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The Influence of Chemical, Organic and Biological Fertilizers on Agrobiological and Antioxidant Properties of Syrian Cephalaria (*Cephalaria Syriaca* L.)

Amir Rahimi [1,*], Sina Siavash Moghaddam [1], Mahdi Ghiyasi [1], Saeid Heydarzadeh [1], Kosar Ghazizadeh [1] and Jelena Popović-Djordjević [2,*]

[1] Department of Agronomy, Faculty of Agriculture, Urmia University, Urmia 5756151818, Iran; ss.moghaddam@urmia.ac.ir or sinamoghaddam2003@gmail.com (S.S.M.); m.ghiasi@urmia.ac.ir (M.G.); s.heydarzadeh@urmia.ac.ir (S.H.); kosar.ghaziizadeh@gmail.com (K.G.)

[2] Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, 11000 Belgrade, Serbia

* Correspondence: e.rahimi@urmia.ac.ir or emir10357@gmail.com (A.R.); jelenadj@agrif.bg.ac.rs (J.P.-D.); Tel.: +989-143-436080 (A.R.); Tel.: +381-11-4413-142 (J.P-D.)

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Abstract: Since chemical fertilizers pollute soil, water and crops, conscientious agricultural producers seek alternatives to chemical fertilizers. Biological fertilizers are considered a reliable alternative for improving soil productivity and plant growth in sustainable agriculture. The response of some agrobiological and antioxidant properties of Syrian cephalaria (*Cephalaria syriaca* L.) to different fertilizer sources was explored in an experiment which included: (i) mycorrhiza + manure; (ii) mycorrhiza + vermicompost; (iii) mycorrhiza + Azotobacter; (iv) mycorrhiza + chemical fertilizer; (v) mycorrhiza; and (vi) control. The results showed that the highest seed yield, biological yield, oil percentage yield, were observed in plants treated with mycorrhiza + vermicompost, whereas the highest 1000-seed weight was obtained from the application of mycorrhiza + manure. With respect to photosynthesizing pigments, the application of mycorrhiza + vermicompost increased chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid content as well as total phenols, total flavonoids and DPPH antioxidant activity as compared to control (unfertilized) plants. The mixed application of different fertilizer sources influenced the uptake of trace elements (Fe, Zn and Cu) optimally. In the light of the obtained results for the agrobiological and antioxidant properties of Syrian cephalaria, in most of the measured traits, there is no significant difference between manure, vermicompost and chemical fertilizers in combination with mycorrhiza. Hence the use of organic and biological inputs instead of chemical fertilizer for improving crop efficiency and quality with the aim of alleviating pollution and accomplishing sustainable agriculture is highly encouraging.

Keywords: manure; mycorrhiza; oil; total phenols; trace elements

1. Introduction

Syrian cephalaria (*Cephalaria syriaca* L.) is an annual plant that grows to the height of 40–110 cm in natural conditions. The taproot of this species can penetrate 60–120 cm into the soil. The hollow and strong stems of the plant grow vertically. The stem and leaves are covered with 4–5 mm hairs. The species has a high branching potential. Since reproductive organs appear at the end of the main branch and auxiliary branches, there is a direct relationship between seed yield and branch number. The reciprocal leaves are serrate in different dimensions and dark green color. The flowers are seen in purple or pink. The species is widely distributed across Turkey, as well as in other European regions like southern France and southern Spain and in northern Africa. It is chilling resistant and can grow in infertile soils [1]. It has been found that different species of this genus contain plenty of triterpenes, flavonoids, glycosides and alkaloids, so they are extensively used in the pharmaceutical industry [2]. The species is also considered an oilseed because it contains about 21–26 percent oil [3]. The oil extracted from the seeds of this plant has a pleasant smell and is yellowish-green in color.

Although the application of organic and biological fertilizers has a long history in agriculture, it has provoked the interests in recent years with the recognition of the very harmful environmental impacts of chemical fertilizers and the serious consideration of the sustainable and organic farming [4]. It is unlikely to accomplish the goals of sustainable and organic agriculture without paying a serious attention to soil biodiversity. Most soil-borne microorganisms play a vital role in converting organic matters into minerals and supplying food requirements of the plants. In addition, some microorganisms are crucial for soil fertility by the role they play in biological fixation of nitrogen and the conversion of some nutrients from unavailable to available form [5]. Mycorrhizal fungi and their symbiosis with plants have various effects on the improvement of plant growth and development so that they can change plant water relations and enhance the drought resistance or tolerance of the host plant [6]. Mycorrhiza fungi influence the absorption of nutrients like phosphorous and nitrogen and water uptake under stressful conditions and the synthesis of plant hormones, alleviate the impacts of environmental stresses, improve resistance to plant pathogens, mitigate root damages, affect soil aggregation, intensify the biological fixation of nitrogen and improve quantitative traits [7]. The more resistance to drought stress induced by mycorrhizal fungi can be related to the increase in leaf photosynthesis rate, the accumulation of non-structural carbohydrates and the decline of osmotic potential [8]. Known as an aerobic and physiological diazotroph, Azotobacter fixes air nitrogen and makes a balance in the uptake of macro and microelements by the plant and, in addition, it synthesizes growth stimulators, such as growth regulating hormones like auxin, different amino acids and so on and thereby it improves the growth and development plant roots and shoots, protects plant roots against soil-borne pathogens and increases high-quality yield per ha [9].

An organic fertilizer source can be exemplified in vermicompost. Vermicompost is a microbiologically active organic compound that is a rich source of macro- and micro-nutrients and is produced by the interaction of earthworms and microorganisms that decompose organic matter [10]. The application of vermicompost in sustainable agriculture improves soil porosity and availability of nutrients [11]. Vermicompost abounds with different microorganisms that release some organic acids, like oxalic acid, leading to the solubility of such nutrients as K and P; also, the increase in soil N content may be related to more activity of phosphatase and protease acids in vermicompost-treated soils [12]. Vermicompost is rich in growth hormones and vitamins, which increases soil microbial population and contributes to the long-term preservation of nutrients without any adverse impacts on the environment [13]. Animal manures are an organic source of nutrients for the sustainable production of the plants. They satisfy plant nutrient requirements and contribute to the increase in soil organic matter, nutrient absorbability by the plants and the maintenance of relative N balance, thereby increasing seed germination percentage and root and stem growth and development [14].

This study aimed to analyze variation of agrobiological and antioxidant properties of Syrian cephalaria without the application of harmful chemical inputs. Hence, manure and vermicompost as organic fertilizer, and mycorrhiza and azotobacter as biofertilizer, can serve as alternatives to mineral fertilizers for improving plant properties.

2. Materials and Methods

2.1. Experimental Design

The study was carried out on the basis of a randomized complete block design with six treatments and three replications, in plots of an area of 6 m^2, at research farm of Urmia University (Lat. 37°31′ N., Long. 45°02′ E., Alt. 1320 m.), during the 2016-2017 growing season. The land was plowed at the optimum moisture level (field capacity) and leveled. Organic, Phosphorus and Potassium fertilizers were used at pre-sowing in autumn, according to soil analysis and farrowed in 50 cm. The experimental treatments were composed of two biological fertilizers and their mixture as well as vermicompost (6.8 ton ha^{-1}), manure (6.3 ton ha^{-1}) and chemical fertilizer (Urea: 110 kg ha^{-1} + Triple superphosphate: 60 kg ha^{-1} + Potassium sulfate: 50 kg ha^{-1} + Micronutrients: 23 kg ha^{-1}), including (a) mycorrhiza fungus + manure, (b) mycorrhiza + vermicompost, (c) mycorrhiza + Azotobacter, (d) mycorrhiza + chemical fertilizer, (e) mycorrhiza and (f) control. The fertilizer treatments were applied to the plots as per the research plan. The fertilizers were used according to the fertilizers program of the crops which have same canopy, based on the soil analyses of the research farm. The seeds of Syrian cephalaria were supplied by the food industry group of Ankara University. One hour before sowing, the seeds were inoculated with Azotobacter biological fertilizer, which contained an effective N fixing bacterium from *Aztobacter vinelandi* (concentration formulated product: 10^9 CFU/g), as per the guidelines of Green Biotech Ltd. firm. To this end, the package content was mixed with water and it was sprayed on the seeds so long that a uniform cover was formed on the seeds. Then, the seeds were dried in shadow before sowing. The soil containing the mycorrhiza fungi (*Rhizophagus intraradices*) (prepared in Organic Plant Protection Clinics, Asababad County, Hamedan, Iran; concentration formulated product: 300 CFU/g) in the amount of 35 g plant^{-1} was placed under the seeds before sowing. Between-row spacing was set at 25 cm and on-row spacing at 10 cm. The seeds were sown in mid-March. Urea fertilizer was used two times (pre-sowing in mid-March and mid-May), according to soil analysis. They had 94% vigor. Before the trial, soil of research farm was sampled at the depth 0–30 cm to determine its physical and chemical properties (Table 1). Along with that, the basic physico-chemical properties of organic fertilizers used in the trial are given (Table 2), as well as the outdoors climatic data of the experimental city Urmia (Table 3).

Table 1. Some physico-chemical properties of soil in the studied region.

EC	pH	Texture	Clay	Silt	Sand	Calcium Carbon Equivalent	SP [1]
					%		
1.37	7.81	Clay loam	43	35	22	15.83	55
N	**Organic Carbon**	**Mn**	**B**	**Zn**	**Fe**	**K**	**P**
	%			mg kg^{-1}			
0.06	1.18	11.5	0.3	1.0	9.1	297	9.1

[1] SP- Saturation percentage.

Table 2. Some physico-chemical properties of organic fertilizers used in the trial.

	K (%)	P (%)	N (%)	OM [1] (%)	EC [2] (dSm^{-1})	pH
Manure	1.1	1.14	1.69	63	8.94	7.57
Vermicompost	3.4	1.64	1.57	52	6.41	8.11

[1] OM- Organic Matter; [2] EC-Electrical Conductivity.

Table 3. The outdoors climatic data of the experimental city *.

Month	Year	Average Relative Humidity (%)	Average Monthly Temperature ($^{\circ}$C)	Monthly Precipitation (mm)
January	2017	45.7	−4.4	4.4
February	2017	65.4	−4.2	39
March	2017	54.8	6.3	20.4
April	2017	56	11.6	59.9
May	2017	52	17.6	11.9
June	2017	47.3	22.7	0
July	2017	40.7	26.3	0.1
August	2017	52.4	25.2	0.6
September	2017	63	21.1	0

Table 3. *Cont.*

Month	Year	Average Relative Humidity (%)	Average Monthly Temperature (°C)	Monthly Precipitation (mm)
October	2017	69.4	12.6	1.8
November	2017	73.0	6.3	38.4
December	2017	48.3	1.7	6.8

* The government meteorological association of Iran.

2.2. Measurement of Growth Parameters

All agronomic practices were performed uniformly for all treatments. After full maturity (in late July), all experimental treatments were separately harvested, and their yield and yield components were recorded. The agronomic traits of seed yield, biological yield, harvest index and 1000-seed weight were recorded on 10 plants per plot at physiological maturity and during harvest. The samples were first oven-dried at 70°C for 24 hours and then, they were examined. Traits such as chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid were determined as reported in literature [15]; the samples were obtained from upper evolved young leaves at the end of flowering stage and two days after irrigation, in the afternoon.

$$\begin{aligned}\text{Chlorophyll } a &= 11.24 \times A_{662} - 2.04 \times A_{645}\\ \text{Chlorophyll } b &= 20.13 \times A_{645} - 4.19 \times A_{662}\\ \text{Total chlorophyll} &= 7.05 \times A_{662} + 18.09 \times A_{645}\end{aligned}$$

$$\text{Carotenoid} = \frac{(1000 \times A_{470} - 1.90 \times \text{chlorophyll } a - 63.14 \times \text{chlorophyll } b)}{214}$$

Seed oil percentage was estimated by hot extraction method as per AOAC Official Method 972.28 (41.1.22) using the Soxhlet extractor [4].

2.3. Determination of Total Phenol and Flavonoid Contents and Anti-Oxidant Activity

Total phenol content of the seeds was determined using the Folin-Ciocalteu reagent [16]. Also, flavonoid content in the extracts was estimated by the literature procedure [17]. To measure antioxidant activity, the readings of sample absorption was converted to DPPH free radical inhibition percentage [18], by the following equation:

$$\text{Free radical inhibition percentage} = \frac{(\text{sample absorption} - \text{control absorption})}{\text{control absorption}} \times 100$$

2.4. Trace Elements Analysis

The absorption of trace elements was determined in fresh digestion extract using their standards with an atomic absorption device (Shimadzu-Tokyo-Japan AA6300) they were read in mg L^{-1} and were calculated in mg kg^{-1} [19,20].

2.5. Statistical Analysis

After the data were normalized, they were subjected to the combined analysis by the SAS 9.1 software package. Also, means were compared by Duncan's Multiple Range Test at the $p < 0.05$ level.

3. Results

3.1. Agrobiological Properties

The significant differences were observed for studied agrobiological properties which can be attributed to the application of different fertilizers ($p < 0.05$). The results are summarized in Table 4. The highest 1000-seed weight was 15.88 g observed in plants treated with mycorrhiza fungi + manure but this treatment did not significantly differ ($p < 0.05$) from mycorrhiza fungi + vermicompost. The lowest 1000-seed weight of 11.99 g was obtained from control (no fertilizer use). However, the application of mycorrhiza fungi alone and control treatment had similar impacts on this trait.

The highest biological yield of 26.98 g plant^{-1} was related to the integrated use of mycorrhiza and vermicompost so the integrated application of various fertilizer sources had a similar effect on the biological yield of Syrian cephalaria. The control treatment exhibited the lowest biological yield of 16.86 g plant^{-1}.

Seed yield was also influenced by fertilizer treatments and the highest seed yield of 12.07 g plant^{-1} was related the integrated application of mycorrhiza fungi and vermicompost, whereas the lowest one (5.99 g plant^{-1}) was observed in control (unfertilized) plants.

The highest harvest index of 44.75% was obtained from the plants treated with mycorrhiza fungi + vermicompost. The mixed application of mycorrhizal fungi with organic, biological and chemical fertilizers had the similar effect on seed harvest index. The lowest harvest index was 36.17% observed in control (unfertilized) treatment ($p < 0.05$).

Plants treated with mycorrhizal fungi + vermicompost produced the highest oil percentage of 25.15% but it did not differ significantly from the mixed application of mycorrhizal fungi and organic, biological and chemical fertilizers. Unfertilized plants showed the lowest oil percentage of 19.32%.

Means comparison revealed that the integrated treatment of mycorrhiza fungi + vermicompost was related to the highest oil yield of 2.99 g plant^{-1} but it did not differ significantly from the application of mycorrhiza fungi + manure and mycorrhiza fungi + chemical fertilizer. The lowest oil yield of 1.18 g plant^{-1} was observed in control plants ($p < 0.05$).

It was observed that the treatment of mycorrhiza fungi + vermicompost was related to the highest chlorophyll *a* and carotenoid content of 2.09 g and 15.13 g per g of fresh weight (FW), respectively. The treatments of mycorrhiza fungi + manure and mycorrhiza fungi + Azotobacter had similar impacts on chlorophyll *a* content, showing an insignificant difference with the application of mycorrhiza fungi + vermicompost. Also, the integrated use of mycorrhiza fungi + manure and mycorrhiza fungi + chemical fertilizer had similar impacts on carotenoid content and they did not differ significantly from the mixed application of mycorrhiza fungi + vermicompost. The highest chlorophyll *b* content of 1.97 mg g^{-1} FW and total chlorophyll of 4.06 mg g^{-1} FW were accomplished from the application of mycorrhiza fungi + vermicompost but no statistically significant difference was observed compared to mycorrhiza fungi + manure ($p < 0.05$). Control (unfertilized) plants exhibited the lowest chlorophyll *a* (1.63 mg g^{-1} FW), chlorophyll *b* (1.47 mg g^{-1} FW), total chlorophyll (3.01 mg g^{-1} FW) and carotenoid (9.43 mg g^{-1} FW) content. The significant increase in some fertilizer treatments as compared to control reflects the positive effect of the mixed application of biological, organic and chemical fertilizers on the amount of photosynthesizing pigments.

The results of analysis of variance (ANOVA) showed that studied agrobiological traits were influenced by fertilizer treatments (Table 5).

A significant effect of various fertilization treatments, at $p < 0.01$ level, was observed for 1000-seed weight, seed yield, oil yield, photosynthesizing pigments (chlorophyll *b* and total chlorophyll) and carotenoid, whereas the influence of treatments on biological yield, harvest index, oil percentage and chlorophyll *a* was significant at the $p < 0.05$ level.

Table 4. Agrobiological properties of Syrian cephalaria as influenced by organic and chemical fertilizers.

Treatments	**1000-Seed Weight (g)**	**Biological Yield (g plant^{-1})**	**Seed Yield (g plant^{-1})**	**Harvest Index**	**Oil Percentage**	**Oil Yield (g plant^{-1})**	**Chlorophyll *a* (mg g^{-1} FW)**	**Chlorophyll *b* (mg g^{-1} FW)**	**Total Chlorophyll (mg g^{-1} FW)**	**Carotenoid (mg g^{-1} FW)**
Mycorrhiza + vermicompost	15.79 ± 0.89 ^{a,1}	26.98 ± 2.68 ^{a}	12.07 ± 1.25 ^{a}	44.75 ± 0.86 ^{a}	25.15 ± 0.16 ^{a}	2.99 ± 0.33 ^{a}	2.09 ± 0.22 ^{a}	1.97 ± 0.08 ^{a}	4.06 ± 0.30 ^{a}	15.13 ± 1.42 ^{a}
Mycorrhiza + chemical fertilizer	15.64 ± 0.40 ^{a}	20.93 ± 1.87 ^{ab}	9.86 ± 0.90 ^{abc}	47.09 ± 0.30 ^{a}	23.89 ± 0.46 ^{a}	2.40 ± 0.24 ^{ab}	1.85 ± 0.12 ^{abc}	1.66 ± 0.08 ^{b}	3.51 ± 0.04 ^{b}	13.56 ± 1.37 ^{ab}
Mycorrhiza + manure	15.88 ± 0.57 ^{a}	22.54 ± 3.50 ^{ab}	10.55 ± 1.62 ^{ab}	46.85 ± 2.86 ^{a}	25.02 ± 1.52 ^{a}	2.64 ± 0.45 ^{ab}	2.01 ± 0.09 ^{ab}	1.95 ± 0.12 ^{a}	3.96 ± 0.21 ^{a}	13.74 ± 0.32 ^{ab}
Mycorrhiza + Azotobacter	14.43 ± 0.24 ^{b}	20.77 ± 2.79 ^{ab}	8.98 ± 0.97 ^{bc}	43.40 ± 3.29 ^{a}	23.17 ± 1.76 ^{a}	2.08 ± 0.26 ^{bc}	1.95 ± 0.16 ^{ab}	1.67 ± 0.03 ^{b}	3.63 ± 0.13 ^{b}	12.91 ± 1.43 ^{bc}
Mycorrhiza	12.21 ± 0.14 ^{c}	17.59 ± 4.81 ^{b}	7.59 ± 2.40 ^{cd}	41.20 ± 1.41 ^{ab}	22.00 ± 0.75 ^{ab}	1.68 ± 0.60 ^{cd}	1.80 ± 0.16 ^{bc}	1.51 ± 0.09 ^{b}	3.31 ± 0.08 ^{bc}	11.35 ± 0.19 ^{cd}
Control	11.99 ± 0.80 ^{c}	16.86 ± 2.09 ^{b}	5.99 ± 0.80 ^{d}	36.17 ± 8.33 ^{b}	19.32 ± 4.45 ^{b}	1.18 ± 0.39 ^{d}	1.62 ± 0.11 ^{c}	1.47 ± 0.17 ^{b}	3.10 ± 0.28 ^{c}	9.43 ± 0.92 ^{d}

[1] Results are presented as means ±standard deviation; means with the same letter(s) in each column did not show significant differences at the $p < 0.05$ level.

Table 5. Analysis of variance for the effect of different fertilizer sources on Syrian cephalaria agrobiological properties.

S.O.V.	**df**	**1000-Seed Weight**	**Biological Yield**	**Seed Yield**	**Harvest Index**	**Oil Percentage**	**Oil Yield**	**Chlorophyll *a***	**Chlorophyll *b***	**Total Chlorophyll**	**Carotenoid**
Replication	2	0.28	7.7	2.06	25.54	7.29	0.22	0.05	0.008	0.10	0.77
Treatment	5	9.75 **	31.53 *	14.07 **	50.57 *	14.41 *	1.23 **	0.08 *	0.13 **	0.40 **	12.16 **
Experimental error	10	0.34	9.73	2.05	13.12	3.74	0.14	0.01	0.01	0.02	1.22
C.V. (%)		4.07	15.05	15.62	8.37	8.38	17.34	6.86	6.20	4.63	8.73

ns, * and ** show insignificance and significance at $p < 0.05$ and $p < 0.01$, respectively.

3.2. Antioxidant Properties and Trace Elements Concentrations

The concurrent application of mycorrhiza + vermicompost produced the highest total phenol content (TPC) of 27.89 mg gallic acids equivalents per g DM (dry matter) but this did not differ significantly from the integrated use of mycorrhiza fungi + manure ($p < 0.05$). Control had the lowest TPC of 21.08 mg gallic acids equivalents per g DM (Table 6).

The highest total flavonoid content (TFC) of 0.56 mg quercetin per g DM, was obtained from the application of mycorrhiza fungi + manure and the lowest, 0.45 mg quercetin per g DM, from control (unfertilized) plants ($p < 0.05$, Table 6).

Means comparisons showed that the highest antioxidant activity (DPPH free radical inhibition) of 60.16% was observed in the simultaneous application of mycorrhiza and vermicompost and the lowest was 47.10% observed in control (unfertilized) plants (Table 6). Seemingly, mycorrhiza + manure escalated antioxidant activity of Syrian cephalaria seeds by improving the physical and chemical properties of soil and the gradual release of nutrients for plants.

The highest Fe concentration of 177.22 mg kg^{-1} was obtained from the application of mycorrhiza + vermicompost and the lowest (154.69 mg kg^{-1}) was observed in control. The treatment of mycorrhiza + vermicompost differed from other treatments significantly ($p < 0.05$). Also, all applied fertilizer treatments improved Fe content versus control significantly ($p < 0.05$, Table 6). The application of mycorrhiza + vermicompost was related to the highest Cu content (32.44 mg kg^{-1}) but it did not show a statistically significant difference with mycorrhiza + manure. The lowest Cu content of 22.98 mg kg^{-1} was observed in control plants (Table 6).

According to means comparison, the application of mycorrhiza + vermicompost produced the highest Zn content of 45.41 mg kg^{-1} and its effect was similar to that of mycorrhiza + manure and mycorrhiza + chemical fertilizer. Control treatment had the lowest Zn content of 37.07 mg kg^{-1} ($p < 0.05$, Table 6).

The results of ANOVA revealed that fertilizer treatments significantly influenced the antioxidant properties of Syrian cephalaria (TPC, TFC and DPPH free radical inhibition) ($p < 0.01$). Moreover, the concentrations of trace elements (Fe, Cu and Zn) were significantly affected by fertilizer treatments ($p < 0.01$, Table 7).

Table 6. Antioxidant properties and trace elements concentrations of Syrian cephalaria as influenced by organic and chemical fertilizers.

Treatments	TPC (mg GA g^{-1})	TFC (g quercetin g^{-1})	DPPH Free Radical Inhibition (%)	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Mycorrhiza + vermicompost	27.89 ± 0.26 ^{a,1}	0.54 ± 0.03 ^{ab}	60.16 ± 0.59 ^{a}	177.21 ± 1.98 ^{a}	32.43 ± 0.21 ^{a}	45.41 ± 0.30 ^{a}
Mycorrhiza + chemical fertilizer	26.55 ± 0.66 ^{bc}	0.53 ± 0.09 ^{b}	56.62 ± 0.14 ^{b}	170.43 ± 0.92 ^{c}	27.39 ± 0.02 ^{b}	43.24 ± 0.03 ^{ab}
Mycorrhiza + manure	27.21 ± 0.14 ^{ab}	0.56 ± 0.15 ^{a}	57.07 ± 0.15 ^{b}	173.57 ± 0.48 ^{b}	31.58 ± 0.66 ^{a}	44.22 ± 0.98 ^{ab}
Mycorrhiza + Azotobacter	25.54 ± 0.29 ^{c}	0.53 ± 0.14 ^{b}	51.13 ± 0.20 ^{c}	172.06 ± 0.50 ^{bc}	27.25 ± 0.16 ^{b}	43.05 ± 0.28 ^{b}
Mycorrhiza	23.00 ± 1.21d	0.51 ± 0.01 ^{b}	50.04 ± 0.73 ^{d}	166.82 ± 2.44 ^{d}	26.71 ± 0.28 ^{b}	42.28 ± 0.39 ^{b}
Control	21.08 ± 1.71 ^{e}	0.45 ± 0.04 ^{c}	47.10 ± 1.11 ^{e}	153.69 ± 3.69 ^{e}	22.98 ± 2.23 ^{c}	37.07 ± 3.12 ^{c}

[1] Results are presented as means ±standard deviation (SD); means with the same letter(s) in each column did not show significant differences at the $p < 0.05$ level.

Table 7. Analysis of variance for the effect of different fertilizer sources on antioxidant properties and trace elements content of Syrian cephalaria.

S.O.V.	df	Total Phenol Content	Total Flavonoid Content	DPPH Free Radical Inhibition	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Replication	2	2.56	0.0005	0.99	11.11	1.97	3.88
Treatment	5	21.02 **	0.003 **	75.03 **	203.37 **	36.19 **	25.07 **
Experimental error	10	0.49	0.0002	0.23	2.65	0.71	1.39
C.V. (%)		2.77	2.91	0.90	0.96	3.01	2.77

ns, ** show insignificance and significance at $p < 0.01$, respectively.

4. Discussion

4.1. Agrobiological Properties

Biological fertilizers, especially in water deficit conditions, can improve root growth and the assimilation of photosynthates due to the increased leaf area and higher photosynthesis capacity at the pre-flowering period [21]. On the other hand, by the retention of water, manure provides an appropriate environment for the activity of the bacteria and the uptake of chemical fertilizers [22]. In a study on the effect of biofertilizers + chemical fertilizers on the yield and yield components of pinto beans [23], the highest 1000-seed weight was obtained from the treatment of 50% chemical urea and triple superphosphate + Nitroxin and biosuperphosphate biofertilizer (a set of phosphate solving bacteria). The results support our findings.

The obtained results indicated that the biological yield was higher when the plants were fertilized with a mixture of organic, biological and chemical fertilizers. It has been reported that the integrated application of Nitroxin and chemical N fertilizer improves the biological yield of anise plants and reduces N fertilization rate considerably [24]. With respect to the impact of mixed application of organic, biological and chemical fertilizers on the biological yield of Syrian cephalaria, it can be argued that this mixture improves soil organic content and contributes to higher yield components including the number of auxiliary branches by affecting moisture and nutrient uptake, retention and availability [25,26] thereby enhancing the biological yield. In a similar study on Phyllanthus amarus in field conditions, it was found that the application of manure + an N-fixing Azotobacter species (Azospirillum sp) remarkably increased the yield versus control [27]. The positive effect of vermicompost and manure may be associated with the increase in soil organic matter and also a balanced availability of the micro- and macro-nutrients in the soil.

Integrated application of mycorrhiza fungi and vermicompost gave the best results, which shows the high capability of organic and biological fertilizers in supplying plant needs at an optimal level. The application of manure mixed with the biological fertilizer contributes to maintaining soil moisture and improving nutrient availability to plants, thereby increasing assimilate synthesis, so the application of these fertilizers helps to increase plant yields by enhancing photosynthate synthesis [25,28]. As well, the yield improvement in integrated nutritional methods may be attributed to the increased level of microbial and enzymatic activities [29,30]. The present study showed that seed yield of Syrian cephalaria was higher when nourished with integrated bio-organic, fertilizers than when inoculated just with Mycorrhizal fungi or applied Mycorrhiza + chemical fertilizers.

Harvest index is the ratio of economic (seed) yield to biological yield (dry weight of all shoots) and shows how assimilates are allocated among vegetative structures of the plant and flower. High harvest index is acceptable when it results from the increase in total dry matter produced at the farm, the increase in the share of the economic yield, and/or both of them [31]. It seems that nutrient and water availability at the seed filling phase enhances the harvest index because their availability affects the current photosynthesis favorably. Thus, the deficiency of nutrients, which may occur in plant growth medium, can change the partitioning of photosynthates among plant organs. What is important in dictating the harvest index is plant responses to resource limitations [32].

It has been documented that the simultaneous use of mentioned fertilizers can improve oil percentage of canola by modifying the physico-chemical properties of soil and enhancing nutrient uptake and the resulting increase in CO_2 uptake and photosynthesis [33]. There is a report that when more chemical N fertilizer was applied, more nitrogenous precursors and more proteins were synthesized; as a result, fewer substances were available to be converted to oil; but in case of mixed application of fertilizer, a balance was created between the synthesis of proteins and oil in plant [34]. Since oil yield is the product of seed yield × oil percentage, the significant difference in oil yield may arise from the significant difference in soil seed of different fertilization treatments. The mixed application of organic, biological and chemical fertilizers can directly influence plant growth by enhancing N uptake, the synthesis of phytohormones and the solubility of minerals [29,33]. Consequently, it contributes to high seed yield as well as high oil yield. Oil yield is the main goal in planting and development of oilseeds including Syrian cephalaria.

It seems that when organic, biological and chemical fertilizers are applied simultaneously, the N requirement of the plant is met, and N wastage is reduced. Then, because of the mineralization process, N starts to turn into absorbable form gradually and thereby, the vegetative growth of the plant is improved during the growth period. As such, more chlorophyll is synthesized in plants exposed to integrated fertilizer regime. It has been reported that when plants were fed with biological and chemical fertilizers, they absorbed more N and produced more chlorophyll; then, they exhibited higher sunlight absorption capacity, photosynthate synthesis and growth and yield [35]. In the present study, the fact that the application of organic and biological fertilizers enhanced leaf chlorophyll synthesis and concentration can be attributed to its role in hindering N leaching and its supply [30,36], which improved the synthesis of growth stimulators, soil microbial population and also the availability and more efficient uptake of nutrients [36].

4.2. Antioxidant Properties

Phenol compounds are usually determined by genetic factors and environmental conditions including nutrition [37]. It has been reported that phenol content of fennel was increased by the application of 50% chemical fertilizer + 50% organic fertilizer + biological fertilizer versus control [38]. The increase in the nutrients of soil treated with manure contributes to increasing net photosynthesis rate of the plant and consequently, the activity of enzymes involved in the biosynthesis of starch and protein in the synthesis of secondary compounds is enhanced [39]. Since hydrocarbons are the skeleton needed to synthesize phenol compounds, their increase means the increase in substrate for phenol compounds and this may be attributed to the allocation of more carbons to the shikimate pathway [40].

Flavonoids have antioxidant properties and are involved in the regulation of enzymatic activities and the synthesis of primary metabolites. Flavonoid content in different plant species is associated with some factors such as fertilizer application [41]. It has been documented that the application of organic, biological and chemical fertilizers has some stimulating effects on the accumulation of flavonoids in broccoli [42]. It is well known that high concentration of flavonoid and phenol can be specified by the role of organic fertilizers in biosynthesis that creates an acetate shikimate pathway and contributes to further synthesis of flavonoids and phenols [43]. In fact, mycorrhiza fungi act as the hairs for roots and increase water and nutrient uptake by extending the contact area of the roots with the soil; in addition, the exudation of organic acids and the generation of CO_2 acidified root region and enhanced the uptake of P and micronutrients [44]. It has been reported that the application of organic fertilizer improved the antioxidant activity of fennel by favorably affecting the physical and chemical properties of soil, soil organic matter and nutrient availability to plants [38].

Microorganisms change soil minerals and organic matter from a form to another by their metabolic activities and change the availability of essential nutrients to plants and other organisms. Therefore, nutrient cycle and soil formation play a significant role in decomposing organic matters [45]. The higher Fe content in plants exposed to mycorrhiza + vermicompost may be caused by the capability of vermicompost and mycorrhiza microorganisms to synthesize siderophores. Also, vermicompost provides the plants with nutrients. Research has shown that mycorrhiza caused Fe chelating and increased its uptake and mobilization in peanuts and sorghum by exuding different kinds of siderophores [46]. On the other hand, the simultaneous application of biological fertilizer contributes to the better use of nutrients arising from their synergic relationship [36]. Vermicompost contains beneficial aerobic microorganisms, such as Azotobacter and lacks anaerobic bacteria, fungi and pathogens [47]. Researchers argue that the mixed application of biological and organic fertilizers enhance the activity of acid phosphatase and alkaline phosphatase around roots, bringing about the increase in soil P content and more uptake of N, Zn, Cu and Fe [48,49]. From the obtained results, it can be inferred that the mixed application of organic, biological and chemical fertilizers is an effective approach to modify soil fertility and increase nutrient uptake, which can enhance some quantitative and qualitative traits of Syrian cephalaria. Given the present tendency to reduce the use of chemical fertilizers in order to alleviate the pollution of underground water resources and crops, increase production efficiency and accomplish the goals of sustainable agriculture, it is recommended to apply combined bio-organic fertilizers, instead of chemical fertilizers.

5. Conclusions

Our results showed that the integrated application of mycorrhiza with vermicompost or manure had the greatest impact on improving the quantitative and qualitative traits of Syrian cephalaria and, in comparison with mycorrhiza + chemical fertilizers, most of the traits showed significant differences and some other traits showed no significant differences. Also, the chemical fertilizer was not superior in none of the traits. Hence combined bio-organic fertilizers can be suggested as the best alternative for chemical fertilizers. With respect to the antioxidant properties, the optimal result was obtained from the mixed application of different fertilizer sources. The highest nutrient contents in plant tissues were related to the application of mycorrhiza + vermicompost. It can be inferred from the results that the mixed application of organic, biological and chemical fertilizers is an effective approach to modifying soil fertility and increasing nutrient uptake, which can enhance some quantitative and qualitative traits of Syrian cephalaria. Given the present tendency to reduce the use of chemical fertilizers in order to alleviate the pollution of underground water resources and crops, increase production efficiency and accomplish the goals of sustainable agriculture, it is recommended to apply organic, biological and chemical fertilizers simultaneously.

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