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Original Article

Effects of Chemical and Biological Fertilizers on Growth, Yield and Essential Oil of *Salvia officinalis*

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Abstract

This experiment was conducted in 2012 at the research field of Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran, to study the effect of chemical and biological fertilizers on Sage (*Salvia officinalis* L.) and replacing biofertilizers instead of high doses of chemical fertilizers. The experiment was conducted in factorial in the form of a randomized complete block design with three replications and two factors: chemical nitrogen and phosphorus fertilizers in four levels (N_0P_0, N_0P_{150}, $N_{300}P_0$ and $N_{300}P_{150}$) and biological fertilizers in four levels (non inoculated control, mycorrhizal inoculation with *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe + *Glomus intraradices* N.C. Schenck & G.S. Sm., bacterial inoculation with *Pseudomonas fluorescens*, and dual inoculation with *G. mosseae* +*G. intraradices* + *P. fluorescens*). The measured traits included: plant height, the number of tillers, leaf area, leaf yield, shoot yield, root weight, essential oil percentage and essential oil yield. Results indicated the significant effect of chemical fertilizer on all measured traits except for the number of tillers. Biofertilizer application had also significant effect on all measured traits except for essential oil percentage. The interaction of the two factors had only a significant effect on leaf area and leaf yield. Mean comparison showed that the highest essential oil yield (37.02 kg/ha) was achieved in N_0P_{150}×*Pseudomonas* which was significantly the same as N_0P_{150}×mycorrhizal inoculation and N_0P_{150}×dual inoculation with mycorrhizae + *Pseudomonas*. Generally, results of this experiment indicated that it is possible to replace biofertilizers instead of high doses of chemical fertilizers in order to reduce the need for chemical fertilizers and prevent the associated problems.

Key words: Mycorrhiza, Nitrogen, Phosphorus, *Pseudomonas*, Sage

Introduction

There is an increasing worldwide trend in using medicinal plants to improve human health and to cure diseases; so the medicinal plants are playing more important role in the economy of countries [1]. The quality of agricultural products has always been under attention and the residue of chemical fertilizers in the products is an important factor of the quality. This is more vital for medicinal plants because they are used in cosmetic, hygienic and pharmaceutical industries in addition to the food industries. As the result, in recent years there have been increasing attempts to provide plants nutritional requirements through non-chemical sources. Of course it is not logical to totally remove chemical fertilizers from the agricultural production cycle; however, it looks possible to reduce the need for chemical fertilizers by the application of non-chemical sources such as the biofertilizers [2].

Biological activity in soil is an important index of soil fertility which can be improved by the application of biofertilizers [3]. Mycorrhiza fungi are among the most important microorganisms which can be applied in soil as biofertilizer. They form symbiotic relation with 83% of the

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dicotyledones and 79% of the monocotyledons; only a few field crops are not able to accept mycorrhizal symbiosis [4]. The benefits of mycorrhizal symbiosis for the field crops were first understood in 1885; mycorrhiza fungi improve soil physical, chemical and biological properties. The hyphae of the mycorrhiza increase plant absorptive surface, and penetrate into soil pores and cracks; making more volume of the soil available to plant which consequently increases water and nutrients absorption. Mycorrhizae also increase the availability of phosphorus to plants and improve plant resistance to biotic and abiotic stresses [4-7].

Mycorrhizae fungi are more effective in soils with lower fertility and nutrient content. Hayman [8] attributed the yield improvement of field crops in symbiosis with mycorrhiza to the enhancement of plant absorptive surface and nutrient uptake. Mycorrhizae facilitate nutrients translocation in plant organs and increase plant growth rate; Liderman [9] found that P content was higher in mycorrhizal plants than in the non-mycorrhizal plants. Khaosaad *et al*. [2] also found an enhancement in P content of the shoot of oregano (*Origanum* L.) when inoculated with mycorrhiza. Kohneh *et al*. [10] reported that mycorrhizal inoculation increased P uptake in tea plants. In addition to phosphorus, mycorrhiza may increase plant uptake of Zn, Cu, S, Ca, K, Fe, Mn and Cl [11]. Arriagada *et al*. [12] observed that mycorrhizal inoculation increased N, P and K content in *Eucalyptus globules* compared with the non-inoculated control plants. Improvement of plant nutrient uptake results in the enhancement of plant growth and yield. Copetta *et al*. [13] reported that mycorrhizal symbiosis increased basil growth and yield parameters such as plant height, the number of leaves, leaf area, biomass, the number of tillers and essential oil content. Vinutha [14] also reported that inoculation basil plants with mycorrhiza fungi increased biomass, crop growth rate and essential oil content.

In addition to the mycorrhizae fungi, plant growth promoting rhizobacteria are also beneficial to plants; *Pseudomonas fluorescens* is one of these bacteria. These bacteria affect plant growth through direct and indirect mechanisms. Direct mechanisms are biological nitrogen fixation, enhancement of nutrients availability to plants, production of phytohormones and iron chelating siderophores etc. Indirect mechanisms are the inhibition of pathogens activity and production of antibiotics [15-17]. Behbood *et al*. [18] reported that application of *Pseudomonas fluorescens* bacteria increased potato yield and macro/micronutrients content. Abdul-Jaleel *et al*. [19] found that inoculating *Catharanthus roseus* with *Pseudomonas fluorescens* resulted in the enhancement of biomass under water deficit conditions.

The benefits of biofertilizers are well studied on crop plants; however, low information is in hand about their effects on medicinal plants, especially Sage (*Salvia officinalis* L.). Sage belongs to one of the well known species of the Lamiaceae family. This plant has culinary and medicinal features [20]. Sage is an aromatic, evergreen perennial plant native to the Mediterranean area [21].

The objective of this experiment was to evaluate the possibility of using biofertilizers to reduce the need for chemical fertilizers in sustainable and healthy sage production system.

Material and Methods

This experiment was conducted in 2012 at the research field of Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran (35° 48′ N, 51° E, 1320 m above the sea level). Mean annual precipitation is 235 mm. The minimum and maximum temperature of the test site area is -20 °C and 38 °C, respectively. The soil type was a clay loam (clay, 16%; silt, 40%; sand, 44%) with the pH of 7.36. and EC of 1.33 ds/m. Further soil physico-chemical properties are listed in Table 1.

The experiment was conducted in factorial in the form of a randomized complete block design with three replications and the following treatments:

Chemical fertilizers (factor A). Chemical nitrogen (N) and phosphorus (P) fertilizers in four levels:

A_1 (N_0P_0, control): without any chemical fertilizer application.

A_2 (N_0P_{150}): 150 kg/ha phosphorus fertilizer (triple super phosphate) was applied to the field at the field preparation stage.

A_3 ($N_{300}P_0$): 300 kg/ha nitrogen fertilizer (46% urea) was applied to the field at the field preparation stage.

A_4 ($N_{300}P_{150}$): 300 kg/ha nitrogen fertilizer and 150 kg/ha phosphorus fertilizers were applied to the field at the field preparation stage.

Biofertilizers (factor B). Biofertilizers were in four levels:

B_1 (control): without any bacterial or fungal inoculation.
B_2: 10 g mycorrhizal powder (*G. intraradices*+*G. Mosseae*) with the population of 30-35 spores/g of soil was applied under each transplant when they were being planted in the main field.
B_3: Transplants roots were soaked in *Pseudomonas fluorescens* strain 187 (105 active bacteria/g) inoculation for 20 minutes before planting at the main field.
B_4 (B_2+B_3, dual inoculation): 5 g/plant mycorrhizal powder (*G. intraradices*+*G. Mosseae*) + *P. fluorescens* strain 187.
The selected field had been under fallow for several years. In late spring, the field was prepared using a deep moldboard plow, disk and then a leveler. Plots were 3 × 3 m with 45 cm inter-row spacing and 40 cm on-row spacing. Irrigation was conducted two times a week by furrow method and weeds were controlled manually during the growth period.
Sage (*S. officinalis*) transplants were held out of the greenhouse for two weeks for hardening, prior to planting them in the main field. Then, at the planting time, transplant roots were soaked with the bacterial inoculation and the required mycorrhizal powder was poured in each planting hole.
At the full flowering stage, plant height, the number of tillers, leaf area, leaf yield, shoot yield, root weight, essential oil percentage and essential oil yield were evaluated. To measure root weight, three plants were uprooted from each plot, roots were cleaned from soil, were dried and were weighted. To evaluate leaf area, samples were taken in early morning and leaves were scanned; the area was measured by AutoCAD software [22]. Finally, to study the essential oil, 80 g of the dried tissue plus with 1.5 L water were poured in 2 L Clevenger. Essential oil was produce by hydrodistillation for 2 h. Data were analyzed using SAS 9.1 and means were compared according to the Duncan's multiple range test at P≤0.05.

Results

Plant height

Analysis of variance indicated the significant effect of chemical and biological fertilizer on plant height; however, the effect of their interactions was not significant (Table 2). Mean comparison of chemical fertilizers showed that among the highest value of this trait (59.42 cm) was observed in $N_{300}P_0$ treatment (Table 3). Mean comparison of the biofertilizers indicated that plant height was the highest (61.58 cm) in mycorrhiza + *Pseudomonas* treatment (Table 4). Among the interactions, plant height was the highest (74.3 cm) in $N_{300}P_{150}$ × the dual inoculation (Table 5).

The number of tillers

Results showed that the number of tillers was significantly affected only by biofertilizer; the effects of chemical fertilizer and the interaction of the two factors were not significant on this trait (Table 2). Mean comparison indicated that among the chemical fertilizers, the number of tillers was the highest (9.02) in N_0P_0 (Table 3), and among the biofertilizers, the trait was the highest (10.5) in the dual inoculation of mycorrhiza+*Pseudomonas* (Table 4). Mean comparison of the interactions also showed that the number of tillers was the highest (14.2) in N_0P_0 × the dual inoculation (Table 5).

Table 1 Properties of the test site soil.

SP (%)	TNV (%)	OC (%)	Total N (%)	P_{ava} (ppm)	K_{ava} (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)
24.63	10.1	0.79	0.08	14.4	378.4	7.72	0.5	1.34	17.72	0.464

Table 2 Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares							
		Plant height	The number of tillers	Leaf area	Leaf yield	Shoot yield	Root weight	Essential oil percentage	Essential oil yield
Block	2	ns	*	**	**	**	*	ns	*
Chemical fertilizer	3	**	ns	**	**	**	**	**	**
Biofertilizer	3	**	**	**	**	**	**	ns	**
A × B	9	ns	ns	*	*	ns	ns	ns	ns
Error	30	139.48	0.19	0.16	179.72	1.27	275.83	0.03	90.09
CV (%)	-	23.53	16.1	17.05	17.05	15.56	16.86	21.22	31.98

ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

Table 3 The effect of chemical fertilizers on the measured traits.

Treatments	Plant height (cm)	The number of tillers	Leaf area (m^2)	Leaf yield (kg/ha)	Shoot yield (kg/ha)	Root weight (g/plant)	Essential oil percentage (%)	Essential oil yield (kg/ha)
N_0P_0	38.57 c	9.02 a	2.15 b	3863.6 b	6324.5 b	74.6 b	0.21 b	10.95 b
N_0P_{150}	47.07 bc	8.38 ab	3.2 a	5754.8 a	9448.6 a	109.02 a	0.41 a	26.8 a
$N_{300}P_0$	59.42 a	6.75 b	2.24 b	4029.3 b	6906.1 b	103.66 a	0.19 b	8.01 b
$N_{300}P_{150}$	55.67 ab	7.1 ab	2.03 b	3649.02 b	6153.1 b	106.57 a	0.41 a	15.47 b

Means in a column followed by the same letter are not significantly different at P≤0.01.

Table 4 The effect of biofertilizers on the measured traits.

Treatments	Plant height (cm)	The number of tillers	Leaf area (m^2)	Leaf yield (kg/ha)	Shoot yield (kg/ha)	Root weight (g/plant)	Essential oil percentage (%)	Essential oil yield (kg/ha)
Control	37.4 c	5.51 c	1.54 c	740.9 d	4540.1 d	69.85 d	0.24 a	7.14 b
Mycorrhiza	46.21 bc	6.68 bc	2.21 b	1225.8 c	6576.9 c	86.21 c	0.32 a	14.33 ab
Pseudomonas	55.55 ab	8.57 ab	2.76 a	1501.2 b	8160.7 b	102.51 b	0.41 a	21.17 a
Myco + Pseu	61.58 a	10.5 a	3.1 a	1962.4 a	9554.5 a	135.3 a	0.30 a	18.59 a

Means in a column followed by the same letter are not significantly different at P≤0.01.

Table 5 The effect of the interaction of chemical fertilizer × biofertilizers on the measured traits.

Treatments	Plant height (cm)	The number of tillers	Leaf area (m^2)	Leaf yield (kg/ha)	Shoot yield (kg/ha)	Root weight (g/plant)	Essential oil percentage (%)	Essential oil yield (kg/ha)
A_1B_1	29.8 f	4.0 d	1.06 f	1905.7 f	3233 h	58.9 e	0.10 d	2.06 f
A_1B_2	39.0 d-f	8.0 b-d	1.78 c-f	3198.4 c-f	5512 e-g	69.8 de	0.11 cd	4.05 ef
A_1B_3	44.1 c-f	9.8 b	2.5 c	4481.4 c	7189 de	77.8 c-e	0.33 a-d	16.04 b-f
A_1B_4	41.3 d-f	14.2 a	3.26 ab	5868.8 ab	9363 bc	91.8 b-d	0.32 a-d	21.67 a-e
A_2B_1	35.3 ef	7.4 b-d	2.19 cd	3937.2 cd	5883 e-g	78.2 c-e	0.25 a-d	10.3 c-f
A_2B_2	41.1 d-f	7.7 b-d	3.27 ab	5887.2 ab	9121 b-d	96.18 b-d	0.54 ab	31.73 ab
A_2B_3	53.0 a-e	9.0 bc	3.49 a	6271.8 a	10529 ab	114.4 b	0.61 a	37.02 a
A_2B_4	58.8 a-d	9.3 bc	3.85 a	6923.03 a	12260 a	147.1 a	0.41 a-d	28.15 a-c
A_3B_1	46.6 c-f	4.6 cd	1.27 ef	2283.9 ef	4113 gh	70.5 de	0.27 a-d	5.78 ef
A_3B_2	54.6 a-e	5.2 b-d	1.9 c-e	3401.01 c-e	5930 e-g	88.8 b-e	0.14 cd	4.96 ef
A_3B_3	46.5 a-c	8.4 b-d	2.52 c	4533.6 c	7667 d-e	107.8 bc	0.15 cd	7.27 d-f
A_3B_4	71.8 a-b	8.6 b-d	3.28 ab	5898.9 ab	9913 b	147.4 a	0.21 b-d	14.02 c-f
A_4B_1	71.7 d-f	5.9 b-d	1.65 d-f	2972.5 d-f	4929 f-h	71.7 de	0.36 a-d	10.42 c-f
A_4B_2	50.0 b-e	5.7 b-d	1.9 c-e	3396.6 c-e	5744 e-g	89.9 b-e	0.49 a-c	16.57 b-f
A_4B_3	60.5 a-d	6.9 b-d	2.55 bc	4593.6 bc	7256 de	109.9 b	0.54 ab	24.35 a-d
A_4B_4	74.3 a	9.7 b	2.02 c-e	3633.3 c-e	6681 ef	154.7 a	0.23 a-d	10.53 c-f

A_1, N_0P_0; A_2, N_0P_{150}; A_3, $N_{300}P_0$; A_4, $N_{300}P_{150}$

B_1, non-inoculated control; B_2, Mycorrhiza; B_3, *Pseudomonas*; B_4, Mycorrhiza + *Pseudomonas*

Means in a column followed by the same letter are not significantly different at P≤0.01.

Leaf area and leaf yield

Analysis of variance indicated that leaf area and leaf yield was both significantly affected by chemical fertilizer, biological fertilizer and the interaction of the two factors (Table 2). Mean comparison of the effect of chemical fertilizer (Table 3) showed that leaf area and leaf yield were the highest in N_0P_{150} (3.2 m^2 and 5754.8 kg/ha, respectively.) Studying the mean comparison of biological fertilizers (Table 4) showed that the dual inoculation with mycorrhiza+*Pseudomonas* resulted in the highest leaf area (3.1 m^2) and leaf yield (1962.4 kg/ha). Among the interactions (Table 5), N_0P_{150}×the dual inoculation of mycorrhiza + *Pseudomonas* had the highest leaf area (3.85 m^2) and leaf yield (6923.03 kg/ha).

Shoot yield

Results showed that shoot yield was significantly affected by chemical and biological fertilizers; however, the effect of their interaction was not significant (Table 2). Among the chemical fertilizer treatments, shoot yield was the highest (9448.6 kg/ha) in N_0P_{150} (Table 3) and among the biofertilizers it was the highest (9554.5 kg/ha) in the dual inoculation of mycorrhiza+*Pseudomonas* (Table 4). Studying the mean comparison of the interaction of chemical fertilizer×biological fertilizer indicated that the highest shoot yield (12260 kg/ha) was achieved in N_0P_{150}×the dual inoculation of mycorrhiza+*Pseudomonas* (Table 5).

Root weight

Analysis of variance indicated the significant effect of chemical fertilizer and biological fertilizer on root weight; the effect of their interaction was not significant (Table 2). Mean comparison of the chemical fertilizer levels indicated that root weight was the highest (109.02 g/plant) in N_0P_{150} (Table 3). Mean comparison of the biological fertilizers also showed that the root weight was the highest (135.3 g/plant) in the dual inoculation of mycorrhiza+*Pseudomonas* (Table 4). Among the interactions, root weight was the highest (147.4 g/plant) in the interaction of $N_{300}P_0$×the dual inoculation of mycorrhiza+*Pseudomonas* (Table 5).

Essential oil percentage

Results of our experiment showed that essential oil percentage was significantly affected only by chemical fertilizer; the effect of biological fertilizer and the interaction of the two factors was not significant (Table 2). Mean comparison of the chemical fertilizer levels indicated that the highest essential oil percentage (0.41%) was achieved in both N_0P_{150} and $N_{300}P_{150}$ (Table 3). Studying the mean comparison of biological fertilizers showed that although essential oil percentage was the highest (0.41%) in *Pseudomonas* treatment; however, there were no differences between levels (Table 4). Results indicated that among the interactions, essential oil percentage was the highest (0.61%) in the interaction of N_0P_{150}×*Pseudomonas* (Table 5).

Essential oil yield

Results indicated that chemical fertilizer and biological fertilizer significantly affected essential oil yield; the effect of their interactions was not significant (Table 2). Among the chemical fertilizers, essential oil yield was the highest (26.8 kg/ha) in N_0P_{150} (Table 3), and among the biological fertilizers, essential oil yield was the highest (21.17 kg/ha) in *Pseudomonas* (Table 4). Mean comparison of the interactions also showed that essential oil yield was the highest (37.02 kg/ha) in the interaction of N_0P_{150}×*Pseudomonas* (Table 5).

Discussion

Results of our experiment indicated the significant effect of chemical nitrogen and phosphorus fertilizers on the measured traits. Nitrogen and phosphorus are two macronutrients which are involved in many plant processes. Nitrogen is the main yield limiting mineral nutrient. Nitrogen takes part in many physiological and biochemical plant processes and is a structural component of amino acids, nucleic acids, enzymes and proteins, chlorophyll and cell wall [7,23]. Phosphorus is also a highly required macronutrient; playing vital roles in energy transfer, cell membranes, nucleic acids and other key compounds [7,24]. In our experiment, co-application of nitrogen and phosphorus ($N_{300}P_{150}$ treatment) increased plant height by 44.33% and essential oil yield by 41.28% compared with the control (Table 3). The effect of chemical fertilizers on the growth, yield and essential oil of medicinal plants is observed in other experiments. Rohricht *et al*. [25] observed that shoot yield and essential oil yield of *S. officinalis* increased when 100-150 kg N/ha was applied. Clark and Menary [26] studied the effect of irrigation and nitrogen fertilizer on peppermint and reported that the highest essential oil yield was achieved when 300 kg N/ha was applied. Leiser and Rokman [27] also reported that application of chemical nitrogen fertilizer increased hyssop (*Hyssopus Officinalis* L.) essential oil yield. Kucey *et al*. [28] found that enhancement of available phosphorus content in soil increased plant root system development and shoot growth and consequently plant yield. Similar results were also reported by Afkhami [29] on balm (*Melissa officinalis* L.) and Ajimoddin *et al*. [30] on basil (*Ocimum basilicum* L.).

Results of our experiment also showed the significant effect of fungal and bacterial inoculations on plant growth, yield and essential oil. The symbiosis with mycorrhizae fungi is the most common and frequently occurred symbiosis between microorganisms and plants [4]. The hyphae of mycorrhiza are connected to plant roots and increase the absorptive surface of plant root system. They penetrate into soil pores and cracks; making more volume of the soil available to plant which consequently increases water and nutrients absorption. This will enable plants to better tolerate the environmental stresses such as the drought stress. The hyphae of mycorrhiza release fixed P from soil and make it available to plants [4-7]. Results of our experiment showed that mycorrhizal inoculation increased sage shoot yield by 44.86% and essential oil yield by 100.7% compared with the non-inoculated control (Table 4). Vinutha [14] found an improvement in basil plant biomass, crop growth rate and essential oil content when inoculated with mycorrhiza fungi. In another experiment on basil, Copetta *et al*. [13] also found that mycorrhizal inoculation improved plant height, the number of leaves, leaf area, biomass, the number of tillers and essential oil content. Joshee *et al*. [31] inoculated *Scutellaria integrifolia* L. roots with mycorrhizal fungi and reported that the inoculation increased plant root system development and also increased the ability of plant to grow in low fertile soils; verifying the effect of mycorrhizal symbiosis on plants nutrient absorption capability. Sailo and Bagyaraj [32] tested the effect of mycorrhizal inoculation on *Coleus forskohlii* (Willd) Briq. and observed an improvement in plant height, the number of branches, biomass, phosphorus content and the quality of the essential oil. The effect of mycorrhizal inoculation on plant nutrient absorption is also reported by Khaosaad *et al*. [2] on oregano, Kohneh *et al*. [10] on tea plants and Arriagada *et al*. [12] on eucalyptus.

It was observed in our experiment that in addition to mycorrhiza, *P. fluorescens* inoculation also increased sage growth, yield and essential oil. Pseudomonas bacteria are reported to have plant growth promoting activity. They convert fixed P in soil to soluble forms by releasing organic acids such as formic acid and acetic acid. The mechanisms by which *Pseudomonas* bacteria improve plant growth and yield can be divided into two categories: direct and indirect mechanisms. Direct mechanisms such as biological nitrogen fixation, enhancement of nutrients availability to plants, increasing the available P to plants through enzymatic and non enzymatic phosphate solubilization methods, production of phytohormones such as auxin, cytokinin and gibbereline, exudation of iron chelating siderophores, and finally, the synergistic relation with other microorganisms which improves the efficiency of the symbiotic system. Indirect mechanisms by which *Pseudomonas* bacteria affect plants are the inhibition of pathogens activity and production of antibiotics [15-17,33,34]. In our experiment, the inoculation of *Pseudomonas* increased shoot yield by 79.75% and essential oil yield by 196.50%, compared with the non-inoculated control (Table 4). Abdul-Jaleel *et al*. [19] found that inoculating *Catharanthus roseus* (L.) G.Don with *P. fluorescens* increased plant biomass under water deficit conditions. Behbood *et al*. [18] also reported that application of *P. fluorescens* increased potato yield and macro/micronutrients content. Few researchers have studied the effect of *Pseudomonas* inoculation on medicinal plants; however, there are reports about the effect of this bacterial inoculation on crop plants. Afzal and Asghari [35] found an improvement in wheat growth and yield as the result of *Pseudomonas* application. Studies of Shah *et al*. [36] also showed the application of *Pseudomonas* increased soil P content and affected soybean growth.

The main objective of our experiment, in addition to testing the effect of chemical and biological fertilizers, was to test the possibility of applying biofertilizers instead of the high doses of chemical fertilizers. Results showed that essential oil yield, which is the main objective of production in medicinal plants, was higher in A_1B_4 treatment which was a biological treatment (no chemical fertilizer × dual inoculation with mycorrhiza + *Pseudomonas*) than in A_4B_1 treatment which was a chemical treatment ($N_{300}P_{150}$ × no inoculation) (Table 5). It represents that when production of higher essential oil yield is desired, application of biofertilizers is preferred to chemical fertilizers. However, the highest essential oil yield was achieved when a combination of chemical and biological fertilizers was used (A_2B_3) (Table 5).

References

1. Anwar M, Patra DD, Chand S, Khanuja SPS. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. Commun. Soil Sci. Plant Anal. 2005; 14:1737-1746.
2. Khaosaad T, Vierheilig H, Nell M, Zitterl-Eglseer K, Novak J, Arbuscular mycorrhiza alter the concentration of essential oils in oregano *Origanum* sp., Lamiaceae. Mycorrhiza. 2006;16:443-446.
3. Singh S, Kapoor KK. Inoculation with phosphate solubilizing microorganisms and a vesicular arbuscular mycorrhizal fungus improves dry matter yield and

nutrient uptake by wheat grown in a sandy soil. Biol. Fertil. Soils. 1998;28:139-144.

4. Gianinazzi S. Vesicular-Arbuscular EndoMycorrhizas Technology in Agriculture: from Genes to Byproducts. Birkhauser, Basel, 1991.
5. Cardoso IM, Kuyper TW. Mycorrhizas and tropical soil fertility. Agric., Ecosyst. Environ. 2006;116:72-84.
6. Smith SE, Read DJ. Mycorrhizal Symbioses, 3rd ed. Academic Press, United Kingdom, 2008.
7. Wiedenhoeft AC. Plant Nutrition. Chelsea House Publishers, USA, 2006.
8. Hayman DS. The physiology of VA-endo mycorrhizal symbiosis. Can. J. Bot. 1983;61:944-963.
9. Liderman RG. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology. 1988;78:366-371.
10. Kohneh A, Haghparast Tanha M, Ramezanpour H. Study of the effect of arbuscular mycorrhizal fungi and phosphorus on P uptake of tea plants in a sterilized soil. Iran J. Agric. Sci. 2007;38:11-17.
11. Sharma AK. Biofertilizer for Sustainable Agriculture. Agrobios, India, 2003.
12. Arriagada CA, Herrera MA, Ocampo JA, Beneficial effect of saprobe and arbuscular mycorrhizal fungi on growth of Eucalyptus globules co-cultured with *Glycine max* in soil contaminated with heavy metals. J. Environ. Manage. 2007;84:93-99.
13. Copetta A, Lingua G, Berta G. Effects of three AM fungi on growth, distribution of grandular hairs and essential oil production in *Ocimum basilicum*. Mycorrhiza. 2006;16:485-494.
14. Vinutha T. Biochemical Studies on *Ocimum* sp. Inoculated with Microbial Inoculants. M.Sc. thesis, University of Agricultural Sciences, Bangalore, India. 2005.
15. Glick BR, Patten CL, Holguin G, Penrose DM. Biochemical and Genetic Mechanisms used by Plant Growth Promoting Bacteria. Imperial College Press, London, United Kingdom, 1999.
16. Shimon M, Tirosh T, Glick BR. Plant growth-promoting bacteria confer resistance in tomato plant to salt stress. Plant Physiol. Biochem. 2004;42:565-572.
17. Zahir AZ, Arshad M, Frankenberger WF. Plant growth promoting rhizobacteria: Application and perspectives in agriculture. Adv. Agron. 2004;81:97-168.
18. Behbood M, Golchin A, Besharati H. The effect of phosphorus and *Pseudomonas fluorescens* on yield and quality of *Solanum tuberosum* L. cv. Ageria. Iran J. Soil Water 2012;26:260-271.
19. Abdul-Jaleel C, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, Panneerselvam R. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* underwater deficit stress. Colloids Surf. B. 2007;60: 7-11.
20. Devecchi M. The use of Labiatae of ornamental interest in the design of parks and gardens. Acta Hort. 2006;723:51-57.
21. Santos-Gomes PC, Seabra RM, Andrade PB, Fernandes-Ferreira M. Phenolic antioxidant compounds produced by in vitro shoots of sage (*Salvia officinalis* L.). Plant Sci. 2002;162:981-987.
22. Asiabanpour B, Yousefzadeh Vafaei J. AutoCAD Training. Iran Mosavvar Publications, Tehran, Iran, 1998.
23. Fageria NK. The Use of Nutrients in Crop Plants. CRC Press, USA, 2009.
24. Tiessen, H. Phosphorus in the Global Environment. In: White PJ, Hammond JP (eds). The Ecophysiology of Plant-Phosphorus Interactions. Springer, USA, 2008, pp: 1-8.
25. Rohricht CM, Curunet M, Solf M, The influence of grandaunt nitrogen fertilization application on yield and quality of sage *Salvia officinalis*. Zetischritft fur Arznei and Gewurzpflanzen. 1996;3:117-122.
26. Clark RJ, Menary RC. The effect of irrigation and nitrogen on the yield and composition of peppermint oil. Aust. J. Agric. Res. 1979;31,489-498.
27. Leiser A, Rokman B. Replication between fertilizer, nutrient withdrawal and composition of different medicinal plants in a plot experiment. Kongressband. 1994;9:19-24.
28. Kucey RMN, Janzen HH, Leggett ME. Microbially mediated increases in plant available phosphorus. Adv. Agron. 1998;42:199-225.
29. Afkhami S. Effect of Phosphate Solubilizing Bacterial and Sulfur on Yield and Quality of Balm (*Melissa officinalis* L.). M.Sc. thesis, Islamic Azad University, Karaj branch, Iran, 2013.
30. Ajimoddin I, Vasundhara M, Radhakrishna D, Biradar SL, Rao GGe. Integrated nutrient management studies in sweet basil (*Ocimum basilicum* L.). Indian Perfum. 2005;49:95-101.
31. Joshee N, Mentreddy SR, Yadav K. Mycorrhizal fungi and growth and development of micropropagated *Scutellaria integrifolia* plants. Ind. Crops Prod. 2007;25:169-177.
32. Sailo GL, Bagyaraj DJ. Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. Mycol. Res. 2005;109:795-798.
33. Glick BR. The Enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 1995;41:109-117.
34. Molla AH, Shamsuddin ZH, Halimi MS, Morziah M, Puteh AB. Potential for enhancement of root growth and nodulation of soybean co-inoculation with Azospirillum and Bradyrhizobium in laboratory systems. Soil Biol. Biochem. 2001;33:457-463.
35. Afzal A, Asghari B. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum* L.). Int. J. Agri. Biol. 2008;10:85-88.
36. Shah P, Kakar KM, Zada K. Phosphorus use efficiency of soybean as affected by phosphorus application and inoculation. Plant Nut. 2001;92:670-671.