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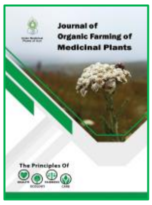

Original Article

Effects of vermicompost and mycorrhizal symbiosis on some morphological traits and seed yield of clary sage (*Salvia sclarea* L.)

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ABSTRACT

In order to study the effect of vermicompost and mycorrhizal fungi on some agronomic characteristics of clary sage (*Salvia sclarea* L.) this research was carried out in Alborz Research Station, Iran's Forestry and Rangelands Research Institute in 2012-2013 under field conditions. The experiment was carried out as a factorial arrangement based on a randomized complete block design with three replications. The factors included vermicompost at four levels (0, 3, 6, and 9 tons ha^{-1}) and mycorrhizal fungi at four levels (no application, application of *Glomus mosseae*, application of *Glomus intraradices*, and combined application of *Glomus mosseae* + *Glomus intraradices*). The results showed that the effect of mycorrhizal fungus and vermicompost on plant height, leaf length, leaf area index, seed yield, and leaf width was significant. The effect of the interaction of treatments on leaf area index, mycorrhizal symbiosis, and 1000 seed weight was significant. The highest seed yield (617.7 kg ha^{-1}) was obtained from the application of 9 tons of vermicompost per hectare. The mean comparison for different mycorrhizal levels showed that all the measured traits had the highest values in the combined inoculation of seeds with *G. mossea* and *G. interaradices*. The highest leaf area index (2.160) was obtained from the combined treatment of 9 tons per hectare of vermicompost and inoculation of seeds with *G. mossea* + *G. interaradices*. The highest mycorrhizal symbiosis (36.67%) was obtained in the combined treatment of 9 tons per hectare of vermicompost and seed inoculation with *G. interaradices*.

1. Introduction

Conventional agricultural systems rely on the use of fossil fuel products such as pesticides and chemical fertilizers and have made a significant contribution in increasing the production rate and reducing the required labor force in the agricultural sector. In addition to providing the food needed by a population growth in the global ecosystem has played an important role (Sharma, 2002b).

The basic solution to reduce toxic residue in human foods, is moving towards sustainable agriculture based on the use of in-farm inputs, including biofertilizers (Arancon et al., 2004; Sharma, 2002a).

One of the main components in sustainable agriculture is the use of biological fertilizers in agricultural ecosystems with the aim of eliminating or significantly reducing the consumption of chemical inputs. Biofertilizers are used to provide nutrients needed by plants in the agroecosystems (Darzi et al., 2006).

Among the types of biofertilizers, mycorrhizal fungi and vermicompost are widely used in sustainable agricultural systems in order to increase the quality and stability of crops and medicinal plants. Mycorrhizal fungi have a symbiotic relationship with the roots of most agricultural plants and through increasing the absorption of nutrients such as phosphorus, nitrogen and some microelements, increasing water absorption, producing plant hormones, reducing the negative impact of environmental stress and increased resistance against pathogenic agents, will improve the growth and yield of plants in sustainable agricultural systems (Sharma, 2002a; Arguello et al., 2006; Arancon et al., 2004).

Ghazi Manas et al. (2013) in study on the effects of different amounts of vermicompost and nitrogen on the quantitative and qualitative yield of German chamomile showed that the effect of vermicompost on the dry flowers weight, percentage of essential oil, dry and fresh flowers yield and chamazulene were significant.

Darzi et al. (2013) showed that the highest amount of essential oil in the seed (4.21%) was obtained when using 10 tons of vermicompost.

Clary sage (*Salvia sclarea* L.) is one of the importance medicinal plants in Iran and the world. The flowers of this plant contain aromatic essential oil, which is widely used in health and cosmetic, food and beverage industries (Omidbeigi, 2000). The studies conducted on mycorrhizal symbiosis in clary sage are very limited. Considering the above-mentioned reasons, this research was conducted with the aim of investigating the effect of vermicompost and mycorrhizal inoculation on some morphologic traits, seed yield and percentage of mycorrhizal symbiosis on sage.

2. Material and Method

2.1 Field experiment

This research was carried out in 2012-2013 in order to study the effect of vermicompost and mycorrhizal fungi on the quantitative and qualitative yield of the clary sage under field conditions at the Alborz Medicinal Plant Research Station in Karaj, Iran. The physical and chemical characteristics of the soil of the experimental site were analyzed before soil preparation (Table 1).

Table 1. Physicochemical properties of farm soil

Texture	Clay (%)	Silt (%)	Sand (%)	phosphorus (ppm)	potassium (ppm)	Total nitrogen%	pH
Loam	20	42	38	8.5	138	0.07	7.9
T.N.V %	**Organic carbon %**	**Electrical conductivity**	**Iron(ds / m)**	**Zinc(ppm)**	**Copper (ppm)**	**Manganese (ppm)**	
11.75	0.68	1.33	1.34	0.62	0.48	10.04	

The experiment was carried out as a factorial arrangement based on a randomized complete block design with three replications. The experimental factors included four amounts of vermicompost (0, 3, 6 and 9 tons ha^{-1}) and four levels of mycorrhizal fungi (no application, application of *Glomus mosseae*, application of *Glomus intraradices*, and combined application of *Glomus mosseae* + *Glomus intraradices*). Mycorrhizal fungus used in this research was prepared from Turan Biotechnology Company.

According to the recommendation of the Soil and Water Research Institute to applying 150 kg of this fungus per hectare in the fields and on the basis of the plant density per unit area in this research, the amount of fungus required for each plant was calculated in the laboratory with a digital scale. After that, the fungus was placed next to the seeds at the time of planting. The vermicompost used in this research was obtained from Beh Saman Company, which physical and chemical characteristics are presented in Table 2.

Table 2. Results of chemical and physical properties of vermicompost.

Electrical conductivity (dS m^{-1})	OM (%)	OC (%)	phosphorus (%)	potassium (%)	Nitrogen (%)
1.1	65	37.7	0.61	3.19	4.92
pH	**Zn (ppm)**	**Mn ppm)**	**Fe (ppm)**	**Moisture (%)**	
7	27-40	15-25	36-50	25	

The nitrogen requirement of sage is reported to be about 100 kg of net nitrogen per hectare (Omidbeigi, 2000). According to 4.92% of nitrogen in vermicompost and the release of nutrients in organic fertilizers in the first year is equal to 35%, about 6 tons per hectare of vermicompost is necessary to meet the nitrogen requirement of the plant.

The dimensions of the experimental plots were 3 × 2.5 meters, the distance between the replications was 2 meters, the distance between the plots was 1 meter. The seeds were obtained from Alborz Research Station and planted on rows at a distance of 30 cm from each other and depth of 3 cm with between-row spacing of 50 cm

Irrigation was repeated every 7 days. The measured traits were included plant height, leaf area index, leaf length and width, percentage of mycorrhizal symbiosis and seed yield. In order to measure traits, two middle rows were taken. Gridline Intersect Method was used to determine the symbiosis percentage of mycorrhizal fungus with roots (Giovannetti, 1980).

2.4 Statistical analysis

The statistical analysis of data obtained from the experiment was performed using SAS software. Duncan's multiple range tests was used to compare the means of treatments and Excel program was used to draw the graphs.

3. Results

Mycorrhiza and vermicompost had significant effects on plant height, leaf length, leaf area index, seed yield and leaf width and their interaction had significant effects on leaf area index, mycorrhizal symbiosis and 1000 seed weight (Table 3).

The highest plant height (114.7 cm) was obtained from the use of 9 tons vermicompost per hectare and the lowest plant height (94.45 cm) was obtained in the control treatment (Table 4).

Also, the combined application of *G. mossea* + *G. interaradices* caused the highest plant height (115.8 cm) (Table 5).

Table 3. Analysis of variance (mean of squares) for the effect of vermicompost and mycorrhizal symbiosis on clary sage (*Salvia sclarea* L.).

S.O.V	df	Height	Leaf Length	Leaf width	LAI	1000 Seed Weight	Seed Yield	Symbiosis
Replication	2	1309.25**	17.88**	21.09**	0.65**	0.12	184436.02**	2.02
Mycorrhiza (M)	3	972.65**	2.46	8.11**	0.28**	0.18	108127.84**	1510.30**
Vermicompost (V)	3	947.06**	6.33**	5.92*	0.86**	4.99**	88974.51**	88.02**
M × V	9	39.36	0.81	1.23	0.12**	0.74**	11281.29	9.21**
Error	30	76.74	0.96	1.51	0.002	0.16	1337.73	2.95
CV (%)	-	8.24	6.82	8.35	2.51	7.30	11.02	6.58

Table 4. Mean comparisons of vermicompost effects on clary sage (*Salvia sclarea* L.)

Vermicompost (ton ha^{-1})	Height (Cm)	Leaf Length (Cm)	Leaf width (Cm)	LAI	1000 Seed Weight (g)	Seed Yield (kg ha^{-1})	Symbiosis (%)
0	97.45b	13.30b	9.84b	1.42d	4.68c	423.9c	23.17c
3	99.90b	14.57a	10.20b	1.69c	5.57b	459.7bc	24.58c
6	113.20a	14.90a	11.45a	1.92b	5.49b	536.5ab	27.39b
9	114.70a	14.71a	10.21b	2.03a	6.25a	617.7a	29.08a

Means followed by similar letters in each column do not significantly differ at α= 5% probability level based on Duncan's test.

Table 5. Mean comparisons of mycorrhizal symbiosis on clary sage (*Salvia sclarea* L.)

Mycorrhiza	Height (Cm)	Leaf Length (Cm)	Leaf width (Cm)	LAI	1000 Seed Weight (g)	Seed Yield (kg ha^{-1})	Symbiosis (%)
Control	94.20c	14.02b	9.64b	1.54c	5.64a	398.80b	9.33c
G.mosseae	106.70b	14.38ab	11.01a	1.84a	5.53a	586.45a	31.08b
G.intraradices	108.50b	14.08b	9.80b	1.80b	5.48a	461.74b	31.00b
G.mosseae+G.intraradices	115.80a	15.01a	11.25a	1.87a	5.34a	590.70a	33.02a

Means followed by similar letters in each column do not significantly differ at α= 5% probability level based on Duncan's test.

The maximum length and width of leaves were obtained from the consumption of 6 tons vermicompost per hectare (Table 4) and the combined application of two types of fungi *G. mossea* + *G. interaradices* (Table 5). The highest seed yield was obtained from the applying of 9 tons vermicompost per hectare (Table 4) and the combined application of two types of mushrooms *G. mossea* + *G. interaradices* (Table 5).

The highest leaf area index (2.160), mycorrhizal symbiosis (36.67 percent) and 1000 seed weight (6.857 grams) were obtained in the combined treatment of 9 tons vermicompost per hectare and the combined application of *G. mossea* + *G. interaradices*, the use of *G. interaradices* and the use of *G. mossea* (Fig. 1-3).

Vermicompost had a significant effect on plant height. According to the previous findings, vermicompost have a positive effect on photosynthesis and dry matter production through increasing water absorption and optimal availability of macro and microelements (Hameeda et al., 2006; Gardezi et al., 2000).

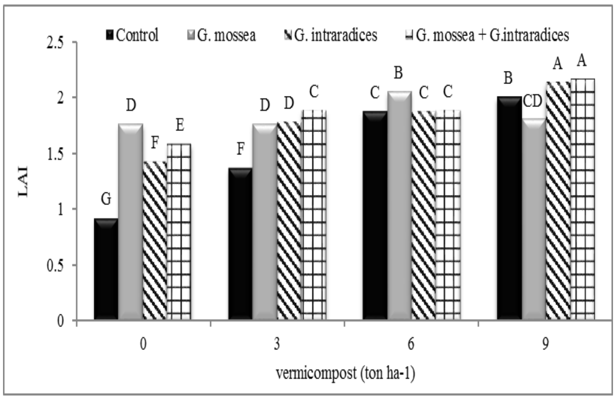

Fig 1. The interaction effect of vermicompost and mycorrhizal symbiosis on the leaf area index of clary sage.

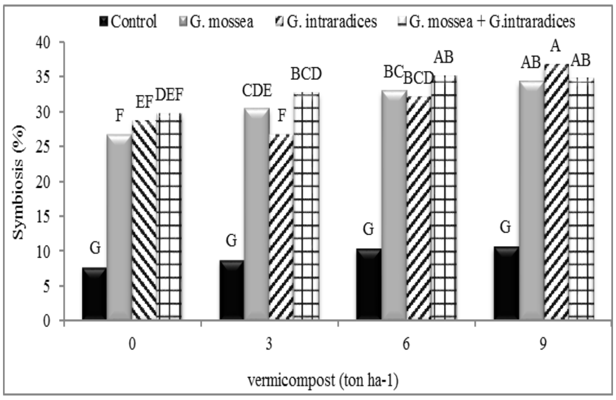

Fig 2. The interaction effect of vermicompost and mycorrhizal symbiosis on symbiosis percentage.

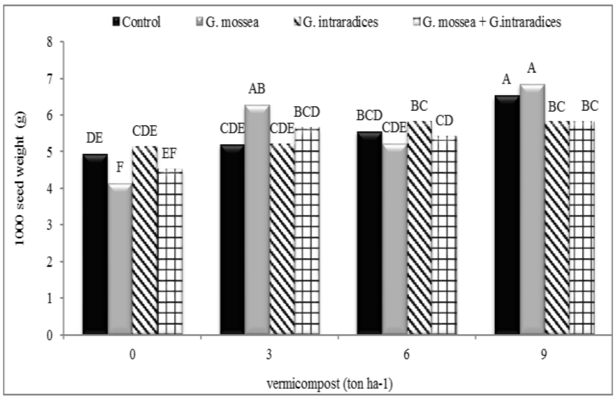

Fig 3. The interaction effect of vermicompost and mycorrhizal symbiosis on 1000 seed weight.

4. Discussion

Vermicompost had a significant effect on plant height. According to the previous findings, vermicompost have a positive effect on photosynthesis and dry matter production through increasing water absorption and optimal availability of macro and microelements (Hameeda et al., 2006; Gardezi et al., 2000).

In a study on the garlic, vermicompost significantly improved plant height (Arguello et al., 2006).

Azizi et al. (2008) also reported the positive effect of vermicompost on the height of chamomile plants. Azizi et al. (2004) also indicated that the use of different levels of vermicompost improved the growth and yield of basil plants. Haj Seyed Hadi et al. (2011) also showed that vermicompost had a positive and significant effect on height and essential oil percentage of chamomile.

Gupta et al. (2002) also observed a significant increase in plant height by inoculating mint plants with mycorrhizal fungi. Mycorrhizal fungi produce the auxin and improve the growth of aerial organs.

The results of Sharifi et al. (2011) on purple basil are also consistent with this research. Abak et al. (2010) studied the several mycorrhizal species on melon and stated that mycorrhizal species increased the growth compared to non-mycorrhizal treatment. Kargar Hajiabadi (2012) showed that *G. intraradices* caused the greatest crown diameter, number of secondary stems, plant height, aerial organ yields and essential oil yield in thyme.

Bachman and Metzger (2008) reported increased leaf area in tomato and French marigold with vermicompost application. The highest leaf area of soybean was recorded in mycorrhizal fungus treatment (Rezvani et al., 2011).

There was a significant difference between percentages of root symbiosis. In this regard, Kapoor et al. (2004) observed that the symbiosis percentage of fennel root in inoculation with two species of mycorrhizal fungi *G. fasiculatum* (84%) and *G.macrocarpum* (80%) was significantly higher than the non-inoculation treatment (10%).

The results of Kapoor et al. (2007) research on dill and cumin showed that the inoculation of the roots of these two plants with different species of mycorrhiza caused a significant increase in the percentage of root symbiosis.

It seems that the nutritional elements in vermicompost have improved the root symbiosis with mycorrhiza by stimulating the growth of the sage root. The results of Gardezi et al. (2000) on *Sesbania emerus* also confirm this result; but in some researches, contradictory results with the present research have been obtained. In this regard, Sainz et al. (1998) observed the applying of vermicompost obtained from urban organic waste, significantly reduced the percentage of root colonization in red clover plants.

Kapoor et al. (2004) stated that the increase in photosynthesis and subsequent improvement in plant dry weight has an important role in the increase of seed weight in inoculated plants.

The increase of 1000 seeds weight in mycorrhiza treatments has been reported by other researchers such as Ilbas and Sahin (2005) on soybean and Ardakani et al. (2000) on wheat.

The findings of many researchers confirm that the presence of biofertilizers in sustainable agricultural systems, especially through the synergistic and intensifying effects, can create a suitable substrate and as a result, the plant's optimal access to nutrient elements improve growth and increase plant biomass (Hazarika et al., 2000); Omar, 1998; Sharma, 2002a; Toro et al., 1997).

The combined use of appropriate amounts of biofertilizers, while improving the biological activities of the soil and the availability and optimal absorption of nutrients by the plant, can increase grain yield, and this effect is more evident in perennial plants (Ouahmane et al., 2006).

The results obtained in this research are consistent with the findings of Toussaint et al. (2007) on basil and Gupta et al. (2002) on mint. In two other studies, which were conducted on wheat and green mung bean under greenhouse conditions, it was found that the use of mycorrhizal inoculum significantly improved the grain yield of the two plants (Singh & Kapoor, 1998).

The result of Ardakani et al. (2000) also showed that the inoculation of wheat roots with mycorrhiza caused a significant increase in grain yield. They attributed this significant effect to the improvement of grain yield components such as the number of grains per spike, the number of spikes per unit area, and the weight of 1000 grains.

Increasing the amount of vermicompost also increased the yield components of sage such as height, inflorescence weight, 1000 seed weight and biomass.

These results can be seen in the research results of Kumawat et al. (2006) on barley, Mba et al. (1996) on chickpeas, and Azizi et al. (2004) on basil. In this regard, in a research conducted by Roy and Singh (2006b), indicated that the use of 10 tons of vermicompost caused a significant increase in grain yield in barley. They found that the use of vermicompost through the stimulation of beneficial soil microorganisms and the continuous and stable supply of mineral elements, especially nitrogen to the plant, increased the yield and yield components.

The study of Arguello et al. (2006) also showed a significant increase in garlic yield. They found that the consumption of vermicompost increased non-structural carbohydrates such as fructan, and subsequently the yield of garlic improved. Also, in a research conducted on chickpea, it was revealed that the consumption of 3 tons of vermicompost per hectare caused a significant increase in grain yield compared to control (Jat & Ahlawat, 2006).

Use of vermicompost through improving the biological properties of the soil such as increasing the microbial biomass and the stable supply of macro elements such as nitrogen and phosphorus as well as the presence of plant growth regulators can improve the growth, development and yield of pepper plant.

Findings of Jordao et al. (2006) on onion, corn, sweet potato, strawberry and lettuce plants are consistent with the results of this research.

5. Conclusion

According to the findings of this study, application of organic inputs (vrmicompost and mycorrhizal symbiosis), and also combined usage of them improved measured traits clary sage by providing nutrient and minerals for plants. Vermicompost prevents nitrogen leaching and keeps water and nutrients around the plant roots, which increases the yield of clary sage. Accordingly, due to environmental protection and social health, biofertilizers could be recommended in sustainable production systems of medicinal plants.

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