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Balm morphology under control of non chemical sources of nutrients

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Abstract

In order to investigate the effect of vermicompost, mycorrhiza and *Piriformospora indica* (semi-mycorrhiza) on the morphology of Balm (*Melissa officinalis*), this experiment was conducted in 2012 in Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran. The experiment was conducted in factorial in the form of a randomized complete block design with three replications. Factors were vermicompost in three levels (0, 5 and 10 t/ha) and inoculation with biofertilizers in four levels including (1) control, (2) inoculation with *Glomus mosseae* + *G. intraradices*, (3) inoculation with *P. indica*, and (4) inoculation with *G. mosseae* + *G. intraradices* + *P. indica*. The measured traits included plant height, the number of lateral branches, inflorescence length, canopy diameter, stem diameter, the number of tillers, leaf width, leaf length and leaf area. Results indicated the significant effect of vermicompost and biofertilizer on nearly all measured traits; however, the effect of their interactions was not significant on any of the measured traits. Mean comparison of vermicompost levels showed that application of 10 t/ha vermicompost was the best treatment; increasing plant height by 11.16%, the number of lateral branches by 39.84% and the number of tillers by 62%. Among the biofertilizers, *G. intraradices* + *G. mosseae* + *P. indica* (Table 3) was the best treatment which increased plant height by 43.32%, the number of lateral branches by 75.61%, the number of tillers by 75.61% and canopy diameter by 36.85%, compared with the control.

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Introduction

Since the side effects of chemical and synthetic medications are revealed, consumption of medicinal plants to cure diseases is increasingly under attention. Species of the *Melissa* genus are perennial and aromatic herbs originating from southern Europe, northern Africa, south of North America and central Asia and northern Iran (Ghahreman, 1992; Majnoon Hoseini and Davazdah Emami, 2007). *Melissa officinalis* L. is the main species of the genus with medicinal uses (Sari and Ceylan, 2002). For 2000 years, balm has been used to cure many diseases and its essential oil has anti bacterial, anti fungal and anti hormonal activities (Bajaj, 1993; Khezri, 2003).

Uncontrolled application of chemical fertilizers to gain high yield in agricultural sector has resulted in various environmental and health problems. Integrated nutrient management aims to provide plants nutritional requirements via different chemical and non chemical sources (Saleh Rastin, 2005). Vermicompost and biofertilizers are an important constituent of integrated nutrient management which can help to reduce the need for chemical fertilizers (Atiyeh *et al*., 2002; Stark *et al*., 2007).

Biofertilizers are various microorganisms including bacteria and fungi with the ability of providing plants with the nutrients. Mycorrhizae are a group of fungi with symbiotic relation with plants. Mycorrhizae are phosphate solubilizing fungi which improve plants growth and yield by facilitating nutrients absorption, increasing root absorptive surface, alleviating the effect of biotic stresses and inducing resistance to plant pathogens (Sharma, 2002; Smith and Read, 2008). Vinutha (2005) observed that inoculating basil with mycorrhiza fungi increased essential oil yield. Ardakani and Mafakheri (2011) also found that mycorrhizal inoculation significantly increased maize height and grain yield.

Vermicompost, a type of compost produced through the digestion system of worms, is an important source of macro and micronutrients, vitamins, enzymes and plant growth promoting hormones. So, it improves soil physical, chemical and biological conditions; resulting in the boost of plants growth (Claudio *et al*., 2009; Prabha *et al*., 2007). Tejad and Gonzalez (2008) tested the effect of vermicompost and animal manure on soil physical condition and found that vermicompost was more effective. Matos and Arrunda (2003) reported that application of vermicompost resulted in the enhancement of Zn, Cu and Br content in soil. Anwar *et al*. (2005) also reported that application of 5 t/ha along with chemical NPK application (50, 25 and 25 kg/ha, respectively) significantly increased basil biomass. Regarding the necessity and benefits of non chemical nutrient management, the objective of this experiment was to evaluate the morphological response of balm (*Melissa officinalis* L.) to different biofertilizers and vermicompost.

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. The research station is 5 km south east of Karaj (35° 48´ N, 51° E, 1320 m above the sea level). The minimum and maximum temperature in the area is -20 and 38°C, respectively. The soil at the test site consisted of 35.71% clay, 38.78% silt and 25.51% sand. The pH of the soil was 7.48 and EC was 1.02 (ds/m). Other physico-chemical properties of the soil are listed in Table 1.

This experiment was conducted in factorial in the form of randomized complete block design with three replications and two factors.

Vermicompost.

In three levels including 0, 5 and 10 t/ha.

Biofertilizer

In four levels including (1) a non inoculated control, (2) *Glomus intraradices* + *G. mosseae*, (3) *Piriformospora indica*, (4) *G. intraradices* + *G. mosseae* + *P. indica*.

Balm (*Melissa officinalis* L.) Seeds were planted in a frame and were transplanted in the main field after 40 days when plants were at 8 leaves stage. Plots were 2 × 3 m with 45 cm inter-row spacing and 40 cm on-row spacing. Vermicompost was added to the soil and mixed with the 30 cm of top soil layer after field preparation. At transplanting time, a suspension of *P. indica* was prepared and roots were soaked it the solution for 12 h. Mycorrhizae were also applied in powder form, 5 g below each plant's root. Irrigation was conducted quickly after transplanting.

Harvest and sampling were conducted at the beginning of flowering and the following traits were measured: plant height, the number of lateral branches, inflorescence length, canopy diameter, stem diameter, the number of tillers and leaf area.
Data were analyzed using SAS and means were compared according to the Duncan's multiple range test.

Results and discussion

Vermicompost

Analysis of variance indicated the significant effect of vermicompost on all measured traits (Table 2). Mean comparison showed that all measured traits had higher values when 10 t/ha vermicompost was applied, except for the leaf area which was higher in the control (Table 3). Application of 10 t/ha vermicompost increased plant height by 11.16%, the number of lateral branches by 39.84% and the number of tillers by 62%; however, reduced leaf area by 21.7% compared with the control (Table 3). On the other hand, there were no significant differences between the effect of 5 and 10 t/ha vermicompost on plant height, canopy diameter, leaf length and leaf area (Table 3).

Vermicompost improves soil porosity, aeration, drainage and water holding capacity; making the rhizosphere more suitable for root development and water and nutrients absorption and resulting in the improvement of plant growth and yield (Darzi *et al.*, 2008; Claudio *et al.*, 2009). Vermicompost facilitates nutrients absorption by forming a complex of them and making them available to plant roots (Rienecke and Vilijoen, 1990). Saeed Nejad and Rezvani Moghaddam (2010) reported that application of vermicompost increased cumin height. In an experiment on marigold (*Tajetes patula*), it was found that vermicompost application increased stem diameter and plant growth; this improvement was attributed to high cation exchange capacity (CEC) of vermicompost (Hidlago *et al.*, 2006). Senthilkumar *et al.* (2004) observed that application of vermicompost increased leaf area in rose. Anwar *et al.* (2005) found that application of 5 t/ha along with chemical NPK application (50, 25 and 25 kg/ha, respectively) significantly increased basil biomass. Fathi *et al.* (2012) also conducted an experiment to test the effect of 10 and 20 t/ha vermicompost on clay sage (*Salvia sclarea*) and found the significant effect on plant biomass and essential oil yield. They reported that application of 20 t/ha vermicompost increased shoot yield by about 27% and essential oil yield by about 29% compared with the control.

Table 1. Physico-chemical properties of the test site soil.

N (%)	P (ppm)	K (ppm)	Ca (Meq/l)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
0.09	8.16	580	9.78	68	0.37	0.42	12.88	3.18

Biofertilizer

Results indicated that biofertilizer significantly affected all measured traits except for stem diameter and leaf area (Table 2). Mean comparison also indicated that nearly all traits were the highest in *Glomus intraradices* + *G. mosseae* + *Piriformospora indica* (Table 3). This treatment increased plant height by 43.32%, the number of lateral branches by 75.61%, the number of tillers by 75.61% and canopy diameter by 36.85%, compared with the control (Table 3). Moreover, there were no significant differences between the effect of different biofertilizer levels on stem diameter and leaf area (Table 3).

Piriformospora indica is an endophyte fungus which was isolated from *Rosopis juliflora* and *Zizyphus nummularia* rhizosphere in India. It colonizes the root of many plants; promoting their growth through inducing resistance to various biotic and abiotic stresses, affecting the metabolism of P, S and N, and increasing the availability of P to plant roots (Oelmuller *et al.*, 2009; Varma *et al.*, 1988). Kumari *et al.* (2003) inoculated cabbage, mustard and spinach with *Piriformospora indica* and found that the fungus improved plants growth and yield.

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

SOV	df	Mean Squares (MS)								
		Plant height	The number of lateral branches	Inflorescence length	Canopy diameter	Stem diameter	The number of tillers	Leaf width	Leaf length	Leaf area
Block	2	**	ns	ns	ns	ns	ns	*	ns	ns
Vermicompost (A)	2	**	**	**	**	**	**	**	**	*
Biofertilizer (B)	3	**	**	**	**	ns	**	**	**	ns
A × B	6	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error	22	3.03	3.28	0.14	14.19	0.005	0.48	0.03	0.10	0.04
CV (%)	-	7.08	17.46	2.49	8.71	20.80	12.89	5.39	7.59	21.97

Ns, non significant; * significant at P≤0.05; **, significant at P≤0.01.

Mycorrhizae are another group of fungi which improve plants growth and yield by solubilizing phosphate, facilitating nutrients absorption, increasing root absorptive surface, alleviating the effect of biotic stresses, inducing resistance to plant pathogens and producing phytohormones (Sharma, 2002; Smith and Read, 2008). Mycorrhizal inoculation may increase plant chlorophyll content as Raiesi and Ghollarata (2006) observed that chlorophyll content was higher in bean plants inoculated with mycorrhizal fungi. Gupta *et al.* (2002) reported that the inoculation with arbuscular mycorrhizal fungi significantly increased colonization rate and yield of mint. Vinutha (2005) found that inoculating basil with mycorrhiza fungi increased essential oil yield. Ardakani and Mafakheri (2011) also found that mycorrhizal inoculation significantly increased maize height and grain yield.

Table 3. The effect of treatments and their interaction on the measured traits.

Treatments	Plant height (cm)	The number of lateral branches	Inflorescence length (cm)	Canopy diameter (cm)	Stem diameter (cm)	The number of tillers	Leaf width (cm)	Leaf length (cm)	Leaf area (m^2)
V_0	22.94b	9.01b	14.67c	40.26b	0.32b	4.29c	3.18c	3.98b	1.06a
V_1	25.21a	9.49b	15.11b	43.89a	0.29b	4.88b	3.35b	4.39a	0.87b
V_2	25.5a	12.6a	16.52a	45.5a	0.41a	6.95a	3.5a	4.49a	0.83b
B_1	20.43c	7.42c	14.34d	36.42c	0.32a	4.51c	3.08c	3.93c	0.894a
B_2	23.43b	9.84b	14.96c	41.58b	0.33a	5.03bc	3.31b	4.16c	0.921a
B_3	25.07b	11.16b	15.7b	45.01b	0.36a	5.64ab	3.43ab	4.37b	0.845a
B_4	29.28a	13.03a	16.74a	49.84a	0.35a	6.31a	3.55a	4.7a	1.047a
$V_0 \times B_1$	19.04d	6.43ef	13.37f	31.36f	0.31bcd	3.14g	2.80d	3.68d	1.03ab
$V_0 \times B_2$	22.83bc	7.62def	14.58de	39.36de	0.33bcd	4.03fg	3.21c	3.98cd	0.98abc
$V_0 \times B_3$	23.95bc	9.88bcd	15.13cd	42.94b-e	0.34a-d	4.65ef	3.33bc	4.08cd	0.95abc
$V_0 \times B_4$	25.93b	12.10abc	15.62c	47.36ab	0.29cd	5.32c-f	3.39abc	4.19cd	1.28a
$V_1 \times B_1$	21.02cd	5.88f	14.17e	37.86e	0.32bcd	4.41ef	3.17c	4.01cd	1.04ab
$V_1 \times B_2$	23.82bc	9.25cde	14.57de	41.62b-e	0.22d	4.48ef	3.29bc	4.17cd	0.90abc
$V_1 \times B_3$	25.99b	10.47bcd	15.19cd	45.13a-d	0.28d	5.13def	3.37abc	4.48abc	0.73bc
$V_1 \times B_4$	30.03a	12.34abc	16.53b	50.95a	0.35a-d	5.51cde	3.56ab	4.92ab	0.84bc
$V_2 \times B_1$	21.24cd	9.96bcd	15.49c	40.03cde	0.32bcd	5.98bcd	3.27bc	4.10cd	0.61c
$V_2 \times B_2$	23.62bc	12.66abc	15.74c	43.77b-e	0.44ab	6.58bc	3.44abc	4.33bc	0.88bc
$V_2 \times B_3$	25.27b	13.14ab	16.79b	46.97abc	0.48a	7.14ab	3.58ab	4.57abc	0.84bc
$V_2 \times B_4$	31.87a	14.66a	18.06a	51.21a	0.42abc	8.10a	3.71a	4.98a	0.99abc

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

The interaction of vermicompost × biofertilizer

Analysis of variance indicate that the interaction of the two factors had no significant on any of the measured traits.

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