

Quantitative and qualitative changes of *Eryngium caeruleum* under the influence of biological factors

Mostafa Koozehgar Kaleji and Mohammadreza Ardakani

Abstract—Objective: Humans have long used volunteer edible for food purposes, but with the advancement of Agricultural science, the use of native wild plants has become less important and today, with increasing population and food shortages, on the one hand, the cost of cultivation and water supply and Suitable agricultural soil, on the other hand, should once again be considered as a secondary resource alongside other food sources. The increasing use of chemical fertilizers has caused irreparable environmental and health damage. *Eryngium caeruleum* is a perennial and native plant that normally grows in autumn and spring. **Materials and methods:** In order to study the impact of different planting densities and biological fertilizers on *Eryngium caeruleum* yield and essential oil content, an experiment was conducted in 2013 in Sari based on a factorial randomized complete block design with 9 treatments and 5 replications. The factors included *Eryngium caeruleum* with 3 density levels of (10 × 30, 20 × 30 and 30 × 30 cm), mycorrhiza at 2 inoculated and non-inoculated levels and *Azotobacter* at 2 levels of inoculated with seeds and non-inoculated. **Results:** The results showed that the mycorrhizal symbiosis treatments and *Azotobacter* application had a significant effect on all measured characteristics and increased the measured characteristics in the experimental group compared to the control group. The amount of naphthalene was obtained from mycorrhizal coexistence of 70.33% and the application of *Azotobacter* 3.33% and control 0.69.

Keywords—Apiaceae, Endemic vegetable, Falcarinol, *Glo-mus mosseae*, Naphthalene

I. INTRODUCTION

Humans have long used volunteer edible plants for nutritional purposes, but considering the progress in the field of Agricultural Sciences, the consumption of native wild plants have gradually lost its importance and on the other hand with the expansion of urbanization, the consumption of these food sources which were once the only source of human food has been completely overlooked. Today, the population boom and food shortages on the one hand and the expensive type of cultivation and suitable soil and agriculture preparation on the other necessitate considering these God-given resources at least as secondary sources with other food sources. The Apiaceae family contains more than 300 genera [1]. These plants are used as medicinal plants, essential oils containing plants and vegetables. Plants of this family, especially the genus *Eryngium*, have great potential for the production of

herbal medicinal products in the future [2]. *Eryngium* is the largest and most controversial taxonomic genus belonging to the Apiaceae family, containing more than 2,500 species, subspecies, and approved species worldwide [3], [1]. Among them, only 23 almost all phytochemical species have been studied [4]. New research shows that different species of *Eryngium* contain different phytochemical compounds and at least 127 compounds have been isolated and identified [4], [5]. Recent studies have shown that plants of this genus contain a variety of nutrients including vitamins, minerals, and proteins [6]. *Eryngium caeruleum* belongs to the Apiaceae family, which has different species in Iran. *Eryngium caeruleum* is one of the most important species of *Eryngium* family in northern Iran used as an endemic vegetable. The cooked leaves of the plant are used as a flavor and also spice in local foods and the dried powdered leaves are also used in stuffed food, soups, yogurt, and olive mix. *Eryngium caeruleum* plants also have medicinal properties (diuretic, appetizing, pain relief, and powerful antioxidants) [7], [8]. The biosynthesis of secondary metabolites in medicinal and aromatic plants depends on genetic, physiological, and environmental factors [9]. Among these factors, the use of biological fertilizers such as plant-enhancing rhizobacteria (PGPR) and arbuscular micro bearing fungi (AMF) as well as organic fertilizers such as vermicompost play an important role in improving yield, reducing the use of chemical fertilizers by improving soil properties in the rhizosphere. Enlargement of root areas, the possibility of changes in the water relations of host plants and the effect on metabolism, rapid activation of the immune system, improving mineral nutrition, and strengthening drought tolerance of host plants, while being environmentally friendly, natural, and beneficial. Some researchers have reported that the water relationships of cloned host plants with AMF and PGPR have improved under moisture stress conditions [10]. Also, the relationship between plant coexistence and root coexistence by fungal micro environmental fungi (AMF) can make a difference in the biosynthesis of its secondary metabolites [11]. AMFs have been associated with about 80% of groundwater plants in natural and cultivated ecosystems, varying in coexistence according to plant genotype [12], [13]. Plants inoculated by AMFs are more tolerant of soil water shortages and use more absorbed water. Also, they improve plant nutrition, development, and essential oil content due to changes in the biosynthesis of secondary metabolites [14], [15]. Biofertilizers based on rhizosphere microorganisms are alternatives to improve soil fertility and increase the productivity of sustainable

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agrosystem systems [16], [17], [18]. Among rhizosphere-dwelling microorganisms, several bacterial species, known as plant-enhancing rhizobacteria (PGPRs), can grow roots and plants. Their mechanisms of action are direct or indirect. And each can act independently with the ability to promote specific physiological responses in plant cells and control biological functions [19]. The inoculation of agricultural products with PGPRs represents only a small part of agricultural practices worldwide. However, several studies have shown that *Azotobacter* and *Azospirillum* enhance plant growth, which can affect the growth and yield of numerous plant species of agricultural and environmental importance [20], [21], [19], [22]. They are not only effective in removing nitrogen. *Azotobacter* spp also has other properties such as growth hormone production, fungal production, siderophore production, and phosphate dissolving properties [23], [24], *Azospirillum* spp. Can regulate and secrete plant growth regulators (phytohormones) such as auxins (acetyl indole-3-acetic acid, IAA), cytokinins, and gibberellins, as well as nitric oxide, which may be the main signals and components. Are plant growth. Advertising effects [19]. Distance and density are very important for achieving optimal production in many vegetables. The choice of plant density is one of the most important factors that affect yield and quality. Appropriate plant distance can achieve optimal yield while plant density or very high plant density reduces yield and quality [25]. Planting density reduces the yield and quality of aromatic plants. Other researchers report that planting density alters the chemical composition of the plant [26], [15], [27], [28]. This study aimed was to investigate the application of mycorrhizal symbiosis and application of *Azotobacter* and different plant densities on the number of essential oil compounds in shoots, its functional components in *Eryngium caeruleum* as a medicinal plant to reducing chemical fertilizers to achieve sustainable agriculture.

II. MATERIALS AND METHODS

Geographical characteristics of the place for performing experiment

The experiment was conducted in fall 2013 in Aboksar, a village near Sari with a geographical location of 53 degrees and 62 minutes east, a latitude of 36 degrees 46 minutes north, and a height of 17.3 meters above sea level, This design was implemented as a factorial in the form of a randomized complete block design with 9 treatments and 5 replication.

Characteristics of experimental plan

The factors include the mycorrhiza *Glomus moseae* on two levels (inoculated and non-inoculated levels with two tons per hectare), *Azotobacter Chroococcum* (non-inoculated and inoculated with seeds at 0.5 liters per hectare CFU = 10^8), different densities of (30×30, 20×30 and 10×30 cm) and 5 tons of vermicomposting per hectare with animal origin were used for each plot. *Azotobacter Chroococcum* was prepared from the soil and water research institute. The size of each plot was about 1.5 × 1.5 square meters and six rows of planting were considered in each plot. The results of soil and organic fertilizer analysis are given in Table 1.

Conservation operation

The planting operations were conducted in November 2013, including irrigation, Weeding weeds. The plant watering was performed once every 4 days after ensuring the plant growth and establishment. To achieve proper density at the 4-5 leaf stage (after the complete plant establishment), the shrubs underwent a thinning process; and finally, the predefined density in each plot density was kept constant.

Sampling during growing season

TO measure some morphological features and essential oil content, vegetative organs were harvested. For this purpose, 8 plants per plot were randomly selected and the height and lateral branches were measured and recorded separately. The selected plants from each plot were harvested manually and separately placed inside the package and were labeled. At maturity, the final characteristics of plant Leaf yield, number of seeds per plant, and 1000 seeds weight were measured. The collected plants were kept in natural conditions away from the sun to dry for ten days. After crushing the plants, 20 grams of samples were mixed with distilled water in The Clevenger apparatus to extract the essential oil. The essential oil extraction time for all the samples was 3 hours. To identify the constituents of the essential oil, the GC / MS gas chromatography, connected to a mass spectrometer 5973 and equipped with a 30-meter HPS column with an internal diameter of 250 micrometers and the stationary phase layer thickness of 0.25 mm, was used. The oven temperature increased from 45 ° C to 250 ° C at 5 ° C per minute and then increased to 280 ° C at 20 ° C per minute. The Helium gas with ionization energy of 70 eV was used. The obtained spectra were compared with standard compounds' mass spectra. The relative percentage of each ingredient component was obtained according to the under-the-curve level in he chromatography spectrum [29].

Data analysis

The data analysis was conducted using SAS software and the mean comparisons were estimated using the LSD test at a 5% probability level.

III. RESULTS AND DISCUSSION

Number of leaves

The variance analysis results (Table 2) showed that the number of leaves in *Eryngium caeruleum* influenced by bio-fertilizer and plant density had a significant effect on the number of leaves ($P < 0.01$). The comparison revealed that the number of leaves per plant increased upon the use of bio-fertilizers so that the highest number of leaves per plant, with a mean of 13.00 leaves belonged to mycorrhizal symbiosis treatments and the lowest with the average of 10.02 leaves pertained to the control group (Figure 2, 3). Shabanzadeh et al. (2009) reported an increased weight, height, and number of leaves for the sunflower inoculated by *Azotobacter*, *Azospirillum* and *Pseudomonas* bio-fertilizers [30]. Also, the study by Hamidi et al (2007) showed that maize seeds inoculated with bio-fertilizers had a higher number of upper leaves per plant which is in line with the results of this study [31].

Flower number

The results of the variance analysis (Table 2) showed that the application of bio-fertilizer had a significant impact on



Fig. 1. Plant life stages *Eryngium caeruleum*

Table 1: Physical and chemical characteristics of soil

	OC	Potassium	Total Nitrogen	Phosphorus	OM	T.N.V	pH	EC	Depth
Texture	(%)	(ppm)	(%)	(ppm)	(%)	(%)	Paste	(dS.m-1)	(cm)
L	1.9	296	0.15	6.7	3.27	27	7.63	0.54	0-30

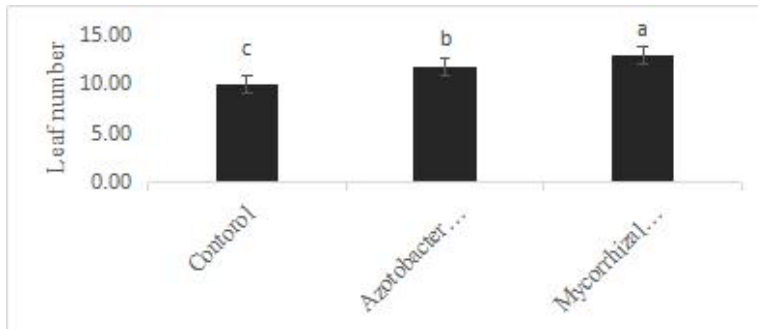


Fig. 2. The effect of Azotobacter and mycorrhiza on the number of leaves

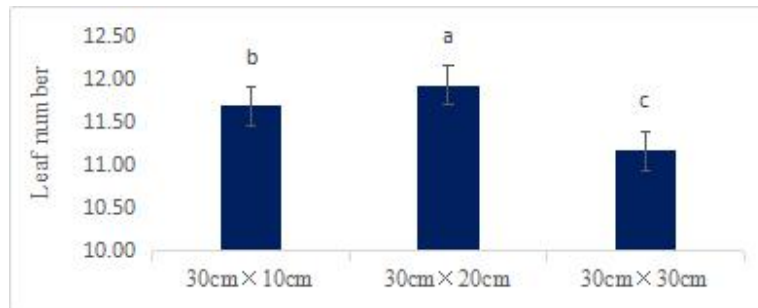


Fig. 3. The effect of different planting densities on the number of leaves

the number of flowers ($P < 0.01$), and the density and density interaction with biological fertilizer did not affect the *Eryngium caeruleum*, flower count. The mean comparison showed the highest and lowest flower counts were observed in mycorrhizal symbiosis treatment with 15.78 and control group with 10.77 (Figure 4). In a two-year the study conducted by Subramanian et al. (2006) on tomatoes, was indicated that the symbiosis of the biological tomato roots with a single species of mycorrhizal fungus significantly increased the flowers count per plant compared to the control group. Increased flowering in this study was attributed to improving the plant's absorption of water and nutrients on account of the mycorrhizal symbiosis impact [32].

Seed number per plant

The variance analysis results (Table 2) showed that the application of bio-fertilizers had a significant impact on the number of seeds per plant ($P < 0.01$). The mean comparison revealed that the highest and lowest numbers of seeds per plant were related to the group treated with mycorrhizal symbiosis and the control group as 873.73 and 624, respectively (Figure 5). Tavakoli Dinani (2008) reported that the inoculation with phosphate biological fertilizer had a significant impact on the yield and quality of two varieties of dill as medicinal plants especially on properties such as plant height, biomass, root length, number of seeds in the plant umbel and bush, biological yield and the amount of the essential oil [33]. The study conducted by Annamalai et al. (2004) also represented a significant increase in seeds due to the consumption of PSB in a medicinal plant called *Phyllanthus amarus* from the Euphorbia family compared to the control group [34].

^{ns}, * and **: non-significant and significant at 5% and 1% probability levels, respectively

Seed weight

The variance analysis results (Table 2) showed that the application of bio-fertilizers has a significant effect on one thousand seed weight ($P < 0.01$). And the density and density interaction with biological fertilizers did not affect one thousand seed weight (Figure 6). Comparing the mean, all fertilizer treatments, compared with the control group, increased one thousand seeds weight. The highest and lowest seed weight was related to the mycorrhizal symbiosis and the control group with 1.19 and 1.11 grams, respectively. In a study conducted by [35], it was shown that fennel root symbiosis with VAM fungi significantly increased the fennel seed weight. [36]

showed that the effect of bio-fertilizer on fennel one thousand seed weight was the highest when inoculated with mycorrhiza.

Root dry weight

The results of the variance analysis (Table 2) showed that the use of biological fertilizer had a significant impact on dry root weight ($P < 0.01$). The mean comparison showed that the highest and lowest dry root weight was related to the mycorrhizal symbiosis and the control group with 4.75 and 1.79 grams, respectively. (Figure 7). [37] stated that the Thyme inoculated with mycorrhizal fungi of *G. intraradices* and *G. mosseae* species increased root dry weight compared to the control. The results of the study on the Thyme grown in the garden and inoculated with mycorrhizal species indicated that the *G. mosseae* and *G. intraradices* increased the root dry weight up to 2.5 times higher than non-mycorrhizal treated groups. [38] stated that the application of vermicomposting significantly increased the root dry weight of tomatoes.

Root length

The data variance analysis results (Table 2) showed that the application of bio-fertilizers had a significant impact on root length ($P < 0.01$). And the interaction of biological fertilizer and plant density had a significant effect on root length at ($P < 0.05$). The interaction means comparison showed that the biological fertilizers had a significant effect on *Eryngium caeruleum* root length, accordingly, the highest and lowest values were related to the mycorrhizal symbiosis treatment with a plant density of 20 \times 30 cm, and the control group as 21.45 cm and 11.38 cm (Figure 8), respectively. [39] reported that plants inoculated with mycorrhiza, produced Auxin which affected the root growth. Producing plant hormone (plant growth substance) and increasing the Enzyme activities, fungi can intensify the plant and root growth leading to higher absorption of the nutrients which provide the ground for averting the drought condition. The mycorrhiza Hyphae extending out are the main factors in supplying the Phosphorus and moisture for the plant [40], [41]. According to the report by [42], the bean root in the presence of vermicomposting witnessed an increase.

(D1: plant density 30 \times 10 cm, D2: plant density 30 \times 20 cm, D3: plant density 30 \times 30 cm)

(C: Control, M: Mycorrhizal symbiosis, A: Azotobacter)

Root diameter

The variance analysis showed that the biological fertilizer application and the interaction had a significant effect on

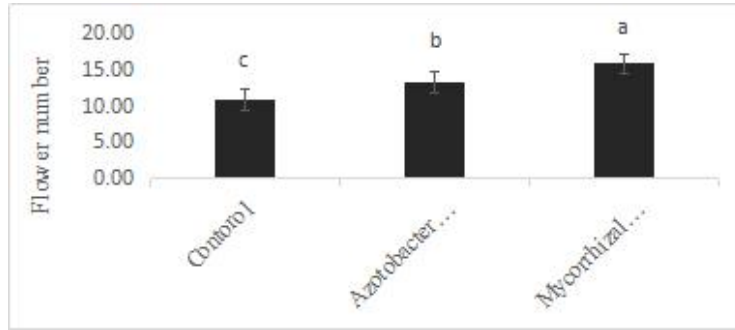


Fig. 4. The effect of Azotobacter and Mycorrhiza on the number of flowers

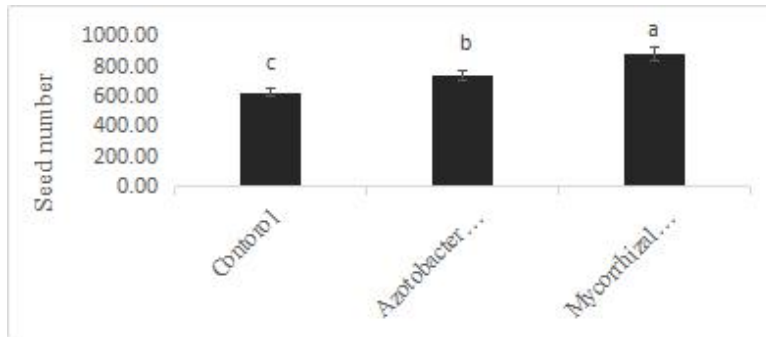


Fig. 5. Effect of Azotobacter and Mycorrhiza on seed number

Table 2: ANOVA results of factors affecting plant traits

S. o V.	df	Leaf number	Flower number	Seed number per plant	1000 seed weight	Root dry weight	Root length	Root diameter	Leaf yield
Replication	4	0.55	4.09	1368.66	0.0002	0.03	2.86	0.01	3.56
Biofertilizer	2	33.63**	94.01**	244630.06**	0.03**	32.92**	361.45**	76.07**	5721429.1**
Plant density	2	2.27**	0.62 ^{ns}	100.06 ^{ns}	0.00006 ^{ns}	0.12 ^{ns}	0.67 ^{ns}	0.06 ^{ns}	19.83**
Interaction of biofertilizer and plant density	4	0.30 ^{ns}	0.45 ^{ns}	1956.13 ^{ns}	0.00006 ^{ns}	0.17 ^{ns}	1.81*	0.15**	0.89 ^{ns}
Error	32	0.38	0.82	1179.64	0.00009	0.08	0.56	0.03	0.69
Coefficient of variations		5.34	6.80	4.60	0.80	8.75	4.59	2.90	0.04

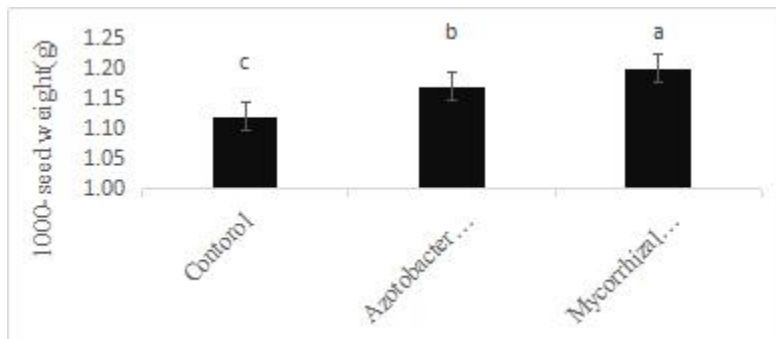


Fig. 6. Effect of Azotobacter and Mycorrhiza on 1000-seed weight

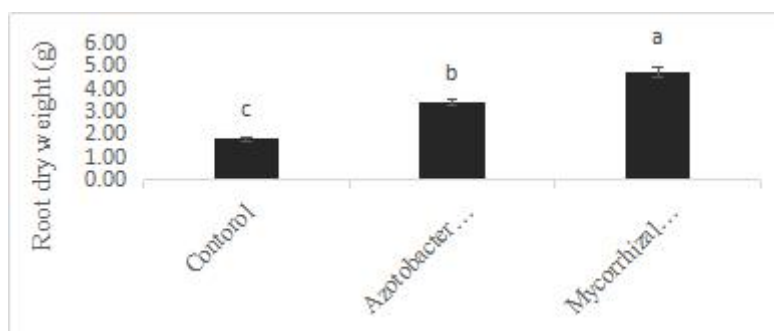


Fig. 7. Effect of Azotobacter and Mycorrhiza on root dry weight

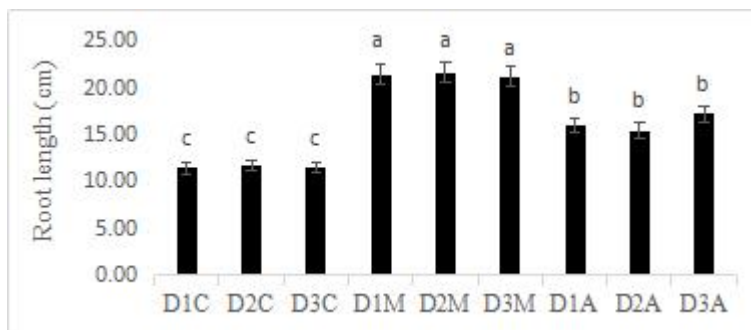


Fig. 8. The effect of different planting densities and application of biological fertilizers on root length

root diameter ($P < 0.01$) (Table 2). The interaction means comparison showed that the biological fertilizer application increased *Eryngium caeruleum* root diameter of which the highest rate belonged to mycorrhizal symbiosis treatment and plant density of 20×30 cm with 8.50 mm compared to that of the control group which was about 3.79 mm (Figure 9). [38] stated that the application of vermicomposting significantly increased tomato root diameter. Accordingly, it can be stated that vermicomposting, due to the presence of microorganisms such as mycorrhiza and its role in increasing the hydraulic conductivity of water in plant roots, is probably increasing the root diameter and dry weight. The vermicomposting increased photosynthesis and shoot growth and subsequently promoted root growth.

(D1: plant density 30×10 cm, D2: plant density 30×20 cm, D3: plant density 30×30 cm)

(C: Control, M: Mycorrhizal symbiosis, A: Azotobacter)

Leaf yield

The analysis of variance results (Table 2) showed that Azotobacter and mycorrhizal application and density had a significant impact on the *Eryngium caeruleum* leaves yield (functioning) ($P < 0.01$). The mean comparison showed that the highest and the lowest yields were witnessed for the mycorrhizal symbiosis treatment and the control group with 2574.52 and 1347.55 kg/ha, respectively (Figure 10). [43] stated that the simultaneous application of mycorrhizal and vermicomposting increased the fresh (green) leaf yield. There is so much evidence to say that plants nourished with mycorrhizal increased photosynthetic rate to provide for their symbiotic needs which are done by increasing the leaf area, increasing the stabilized amount of carbon dioxide per unit

of leaf weight and changing the water and hormonal relations (Valentine et al., 2006). Regarding the fact that the increase in leaf area would expand the leaf yield, studies have shown that mycorrhizal enhanced the plant growth and development by increasing the water absorption and transmission, providing nutrients such as phosphorus and zinc as well as improving and developing the soil microbial content and protecting the plants against pathogens [44], [45].

Essential oil ingredients

The results showed that the highest percentages of ingredients in the control group were D-limonene (1.17%), Cyclohexane (15.75%), and Naphthalene (49.37%), also, most of the ingredients in Azotobacter treatment were Falcarinol (78.52%), Morpholine (5.04%), and Naphthalene (3.33%), accordingly, the ingredients in mycorrhizal symbiosis treatment included Naphthalene (70.33%), Methyl phenyl (3.1%), Falcarinol (1.3%), and Benzaldehyde (2.23%) (Figure 11). In a study conducted by [46], the highest oil yield per hectare, as well as Chamazulene for German chamomile, was witnessed in Phosphate solubilizing bacteria (PSB) and Nitroxin (combination of Azotobacter and Azospirillum) treatments. [47] reported the use of bio-fertilizers to improve the essential oil Anatole and as a result improved the level and quality of fennel essential oil. In another study done by [47], it was indicated that the cilantro inoculation with two species of mycorrhizal fungi significantly increased the quantity and quality of essential oil in a the way that the Geraniol and Linalool values, as important components in the oil mixtures, dramatically increased. The biosynthesis of secondary metabolites in medicinal and aromatic plants depends on genetic, physiological, soil and environmental factors [9] and in partic-

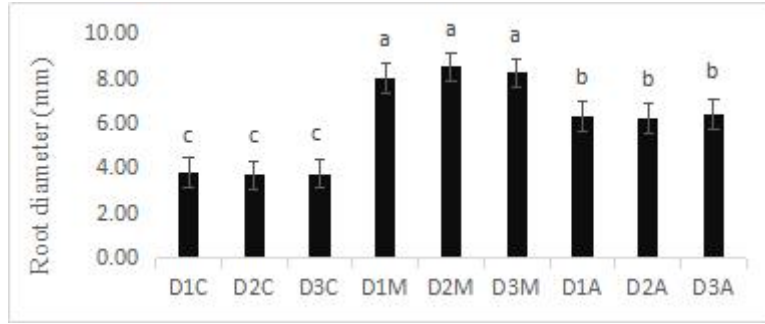


Fig. 9. The effect of different planting densities and application of biological fertilizers on root diameter

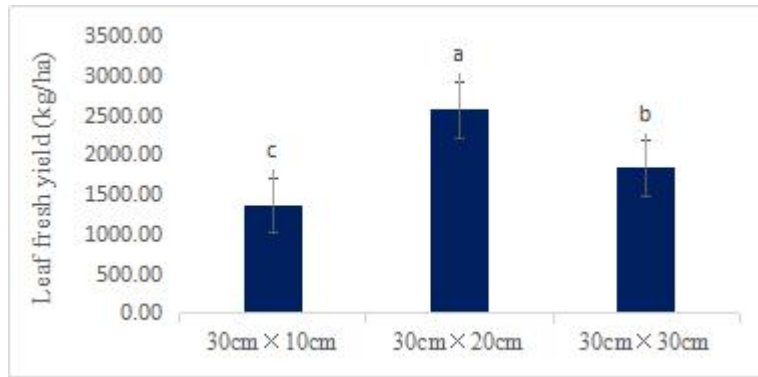


Fig. 10. The effect of different planting densities on fresh weight yield

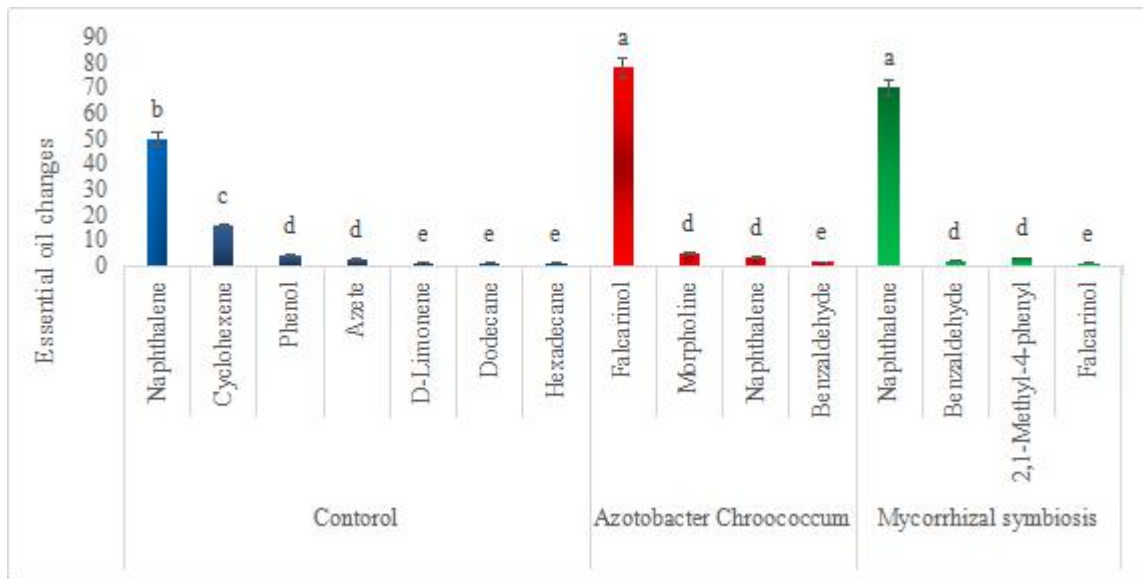


Fig. 11. The effect of using biological fertilizers on the essential oil compounds of *Eryngium caeruleum* plant

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