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Investigation the quality and quantity of *Melissa officinalis* L. under chemical and bio-fertilizers of sulfur

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Abstract

In order to investigate the effect of chemical and bio-fertilizers of sulfur on yield and morphological traits of *Melissa officinalis*, this project was conducted in Research Institute of Forests and Rangelands, Karaj, Iran in 2012. The experiment was conducted as factorial experiment in the form of a randomized complete block design with three replications in filed condition. The main factor was sulfur solubilizing bacteria (*Thiobacillus thioparus)* in two levels: inoculation and lack of inoculation. The sub factor was sulfur fertilizer of bentonite source in two levels (0 and 500kg/ha). The analysis of variance showed that the effect of *Thiobacillus thioparus* was different for plant height, sub stem number and leaves yield ($\alpha \leq 0.05$) and for tillers number, sub-sub stem number, yield of total shoot and stem yield ($\alpha \leq 0.01$), while the effect of sulfur fertilizer was different only for tillers number ($\alpha \leq 0.01$). Effect of chemical and bio-fertilizers were not significant for any of the traits. The comparison showed that bio-fertilizers application had the highest increase in measured traits. So that the highest plant height (35.55cm), sub stem number (34n/p), sub-sub stem number (230n/p), yield of total shoot (604.68kg/ha), leaf yield (429.73kf/ha) and stem yield (174.94kg/ha) belonged to it, Also the highest tiller number observed in chemical fertilizer application. Overall the results indicated that *Thiobacillus thioparus* application significantly increased shoot yield than sulfur chemical fertilizer and suggested micro organisms efficiency and need to pay more attention to them in agricultural systems.

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Introduction

During the last decades, the use of chemical fertilizer and excessive use of them in addition to increasing production costs has been increased chemical fertilizers usage (Nosengo, 2003). So in order to remove pollutants, soil and agricultural products quality, extensive efforts have been initiated in recent years (Kumar *et al.*, 1999). Therefore bio-fertilizers have been introduced as promising combinations for plant nutrition in agricultural system (Subba Rao and Dommergues, 1998). In all of these systems, organic fertilizers (biofertilizers) as a natural alternative to chemical fertilizer play a positive role in fertility increasing to produce sustainable agricultural production (Wu *et al.*, 2005).

Bacteria of *Thiobacillus* genus are the main sulfur oxidizers in soil, that inoculated soil with these bacteria lead to increase in sulfur oxidation speed. In the condition of low population of bacteria in the soil, sulfur application with these bacteria in alkaline and calcareous soils will bring beneficial effects (Wainwright, 1984).

According to Sharafi and his colleagues' results (Sharafi *et al.*, 2010), *Thiobacillus* consumption had significant effect on number of pods per main and sub stem, length of main stem and leaves number per plant and had not significant effect on other traits of *Brassica napus*. But the interaction effect between variety and *Thiobacillus* consumption and lack of consumption was different for seed yield, length of main stem and length of pod in sub stem. According to results, sulfur application significantly increased seed yield and improved traits related to yield of *Arachis hypogaea* L. (Hoseynizadeh-Gashti *et al.*, 2009). Consumption of bentonite sulfur (150kg/ha) significantly increased seed yield, seed yield components, osmotic regulators and essential oil yield of *Nigella sativa* (Rezapour *et al.*, 2011). The use of phosphate soil, sulfur, organic matter and *Thiobacillus* inoculums compared to control treatment increased seed and straw yield of *Brassica napus* with 38 and 70%, respectively. Also after triple superphosphate fertilizer, it had the most seed oil with 39% increase. In general, oil percentage of *Brassica napus* in sulfur-containing treatments was higher than treatments without sulfur (Salimpour *et al.*, 2010). Researchers observed (yadegari and barzgar, 2010) that rate of essential oil and dry matter production of *Melissa officinalis* increased by applying 400-600kg/ha sulfur with 6 weight percent *Thiobacillus* bacteria for providing sulfur and creation favorable environment for micronutrients absorption.

According to bio-fertilizers position in improving soil and agricultural products quality and lack of reported research on the effect of sulfur solubilizing on *Melissa officinalis*, this project was conducted to evaluate the amount and type of sulfur fertilizer consumption for increasing yield of *Melissa officinalis*.

Material and method

In order to investigate the effect of chemical and bio-fertilizers of sulfur on yield and morphological traits of *Melissa officinalis*, this project was conducted in Research Institute of Forests and Rangelands, Karaj, Iran in 2012. The experiment was conducted as factorial experiment in the form of a randomized complete block design with three replications in filed condition.

Used factors

Two factors were used. Factor A: sulfur solubilizing bacteria (*Thiobacillus thioparus)* in two levels: inoculation and lack of inoculation. 6g of *Thiobacillus thioparus* was poured in the holes before seedlings planted. Watering was done immediately after seedlings planted.

Factor B: sulfur fertilizer from bentonite sulfur source in two levels of 0 and 500kg/ha. At the same time by preparing the ground, bentonite sulfur was added to the plots and mixed with soil to the depth of 30cm.

Seedling and land preparation

Seeds were planted in the outdoors treasury in early April and about 35 days later, seedlings were transferred to the main field after land preparation.

Dimensions of plot, spaces between ridges and plant spacing on a line were 2×3m², 45cm and 40cm, respectively. Distance between the plots was 1.5m and distance between blocks was 2.5m. Concurred with seedlings transfer, 40kh/ha nitrogen from urea source was added to the field as base fertilizer. The plots were regularly watered from planting until plant establishments 2 times a week. During growth period necessary tasks such as weeding, etc were done for all the plots the same. Weed control was carried out in 3 stages.

Drying method

After margin removal, harvesting and morphological measurement were done from the middle of plots in flowering stage. Harvested stems were dried in share and airflow. Samples of dried plants in open air were dried in oven at 70°C for 24h and dry matter yield per hectare was calculated for each treatmen.

Statistical analysis

Analysis of variance and means comparison was performed with SAS software. Comparison of means was performed by the Duncan's multiple range tests and the least mean square (lsmeans). Before data analysis, data normalizing test were carried out that for some traits suitable convert (SQRT, Arc Sin) was used.

Results

Analysis of variance showed that *Thiobacillus thioparus* effect was different for plant height, sub stem number and leaf yield (α≤0.05) and for tiller number, sub-subs stem number, yield of total shoot and stem yield (α≤0.01) while sulfur fertilizer was different only for tiller number (α≤0.01). Chemical × bio-fertilizers had no significant effect on any of the traits (Table 1).

Table 1. Analysis of variance of the effect of sulfur solubilizing bacteria and sulfur chemical fertilizer on morphological traits and yield of *Melissa officinalis*.

		Mean Squares (MS)								
SOV	Df	Plant height	Tiller number	Sub stem	Sub-sub stem	Inflorescence length	Canopy diameter	Leaf yield	Stem yield	Shoot yield
Block	2	2.91ns	19.96**	5.86ns	10.59**	66.18**	126.19*	709.55ns	1636.75**	3970.71ns
A	1	12.84*	20.37**	46.11**	14.16**	6.44ns	27ns	11462.68ns	6741.64**	35785.84**
B	1	2.15ns	27.33**	19ns	0.22ns	0.30ns	0.01ns	89.87ns	345.46ns	787.74ns
A×B	1	ns	4.73ns	12.96ns	1.85ns	1.48ns	0.10ns	945.93ns	132.17ns	370.92ns
Error	6	1.08	1.33	4.33	0.90	1.15	18.62	1089.98	90.72	1201.81
CV(%)	-	3.01	7.44	6.42	6.86	6.20	6.81	8.27	6.29	6.30

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01. A, sulfur solubilizing bacteria; B, bentonite sulfur.

Table 2. Effect of sulfur solubilizing bacteria on morphological traits and yield of *Melissa officinalis*.

A	Plant height (cm)	Tiller number (n/p)	Sub Stem (n/p)	Sub-sub stem (n/p)	Inflorescence length (cm)	Canopy diameter (cm)	Leaf yield (kg/ha)	Stem yield (kg/ha)	Shoot yield (kg/ha)
a_1	33.48b	14.23b	30.46b	171.33b	16.61a	61.85a	367.92b	127.54b	495.46b
a_2	35.55a	16.83a	34.39a	230.22a	18.07a	64.85a	429.73a	174.94a	604.68a

Means in a column followed by the same letter are not significantly different at P≤0.01. A, sulfur solubilizing bacteria; a_1, lack of inoculation; a_2, inoculation; n, number; p, plant.

The plant response to sulfur solubilizing

Evaluation the traits in bio-fertilizer consumption of *Thiobacillus thioparus* showed that the highest plant height (35.6cm), tiller number (16.8n/p), sub stem number (34.4n/p), sub-sub stem number (230.2n/p), leaf yield (429.7kg/ha), stem yield (174.9kg/ha) and yield of total shoot (604.7kg/ha) belonged to *Thiobacillus thioparus* consumption. Also there was no significant effect between bio-fertilizer consumption and lack of consumption on inflorescence length and canopy diameter (Table 2).

Table 3. Effect of sulfur on morphological traits and yield of *Melissa officinalis*.

B	Plant height (cm)	Tiller number (n/p)	Sub Stem (n/p)	Sub-sub stem (n/p)	Inflorescence length (cm)	Canopy diameter (cm)	Leaf yield (kg/ha)	Stem yield (kg/ha)	Shoot yield (kg/ha)
b_1	34.09a	14.02b	31.17a	208.14a	17.18a	63.31a	396.09a	145.87a	541.97a
b_2	34.94a	17.04a	38.68a	193.40a	17.50a	63.38a	401.56a	156.61a	558.18a

Means in a column followed by the same letter are not significantly different at $P\leq0.01$. B, sulfur; b_1, lack of consumption; b_2, consumption; n, number; p, plant.

The plant response to bentonite sulfur

Results indicated that sulfur consumption had the highest tiller number with 17n/p. Also there was no significant effect between sulfur consumption and lack of consumption on any measured traits (Table 3).

Table 4. Effect of correlation between measured traits.

Traits	Plant height	Tiller number	Sub stem	Sub-sub stem	Canopy diameter	Inflorescence length	Shoot yield	Leaf yield	Stem yield
Plant height	1								
Tiller number	0.30ns	1							
Sub stem	0.32ns	0.60*	1						
Sub-sub stem	-0.04ns	0.47ns	0.65*	1					
Canopy diameter	-0.23ns	0.60*	0.50ns	0.72**	1				
Inflorescence length	-0.04ns	0.71**	0.34ns	0.66*	0.73**	1			
Shoot yield	0.62*	0.69*	0.58*	0.62*	0.42ns	0.57ns	1		
Leaf yield	0.66*	0.54ns	0.40ns	0.44ns	0.22ns	0.41ns	0.94**	1	
Stem yield	0.44ns	0.77**	0.72**	0.74**	0.62*	0.68*	0.88**	0.68**	1

ns, non significant; *, significant at $P\leq0.05$; **, significant at $P\leq0.01$.

Correlation between measured traits

Results of correlation between traits showed (Table 4) that there was significant positive correlation between sub stem number and tiller number (r=0.60*). Sub-sub stem number had positive correlation with sub stem number (r=0.65*). Canopy diameter showed positive correlation with tiller number (r=0.60*) and sub-sub stem number (r=0.72*). There was positive correlation between inflorescence length and tiller number (r=0.71**), sub-sub stem number (r=0.66*) and canopy diameter (r=0.73**). Yield of total shoot showed positive correlation with plant height (r=0.62*), tiller number (r=0.69*), sub stem number (r=0.58*) and sub-sub stem number (r=0.62*). Leaf yield had significant positive correlation with plant height (r=0.66*) and shoot yield (0.94**). Stem yield showed significant positive correlation with tiller number (r=0.77**), sub stem number (r=0.72*), sub-

sub stem number (r=0.74**), canopy diameter (r=0.62*), inflorescence length (r=0.68*), shoot yield (r=0.88**) and leaf yield (r=0.68*).

Discussion

According to the results, consumption of sulfur solubilizing bacteria was effective in increasing yield of *Melissa officinalis* that these matched with Sharafi and his colleagues' results in terms of the solubilizing effect on plant and mismatched with interaction between consumption and lack of consumption (Sharafi *et al.*, 2010). Also mismatch of the results observed in other researchers' results (Hosynzadeh-Gashti *et al.*, 2009; Rezapour *et al.*, 2011). It seems that the short growing period of *Melissa officinalis* from time of fertilizer application until harvesting is the main reason for it. Seedlings transfer were done in late June and harvesting late September. So for bio-fertilizers more effectiveness, two factors are necessary. First, sufficient number of bacteria should be in the soil. Second, plant and bio-fertilizers have enough time up to its activity be observed (Wainwright, 1984). Also lack of significant effect of bentonite sulfur is related to the short growing period of *Melissa officinalis* and plant low need to fertilizer. The results were not adapted with other results (Yadgari and barzgar, 2010; Salimpour *et al.*, 2010). Positive correlation between shoot yield and the morphological traits indicated that any factor which increase plant height, tiller number, sub stem number and sub-sub stem number, will be effective in increasing shoot yield.

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