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Enhancing the Quality and Yield of European Borage (*Borago officinalis*) by Simultaneous Application of Granulated Compost, Vermicompost and Mycorrhiza

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Abstract
The application of organic fertilizers alone or in combination with bio-fertilizers is important for sustainable agriculture, healthy agricultural production and restoring soil quality. Compost, vermicompost and mycorrhiza amendments could improve soil quality and plant yield. However, little is known about their impact on yield and quality of European borage (*Borago officinalis* L.). To evaluate the effects of vermicompost, granulated compost, and mycorrhiza on qualitative and quantitative traits of *B. officinalis* an experiment was carried out based on a randomized complete block design arranged in a factorial experiment with three replications. Organic fertilizers in five levels (0, 5 and 10 t/ha vermicompost and 3 and 6 t/ha granulated compost) and mycorrhiza (*Glomus mosseae*) in two levels (with and without) were considered. It was shown that the effects of organic fertilizer and mycorrhiza application on nitrogen content were significant in such a way that the maximum nitrogen content was obtained by 10 t/ha vermicompost and mycorrhiza inoculation treatment. The main effects of organic fertilizer and mycorrhiza on phosphorus and potassium contents were also significant. The maximum values were obtained when 10 t/ha vermicompost and mycorrhiza were applied together. Total chlorophyll content, flower-bearing branches, mucilage, and phenol were measured in all three harvests. The results indicated that the interaction effect of harvesting time, organic fertilizer, and mycorrhiza was only significant on chlorophyll content. The maximum chlorophyll content (0.958 $mg\ g^{-1}$ fresh weight) was detected in the third harvest when 10 t/ha vermicompost and mycorrhiza were applied. Maximum mucilage content (4.23%), phenol content (5.07%) and flower-bearing branches (57.45 g m^{-2}) were obtained by 10 t/ha vermicompost treatment. The results suggested that chemical fertilizers could be replaced by organic fertilizer and mycorrhiza to reduce soil and water contamination in the agroecosystems.

Keywords: Biofertilizers, Mucilage, Nutrients, Vermicompost, Yield

Introduction

Currently, one-third of human medicine supply comes from medicinal plants (Chakraborty, 2018). Increasing demand of pharmaceutical factories for raw materials and the necessity for conserving natural vegetation have doubled the importance of studies on aromatic and medicinal plants. The cultivation of medicinal plants based on ecological principles guarantees the quality and reduces the risk of side effects on the function of producing medicine (Griffe et al., 2003). In this type of cultivation, plant

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residue, livestock manure, organic and biological fertilizers are used instead of using external inputs, such as chemical fertilizers and pesticides (Naiji and Souri, 2015). Therefore, the global approach to the production of medicinal plants is toward the establishment of such systems and the implementation of environmental management techniques. Among medicinal plants, German borage (*Borago officinalis* L.) is one of the oldest medicinal plants used all over the world. German borage is an annual herb in the flowering plant family Boraginaceae and is native to the Mediterranean region and central Europe (Akbarpour et al., 2014). German borage is bristly or hairy all over its stems and leaves (El hafid et al., 2002). The most important medical effects of German borage on the human body include analgesic, anti-inflammatory and anti-fever as well as antispasmodic, adrenal gland stimulant and astringent (Farhadi et al., 2012). In addition, it is sedative, narcotic, and diuretic. Its consumption also promotes the secretion of sputum, reduces blood pressure, and improves the heart and respiratory systems' function. It has been used for healing common cold, bronchitis, and pneumonia. The German borage is also used for respiratory problems because of its high mucilage content (Asadi-Samani et al., 2014). The growth, yield and chemical composition of German borage highly depend on the type of fertilizer used, climatic conditions, and harvesting time. Conventional agriculture, with the excessive use of chemical inputs, reduces soil organic matter, diminishes soil biological activity, and, finally, reduces product quality (Liu et al., 2009). Furthermore, the extensive use of these chemical fertilizers poses risks to humans and the environment. Therefore, it is essential to pay more attention to natural fertilizers and use them in agroecosystems (Liu et al., 2009; Sinha et al., 2009; Souri et al., 2018). In recent years, the application of organic or biological fertilizers has been recognized as an approach towards sustainable agriculture. Vermicompost is one of the most common organic fertilizers used in sustainable agriculture since it compensates for the lack of organic matter in soil and is regarded as one of the most suitable methods for promoting soil biological activity (Naiji and Souri, 2018). Besides, vermicompost is rich in nitrogen, phosphorus (P), and potassium (K) as well as iron, zinc, copper, and manganese (Pathma and Sakthivel, 2012; Huerta et al., 2010). Soil organic matter guarantee soil health, nutrients' balance, and microflora activity (Knapp et al., 2010). In a study conducted by Singh (2011), the effects of different fertilizer treatments on nutrient uptake in coriander (*Coriander sativum* L.) were investigated and it was reported that vermicompost application could increase yield and oil production. In a similar study, the effect of organic fertilizers on nutrients uptake in anise (*Pimpinella anisum*L.) was evaluated by Khalesro et al. (2012) who found that vermicompost could significantly affect nitrogen, phosphorus, and potassium concentration. In addition, Ayyob et al. (2014) studied the effect of vermicompost on some qualitative and quantitative characteristics of peppermint (*Mentha piperita* L.) and found that vermicompost application increased chlorophyll and phenol content as well as yield. An increase has been reported in mucilage percentage and yield in marshmallow (*Altheae officinalis* L.) with the application of vermicompost (10 t/ha) and urea (Sadeghi et al., 2014). Granulated compost is another type of organic fertilizer that can play an important role in reducing the use of chemical fertilizers and achieving sustainable agriculture. The controlled process of organic matter decomposition by microorganisms in the presence of oxygen is called composing. Granulating is a general term for a particle which has been enlarged and shaped into a sphere with an optimal diameter. Reaching the desired shape and size of the particles, release of the nutrients required by plants and also better mixing with the soil are the advantages of granulated compost (Souri et al., 2019). In order to investigate the effect of organic fertilizers (manure, vermicompost, coffee compost, and mushroom compost) on *Plantago ovata* Forssk, *Alyssum homolocarpum* and *Lepidium perfoilatum* and *Lalementi aiberica* L., an experiment was carried out by Koocheki et al. (2013) who reported that organic fertilizers could significantly increase mucilage percentage. Another way to achieve sustainable agriculture is to take advantage of microorganisms that play an important role in providing the required nutrients for plants (Bhardwaj et al., 2014). Mycorrhiza, as one of the most important biofertilizers, plays an important role in improving soil fertility and the stability of ecosystems (Gosling et al., 2006). These fertilizers are different kinds of soil microorganisms (especially bacteria and fungi) that convert nutrients into their available formats and can be easily utilized by plants and promote root development (Javaid, 2009). It has been shown that the effects of mycorrhiza on the growth and development of basil could increase the phosphorus content and plant yield (Elhindi et al., 2016; Toussaint, 2007). The positive effect of mycorrhiza on root growth, stomatal conductivity, and chlorophyll content in *Rosmarinus officinalis* has been reported (Sanches-blan et al., 2004). It has been shown that the amount of phenol in leaves and roots of mint increased due to mycorrhiza application (Azad khankandi et al., 2013). Considering the side effects of chemical drugs on human health and the harmful effects of chemical fertilizers on the quality of medicinal plants, contamination of water and soil resources, disturbance of nutrient balance in the soil and reduction of agricultural yields are the result of the lack of or toxicity of some elements.

In spite of the significant roles of organic and biofertilizer in natural and agricultural ecosystems and the harmful effects of chemical fertilizers on the environment and the quality of medicinal plants. However, only few studies have considered the effect of organic and biofertilizer amendments on the yield and quality of Borage. Therefore, the current research aimed to investigate the simultaneous effects of granulated compost, vermicompost and mycorrhiza amendments on yield and quality of borage plant, to reduce the use of chemical fertilizers, and to select the suitable alternative system.

Materials and Methods

This study was carried out in the research field of the Faculty of Agriculture, Yasouj University (30° 38′ N and, 51° 32′ E, altitude 1832 m) in 2016. The average yearly precipitation of the research location (over a 30 year period) is 186 mm and the annual mean temperature is 27 °C. The field was kept fallow during the previous year to reduce the endogenous mycorrhizal fungi and to eliminate their propagules, and to allow for the decomposition of the root debris from the previous crop. Before seedbed preparation, soil samples were collected at the depth up to 30 cm, and the pertaining results are presented in Table 1. The experiment was conducted as a factorial mode in a randomized complete block design with three replications. Organic fertilizer at 5 levels (0, 5 and 10 t/ha vermicompost, 3 and 6 t/ha granulated compost) and mycorrhizal fungus at two levels (with (80 kg ha^{-1}) or without mycorrhiza *Glomu smosseae*) were considered. Plots were prepared after plowing and disk-harrowing. The experimental plots consisted of 5 rows. The distance between the rows was 60 cm and seeds were sown 20 cm apart from the rows. Borage seeds were supplied by Pakan Bazr Company (Isfahan, Iran). Mycorrhiza (80 kg ha^{-1}) was mixed with the seeds before sowing, also, certain amounts of vermicompost were spread onto the soil surface and incorporated into the top 15 cm of the soil manually. The seeds were manually sown in April at 1-2 cm depth. The first irrigation was performed immediately after sowing. From seedling emergence to the 4-5 leaf stage, irrigation was performed every two days and, then, it was reduced to weekly irrigation. Weeds were manually controlled. Eight plants were randomly selected from each plot and labeled to be used for sampling. Flower-bearing branches were harvested three times every 7-10 days. Harvesting was done when 50% of the plants bloomed. Flower-bearing branches were shade dried and then weighted. Also, mucilage and phenol contents were determined in flower-bearing branches (Al-Farsi et al., 2005).

Chlorophyll content

Prior to each harvest, chlorophyll from the leaf samples was extracted in 80% acetone solution according to Arnon's method (1967). Extracts were filtrated and total chlorophyll content was measured spectrophotometrically at 645 and 663 nm, respectively. The total chlorophyll content was expressed as mg g^{-1} fresh weight

Mucilage content

The mucilage content was quantified as described by Iranian Herbal Pharmacopeia (2002). The percentage of extracted mucilage was calculated based on the amount of powdered plant used for the extraction process and the amount of dry mucilage obtained as a percentage (%).

Total phenolic content

The total phenolic content was determined by the method described by Liu and Yao, (2007) with minor modification. Briefly, 50 µL of the essential oil (2 mg/mL) was mixed with 550 µL of Folin–Ciocalteu reagent (10% v/v) for 5 min, followed by the addition of 250 µL of Na_2CO_3 (7% w/v). After incubation for 2 h, the absorbance was measured at λ_{max} = 765 nm. Then, the total phenolic content was expressed as mg Gallic acid (Sigma-Aldrich, St Louis, EUA) equivalent per gram of the extract using the standard curve.

Nutrient content

The last harvest was used to take samples for N, P, and K determination. 20 g of sample was taken from each plot. The plant samples were oven-dried at 75 °C for 48 h and then powdered by an electric mill. Then, the total nitrogen was determined by the Kjeldahl method (Novozamsky et al., 1974). P content was measured using the molybdate and vanadate, using a 6505 JenWay spectrophotometer following colorimetrically method, and K content was determined was using a flame-photometer (JenWay PFP7 flame-photometer) through the method described by Chapman et al. (1962).

Statistical analysis

All the data were subjected to analysis of variance followed by means separation through LSD in SAS software. Probability levels of 1% and 5% ($P \leq 0.01$ or 0.05) were used to test the significant differences among the treatments. LS means procedure was used to compare significant interactions.

Results

Leaf mineral content

The results indicated that the interaction effect of organic fertilizer and mycorrhiza on leaf nitrogen content was significant (Table 2). The maximum leaf nitrogen content (1.09%) was obtained when 10 t/ha vermicompost plus mycorrhiza was applied. The minimum level (0.143%) was obtained in the control treatment (Fig. 1).

Table1. Physicochemical properties of soil and organic matter used in the experiments

	Texture	pH	E.C (dS m^{-1})	N	P (%)	K
Vermicompost	-	7.37	1.4	1.4	2.24	0.88
Granulated compost	-	6.50	2.1	2.2	1.5	1.1
Soil	Silty clay	7.80	0. 50	0.5	0.0006	0.015

1, 2 and 3 denote organic matter, total nitrogen, and mineral nitrogen, respectively

The results indicated that the main effects of organic fertilizers and vermicompost on leaf P percentage were significant (Table 2). Mycorrhiza application increased P percentage (Table 3). In addition, the maximum P percentage (0.121%) was obtained from the highest level of organic fertilizer and vermicompost. As it can be observed in Table 3, the application of granulated organic fertilizers or vermicompost significantly increased leaf P percentage.

The main effects of organic fertilizers and mycorrhiza on leaf K content were significant (Table 2). The mycorrhiza inoculation increased the K content by 2.24% compared to the K content in control plants. Furthermore, application of granulated compost or vermicompost increased leaf K percentage in such a way that the maximum K percentage (0.356%) was obtained from the highest level of vermicompost treatment (Table 3).

Table 2. Analysis of variance (mean squares) for the effects of different fertilizers on nutrients absorbing of *B. officinalis*

Source of variance	df	N	P	K
Replication	2	0.009	0.0002	0.00121
Mycorrhiza	1	0.261**	0.00040**	0.00048**
Organic fertilizers	4	0.657**	0.00240**	0.00620**
Mychorrhiza × Organic fertilizers	4	0.036**	0.00002	0.00003
Error	18	0.002	0.00004	0.00007
C.V. (%)	-	8.89	6.24	2.72

** Significant at the 0.01 probability levels

Table 3. Effect of mycorrhiza and organic fertilizers on P and K concentrations of *B.officinalis*

	Treatment Mycorrhiza	P %	K
	Without inoculation (control)	0.100b	0.316b
	Mycorrhiza inoculation	0.107a	0.324a
	Organic fertilizers (t/h)		
	Control	0.075d	0.273e
Vermicompost	5	0.111b	0.325c
	10	0.121a	0.356a
Granulated compost	3	0.090c	0.306d
	6	0.120a	0.341c

Means followed by common letter are not significantly different at the level of 5% (LSD test).

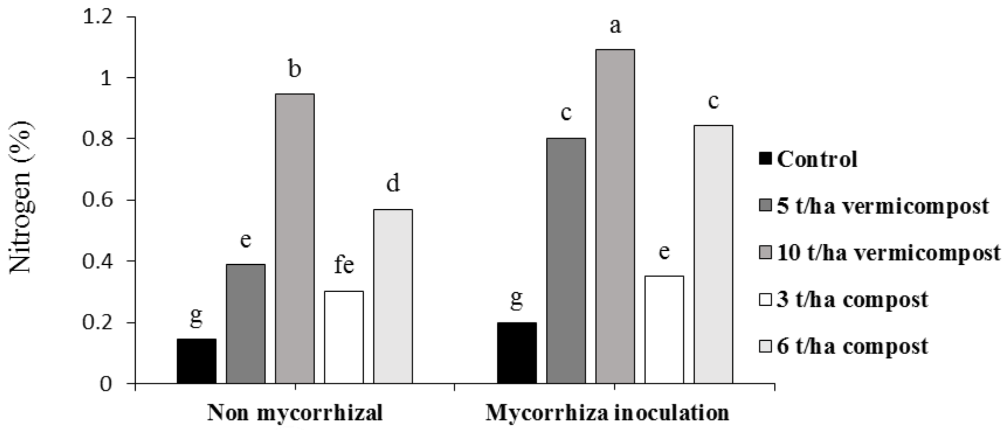

Fig. 1. Effects of organic fertilizers and mycorrhiza on nitrogen content of *B. officinalis* leaf. Means followed by common letters are not significantly different at the level of 5% (LSD test)

Flower-bearing Branches Yield in Each Harvest

The results showed that the main effect of organic fertilizer on flower-bearing branches yield and the interaction effect of mycorrhiza and harvest were significant (Table 4). The maximum dry weight was obtained when 10 t/ha vermicompost was applied (Fig. 2). The interaction between mycorrhiza and harvesting (Fig. 3) showed that multiple harvests did not cause the same trend in the dry weight of flower-bearing branches in such a way that the maximum (69.10 g m^{-2}) dry weight was obtained from the second harvest and mycorrhiza inoculation, whereas the minimum value (37.45 g m^{-2}) pertained to the first harvest and the control treatment.

Total Chlorophyll

The triple interaction among organic fertilizer, mycorrhiza, and harvesting was significant on the total chlorophyll content (Table 4). In all three harvesting rounds, the maximum total chlorophyll was obtained when 10 t/ha vermicompost and mycorrhiza were applied. Furthermore, the third harvest showed the maximum total chlorophyll content (Fig. 4). The maximum chlorophyll content was recorded when 10 t/ha vermicompost was applied in mycorrhiza inoculated plants.

Table 4. Analysis of variance (mean squares) for the effects of different organic fertilizers, mycorrhiza, and harvest time on dry weight, total chlorophyll, mucilage, and total phenol content of *B. officinalis.*

Source of variance	Yield	Total cholorophyll a+b	Mucilage	Total phenol
Replication	48.47	0.00084	1.82	0.839
Mycorrhiza	122.19	0.02659**	0.798**	2.470**
Organic fertilizers	1183.82**	0.10433**	12.237**	6.367**
Mycorrhiza×Organic fertilizers	11.22	0.05071**	0.085	0.131
Error 1	12.17	0.00459	0.118	0.114
Harvesting	6531.72**	0.0588**	0.788**	0.405*
Mycorrhiza×Harvesting	296.16*	0.02184**	0.129	0.032
Organic fertilizers ×Harvesting	138.134	0.00757**	0.084	0.049
Mycorrhiza×Organic fertilizers×Harvesting	132.460	0.02083**	0.067	0.037
Error2	71.493	0.0019	0.076	0.106
C.V. (%)	17.82	4.54	8.72	7.54

*, **, Significant at P< 0.05, P< 0.01 respectively.

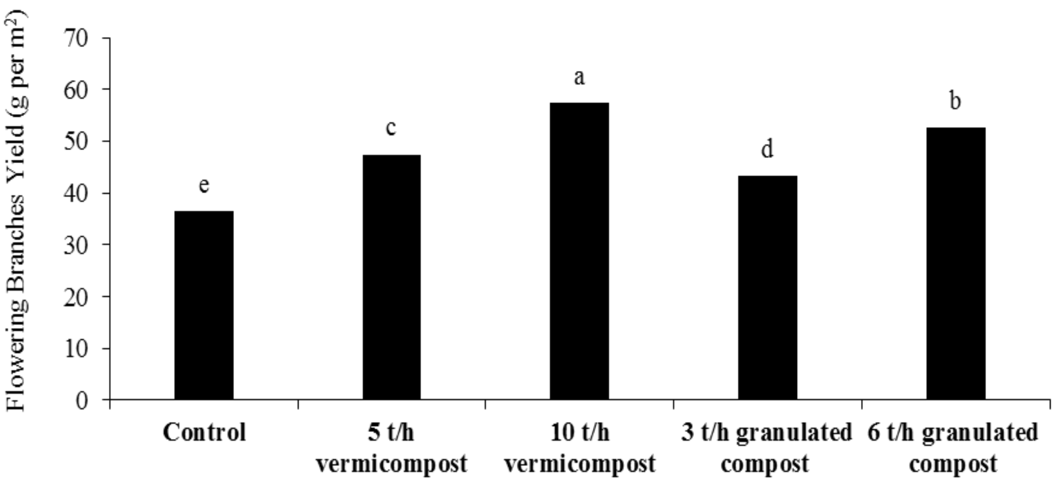

Fig. 2. Effects of the different organic fertilizers on the harvested dry weight of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

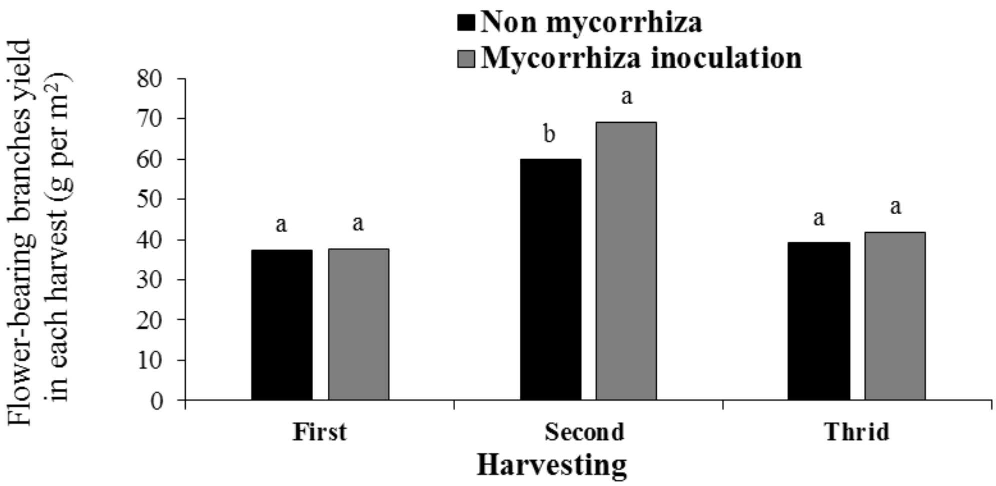

Fig. 3. The interaction effect of harvesting time and mycorrhiza on flower-bearing branches yield of *B. officinalis*. Means followed by common letter are not significantly different at the level of 5% (LSD test).

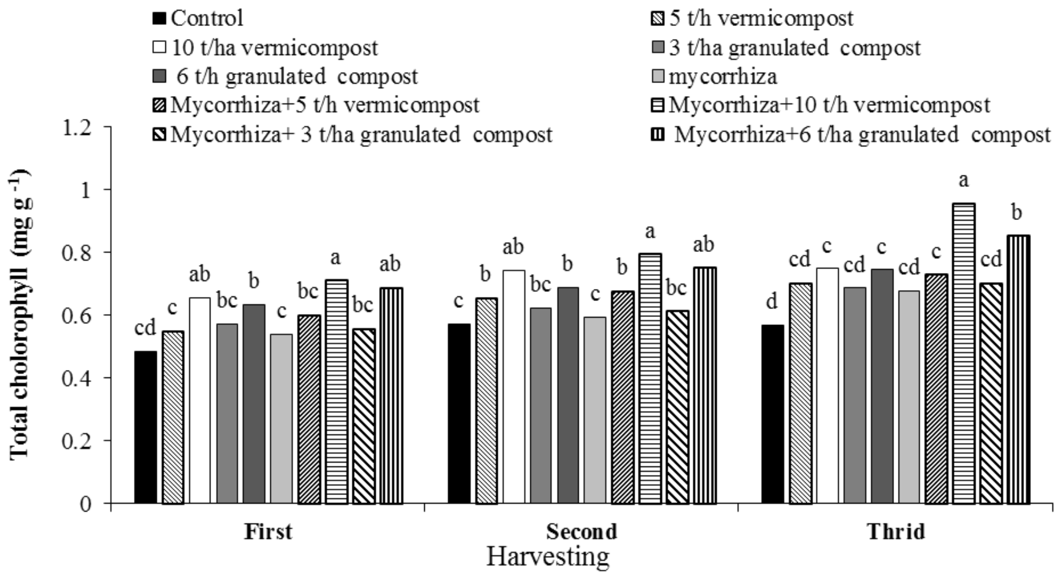

Fig. 4. The interaction effect of organic fertilizers, mycorrhiza and harvesting time on total chlorophyll of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

Mucilage Content

The results showed that the main effect of organic fertilizer, mycorrhiza, and harvesting on the mucilage content of borage plants was significant (Table 4). Mucilage content decreased with the increase in harvesting rounds. The maximum mucilage percentage (3.33%) was obtained from the first harvest, whereas the minimum percentage (3.01%) was obtained from the third harvest (Fig. 5). There is a declining trend dominant over the changes in the percentage of mucilage in inflorescences during the growing season. Mycorrhiza inoculation increased the mucilage percentage (3.26% in mycorrhiza inoculated plants compared to the control treatment 3.07%) (Fig. 6). The maximum mucilage percentage (4.23%) and minimum mucilage percentage (2.2%) were detected in 10 t/ha vermicompost treatment and the control treatment, respectively (Fig. 7).

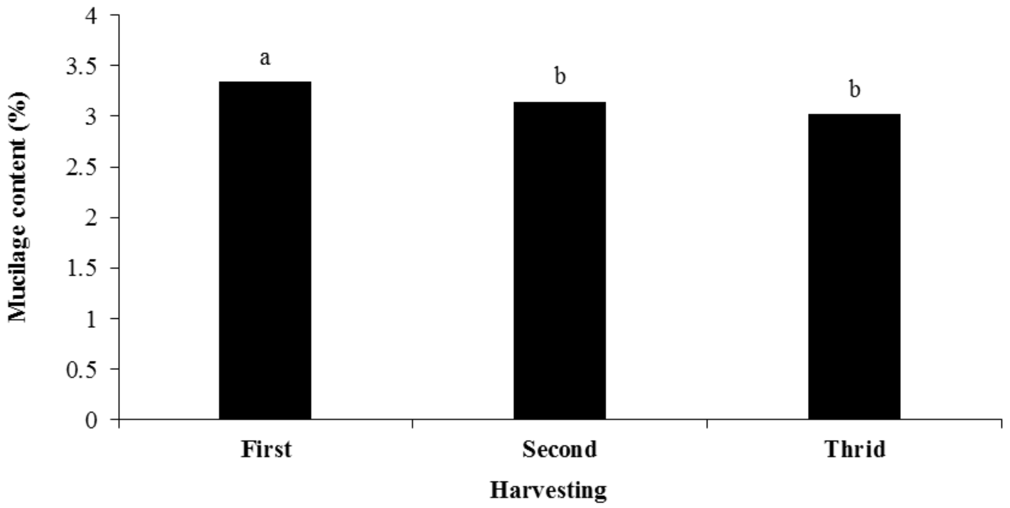

Fig. 5. Effects of harvesting time on mucilage content of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

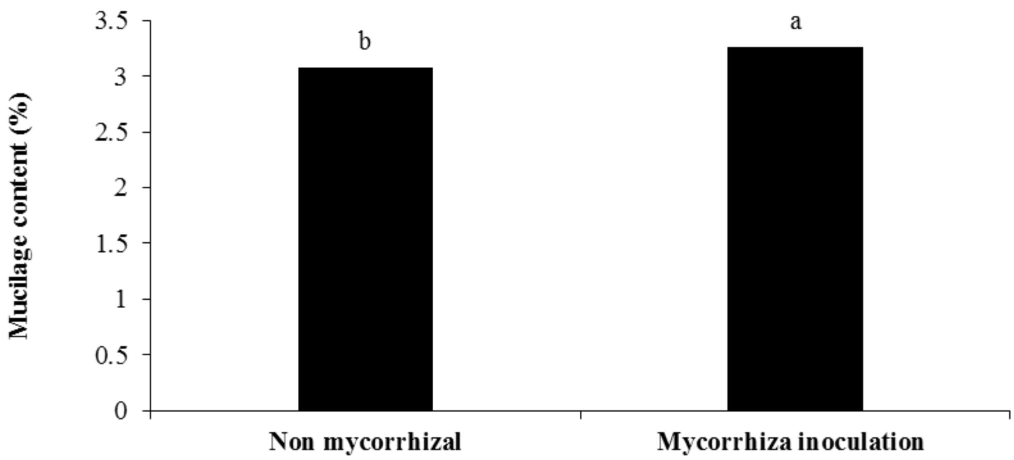

Fig. 6. Effects of mycorrhiza on mucilage content of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

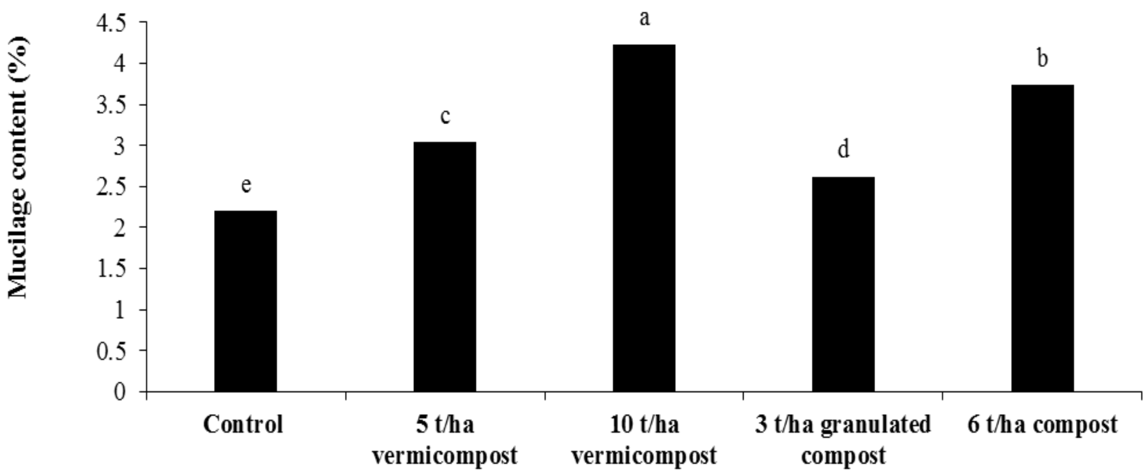

Fig. 7. Effects of organic fertilizers on mucilage content of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

Total Phenol

The analysis of variance indicated that the main effects of organic fertilizer and mycorrhiza on the total phenol content were significant (Table 4). The maximum (4.42%) and minimum (4.19%) total phenol contents belonged to the second and first harvests, respectively (Fig. 8). The application of Mycorrhiza has increased the total phenol content compared to the control treatment (Fig. 9).

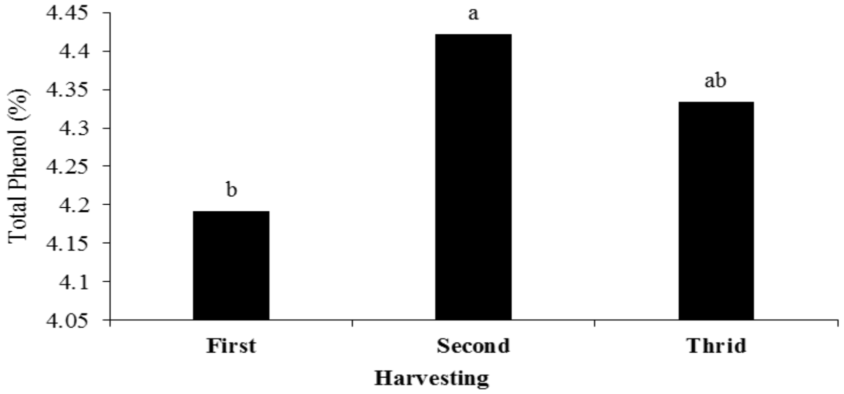

Fig. 8. Effects of harvesting time on phenol content of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

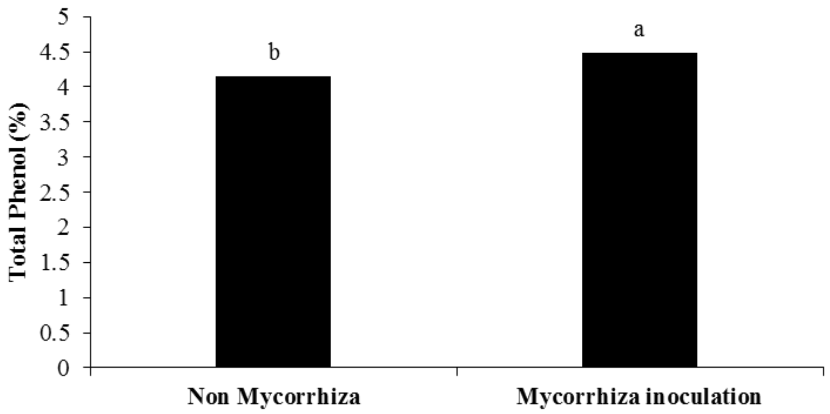

Fig. 9. Effects of mycorrhiza on phenol content of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

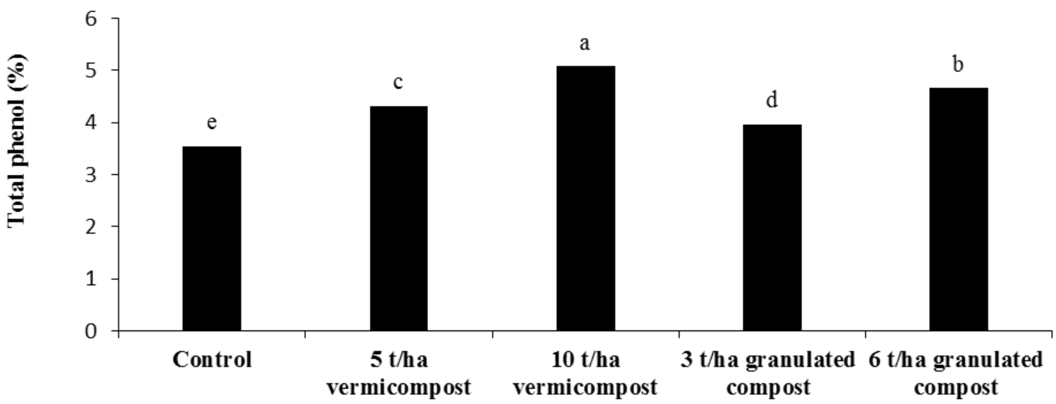

Fig. 10. Effects of the various organic fertilizers on the total phenol of *B. officinalis*. Means followed by common letters are not significantly different at the level of 5% (LSD test).

Discussion

The application of organic fertilizers, whether granulated or vermicompost, increased leaf nitrogen content; however, when these fertilizers were used in combination with mycorrhiza, the increase in nitrogen content was more pronounced. There was also a synergistic effect between organic fertilizers and mycorrhiza on nitrogen content so that the maximum effect was observed when 10 t/ha vermicompost and mycorrhiza were applied at the same time. Since nitrogen is rapidly leached from the soil, vermicompost and compost could provide more nitrogen for the plants and mycorrhizal fungi could increase the root absorption surface area, which allowed the plants to utilize nutrients and water in the soil more efficiently (Roesty et al., 2006). Moreover, it has been reported that vermicompost increases nitrogen content and plant growth by improving soil structure, increasing soil moisture content, promoting soil biological activity, and impacting plant growth that promotes hormones (Pareek et al., 2016; Naiji and Souri, 2018). The related literature shows that the positive effects of vermicompost are attributed to improved soil microbial activity (Atiyeh, 2000), adjusted pH, and increased soil water retention capacity (Mcginnis et al., 2003). It has also been reported that mycorrhiza affects glutamine synthetase, arginine, and urease enzyme activity. Arginine and urease are two key compounds that play a critical role in nitrogen transfer from fungus mycelium to plant roots (Cruz et al., 2007).

Several mechanisms have been proposed for increasing P absorption by the plants inoculated with mycorrhiza; for example, solubilizing soil P by organic acids and phosphate enzyme (Chen et al., 2001), increasing the mobility of P by mycorrhiza mycelium and its strong desire for phosphate ions compared to the plant roots (Mukerji and Chamola 2003), and exploring more volumes of soil are among these methods (Feng et al., 2002). An increase in leaf P content in Marjoram has been previously reported by Khaosaad et al. (2006) who reported that the increase in P content is directly related to mycorrhiza symbiosis. It seems that increase in P content due to vermicompost application is a result of improving of nutrient uptake and microbial activity as reported by Amooaghaie and Golmohammadi (2017) on *Thymus vulgaris*. Furthermore, by adding organic fertilizer to a cultivating system, the humus covers the surface of clay particles and prevents the P stabilization. The presence of P in vermicompost, which is gradually mineralized and absorbed by the plant, is effective in increasing P absorption by the plants. In addition, the production of organic acids, such as citric acid as a result of the decomposition of organic fertilizers can be effective in reducing the pH of calcareous soils and, thereby, may increase P availability in such soils (Latifah et al., 2017).

Mycorrhiza increases root volume and makes soil K more available for the plants. An increase in leaf K percentage in clover has been previously found by Shockley et al. (2004). The increase in K percentage in mycorrhiza inoculated plants has also been reported by Olsson et al. (2008, 2011) who reported that there is a strong relationship between P and K uptake in inoculated plants. K percentage in the third level of vermicompost was revealed to be 33.77% more than that in the first level and 12.96% more than that in the second level, which might be due to the improved soil quality, increased cation exchange capacity, and reduced K leaching. Similar results have been found regarding the effect of vermicompost on seed K content in fennel and tomato plants (Zaller 2007). Improved soil microbial activity, increased plant growth regulators and nutrients uptake, especially K on account of vermicompost treatment, are considered as the main reasons for increasing leaf K content. K is one of the essential elements whose deficiency considerably reduces plant growth. An increase in leaf K content due to mycorrhiza application has been reported by Garcia and Zimmermann (2014).

In the present study, the application of vermicompost increased dry weight. It seems that vermicompost increases soil moisture content and the available nitrogen for the plant; thereby, it creates more favorable conditions for growth, which, in turn, enhances the growth of stems and leaves. These results are in agreement with those found by El-Gendy et al. (2001) in basil where it was indicated that the combination of biofertilizers, chemical, and organic fertilizers as an alternative to chemical fertilizers could increase flower-brearing branch dry weight and yield through improving rhizosphere conditions. In indeterminate species, vegetative growth continues after entering reproductive growth; hence, borage plants increase the leaf area and the number of lateral branches after the first harvest. In the second harvest, the effect of mycorrhiza on yield was more pronounced. It appears that mycorrhiza has improved its symbiosis with the plants just before the second harvest, but this symbiosis decreased afterward due to the less energy provided by the plants as they used their energy to produce more branches.

Vermicompost is rich in humic materials and absorbable elements, such as nitrogen for plants. Due to the presence of nitrogen in the chlorophyll structure, there is a positive and significant correlation between leaf nitrogen and chlorophyll content (Souri et al., 2019). In a study, urea was used as a source of nitrogen and the results showed that chlorophyll ratio was higher for nitrogen treatments compared to fewer nitrogen treatments (Jeffries et al., 2003). Nitrogen was reported as a key element in the production of green pigmentation and chlorophyll in the plants (Shah et al., 2004). Fertilizers used in this study have been shown to increase chlorophyll content by increasing nitrogen availability. Due to the gradual release of nitrogen in organic fertilizers and the positive effect of mycorrhizal on expanding root system during the growing season, nitrogen uptake and chlorophyll synthesis in the second and third harvests were by far higher than those in the first harvest. Baranauskiene et al. (2003) have pointed out that nitrogen increases chlorophyll content and photosynthesis rate. Moreover, vermicompost and compost due to their richness in microelements, especially iron, increase chlorophyll and photosynthesis in treated plants (Hosseinzadeh et al., 2015). It can be concluded that the application of mycorrhiza and vermicompost leads to an increase in chlorophyll and carotenoid content by increasing nitrogen uptake. The maximum chlorophyll content was recorded when 10 t/ha vermicompost was applied in mycorrhiza inoculated plants.

There is mostly a declining trend dominant over the changes in the percentage of mucilage in inflorescences during the growing season. In fact, mucilage may have been used by the plant as a source of energy, as it is made of plant-specific polysaccharides or long chains of sugar molecules. When a plant needs more energy for its metabolic activity, more mucilage will be produced; on the other hand, as plants grow and approach the end of the growth cycle, the mucilage production decreases. These results suggest that mycorrhiza increases water and nutrient availability for the plants and has probably a determining role in increasing the plant's photosynthesis. Therefore, the prevention of yield and biomass loss has produced a significant superiority in mucilage production.

An increase in the mucilage percentage in *Plantago psyllium* due to organic fertilizers has also been reported by Pouryousef et al. (2012). It has been reported that the integrated application of chemical fertilizers and manure increases the mucilage percentage in *Plantago psyllium*. The beneficial effects of organic fertilizers on photosynthesis and the allocation of assimilates increase plant yield and, ultimately, mucilage production (Yadav et al., 2002). In this regard, Hendawy (2008) showed that the application of chemical fertilizer leads to an increase in the number of flowers and mucilage production in *Plantago psyllium*. Similar results have been found by Singh et al. (2003).

The secondary metabolites are essentially made by guiding genetic processes, but their synthesis is influenced by various environmental factors. These factors cause some changes in the growth, development, and also the quantity and quality of the active substances in medicinal plants. The study of environmental and agronomic factors affecting the quantity and quality of secondary metabolites in aromatic and medicinal plants is highly important. It seems that when the plant grows under favorable environmental conditions, the phenol content, as a secondary metabolite, is low. However, when the consecutive harvesting occurs, phenol content increases with respect to the growing season and environmental changes and this leads to higher increase in phenol content in the second harvest. Phenolic compounds, as a group of secondary metabolites, have a diverse and widely distributed chemical structure in plants (Einhellig, 1986) and play a major role in the plant defense system and resistance to environmental stresses (Harborne, 1980).

Laboratory evidence suggests that phenols act as a signal in plant-microbe interactions (Lynn and Chang, 1990). Mycorrhiza, in addition to impacting the growth, can affect the production of secondary metabolites in medicinal plants (Toussaint et al., 2007). Phenol accumulation in mycorrhiza inoculated plants has also been reported by Ling-Lee et al. (1977). Mycorrhizal causes physiological changes in plants by increasing the enzymatic activity (Sakthivel and Karthikeyan, 2015). In plants, secondary metabolites play important ecological roles (Enhelling, 1986). The findings of a study indicated that the maximum phenol content was obtained when *Vinca minor* plants were inoculated with mycorrhiza (Rahmatzadeh and Kazemitabar, 2013). Similar results have been found in artichoke inoculated by *G. mosseae* and *G. intraradices* (Ceccarelli et al., 2010). It has been reported that the application of organic fertilizer increases phenol content in *Solanum lycopersicum* (Sereme et al., 2016). They have also reported that polyphenol biosynthesis is directly affected by organic fertilizers. Similar results have also been reported by Toor and Savage (2005) in tomato. Luján- Hidalgo's et al. (2015) showed that vermicompost increased the total phenol content in *Piper auritum*.

Conclusion

The results obtained from present study showed that organic and biological fertilizers significantly increased the nutrients, yield, mucilage and phenol content in borage plants. Furthermore, the combined use of organic fertilizers and bio-fertilizers in comparison with the separate application of each could improve some of the measured traits. Therefore, applying organic fertilizers along with mycorrhiza to replace chemical fertilizers, in addition to increasing the quantitative and qualitative yields, can be taken in the direction of sustainable agriculture and probably, the reduction of environmental pollution.

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