protocatechuic acid than in control sample CT14 and it is in accordance with Borazan & Bozan, (2013) where was noticed higher decrease of this acid in must-heated wines. There was no statistical signifficante difference in content of phenolic acids comparing samples obtained by classical vinification (CT14) and T60. Contrary to this, applied shorter thermovinification treatman with 80°C, signifficanty changed content of phenolic acids in wine compared with the same control sample CT14 ($p \leq 0,05$) (Graph 1).

Derivates of cinnamic acid found in wine samples CM were extracted in higher content than those found in control sample CC7 where ferulic and caffeic acids were not detected. Derivates of benzoic acid (gallic, protocatechuic, *p*- hydroxybenzoic and vanillic acids) were found in CC7 in higher concentrations than in wine obtained by carbonic maceration (CM) what is in accordance with Pellegrini et al., (2000) who found that content of gallic acid was twice as high as samples obtained by traditional vinification. The most predominat was vanillic acid in both of wine (CC7 and CM) in concentrations 4,3667 mg/l and 3,1993 mg/l. Content of phenolic acids in wine with carbonic maceration (CM) wasn't signifficanty affected, compared with control sample CC7 (Graph 2).



Graph 1. Content of phenolic acids in wine obtained by thermovinification (T60 and control T80) and control sample made by classical vinification (CC14).



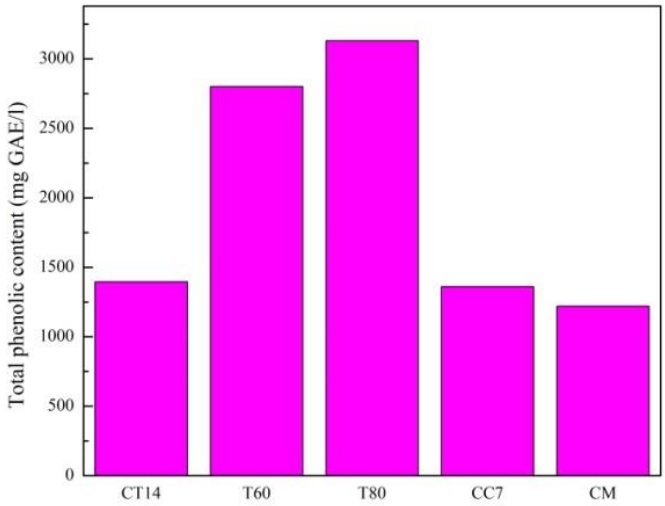
Graph 2. Content of phenolic acids in wine made by carbonic maceration (CM) and control sample made by classical vinification (CC7).

Influence on total phenolic content by thermovinification and carbonic maceration

Samples made by thermovinification contained higher content of TPC than in control sample made by classical vinification (CT14). The highest extracted total phenolic content by thermovinification was observed for TM80 (3130 mg/l) (Graph 3). Thermo-maceration pre-treatment is a time and temperature dependent process that enables to achieve higher final concentrations of phenolics than other extraction methods. Some studies highlighted benefits of moderate temperatures (60–70 °C) for an optimal extraction, since high temperature enhances mass transfer and increases the internal liquid phase which raises the pressure and causes circulation of the solutes through plant membranes (Wojdyło et al., 2021). According to Borazan & Bozan (2013) he highest TPC content was found in wines that were produced under the condition of prefermentative mash heating (2.22–2.72 g GAE/l), followed by the control and enzyme-treated wines what is similar with our investigation.

According to Gómez-Míguez & Heredia (2004) the total polyphenol content was lower, in the carbonic maceration wine (978.30 versus 2219.50 mg/L in traditional vinification) what is in accordance with our results (1220mg/l versus 1360mg/l in classical vinification CC7). This fact seems to show that the degree of skin tissue degradation was insufficient. Results of a multi-year study of Hungarian varieties indicated that carbonic maceration increased the total phenol content of the wines compared to fermentation on the skins. Opposite to this, study with

muscadine cultivars showed no effect or a decrease in total phenols with carbonic maceration (Sacchi et al., 2005). The effect on total phenols was dependent on the varieties present (Pellegrini et al., 2000).



Graph 3. Total phenolic content of control and wine samples made by thermovinification and carbonic maceration.

Conclusion

There are a lot of different technique for vinification such as remontage, pigeage, rack and return, but also it could be applied cold soaking, thermovinification and carbonic maceration. In this paper it was investigated impact of thermovinification (60°C and 80°C) and carbonic maceration on content of non-flavonoid compounds (derivates of benzoic and derivates of cinnamic acid) and it was observed no statistical signifficante difference in content of phenolic acids comparing samples obtained by classical vinification (CT14) and thermovinification T60. Contrary to this, applied shorter thermovinification treatman with 80°C, signifficanty changed content of phenolic acids in wine compared with the same control sample CT14 ($p \leq 0,05$). It was noticed higher content of TPC in wine samples made by thermovinification with 80°C.

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Original scientific paper

Some important fruit characteristics of diverse *Elaeagnus angustifolia* L. genotypes from Coruh valley in Turkey

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Abstract

Russian olive (*Elaeagnus angustifolia*) fruit are used for traditional food and dietary supplements in Eastern Europe, Balkans, Anatolia and near Asia. The specie morphologically included numerous diverse genotypes and also contain large amounts of phytochemicals that may benefit human health. Information on the effect of genotype on some important fruit traits is needed in order to formula better breeding strategies for Russian olive. The main fruit characteristics of Russian olive genotypes were evaluated from 10 native grown plants at Coruh valley in Turkey. Results showed that genotype influenced fruit weight, shape index, peel and flesh color, total phenolic content and antioxidant activity. The fruit weight ranged from 0.96 g (EA6 genotype) to 2.09 g (EA9) genotype. The majority of genotypes had cream flesh color but white cream color was also evident. Total phenolic content varied from 433 mg GAE/100 g FW (EA1) to 604 mg GAE/100 g FW (EA7). Genotypes EA9 and EA3 had significantly higher fruit weight and genotypes EA7 and EA2 had the highest antioxidant capacity, and they may be good selections for producing health Russian olive products.

Key words: Russian olive, composition, analysis.

Introduction

Anatolia is origin or second homeland of a lots of wild edible fruits and one of them is *Elaeagnus angustifolia* L. The plant known as Russian olive or oleaster and is close relatives of sea buckthorn (*Hippophia rhamnoides* L.) because both *Elaeagnus* and *Hippophae* genus belongs to Elaeagnacea family. The family is also included another genus namely *Shepherdia* (Ayaz et al., 1999; Sahan et al., 2013; Kalyoncu et al., 2008). Russian olive has nitrogen fixing

capabilities as sea buckthorn relatives and when planted with fruit trees is said to increase the overall yield of the orchard by 10% whilst themselves producing a yield of berries. Soil under subcanopy Russian olive showed a 55% increase in total nitrogen and nearly four times the amount of available nitrogen compared to soils under *Populus* alone (DeCant, 2008; Singh et al., 2008).

Elaeagnus angustifolia, widely distributed all around the Turkey because plant is valued not only for edible fruits but also ornamental values due to scented flowers and colorful foliage. In Turkey the plants occur diverse altitude and climatic conditions from subtropical the Mediterranean region to mild continental Black Sea Region and Marmara Region even in cold continental East Anatolia Region (Kalyoncu et al., 2008).

In terms of world distribution, *Elaeagnus angustifolia* native to central and western Asia countries included Afghanistan, Pakistan, Uzbekistan, Azerbaijan, south Russia, south Ukraine, Kazakhstan and Iran. It is also introduced to Northern America. It was originally planted in the United States beginning in the late 1800s as an ornamental shrub or small tree.

Russian olive trees bear single seeded ellipsoid or sub-globose fruits. The plant is a long live tree (80 to 100 years) which grows rapidly up to 10 m in height and 30 cm in diameter and starts to fruit after 5 to 6 years (Kiseleva and Chindyaeva, 2011). The plant can tolerate a wide range of harsh environmental conditions such as flood, severe drought, stony, sandy and high salinity or alkalinity of the soils (Asadiar et al., 2013).

Different parts of the Russian olive plant have been used in a variety of medicinal formats, in perfume industries, as well as in wood-work and musical instruments production (Kiseleva and Chindyaeva, 2011). Russian olive fruits have high nutritional values which contain proteins, sugar, vitamins and minerals. The flowers are small, fragrant, and a yellowish-white color and have been used as a source of nectars for honey bees, as well as a flavoring agent in liqueur production (Sahan et al., 2013; Saboonchian et al., 2014).

In literature limited study has been conducted on Russian olives. Thus, the aim of this study was to characterize wild Russian olive genotypes grown in Coruh valley based on their morphological traits and biochemical content.

Material and Methods

Plant material and extraction process

Elaeagnus angustifolia genotypes were grown as wild in Yusufeli district. A total ten Russian olive samples were obtained from alongside Barhal river. Fruits were harvested in the periods

when the berries belonging to genotypes reached full maturity. Around 1 kg Russian olive berries was carefully collected in the wild grown trees in 2020 and transferred quickly to the laboratory. 40 fruits were used for fruit weight, shape index, pedicel length, peel and flesh color determination. Fruit weight and pedicel length were determined by digital balance and digital caliper. For biochemical analyses, pedicels were removed and the fruits (peel+flesh) were freeze-dried. The freeze-dried tissues were then extracted with 200 mL of 1% HCl in methanol. Extraction was carried out under stirring for 48 h, and repeated in triplicate. Extracts were pooled, and this mixture was used for further procedures after deep-freezing (-70 ºC) for no longer than 3 days.

Total polyphenol content

To determine the total phenolic content (TPC) by the Folin-Ciocalteu method, 1 mg of each extract or fraction in 1 mL of methanol, was prepared. Briefly, the extract solution (100 µL) was diluted to 3 mL with distilled water and then oxidized with the Folin-Ciocalteu reagent (500 µL); after 3 min, the reaction was neutralized with Na_2CO_3 20% solution (2 mL) and then allowed to react for 60 min in darkness. The absorbance of the resulting blue colored reaction was measured at 650 nm in a spectrophotometer. The calibration curve was performed with gallic acid (concentration range 0.30–9.00 µg/mL) and the results were expressed as mg of gallic acid equivalents per 100 g of fresh fruit (Singleton and Rossi, 1965).

DPPH free radical scavenging activity

The DPPH radical scavenging capacities of samples were performed according to Materska et al. (2015). Briefly, 200 µL of serially diluted sample or methanol (control) were added to 2.8 mL of 70 µM DPPH methanol solution. After vigorous shaking, the mixture was placed in the dark for 30 min at room temperature, and then absorbance value was determined using a Shimadzu UV-1800 spectrometer at 517 nm. The antioxidant activity was expressed as inhibition percentage (I%). The results were expressed as µmol Trolox/g FW.

Statistical analysis

The study was planned as four replication including 10 bunch and berry per replicate. In the statistical evaluations, Windows SPSS 20 was used and the differences between the means was evaluated by subjecting to ANOVA variance analysis and determined with Duncan multiple comparison test ($p<0.005$).

Results and Discussion

Morphological properties

As shown in Table 1, fruit weight, shape index and pedicel length statistically significantly varied among genotypes at 0.05 level and fruit weight, shape index and pedicel length were found between 0.96-2.09 g, 0.55-0.84 and 2.29-6.34 mm among 10 genotypes of wild Russian olive. The majority of genotypes had cream flesh color and some genotypes had white cream flesh color (Table 1). Peel color were more variable and EA3 and EA9 genotypes had dark orange, EA2, EA6 and EA7 had orange peel color. EA4 and EA5 had light orange peel color while EA1, EA8 and EA10 had orange cream peel color. (Table 1). Previously Uzun et al., (2015) reported quite variable peel color among a large number of Russian olive genotypes in Turkey. They found that orange was found to be the dominant peel color. Fourteen genotypes had orange peel color and 14 of them had an orange-cream peel color. They also determined a light orange-cream color in 11 genotypes. In addition, four genotypes showed a dark orange peel color whereas two genotypes had light orange peel color. Same authors found that flesh color of the genotypes showed lower variation than peel color and majority of the genotypes (44 of 56) had cream pulp color. Variation in the fruit color parameters may be due to genotype, environmental factors and degree of maturity at harvest (Yilmaz et al., 2009).

Table 1. Some important fruit characteristics of *Elaeagnus angustifolia* genotypes

Genotypes	Fruit weight (g)	Shape index	Pedicel length (mm)	Flesh color	Peel color
EA1	1.44	0.55	3.39	White cream	Orange cream
EA2	1.24	0.70	2.29	Cream	Orange
EA3	2.02	0.61	6.11	Cream	Dark orange
EA4	1.30	0.66	5.83	Cream	Light orange
EA5	1.13	0.72	3.89	White cream	Light orange
EA6	0.96	0.65	6.34	Cream	Orange
EA7	1.55	0.68	5.31	Cream	Orange
EA8	1.39	0.84	4.98	White cream	Orange cream
EA9	2.09	0.74	5.44	Cream	Dark orange
EA10	1.32	0.70	5.78	Cream	Orange cream
LSD_{05}	0.17	0.06	0.33		

Uzun et al., (2015) found fruit weight between 0.59-2.56 g, fruit shape index 0.52-0.92 and pedicle lengths betweeen 1.95-7.74 mm among 56 wild grown *Elaeagnus angustifolia* genotypes in Central Anatolia. Cansev et al. (2011) reported fruit weight between 1.71-2.66 g and average fruit shape index 0.60. Our results shows differences with previous studies. Raj et

al. (2010) found fruit weight and shape index between 0.43-0.99 g and 0.62-0.76 among five Russian olive genotypes, respectively in India. In a previous study performed in the province of Isparta in Turkey, the fruit weight of randomly sampled *E. angustifolia* fruits from a local market ranged between 0.96 g and 3.80 g (Akbolat et al., 2008). Likewise, Ersoy et al. (2013) reported an average fruit weight of 2.90 g for selected Russian olive trees.

Moisture, total phenolic content and DPPH values of *Elaeagnus angustifolia* genotypes are given in Table 2. We found statistically significant differences among *Elaeagnus angustifolia* genotypes for moisture content, total phenolic content and DPPH values (Table 2).

The moisture content was found between 24.04% (EA8) and 27.33% (EA5). The highest total phenolic content was the highest in EA7 genotypes as 604 mg GAE/100 g FW, and followed by EA2 (588 mg GAE/100 g FW), EA6 (544 mg GAE/100 g FW) while the lowest total phenolic content was found in EA1 genotype as 433 mg GAE/100 g FW, respectively.

Table 2. Some important fruit biochemical characteristics of *Elaeagnus angustifolia* genotypes

Genotypes	**Moisture (%)**	**Total phenolic content (mg GAE/100 g FW)**	**DPPH (µmol Trolox/g FW)**
EA1	24.41	433	28.18
EA2	26.23	588	23.72
EA3	24.92	479	25.55
EA4	27.04	495	24.70
EA5	27.33	442	28.34
EA6	24.76	544	24.23
EA7	26.06	604	22.11
EA8	24.04	461	27.23
EA9	25.55	487	26.07
EA10	25.11	504	24.89
LSD_{05}	1.02	38	2.31

DPPH values of the genotypes were found between 22.11-28.34 µmol Trolox/g FW. Cansev et al. (2011) reported average moisture content, total phenolic content and DPPH value in *Elaeagnus angustifolia* genotype as 26.48%, 470 mg GAE/100 g and 27.80 µmol Trolox/g, respectively. Hassanzadeh and Hassanpour (2018) evaluated physicochemical characteristics and antioxidant properties of thirty-eight *Elaeagnus angustifolia* L. genotypes in Iran and reported that the total phenolic content of pulp in the studied genotypes was in the range of 250-820 mg GAE/100 g FW, which are in agreement with our study. They also found that the highest antioxidant capacity of peel based on the DPPH assay was seen in KH7 genotype (93.29%) and the lowest was found in the U9 genotype (49.22%).

Conclusion

In conclusion, although a great variation in morphological traits and biochemical value was observed, all the studied *Elaeagnus angustifolia* L. genotypes had showed high diversity and can contribute significantly in a healthy and balanced diet. However, in order to make better use of the existent biodiversity, the selection and improvement of these genotypes for specific end-use of the final product should be implemented according to their special characteristics. Therefore, for fresh market, genotype EA9 and EA3 has very promising properties regarding its bigger fruits. Moreover, considering their high TPC and TPA content, these latter genotypes could be also selected for pharmaceutical applications (dietary supplements, herbal preparations, etc.), especially genotype EA7 which is an underutilized landrace considering its limited farming scale.

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Original scientific paper

Physicochemical and antioxidant properties of three strawberry cultivars and wild strawberry from central Bosnia region

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Abstract

Strawberries contain important amount of bioactive compounds, mostly polyphenols. Aim of this study was to evaluate physical properties and determine amount of polyphenols, anthocyanins and antioxidant activity in 3 different strawberry cultivars (Clery, Marmolada and Arosa) and compare it to the wild strawberry (*Fragaria vesca* L.) from the same area. Total phenolic content was determined by the Folin-Ciocalteu method. Antioxidant activity was determined using ABTS radical scavenging capacity assay and ferric reducing antioxidant potential (FRAP) assay. For determination of total anthocyanin content of all samples pH-differential method was used. There are significant differences in the content of all investigated bioactive compounds among selected strawberry cultivars. The highest phenolic content and antioxidant activity was found in wild strawberry (*Fragaria vesca* L.). Cultivar Clery had slightly higher content of total anthocyanins than wild strawberry fruits. Thus, it can be concluded that wild strawberry is a good source of polyphenols, anthocyanins, and antioxidants.

Key words: Anthocyanins, Antioxidant activity, Polyphenols, Strawberry

Introduction

The garden strawberry (*Fragaria x ananassa* Duch.) is one of the most economically important berry fruit species with a global annual production that exceeds 8.3 Mt (FAOSTAT, 2018). Commercial production of strawberry in Bosnia and Herzegovina has been experiencing

massive expansion in recent decades but with 9827 t in 2018 (FAOSTAT, 2018) it is still significantly behind production in developed countries. The genotypes of a wild strawberry (*Fragaria vesca* L.) in Bosnia and Herzegovina represent the significant genetic resource of importance for the conservation of biological diversity (Skender et al., 2015).

The fruits of cultivated strawberry cultivars are characterized by large size, bright red colour, characteristic aroma, juicy texture, and sweet fruity flavour (Yildiz et al., 2014). They are an important source of various chemical substances such as easily assimilable sugars, as well as organic acids, many vitamins, pectins and fibre. Strawberries are especially valued for their high content of health promoting compounds with antioxidant properties such as vitamin C and phenols (Tulipani et al., 2008; Milivojević et al., 2010; Djilas et al., 2011; Milivojević et al., 2011; Aaby et al., 2012; Yildiz et al., 2014; Skender et al., 2015). The content of these compounds depends on many factors such as variety, ecological conditions, degree of the fruit ripeness, the growth system, agrotechnical and protective measures (Pineli et al., 2011; Tulipani et al., 2011; Skender et al., 2015). It has been reported that number of bioactive compounds vary significantly with genotype (Anttonen et al., 2006).

The aim of this study was to evaluate physical and chemical characteristics of the most grown strawberry cultivars in central Bosnia (Gornji Vakuf-Uskoplje locality) and compare their properties with the wild strawberry growing in the same geographical area.

Materials and Methods

Plant material

In this paper phenological, pomological and chemical properties were obtained of two-year-old strawberry cultivars Clery, Marmolada, Arosa and wild strawberry. Samples of strawberry cultivars were collected in 2018 from intensive plantations in Rajci locality near Gornji Vakuf-Uskoplje, Bosnia and Herzegovina (coordinate (43°56'17.02" N 17°35'17.99" E). Wild strawberry (*Fragaria vesca* L.) was collected from natural population in the immediate vicinity in the same geographic area at an altitude of 670 m. The experiment was set up in a completely randomized block design with three replications per cultivar. Fruits of cultivated strawberry were collected at the first harvest, whereas wild strawberry were harvested at full ripening (full red colour). Collected samples were kept frozen at -20°C until the analysis.

Phenological properties

The testing of phenological fruit parameters were included onset of blossom, end of blossom, start of ripening and end of ripening. The onset of blossom was considered when 25% of flowers were opened. The end of blossom was regarded as flowering of the remaining 20% of the flowers. The start of ripening was determined when fruits show 25% of red-coloured epidermis. The end of ripening was considered as ripening of the remaining 20% of the fruit.

Pomological properties

Average fruit weight was determined on a total 30 fruits per each cultivar in respect to three replications, with ten fruits in each replication. Fruit weights of each cultivar and wild strawberry were measured with a digital balance (Kern 440, Kern and Sohn, Germany) with the accuracy of 0.01 g. Fruit height and width were measured using a digital Vernier caliper with the accuracy of 0.01 mm and results were expressed in millimeters (mm). Fruit index shape was calculated as ratio of maximum height to width, H/W.

Chemical properties

Extraction procedure

20 ml of acidified methanol (0.1% HCl) was added to 4 g of homogenized samples. Subsequently, the samples were subjected to extraction for 60 minutes in a shaker at a rate of 200 rpm at room temperature and then centrifuged.

Determination of total phenols

The total phenolic content was determined with Folin–Ciocalteu method described by Singleton et al. (1999) and as previously described by Kazazic et al. (2016). Gallic acid was used to prepare the standard curve, and the results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight of fruit (mg GA/100 g FW). All measurements were done in triplicate.

Determination of total anthocyanin content

Total anthocyanin content was determined using pH-differential method (Zhishen et al., 1999). Two solutions of fruit samples were prepared, one with 0.5 ml of extracts in 2 ml of potassium chloride buffer (0.025 M, pH 1.0) and other with 0.5 ml of extracts in 2 ml of sodium acetate buffer (0.4 M, pH 4.5). The absorbance was measured simultaneously at 510 nm and 700 nm with spectrophotometer (Genesys 20 thermo spectronic) after 20 min. The total anthocyanin

content was expressed as mg of cyanidin-3-glucoside equivalent per 100 g of fresh weight of fruit (mg CGE/100 g FW).

Ferric reducing antioxidant potential (FRAP) assay

The antioxidant activity was evaluated using FRAP method of Benzine and Strain (1996). FRAP reagent was prepared immediately before use by mixing 20 ml of 300 mM acetate buffer (pH 3.6) with 20 ml of 10 mM TPTZ (2,4,6-tri-(2-pyridyl)-5-triazine) in 40 mM HCl acid with 20 ml of 20 mM $FeCl_3$ (ferric (III)chloride) and 24 ml of distilled water. Absorption of the blue-colored complex was measured at 593 nm against a blank sample (water).

The results were expressed as mM Fe (II) per 100 g of fresh weight of fruit (mM Fe^{2+}/100 g FW FW).

Free Radical Scavenging using the ABTS Radical (Trolox equivalent antioxidant capacity (ABTS)) assay

Determination of the antioxidant activity with $ABTS^{\bullet+}$ reagent (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was carried out following the method of Re et al. (1999), and as described in work by Kazazic et al. (2016). The results were expressed as mg of Trolox equivalent per 100 g of fresh weight of fruit (mg TE/100 g FW).

Statistical analysis

Statistical data processing was performed by variance analysis (ANOVA). Tukey-Kramer test at $p \leq 0.05$ was used to determine if there is significant difference between samples. All experiments for total phenolic content, total anthocyanin content and antioxidant activity using FRAP and ABTS assay were conducted in triplicates. The values are expressed as the mean ± standard deviation (SD). Correlations between total phenolics, anthocyanins and antioxidant activity (FRAP and ABTS method) were determined using Pearson′s Correlation Coefficient Test.

Results and Discussion

Based on the data from Table 1, the starting phase of flowering happens in the period from 12th till 20th of April. The earliest beginning (12.04.) and end of flowering (12.5.) was recorded in the cultivar Clery, then Marmolada (beginning 15.04.; end 18.05.), after that cultivar Arosa (beginning 18.04.; end 22.05.), and latest in the *Fragaria vesca* L. (20.4. beginning 28.05.). Beginning of flowering of all cultivated cultivars was within 6 days which is consistent with

the results obtained by Bogunović et al. (2015). According to Milivojević et al. (2006) cultivars Clery (22.03. to 01.05.) and Arosa (04.04. to 11.05.) flowered earlier compared to our results. Bogunović et al. (2015) also reported earlier flowering of cultivar Clery (19.04.-17.05.). The time of flowering depend on cultivar but is also influenced by climatic conditions.

Table 1. Phenological observation results of studied strawberry cultivars and wild strawberry

Cultivar	Start of blossom	End of blossom	Start of ripening	End of ripening
Clery	12.04.	12.05.	08.05.	20.06.
Marmolada	15.04.	18.05.	15.05.	17.06.
Arosa	18.04.	22.05.	17.05.	16.06.
Fragaria vesca L.	20.04.	28.05.	25.05.	26.06.

Table 2 shows the results of fruit weight, height, and width, where the differences between strawberry cultivars and wild strawberry (*Fragaria vesca* L.) are clearly noticed. The strawberry fruit weight is a quantitative trait and depends on genetic predisposition of the cultivars (Whitaker et al., 2011) and environmental factors such as planting time, temperature, and relative humidity fluctuations (Ruan et al., 2011).

The wild strawberry had the significantly lower average fruit weight compared to investigated cultivars of strawberry (Tukey test, p=0.05). Fruit weight of Arosa, Clery and Marmolada cultivars are 16.08 g, 15.04 g, 15.02 g, respectively. There was no significant difference in fruit weight between three investigated cultivars.

Table 2. Pomological observation results of studied strawberry cultivars and wild strawberry

Cultivar	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Fruit shape index
Clery	15.04±3.41a	33.45±4.39a	26.16±4.58a	1.28±0.19
Marmolada	15.02±4.62a	31.09±2.39a	25.21±4.93a	1.27±0.22
Arosa	16.08±4.37a	30.7±4.77b	23.83±4.65ab	1.31±0.20
Fragaria vesca L.	1.02±0.58b	10.25±0.19c	9.82±0.57c	1.05±0.06

The results of the fruit weight in wild strawberry deviates from the results obtained by Skender et al. (2015) and Milivojević et al. (2010) who obtained significantly lower values of wild strawberry fruit weight. The fruit weight depends on cultivar as well as on agroecological conditions.

The highest value of fruit height (33.45 mm) and width (26.16 mm) was determined in the cultivar Clery, then cultivar Marmolada (height 31.09 mm; width 25.21 mm) and the lowest in

cultivar Arosa (height 30.7 mm; width 23.83 mm). Regarding the fruit height of wild strawberry, our results are similar to the results by Skender et al. (2015) (11.43 mm) and Labokas and Bagdonaitë (2005) (10.7 mm) and slightly lower related to the results reported by Milivojević et al. (2010) (12.39 mm). Our results of the fruit width of wild strawberry (9.82 mm) are comparable to the results by Skender et al. (2015) (8.9 mm) and Milivojević et al. (2010) (10.12 mm) and slightly lower than results by Labokas and Bagdonaitë (2005) (13.0 mm).

Obtained values of fruit weight and height of cultivar Clery are similar to the results reported by Dobričević et al. (2014). and Milivojević et al. (2006) (14.4 g). Our results of Clery fruit height are slightly higher than results by Skender et al. (2015) (29.13 mm), while Dobričević et al. (2014) registered slightly higher average fruit width.

The investigated cultivar Arosa had fruit weight, height and width in accordance with results reported by Skender et al. (2015), while Milivojević et al. (2015) obtained significantly higher value of the fruit weight. The results of fruit weight of Marmolada cultivar (15.02 g) are in accordance with the results reported by Milivojević et al. (2006) but significantly higher than results reported by Skender et al. (2015). Skender et al. (2015) reported values 28.20 mm for the fruit height and 32.97 mm for the fruit width of the cultivar Marmolada and these results are in accordance with our research.

Our results of the fruit shape index are comparable to the results by Milivojević et al., 2010 and Milivojević et al., 2015).

The results presented in Table 3. demonstrated that wild strawberry contained highest total phenolic content of all investigated samples (443.57 mg GA/100 g FW). The total phenolic content of the cultivated strawberries ranged from the 187.42 mg GA/100 g FW for Marmolada cultivar, followed by Arosa (178.22 mg GA/100 g FW) and Clery (166.76 mg GA/100 g FW). The content of polyphenols was affected by cultivar. According to research by Yildiz et al. (2014) the content of total phenols in 15 wild and cultivated strawberry fruits from Turkey ranged from 138-228 mg GA/100 g FW. Analysis of the content of total phenols in Serbia has shown 486 mg GA/100 g in wild strawberry, and 109 mg GA/100 g in Marmolada cultivar (Milivojević et al., 2011). Djilas et al. (2011) reported a total phenol content in the fruits of the Marmolada cultivar of 228.04 mg GA/100 g FW, while slightly lower values were reported for the Clery cultivar (170.75 mg GA/100 g FW) which is in accordance with our results. Microclimatic conditions (temperature, humidity, and light) can affect the synthesis of phenolic compounds in plants, as stated by Shin et al. (2007).

Table 3. The content of total phenolics (TP), total anthocyanins (TA) and antioxidant activity using FRAP and ABTS method of studied strawberry cultivars and wild strawberry

Cultivar	TP (mg GA/100 g FW)	TA (mg CGE/100 g FW)	Antioxidant activity	
			FRAP (mM Fe^{2+}/100 g FW)	ABTS (mmol TE/100 g FW)
Clery	166.76±1.13	24.34±0.29	1.10±0.10	0.19±0.003
Marmolada	187.42±3.60	14.74±0.26	1.07±0.02	0.20±0.006
Arosa	178.22±4.49	14.70±0.18	1.45±0.31	0.20±0.03
Fragaria vesca L.	443.57±4.14	23.78±0.54	2.16±0.08	0.40±0.006

According to the data from Table 3, the content of total anthocyanins in the cultivar Clery had slightly higher value (24.34 mg/100 g FW) compared to the other two cultivars (Arosa 14.70 mg/100 g FW and Marmolada 14.74 mg/100 g FW). Slightly lower values of total anthocyanins were measured in wild strawberry fruits (23.78 mg/100 g FW) compared to the Clery cultivar. These data are consistent with previous reports. Concentration of anthocyanins, in fruits of 27 cultivars of strawberry (*Fragaria x ananassa* Duch.) grown in Norway, varied from 8.5 to 65.9 mg/100 g of FW (Aaby et al., 2012). Voca et al., (2008) reported data on the total anthocyanin content of the Clery cultivar of 181.85 mg/kg, which is slightly lower compared to the results of this study. Djilas et al. (2011) reported total anthocyanin values of 32.0 mg/100 g FW for the Marmolada cultivar and 36 mg of cyanidin-3-glucoside / 100 g FW for the Clery cultivar. Yildiz et al. (2014) measured the average content of total anthocyanins in the fruits of 15 wild and cultivated strawberries of 28.62-53.51 mg/100 g FW, which is significantly higher compared to our results. Differences in concentration of anthocyanins found by different authors might be due to the use of different varieties and different extraction solvents. Anthocyanin content in strawberries is also affected by the cultivar, ripening, storage conditions, and other components.

As demonstrated in Table 3, wild strawberry had slightly higher antioxidant activity (2.16 mM Fe^{2+}/100 g FW) compared to cultivated strawberries determined by FRAP method. The following values were recorded: Clery (1.10 mM Fe^{2+}/100 g FW), Marmolada (1.07 mM Fe^{2+}/100 g FW) and Arosa (1.45 mM Fe^{2+}/100 g FW).

Antioxidant activity determined in 15 wild and cultivated strawberry cultivars of *Fragaria vesca* L. by FRAP method by Yildiz et al. (2014) ranged from 31.5–49.11 μmol/g FW. Montenegro at al. (2016) reported antioxidant activity of *Fragaria vesca* L. in the range of approx. 8 to 26 μmol ferrous sulfate/g FW, which is in accordance with our results. Halvoresen

et al. (2002) analyzed antioxidant activity in three samples of *Fragaria vesca* L. from different geographical regions and obtained mean value of 6.88 mmol/100 g FW.

Antioxidant activity determined by ABTS method was in range from 0.19 mmol TE/100 g FW for Clery to 0.40 mmol TE/100 g FW for wild strawberry. Using ABTS, Voća et al. (2008) report a data of 3.53 mmol TE/kg FW in Clery cultivar. Regarding wild strawberry (*Fragaria vesca* L.), 33.17 µmol TE/g FW was found by Scalzo et al. (2005) and 11.34 µmol TE/g FW by Pellegrini et al. (2003) using this method.

Remberg et al. (2007) state that antioxidant activity is conditioned by the type, variety, and size of the fruit, whereby berries with smaller fruits showed higher values of antioxidant activity. This is confirmed by the results of the antioxidant activity of the fruit in the examined wild berry fruit species. Different assays can be used for assessment of antioxidant activity and they can be divided in two groups: assays using organic radical producers (ABTS, DPPH, DMPD, PCL) and assay using metal ions for oxidation (FRAP). Antioxidant activity is very dependent on the free radical used for determination therefore one should use different free radicals. FRAP is method which is not based on selective reduction so it should be preferred. When comparing results from different studies we need to have in mind that solvents, temperature, time of extraction influence extraction yields and antioxidant activity of extracts.

The correlation coefficient between the total phenolic content and antioxidant activity was determined (Table 4). From the results, the strongest positive correlation was found between total phenolic content and antioxidant activity determined using ABTS radical (r = 0.994). Strong positive correlation also exists between total phenols and the antioxidant activity determined by FRAP (r = 0.893). The content of total phenols in relation to the content of anthocyanins showed no correlation (r = 0.491). Ferreyra et al. (2007) reported positive correlation of antioxidant activity and total phenolic content but no correlation of antioxidant activity with the concentration of anthocyanins. Poor correlation between anthocyanins and antioxidant activity determined by ABTS and FRAP assays was reported by Oszmiański and Wojdylo (2009).

Table 4. Pearson correlation between total phenolics, anthocyanins and antioxidant activity (FRAP and ABTS method)

	Total phenolics	FRAP	ABTS	Total anthocyanins
Total phenolics	1			
FRAP	0.8934	1		
ABTS	0.9936	0.8662	1	
Total anthocyanins	0.4915	0.3641	0.5134	1

Conclusion

The time of flowering and pomological characteristics depend on cultivar, agroecological and climate conditions.

The obtained results showed that there are significant differences in the content of investigated bioactive compounds among selected strawberry cultivars in Herzegovina region. We found positive correlation between the total phenol content and antioxidant activity of investigated strawberry cultivars, while no correlation was detected between the content of total phenols and anthocyanins. Highest content of total phenols and antioxidant activity were detected in wild strawberry fruit indicated wild strawberries as valuable source with respect to examined polyphenolic compounds. Further investigations are required to give precise evaluation and characterization of bioactive molecules in these fruit cultivars.

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Original scientific paper

Morphological definition populations of *Allium ursinum* L. from the western part of the Republic of Serbia

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Abstract

In Western Serbia, at an altitude of 80 to 1211 m, thirteen populations of wild garlic (*Allium ursinum* L.) were discovered at different localities in eight different soil types. Plant material was sampled from each of the thirteen localities by the method of random sampling. Morphological parameters were measured on the sampled plant material: length of aboveground and underground part of the plant, bulb thickness, weight, length, leaf width, length and thickness of the flower stalk, with the aim of assessing the locality that has the most productive morphological parameters. The populations of *Allium ursinum* growing on the mountains Povlen and Rudnik have the most productive morphological parameters. It can be said that wild garlic in these two localities is the most adequate plant material for harvesting fresh plant material, which will be used for other research in agronomy.

Key words: bulbs, morphometric data, populations, wild garlic

Introduction

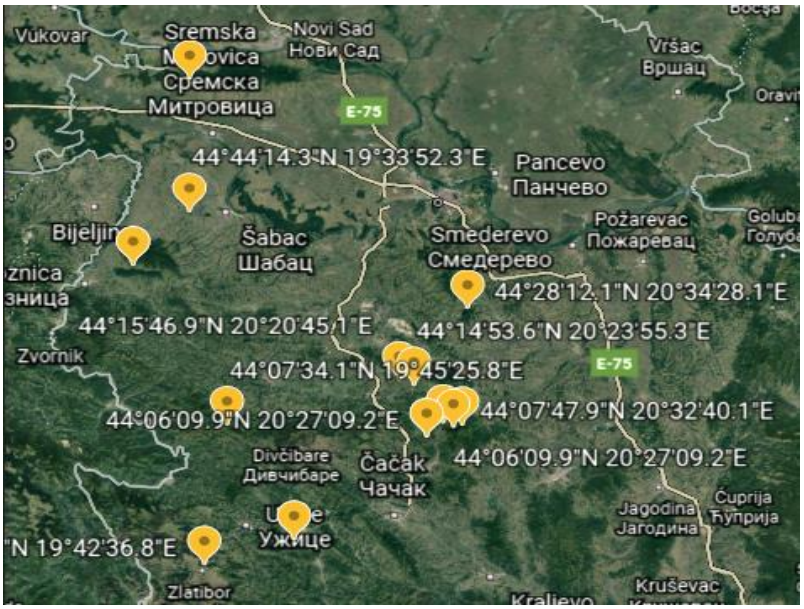
Allium ursinum L., known as wild garlic, ramsons, broad-leaved garlic or bear's garlic, is a perennial herbaceous plant that has been used in traditional medicine for centuries (Sendl et al., 1992). In the past, this plant species belonged to the family *Alliaceae*, but the modern systematics classifies it in the family *Amaryllidaceae* (Friesen et al., 2006; Chase et al., 2009; Govaerts 2011). Plants of the genus *Allium* are most often used in nutrition and treatment

(Benkeblia and Lanzotti, 2007). Their edible parts are used to treat and prevent a large number of diseases (Gorinstein et al., 2007; Sabha et al., 2012), including cancers (Sengupta et al., 2004), elevated cholesterol levels, various inflammations, diabetes and obesity (Kalayarasan et al., 2009). The healing properties of wild garlic result from the activity of its secondary metabolites (Boscher et al., 1995; Yoo and Pike 1998), which accumulate mainly in the leaves and roots of this plant species, but are also present in its other plant parts. The most common ones are sulphur and phenolic compounds (Smeets et al., 1997), but their quantitative and qualitative properties are not stable (Schmit et al., 2002). Their amounts mostly depend on the harvest date, but the manner of processing of plant raw material is also important. The highest content of sulphur compounds is present in the leaf (Smeets et al., 1997) in the phase prior to the formation of the inflorescences (Schmitt et al., 2002, 2005). The highest content of phenolic compounds is present in bulbs (Smeets et al., 1997) in the phase following the seed formation and extinction of the aboveground plant part (Sobolewska et al., 2015). Wild garlic populations are widespread throughout Europe and Asia, both at high and low altitudes (Oborny et al., 2011). For productive growth and development, the plants require high humidity, as well as moist and well-drained humus soils (Szafer and Zarzycki, 1972; Oborny et al., 2011) although there have been cases of growing on dry, shallow and insufficiently drained soils, characterized by high humidity (Eggert, 1992). In addition to air humidity, a high concentration of aluminium in the soil solution is also considered a key to the growth and development of wild garlic (Andersson 1993). Wild garlic is a spring geophyte (Ernst, 1979). The bulb is fleshy and elongated from 1.5 to 6.5 cm (Ellenberg, 1988), while the aboveground part can grow up to 50 cm, and it consists of an upright inflorescence shoot surrounded by elliptical leaves (Szafer and Zarzycki, 1972; Morschhauser et al., 2009; Oborny et al. 2011). Flowers may produce a large number of seeds whose germination may last up to five years (Ernst, 1979).

This study aimed to find wild garlic populations at their natural stands in part of the territory of the Republic of Serbia and to estimate and compare their morphological data as it will enhance the selection of the most representative wild garlic populations for further agronomical studies.

Material and Methods

For this research, fresh plant material of wild garlic was collected from the natural stands at 13 different localities in Western Serbia (Picture 1).



Picture 1. Natural stands of wild garlic populations in Western Serbia included in the study.

Geographical data of all localities of wild garlic populations included in this study were determined by the use of a GPS device, while the data with regard to the soil type at studied localities were determined according to the soil map of Serbia (Škorić et al., 1985) (Table 1).

Table 1. Data for 13 natural stands of wild garlic populations in Western Serbia.

No.	Name of the locality	Geographical coordinates		Altitude (m)	Soil type
		Longitude	Latitude		
1	Povlen Mnt.	44°07'34.1"N	19°45'25.8"E	1211	Ranker
2	Zlatibor Mnt.	43°43'58.2"N	19°42'36.8"E	950	District cambisol
3	Rudnik Mnt.(1)	44°07'47.9"N	20°32'40.1"E	924	Ranker
4	Rudnik Mnt. (2)	44°08'31.2"N	20°30'29.1"E	876	Ranker
5	Kosmaj Mnt.	44°28'12.1"N	20°34'28.1"E	618	Luvisol
6	Zlakusa	43°48'30.5"N	20°00'41.8"E	545	Rendzina
7	Šilopaj	44°06'09.9"N	20°27'09.2"E	460	Eutric cambisol
8	Stragari	43°39'12.1"N	21°07'00.4"E	291	Ranker
9	Belanovica	44°14'53.6"N	20°23'55.3"E	234	Eutric cambisol
10	Ležimir	45°07'42.7"N	19°31'38.5"E	190	Chernozem
11	Iverak	44°34'42.8"N	19°22'42.8"E	160	Luvisol
12	Ljig	44015'46.9"N	20°20'45.1"E	148	Pseudogley
13	Slepčević	44°44'14.3"N	19°33'52.3"E	80	Smonica

Plant material was sampled during the vegetation period at different time intervals, in the period from February to June 2020. The sampling time of the plant material mostly depended on the locality and the part of the plant to be sampled.

Whole fresh plants (30 per locality) were sampled before flowering; at sites 6–13, in the second part of March, while at sites 1–5, at the beginning of April 2020 (Table 1). The inflorescence shoots (30 per population) were sampled at the same localities during their full bloom, which happened during June, 2020 (at localities 6–13, in the first week of June, while at localities 1–5, in the second part of June). All measurements on sampled material were performed in the agronomy laboratory of the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia. In the laboratory conditions, the following morphometric measurements on fresh plant material were performed (30 plants per location): the lengths of the whole plant, of the inflorescence shoot and of the underground plant part, as well as the length, width and number of leaves (on each of the 30 plants), the number of flowers per inflorescence (30 inflorescences per location), the bulb thickness, and the inflorescence shoot thickness at its top and at its bottom. Fresh weight measurements were also performed (30 plants per population), including the whole plants, the leaves and the bulbs. All measurements were performed in four samples, using a calibrated analytical balance and a micrometre device (ruler and subler). All results obtained in this study were processed by analysis of variance (ANOVA) using the statistical software SPSS (IBM SPSS Statistics 22). Testing the significance of the difference of arithmetic means of populations by localities for each morphological parameter was done by Duncan's test at the 5% level of significance.

Results and Discussion

Results of statistical analysis of all measured morphometric data and biomasses of studied fresh Wild garlic populations are presented in Table 2. The mean values of the measured morphological parameters define the productivity of each of the studied population of wild garlic at its natural stand.

In the available literature, differences in studied morphological parameters of wild garlic at its natural stands are usually explained either by the variations in altitude, soil type or climatic factors (or by a combination of them). For instance, Todorović et al., (2006) attributed differences in the morphological features to the altitude. Namely, at higher altitudes, wild garlic populations showed better morphological characteristics as well as a higher content of macro and microelements in leaves. Similar to the investigation of Eggert's (1972), who studied

morphological parameters of *A. ursinum* subsp. *ursinum* populations deriving from different localities in Germany, in our study, differences between all studied populations were observed (Table 2). In addition, our population from the highest altitude (Povlen Mountain) proved to be the most excelling one, as it was characterized by the highest whole plant biomass (11.97 g) and length (42.7 cm), the highest bulb thickness (7.72 mm) and the number of leaves per plant (2.07), as well as by the highest leaf fresh weight (4.93 g), length (16.19 cm) and width (6.05 cm). Based on data on the number of flowers per inflorescence, the most excellent populations came also from higher altitudes; from the Rudnik Mountain – 1 (17.90 flowers per inflorescence) and from the Povlen Mountain (17.60 flowers per inflorescence). On the other hand, populations from localities at the lowest three altitudes (Iverak, Belanovica and Ljig) comprised plants with generally smaller habitus (Table 2). As for the soil, according to Tutin (1957), wild garlic populations spontaneously grow on heavy, drained loamy soils. Kaja (2012) characterised plant preferences for the soil type as damp but well-drained and shady such as in deciduous forests, usually with a high humus content, whereas Grime (1988) suggested the plants preferring the ranker soil type. All these findings are in agreement with our data, though in our study, populations prevailingly occurred on pseudogley (38,46%) but also spontaneously grew on other soil types (Table 1). According to Kaja et al. (2012), *Allium ursinum* has only two subspecies, subsp. *ucrainicum* and subsp. *ursinum*, which do not grow at the same localities. The subsp. *ucrainicum* is distributed mainly in the southwestern and northern parts of Germany and also in northern Poland. The distribution range of subsp. *ursinum* includes Austria, the Czech Republic, Poland, Slovakia, Hungary, Slovenia, Croatia, Serbia, Bosnia and Herzegovina, Montenegro, North Macedonia, Kosovo, Bulgaria, Romania, Ukraine, Belarus, the European part of Russia. This is in agreement with our data, as we observed that all populations found in western Serbia did not have scabrous pedicels with numerous papillae, which is, according to Tutin (1957), a confirmation that they belong to subsp. *ursinum*.

Table 2. Morphometric data and biomasses of Wild garlic populations collected from 13 natural stands in Western Serbia.

Morphological parameters		*Allium ursinum* natural stands												
		Higher altitude localities (above 500 asl)						**Lower altitude localities** (below 500 m asl)						
		Povlen	Zlatibor	Rudnik		Kosmaj	Zlakusa	Šilopaj	Stragari	Belanovica	Ležimir	Iverak	Ljig	Slepčević
				1	2									
Whole plant	weight (g)	11.97 e	3.12 cd	3.35 d	3.07 cd	1.96 abc	2.12 abc	1.78 ab	3.17 cd	1.16 a	3.58 d	0.95 a	1.10 a	2.40 bcd
	length (cm)	42.70 f	36.07 e	31.83 d	29.00 cd	27.63 c	28.20 c	23.93 b	30.40 c	21.60 ab	28.27 c	20.62 a	20.70 a	28.46 c
Aboveground plant part	length (cm)	25.07 f	21.23 e	19.73 de	18.60 cd	16.10 b	18.13 cd	15.83 b	18.50 cd	12.13 a	17.20 bc	12.08 a	11.63 a	17.36 d
Bulb	thickness (mm)	7.72 g	5.88 ef	6.50 f	5.70 de	4.92 bc	6.02 ef	5.72 de	5.54 cde	4.97 bc	5.02b ce	0.90 a	4.47 b	5.68 de
Leaf	number of leaves per plant	2.07 e	1.23 bc	1.17 a	1.23 bc	1.23 bc	1.03 a	1.10 a	1.10 a	1.13 a	1.67 d	1.07 a	1.10 a	1.47 cd
	weight (g)	4.93 g	1.26 ef	1.23 ef	1.12 cef	1.22 ef	1.15 cef	0.84 bce	1.23 ef	0.68 abc	1.52 f	0.31 a	0.42 ab	1.09 cef
	length (cm)	16.19 h	13.15 fg	13.88 f	13.80 f	12.26 cdf	11.66 bcd	10.93 b	12.40 df	9.67 a	11.09 bc	8.53 a	8.74 a	12.26 cdf
	width (cm)	6.05 e	3.83 c	4.58 d	4.22 cd	3.22 b	3.27 b	3.16 b	4.20 cd	3.13 b	3.97 c	2.91 b	2.39 a	3.23 b
Inflorescence stalk	length (cm)	40.23 e	32.38 abcde	41.63 e	38.13 cde	35.43 bcde	26.67 abc	53.43 g	27.32 abcde	27.52 abcde	39.22 de	21.02 a	27.07 abcd	31.57 ab
	thickness at the bottom (mm)	4.30 j	3.12 de	3.97 ij	3.92 hij	3.50 efg	2.93 cd	3.50 efg	3.60 ghi	2.68 c	4.25 j	1.89 b	3.18 def	3.03 a
	thickness at the top (mm)	1.93 hi	1.48 e	1.90 hi	1.83 ghi	1.57 ef	1.18 c	1.75 fgh	1.62 efg	1.13 c	2.00 j	0.58 b	1.48 e	1.16 a
Inflorescence	number of flowers	17.60 efg	15.37 cefg	17.90 ef	17.00 efg	14.50 cdef	14.30 cefg	13.13 bcef	10.50 abc	12.63 bce	18.57 f	7.40 a	11.67 abc	11.37 ab

The variability in morphological traits between studied wild garlic populations depended mostly on altitude and climatic features of their natural stands. According to Kaja et al. (2012), *A. ursinum* subsp. *ucrainicum* mainly grows at altitudes above 700 m a.s.l. while the subsp. *ursinum* below 700 m above sea level. However, the wild populations of subsp. *ursinum* found in the western part of the Republic of Serbia were equally distributed at altitudes ranging from 80 to 1120 m above sea level (Table 1).

Conclusion

Based on the obtained results of this research, we can say that in different localities of Western Serbia, wild *Allium ursinum* grows on different types of soil, which is characterized by different morphological parameters. By measuring the morphological parameters of the plant material *Allium ursinum* sampled from 13 localities and analyzing their results, it can be concluded that *Allium ursinum* growing on the mountains Povlen and Rudnik has the best morphological parameters. This research provided useful information on the localities where *Allium ursinum* grows together with its morphological parameters. In case of the need for fresh plant material useful in nutrition, medicine and other agronomic research, it is recommended to harvest from the mountains of Povlen and Rudnik.

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Original scientific paper

Damages to agricultural crops caused by an increase in the number of wild boars in the hunting ground "Kutlavica"

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Abstract

The problem of damage to agricultural crops from wildlife is evident every year, and, in many cases it is the subject of litigation between farmers and hunting ground users to compensate for the damage caused and in most cases both sides are dissatisfied with the epilogue. Damage to fields with cereals most often occurs on cornfields, during the milk phase of grain maturity, and the fields where early hybrids are grown, which are the first to mature, are most affected. Damage is caused by breaking (felling) the tree and consuming the corn class. The aim of the research was to determine in what way and to what extent wild boars, bred in the hunting ground, cause damage to agricultural crops. Research on the amount of damage done was performed during the hunting seasons 2015/2016, 2016/2017, 2017/2018, 2018/2019 and 2019/2020 in the hunting ground LU "Kutlavica" in Manojlovac. A nalysis demands for damages to agricultural crops, take the data on the damage caused to the corn, potato fields and fruit trees in the plots of physical persons from five locations within the hunting ground " Kutlavica ": KO Donja Kupinovica, KO Gornja Kupinovica, KO Jersenovo, KO Orašac, KO Jelasnica. During the data analysis, the correlation coefficient and standard deviation were determined, the statistical significance between the number of requests caused by those claims and the number of heads of wild boar, as well as a statistically significant correlation between these two variables at the level of $P < 0, 05$. Maintaining the optimal number of game, planned sowing of cereals, raising the level of protection of agricultural crops, winter feeding and the use of new IT technologies in the hunting ground, are key solutions in preventing the damage caused by wild boars.

Key words: wild boar, damages, maize, claim, compensation

Introduction

The problem of damage to agricultural crops from wildlife is evident every year, in many cases it is the subject of litigation between farmers and hunting ground users to compensate for the damage caused and in most cases both sides are dissatisfied with the epilogue.

The Law wildlife and hunting clearly prescribes the right to compensation. In many cases, farmers are not aware of what protection measures on plots they need to take in order to protect agricultural crops. The most common problem, which is present in hunting grounds, are plots of small areas that are sown with the sown with maize culture and it is practically impossible to adequately protect all plots (Gajović, 1988). The increase in the number of wild boars in hunting grounds is the reason that the damage from wild animals is becoming more drastic on agricultural crops. The hunters and farmers must take appropriate measures to protect the agricultural crops from wild animals.

Wild boar causes damage of various sizes and intensities. Damage occurs in those areas where hunting grounds lean on agricultural land. The most common damage from wild boar occurs during sowing and ripening of agricultural crops. It causes damage by plowing, digging, trampling young crops and consuming seeds (Kostić, 1988).

Damage caused by wild boars to agricultural crops can be divided into: damage to areas under cereals, damage to potato fields and damage to meadows and orchards. Damage to fields with cereals most often occurs on cornfields, during the milk phase of grain maturity, and the fields where early hybrids are grown, which are the first to mature, are most affected. Damage is caused by breaking (felling) the tree and consuming the corn class. However, the damage from consumption is much less than the losses caused by breaking and trampling plants. It should be taken into account that at the same time and on the same surface, 30-40 animals of this species, of different sex and age, can be found at once. Damage to cereal fields occurs primarily on plots where the previous crop was corn. When combining corn, a small number of cobs are not harvested by machine, but remain behind the combine on the ground, and after harvest. Thanks to their extraordinary sense of smell, wild boars feel the fermented (pickled) grain or cobs under the ground and dig to it, causing more or less damage to the fields with grain. The possibility of occurrence and the magnitude of these damages depend on the position of the plot in relation to forest complexes or places where wild boars are most often kept.

Damage to potato fields usually occurs in the first days after sowing this crop, but it can occur until the moment the potatoes are removed. The most severe spring damage is when the sown

crop is destroyed, and there is no time for another sowing. However, the damage caused during the year is no less significant, given the fact that the crops sown are in a state of development. Damage to meadows in the largest percentage occurs in spring and early summer, although they were also recorded in winter. The main reason for this is the intensive development of insects (earthworm, etc.), which the wild boar, with its developed sense of smell, precisely locates as food and finds it digging. The consequences are plowed meadows on which significantly reduced the yield of grass mass, as well as the necessity of their conduct. The damage caused to agricultural plots in the largest percentage occurs along the edge of the forest or in its vicinity. In the mountainous parts of the hunting ground of the plots are very small, 0.1 - 0.3 ha, so that the protection measures by the owners of plots and generally not undertaken. In the hilly parts of the investigated hunting grounds, even greater damages were observed on agricultural areas (Karankić-Mirić, 2009). The magnitude of the damage largely depends on the population density of wild boar. Harmful effects of wild boar in forestry can be manifested by consuming oak and beech acorns, and thus reducing the number of germinating seeds. Damage to plots planted with oak and beech acorns can be caused by plucking and damaging young plants during digging. In addition, fungal diseases can intensify due to damage to tree roots. In addition, they can affect the stimulation of grass and create conditions for rejuvenation of unwanted species.

Wild animals are conceptually defined by the Law wildlife and hunting ("Official Gazette of RS", No. 18/2010 and 95/2018). According to the provisions of Article 4, para. 1. point . 4 of this law "game is a species of wild mammals and birds determined by law". In the literature, we find "that these are the animals that were or can become the subject of hunting, and which live freely in nature " (Karankić-Mirić, 2009). The user of a hunting ground (Petrović, 1996) can be a legal entity that meets the conditions for conducting hunting management in accordance with the provisions of the law. The user of a hunting ground can be a legal entity established as a public enterprise, a company, another form of enterprise, as well as hunting associations established to operate in accordance with the law (Počuča, 2008).

The question of liability for damage caused by protected wild animals saved by the Law wildlife and hunting and the Ordinance on measures to prevent damage to wildlife and the procedure and method of determining damages, and certain specific damage caused by wild animals also apply to the Law on Obligatory Relations (Gajović 1988). The damage that wild animals (wild boar) cause to the property and body of people is responsible according to the principles of objective responsibility. It will be the same in the case of wild boar, ie. damage

caused by wild animals to agricultural and forest crops (Urošević et al., 2010). The subject of liability for this type of damage is the organization that manages the hunting ground (hunting ground user). In order to be able to talk about compensation for damage, the presumption is the existence of damage. These are damages that originate from wild animals, which wild animals can cause to people or property on the land, waters and forests where the hunting ground is located, as well as on the areas outside the hunting ground where the game is located. These damages are different in content and depend on the specific game. A wild boar can dig over a plot of land with vegetables or run over and trample the whole plot sown with cereals (Popović, 2006).

When they cause damage to an agricultural producer is of crucial importance, given the specificity of the charges, first to know the dates in which to pay and who can apply for compensation. According to Article 89, paragraph 1 of the Law wildlife and hunting, a legal or natural person to whom the game has caused damage may submit a request for compensation to the user of the hunting ground, within 10 days from the date of the damage. It is advisable to state when and which game caused damage to the injured party, with a description of the damage and the amount, if the injured party was allowed to do so (Radovanov et al., 2009).

The aim of the research was to determine in what way and to what extent wild pigs, which are bred in the hunting ground, cause damage to agricultural crops (Popović, 2006).

Material and Methods

The research was conducted during the hunting seasons 2015/2016, 2016/2017, 2017/2018, 2018/2019 end 2019/2020 in the hunting ground LU "Kutlavica" in Manojlovac. The number of wild boars, sex, age, determined by spring counting, was registered. Also, analysis of demands for compensation to agricultural crops, take the data on the damage caused to the corn, potato fields and fruit trees by individuals from five locations within the hunting ground " Kutlavica ": KO Donja Kupinovica, KO Gornja Kupinovica, KO Jersenovo, KO Orašac, KO Jelasnica. In addition, data were taken on the introduction of corn into the hunting ground with the aim of feeding wild boars. The SPSS Statistics program was used for data analysis, and descriptive data analysis and correlations were performed, ie the mutual relationship of variables.

Results and Discussion

A review of annual management plans shows that the total number of wild boars in the entire territory of the hunting ground "Kutlavica" increases every year, primarily in areas where it is possible to organize agricultural production of nutrients in grain.

Table 1. Number of wild boar heads by Cadastral Municipalities (heads)

Year	KO (Cadastral Municipalities)											
	Total wild boar	**%**	**Crkovnica**	**%**	**Gornja Kupinovica**	**%**	**Jarsenovo**	**%**	**Jašunja**	**%**	**Stupnica**	**%**
2015/2016	28 / 18*	64.3	4	22.2	5	27.7	4	22.2	2	11.1	3	16.6
2016/2017	26 / 16*	61.5	3	1 8.7	6	37.7	5	31.2	0	0	2	12.4
2017/2018	30 / 21*	70.0	3	14.3	6	28.5	6	28.6	2	9.5	4	19.1
2018/2019	30 / 22*	73.3	4	18.2	6	27.3	5	22.7	2	9.1	5	22.7
2019/2020	32 / 24*	75.0	6	25.0	9	37.5	7	29.1	5	20.8	7	29.1

*Source: Annual management plan of LU "Manojlovce". *- total number of wild boars /number of wild boars in the area*

The reasons for the increase in the number of wild boars are found in good environmental conditions and the presence of food in the field. Good feeding and ambient conditions, especially during the winter period, conditioned good reproductive characteristics of sows and a larger number of pigs.

The increased number of wild boars and the greater need for food conditioned the movement and migration of pigs for food, and in that way they caused damage to crops, field and fruit crops. Farmers and users of hunting grounds are certainly responsible for the damage caused to agricultural crops, as Petrovic (1996) stated in his research.

Table 2. Total number of submitted claims for compensation for damages (pieces)

Year	KO (Cadastral Municipalities)											
	Total requests	**%**	**Crkovnica**	**%**	**Gornja Kupinovica**	**%**	**Jarsenovo**	**%**	**Jašunja**	**%**	**Stupnica**	**%**
2015/2016	14/ 11*	78.6	2	18.2	3	27.3	3	27.3	1	9.1	2	18.1
2016/2017	12/ 9*	75.0	1	11.1	4	44.4	3	33.3	0	0	1	1.2
2017/2018	15/ 12*	80.0	1	8.3	5	41.6	4	33.3	1	8.3	1	8.5
2018/2019	18/ 15*	83.3	3	20.0	4	26.6	5	33.3	1	6.6	2	13.5
2019/2020	28/25*	89.3	4	16.0	7	28.0	5	20.0	4	16.0	5	20.0

*Source: Annual management plan of LU "Manojlovce". *- total number of requests / number of requests in the area*

The number of reported damages is constantly increasing as the number of wild boar has increased, which clearly indicates that there is a correlation between the number of claims for damages and the number of wild boar. For many years, farmers could not or were not aware that they could file a claim and seek compensation for the damage caused to them. Popović et al. (2008) came to similar findings in their study.

Table 3. Amount of damage caused to agricultural crops (dinars)

Year	Damages caused (dinars)							
	Total damages (dinars)	%	Corn	%	Potatoes	%	Fruit trees	%
2015/2016	102000.00 / 82000.00*	80.4	77000.00	9 3.9	0.00	0	5000.00	6.10
2016/2017	84000.00 / 74000.00*	88.1	67000.00	90.5	7000.00	9.5	0.00	0
2017/2018	112000.00 / 93000.00*	83.0	81000.00	87.1	12000.00	12.9	0.00	0
2018/2019	134000.00 / 102000.00*	76.1	94000.00	92.1	0.00	0	8000.00	7.9
2019/2020	157000.00 / 139000.00*	88.5	124000.00	89.2	3440.00	2.4	11560.00	8.4

Source: Annual management plan of LU "Manojlovce".- total number of requests / number of requests in the area*

The amount of damage reported by agricultural producers was justified because they arose from liability for damage caused by animals, said Radovanov (2009) in his research, which was also presented by the authors in this paper. The amount of damages that were reported was higher and the financial amounts for compensation were constantly increased.
Statistical processing of the data clearly shows that the standard deviation is also positive when observing the number of wild boar, as well as the number of claims for damages(Table 4).

Table 4. Descriptive Statistics

	Mean	SD
Number wild boar	20.20	3,194
Number of damages	14.40	6,309

Observing the correlative relationship between the variables, the analysis of data determined the statistical significance at the level of P = 0.05, which is shown in.

Table 5. Correlations

		Number wild boar	Number of damages
Number wild boar	Pearson Correlation	1	.864
	Sig.		.059
	N	5	5
Number of damages	Pearson Correlation	.864	1
	Sig.	.059	
	N	5	5

Statistical significance at level P = 0.05

Conclusion

The total number of wild pigs on the entire territory of the hunting ground "Kutlavica" is increasing every year, primarily in areas where it is possible to organize agricultural production of nutrients in grain. In the hunting ground "Kutlavica", the number of wild pigs can be seen increasing. The reason should be sought in the diverse offer of plant crops and the observed area.

A larger number of wild boar causes their greater presence in the field, which causes a larger number of damages. Monitoring the movement of wildlife and the organization of hunting for the protection of agricultural crops in a large number of hunting grounds reduce the damage to farmers on agricultural crops; Maintaining the number of game to the optimal number determined by the planning document.

Providing sufficient food and water in the hunting ground during the hunting year for all game species in the hunting ground, especially during the winter period and natural disasters, immediately before sowing and planting, as well as during ripening of crops and fruits directly affects the number of damage in the field.

Providing assistance and information to property owners and users regarding the protection of human life and health and property from wild animals, as well as the selection of certain crops for sowing, planting and procurement of means to prevent damage from wild animals, information through the media, advertising on local bulletin boards and secondly, it is of great importance to compensate for the damage in the fields.

Only greater education and joint work of farmers and hunting ground users will reduce the damage to agricultural crops from wild animals, and thus the dissatisfaction of farmers.

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Štete na poljoprivrednim usevima prouzrokovane povećanjem broja divljih svinja u lovištu "Kutlavica"

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Sažetak

Problem šteta na poljoprivrednim kultura od divljači svake godine evidentan je, u mnogo slučajeva predmet je sudskih sporova između poljoprivrednika i korisnika lovišta za nadohnadu pričinjene štete i u većini slučajeva obe strane su nezadovoljne epilogom. Štete na poljima sa žitima najčešće nastaju na kukuruzištima, u periodu mlečne faze zrelosti zrna, a najviše su pogođene njive na kojima se gaje rani hibridi, koji prvi i sazrevaju. Štete nastaju lomljenjem (obaranjem) stabla i konzumiranjem ploda kukuruza. Cilj istraživanja bio je da se utvrdi na koji način i u kolikoj meri divlje svinje, koje se gaje u lovištu, pričinjavaju štete na poljoprivrednim kulturama. Istraživanje o visini napravljenih šteta je obavljeno tokom lovnih sezona 2015/2016., 2016/2017., 2017/2018, 2018/2019 i 2019/2020 u lovištu LU “Kutlavica” u Manojlovcu. Analizom zahteva za nadoknadu štete na poljoprivrednim usevima, uzeti su podaci o pričinjenim štetama na kukuruzu, krompiristima i voćkaricama na parcelama fizičkih lica sa pet lokacija unutar lovišta „Kutlavica“: KO Donja Kupinovica, KO Gornja Kupinovica, KO Jersenovo, KO Orašac, KO Jelašnica. Sproveđenjem deskriptivne analize podataka i korelacijom, utvrđena je statistička značajnost između broja zahteva pričinjenih šteta i broja grla divljih svinja, kao i statistički značajna korelacija između ove dve varijable na nivou $P<0.05$. Održavanje optimalnog brojnog stanja divljači, plansko sejanje žitarica, podizanje stepena zaštite poljoprivrednih kultura, zimska prihrana i korišćenje novih IT tehnologija u lovištu, ključna su rešenja u sprečavanju šteta koje pričinjavaju divlje svinje.

Ključne reči: divlje svinje, štete, kukuruz, zahtev, nadoknada.

Original scientific paper

Microbiological status of drinking water on farms in the Republic of Srpska (B&H) in the period 2018-2020 in relation to the examined parameters

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Abstract

Water is essential for life, and a satisfactory (adequate, safe and accessible) supply must be available to all. No source of water that is intended for human consumption can be assumed to be free from pollution. Zero-probability level of microbiological contamination of drinking water does not exist. The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard.

The experiment used drinking water samples originating from domestic animal farms from the territory of Republika Srpska (B&H) sampled in the period 2018-2020. A total of 645 samples were examined.

The aim of this study is to determine the microbiological status of drinking water on farms of domestic animals in the Republic of Srpska (B&H) in relation to the examined parameters, in order to see the real risks to animal health and give recommendations for improvement.

For microbiological testing of sterilized milk were used methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1/A1.

The analysis revealed a significantly higher number of unsatisfactory samples of well water in relation to the water supply system. The obtained results indicate significant fecal contamination of water, especially with intestinal enterococci and coliforms, and less with *E. coli*.

The microbiological status of water on farms in the Republic of Srpska (B&H) in the period 2018-2020 is significantly improved compared to previous years, but still unsatisfactory given the large number of unsatisfactory samples. However, given the fact that over a third of farms

are supplied with water originating from wells, there is a high risk to the microbiological status of the water, given that well water is not under constant monitoring.

Key words: 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).

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and may be subject to natural or manufactured sources of pollution that may result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination (EA, 1998; WAA, 1988).

Zero-probability level of microbiological contamination of drinking water does not exist. 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”. Minimum requirements for parametric values used to assess the quality of water intended for human consumption is 0/100 ml for *E. coli* and intestinal enterococci.

In Republic of Srpska (B&H) the limit value for total count on 22°C 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 (Regulation, 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 (protozoa, parasite eggs) capable of surviving for different periods, and raise the health risk for many people throughout the world. In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use. 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). On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health (EA, 2002a). These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests. Indicator organisms are used to assess the microbiological quality of water.

Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated (Edberg, 2000).

The use of indicator bacteria, in particular *Escherichia coli* (*E. coli*) and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen et al., 2000).

The key criteria for ideal bacterial indicators of faecal pollution are that they should be universally present in large numbers in the faeces of human and other warm-blooded animals. They should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*. The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* bacteria are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin (Edberg, 2000; WHO, 1993) and it can represent up to 95 % of the Enterobacteriaceae found in faeces (Waite, 1985).

Coliform bacteria belong to the family *Enterobacteriaceae* and share similar cultural characteristics. Typical genera encountered in water supplies are *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia* and *Yersinia*. When coliform bacteria are isolated from drinking water supplies it is often useful to determine which species of coliform bacteria

are present, particularly if problems recur, in order to determine the source and significance of the coliform bacteria being recovered (EA, 2002a).

E. coli is a coliform bacteria and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water (EA, 2002b).

E. coli occurs in the faeces of all mammals, often in high numbers (up to 10^9 per gram of faeces). This widespread faecal occurrence, coupled with methods that for the recovery and enumeration of *E. coli* are relatively simple to conduct, has contributed to the detection of this bacteria as the cornerstone of microbiological water quality assessment for over 100 years (Edberg, 2000; WHO, 1993).

Enterococci include a number of species that occur in the faeces of humans and warmblooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution. Enterococci of faecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and coliform bacteria (WHO, 1993).

The species of enterococci that occur in faeces and, therefore, are more likely to be found in polluted waters, can be divided into two main groups. The first includes *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans*. These organisms are normally present in the faeces of humans and various animals. The second group includes *Streptococcus bovis*, *Streptococcus equinus* and *Enterococcus avium*. These organisms are not normally found in human faeces. The identification of species may, therefore, give an indication of the source of contamination (EA, 2002c). Wild animals and birds can also be natural sources of zoonotic pathogens.

Tests for colony count bacteria growing at 37°C and 22°C enable a count to be determined of the heterotrophic bacterial population of the water (EA, 2002a). The bacteria grown in these tests are not indicators of faecal contamination, although historically, the count at 37°C was taken to give an indication of faecal contamination. It is well recognised, however, that only a small fraction of the viable bacterial population present in water is enumerated by the procedures normally employed. Despite this, monitoring of water supplies for colony count bacteria can be useful for monitoring trends in water quality or detecting sudden changes in quality. If levels are substantially increased relative to normal values, there may be cause for concern.

The territorial and economic organization of the Republic of Srpska (B&H) 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. The highest precipitation is in the peripheral mountainous parts of Republic of Srpska, the least populated and developed.

Kalaba et al. (2020) found that in the Republic of Srpska (B&H) in the period 2015-2017 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.

In a study that included microbiological analysis of water on farms in the Republic of Srpska (B&H), Kalaba et al. (2015) found that enterococci are most prevalent in water from the region of Prijedor, Banja Luka and Doboj, *E. coli* in the region of Doboj, Prijedor and Bijeljina, and coliforms in the region of Prijedor.

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). All unsatisfactory water samples originate from wells. Also, the study analyzes the water originating from cattle farms and milk collection points in Croatia, showed the presence of an increased total count and the presence of pathogenic bacteria in the level 20% TC22°C, 20% TC 37°C, 5% *E. coli*, 5% CB i 12% EC (Denžić et al., 2013), 48% TC22°C, 55% TC 37°C, 20% *E. coli*, 32% CB i 41% EC (Denžić et al., 2012) i 71% TC22°C, 63% TC 37°C, 45% *E. coli*, 61% CB i 54% EC (Jaki et al., 2010).

The aim of this study is to determine the microbiological status of drinking water on farms of domestic animals in the Republic of Srpska (B&H) in relation to the examined parameters, in order to see the real risks to animal health and give recommendations for improvement.

Materials and Methods

The experiment used drinking water samples originating from domestic animal farms from the territory of Republika Srpska (B&H) sampled in the period 2018-2020. A total of 645 samples were examined (384 in 2018, 160 in 2019 and 101 in 2020).

Laboratory testing of samples was performed at the Public Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka.

Microbiological examination was carried out according to the Regulation (2017). This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C (TC 22°C) and 37°C (TC 37°C) according to BAS EN ISO 6222 (ISBIH, 2003), coliform bacteria (CB) and *E. coli* according to BAS EN ISO 9308-1/A1 (ISBIH, 2018) and intestinal enterococci (EC) according to BAS EN ISO 7899-2 (ISBIH, 2003).

In our research and in the statistical 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

When it comes to the representation of samples in relation to the category, 61.90% of samples was from water supply system and 38.10% from wells. Figure 1 shows the average regional representation of samples originating from water supply system in % for the period 2018-2020 years.

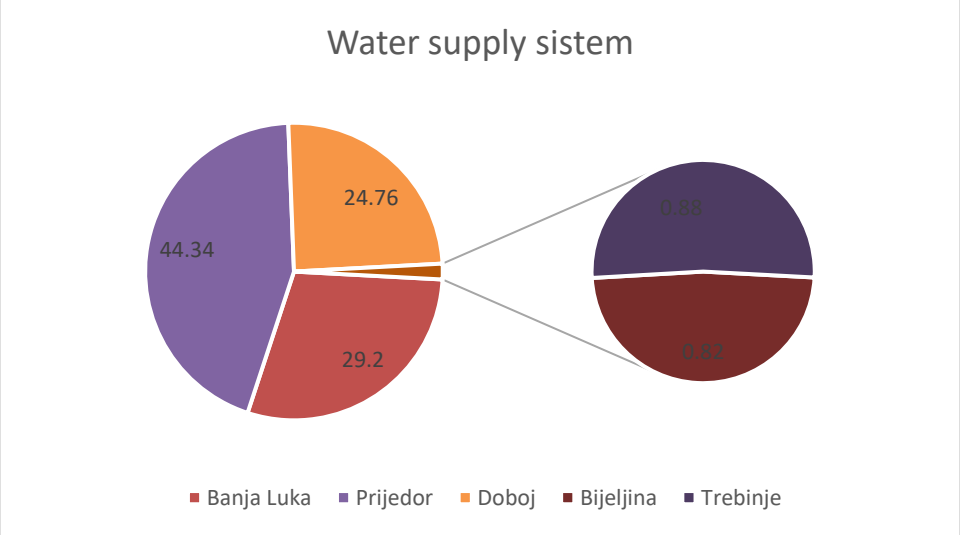

Figure 1. Average regional representation of samples originating from water supply system in % for the period 2018-2020

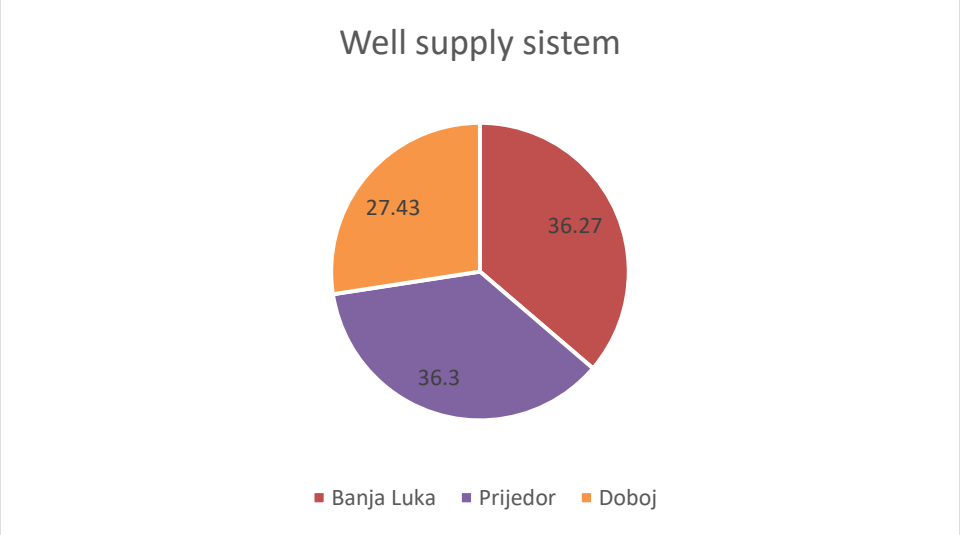

Figure 2. shows the average regional representation of samples originating from well supply system in % for the period 2018-2020 years

Most of the analyzed samples come from regions with the highest concentration of domestic animal farms (Banja Luka, Prijedor and Doboj). The analyzed number of samples originating from the region of Bijeljina and Trebinje is negligibly small, so it was left out of the discussion, while none of the samples analyzed from the region of East Sarajevo.

Table 1 shows the average test results of the total number of samples by region in % in relation to the test parameter for the period 2018-2020.

Table 1. Test results of total number of samples by regions in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	20.08±9.74	25.56±11.70	15.38±3.98	9.99±2.66	11.86±5.24
Prijedor	13.97±4.76	16.69±7.39	10.81±5.55	5.49±1.15	8.24±2.01
Doboj	8.47±4.26	10.47±5.72	5.34±1.28	3.12±1.18	6.03±1.13
Bijeljina	16.67±3.28	50±5.28	16.67±3.28	16.67±3.28	50±5.28
Trebinje	33.33±3.85	-	-	-	-

When it comes to the regional presence of pathogenic bacteria in the water, the obtained results are in accordance with the results of the Kalaba et al. (2015). The largest nuber of pathogens and total count at 22°C and 37°C is in the Banja Luka region, while the better situation is in the Doboj region.

All types of water sources may be subjected to contamination by agricultural activity (EA, 2002a). 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. These include for example, leaking hatches on service reservoirs, contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross connections and back-flow effects. Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognised sources of these organisms.

According to the WHO (2011), *E. coli* are the only true indicator of faecal contamination; they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Faecal streptococci represent evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms (WHO, 1996). The obtained results indicate significant fecal contamination of water, especially with intestinal enterococci and coliforms, and less with *E. coli*.

Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. 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 (EA, 2002a). The test results indicate that the causes of microbiological water malfunction 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.

Kalaba et al. (2015) state that 30.50% of samples originating from water supply system and 76.72% of samples from wells are unsatisfactory. 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), Denžić et. al (2012) and in accordance with the results Denžić et al. (2013).

Tables 2 and 3 show the results of testing water from water supply system and well water by region in % in relation to the test parameter for the period 2018-2020. years.

Table 2. Test results of water from water supply system by regions in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	9.91±4.33	12.51±3.25	4.98±1.43	2.60±0.43	2.94±0.37
Prijedor	7.14±2.83	7.94±4.21	4.07±1.06	1.67±0.34	3.53±0.68
Doboj	2.96±1.08	6.30±2.23	1.85±0.36	1.85±0.22	1.85±0.22
Bijeljina	16.67±3.28	50±5.28	16.67±3.28	16.67±3.28	50±5.28
Trebinje	33.33±3.85	-	-	-	-

Table 3. Test results of well water by regions in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	32.84±8.60	44.09±8.50	26.69±2.71	21.03±8.36	19.70±6.88
Prijedor	26.51±3.03	31.10±7.88	21.70±5.72	13.92±3.49	21.14±5.56
Doboj	16.14±3.26	16.14±3.26	10.29±3.07	4.74±0.28	11.44±4.54
Trebinje	33.33±3.85	-	-	-	-

The obtained results for water supply system differ significantly and are better in relation to the results Denžić et al. (2013), while the results obtained for well water are in accordance with the results Denžić Lugomer et al. (2019) and Kiš et al. (2017).

There are large differences between water and quality control systems for water destined for human or animal consumption (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.

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.

It is incorrect to state that drinking water distribution and delivery systems should be sterile, the active growth of microorganisms is considered indicative of failures in water processing units or distribution. Adapted bacteria can grow even in oligotrophic systems, such as distilled water (Gottschal, 1992; Poindexter, 1987; Roszak and Colwell, 1987).

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 (Regulation, 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.

Conclusion

The microbiological status of water on farms in the Republic of Srpska (B&H) in the period 2018-2020 is significantly improved compared to previous years, but still unsatisfactory given the large number of unsatisfactory samples. However, given the fact that over a third of farms are supplied with water originating from wells, there is a high risk to the microbiological status of the water, given that well water is not under constant monitoring. This is especially important having in mind the fact that the test results indicate that the causes of microbiological water malfunction come from animals. In order to gain a complete insight into the microbiological status of water on farms in the Republic of Srpska (B&H), it is necessary to comprehensively

and routinely test water samples originating from all regions, in proportion to the distribution of farms. Having in mind the importance of water for the life and health of animals, it would be justified to perform microbiological analysis of water from farms at least once, optimally twice a year.

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Original scientific paper

Influence of maximum daily temperature and temperature-humidity index on pig growth in fattening

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Abstract

The aim of the study was to determine how the average growth and conversion of food changes during the year, i.e. how the appetite and final weight of pigs are affected by the maximum daily temperature, and what is the influence of the temperature-humidity index (THI). The testing period consists of comparing two rounds of fattening: summer and winter. Three litters entered the test, where they are the same sows aged 3 years. All the sows are of the same breed "YU LANDRAS" and the breeding material was used from the same boar breed "GREAT YORKSHIRE". Boar was used in both cycles in all sows. The summer period of fattening began at the end of May 2018, and the winter period of fattening began at the end of November 2018. The piglets entered the shelter three days apart with an average body weight of 25 kg and came out of fattening at the end of September with an average body weight of 105 kg. Piglets entered the fattening farm three days apart with an average body weight of 25 kg. and came out of fattening at the end of March 2019 with an average body weight of 105 kg. The results of the research show that the growth of piglets was the largest, 789.83 gr. in the period when the temperature in the building was between 16^{o}-26^{o}C. Also, the same studies showed a correlation between temperature and food consumption, because piglets consumed less food (2.08 kg) in the period of high temperatures than in the conditions of ideal temperature (2.21 kg). It was THI that had a favourable effect on piglet growth during the period of optimal temperature.

Key words: piglets, growth, food, THI, temperature

Introduction

Meat production is related to a number of problems that a modern farmer must take into account. This primarily refers to the choice of breed, preservation of pig health, proper insemination and biological factors. However, a special place in this regulation belongs to the maintenance of optimal ambient conditions during fattening. Because of all this, the problem of slaughterhouse production optimization is solved multidisciplinary (Aaslyng et al., 2001). This is a problem, but also a challenge for all those involved in meat production. The development of pig production also influenced the knowledge about the importance of stress in fattening pigs. Stres as a natural reaction of the organism to the action of some environmental factor in animals leads to the appearance of the so-called "porcine stress syndrome (PSS)". A syndrome that clearly affects the qualitative and quantitative characteristics of meat at slaughter (Adeola et al., 1992). Pigs as animals easily susceptible to stress reactions, especially pigs of pronounced meatiness such as the "Pietren" breed, largely depend on the ambient conditions.

Ambient conditions for keeping pigs are one of the basic problems, but also stressors in fattening pigs. On farms animals are often kept in an area where their ability to move is limited (De Jong C. et al., 2000), and due to their physiology, they require an extremely high level of ventilation in buildings, which is often the main cause of heat stress in animals. It is also known that pigs are animals with a congenital cannibalistic disorder which further increases the level of stress in the animal (Cameron et al., 1999). Due to all the above, pigs require exceptional housing conditions, which increases investments in the production itself.

Heat stress is certainly one of the basic stressors that causes large losses in pig farming (Driessen B. and Geers R., 2000). The main source of heat in the building is the outside temperature, which we cannot influence to a great extent, but on the other hand, we can completely influence the temperature in the building. Unlike other animals, the optimal temperature in fattening pigs is slightly higher and ranges around 18°-22°C. However, the first signs of overheating, like decreased appetite, weight loss, increased aggression were noticed when temperatures are above 25°C. Elevated temperature in combination with increased humidity (over optimal relative humidity that in pigs ranges from 60 to 65%), leads initially to weight loss, then violation of quantitative and qualitative characteristics of meat and finally to health disorders that can lead to lethal end in severe cases. Low but also high temperatures that go beyond the optimal limits in pigs of different ages lead to different disorders. Fatteners at the beginning of fattening are more dependent on higher temperatures,

so the cold bothers them more, while the animals in the second part of fattening after 60 kg of body weight will have a higher load of high temperatures (Grandin T., 2003). An additional problem with fatteners is the increased relative humidity. The combination of these two factors is expressed as a temperature-humidity index and represents the basic parameter of atmospheric/microclimate factors at the level of stress in animals (English et al., 1988).

The value of the index in the literature is divided into several levels, as follows:

- intensity level up to 74: no effect on the occurrence of stress;
- value from 75 to 78: the first signs of stress were observed, which are reflected in a slightly increased nervousness, decreased appetite, increased need for fluid intake;
- value from 79 to 83: serious disturbances in appetite were observed, the level of aggression was clearly expressed, the appearance of cannibalism towards weaker throats in boxing, in a later phase severe depression;
- value over 84: if the number of days is continuously high, deaths due to overheating are inevitable (Febbraio MA et al., 1996).

The aim of the study was to determine how the average growth and conversion of food changes during the year, i.e. how the appetite and final weight of pigs are affected by the maximum daily temperature, as well as what is the influence of the temperature-humidity index –THI (Temperature Humidity Index, Armstrong 1994; Bolmanova et al. 2007).

Material and Methods

The experimental testing was performed in production facilities on the family farm "Janjić" in the village of Donja Jajna. During the examination period, which lasted 119 days per shift, a comparison of two fattening shifts was performed: summer and winter. Three litters of piglets entered the study, a total of 29 piglets, whose origin is from the same sow aged 3 years. The sows used as piglets are of the "JU LANDRAS" breed, and the breeding material was used from the same boar of the "GREAT YORKSHIRE" breed. The summer period of fattening began at the end of May 2018. Piglets entered the fattening farm three days apart with an average body weight of 25.4 kg and came out of fattening at the end of September with an average body weight of 105.2 kg. The winter period of fattening began at the end of November 2018. Piglets entered the fattening farm three days apart with an average body weight of 25.2 kg and came out of fattening at the end of March 2019 with an average body weight of 105.6 kg. The average number of piglets in the examined litters was 28 piglets , in both examined periods. In this paper, the influence of maximum temperature and THI on the amount of food

consumed and daily gain of piglets was examined. The values of maximum daily temperatures and relative humidity were measured daily in the building itself (professional humidity and temperature meter FLUS ET-951), while the value of the temperature-humidity index was calculated based on a well-known formula taken from the literature.

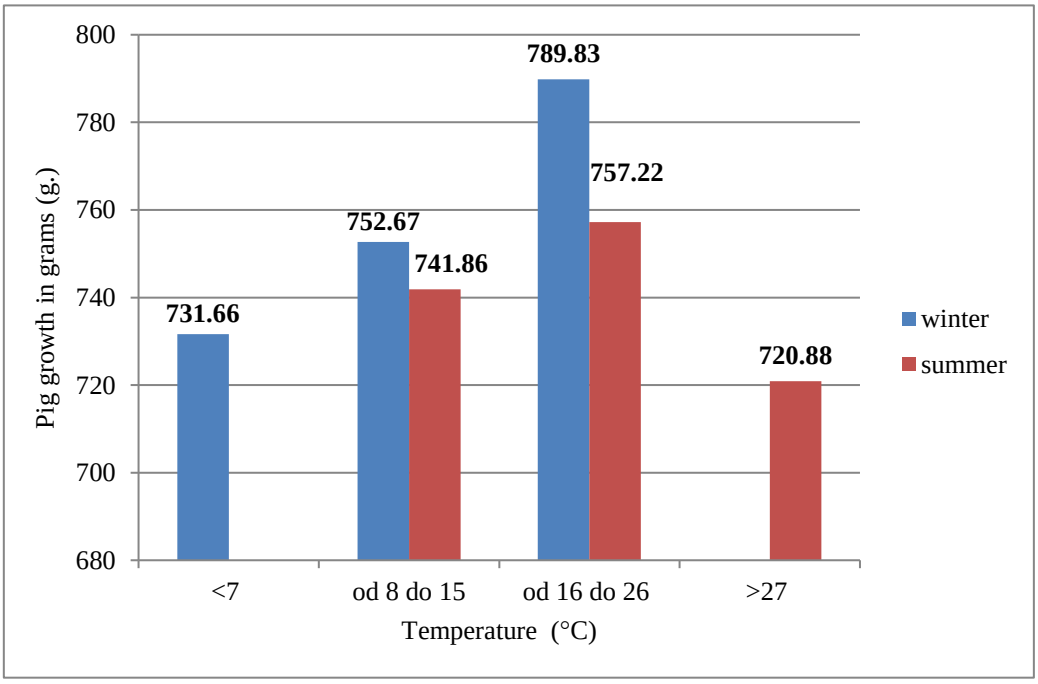
THI was calculated using following formula:

$$THI = (0{,}8\,Tmax) + \left(\frac{\varphi}{100}\right)(Tmax - 14{,}4) + 46{,}4$$

φ – relative humidity.

Results and Discussion

The influence of maximum daily temperatures and temperature-humidity index on the average daily gain in fattening pigs and the average daily amount the food consumed per head is shown in the graphs:



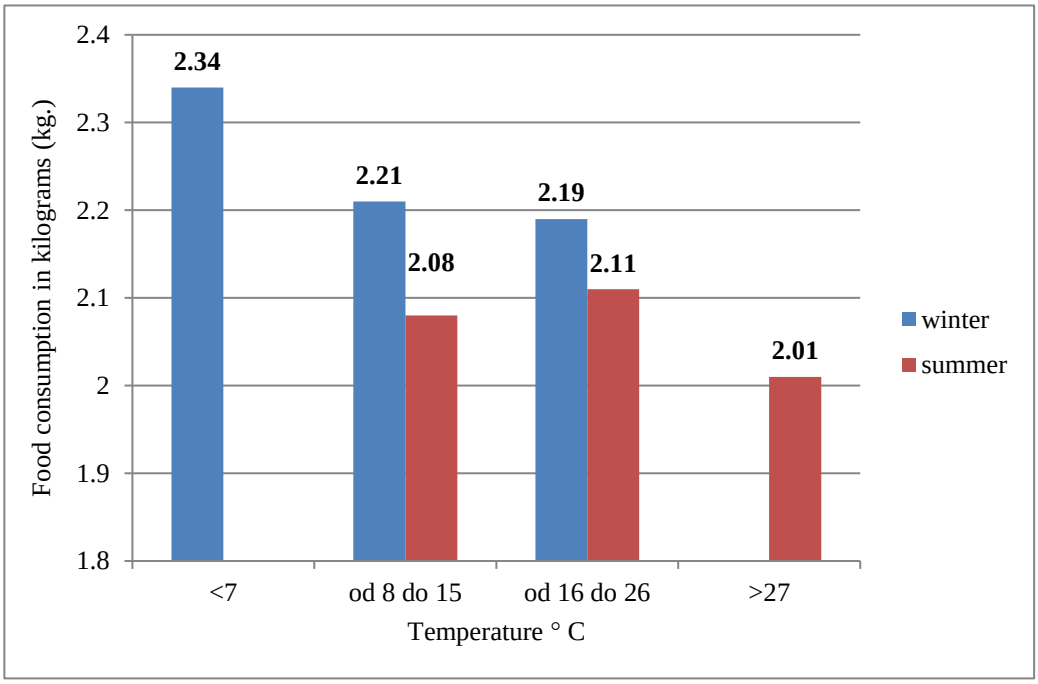
Graph 1. Influence of maximum daily temperatures on the average daily gain per head g/day

(Source: Research results 2018)

Graph 1. shows the results according to which it was determined that in the examined litter at a temperature of less than 7°C, the average increase in the winter months was 731.66 g per day per head. On days when the maximum temperature ranged from 8° to 15°C in the winter months, the average daily gain per head was 752.67 g, while in the same interval but in the

summer months, the average daily gain per head was 741.86 g, which was confirmed during the research of Milovanović and other authors (2009) in their work.

In the days when the maximum temperature ranged from 16° to 26°C in the winter months, the average daily gain per head was 789.83 g, while in the same interval but in the summer months, the average daily gain per head was 757.22 g. In the days when the maximum temperature was in the interval over 27° C in the summer months, the average daily gain per head was 720.88 g.

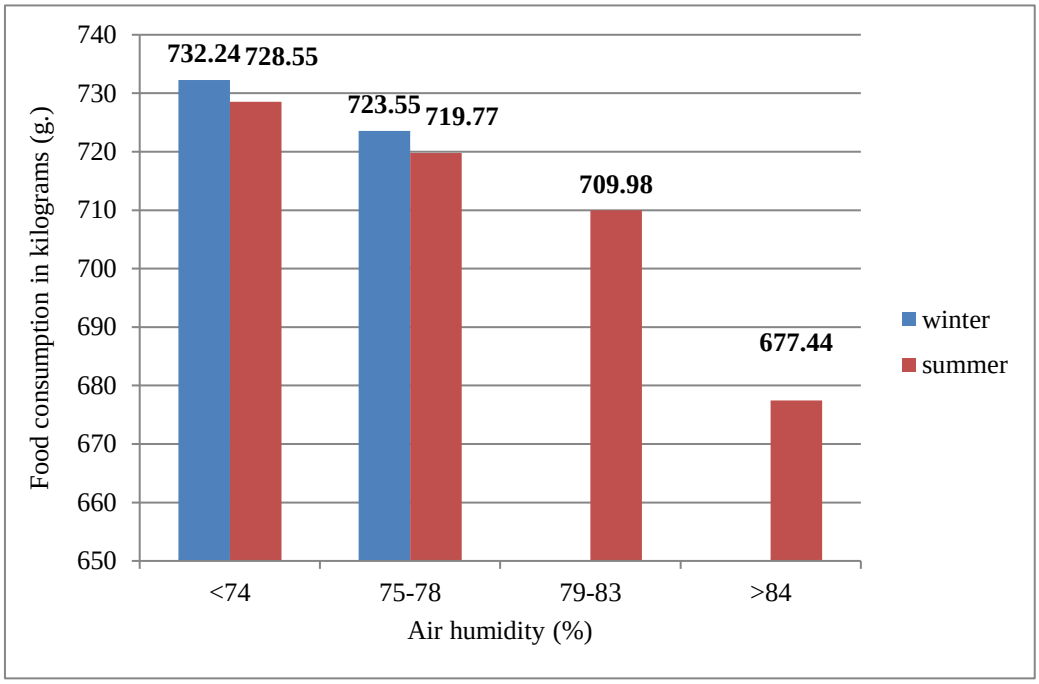


Graph 2. Influence of maximum daily temperatures on daily food consumption per head kg/day

(Source: Research results 2018)

The results presented by Noguera et al (2002) in their research were evidence that food consumption in winter is higher compared to summer due to the energy needs of piglets. Graph 2 shows the results according to which it was determined that in the examined litter at a temperature of less than 7 ° C, the average food consumption in the winter months was 2.34 kg per day per head. On days when the maximum temperature ranged from 8 to 15 ° C in the winter months, the average daily food consumption per head was 2.21 kg, while in the same interval but in the summer months, the average daily food consumption per head was 2.08 kg. On days when the maximum temperature ranged from 16 to 26 ° C in the winter months, the average daily food consumption per head was 2.19 kg while in the same interval but in the

summer months the average daily food consumption per head was 2.11 kg. On days when the maximum temperature was in the interval over 27 ° C in the summer months, the average daily food consumption per head was 2.01 kg. Similar results were obtained by Weary et al., (1998) in their research.



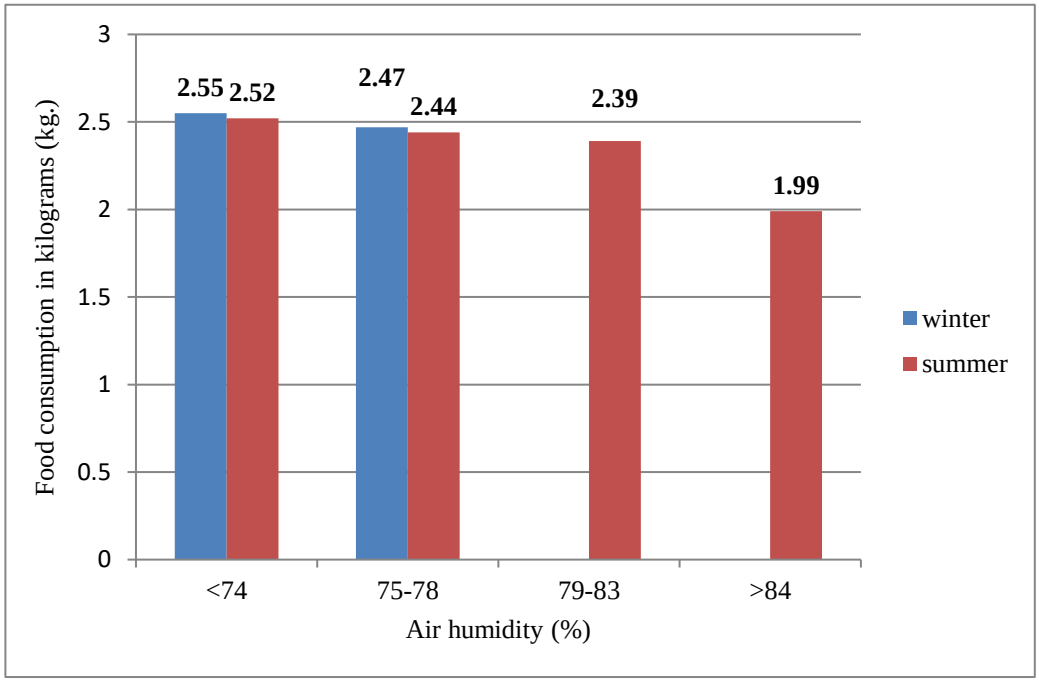
Graph 3. Influence of temperature-humidity index on average daily gain per head g/day

(Source: Research results 2018)

Graph 3 shows the results according to which it was determined that in the examined litter at the value of the temperature-humidity index less than 74, the average increase in the winter months was 732.24 g per day per head. In the days when THI was in the interval of 75-78 in the winter months, the average daily gain per head was 723.55 g, while in the same interval but in the summer months, the average daily gain per head was 718.77 g. Similar results were published by Teodorović and Radović (2002) in their scientific research.

In the days when THI was in the interval of 79-83 in the summer months, the average daily gain per head was 709.98 g. and on days when THI was in the interval over 84 in the summer months, the average daily gain per head was 677.44 gr.

Investigating the correlation between fattening and reproductive traits of sows, Radovic et al., (2008) showed that ambient conditions, temperature and humidity have a direct impact on the amount of food consumed and the achieved yield in fattening.



Graph 4. Influence of temperature-humidity index on daily food consumption per head kg/day
(Source: Research results 2018)

Graph 4 shows the results according to which it was determined that in the examined litter at the value of the temperature-humidity index less than 74, the average daily food consumption in the winter months was 2.55 kg per day per head. While in the summer months, it averaged 2.52 kg per head per day. In the days when THI was in the interval of 75-78 in the winter months, the average daily food consumption was 2.47 kg, while in the same interval, but in the summer months, it was 2.44 kg per day per head.

On days when THI was in the interval of 79-83 in the summer months, the average daily food consumption was 2.39 kg per head, and on days when THI was in the interval over 84 in the summer months, the average daily food consumption per head was 1.99 kg.

Conclusion

Based on the obtained and presented results on the graphs, the following conclusions can be drawn:

- The most favourable interval for the growth of piglets in fattening is the interval when the maximum daily temperature is in the range from 16 to 26 ° C, 27 ° N.

- The highest appetite in fattening is at the lowest temperatures below 7 ° C, while the lowest is on days when the maximum daily temperature exceeded 27 ° C.
- However, increased appetite and the highest food consumption on days when the maximum daily air temperature did not exceed 7 ° C, does not mean the highest daily gain. The reason for this phenomenon is certainly the need of the throat for energy consumption for metabolic needs, i.e. for self-heating.
- With the increase of the value of the temperature-humidity index, there is an increased stress which adversely affects both the daily consumption of food and the daily growth of the throat. On days when the index value is over 84, daily food consumption has almost halved compared to days without stress intensity.

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Original scientific paper

Pedigree analysis of Lipizzan stallions: generation interval and inbreeding

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Abstract

Pedigree analysis is an important tool for the assessment of population structure and inbreeding levels, which are important for closed populations under high selection pressure, such is the stud Vučijak. The aim of this research is to analyze the level of inbreeding of six Lipizzan stallion lines, as well as the length of the generation interval, in order to assess the selection intensity. Information on five generation pedigrees of 44 Lipizzan stallions was used to calculated the coefficient of inbreeding (F). Further, the total generation intervals was calculated (TGI) according pedigree data for 304 stallions. Of the total number of stallions, 149 were subsequently introduced into reproduction and for these animals also was calculated generations interval (GI). The F ranged from 0.012 (Favory) to 0.034 (Maestoso). The total generation interval ranged from 9.21 (Pluto) to 13.39 (Neapolitano). The lowest GI was for the Siglavy (9.32) and the longest for the Neapolitano stallion lines (12.50). The analysis of variance showed that there are significant differences between six stallion lines. The results can be used for managing mating program on the stud, and also for implementation of conservation strategy of the Lipizzan horses.

Key words: inbreeding, generation interval, stallion lines, population structure

Introduction

Lipizzan horse characterized long history of existence, strict and systemic selection during almost 450 years, whit great morphological and genetic variability. The base population of the Lipizzan breed is structured into a number of rather large, mostly state owned, studs with

limited exchange of horses over the last few decades (Zechner et al., 2002). Currently, Lipizzan horses are kept in the national stud farms in Austria, Hungary, Slovenia, Croatia, Slovakia, Italy, Romania, Bosnia and Herzegovina and Serbia, as well as private breeding organizations. According to LIF, there is an eleven national stud farms and breeding organizations from 16 countries of Europe, America, Australia and Africa. The total number of Lipizzan horses on national stud farms as well as private breeding is 11.602 horses (LIF, 2019). Considering this low number and long history of breeding in a more or less closed population, the population can be considered genetically small (Zechner et al., 2001).

Lipizzan horses are one of most valuable animal genetic resources from Bosnia and Herzegovina (Rogic et al., 2018). National stud Vučijak is the only Lipizzan stud in Bosnia and Herzegovina, established in 1946. According stud books, it can be seen that the founder's horse was introduced from the territory of the Croatia (Popadić et al., 2019). Subsequently, stallions and mares from Slovenia and Serbia was introduced to the stud Vučijak. The primary goal of establishing the stud was to improve the existing horse population in North Bosnia, respectively to get the horse with smaller body frame suitable for driving and carrying (Rogic et al., 2018). In the last decades there was a modification of the goals of horse breeding (Borowska and Szwaczkowski, 2014). Currently, the aims of breeding program are, or sport horse breeding, or conservation of national breeds, which is case with stud Vučijak.

In animal breeding, genealogical information is important for genetic evaluation and genetic variability (de Rochambeau et al., 2000). Analyzing of the pedigree information is an important tool for the identifications of genetic diversity and changes that occur from generation to generation (Bokor et al., 2013). Additionally, pedigree analysis can enable us to estimate the population structure and inbreeding levels, which are important for populations under high selective pressure and closed stud books for many years (Gutiérrez et al., 2005).

The objective of present study was to analyze the level of inbreeding of six Lipizzan lines, as well as the length of the generation interval, in order to assess the selection intensity. The obtained results can be used for managing mating program on the stud, and also for implementation of conservation strategy of the Lipizzan horses.

Materials and Methods

Information from current and older stud book of Vučijak were primary sources of information. The secondary source of data was "Stud Book of Lipizzan horse from stud Vučijak 1946-2007"

(Stojanović et al., 2006). National stud Vučijak breeds 6 stallion lines: Neapolitano, Favory, Conversano, Siglavy, Pluto, Maestoso.

Inbreeding coefficient (F)

Every animal, whit complete pedigrees back 5 generations, was used. Pedigrees collected for all stallions currently living on the stud, and pedigrees of total 44 stallions ware used. For every animal the inbreeding coefficient was calculated according to the Wright (1931).

Generation intervals

Pedigrees collected for the period of 1946. to 2017. The total generation intervals ware calculated (TGI) according pedigrees of 304 stallions. Total generation interval (TDI) defined as the average age of parents at the birth of their offspring, and he was calculated for every animal, whether or not it was later introduced into reproduction.

Based on the pedigree data, it was concluded that of the total number of stallions, 149 were later introduced into reproduction. For these stallions also calculated generation interval (GI), defined as the average age of the parents at the time of birth of their offspring (James, 1977).

Statistical analysis

A simple analysis of variance was used to determine the difference in the coefficient of inbreeding and generation intervals between stallion lines, whereby the F-test was calculated. The significance of the difference was tested based on the Duncan test at the significance level of 0.05. Animals whit F=0 were not taken into statistical data processing. The statistical program SPSS17 was used for data processing.

Results and Discussion

The F between sire lines in the Lipizzan horse from stud Vučijak

In order to understand the differences between inbreeding level, the analysed stallions are divided into 6 groups of stallion lines. The simple analysis of variance was performed (Tab. 1).

Table 1. Analysis of the variance for F for the six stallion lines

Stallion Lines	*F*	*F calculated*	*F tab*	
			0.05	0.01
Pluto	0.016			
Siglavy	0.017			
Maestoso	0.034	2.72*	2.53	3.70
Neapolitano	0.017			
Favory	0.012			
Conversano	0.033			

The value of F-test (2.72) indicates that there is a statistically significant difference between the average values of the inbreeding coefficient of the six sire lines. The lowest F was found in the Favory (0.012), and the highest in the Conversano (0.033) and Maestoso (0.034) stallion lines.

Table 2. Duncan test of differences for F between the six stallion lines

	Pluto	*Siglavy*	*Maestoso*	*Neapolitano*	*Favory*	*Conversano*
Pluto	-	ns	*	ns	*	*
Siglavy	-	-	*	ns	*	*
Maestoso	-	-	-	*	*	ns
Neapolitano	-	-	-	-	*	*
Favory	-	-	-	-	-	*

* significant, ns – none significant

Duncan test showed that mean value of the inbreeding coefficient of the Favory was significantly different from the other stallion lines. The F of the Conversano was significantly different in relation to the mean value of other stallion lines, except the Maestoso. The F of the Neapolitano was significantly different in relation to the Favory, Conversano and Maestoso stallion lines. The Favory stallion line is the least influenced by inbreeding in relation to the other stallion lines of the stud Vučijak, approximately, stallion lines of Conversano and Maestoso are the most influenced (Tab. 2).

When calculating the inbreeding coefficient of an individual, or average inbreeding coefficient of population, the lenght and completeness of a pedigree have a large effect on the outcome of calculation (Cothran et al., 1984). According Zechner et al. (2002) in the eight Lipizzan national stud, using five generation pedigrees for calculating inbreeding coefficients leads to value around 2%, which is in accordance with obtained results. Only Conversano and Maestoso stallion lines show significantly higher inbreeding as a results of the management strategy which was mainly concerned with conservation of stallion and mare lines.

The generation intervals between stallion lines in the Lipizzan horse from stud Vučijak

The total generation interval (TDI) was calculated for every stallion. In order to understand the differences between mean values of total generation intervals, the simple analysis of the variance was calculated (Tab. 3).

Table 3. Analysis of the variance for TDI for the six stallion lines

Stallion Lines	*PDR*	*F calculated*	*F tab*	
			0.05	0.01
Pluto	9,21			
Siglavy	9,48			
Maestoso	10,56	5,40*	2,21	3,02
Neapolitano	13,39			
Favory	9,28			
Conversano	10,39			

The lowest TDI was found in the Pluto (9.21), and the highest in the Neapolitano (13.39) stallion lines. The calculated F-test (5.40) indicates that there is a significantly different between the average values of the TDI of the six stallion lines.

Tab. 4. Duncan test of differences for TDI between the six stallion lines

	Pluto	*Siglavy*	*Maestoso*	*Neapolitano*	*Favory*	*Conversano*
Pluto	-	ns	ns	*	ns	ns
Siglavy	-	-	ns	*	ns	ns
Maestoso	-	-	-	ns	ns	ns
Neapolitano	-	-	-	-	*	*
Favory	-	-	-	-	-	ns

* significant, ns – none significant

Duncan test showed that mean value of the TDI of the Neapolitano was significantly different from the other stallion lines, expect Maestoso. Based on pedigrees, it can be seen that the youngest stallions of the Pluto stallion line ware introduced into reproduction whit three years old, and the maximum use in reproduction is up to 18 years. From other side, stallion Neapolitano Bascstelze was excluded from reproduction at the 30 years old, and was the oldest stallion used in reproduction. This may explain the differences obtained in this work.

Table 5. Analysis of the variance for GI for the six stallion lines

Stallion Lines	*GI*	*F calculated*	*F tab*	
			0.05	0.01
Pluto	8,39			
Siglavy	9,32			
Maestoso	10,54	2,47*	2,21	3,02
Neapolitano	12,50			
Favory	8,51			
Conversano	9,88			

The lowest GI was found in the Pluto (8.39), and the highest in the Neapolitano (12.50) stallion lines. The calculated F-test (2.47) indicates that there is a significantly different between the average values of the GI of the six stallion lines.

Tab. 6. Duncan test of differences for GI between the six stallion lines

	Pluto	*Siglavy*	*Maestoso*	*Neapolitano*	*Favory*	*Conversano*
Pluto	-	ns	*	*	ns	*
Siglavy	-	-	*	*	ns	*
Maestoso	-	-	-	*	*	ns
Neapolitano	-	-	-	-	*	*
Favory	-	-	-	-	-	*

* significant, ns – none significant

Duncan test showed that mean value of the GI of the Neapolitano was significantly different from the other stallion lines. The GI of the Conversano and Maestoso was significantly different from other stallion lines, expect among themselves. The smaller number of stallions used in data processing of generation interval, which can be reason for lower obtained values and more significant differences in GI between stallion lines. According Čačić and Curik (2014) in the eleven Lipizzan national stud, the average value of TDI ranged from 9.6 to 14.6, and GI from 9.4 to 13.4, which is in accordance with obtained results.

Low values of generation intervals were contributed by selection. Selection on the stud Vučijak was mainly carried out according on origin and morphological characteristics, and less according value of progeny testing. Also, a small number of stallions in breeding prevents the elimination of inferior stallions from breeding, so most stallions are introduced into reproduction. Further, in our country there is no procedure and defined system of training and testing of stallions in order to obtain credible data for the selection of the highest quality stallions Just non-implementation of detailed testing stallions leads to a significant reduction in the length of the generation interval.

Conclusion

In conclusion, the obtained results indicated that Lipizzan stallions from stud Vucijak are similar with other national European stud, according inbreeding level and length of generation intervals. The higher inbreeding level and shorter generation intervals as a result of the management strategy which was mainly concerned with conservation of stallion and mare lines. In the future is a necessary to work on procedure and defined system of training and testing of stallions in order to obtain credible data for the selection of the highest quality stallions and planned breeding in order to increase the safety of assessment in achieving genetic progress. Also, it is necessary to do a complete analysis of the pedigree of the entire population, which would include other parameters for assessing the genetic and population structure of the Lipizzan horses from stud Vučijak.

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Original scientific paper

Effects of different light intensity on the growth of rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Abstract

The experiment of determining effect of different light intensity on the growth of rainbow trout (*Oncorhynchus mykiss*) fingerlings it was realized in the aquaculture laboratory at the Faculty of Agriculture in Banja Luka, for a period of 30 days. Experiment was set up in 3 groups (G_1, G_2, and G_3) with three repetitions. A total of 450 rainbow trout fingerlings (150 individuals/group), with an average individual body weight of 1.60-1.69 g, were inhabited. The light intensity (lx) during the experiment averaged 2.92 lx in G_1, 17.32 lx in G_2, and 196.22 lx in G_3, during 24 hours. Light intensity (lx) was measured once per day above the water surface using the Light Meter PCE-MLM1 device. Length and individual fingerlings mass was determined by measuring the individuals at the start and at the end of the experiment (random sample 30 fingerlings/group). The goal of the experiment was to determine the effects of different light intensity ($G_{1(2.92\ lx)}$, $G_{2(17.32\ lx)}$, $G_{3(196.22\ lx)}$) on growth of the rainbow trout (*Oncorhynchus mykiss*) fingerling. In order to determine the effects of different light intensities based on the obtained results, the specific growth rate (SGR) was analyzed, thermal unit growth coefficient (TGC), condition factor (CF), feed conversion ratio (FCR), relative body weight gain (BWG) and survival rate (%) rainbow trout fingerlings. The largest biomass gain (%), SGR, TGC, survival (%) and the lowest FCR were found in group $G_{1(2,92\ lx)}$, but the differences found in the examined growth characteristics were not statistically significant ($p>0.05$).

Key words: light intensity, growth, rainbow trout, fingerlings

Introduction

The success of farming different age categories of rainbow trout and other fish species depends on many factors that affect the achievement of good growth, feed utilization and health status of farmed fish. In production conditions, human influence on some ecological factors of the breeding environment is limited. However, when it comes to the rearing of juvenile rainbow trout, which is realized in closed spaces, there is a possibility of managing most of the factors that affect the growth of juveniles, including the intensity of light. In general, salmonid hatcheries are, as a rule, darkened areas because direct sunlight has a negative effect on the eggs of salmonid fish species and its embryonic development. Therefore, artificial light is used in hatcheries to perform daily tasks during embryonic development, and it is especially needed during the initial feding rainbow trout. Light is essential for the normal life, growth and development of most plants and animals, with the exception of some species that inhabit deep parts of the sea (Boeuf & Le Bail, 1999). Fish need light to synchronize all life phases, growth and survival (Villamizar et al. 2011, Delabbio, 2015), and we can observe it from the aspect of color spectrum, intensity and photoperiod (Boeuf & Le Bail, 1999). Boeuf & Le Bail (1999) state that light intensity is probably not very important in stimulating growth, unlike the photoperiod, but they emphasize the importance that the effect of light on fish is manifested by better utilization of feed, and not only by stimulating feed intake. Too intense light can affect fish stressfully or even deadly (Boeuf & Le Bail, 1999). Stefansson et al. (1993) state that the light intensity in the tested range from 27 to 715 lx does not significantly affect the growth of adult salmon, while Oppedal et al. (1997) report that adult Atlantic salmon farmed in cages in the sea at high light intensity (22.2 $W \cdot m^{-2}$) has significantly better growth. Wallace et al. (1988) state that lake Arctic char (*Salvelinus alpinus*) with an average weight of 1.4 g grew best at a light intensity of 50 lx, with the lowest mortality compared to other treatments with 700, 200 and 10 lx light intensities, while growth of juvenile salmon weighing 0.7 g farmed for 35 days, better at a light intensity of 700 lx compared to 10, 50 and 200 lx. Taylor et al. (2006) in the results of three years of field research state that exposure to higher light intensity seems to promote faster growth and feed efficiency at all stages of rainbow trout farming. The goal of this study was to determine the effects of different light intensities ($G_{1(2,92\ lx)}$, $G_{2(17,32\ lx)}$, $G_{3(196,22\ lx)}$) on the growth of rainbow trout (*Oncorhynchus mykiss*) fingerlings.

Material and Methods

The experiment of determining effect of different light intensity on the growth of rainbow trout (*Oncorhynchus mykiss*) fingerlings realized in the aquaculture laboratory at the Faculty of Agriculture in Banja Luka, for 30 days. Experiment was set up in 3 groups (G_1, G_2, and G_3) with three repetitions in aquariums with a volume of 65 l/aquarium (58 cm x 33 cm x 34 cm). A total of 450 rainbow trout (150 individuals / groups) fingerlings, with an average individual body weight of 1.60-1.69 g, were inhabited. The aquariums were covered with dark foil on the sides, and the light (white) reached the aquariums from the water surface. The light intensity (lx) during the experiment averaged 2,92 lx in G_1, 17.32 lx in G_2, and 196,22 lx in G_3, during 24 hours. Light intensity (lx) was measured once a day just above the water surface with the Light Meter PCE-MLM1. At the beginning and at the end of the experiment, BW - individual body weight (g), TL - total body length (mm), FL - fork length (mm) and SL - standard body length (mm) of the rainbow trout fingerlings (30 fingerlings/group) were measured. Individual body weight of the fingerlings and daily amounts of feed were measured with a digital Kern scale (load capacity: 600g; precision: 0.01g), and body length with a digital movable scale UNIOR 270A (precision: 0.01 mm). Water temperature (^oC), dissolved oxygen content in water (mg/l) and water oxygen saturation (%) were measured daily with an oximeter (Oxi 330i / SET 2B20-0011 WTW), and water pH was measured once a week with a pH meter (pH 330i / SET 2A20-1011 WTW). The Atman HP-4000 air compressor was used for aeration of water in all aquariums. Nutra 0 (Skreting) feed of the following composition was used for feeding the rainbow trout fingerlings: crude protein 54%, crude fats 18%, crude fiber 1%, crude ash 9.5%, digestible energy 18.8 MJ/kg. Daily dietary norms in all groups were the same, and amounted to 4%. Based on the obtained results, the following parameters were determined: specific growth rate (SGR), thermal unit growth coefficient (TGC), condition factor (CF), feed conversion ratio (FCR), relative body weight gain (BWG) and survival rate (%) of rainbow trout fingerlings using the following formulas:

$$SGR = (\ln FBW - \ln IBW)/D \times 100$$

SGR – specific growth rate; FBW – final body weight (g);
IBW – initial body weight (g); ln – natural logarithm (e = 2,7183); D – number of days.

$$TGC = (FBW^{1/3} - IBW^{1/3})/(TxD) \times 100$$

TGC – thermal unit growth coefficient; FBW – final body weight (g); IBW – initial body weight (g); T – water temperature (°C); D – number of days.

$$CF = (BW/L^3) \times 100$$

CF – condition factor; BW – body weight (g); L – fork length (cm).

$$FCR = F/G$$

FCR – feed conversion ratio; F – feed consumption (kg); G – gain mass (kg).

$$BWG = [(FBW-IBW) / IBW] *100$$

BWG – body weight gain; FBW – final body weight (g); IBW – initial body weight (g).

$$\text{Survival rate } (\%) = (N_t/N_0) \times 100$$

N_t – number of fish at the end (n); N_0 – number of the fish at the start (n).

The obtained results were statistically processed using the statistical programs SPSS17 and MS Excel. The processing includes descriptive statistics and a simple analysis of variance to determine the significance of differences.

Results and Discussion

The results of measuring the basic parameters of water quality during the experiment are shown in Table 1. The presented data shows similar tendencies of the analyzed parameters of water quality in all groups. The water temperature was high for growing juvenile rainbow trout, and the oxygen content was lower.

Tabel 1. Results of basic water quality paremeters (average±SD and coefficient of variation – CV) during the experiment

Parameter	$G_{1(2.92\ lx)}$		$G_{2(17.32\ lx)}$		$G_{3(196.22\ lx)}$	
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV
T (°C)	16.49±0.53	3.19	16.69±0.41	2.47	16.59±0.55	3.31
O_2 (mg/l)	7.21±0.64	8.89	7.27±0.65	8.90	7.61±0.67	8.87
O_2 (%)	74.86±6.74	9.01	75.62±6.46	8.55	78.96±6.63	8.39
pH	7.38±0.13	1.75	7.38±0.05	0.69	7.34±0.15	2.03

It was found that there was no statistically significant difference between the environments ($p>0.05$) of water temperature ($F_{2,6} = 0.657$; $p = 0.552$), the content of dissolved oxygen in water ($F_{2,6} = 1.078$; $p = 0.398$), oxygen saturation of water ($F_{2,6}=1.185$; $p= 0.368$) and pH of water ($F_{2,6} = 0.285$; $p = 0.761$) between the observed groups during the experiment.

Table 2 shows the average weights, body lengths and other analyzed indicators of rainbow trout fingerlings growth and feed utilization ± standard deviation (SD).

Table 2. Overview of rainbow trout fingerlings growth characteristics before and after exposure to different light intensities

Parameter	$G_{1(2.92\ lx)}$		$G_{2(17.32\ lx)}$		$G_{3(196.22\ lx)}$	
	Initial	Final	Initial	Final	Initial	Final
TL (mm)	55.33±3.49	84.75±11.09	54.91±5.06	83.25±11.72	56.29±3.34	84.92±10.68
FL (mm)	52.92±3.18	81.02±10.84	52.07±4.95	79.55±11.34	53.24±3.22	80.89±11.07
SL (mm)	47.59±3.24	74.82±10.37	46.65±4.21	73.42±10.73	47.97±3.00	74.51±9.69
BW (g)	1.60±0.34	7.18±2.85	1.60±0.47	6.61±2.73	1.69±0.35	7.42±2.56
CF	1.08±0.01	1.35±0.02	1.20±0.13	1.31±0.05	1.12±0.04	1.40±0.08
BWG (%)	347.22±25.78		317.44±91.20		337.70±58.66	
SGR (% day^{-1})	4.99±0.20		4.71±0.77		4.90±0.45	
TGC	0.153±0.01		0.141±0.03		0.151±0.02	
FCR	0.57±0.07		0.77±0.27		0.60±0.11	
Survival rate (%)	96.00±3.46		89.33±5.77		93.33±5.03	

There was no statistically significant difference ($p>0.05$) at the beginning and end of the experiment

At the beginning of the experiment, the average values of TL, FL, SL and BW were similar and no statistically significant difference was found in the averages ($p>0.05$) of the total body length ($F_{2,87} = 0.923$; $p = 0.401$), fork length ($F_{2,87} = 0.739$; $p = 0.480$), standard body length ($F_{2,87} = 1.118$; $p = 0.332$) and individual body weight ($F_{2,87} = 0.537$; $p = 0.586$). Also, in the end, no statistically significant difference was found in the averages ($p > 0.05$) of the total body length ($F_{2,87} = 0.202$; $p = 0.817$), body length to the tail fin fork ($F_{2,87} = 0.161$; $p = 0.851$), standard body length ($F_{2,87} = 0.154$; $p = 0.858$) and individual body weights ($F_{2,87} = 0.696$; $p = 0.501$).

The condition factor of rainbow trout fingerlings is higher in the end, compared to the beginning when the condition factor was significantly lower, and indicates good nutrition of rainbow trout fingerlings in all groups. At the beginning, no statistically significant difference ($p>0.05$) of condition factor was found ($F_{2,6} = 2.02$; $p = 0.213$), and in the end there was also no significant difference in CF between the observed groups ($F_{2,6} = 2.232$; $p = 0.189$). Dekić et al. (2016) states that the factor of fish condition is one of the ways to monitor the influence of environmental factors on fish. Although the highest increase in body (%) was recorded in group $G_{1(2,92\ lx)}$, no statistically significant difference ($\alpha = 0.05$, $F_{2,6} = 1.168$; $p = 0.850$) was found between the observed groups. There was no statistically significant difference ($p>0.05$) SGR (($F_{2,6} = 0.23$; $p = 0.806$) and the TGC between the observed groups ($F_{2,6} = 0.397$; $p = 0.689$), but the highest values of SGR and TGC are present in individuals from group $G_{1(2,92\ lx)}$ with the lowest light intensity. Dadfar et al. (2017) states that in the experiment of the effects of light color on the condition factor and SGR, at the photoperiod 14: 8 (L:D), the highest two

months old rainbow trout condition factor under light yellow was achieved (CF = 1.19) and white (CF = 1.17) and SGR under light yellow (SGR = 3.60) and white (SGR = 3.54), which is significantly lower than the results obtained in this experiment which can be attributed to the higher water temperature. The feed conversion coefficient is the lowest in group $G_{1(2,92\ lx)}$ with the lowest light intensity, but without significant differences ($p>0.05$) between the observed groups ($F_{2,6} = 1.136$; $p = 0.382$).

Survival rate (%) of the rainbow trout fingerlings was highest in the group with the lowest light intensity, $G_{1(2,92\ lx)}$ (96%), with no statistically significant difference ($p>0.05$) between the observed groups ($F_{2,6} = 1.434$; $p = 0.310$).

The highest SGR, TGC, body weight gain, survival rate and lowest FCR in low light conditions (24 hours) were recorded in group $G_{1(2,92\ lx)}$, the lowest light intensity, which can be related to the statement of Boeuf & Le Bail (1999) that it can be assumed that these effects may be related to less stressful conditions for fish, ie that the interpretation of the results of the effects of light intensity on growth should be approached carefully, because they depends on breeding conditions and fish stress. Variations in these indicators are the lowest compared to the other two groups. Sönmez et al (2009) states that the rainbow trout fingerlings feding in different photoperiod regimes has the highest growth rate and the best feed conversion ratio in juveniles at 16 hours light and 8 hours dark. Savić & Mikavica (2013) state that by cultivating a rainbow trout of similar size in different diets at photoperiod 8:16 (L : D), CF, SGR and TGC are lower compared to this study, which may be the result of lower water temperature, regime nutrition or lighting. Noble et al. (2005) states that the TGC of rainbow trout is higher in individuals at 50 lx than in those at 700 lx, but this difference is not significant, and the light intensity does not significantly affect feed spoilage. Examining the effect of four different light intensities (0.07; 1.0; 50.0 and 700 lx) on the self-feeding ability of rainbow trout Mizusawa et al (2007) state that light intensity did not significantly affect self-feeding ability. Ergün et al (2003) and Barimani et al (2013) recommend continuous illumination, ie a longer photoperiod (light : dark) to achieve better growth and a lower juvenile rainbow trout feed conversion rate. Newman et al (2015) present research data on the effects of artificial light at night (ALAN) on Atlantic salmon in which they state that artificial light during the night affects salmon behavior disorder and that it is unclear which physiological processes are behind the observed behavior modifications.

Conclusion

In general, it can be said that a significant increase in rainbow trout fingerlings growth was achieved in all groups, which is indicated by the average values of length and weight, CF, weight increase (%), SGR, TGC, FCR. The highest BWG (%), SGR, TGC, survival, and lowest FCR were found in group $G_{1(2.92\ lx)}$. However, different light intensities (2.92 lx, 17.32 lx and 196.22 lx) did not affect the appearance of a significant difference ($p > 0.05$) growth characteristics of the rainbow trout (*Oncorhynchus mykiss*) fingerlings.

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Original scientific paper

Comparison of the antibacterial effect of manuka honey and domestic acacia honey

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Abstract

The global problem of bacterial resistance to antibacterial drugs is the reason for the search for new antibacterial drugs. Honey has good antibacterial properties and therefore much is expected of honey that has an antibacterial effect especially on bacteria that are resistant to the weight of the drugs used. Several components present in honey are responsible for the antibacterial activity of honey in concentrations that differ in different types of honey. The aim of this work is to compare the antibacterial properties of domestic acacia honey with manuka honey (*Leptospermum scoparium*), which has a standardized level of antibacterial activity.

For testing the antimicrobial activity of manuka honey and acacia honey it was used clinically isolates bacteria *Streptococcus* group D, *Escherichia coli, Staphylococcus aureus, Salmonella enterica* and *Salmonella typhymurium* from collection of Public Institute Veterinary Institute Republic of Srpska "Dr Vaso Butozan", Banja Luka and reference *Staphylococcus aureus* WDCM 00034. Bacterial cultures were prepared by incubating at 37°C in nutrient broth for 18 hours. The agar diffusion method was used. Disks with a diameter of 9 mm were placed on Petri dishes on Müeller-Hinton agar, previously seeded with 100µl of bacterial suspension with a concentration of 10^5 CFU/ml.

The results showed that both analysed honey samples have good antibacterial activity against the tested pathogens with inhibition zones from 25.33 mm to 37.33 mm for manuka honey, and 28.00 mm to 37.00 mm for acacia honey. Both honeys showed a bactericidal effect on Gram-positive bacteria and a bacteriostatic effect on Gram-negative bacteria

Key words: manuka honey, acacia honey, antibacterial activity

Introduction

In traditional medicine, honey occupies a very important place for the treatment of many diseases. Evidence of the centuries-old use of honey is over 8000 years old and is described in drawings from the Stone Age. The ancient Egyptians, Assyrians, Chinese, Greeks, and Romans used honey to treat wounds and digestive problems, of which there is numerousevidence (the Vedas, about 5,000 years old, the Qur'an, the Talmud, the Bible, the holy books of China, India, Persia, and Egypt, Egyptian papyrus1900-1250 BC, Sumerian clay tablets, 6200 BC) (Zumla and Lulat, 1989; Jones, 2009).

With the discovery of antimicrobial drugs, the healing properties of honey are neglected. Excessive use of antimicrobial drugs has led to the emergence of resistant bacteria and in the 20th century began a more intensive study of honey, especially its antimicrobial properties, and the ability to accelerate wound healing. Due to its inhibitory action, honey is called "inhibin", and hydrogen peroxide formed by glucose oxidase process has been identified as "inhibin" (White et al., 1963).

Manuka honey is a monofloral honey obtained from the manuka plant, *Leptospermum scoparium*, family *Myrtaceae*, which grows as a shrub or tree (2 to 4 m) in New Zealand and Eastern Australia. Some types of manuka honey naturally have a very stable and strong antibacterial activity that is not found in other types of honey. In all species at different levels, there is hydrogen peroxide, which is produced by one enzyme that bees add to nectar. In manuka honey there is a non-peroxide activity that originates from the nectar of the flowers of the manuka plant and it is completely different from the activity of hydrogen peroxide which is found at different levels in most types of honey (Molan, 2008). Non-peroxide activity of honey is stable, not lost by standing, has a strong effect in undiluted honey because it has greater potency of antibacterial action penetrating deeper into infected tissue, is always active, diffuses deeper into tissue and has a stronger effect on some pathogens (*Escherichia coli*, *Enterococci*, *Helicobacter pylori*).

The problem of resistance of microorganisms to antimicrobial drugs has increased the interest of the scientific public for manuka honey as an active substance, especially due to its antioxidant and antibacterial properties in wound treatment (Alvarez-Suarez et al., 2013; Alvarez-Suarez et al., 2014).

The mechanism of antibacterial activity of manuka honey is complex and has not been fully elucidated. It can be divided into two groups of mechanisms by which it has an antibacterial effect. The first group includes mechanisms of antibacterial activity based on its

physicochemical properties (osmolarity, viscosity, pH value, or acidity). The second group of mechanisms of antibacterial activity of honey is based on chemical compounds that are present in it (hydrogen peroxide, methylglyoxal and antimicrobial peptide bee defensin) (Molan, 1992).

A key factor in the antibacterial activity of manuka honey is methylglyoxal (MGO) (non-peroxide activity), present in high concentrations (38 mg/kg to 1541 mg/kg), and catalase from body fluids and serum does not degrade it. MGO is also present in honey derived from other honey plants, but its concentration did not exceed 24 mg/kg (Mavric et al., 2008; Kwakman et al., 2010). Non-peroxide activity also depends on the type of manuka plant from which honey originated and on the proportion of manuka nectar in honey. Other phytochemicals, especially phenols, are essential for antimicrobial activity (Atrott, 2013). The presence of MGO in manuka honey contributes to its uniqueness and is called Unique Manuca Factor (UMF®). The UMF label guarantees that the honey has been laboratory tested, its originality and origin. Also, the UMF label guarantees the presence of non-peroxide activity that is not found in any other type of honey. The higher the UMF, the higher the healing properties of manuka honey (e.g., UMF factor 5-9, UMF factor 10-14, UMF factor 15+).

The antibacterial activity of manuka honey has been confirmed in various pathogens, including those resistant to first-line antibacterial drugs (*Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhimurium, Proteus mirabilis, Shigella flexneri, Enterobacter cloacae, Salmonella enterica* serovar *Typhi, Campylobacter spp., Helicobacter pylori,Staphylococcus aureus, Streptococcus pyogenes,Methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus agalactiae, coagulase negative Staphylococcus aureus,* hemolytic streptococci, *Streptococcus mutans, Streptococcus sobrinus, Actinomyces viscosus, vancomycin-resistant enterococci* (VRE)etc.) as evidenced by numerous studies (Lin et al., 2017; White, 2016; Ahmed and Othman, 2013; Majtan et al., 2011; Lin et al., 2011; Visavadia et al., 2006; Sherlock et al., 2010).

In clinical examination honey has been shown to act on infected wounds that are resistant to conventional antibiotics and antiseptics. *In vitro* studies have shown that honey acts against multidrug-resistant microorganisms such as MRSA, VRE and other multidrug-resistant Gram-negative microorganisms, including *Pseudomonas aeruginosa (*George and Cutting, 2007).

Acacia honey is highly valued among consumers due to its properties and is one of the most valued types of honey that can be found on the European market (Persano Oddo and Piro, 2004). It is extremely light in color (almost colorless), has a faint odor and a pleasantly mild

taste. Due to the higher content of fructose in relation to glucose, it crystallizes slowly. The presence of even small amounts of foreign nectar or honeydew ingredients can impair the properties of acacia honey and make it less acceptable to consumers (Persano Oddo and Piro, 2004). This type of honey suits most people because its aroma is practically not felt in drinks. It is an excellent choice for people who are recovering from an illness. It is also used for problems of the cardiovascular system and constipation, it helps with insomnia, nervousness and tension.

The aim of this paper is to compare the antibacterial properties of domestic acacia honey with manuka honey, which has a standardized level of antibacterial activity and to determine the type of antibacterial activity.

Materials and Methods

In this study we used Manuka honey UMF 15+ originating from New Zealand, that was purchased in a commercial pharmacy Žužemberk (Grajski square 41 Žužemberk, Slovenia and acacia honey originating from Republic of Srpska (Bosnia and Herzegovina).

For testing the antimicrobial activity of manuka honey and acacia honey it was used clinically isolates bacteria *Streptococcus* group D, *Escherichia coli, Staphylococcus aureus, Salmonella enterica* i *Salmonella typhymurium* from collection of Public Institute Veterinary Institute Republic of Srpska "Dr. Vaso Butozan" Banja Luka and reference culture *Staphylococcus aureus* WDCM 00034. Bacterial cultures were prepared by incubating at 37°C in nutrient broth for 18 hours.

The agar diffusion method was used. Disks with a diameter of 9 mm were placed on Petri dishes with Müeller-Hinton agar, previously seeded with 100 µl of bacterial suspension with a concentration of 10^5 CFU/ml. The discs were filled with 100µl of honey. Thereafter, the Petri plates were refrigerated for 30 minutes to diffuse the sample into the medium and then incubated at 37°C for 24±3 hours. Three repetitions were performed for each honey sample. After incubation, the results were read by measuring the diameter of the inhibition zone and the mean and standard deviation were calculated.

To determine whether honey has a bactericidal or bacteriostatic effect, a small piece of agar was taken from the inhibition zone and added to the nutrient broth, which was then incubated at 37°C for 24±3 hours. If the broth was a blur after incubation, the effect of honey is considered to be bacteriostatic, ie if the broth has remained clear, the action of honey is bactericidal.

Results and Discussion

In addition to sensory and physicochemical properties antibacterial properties are one of the main characteristics of honey quality. The results of the antibacterial activity of manuka and acacia honey are presented in Table 1.

Table 1. Antibacterial action of manuka and acacia honey

Pathogenic bacteria	Inhibition zone in mm $\bar{x}\pm\delta$	
	Manuka honey	Acaciahoney
Streptococcus group D	37 ± 3.61	34 ± 1
Staphylococcus aureus	34 ± 3.61	32.67 ± 4.51
Escherichia coli	31.33 ± 2.31	33.67 ± 2.08
Salmonella enterica	30.33 ± 2.52	33.67 ± 1.16
Salmonella typhimurium	27.33 ± 4.62	31 ± 3.61
Staphylococcus aureus WDCM 00034	33.33 ± 1.53	37.67 ± 2.52

Both analyzed honey samples showed good antibacterial activity against the tested bacteria, and the zone of inhibition ranged from 27.33-37 mm for manuka honey, while for acacia honey it was 31-37.67 mm. Manuka honey showed a stronger effect than acacia honey on *Staphylococcus aureus* while acacia honey had a stronger antibacterial activity against other tested bacteria. Acacia honey had the weakest effect on *Staphylococcus aureus*, which is in accordance with the results Dugalić-Vrndić et al. (2005). Both honeys showed higher antibacterial activity against Gram-positive than against Gram-negative bacteria, which is in accordance with the results of Kalaba et al. (2020). The difference in sensitivity to honey and other antibacterial agents between Gram-positive and Gram-negative bacteria may be due to the structure of the cell wall. Gram-positive bacteria do not have an outer membrane that protects the peptidoglycan layer from Gram-negative bacteria, which facilitates the penetration of antibacterial agents and causes damage (Madigan et al.,2015).

The results of this research are in accordance with numerous studies in which the antibacterial activity of honey originating from different parts of the world has been examined and confirm the pronounced antibacterial activity against the tested pathogens(Linet al., 2017; Khalil, 2006; Alvarez-Suarez et al., 2013; Anthimidou and Mossialos, 2013). The results of this research confirm the thesis that the antibacterial activity of honey varies and that it depends on the botanical and geographical origin of honey. Laboratory research has shown that honey can slow the growth of bacteria such as *Escherichia coli* and *Salmonell* and successfully fights bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are common in hospitals, medical and veterinary clinics (Lu et al., 2013; Lu et al., 2014).

Differences in zones of inhibition may also be a consequence of the method used. The agar-diffusion method, which tests the susceptibility of *Staphylococcus aureus* to antibacterial drugs is currently the most commonly used method for assessing the antibacterial activity of honey. The limiting factor is that different types of bacteria are differently sensitive to different types of honey. In addition, the agar-diffusion method evaluates the activity of honey according to the size of the growth inhibition zone, which depends on the antibacterial activity, but also on the rate of diffusion of antibacterial components through the agar. The antibacterial activity of honey can be incorrectly defined as low, due to compounds of relatively high molecular weight whose diffusion through agar is limited (Kwakman et al., 2010; Kwakman et al., 2011; Allen et al., 1991). If the antibacterial activity of honey was tested by a standard method, then the inhibitory activity of different types of honey could be compared (Molan, 1992).

Due to the influence of various factors on the antibacterial properties of honey, it is not possible to accurately predict how much antibacterial activity a honey will have, so if honey is used for therapeutic purposes, it is necessary to pre-test the antibacterial properties (Gobin et al., 2014; Sherlock et al., 2010). The antibacterial activity of honey can differ significantly in different bacterial species, even among related bacteria, as in this case.

Honey inhibits bacterial growth and may have bactericidal or bacteriostatic activity.

Figure 1. shows the results of testing the type of antibacterial action of manuka and acacia honey.

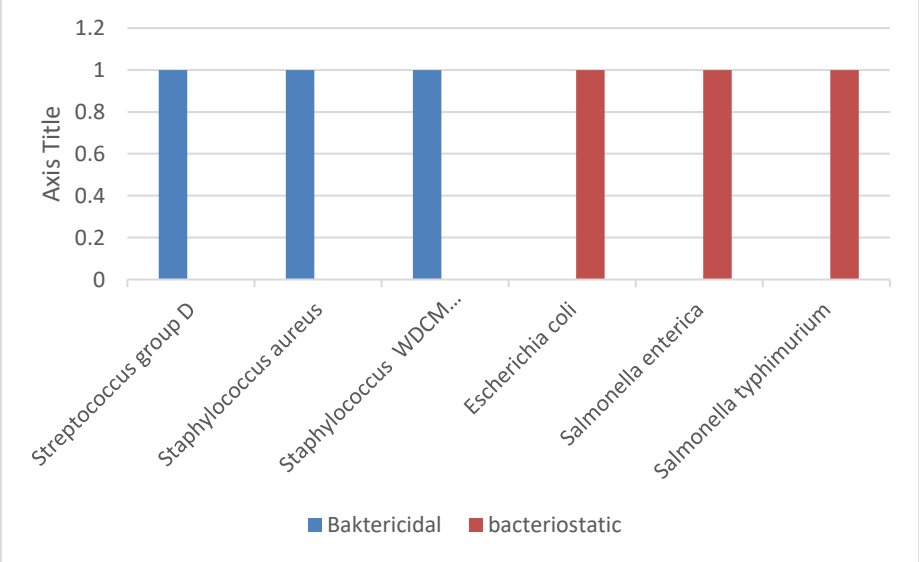

Figure 1. Type of antibacterial action of manuka and acacia honey

Both honeys showed a bactericidal effect on Gram-positive bacteria and a bacteriostatic effect on Gram-negative bacteria. The obtained results are in accordance with the results Kalaba et al. (2020) which were related with research of the antibacterial action of four types of homemade honey and Manuka honey.

Conclusion

The results showed that both analysed honey samples have good antibacterial activity against the tested pathogens with inhibition zones from 25.33 mm to 37.33 mm for manuka honey and 28.00 mm to 37.00 mm for acacia honey.

The study found that manuka and acacia honey show good antibacterial activity against clinical isolates of bacteria *Streptococcus* group D, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Salmonella typhymurium* and reference strain *Staphylococcus aureus* WDCM 00034 and acacia honey according to group D *Streptococcus* and *Escherichia coli*. Manuka and acacia honey showed higher antibacterial activity against Gram-positive than against Gram-negative bacteria, with bactericidal activity being slightly more pronounced in manuka honey.

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Review paper

Review of Geographical Indications schemes in South East Europe

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Abstract

The aim of this paper is to analyse the situation in the field of foodstuff geographical indications (GI) in South and Eastern Europe (SEE), the state of play related to the harmonization of the geographical indication with the EU acquis and to draw recommendations for future development of the geographical indication in South and Eastern Europe. Despite the large number of traditional products, suitable agroecological condition for production, this potential is not used so far. For small farms which is dominating in SEE, path for improved competitiveness is not in the production of high-yield production of average quality products, but rather in the added value products such as a GIs. The methodology used in this paper is mainly descriptive statistical and comparative analyse. According to the results of this paper, the main reasons for the underdevelopment of GIs in SEE are: in the unharmonized legal framework of most countries/territory with the EU, poorly developed system of producer organizations, lack of flexible registration environment for small processing capacities, lack of systemic GI support measures etc. The analysis showed that all countries/territory have established a legal framework related to GIs, but with the exception of Montenegro, these laws are not fully harmonized with the EU. The analysis showed that due to the poor visibility of these products, producers and consumers do not benefit much from previous GIs registered products. There is no single product from SEE registered in the EU. Recommendations for further development of GIs in SEE include: full harmonization of legislation with the EU,

[*] This designation is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ Opinion of the Kosovo declaration of indepedence.

introduction and support for the development of producer's organizations, introduction of flexible conditions for registration of small processing capacities, system support for GI producers and processors, support for registration of national GIs at EU level.

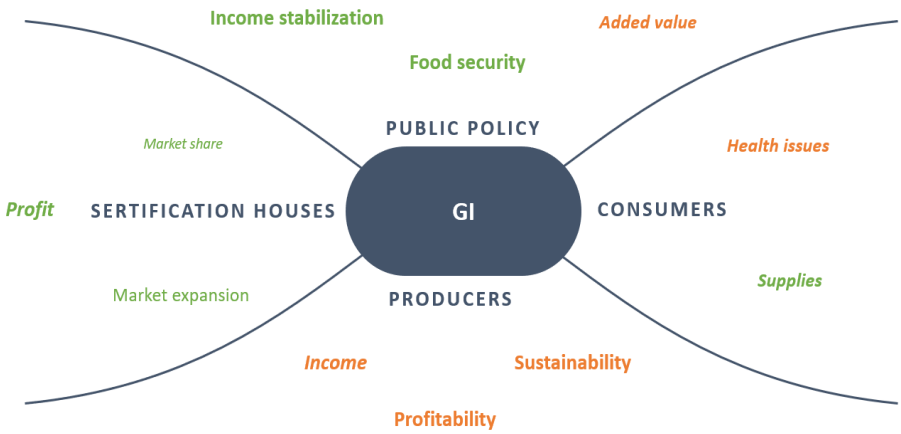
Key words: Geographical indication, Protected Designation of Origin, Protected Geographical Indication, Traditional Specialties Guaranteed

Introduction

Foodstuff quality schemes aim to provide consumers with proof that a product has been produced according to a certain methodology, in a certain area or that it contains certain characteristics. Thus, geographical indications also aim to indicate traditional products that are produced/processed in a certain area or, in the case of TSG, produced according to a traditional recipe.

A number of researches indicate how GIs importance for the agricultural sectors (Arfini et al., 2019; Barjolle, 2010; Gracia et al., 2007; Raimondi et al. 2018; FAO, 2008; WIPO 2018, Paraušić et al., 2020; Kljajić aet al., 2013; Janković et al., 2018; Popović et al., 2018).

According to the above, GIs are important for farmers, consumers and other students in the GIs system (Scheme 1).



Scheme 1. Importance of GIs for different stakeholders

Source: Authors'

The following countries/territories are analysed in this paper: Albania, Bosnia and Herzegovina, Kosovo*, North Macedonia, Montenegro, and Serbia.

The paper has three main objectives:

1) To determine the current situation regarding foodstuff GIs in the specified SEE countries / territories.
2) To determine the degree of harmonization achieved in this advertisement with the EU.
3) To identify obstacles and provide recommendations for further development of GIs in SEE.

Three main GIs schemes are prescribed in the EU: PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Specialty Guaranteed). In addition to the above, there are also optional GIs schemes.

For all SEE countries, the legal framework related to the EU in GIs is important for two reasons, firstly in the process of harmonization with the EU acquis they are obliged to harmonize with this area and secondly harmonization with the EU legal framework enables protection of national GIS products at EU level. Currently there is no GIs products from SEE registered in the EU.

GIs legal framework in EU

- Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs;
- Regulation (EC) No 1308/2013 on the protection of geographical indications for wine;
- Regulation (EC) No 251/2014 on the protection of geographical indications for aromatised wine;
- Regulation (EC) No 787/2019 on the protection of geographical indications for spirit drinks;

SEE countries are rich in high quality traditional products with high market potential. Main reason for GIs potential are not used in full scale is in the inadequate legal framework, orientation to the national GIs product registration with poor visibility instead of EU registration, lack of necessary infrastructure for GIs development.

On the contrary EU has well established legal framework with common EU registration and GIs labels.

Why GIs are important for SEE?

1) A large number of traditional products and good production conditions.
2) In all SEE countries process of depopulation of rural areas is present, so the special importance of GIs in improving the profitability of agricultural production on small farms and population retention in rural areas

3) Small size farms in SEE are usually unable to compete in economies of scale, so its completeness should be based on "value-added" products, such as GI.

Material and Methods

The methodology used in this paper is consist on:

- Desk research;
- Literature rewove,
- Comparative analyse.

Main data sources are national statistics databases and ministries of agriculture's data.

Results and Discussion

All analysed SEE countries have an established legal framework regarding foodstuff GIs. Table 1 presents the legal framework, the competent institution and the degree of harmonization with the EU legal framework.

Table 1. Legal framework in 2020, competent institution and degree of harmonization with the EU legal framework in SEE

Country/ territory	Adopted laws GIs quality schemes	Compliance with EU acquis	Competent Authority
ALB	Law on the quality scheme of agricultural and foodstuff (OG 8/2019)	In line, should be fully harmonized	Ministry of Agriculture and Rural Development
BIH	Rulebook on quality systems for food products (OG 90/18)	Partially	Food Safety Agency of BiH
KOS*	Law on Geographical Indications and Designations of Origin set out the rules of the GI protection in general (OG 05/L-051) in force	Partially	Intellectual Property Agency and the Ministry of Trade and Industry
MNE	Law on quality schemes of agricultural and foodstuffs (OG 01-347/2)	Fully harmonized	Ministry of Agriculture and Rural Development
MKD	Law on the Quality of Agricultural Products (140/2010, 53/2011, 55/2012, 106/2013, 116/2015, 149/2015; 193/2015)	Partially	Ministry of Agriculture, Forestry and Water Economy
SER	Law on Indications of Geographical Origin (Official Gazette of RS, no. 18/2010).	Not harmonized	Intellectual Property Office with support of the Ministry of Agriculture, Forestry and water Management

Source: Authors'

In this part, the situation in the field of GIs in each of the SEE countries is analysed separately.

Geographical indications schemes in Serbia

Agriculture represents one of most important sectors in Serbian economy.

Serbian legal framework is not harmonized with EU. Main unconformity lie in the institutional framework where Intellectual property office is in the charge for foodstuff GIs supported by the MAFWM. Second unconformity is found in fact that party not involving in the production of GIs can fill application for recognition (i.e. chamber of commerce, local municipalities etc.), while EU rules allowing only group of producers which are producing that traditional products to apply for recognition. Third there is no proper objection procedure within the registration of GIs products.

Regarding the labelling of the GIs foodstuff in Serbia, self-adhesive stamps made by the National Bank of Serbia are used. This way of marking has proven to be less efficient since it requires additional costs for producers, stamps are not suitable for gluing on individual packages, etc.

PDO PGI

Picture 1. PDO and PGI self-abrasive labels

Currently there is 36 registered PDO products and 10 PGI. Most of the products are without users.

Geographical indications schemes in Bosnia and Herzegovina

BIH made significant progress in GIs since 2018 with adoption of the Rulebook on quality systems for food products (OG 90/18) creating legal framework almost fully aligned with EU.

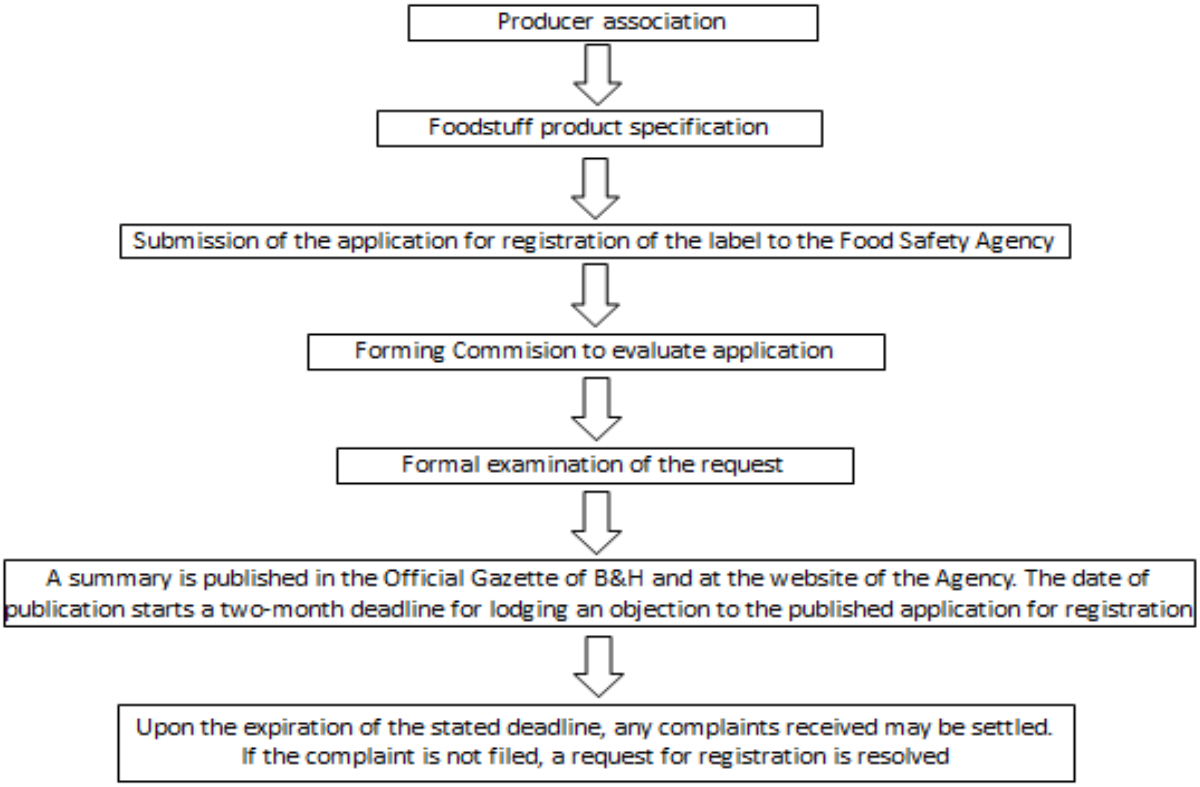
Picture 2. GIs labels in BIH

Three areas have been identified that are not fully in line with the acquis (SWG, 2020):

1) GIs quality system for agricultural products is not established.

2) Optional quality schemes are not established.
3) If the original name is in other alphabet than Latin, it will need to be prescribe to have Latin translation.

BIH has GIs registration procedure in line with EU (Scheme 2).



Scheme 2. GIs registration process in BIH

Source: SWG, 2020

Four GIs products registered in BIH and one in the procedure.

Geographical indications schemes in Montenegro

Only fully harmonized legal framework with EU is found in Montenegro.

Legal framework governing GIs is:

- Law on quality schemes of agricultural and foodstuffs (Official Gazette of Montenegro, No 01-347/2);

Beside PDO; PGI and TSG, Montenegro introduced optional quality marks: Higher quality, Mountain product; From my farm.

Picture 3. GIs labels in Montenegro

Registration procedure is in accordance with EU.

Registration procedure is as follow:

- GIs registration is in the competence of responsibility of the Ministry of Agriculture and Rural Development.
- The GIs registration application can be submitted only with producer's association.
- First step is to submit product specification to the Ministry of Agriculture and Rural Development.
- The Ministry of Agriculture and Rural Development is appointing Commission for application examination.
- In the case of Commission' positive decision next step I public announcement and possibility for objecting the application.

Seven GIs products are registered so far.

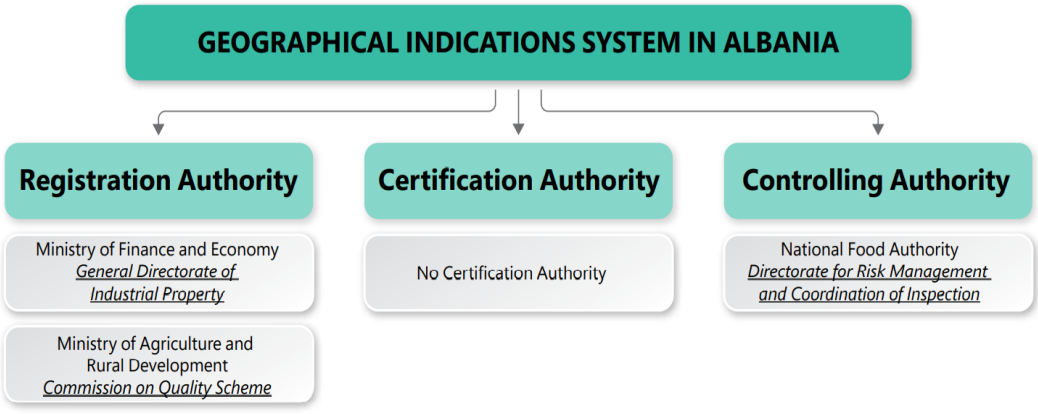
Geographical indications schemes in Albania

Albania has established legal framework allowing PDO, PGI and TSG GIs marks. So far there are 22 registered products.

Picture 4. GIs labels in Albania
Source: Ministry for Europe and foreign affairs Republic of Albania (2019)

As other SEE countries/territory Albania has large number of well-known traditional foodstuff, low farmland and excellent conditions for GIs development. As in the other analysed countries Albanian' farmers are not using GIs potential. Main reasons are in not harmonized legal framework with EU Acquis, poor GIs visibility, absence of the flexible procedures for registration of the small foodstuff processing capacities.

At the scheme 3 is presented GIs products registration procedure in Albania. It can be concluded that process is not aligned with EU, and certification authorities are not appointed.

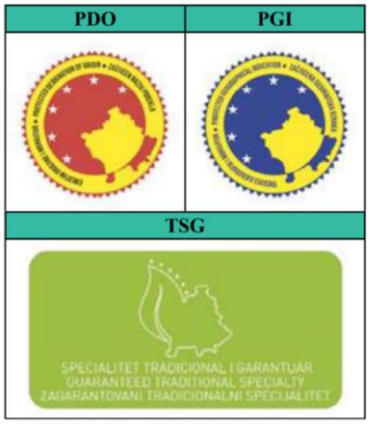


Scheme 3. GIs registration process in Albania
Source: SWG, 2020

GIs products visibility is poor. The website of the Ministry of Agriculture and Rural Development needs to be updated with GIs products specifications.

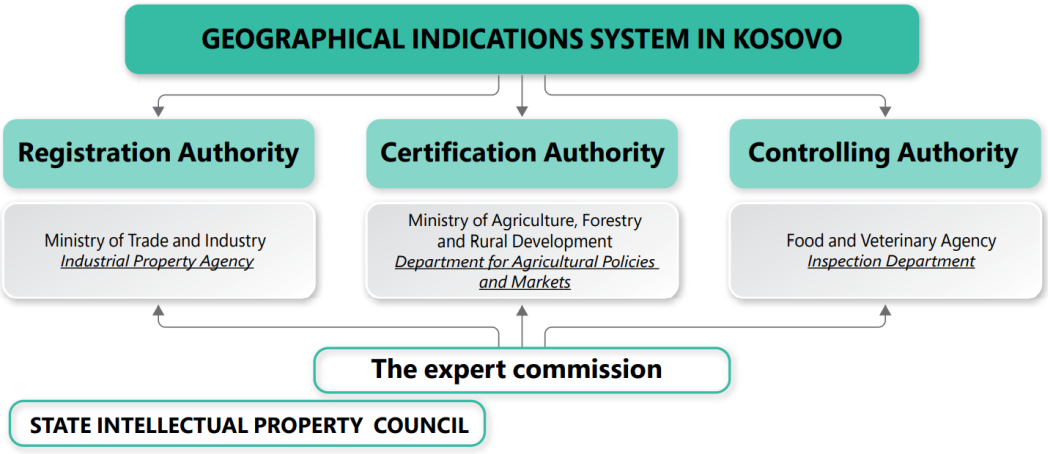
Geographical indications schemes in Kosovo*

Kosovo* has established legal framework allowing PDO, PGI and TSG GIs marks. There are no registered products but Sharri Cheese as PDO and Rahoveci Wine as PGI in the registration procedure.



Picture 5. GIs labels in Kosovo*
Source: SWG, 2020

At the scheme 4 is presented GIs products registration procedure in Kosovo*. It can be concluded that process is not aligned with EU, and certification authorities are not appointed. There are multiple institutions in charge of GIs which is proven not to be efficient institutional framework.



Scheme 4. GIs registration process in Kosovo*
Source: SWG, 2020

Geographical indications schemes in North Macedonia

North Macedonia has established legal framework allowing PDO, PGI and TSG GIs marksThere is one PDO registered - Ohrid cherry.

Picture 6. GIs labels in North Macedonia
Source: SWG, 2020

North Macedonian GIs registration procedure is harmonized with EU. Only group of farmers who producing GIs product is eligible to initiate registration procedure, there is proper objection procedure etc. (Scheme 5).

REGISTRATION PROCEDURE
Association or group of farmers decide to protect product (legal entity)
Elaborate or specification of the product
Single document (for PDO and PGI)
Fill the Application form
Request with the required documentation submit to MAFWE (name and address of the association, identification number, representative of the group etc.)
MAFWE-nominate a Commission for control of the delivered documentation (experts from the field +administration)
if is positive opinion/ publish on the web side of MAFWE/ 6 months
If there is no objection
Product is imported in the Register of PDO. PGI or TSG
All producers should be registered individually
Producers are officially imported into the Register of Producers Each producer to be entered in the registry should have undergone the procedure for verification of compliance with the elaborate and / or the specification, according to a predefined control plan.
Regular control of the product and manufacturing process by competent bodies
Announcement when Producers started with production of the protected product (determination of the conformity of production, processing and the product with the specification)

Scheme 5. GIs registration process in North Macedonia

Source: SWG, 2020

Obstacle in the implementation and control of the GIs is in the lack of the interest from the certification bodies to take part in the GIs due to the small market, so Ministry of agriculture was forced to take that obligation.

North Macedonia made significant progress in harmonizing legal framework with EU, but there is some minor unconformities i.e.:

- Optional quality terms are not in compliance with EU.
- Product specification is missing in certain provisions.
- Animal products are lacking in provision on quality of feed;
- Traceability the product is not aligned fully with EU.
- Some provisions regarding GIs control are not prescribed.

Obstacles to the development of geographical indications in SEE

The most important question within this paper is if the conditions are good for GIs in SEE countries why this foodstuff quality scheme is not developed and what are the limitations.

According to the analyses all SEE countries/territory are facing similar challenges.

Those are most important limitation which need to be addressed in order to develop GIS in SEE:

1) Inconsistency of the institutional and legislative framework with the EU;
2) Difficult registration of small processing capacities;
3) Small number and low activity of interest associations of agricultural producers (PO and PG have not been established);
4) Absence of systemic long-term support measures;
5) Poor “visibility” of GI products;
6) Poor information of producers / consumers;
7) Weak connection of products with geographical indication with rural tourism;
8) Lack of cooperation in the registration of cross-border GI products;

Conclusion

According to the results of this paper, the main reasons for the underdevelopment of GIs in SEE are: in the unharmonized legal framework of most countries/territory with the EU, poorly developed system of producer organizations, lack of flexible registration environment for small processing capacities, lack of systemic GI support measures etc. The analysis showed that all countries/territory have established a legal framework related to GIs, but with the exception of Montenegro, these laws are not fully harmonized with the EU. The analysis showed that due

to the poor visibility of these products, producers and consumers do not benefit much from previous GIs registered products. There is no single product from SEE registered in the EU. Main recommendations for development of the GIs in SEE are:

1) Harmonization of the Institutional and Legislative Framework with the EU;
2) Flexibility and deviations from food safety requirements for small processing facilities;
3) Introduction of long-term systemic support measures;
4) Establishment and support of producer organizations and producer groups;
5) Assistance to producers in obtaining EU registration;
6) Initiatives for cross-border registration of geographical indications;
7) Connecting GIs foodstuff with rural tourism;
8) Establishment of regular annual meetings of agricultural policy makers from WB countries related to public quality standards (geographical origin and organic agriculture);

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Original scientific paper

Change of family farm production type in terms of increasing economic business results

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Abstract

Agricultural producers are in a constant dilemma of how to respond to modern economy challenges without compromising their businesses and consequently their own existence. Also, unstable market and economic conditions are the main cause of why farmers hesitate to make any large investments. Considering the aim of this research, family farm model was used to optimize sowing structure and test the possibilities of introducing new lines of production, to diversify production and provide better economic results, without investing in new fixed assets. The results obtained in this work show that the change of production type (introducing vegetable production) would ensure a increasing of gross margin for almost 200%. In addition, the change of production type would also lead to an increased engagement of permanently employed workers by more than 3.5 times and mechanization for about 20%.

Key words: production type, family farms, economic results

Introduction

The most of crop production in the Republic of Serbia is realized on family farms of AP Vojvodina (Munćan et al. 2010; Todorović, 2018). High prevalence of small family farms, low level of agricultural technology utilization, outdated mechanization and a negligible percentage of irrigated areas have a strong impact on the economic results of these farms (Munćan, 2016). However, numerous studies have shown that there are large time reserves in the engagement of labor and mechanization, so going further with production rationalization, should partially neutralized unfavorable economic indicators.

Therefore, based on data obtained from family farm located in the territory of South Banat, a study was conducted to examine the possibility of increasing the economic business results by partially changing production type. The starting point was the assumption that changing production type, by including the production of vegetables, would improve the economic business results of the observed family farm. Production of vegetables, as one of the most intensive branches of plant production, was introduced into the production structure, because it enabled more rational and intensive use of land, greater engagement of labor and mechanization, and it contributed to increase of economic efficiency due to high yields and high product prices. Farmers can sell their vegetables through different marketing channels ranging from the farm gate, restaurants, wholesalers and supermarkets. The choice of an outlet may depend among other things on the agro-ecological location of the channels and whether the farmer is able to meet market requirements such as quality, safety standards and consistent supply (Masuku and Xaba, 2013). With other branches of agriculture (field farming, fruit farming, viticulture) and group of extractive food industry (industry for production of sugar, flour, vegetable oils, vegetable fat, etc.), vegetable industry belongs to the basic branches of food industry and has the status of inevitable segment of the overall food economy (Puškarić and Jovanović, 2020).

The method of linear programming was used to optimize current structure of crop production, as well as the suggested crop and vegetable production.

The obtained optimization results showed that vegetable production conjoined with crop production could significantly increase the economic results of family farms. Also, in accordance with market requirements, suggested production rationalization could represent development direction for small family farms.

Matherial and Method

The main goal of the conducted research was to explore opportunities for increase of economic results through change of production type, based on data obtained from a family farm with 14.1 hectares of arable land located in the territory of South Banat.

Considering the research was conducted on a family farm, as a basic organizational unit in agricultural production, which does not allow experimenting with, the modeling method was used to carry out this research.

The use of model, as basic research method, provides a solution for organizing production, as the most demanding problem in agriculture, considering all the potential advantages and

disadvantages of a certain production system (Krstić, Smiljić, 2003). The mathematical method used to experiment with defined models was the method of linear programming. Based on available labor and mechanization, and in accordance with agro-technical norms and restrictions, the optimization of the production structure was performed by applying this method before and after the change of production type.

In line with the aim of the research, two models have been defined to optimize the production structure. The first model was used to optimize the existing structure of crop production, which included only three crops. The second model was used to optimize crop and vegetable production, since it included two lines of vegetable production in addition to three already existing lines of crop production.

The models were designed so that the constraints relate to the size of the arable land, crop rotation conditions, available labor and mechanization capacities. Gross margin was used as a linear function to be maximized.

Gross margin represents one of the most suitable economic business indicators for family farms, because it determines how much farmers gain or lose by investing funds (Ivkov et al., 2008). In addition, the gross margin provides the base for continuous business monitoring, product competitiveness improvement and profitability increase, for each line of production, as well as total farm production. Therefore, as an indicator of profitability, the higher the gross margin, the more efficient a given operation is in generating profit from operating costs involved in production (Wei at al., 2020). Accordingly, the gross margin is a good starting point for quality planning (Pejanović, 2009).

By using the gross margin as the optimality criterion to be maximized, the negative impact of fixed costs distribution on the assumed production activities, that may cause some incorrect solutions, is eliminated (Novković et al., 2008)

Results and Discussion

One of the main goals of this research was to determine the optimal production structure on family farms that provides complete use of all available resources in order to achieve maximal financial results, with regard to numerous biotechnical, technical, technological, production and market constraints. Based on data collected through the survey, concerning the conditions and results of production, family farm model, with crop production solely, was defined. The design of the first model was based on the following indicators:

- farm size is 14.1 hectares of arable land,

• one active member is permanently engaged on the farm, while another active member is engaged in daily operations to a maximum of 50% of working hours (partially employed outside the farm),

• the farm has one tractor and all necessary farm machines, implements and tools for crop production,

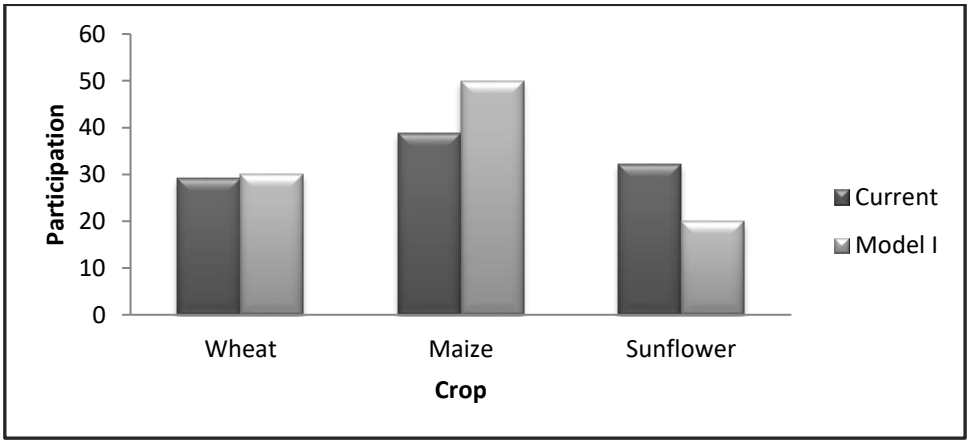
• during the harvest, the services of a combine are engaged,

• farm production is organized entirely on their own land,

• three crops are represented in the sowing structure: corn on average 38.7%, wheat on 29.1% and sunflower on 32.2% of arable land,

• the average total gross margin is 808,027.35 RSD.

Relying on the defined assumptions, first model was constructed and an optimal solution for the sowing structure in crop production was obtained (Graph 1).



Graph 1. Changes in sowing structure

Source: Created by the authors based on results of the optimization

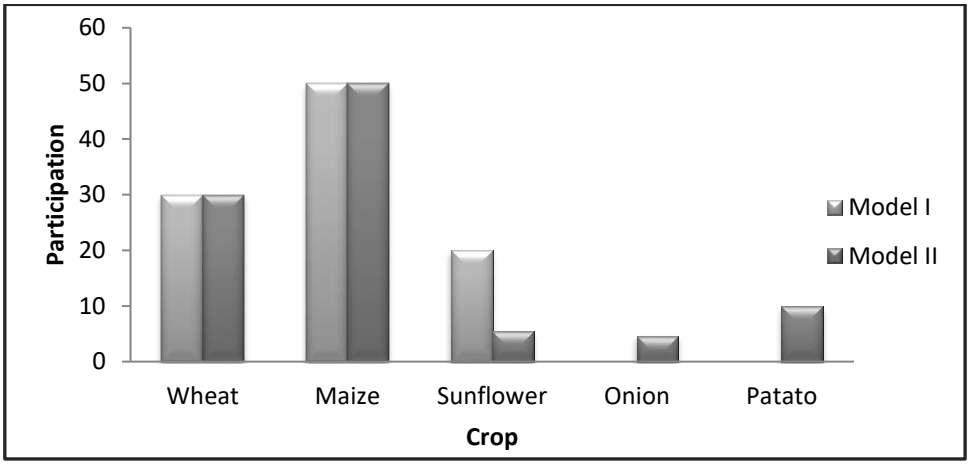
The results obtained by optimizing the production structure in the first model (solely crop production) have showed following optimal sowing structure: 30% of arable land under wheat, 50% under corn and 20% under sunflower. Optimization of sowing structure indicates an increase of areas under corn and wheat, and reduction of areas under sunflower.

Obtained sowing structure anticipates an increase in farm's total gross margin by 17,045.37 RSD, or about 2% more than current total gross margin. Also, acquired sowing structure would not lead to major changes in distribution of time used for working process, nor the appearance of labor peaks, so it could be concluded that even after optimizing the sowing structure, significant time reserves of labor and mechanization usage remain.

Therefore, another model was constructed to examine the possibilities of increasing economic results by changing production type, through the inclusion of vegetable production in the sowing structure, starting from previously defined assumptions.

The second model implies additional activities, concerning lines of vegetable production (onion and potato), anticipating maximal share in sowing structure, in terms of more rational utilization of available labor and mechanization capacities, as well as business results improvement.

While defining the second model, it was assumed that the lines of vegetable production would have the same prospects as lines of crop production for being included in crop rotation, no side production would be organized, and production technology would be harmonized with agroecological conditions in farm's environment and with available mechanization and labor capacities.



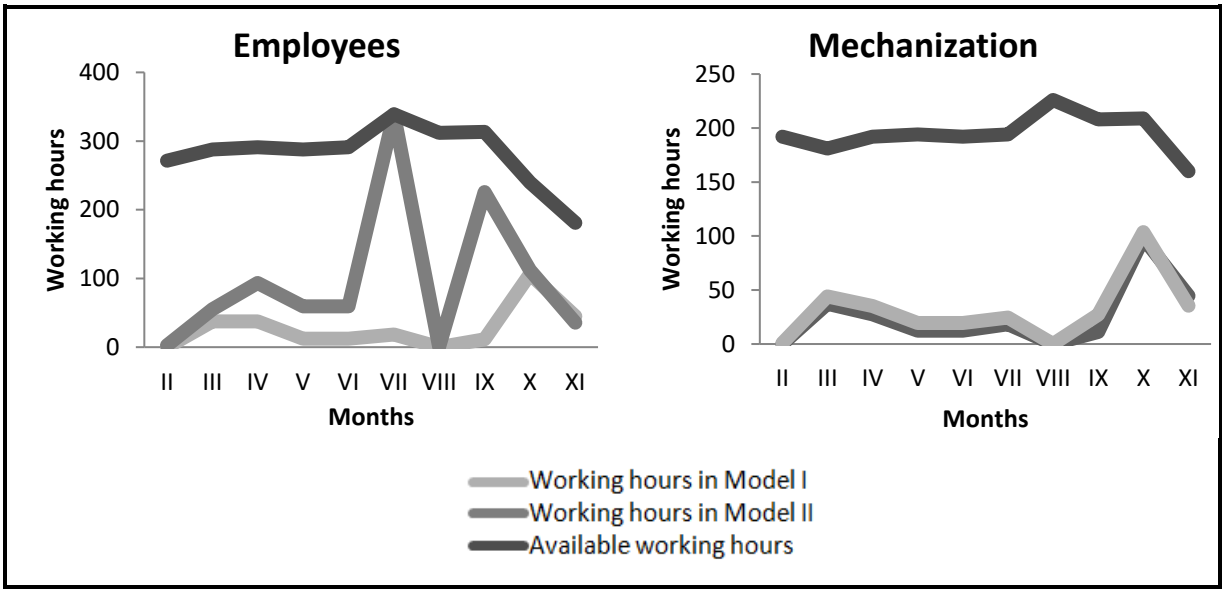
Graph 2. Sowing structure after the change of production type

Source: Created by the authors based on results of the optimization

The results obtained by optimizing the second model (Graph 2), showed that all lines of production are represented in the sowing structure. It can also be concluded that vegetable production entered the sowing structure at the expense of areas under sunflower, while wheat and corn are represented in the same percentage as in the first optimal solution.

From the economic and organizational aspects, the introduction of vegetable production in the sowing structure would mean a significant increase in gross margin. Therefore, the total gross margin would increase by 841,871.48 RSD, which is twice as much as the gross margin obtained by optimizing solely crop production. Consequently, the inclusion of vegetable production in the optimal solution would cause significant changes in distribution of time used for labor and mechanization engagement (Chart 3).

Analyzing available funds of working hours has special importance in terms of the degree of their utilization, concerning employees and certain categories of mechanization, because it implies to possibility of performing planned operations in optimal timeframe (Novković et al., 2008).



Graph 3. Changes in working hours of employees and mechanization after the change in direction of production

Source: Created by the authors based on results of the optimization

The change of the production type on the observed family farm would cause the percentage of labor utilization to increase notably, with appearance of labor peaks in July. In the first model, total available labor utilization was slightly less than 10%, while in the second model it has reached almost 35%.

On the other hand, mechanization capacity still wasn't fully used, so that the degree of utilization concerning mechanization ranged from 13%, in the first, to 16% in the second model.

By comparing the results obtained by optimizing the first and second models, it was established that the change of production type would ensure a significantly higher gross margin, both in total and per ha. In addition, the change of production type would also lead to an increased engagement of permanently employed workers by more than 3.5 times and mechanization for about 20%.

However, productivity per working hour of employee would notably decrease, so that the gross margin per working hour would be reduced by almost 45%.

Conclusion

Based on formulated mathematical models and defined optimality criteria, solutions were obtained that indicate significant opportunities to increase the economic results of the family farm by changing production type. Results gained through solving formulated models implicate the following conclusions:

• Family farms have sufficient funds concerning working hours of mechanization and active members permanently employed in agriculture. Therefore, the stability of the solutions obtained in the optimization process would not be jeopardized.

• By changing production type, the percentage of labor and mechanization utilization would increase by almost 25% and 20%, respectively.

• Including new lines of vegetable production into the sowing structure (Model II) would ensure that the total gross margin doubles compared to the one obtained in optimal solution for the model with solely crop production, although its amount per working hour of employee would be considerably reduced.

Reviewing results obtained in the conducted research, the question of the motive for the transition to combined crop and vegetable production arises. However, the significant increase in farm's gross margin, accompanied by the growth of its economic power, certainly exceeds the intensified engagement of labor in the production process.

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Promena smera proizvodnje porodičnih gazdinstava u funkciji povećanja ekonomskih rezultata poslovanja[1]

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Sažetak

Poljoprivredni proizvođači su u konstantnoj dilemi kako da odgovore na izazove savremenog privređivanja, a da pri tome ne ugoze sopstvenu egzistenciju i poslovanje. Takođe, nestabilni tržišni i ekonomski uslovi su osnovni razlog zbog čega se gazdinstva ne usuđuju na velike investicije. Imajući to u vidu, cilj ovog istraživanja je da se, koristeći model porodičnog gazdinstva, izvrši optimiranje strukture setve i provere mogućnosti uvođenja novih linija proizvodnje, koje bi trebalo da diverzifikuju proizvodnju i obezbede bolje ekonomske rezultate, bez ulaganja u nova osnovna sredstva.

Rezultati dobijeni u ovom radu pokazuju da bi promena vrste proizvodnje (uvodjenje povrtarske proizvodnje) obezbedila povećanje bruto marže za skoro 200%. Pored toga, promena tipa proizvodnje takođe bi dovela do povećanog angažovanja stalno zaposlenih radnika za više od 3,5 puta I mehanizacije za oko 20%.

Ključne riječi: smer proizvodnje, porodična gazdinstva, ekonomski rezultati

[1] Rad nastao kao rezultat istraživanja u okviru "Ugovora o realizaciji i finansiranju naučnoistraživačkog rada u 2021. godini između Poljoprvrednog fakulteta u Beogradu i Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije", evidencioni broj ugovora: 451-03-9/2021-14/ 200116

Original scientific paper

Financial aspects of potato production on farms in the Republic of Serbia

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Abstract

Vegetable production in the Republic of Serbia is performed on about 130,000 hectares that is about 3.5% of total plant production. The most common vegetable in Serbia is potato, which is grown on about 30,000 hectares. The goal of the paper is to show the financial aspects of potato production on farms in Republic of Serbia in the 2015-2019 period by utilization of calculation based on variable costs. The data for analysis have been collected on 323 farms (survey done on selected farms conducted by the Institute for Science Application in Agriculture - IPN). By utilization of sensitivity analysis it has been also shown in the paper the impact of prices and yields on amount of gross margin in potato production. The obtained results indicate a constant increase of the gross margin amount in the potato production in the analyzed period, as well as that changes in prices and yields have a significant impact on the gross margin in potato production.

Key words: gross margin, potato, farms, Serbia

Introduction

Vegetable production is present in almost all regions of the Republic of Serbia, with numerous vegetable crops that are grown. Production is performed both in the field and in the green houses, depending on the conditions of production, with the field production being more prevalent (Ljiljanić and Rajić, 2018). Vegetable production is a highly intensive and profitable branch of agricultural production, so it can have a significant impact on development of agricultural sector, but this production also significantly depends on the degree of overall economic development (Paunović, 2016). Potato is a vegetable crop that is traditionally used in the Republic of Serbia in the preparation of national dishes, and therefore it is significantly

used in the nutrition (Ilin et al., 2014). This is confirmed by the fact that potatoes are vegetables that are grown on the largest areas in Serbia. Potatoes are grown on 30,000 hectares, with average yields of about 17 t/ha (Statistical Office of the Republic of Serbia). Significant part of the total potato production in Serbia is realized in the regions of Šumadija and Western Serbia, as well as in the region of Southern and Eastern Serbia, since those regions have the most favourable conditions for the potato growing. However, it has to be noted that the highest average yields have been achieved in the territory of Vojvodina, where mainly industrial potatoes are produced (Vlahović, 2015). The total yields of potatoes varied in the analyzed 2015–2019 period and amounted to maximum 700,000 tons (Statistical Office of the Republic of Serbia).

Matherial and Method

The methods used in the analysis presented in the paper are the following: survey, the direct costing calculation, desk research and descriptive method. Financial performance indicators are universal measures of the profitability in any production. In agricultural production, financial performance indicators are most often calculated by using the data from the analytical calculation of the full cost price or by direct costing calculation. For the family farms, which are the subject of the present analysis, the direct costing calculations based on variable costs are most often used to determine the optimal volume and structure of production, while indicators obtained from this calculation can be used as a good financial instrument for business decisions (Gogić, 2014). The direct costing calculation determines the following basic financial indicators of production: production value, variable costs and gross margin. The general formula for calculating gross margin is as follows:

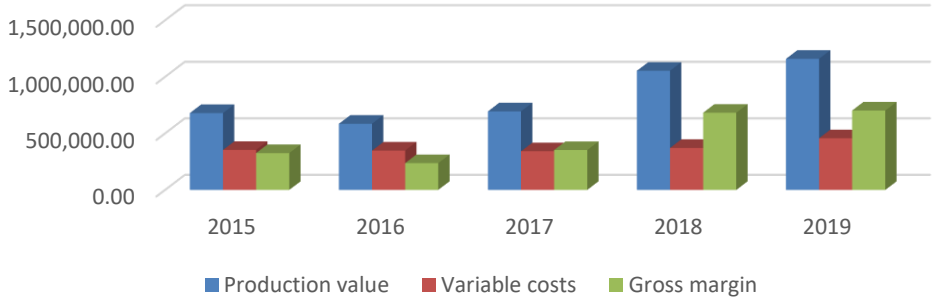
$$PV - VC = GM$$

PV - production value VC – variable costs GM – gross margin

Operating performance is expressed through gross margin, which represents the difference between the production value and variable costs (Savić et al., 2020). Calculation of gross margin represents the fast and efficient way of calculating an indicator used for comparing different production lines on a family farm and for selection of the most profitable one (Andrić, 1998; Tomić et al., 2013). The aim of this paper is to present financial indicators in potato production in Serbia by using the method of direct cost calculation.

Results and Discussion

On the basis of direct cost calculations there have been calculated the average annual amounts of production value, variable costs and gross margin in the potato production on family farms in the Republic of Serbia for the analyzed period (2015-2019).



Source: Authors' calculation based on IPN survey data

Figure 1. Financial indicators of potato production on family farms in Serbia for the 2015-2019 period (RSD[1]/ha)

Figure 1 presents the dynamics of potato production financial indicators in the 2015-2019 period, based on direct costing calculation and calculated for 1 ha area. It can be concluded that the highest values of all financial indicators were recorded in 2019. The lowest production value and gross margin were recorded in 2016, while variable costs were the lowest in 2017.

Table 1: Financial indicators, base and chain indices in the potato production for the 2015-2019 period (calculated for 1 ha)

Indicator	2015	2016	2017	2018	2019
Production value (RSD)	679,536.42	584,904.26	694,720.93	1,053,519.23	1,159,060.56
BI* (%)	-	86.07	102.23	155.03	170.57
CI** (%)	-	86.07	118.78	151.65	110.02
Variable costs (RSD)	353,757.43	347,986.69	342,122.91	371,163.02	456,943.02
BI* (%)	-	98.37	96.71	104.92	129.17
CI** (%)	-	98.37	98.31	108.49	123.11
Gross margin (RSD)	325,778.99	236,917.57	352,598.02	682,356.21	702,117.54
BI* (%)	-	72.72	108.23	209.45	215.52
CI** (%)	-	72.72	148.83	193.52	102.90
Share of the gross margin in the production value (%)	47.94	40.51	50.75	64.77	60.58

[1] RSD - Republic of Serbia Dinar
*BI – base indices
**CI – chain indices
Source: Authors' calculation based on IPN survey data

In Table 1 there are shown the most significant financial indicators in potato production for the 2015-2019. period, obtained from the direct costing calculation (calculated for 1 ha area). The

oscillations in the value of production by individual analyzed years occured due to changes in the average yield and price of potatoes. The value of potato production varied significantly in the analyzed period. The lowest production value was recorded in 2016, when it amounted to 584,904.26 RSD/ha, while it was the highest in 2019 (1,159,060.56 RSD/ha). Observing the base indices, it can be concluded that in 2018 production value increased by 55.03% compared to the 2015 base year. In 2019 production value increased by 70.57% compared to the base year and this was the highest production value achieved in the analysed period. Indicators obtained on the basis of chain indices show that in 2016, compared to 2015, there was a decrease in the production value by 13.93%, while in other analyzed years a continuous increase was recorded. The largest increase between the analyzed years was in 2018, when the value of potato production was by 51.65% higher compared to 2017.

In contrast to the amount of production value, variable costs did not have significant changes, except in 2019, when they were slightly higher as compared to previous years. In 2019, the variable costs of potato production were 456,943.02 RSD/ha. In other analyzed years, the variable costs amounted to about 350,000 RSD/ha, as also indicated by the base and chain indices.

Taken into account that the amounts of variable costs had smaller changes in analyzed years, it can be concluded that the gross margin was mostly influenced by the value of production, more precisely by the yields and prices of potatoes. The obtained results confirm this fact, so in 2019 the largest amount of gross margin was recorded (702,117.54 RSD/ha). The base indices show that the gross margin in 2019 was by 115.52% higher compared to the indices in the base year (2015). The chain indices show that the largest changes in analyzed period were recorded in 2017 and 2018. In 2017 the gross margin was by 48.83% higher compared to 2016, while in 2018 an increase was 93.52% compared to 2017.

An indicator that can represent the change in profitability of the family farm is the share of gross margin in the production value. Depending on how the value of gross margin moves, and thus the value of production and variable costs, this indicator also changes. The higher this indicator, the lower the share of production costs, which should be the goal of every family farm. Based on the obtained financial indicators of potato production on the family farms in the Republic of Serbia for the 2015-2019 period, it can be concluded that this indicator was the highest in 2018, when the gross margin was 64.77% of the value of production. In 2018, this indicator was higher than in 2019, although in 2019 the highest amount of gross margin and production value was achieved, but the variable costs were also higher and they had a larger

share in the production value. The smallest share of gross margin in the production value was recorded in 2016, i.e. it amounted to 40.51% of production value.

One of the elements that affect the value of production, and thus the gross margin of potatoes as well, is the selling price of potatoes. In the analyzed period, the selling price of potatoes realized by the surveyed farms, ranged from 22.44 RSD/kg to 33.93 RSD/kg. The lowest price was in 2016, while the highest one was in 2019. In 2018, the price was close to the price in 2019 (33.24 RSD/ kg), in 2015 it was 27.32 RSD/kg, and in 2017 it was 26.26 RSD/kg. Comparing the average annual prices of potato with the average amount of gross margins, it can be concluded that the highest gross margins were recorded in the years when the price of potatoes was the highest.

Since it was determined that the yields and prices have the significant impact on the value of the gross margin in potato production, the sensitivity analysis has been done. The sensitivity analysis shows how the gross margin of potato production varies due to changes in the factors that influence the value of production. It was analyzed how changes in prices and yields affect the changes of the gross margin. The sensitivity analysis took into account the absolute changes in prices and yields, when the specified parameters change by 10% and 20% respectively.

Table 2 shows the sensitivity analysis of the gross margin in potato production for the 2015-2019 period, due to the changes in prices and yields, based on the five-year average prices, yields and gross margins achieved on the surveyed farms.

Table 2. Sensitivity analysis of the potato production gross margin to the price and yield changes

		Price (RSD/kg)				
		-20%	-10%	Average	+10%	+20%
Yield (kg/ha)		**22.91**	**25.78**	**28.64**	**31.50**	**34.37**
-20%	**23,083.28**	162,456.36	228,566.87	294,677.39	360,787.90	426,898.41
-10%	**25,968.69**	228,566.87	302,941.20	377,315.53	451,689.86	526,064.18
Average	**28,854.10**	294,677.39	377,315.53	**459,953.67**	542,591.81	625,229.95
+10%	**31,739.51**	360,787.90	451,689.86	542,591.81	633,493.77	724,395.73
+20%	**34,624.92**	426,898.41	526,064.18	625,229.95	724,395.73	823,561.50

Source: Analysis performed by the authors, based on IPN survey data

Based on the collected data of gross margins on the farms where potatoes was the dominant crop in the 2015-2019 period, the average price, average yield and average gross margin of the potato production were calculated. The average price of potatoes for this five-year period was

28.64 RSD/kg, the average yield was 28,854.10 kg/ha, while the average gross margin was 459,953.67 RSD/ha. As it could be expected, 20% reduction in the price and yield of potatoes would drastically reduce the gross margin of the potato production (by more than 60%), while 20% increase in price and yield would lead to an increase in gross margin by almost 80%. Based on the realized analysis, it can be concluded that changes in prices and yields significantly affect the gross margin value of the potato production.

Potato is grown in the Republic of Serbia on the largest areas compared to other vegetable crops. However, areas under potatoes show a declining trend. In 2015, potatoes were grown on 42,000 ha, while in 2019 that area was reduced to 34,000 ha. Since the areas were decreasing, the total annual production was lower. In 2019 the average yield of potetoe was significantly increased, it reached 20.60 t/ha, so the total production exceeded the level of production from 2015, when the largest areas under potato were recorded. Analysis of realized gross margins in potato production on family farms in the Republic of Serbia in the 2015-2019 period shows that the amount of gross margin in 2019 was twice as high as in 2015 and amounted to 702,117.54 RSD/ha. Prices and yields had a significant influence on the gross margin in potatoe production, which can be concluded from the analysis of the gross margin sensitivity to changes in prices and yields in potato production. Sensitivity analysis indicates that an increase in the price and yield of potatoes by 20% led to an increase in gross margin in potato production by 80%, while a decrease in price and yield by 20% led to a decrease in gross margin by 60%.

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Original scientific paper

The reconsideration of cooperative principles – the pillar of development or a limiting factor?

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Abstract

Cooperative principles are the milestone for identifying cooperative organisations around the world. Throughout history, cooperative principles have changed and adapted to the various forms of cooperatives. Finally, in 1995, the so-called seven basic cooperative principles were established. The paper presents a theoretical analysis of the problem of the implementation of cooperative principles. This paper aims to examine whether it is possible and justified to insist on the firm implementation of the principles in the regular daily business of cooperatives, what limits their implementation, and the potential solutions to overcome these difficulties. The research indicates that the necessity of cooperative principles is indisputable but that there are significantly conflicting views on how they should be applied in practice. It may be concluded that it is necessary to insist on the very idea underlying the cooperatives, that is, that the principles should be viewed as a whole. Certain inevitable exceptions from the cooperative principles in everyday business can be tolerated, but it is necessary to insist on their unwavering application in the long run.

Key words: cooperative principles, implementation, business, evolution

Introduction

At the XXXI Congress of the International Co-operative Association held in 1995, on the centenary of this organisation, the Cooperative Identity Statement was adopted, defining elements of cooperative identity, namely: definition of cooperative, cooperative values and cooperative principles. The significance of these elements is reflected in the fact that they strive

to unite national cooperative movements, regardless of the social and political organisation of the country, level of development, cooperative legislation, type and number of cooperatives. The prevailing view in the cooperative literature is that the principles aim to explain the difference between cooperatives and other forms of legal entities (Dunn, 1988; Royer, 1992; Zeuli and Radel, 2005; Novkovic, 2008).

Rethinking cooperative principles – Literature preview

The analysis of cooperative principles and their significance for the cooperative movement does not doubt the cooperative identity but is aimed at strengthening and reflecting the vitality of cooperative organizations. At the same time, the revision of cooperative principles is important for cooperatives themselves. The principles respect not only the needs of cooperatives, but also the environment in which they operate. Therefore, the principles are continuously reviewed and changed.

There is an agreement in the literature that the first four represent essential cooperative principles, without which the cooperative would lose its identity (Cook 1993; COPAC, 2008). They guarantee the conditions under which the members own and manage the cooperative, as well as how they benefit from its operations. These principles are aimed at meeting the needs of members. The following three principles are also important. The principle of education is important from the aspect of effective membership, it is a commitment to continuous training of members so that they can take an active part in the work of the cooperative, and is, therefore, a prerequisite for democratic control. The principle of cooperation among cooperatives directs them to work together and achieve a better position on the market and it enables them to serve their members more effectively. The last principle indicates the need for cooperatives to be active members of the community and is the starting point of social responsibility of the cooperative. The last three principles are, therefore, the source of the social role of cooperatives.

In addition to analysing the principles themselves, significant attention in the literature is paid to how they are applied in practice. Zeuli and Radel (2005) point out that the implementation and understanding of principles vary from cooperative to cooperative. McNamara (2007) argues that cooperatives do not have to respect the cooperative identity but that if they do not apply cooperative principles, they lose certain comparative advantages. In other words, if cooperatives behave like profit-oriented companies, they lose the loyalty of their members. Dunn (1988) categorically claims that deviations of cooperative principles are inevitable in practice and that flexibility in their application is necessary. Nikolić (2009; 2014) concludes

that in everyday business, the essence of cooperative principles must be taken into account, while certain deviations cannot be ignored. Nilsson (1996) believes that the complete application of cooperative values and principles can be discussed only from a scientific perspective. Avsec (2009) claims that the deviation of principles is inevitable and that it is necessary to consider which principles can be deviated so that it doesn't endanger the cooperative nature. Novkovic (2008) goes even further and says that legal requirements usually stipulate that cooperatives respect only the common capital ownership and democratic control and that co-ops are free to change principles as needed.

Considering the previously stated, it is extremely important to analyse how individual principles are applied in practice, what limits their consistent respect, and the consequences of deviating from these principles.

Material and Methods

The paper is theoretical, so desk research methods were applied. An analysis of a number of papers on related topics was performed, and attitudes about implementing cooperative principles in practice were systematised. Accordingly, the method of analysis and synthesis was applied, i.e. based on the existing secondary data and previous research, certain conclusions were drawn. The main source comprised papers dealing with cooperatives in Serbia and abroad, with special emphasis on understanding cooperative principles, their role in preserving the identity of the cooperative and the problems that these organisations face when applying principles in everyday business decisions.

Results and Discussions

In cooperative theory, there is an agreement that cooperative principles are the basis that distinguishes cooperatives from other forms of economic organisations, especially profit-oriented companies. In the last few decades, there has been more theoretical and empirical evidence that implementation of cooperative principles is related to the financial success of cooperatives (Nikolić, 2014; Rixon, 2013). Monitoring the implementation of cooperative principles in practice is therefore extremely important not only to preserve the cooperative identity but also because the success of cooperatives depends on them.

1. Voluntary and Open Membership

This principle is also called the principle of neutrality. It belongs to the group of unchangeable principles that have existed since the founding of the first cooperatives. It implies that

cooperatives are voluntary organisations without any coercion when entering and leaving the membership, but also that anyone willing to accept the responsibilities of membership can join a cooperative. A number of European countries do not impose any restrictions on membership in the laws governing the cooperative sector (such as Bulgaria, Romania, Germany), while others prescribe some procedures for acquiring membership in accordance with the co-op rules. For example, the Latvian Co-operative Societies Law (Latvijas Vestnesis, 48/49 1109/1110) defines that person who utilise the services of a cooperative recognise and comply with the rules of the association, and have made an investment in equity capital and other payments requested by the articles of association, can become a member. Furthermore, the law stipulates that all farmers who meet the above conditions cannot be rejected, unless they have previously been excluded from another cooperative. The Finnish Co-operative Act (1488/2001, Osuuskuntalaki) defines that anyone who meets the accession criteria can become a co-op member, but also that a potential member can be rejected if necessary due to the nature or scope of the business. New generation cooperatives are known for practising the so-called closed membership, that is, they prescribe high equity capital, which *per se* represents a kind of restriction on joining the cooperative. In addition, after collecting the required amount of capital or the required level of raw material that can be processed in the existing processing capacities, the new generation cooperatives do not accept new members. Similarly, changes in the external environment can force cooperatives to limit membership. Bijman et al. (2012) report that dairy cooperatives in the EU, when facing the quotas in production, decided to refrain from an open membership policy in order to control production levels, although it means deviation from traditional cooperative principles.

Voluntary membership is widely implemented today, although in the period after the Second World War, in the Eastern Bloc countries, there was a deviation from this principle. However, there are doubts about open membership. Open membership essentially implies the freedom of entry and exit from the cooperative of a group of people, defined by certain characteristics: previously, it could be nationality, and today occupation, possession of agricultural land or other characteristics. In fact, the explanation of the first cooperative principle states that anyone can join a co-op and that they do not discriminate based on gender, social, racial, political or religious factors. However, certain restrictions can be defined. One of the possible restrictions on membership for an agricultural cooperative may be that a potential member should be engaged in agricultural production or own agricultural land.

2. Democratic Member Control

The second cooperative principle belongs to the group of essential principles and has existed since the founding of the first cooperatives, through all three generations of cooperative principles. It was created as a counter-reaction to profit-oriented companies in which decision-making is in the hands of a small number of people, i.e. it is related to the level of investment. It focuses on cooperative management, where all members have the equal right to participate in business decision-making and the creation of business and investment policies of cooperatives (Zakić et al., 2018; Nikolić, 2018). This means that all members are awarded one vote, regardless of the investment level.

In most European countries, as a rule, the principle "one member - one vote" is applied (Bijman et al., 2012). Strict implementation of this rule is guaranteed, for example, in Greece, Bulgaria, Croatia, Romania, Montenegro and Serbia. However, in some cooperatives, members with a larger number of votes can be found. Cook (1993) states that members should manage the cooperative on a democratic basis and that the number of votes should not be related to the formation of capital, i.e. investments in the cooperative, but it can be linked to the use of cooperative services. For example, in Austria, the number of votes may depend on the extent of the business transaction with the cooperative. In some countries, the allocation of a larger number of votes is related to the membership of legal entities. In Belgium, legal entities that are members of cooperative may have more than one vote, with a limit of 10% of the total number of votes. In Finland, a member of a cooperative can have a higher number of votes only in those cooperatives where the majority of members are legal entities. Special categories of members can also have larger number of votes. For example, in Italy and France, special types of members called ‘investor members’ can have a larger number of votes, although usually with certain restrictions. Investor members represent a special category of cooperative members who do not satisfy the dual nature of membership: they do not participate in the business of a cooperative but only invest capital (Nikolić, 2009).

Royer (1992) agrees that voting rights can be appointed according to the risk assumed, and since it is done proportionally to their transaction with cooperative, the number of votes should be based on that as well. This means that voting rights may be associated with the risk taken in business, and the basis for this way of granting voting rights is the scope of business through the cooperative. Regardless of the motive for awarding a larger number of votes to individual members, it is extremely important not to allow individuals or groups of members to take over the control in decision-making in the cooperative, as this would undermine the democratic basis on which they rest.

3. Members' Economic Participation

The principle of economic participation of members in the work of the cooperatives primarily regulates the process of forming cooperative capital, while its secondary interpretation refers to the sensitive issue of surplus distribution in the cooperative (Nikolić, 2009).

One of the most important issues of establishing and business performance of cooperatives is the formation of cooperative capital. Since the early days, cooperatives have relied on membership to provide the necessary capital. With the development of financial markets, other opportunities for the creation of capital have opened up for cooperatives. The limiting factors in this process are cooperative values and principles representing the guardian of the cooperative's identity (Nikolić, 2018). Moving away from cooperative principles and neglecting the true nature of cooperatives resulted in the problem of cooperative property (Nikolić and Arsenijević, 2015). Therefore, the formation of cooperative capital in accordance with the respect of cooperative principles, as an extremely important issue, is often regulated by law, as was the case in Serbia in the early 1990s (Simmons and Nikolić, 2016).

The third principle also applies to the distribution of surplus. Unlike profit-oriented organisations where the profit is divided in proportion to the amount of investment, in cooperatives, the surplus is divided to members in proportion to the volume of business with the cooperative (Nikolić, 2018). However, this principle occasionally suffers from certain deviations. Although such distribution of surplus is desirable, it is not always dominant, i.e. it is possible to divide the surplus in cooperatives by another "key", such as the type of membership or (significantly less desirable) combined with the level of investment. This way of distributing the surplus can create problems, especially if the co-op members do not know enough about the cooperative principles, i.e. they do not understand the need to preserve the cooperative nature (Nikolić, 2020).

4. Autonomy and Independence

The fourth cooperative principle indicates the need for cooperatives to preserve autonomy and democratic member control. Historically, this was not always the case, especially in countries with a centrally planned economic system. In the modern market, cooperatives are increasingly engaged in business relations with companies from other sectors, and it is essential to support the freedom of cooperatives to control their business regardless of business partners (Nikolić, 2009).

The autonomy and independence of cooperatives should also be respected within the cooperative movement. Namely, cooperatives must be free to decide independently on business

activities and association with other cooperatives and cooperative unions. In some countries, mandatory membership in cooperative unions is stipulated, which is often associated with cooperative audit (Zakić et al., 2018). Given the need and importance of conducting a cooperative audit, this cannot be considered a violation of the fourth cooperative principle.

It is especially important to insist on the autonomy of the cooperative sector from the government. Namely, in times of great crises, it is not uncommon for the state to use available funds to help the cooperative sector. There are numerous examples of government interventions aimed at supporting cooperatives. In the early 1930s, state-sponsored cooperative bank was established in the United States of America to provide favourable sources of funding to the poorest. In 1990s more than 1,200 workers' cooperatives were established in Finland, with government support, in order to reduce unemployment. At the beginning of the XXI century, in Argentina the government-supported workers' cooperatives were founded and they took over 200 companies after the bankruptcy (Zakić and Nikolić, 2018). Therefore, it is very important to establish a sensitive boundary between support for cooperatives and violation of the fourth cooperative principle.

5. Education, Training and Information

The fifth cooperative principle explains that cooperatives provide education and training for their members, managers, employees and elected representatives so that they can effectively contribute to the development of their cooperative. In other words, it is aimed at creating an effective membership, which is a prerequisite for the democratic control of cooperatives. In addition to this internal aspect, co-ops inform the general public, particularly young people and decision-makers, about the importance of cooperatives.

Education in the sense of this principle does not mean a mere distribution of information to members but an understanding of the cooperative idea and goals. Education and training of cooperative members, managers and employees enables the development of skills needed to carry out their individual activities in a responsible and productive way, ultimately contributing to the cooperative itself (Nikolić, 2009).

Despite the fact that the fifth principle belongs to the group of the oldest cooperative principles, not enough attention is paid to it. Dunn (1988) states that this principle is needed only to the extent that modern cooperative principles can be applied in practice, that is, to enable cooperative members to exercise their management rights.

6. Cooperation among cooperatives

The need for cooperation among cooperatives has been noticed since the time of the first cooperatives, and was proclaimed as a principle in 1966. It implies the freedom of cooperatives to associate and assume various forms of cooperative organizations. It can be said that it represents a business strategy without which cooperatives would be economically more vulnerable (Nikolić, 2014). Since cooperatives on the modern market face competition from profit-oriented organizations, it is important that cooperatives, through associations, strengthen their potential.

Research shows that the absence of cooperatives in villages, i.e. the great distance from existing cooperatives, is one of the main reasons why farmers do not join cooperatives (Nikolić and Arsenijević, 2015). In addition, agricultural cooperatives can hardly survive alone and they need adequate sources of funding, which can primarily be provided by saving and credit cooperatives. In some countries, the survival of cooperatives has been threatened by the existence of "quasi-cooperatives" in previous decades, which has shaken faith in cooperatives as organisations (Nikolić et al., 2018). The existence of various forms of true cooperatives that respect cooperative values and principles and that are focused on meeting the needs of their members is the basis for the implementation of the sixth cooperative principle.

7. Concern for Community

This principle was first introduced in 1995 and belongs to the group of "younger" co-ops principles. It indicates the connection between the members of the cooperative, the environment from which they come, and the cooperatives themselves (Nikolić, 2009), i.e. it is connected with the sustainable development and the goals of the International Co-operative Alliance (Nikolić, 2014). It was developed among cooperatives in response to growing pressure to conduct business in a sustainable manner.

This principle is not limited exclusively to cooperatives, because in recent years, there has been an increasing interest in corporate social responsibility (Nikolić, 2017). In the modern market, corporate social responsibility is becoming imperative for the company’s success, and it is associated with consumer attitudes towards the products and the company itself.

Conclusions

Cooperative principles are a segment of the cooperative sector that is most often analysed in the literature. There is almost no scientific paper dealing with co-ops and not at least mentioning cooperative principles. This is a result of the fact that without knowledge and

respect for cooperative principles, cooperatives lose the opportunity to distance themselves from other legal entities in the modern market.

However, a firm reliance on cooperative principles is rarely mentioned in the literature, while more sources claim that distortion of these principles is inevitable. This is probably the case because cooperatives are not legally constrained to apply those principles due to the fact that the International Cooperative Alliance, who adopted cooperative principles, has no legal but only advisory role. Even so, the survival, competitiveness and success of cooperatives strongly depend on conducting business in line with cooperative principles.

The paper analyses the way in which cooperative principles are manifested in the business of cooperatives, difficulties co-ops face in respecting these principles and consequences of non-compliance with the principles on the position and success of cooperatives. It is not possible to decide on one principle being more important than the others, because their strength is precisely in the synergy of all principles. There are differences in the interpretation and consistency of respect for cooperative principles in European cooperative practice, which is to some extent included in the laws governing this area. Deviation from strict compliance with the principles is practically inevitable in the modern market. The essence, however, is to ensure, in the long run, that cooperatives perform their business in line with the principles widely recognised today in order to maintain the spirit of cooperatives, to avoid consequences as well as loss of trust in these organizations.

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Original scientific paper

Forecasting of plum production in Republic of Srpska

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Abstract

In this paper, a quantitative research method was used in order to forecast the trend of plum production parameters movement in Republic of Srpska for the period from 2020 to 2024. For this purpose, a quadratic trend model, as the most appropriate for the analyzed twenty-year data series (2000-2019), was used. The results of the research show that in the five-year forecast period not only the growth of fruit-bearing plum trees can be expected, but also a continuing decline of production and yield. Such results can serve as a way of considering the adoption of some of the strategic decisions in this production.

Key words: Forecasting, Trend, Plum, Republic of Srpska

Introduction

Plum is the most important fruit in Republic of Srpska and also Bosnia and Herzegovina. This is supported by the fact that the plum is the most common fruit in Republic of Srpska and that its production of 73,736 t in 2019 was higher by slightly over 8,300 t than the apple production, which is the second fruit in Republic of Srpska with the production of 64,425 t in the same year (https://www.rzs.rs.ba). The ancient Slavs grew plums in their homeland, although the true origin of this culture is from Asia Minor (Drkenda et al., 2010). Plums are valued for fresh consumption and also for the fact that various products can be obtained from them (jams, compotes, jellies, candied fruit, frozen fruit, liqueurs, brandies) (Butac, 2010). They are the type of fruit with the highest nutritional value and with a high content of carbohydrates, minerals and vitamins that stimulate the human body health (Milošević & Milošević, 2018; Botu & Botu, 2007).

The economic importance of plum production is reflected in enabling entry into new markets, increasing employment and the degree of capacity utilization in agriculture and the food industry, which encourages the development of entrepreneurship and the national economy (Prodanović, 2015). The economics of plum production is determined by numerous factors. The most important ones are: variety selection, location, application of agro and pomotechnical measures, production costs and market prices (Prodanović et al., 2017). In addition, modern plum growing systems imply technology that allows fast achievement of full fertility, regular fertility and high quality of fruit (Glišić et al., 2016). Some varieties have a special significance in processing such as the variety known as Požegača (Madžarka, Savka) in the Balkan (Cvetković and Glišić, 2020).

Global plum production is at the level of 12,601,312 t. The largest producer is China with more than half of the world's production (6,995,738 t). China is followed by Romania with 692,670 t, as well as Serbia with 558,930 t in 2019. The largest exporter of this fruit in 2019 was Chile with 155,217 t of exports, while the largest importer of plums in the same year was also its largest producer, China (together with Hong Kong), with a volume of 85,115 t of imports. Regarding the participation in global production, it can be noted that the production of plums in Bosnia and Herzegovina is minimal and amounts only 0.47%, or 59,970 t in 2019 (http://www.fao.org/faostat/en/#home).

Due to the economic importance that plum has, greater emphasis should be placed on forecasting the movement of its production parameters. Thus, Mutavdžić (2010) considers that in the market conditions, successful production depends on monitoring, analysis and forecast of results and the most important factors that affect it. In their previous research, some of the authors have dealt with the analysis of fruit production (Maksimović, 2012; Keserović et al., 2014; Vlahović et al., 2015; Milić et al., 2016, Stanković and Vaško, 2018; Užar et al., 2019). Nedeljković and Potrebić (2020) used the evaluated trend model to forecast apple production in Republic of Srpska. Namely, they found that the growth of fruit-bearing trees and apple production could be expected in the three-year forecast period (2019-2021). On the contrary, a decrease of yield of this fruit can also be expected.

The subject of this paper is the analysis of the movement of plum production in Republic of Srpska, which would aim to select an appropriate trend model that would forecast the movement of production parameters of this fruit in the next five years.

Material and Methods

Available data from the Institute of Statistics of Republic of Srpska, as well as the FAOSTAT database, were used as the source of the research. Apart from the standard indicators of descriptive statistics (average, interval of variation, standard deviation and coefficient of variation), linear, quadratic and exponential trend models were used for the observed twenty-year period as well as for the five-year forecast (2020-2024).

The linear trend model can be obtained using the following expression:

$$Yt = \beta_0 + \beta_1 t + e_t$$

The quadratic trend model is obtained using the following expression:

$$Yt = \beta_0 + \beta_1 \times t + \beta_2 t^2 + e_t$$

The model of the exponential trend is obtained using the following expression:

$$Yt = \beta_0 \times \beta^t{}_1 \times e_t$$

Three accuracy indicators were used to select the appropriate forecasting model: mean absolute percentage error (MAPE), then mean absolute deviation (MAD) and mean squared deviation (MSD) (Makridakis & Hibon, 2000; Goodwin & Lawton, 1999; Sidik, 2010). According to Karim et al. (2010), the lowest values of all accuracy parameters recommend the selection of the appropriate forecasting model.

MAPE (Mean Absolute Percentage Error) is a forecasting method used in time series where periodicity is particularly observed (Nedeljković & Vujić, 2020). It is expressed by the following formula:

$$\text{MAPE} = \frac{1}{n}\sum \text{I(yt} - \hat{\text{y}}\text{t)/yt I100}$$

Mean Absolute Deviation (MAD) represents a dispersing method that is created as a deviation of the modality from the representative parameter (Nedeljković & Vujić, 2020). It is obtained on the basis of the following formula:

$$\text{MAD} = \frac{1}{n}\sum \text{Iyt} - \hat{\text{y}}\text{tI}$$

MSD is the Mean Squared Deviation that represents the mathematical expectation of how well the arithmetic mean represents the results obtained (Nedeljković & Vujić, 2020). It is expressed on the basis of the following formula:

$$\text{MSD} = \frac{1}{n}\sum (\text{yt} - \hat{\text{y}}\text{t})^2$$

Results and Discussion

The average number of fruit-bearing plum trees in Republic of Srpska in the observed period was almost 5.870 with a very stable trend expressed through the achieved coefficient of variation of 3.74%. As opposed to the trend in the number of fruit-bearing trees, plum production showed a significant variation (cv = 34.18%) with an average of 75,859 t. The maximum production of plums was recorded in 2013 and amounted to 133,581 t. Like production, the yield of plums has a significant and identical variation throughout the analyzed period. The highest yield of over 22 kg per tree was achieved in the same year when the highest production of this fruit was recorded (Table 1).

Table 1. Dynamics of plum production in Republic of Srpska (2000-2019)

Production parameters	Average	Interval of variation		Standard deviation	Coefficient of variation (%)
		Min.	Max.		
Fruit-bearing trees	5.869,10	5.574	6.267	219,299	3,74
Production (t)	75.858,80	26.857	133.581	25929,68	34,18
Yield (kg per tree)	12,92	4,70	22,20	4,315	33,40

Source: Data taken from IS RS

The analysis of the accuracy measure of the evaluated three trend models shows that the quadratic trend model is appropriate for forecasting the movement of the number of fruit-bearing plum trees in the next five years. Namely, the quadratic trend model has the lowest recorded parameter values (MAPE, MAD, MSD) (Table 2).

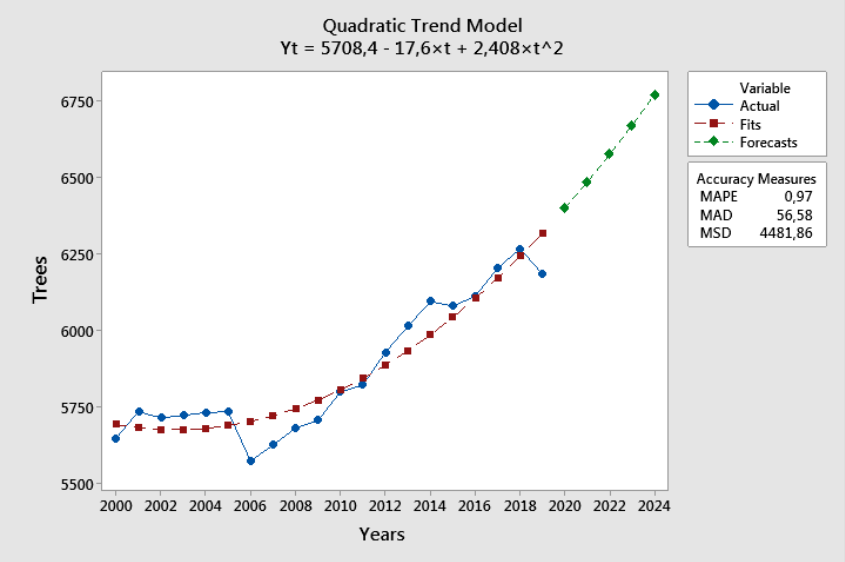
Table 2. Accuracy indicators

Trend model	Parameters		
	MAPE	MAD	MSD
Linear	1,43	83,03	9570,58
Exponential	1,41	81,55	9198,92
Quadratic	**0,97**	**56,58**	**4481,86**

Source: The author's calculation

The following Graph 1 shows the movement of fruit-bearing plum trees in the next five years (2020-2024) and also its actual and default values, while **Table 3** shows the estimated number of fruit-bearing plum trees in Republic of Srpska for the next five years. In the same table, an increase in the number of fruit-bearing trees year after year of the forecast period can be

noticed. The estimated number of fruit-bearing trees in the last year of the forecast period (2024) should be slightly higher than 900.



Graph 1. Movement of the number of fruit-bearing plum trees

Table 3. Forecasting of fruit-bearing plum trees

Period	Estimated value (fruit-bearing trees)
2020	6400,55
2021	6486,48
2022	6577,22
2023	6672,78
2024	6773,16

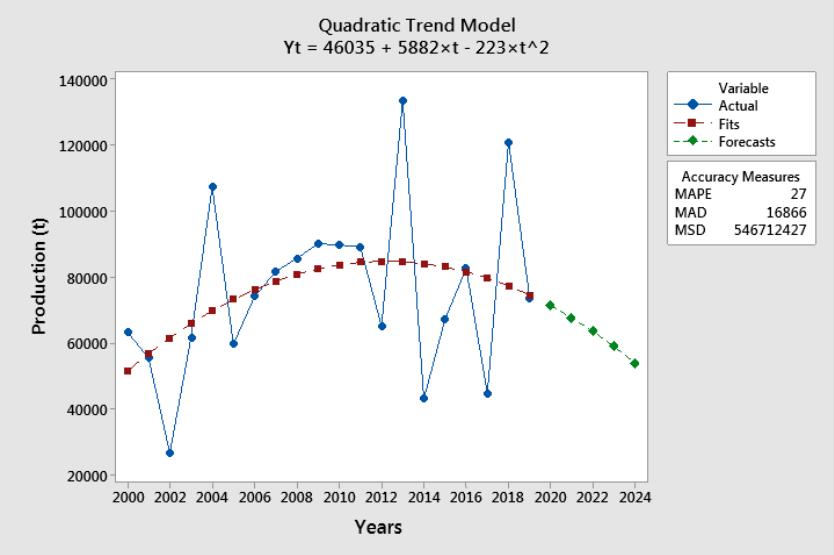
Source: The author's calculation

As far as the fruit-bearing plum trees and also their production is concerned, a quadratic trend model, which is used for forecasting, has the lowest accuracy parameters. (**Table 4**) Graph 2 visualizes plum production movement in Republic of Srpska for the next five years. The data in Table 5 show that production, unlike the number of trees, will continuously fall, and that in the last year of forecasting, it will be at 71% of average production from the period of analysis, 53,983 t.

Table 4. Accuracy indicators

Trend model	Parameters		
	MAPE	MAD	MSD
Linear	31	18962	590195494
Exponential	29	19284	611356533
Quadratic	**27**	**16866**	**546712427**

Source: The author's calculation



Graph 2. Movement of plum production

Table 5. Forecasting of plum production

Period	Estimated value (t)
2020	71407,1
2021	67718,8
2022	63585,3
2023	59006,8
2024	53983,0

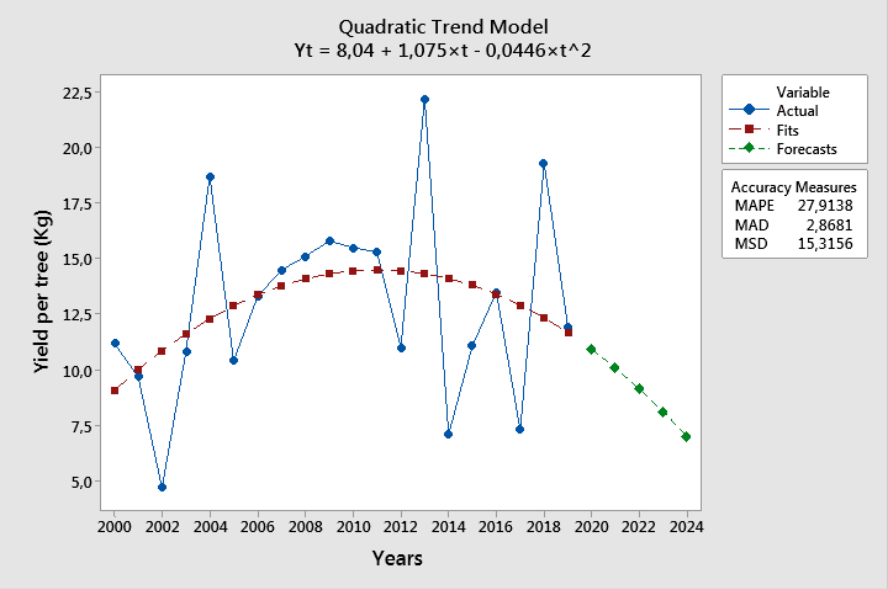
Source: The author's calculation

Also, trend model that is the most appropriate for forecasting of plum yield is the quadratic model due to its lowest values of achieved parameters. (Table 6) The trajectory that shows the decline of plum yield in the observed period, and its further continuation of the constant decline in the next forecast period can be seen in Graph 3. At the end of the forecast period, 7 kg per tree can be expected, which is almost 6 kg per tree less than the recorded yield in the twenty-year observation period (Table 7).

Table 6. Accuracy indicators

Trend model	**Parameters**		
	MAPE	MAD	MSD
Linear	32,0558	3,3351	17,0652
Exponential	30,3354	3,3431	17,6493
Quadratic	**27,9138**	**2,8681**	**15,3156**

Source: The author's calculation



Graph 3. Movement of plum yield

Table 7. Forecasting of plum yields

Period	Estimated value (kg per tree)
2020	10,92
2021	10,07
2022	9,14
2023	8,11
2024	7,00

Source: The author's calculation

Conclusion

From all the above, it can be concluded that in Republic of Srpska the plum represents fruit that has the highest production but minimal participation in global production. In the observed period, plums have large fluctuations in production and yield. Based on the choice of the appropriate trend model, in this case a quadratic trend model, we can expect further growth of fruit-bearing plum trees to the level of 6,773 in 2024. Additionally, a decline of production to 53,983 t, as well as a decline of yield to 7 kg/tree in the last year of the forecast period, is predicted. Even though the number of the fruit-bearing trees has the tendency to increase, it is evident that the decline in yield, caused by poor sortiment and unfavourable weather conditions in a certain period of time, is accompanied by a decline in production of this fruit in Republic of Srpska. Precisely because of its economic importance, further research should be developed in the direction of determining other influences on the plum production trend in Republic of

Srpska, and this research should be used to make better strategic decisions that would concern further progress in this branch of agriculture.

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Predviđanje proizvodnje šljive u Republici Srpskoj

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Sažetak

U radu je primjenom kvantitativne metode istraživanja predviđen trend kretanja proizvodnih parametara šljive u Republici Srpskoj za period 2020-2024.godina. U tu svrhu je korišćen kvadratni trend model, kao najpogodniji za analiziranu dvadesetogodišnju seriju podataka (2000-2019). Rezultati istraživanja pokazuju da se u petogodišnjem periodu predviđanja može očekivati rast rodnih stabala šljive ali i konstantan pad proizvodnje i prinosa. Rezultati istraživanja mogu poslužiti u svrhu razmatranja donošenja nekih od strateških odluka u ovoj proizvodnji.

Ključne riječi: predviđanje, trend, šljiva, Republika Srpska

Original scientific paper

Financial analysis of agricultural cooperatives in the Republic of Srpska

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Abstract

The subject of the research is the financial analysis of cooperatives' registered as agricultural businesses in the Republic of Srpska from 2014-2018. The analysis is based on the publically available financial statements and includes absolute and relative indicators of financial position and performance. According to their number, cooperatives make almost 1/3 of total agricultural businesses in the Republic of Srpska. Average revenues of all cooperatives represent 12.3% of total revenues of the whole agricultural sector. Their assets make almost 10% of total agricultural sector's assets, while cooperatives' equity represents 18.5% of total equity of the whole sector. Considering the relative financial indicators, only profitability ratios were weaker than ratios of other agricultural companies. However, this should not be significantly alarming as cooperatives' business motives differ from the motives of profit-oriented companies. Since other ratios (liquidity and solvency ratios) were similar or better than those of other companies were, we can conclude that the financial position of agricultural cooperatives in general is not worse than the position of other agribusinesses. Nevertheless, to be more attractive for individual farmers, cooperatives need to increase their performance.

Key words: agricultural cooperatives, financial analysis, performance, indicators

Introduction

Cooperatives play a prominent role in the agricultural sector, both in developed and developing countries. For example, in EU, there are more than 50,000 cooperative enterprises, employing more than 650,000 people and making 347 € billion in annual turnover (Cooperatives Europe, 2015). In the Republic of Srpska (RS), cooperatives make almost 1/3 of total agribusinesses in the RS, their average annual revenues represent 12.3% of total agricultural sector's revenues,

while cooperatives' equity represents 18.5% of total equity of the whole sector.

Traditionally agricultural cooperatives have been considered as a vehicle for economic development, because they enable small producers to capture economies of size and improve their marketing power. They are especially important in countries where family farming is a dominant form of agricultural organization (Tortia et al., 2013) such is the case in the RS. Because of their importance, many governments actively promote and assist agricultural cooperatives, but this support is based on the evaluation and monitoring of cooperatives performance.

In today's complex and global marketplace, it is crucial that management understand the financial situation of their cooperative. Regular review of financial statements is necessary to make prudent business decisions, to protect the equity invested by members, and to make sound long-term strategic plans. Pokharel et al. (2019) argues that because of the importance of agricultural cooperatives to farmers and the agricultural economy, the financial performance of cooperatives is worth of empirical analysis in a rapidly changing agricultural economy. In addition, a cooperative must position and protect the business for short-run and long run sustainability by adhering to a balance sheet management philosophy that manages both liquidity and solvency (Barton et al., 2011).

Several studies have examined the financial position and performance of agricultural cooperatives and agribusinesses by using financial ratios, such are: Broz & Švaljek, 2019; Pokharel et al., 2019; Burns et al, 2015; Lerman & Parliament, 1990, and other. They all agree that cooperatives suffer from low profitability and some of them emphasize that the performance of agricultural cooperatives should be analyzed by using measures specific for cooperatives (see: Pokharel et al., 2019). On the other side, evidence on financial analysis of agricultural cooperatives in the RS and B&H are limitted. Regarding similar researches in the RS, Stojanović & Stojanović (2015) carried out the analysis of the general financial position of the agricultural sector in the RS, as a whole, for the period 2010-2012. Vaško et al. (2016) analyzed revenues, costs and business results of RS' agricultural companies in the 2007-2014 period, as well as for the period 2015-2018 (Agriculture Cooperatives Development Program, 2019-2025). Kulelija et al. (2016) analyzed the liquidity of 153 firms from the agribusiness sector in B&H in the 2008-2014 period. Vaško et al. (2018) compared financial performance of the companies in the agricultural sector and food industry in the RS while Stojanović (2019) compared the performance of crop, livestock and mixed agricultural producers in the Republic of Srpska for the period 2010-2015. In this sense, this paper is the first research that deals with

an in-depth analysis of the profitability of cooperatives in agriculture compared to other forms of business organization.

Materials and Methods

Subject of the research were 83 active agriculture cooperatives, meaning the ones that submitted their financial reports on a regular basis within time framework of 2014 and 2018 year. Cooperative membership in these 83 active cooperatives counts only 1,396 cooperators or 17 cooperative members per cooperative on average. According to Cooperative Union database, cooperatives perform business cooperation with 3,578 subcontractors (nonmembers) or 43 subcontractors per cooperative on average. The number of employees is 337 for all 83 active cooperatives, or 4 employees per cooperative on average.

Two essential financial statements used for the purposes of analysis of cooperatives financial reports are balance sheet and income statement (profit and loss account). Together, these two reports provide a picture of the cooperative's financial position, its performance, and its ability to meet its financial obligations. In order to perform the comprehensive financial analysis of agricultural cooperatives in the RS, we have used absolute indicators and relative indicators, i.e. financial ratios. Absolute indicators are selected figures from balance sheet and income statement such as: total revenues, average revenue per agribusiness, total net profit, profit before taxes, operating profit, total assets, total liabilities, average liabilities per agribusiness and equity. Financial ratios include three groups of ratios: profitability ratios, liquidity ratios and solvency ratios. Formulas for calculating these ratios are commonly known, but we refer to those that can be found in Žager & Žager (1999), Bragg (2002), Wheeling (2008), Gibson (2009), Kramer & Johnson (2009), Ivaniš & Nešić (2011), Rodić et al. (2011) and Mikerević (2011). Financial analysis also included trend analysis, as well as the comparative analysis.

Results and Discussion

Trend analysis of absolute financial figures

The trend analysis included absolute financial figures from income statement and balance sheet and covered the five-year period (2014–2018). It referred to cooperatives and other agribusinesses that constitute total agricultural sector in the RS. The number of cooperatives varied from 84 to 104 during the period and they represent 1/3 of total agricultural businesses

in the RS[2]. Table 1 presents the selected figures from publically available financial statements for cooperatives in the five-year period.

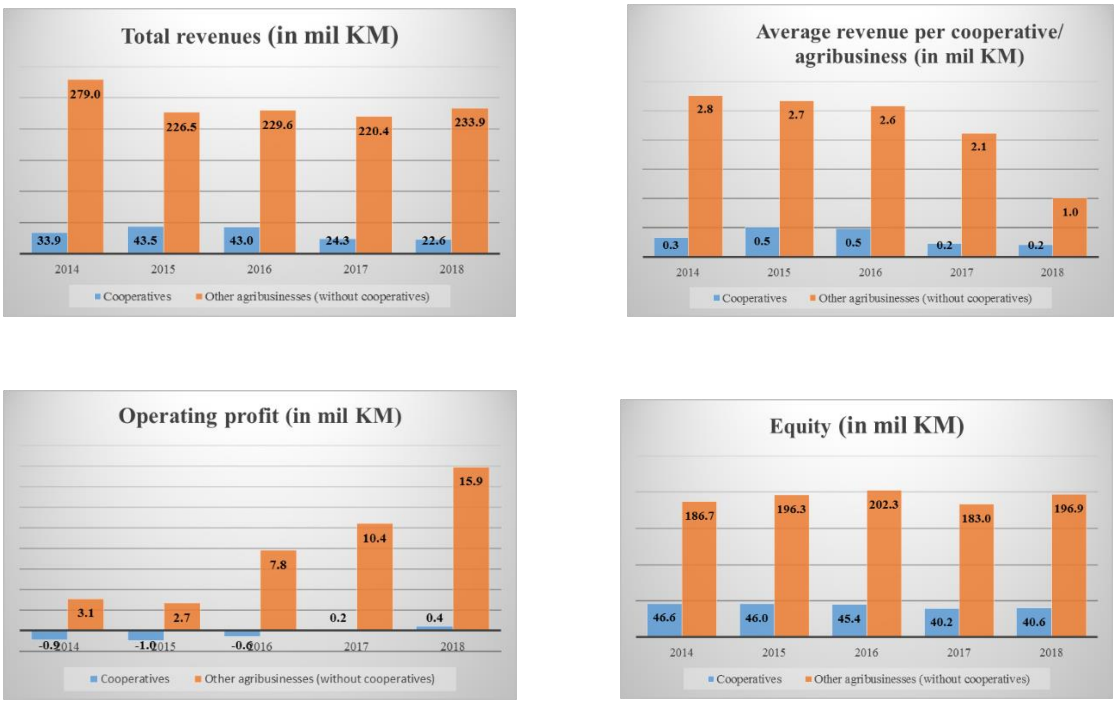
Table 1. Selected balance sheet and income statements figures of cooperatives, 2014-2018 (in mil KM)

Income statement and balance sheet figures	2014	2015	2016	2017	2018	Average, all cooperatives (2014-2018)
Total revenues	33.9	43.5	43.0	24.3	22.6	33.5
Average revenue (per cooperative/agribusiness)	0.3	0.5	0.5	0.2	0.2	0.4
Total net profit (loss)	-1.8	-3.4	-1.3	-1.1	0.5	-1.4
Profit (loss) before taxes	-1.7	-3.3	-1.2	-1.0	0.6	-1.3
Operating profit (loss)	-0.9	-1.0	-0.6	0.2	0.4	-0.4
Total assets	95.4	94.4	96.1	74.0	74.3	86.8
Total liabilities	46.0	45.9	48.2	31.3	31.2	40.5
Average liabilities (per cooperative)	0.5	0.5	0.5	0.3	0.3	0.4
Total equity	46.6	46.0	45.4	40.2	40.6	43.7

As we can see in Table 1, cooperatives had the largest revenues in 2015 and 2016. However, they were not profitable in these years. The structure of operating revenues is dominated by revenues from the sale of goods (70% on average) and revenues from the sale of products (22% on average). A trend of decreasing the importance of trade in other people's goods and increasing the share of own-produced products in total revenues, is observed (Ministry of Agriculture, 2019). During the period, their assets decreased, as well as the liabilities, while equity increased. Their profitability improved in last two years reaching total net profit of 11.9 mil KM in 2018. It is interesting to mention that 48 cooperatives, on average, realize net profits during the period. Total assets of cooperatives make 9.9% of total agricultural sector's assets and amounts to 86.8 mil KM on average.

Graph 1 shows that average total revenues of cooperatives were 7 times less than other businesses' revenues in the period 2014-2018. They amounted to 33.5 mil KM on average, which represents 12.3% of revenues of total agricultural sector. Average revenues per cooperative moved between 0.2 and 0.5 mil KM and they were 6 times less than those of other agribusinesses were.

[2] The number of other agribusinesses (excluding cooperatives) was between 225 and 247.



Graph 1. Comparative analysis of total revenues, average revenues per entity, operating profit and equity between cooperatives and other agribusinesses (without cooperatives), 2014-2018.

Cooperatives' operating profit was significantly weaker than other agribusinesses operating profit and it was positive only in the last two years. Total equity of cooperatives decreased during the period for 6% and, on average, it represents 18.5% of total agricultural sector's equity.

Financial ratios analysis

It is not possible to make informed business decisions, if the financial statements are not properly interpreted and analyzed. Comparisons across time and with sector's averages are an important piece of the analysis. However, care needs to be taken when making comparisons. Since size and volume make comparing different cooperative businesses difficult, analysts often use financial ratios. For the purposes of our financial analysis, we have chosen three categories of ratios: profitability, liquidity and solvency. Table 2 shows the comparison of performance and financial position between cooperatives and other agribusinesses (without cooperatives) measured by the selected financial ratios.

We can see in Table 2 that profitability ratios of cooperatives (ROA -1%, ROE 1%) are weaker then the performance measures of other agribusinesses (ROA 0%, ROE 4%). These ratios are also weaker than the profitability ratios of Croatian agricultural cooperatives (Broz & Švaljek,

2019)[3]. Although cooperatives' business motives differ from the motives of profit-oriented companies, such as investors' owned agricultural companies, this could be an obstacle in the market competition and could threaten the attractiveness of cooperatives as a form of business organization. Therefore, future research should be focused on the comparison of performance between individual farms and cooperatives in order to investigate the justification of cooperatives establishment and advantages of this organizational form.

Table 2. Average financial ratios of cooperatives and other agribusinesses, 2014-2018

	Cooperatives average (2014-2018)	Other agribusinesses average (2014-2018)
Profitability ratios		
Return on assets – ROA (in %)	-1%	0%
Return on equity – ROE (in %)	1%	4%
Liquidity ratios		
Current ratio	0.9	1.0
Quick ratio	0.6	0.5
Interest coverage ratio (in %)	0%	190%
Inventory turnover (in days)	35	68
Average receivable collection period (in days)	97	77
Average accounts payable days	62	112
Solvency ratios		
Long-term debt to equity ratio	0.0	0.0
Total liabilities to assets ratio (in %)	62%	69%
Equity to assets ratio (in %)	37%	31%
Financial stability ratio (Long-term assets to (equity + long-term liabilities))	0.9	0.8
Assets to total liabilties ratio	1.6	1.4

Current and quick ratios are on a similar level for cooperatives and other agribusinesses, as well as those of agricultural cooperatives in Croatia (Broz & Švaljek, 2019)[4]. Nevertheless, activity ratios, used to measure liquidity, are better with other agribusinesses since they have shorter period for receivables collection and longer period for liabilities payments, although inventory turnover is shorter with cooperatives.[5] In addition, interest coverage ratio is much higher with other agribusinesses, which is in line with the fact that they earn higher operating profits. However, since debt to equity ratio is zero on average, for both groups of

[3] For example, in 2017, these ratios for Croatian cooperatives were 0.4 (ROA) and 1.2 (ROE).
[4] In 2017, these ratios for Croatian cooperatives were the same.
[5] For example, in 2017, these indicators for Croatian cooperatives were 145 days for inventory turnover, 70 day for receivables collection and 161 days for liabilities payments. This means that the cooperatives in the RS are in worse position when it comes to receivables collection and liabilities payments periods (Broz & Švaljek, 2019).

agribusinesses, this means that agricultural sector, in general, is not financed through long-term debt.

Regarding other solvency ratios, we can see that total indebtedness (total liabilities to total assets) is less with cooperatives since 62% of their assets is financed through liabilities (69% with other agribusinesses). Accordingly, total assets are higher than total liabilities with cooperatives (cooperatives' ratio is 1.6 and for other agribusinesses it is 1.4). Finally, equity to assets ratio show that cooperatives' assets are covered by equity to a higher extent (with 37%[6]) than those of other agribusinesses are (31%).

Albeit cooperatives have lower profitability ratios[7], since other ratios (liquidity and solvency) were similar or better than those of other companies were, we can conclude that the financial position of agricultural cooperatives in general is not worse than the financial position of other agribusinesses.

Conclusion

Today, cooperative all over the world face the challenges of the internationalization of markets, of innovation, of environmental conservation and of food security. In the context of trade liberalization and globalization, the cooperative, as a form of agricultural production organization, is considered one of the best instruments of self-protection for small farmers mainly due to its self-help concept and member's participation. That is the reason why governments should actively promote and assist agricultural cooperatives. However, justification of continued public support of the cooperative form of organization requires evaluation and monitoring of cooperative performance. In addition, identifying the causes of financial difficulties for agricultural cooperatives provides guidance to managers that affects their strategic planning.

As it is the case in other countries, cooperatives in the Republic of Srpska face similar challenges. Cooperatives need adequate capital to function efficiently and to grow. They need reserves for depreciation and unpredictable contingencies. Not only is it important to have sufficient capital initially, but it is also vital for daily operations and growth. As we could see from our research, long-term debt is not available to the agribusinesses in the Republic of Srpska in general and this is the challenge for both cooperatives and the government.

[6] This is quite similar to the Croatian agricultural cooperatives (37.3%) (Broz & Švaljek, 2019).
[7] Which has also been confirmed by other researches and analysis (Broz & Švaljek, 2019; Pokharel et al., 2018; Lerman & Parliament, 1990)

The second challenge, related to the former one, is the need to be profitable in order to finance much-needed assets and maintain a strong balance sheet. Since cooperatives cannot rely on long-term financing, or larger investments from their members, their ability to generate cash flow through operations is essential implying that maintaining and improving profitability is critical as well. Eventually, profits resulting from the overall financial performance of these cooperatives benefits all stakeholders.

In the Republic of Srpska, the implementation of the law of inheritance and customary law resulted in many small and medium sized properties. Such fragmentation of land does not enable small producers to capture economies of size and achieve better market position. Since family farming is a dominant form of agricultural organization, there is a significant unused economic potential based on joining individual farmers in cooperatives. Although, the establishment of cooperatives could be the solution for small farms to achieve critical size and provide larger capital for investments, cooperatives in the RS need to achieve better performance in order to be more attractive and enable its members to achieve their goals. However, the success of cooperative performance can be improved by creating a more favorable business environment for cooperatives by applying a stimulating tax policy towards cooperatives, greater co-financing of cooperative capital investments, as well as by innovating cooperative legislation in relation to cooperative property.

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Финансијска анализа пољопривредних задруга у Републици Српској

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Сажетак

Предмет нашег истраживања јесте финансијска анализа задруга које су биле регистроване као пољопривредна друштва у Републици Српској од 2014-2018. Анализа се заснива на јавно доступним финансијским извјештајима и укључује апсолутне и релативне показатеље финансијског положаја и успјешности. Према њиховом броју, задруге чине скоро 1/3 укупног броја пољопривредних друштава у Републици Српској. Просјечни приходи свих задруга представљају 12.3% укупних прихода читавог пољопривредног сектора. Њихова актива чини скоро 10%, док капитал задруга представља 18.5% укупног капитала читавог пољопривредног сектора. Посматрајући њихове релативне финансијске показатеље, само су показатељи профитабилности били лошији од показатеља осталих пољопривредних предузећа. Међутим, ово не би требало да буде посебно алармантно с обзиром да се пословни мотиви задруга разликују од мотива профитно орјентисаних предузећа. Имајући у виду да су остали показатељи (ликвидности и солвентности) били слични или чак, у неким случајевима, бољи од показатеља осталих друштава, не можемо закључити да је финансијски положај пољопривредних задруга генерално лошији од положаја осталих друштава. Ипак, да би биле привукле газдинства да се удружују, пољопривредне задруге би морале да повећају своју успјешност пословања.

Кључне ријечи: пољопривредне задруге, финансијска анализа, успјешност, показатељи

Professional paper

Position and perspectives of sustainable development of horticultural production in the Republic of Serbia

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Abstract

The deep economic crisis in which Serbia finds itself today is a consequence of the insufficient adaptability of the Serbian economy to the changes that have occurred in the world economy, as well as the insufficient and inadequately used resources it owns. In the long run, economic recovery will not be possible without a thorough restructuring and inclusion in new economic flows. As such, agriculture stands out as the support of the national economy, which can be a key factor in economic prosperity. The production of horticultural plants has a very important place in modern agricultural production and trade and is one of the most intensive agricultural activities. It can be organized on smaller areas, enables the engagement of a larger number of people, which has the effect of reducing unemployment, and in addition to harmonizing with European and world standards, it can be a key factor in economic prosperity. Therefore, in the future, we must look at horticultural production in Serbia from the aspect of a *perspective life occupation*.

Key words: agriculture, horticulture, perspectives, Serbia, sustainable

Introduction

Serbia has the most suitable climate conditions for the development of agricultural production in Europe. According to (*SANU*, 2020) agricultural land covers 65.21% of its territory, which is 5,060,000 ha of agricultural land. Per capita, Serbia currently has 0.46 ha of arable land, which is twice as much as the world average of 0.27 ha (*Moravčević et al.,* 2019). In the structure of use, agricultural land is used by family farms, farms of legal or private entities and

agricultural cooperatives. Of the total agricultural area, 65.1% are arable land and gardens, orchards (4.7%), vineyards (0.6%), meadows and pastures (18.5%) and others (*SANU*, 2020). It is considered that between 200,000-350,000 ha of arable land and pastures are not used. According to the average size of the farm and the number of plots per farm, Serbia is one of the European countries with the most unsuitable ownership structure and division, which is an obstacle not only for economical and profitable production, but also for the application of modern technical and technological advances of today.

Soil fertility, in terms of quality from 1st to 4th class, makes 4 million ha, which is 45.5% of the total area of the territory of Serbia together with Kosovo and Metohija. The most fertile lands of the 1st and 2nd class are located in Vojvodina, Macva, Posavina, river valleys of Central Serbia and Kosovo and Metohija and as such are suitable for all types of highly productive plant production, which includes horticultural production (*SANU*, 2020). The deep economic crisis in which Serbia finds itself today is a consequence of the insufficient adjustment of the Serbian economy to the changes that have occurred in the world economy, as well as the insufficient and inadequately used resources it owns. The Republic of Serbia does not have a modern economy which follows the free market model. The largest sector of economy is the service sector which accounts for 63.8% of GDP, and the agricultural sector is right after the industrial sector and it makes for 12.7% of GDP (*Vukša et al.,* 2018). In the long run, economic recovery will not be possible without thorough restructuring and inclusion in new economic flows. Agriculture is one of the pillars of the economy at the national level in the creation of total gross domestic product. The production of horticultural plants has a very important place in modern agricultural production and trade and is one of the most intensive agricultural activities. It can be organized in smaller areas, enables the engagement of a larger number of people, which reduces unemployment, and in addition to harmonization with European and world standards, can be a key factor in economic prosperity.

The position of horticultural production in Serbia

Horticultural production is a part of agricultural plant production that has a very important place in modern agricultural production and trade. Its significance is reflected in the facts that the products obtained in this part of agricultural production are necessary not only as everyday food and for obtaining raw materials for the food and pharmaceutical industry, but are also of great importance in the urbanization. It consists of vegetable, flower, fruit, vineyard and nursery production, as well as the production of medicinal, aromatic and spice plants. This type

of production is considered to be the most intensive agricultural activity. It is constantly growing, improving and modernizing, to which urbanization and the growth of the living standard of the population also contribute. Horticultural production in Serbia takes place both outdoors and in a protected area (greenhouses, hothouses). In Serbia, the largest percentage of the horticultural production is organized by family farms (individuals), which are the main carriers of agricultural production as a whole. There are about 630 thousand registered agricultural farms in Serbia, which cultivate 2.8 million ha of that total area of agricultural land (3.5 million ha) (*Moravčević et al.,* 2019).

The most intensive part of horticultural production in Serbia is vegetable production (*SANU*, 2020). It is organized on more than 120 000 ha (*Lazić et al.,* 2019). Production takes place, in the open field and as intensive production in greenhouses or tunnels. In the open field, production takes place on about 100 000 ha, of which 38 472 ha are under potatoes (*SANU*, 2020). According to census of agriculture from 2012, areas under greenhouses were 2 500 ha. However, it is estimated that there are more then 10 000 ha of protected areas (greenhouses, tunnels), and only a few tens of hectares of greenhouses, who are over 40 years old (*Moravčević et al.,* 2019). The production of flower and decorative planting material has marked a significant development in recent years, thanks to the demand throughout the year (*Vujošević and Popović,* 2017), (*Mićanović et al.,* 2019). Flower production is organized both in the open field and in a protected area (Table 1). About 2 125 farms on the area of 382.24 ha are engaged in the production of flowers and ornamental plants in Serbia. Floriculture as a special type of horticultural production in our country which is gaining in importance. The areas of open space under flowers are 263 ha and are estimated at 600 ha (*Mićanović et al.,* 2019). The main production places for flower production in Serbia are: Belgrade region with 40.76 ha, Macva region with 19.44 ha, Rasina region with 33.41 ha and Sumadija region with 11.58 ha (*Vujošević and Popović*, 2017). In the facilities of the protected area (greenhouses, tunnels), production is performed on small areas, 119 ha (Tab.1), which are usually the size of 200-400 m^2 (*SANU*, 2020). There is almost no production of flowers in modern glasshouses, which is a consequence of the high costs of building larger facilities and their maintenance, as well as small state incentives. Only 759 ha of the total area under the protected space, are heated (*Lazić et al.,* 2019).

Table 1. Areas under flowers (open field and protected space) in Serbia

	≤ 1 ha	1,01 – 2 ha	2,01 – 5 ha	5,01 – 10 ha	10,01 – 50 ha	≥ 50 ha	TOTAL
Area (ha)	106	63	90	60	59	4	**382**
Protected space (ha)	42	18	24	23	10	2	**119**
Open field (ha)	64	45	66	37	49	2	**263**

Data source: Republic Statistical Office, January 2016

The orchards in Serbia occupy 163.310 ha of agricultural land (4.7%), while the area under vineyards is much smaller and amounts to about 25 000 ha (*Lukić, et al.*, 2016), (*SANU*, 2020). This part of horticultural production also takes place mainly on private agricultural farms, which is still at a low technological level because the production is subject to the influence of weather conditions. According to the data from 2017, the production of fruit in Serbia took place on an area of 175.863 ha, and grapes on an area of 21 201 ha (*SANU*, 2020). The production of medicinal plants and aromatic herbs takes place on areas that are estimated at only 3 500 ha (*Moafa*, 2020) of which the largest areas are in Vojvodina. Production takes place on family farms or through free-range collection. It is estimated that there are about 4 000 organized households of collectors, which makes about 12 000 registered collectors (*Turudija-Živanović*, 2010). In terms of biodiversity, Serbia is ranked among the 158 best centres in the world, with the highest benefits for intensive production of medicinal, aromatic and spicy herbs (*Moafa*, 2020). More than 700 plant species with medicinal properties have been identified in the rich flora, while 400 species of medicinal plants have been registered. Out of the total number of registered medicinal plant species, 280 of them are traded as industrial raw materials which are used for further production of medicines and cosmetics industry (*Filipović and Popović*, 2014), (*Moafa*, 2020). Italian experts estimate that due to very suitable natural conditions, organic production of medicinal, aromatic and herbal plants can be organized in Serbia on areas of up to 300 000 ha (*Filipović and Popović*, 2014).

The most important vegetable crops grown in Serbia are peppers, beans, cabbage, tomatoes and peas. Of the flower crops, roses, gladioli, chrysanthemums and lilies are mostly grown in open areas, while the production of seasonal flower seedlings in autumn or winter-spring production and some cut flowers such as calla, roses, chrysanthemum (*Vujošević and Popović*, 2017). Of the fruit species, apples (20% of the total area), plums, peaches, apricots, cherries, sour cherries, walnuts, hazelnuts, raspberries and strawberries are mostly grown (*Lukić et al.,* 2016). In the structure of vineyards, wine grape varieties dominate, over 70% of the area.

Slightly more than 230 producers are engaged in wine production, which annually produces on average about 0.3 million hectolitres of wine (*Lukić et al.,* 2016). Plantation cultivation of medicinal, aromatic and herbal plants accounts for about 50% of the total turnover, while the remaining 50% is achieved by collecting them from free flora (*Golijan*, 2016). The most common cultivated species are chamomile, lemon balm, valerian, thyme, coriander, etc. In recent years, organic production has become very popular, which achieves a higher price of raw materials compared to conventional production, from 20-30% to 100% (*Stepanović and Radanović*, 2011).

The foreign trade balance of vegetables and fruits during 2020 was the largest in the last five years (https://comtrade.un.org/). Compared to 2019, the value of exports increased by 12% and amounted to 864 million euros (Tab. 2). In the structure of exports during 2020, fruits participated with 83%, and vegetables with 17%. In total exported quantity, were 53% of frozen fruits and vegetables, 32% of fresh fruits and vegetables and 15% as dried, canned, processed vegetables and juices (Tab. 2).

Table 2. Foreign trade balance of fruit and vegetable exports in Serbia (2019-2020)

Fruit/Vegetables	Total exports (%) **2019**	Value in EUR	Total exports (%) **2020**	Value in EUR	**Growth (%)** 2020/2019
Fresh	33.19	256 029 942	32.21	278 311 555	8.70
Frozen	50.29	387 933 416	52.88	456 854 721	17.77
Others	16.52	127 400 089	14.91	128 851 754	1.14
TOTAL	100	**771 363 447**	100	**864 018 031**	**12.01**

Data source: https://comtrade.un.org/

The largest surplus is realized by the export of industrial vegetables, namely peas, green beans and sweet corn (in the total annual amount of EUR 20 million) as well as peppers whose exports are higher than imports (https://www.nationmaster.com), (https://www.stat.gov.rs). The largest deficits are present in tomatoes, beans, garlic, cabbage, pumpkins and spinach (*Moravčević et al.*, 2019). During 2020 also, there was an increase in exports of organic vegetables by 30% compared to 2019 (https://comtrade.un.org/). Exports amounted to 37.5 million EUR (https://comtrade.un.org/). The importance of vegetable production is also indicated by the fact that the production of vegetables in the open field provides 5-8 times higher production value compared to the value achieved by wheat production, while the production of vegetables in protected spaces achieves a higher value of 190-250 times (*Vlahović, et al.,* 2010). In the structure of exports, 4 fruit species dominate: raspberry, apple, cherry and peach (*SANU*, 2020). In the foreign trade balance, only 0.1 million hectolitres of wine are exported, while it is

imported in almost the same quantity as it is produced in Serbia (0.3 million hectolitres). In recent years, there has been a gradual increase in wine exports (*SANU*, 2020). In 2019, the value of wine exports was 9% higher than in 2018, but that value of exports decreased by 17% in 2020 (https://comtrade.un.org/). During 2020, also the value of wine imports decreased by 2% compared to 2019 (www.stat.gov.rs). The value of foreign trade in flowers and other ornamental plants in the first six months of 2018 (*Mićanović et al.,* 2019) amounted to a total of EUR 7.4 million (4.346 tons). The value of exports amounted to EUR 2.8 million (2.545 tons), while the value of imports amounted to EUR 4.6 million (1.801 tons). The group "*live flowers*" is exported the most (91%), while planting material is mostly imported 39%, cut flowers 32% and "*live flowers*" 22% (*Mićanović et al.,* 2019). The total realized foreign trade of medicinal and aromatic herbs during 2012, amounted to 22.9 million dollars, of which the value of exports amounted to 15.2 million dollars, and imports to 7.7 million dollars.

The most important foreign trade partners of Serbia in the trade of vegetables, fruits, wine, flowers and medicinal aromatic and herbs are the Russian Federation, EU countries and the Central European Free Trade Area (CEFTA), USA, Canada and the Middle East (*Vujošević and Popović,* 2017), (https://comtrade.un.org/), (https://www.stat.gov.rs). The largest percentage of flowers and medicinal and aromatic herbs are exported to EU countries and CEFTA countries (more than 80%), and only 5-10% to other countries (*Vujošević and Popović,* 2017), (https://www.stat.gov.rs).

Perspectives of sustainable development of horticultural production in serbia

Agricultural production in Serbia today is at the level of the average of underdeveloped countries, which is paradoxical considering the existing human and natural resources. This is a consequence of the obsolescence of technical means and the age structure of the population engaged in agriculture (*Lukić et al.,* 2016). Also, the problems are reflected in the weak organization, insufficient connection of science and practice, lower product quality, fluctuating yields, underdeveloped market.

Horticultural production in the economic system should become a profitable production branch in Serbia. The goals in agricultural horticultural production in Serbia may be the similar to those in developed countries but not the same because it can lead to disruptions in production, or stagnation of some types of production. Also, it could come weak market competitiveness and production losses. Adaptation of agriculture to resources, application of new knowledge through the involvement of scientific institutions in the study and programming of agricultural horticultural production with the improvement of technical and technological measures and

intensive international cooperation are the key basis for developing of an appropriate model of Serbian agricultural horticultural production (*Knežević and Mićanović*, 2013).

Each state has the responsibility to define the framework of political and institutional changes that contribute to more efficient development of the agricultural sector, above all to respond to current challenges with a stable, long-term and efficient policy. All the strategies adopted so far, in the last more than 25 years, to solve these systemic problems are completely identical and have not given the expected results (*Moravčević et al.,* 2019). There is also the latest proposed Strategy, as a "new beginning" of the promoted National Program for the Revival of Rural Villages of Serbia, adopted in 2020 as in all previous ones, defines the problems of agricultural production and proposed solutions that are "already seen". As there is no more time to wait, from today, as Nikola Tesla said, "we will not work for the present, but for the future"! This future can be made as a first step, by updating the existing or creating new databases related to the registration of agricultural farms that deal with a specific type of horticultural production, so that they can be monitored, analysed and improved, which do not exist today. Also, creating systemic preconditions for demographic renewal of villages in Serbia, with the development of rural infrastructure. Improving agriculture in general, and thus horticultural production in Serbia and sustainable economic, social and cultural development of villages in Serbia today is no longer possible without a fundamental change in the systemic and legal framework in areas of vital importance to people living in agriculture and living in villages. Significant increase in subsidies with the providing of more favourable credit lines for the purchase of necessary equipment for specific types of horticultural production (modern protected facilities, specialized machinery, irrigation equipment, vehicles, refrigerators, dryers) as well as funds for marketing, trade and services. Institutional encouragement of forming or strengthening of already existing associations of producers for each area of horticultural production (vegetables, florists, nurseries, fruit growers, winegrowers and producers and collectors of medicinal aromatic and herbs plants). The state can also regulate the use of renewable energy sources (geothermal water, biomass) by its laws and acts, then the transfer of knowledge between scientific institutions dealing with specific areas of horticultural production, and producers, be they farmers or industry.

There is a lot of possibilities for improving horticultural production in Serbia. Its greatest resource is the individual producer, and if the state preserves that resource, it can count on the sustainability and survival of the village and thus on horticultural production from the aspect of a *perspective life occupation*.

Conclusion

Applied science is the basis for the development of a competitive economy, especially in the field of agriculture. On the one hand, these are natural resources that the Republic of Serbia possesses and they represent a huge export potential, and on the other hand, there are the potentials of the scientific research and innovation sector. Work on establishing and strengthening cooperation between science and economy through the encouragement of research programs focused on the commercial application of knowledge and technology is one of the key conditions. Therefore, it is necessary to put all resources in the function of initiating and improving economic growth in the field of horticultural production.

The aim is not only to increase the number of agricultural producers in these areas of horticultural production (vegetables, floriculture, nurseries, fruit growing, viticulture, medicinal, aromatic and herbs plant) but that all these productions take place using modern technologies, on larger areas, which would achieve a higher yield and get the appropriate quality of the final products (fruits, flowers, medicinal raw materials). This could meet not only the needs of the domestic market, but also significantly contribute to increasing the export of products. The potential of the Republic of Serbia has been significantly increased through the process of liberalization of trade relations between Serbia and the world, so that the total market has increased to 1 billion people. Education and training of producers from the point of view not only of improving the general importance of knowledge for improving the competitiveness of products and services, but also adapting to European and world standards, as well as the possibilities of interconnection and networking. Also, involvement in development, research and commercial programs and projects at the domestic and international level.

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