

Inoculation with mycorrhiza and *Azotobacter chroococcum* affects the quantitative and qualitative characteristics of *Eryngium caeruleum* at different planting densities

La inoculación con micorrizas y *Azotobacter chroococcum* afecta las características cuantitativas y cualitativas de *Eryngium caeruleum* a diferentes densidades de siembra

Mostafa Koozehgar Kaleji^{1*}, Mohammad Reza Ardakani², and Sheida Khoshnati¹

ABSTRACT

Eryngium caeruleum is a perennial native plant that grows under diverse climatic conditions of Iran. This study aimed to investigate the effect of mycorrhizal and *Azotobacter* inoculation on the quantitative and qualitative characteristics of *E. caeruleum* at various planting densities. Factors included three levels of plant density (10×30 cm, 20×30 cm, and 30×30 cm), two levels of seed inoculation with Vesicular-Arbuscular Mycorrhiza (VAM) fungi (inoculation with *Glomus mosseae* and without inoculation), and two levels of seed inoculation with *Azotobacter chroococcum* (with and without inoculation). The application of VAM fungi and *A. chroococcum* significantly affected the measured traits. The interaction effect of plant density×mycorrhizal application and plant density×*Azotobacter* application on dry leaf weight was significant at a 1% level. The total leaf dry weight for the VAM treatments at a 30×30 cm plant density was 2.93 g. Also, the application of mycorrhizal fungus increased the essential oil percentage, the essential oil yield, and phosphorus concentration in the aerial organs by 132.68%, 100%, and 137.5%, respectively, compared to the control treatment. The simultaneous application of *A. chroococcum* and VAM improved the quantity and quality of the yield components of *E. caeruleum* by increasing the availability of mineral nutrients.

Key words: oil crop, endemic herb, free-living N₂ fixers, *Glomus mosseae*, local cuisine.

RESUMEN

Eryngium caeruleum es una planta perenne nativa que crece en diversas condiciones climáticas de Irán. Este estudio tuvo como objetivo investigar el efecto de la inoculación de micorrizas y *Azotobacter* sobre las características cuantitativas y cualitativas de *E. caeruleum* en diferentes densidades de siembra. Los factores incluyeron tres niveles de densidad de plantas (10×30 cm, 20×30 cm y 30×30 cm), dos niveles de inoculación de semillas con hongos Vesículo-Arbuscular Micorriza (VAM) (inoculación con *Glomus mosseae* y sin inoculación) y dos niveles de inoculación de semillas con *Azotobacter chroococcum* (con y sin inoculación). La aplicación de hongos VAM y *A. chroococcum* afectó significativamente las características medidas. El efecto de interacción densidad de plantas×aplicación de micorrizas y densidad de plantas×aplicación de *Azotobacter* sobre el peso seco de la hoja fue significativo a un nivel del 1%. El peso seco total de las hojas para los tratamientos VAM a una densidad de plantas de 30×30 cm fue de 2.93 g. Además, la aplicación del hongo micorrízico incrementó el porcentaje de aceite esencial, el rendimiento de aceite esencial y la concentración de fósforo en los órganos aéreos en 132.68%, 100% y 137.5%, respectivamente, en comparación con el tratamiento control. La aplicación simultánea de *A. chroococcum* y hongos VAM mejoró la cantidad y calidad de los componentes del rendimiento en *E. caeruleum* al aumentar la disponibilidad de nutrientes minerales.

Palabras clave: cultivo oleaginoso, hierba endémica, fijadores de N₂ de vida libre, *Glomus mosseae*, gastronomía local.

Introduction

Eryngium caeruleum is one of the endemic plants of Iran with almost unknown potential that is neglected. It is an herbaceous perennial plant growing in northern Iran. *E. caeruleum* belongs to the family Apiaceae, represented by different species in the country. The plants of this family,

especially of the genus *Eryngium*, could have a great potential for the future production of herbal medicines. This plant is used as a medicinal plant and as a vegetable. The leaves of this plant are used to flavor cooked vegetables in various local dishes and used in soups or consumed mixed with yogurt (Khoshbakht *et al.*, 2007). Few studies have reported the content of total phenolic compounds and

Received for publication: December 16, 2022. Accepted for publication: July 17, 2023.

Doi: 10.15446/agron.colomb.v41n2.106390

¹ Department of Agronomy, Karaj Branch Islamic Azad University, Karaj (Iran).

² Department of Agronomy and Plant Breeding, Karaj Branch Islamic Azad University, Karaj (Iran).

* Corresponding author: mostafa.koozehgar@gmail.com



total flavonoids, and antioxidant properties of the alcoholic extract of the leaves (Dehghan *et al.*, 2016). This plant is widely used in the medicine and food industry as a diuretic and appetite stimulant (Hashemabadi & Kaviani, 2011). The shoots and roots contain terpenoids, saponins, flavonoids, phenolic acids, polystyrene, and other biologically active compounds (Hashemabadi & Kaviani, 2011; Erdem *et al.*, 2015). Various species of *Eryngium* contain numerous phytochemical compounds, and at least 127 compounds have been isolated and identified (Wang *et al.*, 2012; Erdem *et al.*, 2015). In addition, the plants of this genus contain various nutrients, including vitamins, minerals, and proteins (Paul *et al.*, 2011).

Using chemical fertilizers in intensive agriculture may cause damage to plant production, soil health, and environment (Anwar *et al.*, 2005). One of food security's most essential tasks is finding a suitable alternative to mineral fertilizers. The employment of these fertilizers has also increased climate pollution (Elser & Bennett, 2011; Tubiello *et al.*, 2015; Bauer *et al.*, 2016). Moreover, biodiversity has decreased significantly in agriculture due to habitat loss (Tilman *et al.*, 2017). The microorganisms and bacteria in soil affect the cycling of mineral nutrients such as nitrogen, sulfur, and phosphorus (Kumar *et al.*, 2018). Considering the beneficial microbial activities, biofertilizers have significant potential to increase the health and productivity of plants and reduce the need for synthetic fertilizers. Arbuscular mycorrhizal fungi (VAM) can form a symbiotic association with plants that benefits both partners through the acquisition and uptake of mineral nutrients, especially phosphorus, from the soil (Barea *et al.*, 2011; Hoseinzade *et al.*, 2016). Mycorrhiza, as a critical component in ecosystems, positively affects quantitative and qualitative characteristics of plants (Harrier & Watson, 2004; Gosling *et al.*, 2006). Examples of the role of VAM in agricultural ecosystems include increasing the active surface area of the root system for better uptake of nutrients from soil, especially under phosphorus deficiency (Kapoor *et al.*, 2007), increasing photosynthesis (Copetta *et al.*, 2006), increasing resistance to drought, salinity, and resistance to pests and diseases (Feng *et al.*, 2002; Pinior *et al.*, 2005; Samarbakhsh *et al.*, 2009), improving soil structure (Celik *et al.*, 2004), and increasing the activity of N₂-fixing bacteria (Antunes *et al.*, 2006). The variable performance of mycorrhizal fungi depends on the host plant; many studies have reported the positive effects of symbiosis of plants with mycorrhizal fungi (Jansa *et al.*, 2008; Pellegrino & Bedini, 2014; Derkowska *et al.*, 2015; Palencia *et al.*, 2015). Koozehgar Kaleji *et al.*

(2021) found that using mycorrhiza and organic fertilizers increased the quantitative and qualitative yield of *Nasturtium officinale*.

Azotobacter is a free-living N₂-fixing bacterium that produces various siderophores and can increase the absorption capacity of Zn, Fe, and Mo by plants, as well as the solubility of phosphorus from insoluble compounds; the use of these microorganisms constitutes one of the most effective ways to improve nutrient mobility and absorption (Mrkovacki *et al.*, 2001). *Azotobacter* are free-living rhizobacteria that promote plant growth by producing auxins, gibberellins, and cytokinins, and making mineral nutrients, especially nitrogen, available to plants (Jnawali *et al.*, 2015). Inoculation with different *Azotobacter* species increased nutrient availability and synthesis of biologically active compounds, thus, positively affecting plant growth and yield (Rojas-Tapias *et al.*, 2012; Delshadi *et al.*, 2017; Turan *et al.*, 2017; Rodrigues *et al.*, 2018).

Increasing planting density increases competition between plants for growth factors such as adequate space for branching and root growth and the acquisition of light, mineral nutrients, and moisture (Bairagi, 2014). Ahmad and Abdulla (2016) compared the effects of three planting densities (20×20 cm, 30×30 cm, and 40×40 cm) on fenugreek and observed the highest plant dry weight at the highest plant density (40×40 cm). This study evaluated the effects of the joint application of *Azotobacter chroococcum* and VAM with various plant densities on growth performance and shoot nutrient content of *E. caeruleum*.

Materials and methods

The experiment was conducted in 2016-2017 in Aboksar, a village in Mazandaran province (northern part of Iran) located at 53°62' E, 36°46' N, and an elevation of 17.3 m a.s.l. with moderate climate (Tab. 1) in a factorial randomized block design with five replicates with 685 plants per replicate and a total 3425 plants.

Factors included three levels of planting density of the *E. caeruleum* plants (10×30 cm, 20×30 cm, and 30×30 cm), two levels of seed inoculation with VAM fungi (without and with inoculation with *Glomus mosseae*, a soil-based inoculum of a local isolate, which consisted of 1200 spores/100 g of soil) and two levels of seed inoculation with *Azotobacter chroococcum* (without and with inoculation); a bacterial culture was produced by the Soil and Water Research Institute, Iran Ministry of Agriculture, using 0.5 L ha⁻¹ at the time point (CFU=108). Seeds from

TABLE 1. Climate conditions of the experiment site.

Year	Month	Air temperature (°C)			Total rainfall (mm)	Relative air humidity (%)			Solar radiation (MJ m ⁻²)	Total hours of sunshine
		Average	Max	Min		Average	Max	Min		
2016	September	21.6	26.7	16.5	205.9	76	95	57	448.3	184.5
	October	15.2	19.5	10.8	89.7	79	96	61	407.6	147.7
	November	10.7	15.3	6.1	133.2	79	96	62	261	144.1
	December	7.2	12.5	1.9	5.7	76	94	57	270	166.9
2017	January	5.2	11.5	1.4	25.4	79	95	60	259.4	153.4
	February	11.0	15.5	6.4	26.6	77	94	59	349.3	124.8
	March	14.2	19.8	8.6	53.3	76	97	54	407.2	217.4
	April	21.3	27.2	15.7	4.4	70	92	48	532.6	232.7
	May	25.3	30.8	20.0	136.1	72	94	50	636.2	273.6
	June	27.4	31.8	23.1	5.2	73	93	53	667.4	226.3
	July	28.1	33.8	22.5	8.8	67	90	44	615.5	309.9
	August	27.6	32.4	22.8	1.2	71	90	52	714.4	213.7

wild plants were surface sterilized with 0.5% sodium hypochlorite for 3 min and then washed thoroughly before sowing. For the VAM inoculation treatments, a thin layer of 5 g of *G. mosseae* inoculum containing 1200 spores per 100 g of soil was spread 2 cm below the soil surface; then, the seeds were sown separately in each plot. A vermicompost of 5 t ha⁻¹ (animal origin) was calculated and applied on each plot. The size of each plot was 1.5×1.5 m², and each plot was planted with six rows. The results of the loam soil (Entisols according to the USDA soil classification) and organic manure analysis are shown in Table 2. In the first stage, the basic tests include the determination of soil texture by the hydrometric method (Bouyoucos, 1962), organic carbon by the Walkley and Black method (Nelson, 1982), equivalent calcium carbonate by back titration method, and the electrical conductivity of saturated extract and pH of saturated soil paste (Page *et al.*, 1982).

Planting operations were carried out in November 2016, including irrigation, thinning, and weeding. Watering of the plants was done every 4 d. The plants were thinned to achieve adequate density at the 4-5 leaf stage (after the

plants were fully established). Finally, the predefined plant density was kept constant in each plot. Vegetative organs were harvested to measure morphological characteristics and essential oil content at the end of the vegetative period (formation of the flowering stem). For this purpose, eight plants per plot were randomly selected, and leaf area and dry weight of leaves were measured and recorded separately. The relative chlorophyll content in plants was measured in leaves of the highest expansion at the pre-flowering stage using SPAD 502 PLUS. The selected plants of each plot were manually harvested and separately placed in the package and labeled. The harvested plants were dried for 10 d under natural conditions without exposure to sunlight. After crushing the leaves, 20 g of the leaf samples were mixed with distilled water in the Clevenger apparatus (Sina Glass, Tehran, Iran) to extract the essential oil. The extraction time for the essential oil was 3 h for all samples. After the oil was dehydrated with sodium sulfate, the oil content was determined as a percentage. To determine nutrient concentrations, the collected leaves were dried and pulverized with an electric mill and digested with sulfuric acid, salicylic acid,

TABLE 2. Physical and chemical properties of experiment soil and chemical properties of vermicompost.

Soil	Texture	OC (%)	Potassium (mg kg ⁻¹)	Nitrogen (%)	Phosphorus (mg kg ⁻¹)	OM (%)	Lime (%)	pH	EC (dS m ⁻¹)	Depth (cm)
	Loam	1.9	296	0.15	6.7	3.27	27	7.63	0.54	0-30
Vermicompost	Nitrogen	Phosphorus (%)	Potassium (%)	Copper (mg kg ⁻¹)	Zinc (mg kg ⁻¹)	Manganese (mg kg ⁻¹)	Iron (mg kg ⁻¹)	Magnesium (%)	Calcium (%)	OC (%)
	1.3	0.98	0.45	0.98	57.7	501	1069	0.67	2.85	16.8

OC – organic carbon contents; OM – soil organic matter contents; EC – electrical conductivity.

hydrogen peroxide, and selenium to prepare the extracts (Emami, 1996). The leaf area was measured using ImageJ software. The amount of essential oil was weighed with a balance of 0.0001 g and calculated as the percentage and essential oil yield in kg ha⁻¹. Nitrogen content was determined using titration after distillation in the Kjeltac Autoanalyzer (Bremner & Mulvaney, 1982). The amount of phosphorus was analyzed by the colorimetric method (yellow molybdenite vanadate) (Emami, 1996). Foliar contents of iron, copper, zinc, manganese, and potassium were measured by atomic absorption, and potassium content was analyzed using a flame emission photometer Jenway™ PFP7 (Emami, 1996). Data were statistically analyzed by analysis of variance (ANOVA) using SAS software v 9.4, and mean comparisons were estimated at a probability level of 5% using the LSD test.

Results

Leaf dry weight

Analysis of variance (Tab. 3) showed that application of biofertilizer and the interaction of plant density and biofertilizer had a significant effect on the dry weight of leaves ($P<0.01$). The mean comparisons showed that the highest dry weights were 2.93 g when treated with VAM at a plant density of 30×30 cm (Tab. 4).

Leaf area

According to the analysis of variance (Tab. 3), the application of *Azotobacter* and VAM had a significant effect on leaf area ($P<0.01$), and density had a significant effect ($P<0.01$). Based on the mean comparison data, all fertilizer treatments increased leaf area compared to the control group.

TABLE 3. Analysis of variance with the effects of biofertilizers on *Eryngium caeruleum* traits.

Sources	df	Leaf area	Leaf dry weight	Essential oil percentage	Essential oil yield	Chlorophyll content
Replicate	4	4538.11	0.39	22.77	6.11	0.631
Density	2	15181.54**	1.01**	10.33 ^{ns}	0.00 ^{ns}	2.18 ^{ns}
VAM fungi	1	1715692.23**	5.19**	702.76**	0.348**	59300.74**
<i>Azotobacter</i>	1	162415.63**	5.87**	3484.09**	1.23**	107964.003**
Density and VAM fungi	2	7587.73 ^{ns}	1.28**	15.16 ^{ns}	0.00 ^{ns}	0.139 ^{ns}
Density and <i>Azotobacter</i>	2	798.592 ^{ns}	0.576**	18.81 ^{ns}	0.00 ^{ns}	2.16 ^{ns}
Error	32	3436.91	0.04	7.77	5.73	1.98
Coefficient of variations		9.85	9.41	0.97	0.24	6.19

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

TABLE 4. Mean comparison effect of biofertilizers on *Eryngium caeruleum* traits.

Treatment		Leaf area (cm ²)	Leaf dry weight (g)	Chlorophyll content (SPAD units)	Essential oil percentage	Essential oil yield (kg ha ⁻¹)
Plant density × without biofertilizer (Control)	D ₁	411.63 e	1.60 d	32.02 d	0.41 c	90.48 c
	D ₂	403.47 e	2.18 b	36.04 c	0.41 c	90.62 c
	D ₃	387.90 f	2.03 c	34.20 c	0.42 bc	90.78 c
Plant density × inoculation with <i>Azotobacter</i>	D ₁ A ₁	555.07 c	2.60 a	42.66 b	0.63 b	181.56 b
	D ₂ A ₁	569.38 c	2.00 c	44.70 b	0.63 b	179.83 b
	D ₃ A ₁	518.22 d	2.64 a	43.94 b	0.63 b	181.43 b
Plant density × inoculation with <i>Glomus mosseae</i>	D ₁ V ₁	846.32 b	2.04 c	54.78 a	0.82 a	210.26 a
	D ₂ V ₁	944.19 a	2.84 a	56.80 a	0.82 a	210.53 a
	D ₃ V ₁	845.55 b	2.93 a	55.34 a	0.81 a	210.80 a

Means in each column followed by similar letters are not significantly different at the 5% probability level- using LSD Multiple Range Test.

D₁: plant density 30×10 cm, D₂: plant density 30×20 cm, D₃: plant density 30×30 cm (without biofertilizer).

D₁A₁: plant density 30×10 cm × inoculation with *Azotobacter*, D₂A₁: plant density 30×20 cm × inoculation with *Azotobacter*, D₃A₁: plant density 30×30 cm × inoculation with *Azotobacter*.

D₁V₁: plant density 30×10 cm × inoculation with *G. mosseae*, D₂V₁: plant density 30×20 cm × inoculation with *G. mosseae*, D₃V₁: plant density 30×30 cm × inoculation with *G. mosseae*.

The highest and the lowest leaf area was recorded in the VAM treatment (944.19 cm²) and the control group (403.47 cm²), respectively (Fig. 1).

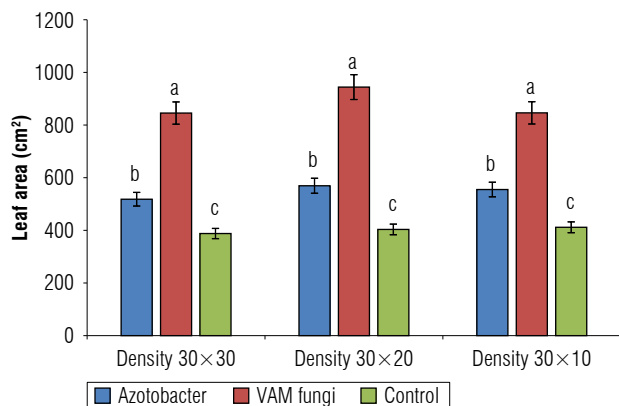


FIGURE 1. Leaf area of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Essential oil content

Analysis of variance (Tab. 3) showed that the VAM, and *Azotobacter* had a significant effect on essential oil content in leaves ($P<0.01$). The comparison of the mean interaction showed that the highest content was found in the treatments with VAM and plant density of 10×30 cm and 20×30 cm with 0.82% and the lowest in the control treatment with 0.41% (Fig. 2).

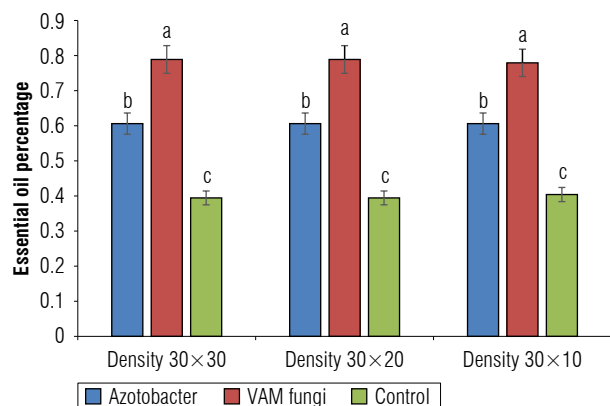


FIGURE 2. Essential oil percentage in leaves of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Essential oil yield

The results of the analysis of variance (Tab. 3) showed that the different fertilizer treatments had a significant effect on the essential oil yield of *E. caeruleum* ($P<0.01$). A comparison of mean values showed that the VAM treatments gave the highest oil yield with an average of 210.53 kg ha⁻¹, and the control treatment gave the lowest with an average of 90.48 kg⁻¹ (Fig. 3).

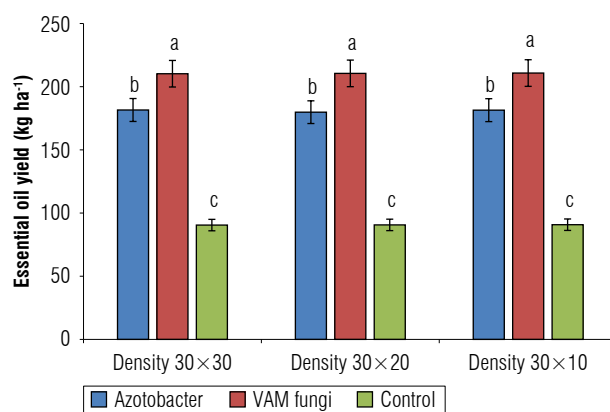


FIGURE 3. Essential oil yield in *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Chlorophyll content

The results of the analysis of variance (Tab. 3) showed that the application of biofertilizer had a significant effect on the chlorophyll index ($P<0.01$). Plant density and its interaction with biofertilizer did not affect relative chlorophyll content. The mean comparison results showed that the highest and lowest chlorophyll content were found in the VAM treatment and control treatment at 56.80 and 32.02, respectively (Fig. 4).

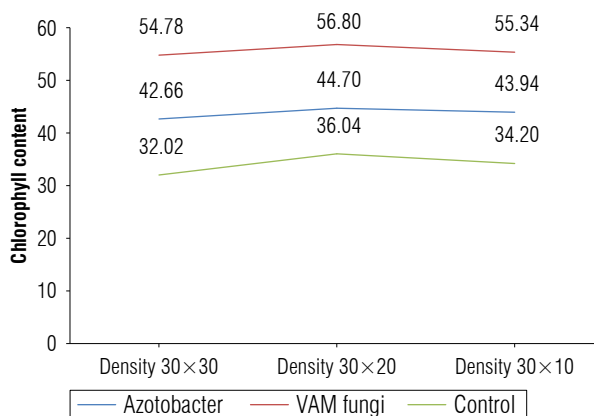


FIGURE 4. Chlorophyll content (SPAD units) in *Eryngium caeruleum* leaves after applying biofertilizers at three planting densities (cm).

Mineral nutrient uptake

Analysis of variance (Tab. 5) showed that the application of biological fertilizers significantly increased the nutrient content in *E. caeruleum* ($P<0.01$). The mean comparison results showed that the highest and lowest nitrogen content was found in the *Azotobacter* treatment and control treatment with 0.71% and 0.51%, respectively (Fig. 5). Similarly, the highest and lowest phosphorus contents were found in the VAM treatment at 0.19% and the control treatment at 0.08% (Fig. 6). Compared to the control treatment,

inoculation with VAM and *Azotobacter* increased the concentration of iron, manganese, zinc, and copper by 1749.5, 180.9, 25.22, 14.02 mg kg⁻¹, respectively, and magnesium by

0.11%. The average comparison also showed that the VAM treatment had the highest contents of potassium (0.599%) and calcium (2.88%) (Tab. 6).

TABLE 5. Analysis of variance with the effects of biofertilizers on foliar nutrient content of *Eryngium caeruleum*.

Sources	df	Nitrogen	Phosphorus	Potassium	Magnesium	Iron	Zinc	Copper	Manganese	Calcium
Replicate	4	0.001	0.19	0.00	0.05	0.08	0.37	0.26	1.65	0.54
Density	2	1.000 ^{ns}	0.086*	0.009**	0.089 ^{ns}	0.81 ^{ns}	0.948 ^{ns}	0.43 ^{ns}	49.87**	0.89 ^{ns}
VAM fungi	1	0.001 ^{ns}	1.44**	0.001**	0.690**	1597828.40**	186.00**	207.50**	19278.67**	0.16 ^{ns}
<i>Azotobacter</i>	1	0.236**	1.29**	0.001**	0.016 ^{ns}	2587438.27**	103.78**	1.82**	74710.28**	0.69**
Density and VAM fungi	2	0.001 ^{ns}	0.52**	0.001**	0.27 ^{ns}	0.440*	0.507**	0.30**	3.87**	0.49 ^{ns}
Density and <i>Azotobacter</i>	2	0.001 ^{ns}	1.02**	0.000 ^{ns}	0.049 ^{ns}	0.361 ^{ns}	0.22**	0.284**	6.82**	0.02 ^{ns}
Error	32	0.11	0.02	0.004	0.82	0.129	0.44	0.43	0.59	0.08
Coefficient of variations		1.39	3.81	0.85	6.98	0.02	0.86	2.08	0.46	0.35

ns, * and **: non-significant and significant at 5% and 1% probability levels, respectively.

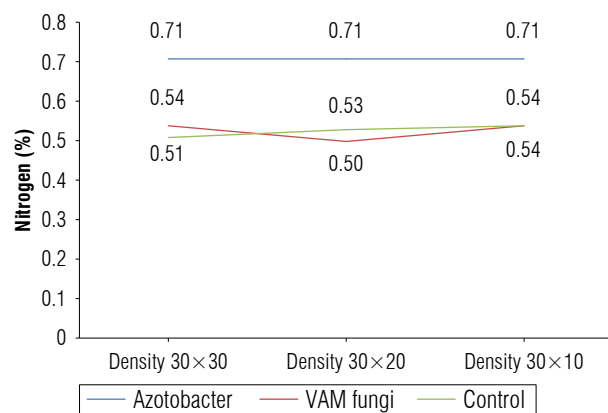


FIGURE 5. Foliar nitrogen contents of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm).

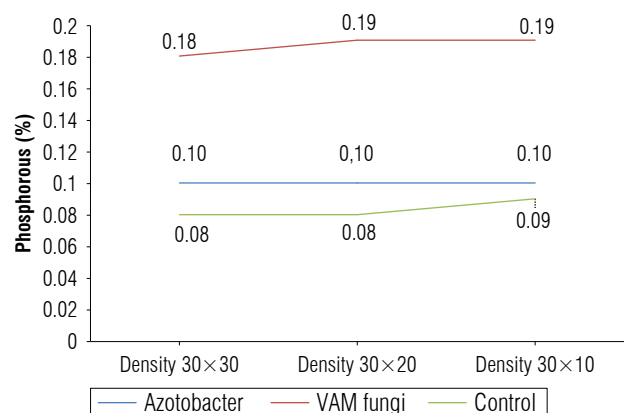


FIGURE 6. Foliar phosphorus contents of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm).

TABLE 6. Mean comparison of nutrient content in leaves of *Eryngium caeruleum* under the influence of biological fertilizer application at different planting densities.

Treatment		Nitrogen (%)	Calcium (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Iron (mg kg ⁻¹)	Zinc (mg kg ⁻¹)	Copper (mg kg ⁻¹)	Manganese (mg kg ⁻¹)
Plant density × without biofertilizer (Control)	D ₁	0.51 c	1.77 e	0.08 c	0.564 c	0.07 b	1161.5 c	22.00 c	8.79 c	77.69 c
	D ₂	0.53 b	1.80 d	0.08 c	0.608 a	0.07 b	1160.0 c	22.26 c	8.82 c	78.24 c
	D ₃	0.54 b	1.80 d	0.09 c	0.566 c	0.08 b	1161.3 c	22.19 c	8.77 c	78.96 c
Plant density × inoculation with <i>Azotobacter</i>	D ₁ A ₁	0.71 a	2.88 a	0.10 b	0.556 d	0.10 a	1748.2 a	24.48 b	9.1 b	175.84 a
	D ₂ A ₁	0.71 a	2.88 a	0.10 b	0.558 d	0.11 a	1749.5 a	25.18 a	9.0 b	179.28 a
	D ₃ A ₂	0.71 a	2.88 a	0.10 b	0.560 d	0.11 a	1749.3 a	25.22 a	9.2 b	180.9 a
Plant density × inoculation with <i>G. mosseae</i>	D ₁ V ₁	0.54 b	2.42 b	0.18 a	0.571 b	0.10 a	1622.4 b	23.50 b	13.58 a	128.08 b
	D ₂ V ₁	0.54 b	2.42 b	0.19 a	0.581 b	0.9 a	1623.2 b	24.00 a	13.74 a	128.68 b
	D ₃ V ₁	0.54 b	2.38 c	0.19 a	0.599 a	0.10 a	1622.6 b	23.60 b	14.02 a	131.94 b

Means in each column followed by similar letters are not significantly different at the %5 probability level using LSD Multiple Range Test.

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

D₁A₁: plant density 30×10 cm × inoculation with *Azotobacter*, D₂A₁: plant density 30×20 cm × inoculation with *Azotobacter*, D₃A₂: plant density 30×30 cm × inoculation with *Azotobacter*.

D₁V₁: plant density 30×10 cm × inoculation with *G. mosseae*, D₂V₁: plant density 30×20 cm × inoculation with *G. mosseae*, D₃V₁: plant density 30×30 cm × inoculation with *G. mosseae*.

Discussion

The application of VAM and *Azotobacter* significantly affected the characteristics of *E. caeruleum*, including morphological characteristics and dry weight, leaf area, chlorophyll content, percent essential oil, essential oil yield, and nutrient uptake (Tab. 3). Vermicompost with high porosity can absorb and store considerable amounts of minerals and release them gradually. In addition, its high water-holding capacity (Arancon *et al.*, 2004) affects the growth of the studied plants. Furthermore, the application of vermicompost, VAM, and *Azotobacter* increases the growth of the plants and causes a significant difference compared to the control group due to the release of macro and microelements, vitamins, enzymes, and growth-promoting hormones, as well as the increase in the number of efficient soil microorganisms. As shown in Tables 5 and 6, the application of VAM, *Azotobacter*, and vermicompost improved the morphological characteristics compared to the control group. Table 4 also shows that all measured traits improved when treated with VAM and *Azotobacter*. The results of this study are consistent with the findings of Koozehgar Kaleji and Ardakani (2018) and Koozehgar Kaleji and Ardakani (2019), who reported an increase in leaf area, essential oil percentage, essential oil yield, and dry leaf weight of *E. caeruleum* M. Bieb and *Froriepia subpinnata* with the use of organic fertilizers and mycorrhiza. Mycorrhizal symbiosis of fungi with plants can increase nutrient uptake by the plants and yield (Chen *et al.*, 2017). Other experimental results on tomato plants induced by VAM inoculation showed that leaf area and nitrogen, potassium, calcium, and phosphorus content increased (Balliu *et al.*, 2015). Another study observed that the application of *Azotobacter* and mycorrhiza increased the chlorophyll content of the plant *Prosopis chilensis* ([Molina] C.E. Hughes & G.P. Lewis) compared to the control (Faramawy, 2014). Determining the optimum plant density could be critical for plant growth and yield. Optimal plant density prevents inter-plant competition and increases product and final dry matter by increasing light, nutrient, and water uptake efficiency and reducing evapotranspiration. The increase can be attributed to the uptake of nutrients, especially phosphorus (Larimi *et al.*, 2014). Other studies have shown that *G. mosseae* increases the essential oil content of *Origanum majorana* (L.) (Khaosaad *et al.*, 2006). Also, two species of mycorrhizal fungi (*Glomus fasciculatum* [Rhizoglomus *fasciculatum* (Thaxter) Sieverding, G.A. Silva & Oehl], *Glomus macrocarpum* Tul. & C. Tul.) increased the growth and essential oil concentration of fennel (Kapoor *et al.*, 2004) and coriander (Kapoor *et al.*, 2002). Using biofertilizers increased the yield and essential oil content of *Ocimum*

basilicum L. (Al-Mansour *et al.*, 2018). Since the essential oil yield is related to the percentage of essential oil and the dry weight of the plant, any increase in these two parameters may result in a higher yield of oil. Rueda *et al.* (2016) showed that inoculation of *Azospirillum* and *Azotobacter* with *Fragaria vesca* L. increased plant height, dry weight of roots and shoots, leaf area, chlorophyll content, nutrient content, and plant yield. Tang *et al.* (2009) found that maize inoculated with *G. mosseae* had higher chlorophyll synthesis, significantly improving plant photosynthesis. This improvement was attributed to increased nitrogen uptake by inoculated plants. Inoculation of *A. chroococcum*, *Pseudomonas putida* (Trevisan), *Bacillus polymyxa* or *Paenibacillus polymyxa* (Prazmowski), and VAM significantly increased root and shoot biomass, chlorophyll, and NPK content in plants (Vafadar *et al.*, 2014). The results on phosphorus, copper, zinc, and manganese agree with the results of Koozehgar Kaleji and Ardakani (2018). Another study showed that the effect of mycorrhizal inoculation on the concentrations of iron, manganese, and copper was significant ($P < 0.01$); inoculation with VAM fungi increased the concentrations of nutrients such as iron, manganese, zinc, and copper by 142, 67.6, 21, and 12 units, respectively, compared to the control treatment (Ortas & Bykova, 2018). The results of this study are consistent with the findings of Zhang *et al.* (2016), who found that inoculation of mycorrhiza increased phosphorus contents in plants. Root colonization by VAM and bacteria can increase root length and surface area, thus, increasing water and nutrient uptake, especially under drought conditions (Marulanda *et al.*, 2006; Marulanda *et al.*, 2009). Other research results showed that inoculation with mycorrhizal fungi increased the absorption of nitrogen, phosphorus, potassium, zinc, iron, and copper in sesame plants compared to the control (Askari *et al.*, 2018).

Conclusions

Application of VAM and *Azotobacter* treatments at different plant densities increased the dry weight of leaves, essential oil yield, leaf area, and oil content. In addition, increasing plant density increased yield components. Increases in yield and yield components of *E. caeruleum* due to the use of a biological fertilizer can be attributed to the increased absorption of nutrients, such as nitrogen, phosphorus, potassium, and a microbial population in the rhizosphere in the soil. Inoculating the seed with growth-promoting bacteria apparently increased the growth of *E. caeruleum* by creating a cycle that provided nutrients through the production of fungal mycelia, which increased nutrient uptake by the roots and indicated enhanced

microbial activity in the soil. Additional tests showed that mycorrhizal symbiosis, vermicompost and *Azotobacter* applications increased plant growth and yield. The study show that the combination of low-input systems, free-living nitrogen-fixing bacteria, and VAM could be a suitable alternative to chemical fertilizers and complete fertilizers, especially in low-input systems or organic farming.

Acknowledgments

We thank all those who helped us to implement these experiments. This research was not explicitly funded.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

MK and MRA designed the experiments, MK carried out field and laboratory experiments, SK contributed to the data analysis, and MK, MRA, and SK wrote the article. All authors reviewed the final version of the manuscript.

Literature cited

- Ahmad, M. K., & Abdulla, A. R. (2016). The effect of plant density on the growth and seed yield of fenugreek (*Trigonella foenum-graecum* L.). *Journal of Zankoy Sulaimani*, 18(4), 85–90.
- Al-Mansour, B., Kalaivanan, D., Suryanarayana, M. A., Umesha, K., & Nair, A. K. (2018). Influence of organic and inorganic fertilizers on yield and quality of sweet basil (*Ocimum basilicum* L.). *Journal of Spices and Aromatic Crops*, 27(1), 38–44. <https://doi.org/10.25081/josac.2018.v27.i1.1013>
- Antunes, P. M., Deaville, D., & Goss, M. J. (2006). Effect of two AMF life strategies on the tripartite symbiosis with *Bradyrhizobium japonicum* and soybean. *Mycorrhiza*, 16(3), 167–173. <https://doi.org/10.1007/s00572-005-0028-3>
- Anwar, M., Patra, D. D., Chand, S., Alpesh, K., Naqvi, A. A., & Khanuja, S. P. S. (2005). Effect of organic manures and inorganic fertilizer on growth, herb and yield, nutrient accumulation, and oil quality of French basil. *Communications in Soil Science and Plant Analysis*, 36(13–14), 1737–1746. <https://doi.org/10.1081/CSS-200062434>
- Arancon, N. Q., Edwards, C. A., Atiyeh, R., & Metzger, J. D. (2004). Effects of vermicomposts produced from food wasted on the growth and yields of greenhouse peppers. *Biore-source Technology*, 93(2), 139–144. <https://doi.org/10.1016/j.biortech.2003.10.015>
- Askari, A., Ardakani, M. R., Vazan, S., Paknejad, F., & Hosseini, Y. (2018). The effect of mycorrhizal symbiosis and seed priming on the amount of chlorophyll index and absorption of nutrients under drought stress in sesame plant under field conditions. *Applied Ecology and Environmental Research*, 16(1), 335–357. https://doi.org/10.15666/aer/1601_335357
- Bairagi, S. K. (2014). Effect of different doses of phosphorus and row spacing on the yield and quality of fenugreek (*Trigonella foenum-graecum* L.) seed. *The Asian Journal of Horticulture*, 9(2), 338–341. <https://doi.org/10.15740/HAS/TAJH/9.2/338-341>
- Balliu, A., Sallaku, G., & Rewald, B. (2015). AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability*, 7(12), 15967–15981. <https://doi.org/10.3390/su71215799>
- Barea, J. M., Palenzuela, J., Cornejo, P., Sánchez-Castro, I., Navarro-Fernández, C., López-García, A., Estrada, B., Azcón, R., Ferrol, N., & Azcón-Aguilar, C. (2011). Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. *Journal of Arid Environments*, 75(12), 1292–1301. <https://doi.org/10.1016/j.jaridenv.2011.06.001>
- Bauer, S. E., Tsigaridis, K., & Miller, R. (2016). Significant atmospheric aerosol pollution caused by world food cultivation. *Geophysical Research Letters*, 43(10), 5394–5400. <https://doi.org/10.1002/2016GL068354>
- Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal*, 54, 464–465.
- Bremner, J. M., & Mulvaney, C. S. (1982). Nitrogen-total. In A. L. Page, R. H. Miller, & D. R. Keeney (Eds.), *Methods of soil analysis. Part 2. Chemical and microbiological properties* (pp. 595–624). American Society of Agronomy, Soil Science Society of America, Inc. Book Series. Madison, Wisconsin. <https://doi.org/10.2134/agronmonogr9.2.2ed>
- Celik, I., Ortas, I., & Kilic, S. (2004). Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil and Tillage Research*, 78(1), 59–67. <https://doi.org/10.1016/j.still.2004.02.012>
- Chen, S., Zhao, H., Zou, C., Li, Y., Chen, Y., Wang, Z., Jiang, Y., Liu, A., Zhao, P., Wang, M., & Ahammed, G. J. (2017). Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. *Frontiers in Microbiology*, 8, Article 2516. <https://doi.org/10.3389/fmicb.2017.02516>
- Copetta, A., Lingua, G., & Berta, G. (2006). Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. *Genovese*. *Mycorrhiza*, 16, 485–494. <https://doi.org/10.1007/s00572-006-0065-6>
- Dehghan, H., Sarrafi, Y., & Salehi, P. (2016). Antioxidant and anti-diabetic activities of 11 herbal plants from Hyrcania region, Iran. *Journal of Food and Drug Analysis*, 24(1), 179–188. <https://doi.org/10.1016/j.jfda.2015.06.010>
- Delshadi, S., Ebrahimi, M., & Shirmohammadi, E. (2017). Effectiveness of plant growth promoting rhizobacteria on *Bromus tomentellus* Boiss seed germination, growth and nutrients uptake under drought stress. *South African Journal of Botany*, 113, 11–18. <https://doi.org/10.1016/j.sajb.2017.07.006>
- Derkowska, E., Sas Paszt, L., Harbuzov, A., & Sumorok, B. (2015). Root growth, mycorrhizal frequency and soil microorganisms in strawberry as affected by biopreparations. *Journal of Advances in Microbiology*, 5(1), 65–73. <https://doi.org/10.4236/aim.2015.51007>
- Elser, J., & Bennett, E. (2011). A broken biogeochemical cycle. *Nature*, 478, 29–31. <https://doi.org/10.1038/478029a>
- Emami, A. (1996). *Plant decomposition methods* (Vol. 1, Technical Leaflet No. 982). Soil and Water Research Institute (In Persian).

- Erdem, S. A., Nabavi, S. F., Orhan, I. E., Daglia, M., Izadi, M., & Nabavi, S. M. (2015). Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus *Eryngium*. *DARU Journal of Pharmaceutical Sciences*, 23(1), Article 53. <https://doi.org/10.1186/s40199-015-0136-3>
- Faramawy, F. M. K. (2014). Response of *Prosopis chilensis* to biofertilization under calcareous soil of RasSudr. 2 – Pod production. *Annals of Agricultural Sciences*, 59(2), 263–271. <https://doi.org/10.1016/j.aos.2014.11.015>
- Feng, G., Zhang, F., Li, X., Tian, C., Tang, C., & Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*, 12, 185–190. <https://doi.org/10.1007/s00572-002-0170-0>
- Gosling, P., Hodge, A., Goodlass, G., & Bending, G. D. (2006). Arbuscular mycorrhiza fungi and organic farming. *Agriculture, Ecosystems & Environment*, 113(1-4), 17–35. <https://doi.org/10.1016/j.agee.2005.09.009>
- Harrier, L. A., & Watson, C. A. (2004). The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Management Science*, 60(2), 149–157. <https://doi.org/10.1002/ps.820>
- Hashemabadi, D., & Kaviani, B. (2011). Chemical constituents of essential oils extracted from the leaves and stems of *Eryngium caucasicum* Trautv. from Iran. *Journal of Essential Oil Bearing Plants*, 14(6), 693–698. <https://doi.org/10.1080/0972060X.2011.10643991>
- Hoseinzade, H., Ardakani, M. R., Shahdi, A., Asadi Rahmani, H., Noormohammadi, G., & Miransari, M. (2016). Rice (*Oryza sativa* L.) nutrient management using mycorrhizal fungi and endophytic *Herbaspirillum seropedicae*. *Journal of Integrative Agriculture*, 15(6), 1385–1394. [https://doi.org/10.1016/S2095-3119\(15\)61241-2](https://doi.org/10.1016/S2095-3119(15)61241-2)
- Jansa, J., Smith, F. A., & Smith, S. E. (2008). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist*, 177(3), 779–789. <https://doi.org/10.1111/j.1469-8137.2007.02294.x>
- Jnawali, A. D., Ojha, R. B., & Marahatta, S. (2015). Role of *Azotobacter* in soil fertility and sustainability—a review. *Advances in Plants & Agriculture Research*, 2(6), 250–253. <https://doi.org/10.15406/apar.2015.02.00069>
- Kapoor, R., Chaudhary, V., & Bhatnagar, A. K. (2007). Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza*, 17, 581–587. <https://doi.org/10.1007/s00572-007-0135-4>
- Kapoor, R., Giri, B., & Mukerji, K. G. (2002). Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil. *Journal of the Science of Food and Agriculture*, 82(4), 339–342. <https://doi.org/10.1002/jsfa.1039>
- Kapoor, R., Giri, B., & Mukerji, K. G. (2004). Improved growth and essential oil yield and quality in *Foeniculum vulgare* mill on mycorrhizal inoculation supplemented with P-fertilizer. *Bioresource Technology*, 93(3), 307–311. <https://doi.org/10.1016/j.biortech.2003.10.028>
- Khaosaad, T., Vierheilig, H., Nell, M., Zitterl-Eglseder, K., & Novak, J. (2006). Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). *Mycorrhiza*, 16(6), 443–446. <https://doi.org/10.1007/s00572-006-0062-9>
- Khoshbakht, K., Hammer, K., & Pistrick, K. (2007). *Eryngium caucasicum* Trautv. cultivated as a vegetable in the Elburz Mountains (Northern Iran). *Genetic Resources and Crop Evolution*, 54(2), 445–448. <https://doi.org/10.1007/s10722-006-9121-5>
- Koozehgar Kaleji, M., & Ardakani, M. R. (2018). Quantitative and qualitative performance of *Froriepia subpinnata* as affected by mycorrhizal symbiosis, compost tea, and vermicompost. *Iranian Journal of Plant Physiology*, 8(3), 2457–2467. <https://doi.org/10.30495/IJPP.2018.540990>
- Koozehgar Kaleji, M., & Ardakani, M. R. (2019). Effects of organic fertilizers application on yield and yield components of *Eryngium caeruleum* M. Bieb. affected by mycorrhizal symbiosis. *Iranian Journal of Medicinal and Aromatic Plants Research*, 34(6), 924–935. <https://doi.org/10.22092/ijmapr.2019.120403.2235>
- Koozehgar Kaleji, M., Ardakani, M. R., Abedini Aboksari, H., Bandegani Rooieen, M., & Khosniat, S. H. (2021). Effect of mycorrhizal symbiosis and application of organic fertilizers on photosynthetic pigments, yield and nutrient content *Nasturtium officinale*. *Crop Production*, 14(1), 87–102. <https://doi.org/10.22069/EJCP.2021.18599.2379>
- Kumar, V., Kumar Dubedi Anal, A., & Nath, V. (2018). Growth response of litchi to arbuscular mycorrhizal co-inoculation with *Trichoderma viride*, *Azotobacter chroococcum* and *Bacillus megatarium*. *Indian Phytopathology*, 71, 65–74. <https://doi.org/10.1007/s42360-018-0010-6>
- Larimi, S. B., Shakiba, M., Mohammadinasab, A. D., & Vahed, M. M. (2014). Changes in nitrogen and chlorophyll density and leaf area of sweet basil (*Ocimum basilicum* L.) affected by biofertilizer and nitrogen application. *International Journal of Biosciences*, 5(9), 256–265. <http://doi.org/10.12692/ijb/5.9.256-265>
- Marulanda, A., Barea, J. N., & Azcón, R. (2006). An indigenous drought-tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. *Microbial Ecology*, 52, 670–678. <https://doi.org/10.1007/s00248-006-9078-0>
- Marulanda, A., Barea, J.-M., & Azcón, R. (2009). Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *Journal of Plant Growth Regulation*, 28, 115–124. <https://doi.org/10.1007/s00344-009-9079-6>
- Mrkovacki, N., & Milic, V. (2001). Use of *Azotobacter chroococcum* as potentially useful in agricultural application. *Annals of Microbiology*, 51(2), 145–158.
- Nelson, R. E. (1982). Carbonate and gypsum. In A. L. Page (Ed.), *Methods of soil analysis. Part 2. chemical and microbiological properties* (pp. 181–197). American Society of Agronomy, Soil Science Society of America.
- Ortas, I., & Bykova, A. (2018). The effect of mycorrhiza inoculation and phosphorus application on phosphorus efficiency of wheat plants. *Communications in Soil Science and Plant Analysis*, 49(10), 1199–1207. <https://doi.org/10.1080/00103624.2018.1455849>

- Page, A. L., Miller, R. H., & Kenney, D. R. (1982). *Methods of soil analysis. Part 2. Chemical and microbiological properties* (2nd ed.). Agronomy Society of America, Soil Science Society of America.
- Palencia, P., Martínez, F., Pestana, M., Oliveira, J. A., & Correia, P. J. (2015). Effect of *Bacillus velezensis* and *Glomus intraradices* on fruit quality and growth parameters in strawberry soilless growing system. *The Horticulture Journal*, 84(2), 122–130. <https://doi.org/10.2503/hortj.MI-002>
- Paul, J. H. A., Seaforth, C. E., & Tikasingh, T. (2011). *Eryngium foetidum* L.: A review. *Fitoterapia*, 82(3), 302–308. <https://doi.org/10.1016/j.fitote.2010.11.010>
- Pellegrino, E., & Bedini, S. (2014). Enhancing ecosystem services in sustainable agriculture: biofertilization and biofortification of chickpea (*Cicer arietinum* L.) by arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 68, 429–439. <https://doi.org/10.1016/j.soilbio.2013.09.030>
- Pinior, A., Grunewaldt-Stocker, G., von Alten, H., & Strasser, R. J. (2005). Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll *a* fluorescence, praline content and visual scoring. *Mycorrhiza*, 15(8), 596–605. <https://doi.org/10.1007/s00572-005-0001-1>
- Rodrigues, M. Â., Ladeira, L. C., & Arrobas, M. (2018). *Azotobacter*-enriched organic manures to increase nitrogen fixation and crop productivity. *European Journal of Agronomy*, 93, 88–94. <https://doi.org/10.1016/j.eja.2018.01.002>
- Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D., & Bonilla, R. (2012). Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Applied Soil Ecology*, 61, 264–272. <https://doi.org/10.1016/j.apsoil.2012.01.006>
- Rueda, D., Valencia, G., Soria, N., Rueda, B. B., Manjunatha, B., Kundapur, R. R., & Selvanayagam, M. (2016). Effect of *Azospirillum* spp. and *Azotobacter* spp. on the growth and yield of strawberry (*Fragaria vesca*) in hydroponic system under different nitrogen levels. *Journal of Applied Pharmaceutical Science*, 6(1), 48–54. <https://doi.org/10.7324/JAPS.2016.600108>
- Samarbakhsh, S., Rejali, F., Ardakani, M. R., Pak Nejad, F., & Miransari, M. (2009). The combined effects of fungicides and arbuscular mycorrhiza on corn (*Zea mays* L.) growth and yield under field conditions. *Journal of Biological Sciences*, 9(4), 372–376. <https://doi.org/10.3923/jbs.2009.372.376>
- Tang, M., Chen, H., Huang, J. C., & Tian, Z. Q. (2009). AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. *Soil Biology and Biochemistry*, 41(5), 936–940. <https://doi.org/10.1016/j.soilbio.2008.11.007>
- Tilman, D., Clark, M., Williams, D. R., Kimmel, K., Polasky, S., & Packer, C. (2017). Future threats to biodiversity and pathways to their prevention. *Nature*, 546, 73–81. <https://doi.org/10.1038/nature22900>
- Tubiello, F. N., Salvatore, M., Ferrara, A. F., House, J., Federici, S., Rossi, S., Biancalani, R., Condor Golec, R. D., Jacobs, H., Flammini, A., Prosperi, P., Cardenas-Galindo, P., Schmidhuber, J., Sanz Sanchez, M. J., Srivastava, N., & Smith, P. (2015). The contribution of agriculture, forestry and other land use activities to global warming, 1990–2012. *Global Change Biology*, 21(7), 2655–2660. <https://doi.org/10.1111/gcb.12865>
- Turan, M., Yildirim, E., Kitir, N., Unek, C., Nikerel, E., Ozdemir, B. S., Güneş, A., & Mokhtari, N. E. P. (2017). Beneficial role of plant growth-promoting bacteria in vegetable production under abiotic stress. In A. Zaidi, & M. S. Khan (Eds.), *Microbial strategies for vegetable production* (pp. 151–166). Springer Science Reviews, Springer. https://doi.org/10.1007/978-3-319-54401-4_7
- Vafadar, F., Amooaghaie, R., & Otrushy, M. (2014). Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *Journal of Plant Interactions*, 9(1), 128–136. <https://doi.org/10.1080/17429145.2013.779035>
- Wang, P., Su, Z., Yuan, W., Deng, G., & Li, S. (2012). Phytochemical constituents and pharmacological activities of *Eryngium* L. (Apiaceae). *Pharmaceutical Crops*, 3, 99–120. <https://doi.org/10.2174/2210290601203010099>
- Zhang, X., Wang, L., Ma, F., Yang, J., & Su, M. (2016). Effects of arbuscular mycorrhizal fungi inoculation on carbon and nitrogen distribution and grain yield and nutritional quality in rice (*Oryza sativa* L.). *Journal of the Science of Food and Agriculture*, 97(9), 2919–2925. <https://doi.org/10.1002/jsfa.8129>