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The effect of Sulphur and *Thiobacillus* on nutrient availability, vegetative growth and essence production in lemon balm (*Melissa officinalis* L.)

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ABSTRACT

To study the effect of *Thiobacillus*, Sulphur and Organic material on vegetative growth and essence production in lemon balm (*Melissa officinalis* L.) a Complete Randomized Design was conducted in pots in field condition at Shahrekord, Iran on 2008. The factors were ten soil treatments inclusive 200,400 and600Kg/ha Sulphur, 200,400 and600Kg/ha Sulphur+*Thiobacillus,*200,400 and600Kg/ha Sulphur+ *Thiobacillus+* Organic material and without application of Sulphur, *Thiobacillus* and Organic material. The results revealed that there were significant differences among treatments in Copper, Zinc, Iron and Manganese content in soil after harvesting and fresh weight, dry weight and number of lateral stems in plants. Differences between Essence content in dry plants in various treatments were significant too. Treatment of 400Kg/ha Sulphur+*Thiobacillus*+Organic material and Control demonstrated the highest and lowest Copper and Manganese content in soil after harvesting and fresh weight, dry weight and lateral stems in plants respectively.

Key words: lemon balm (*Melissa officinalis* L.), essence, fresh herb, dried plant, Sulphur, *Thiobacillus*, Organic material

INTRODUCTION

Thiobacilli are a group of gram-negative chemoauto-trophic bacteria that can obtain energy for growth from the oxidation of a variety of inorganic sulphur compounds. *Thiobacillus*, an obligately autotrophic species, can also oxidize ferrous ion. This organism is very important in many mineral leaching operations, especially in bacterial leaching of sulphide ores for the recovery of several metals (Donati *et al*., 1997). *Thiobacillus* increased the level of protein phosphorylation and induced by the copper and other heavy metals (Teresa *et al*., 2000). This bacteria is an acidophilic iron oxidising bacterium that can be found naturally in acid mine drainage. These bacteria catalyse the oxidation of iron pyrites to ferric sulphate and sulphuric acid, increasing the reaction rate for chemical oxidation. At the iron oxidation step in such a system, a high density of cells of iron-oxidising bacteria is essential for rapid iron oxidation (Gomez *et al*., 2000).

Sulphur (S) being one of the essential plant nutrients accounting to about 10% of the total N content (Haneklaus *et al*., 2003) had received little attention for many years, since fertilizers and atmospheric inputs supplied the soils with adequate amounts (Scherer, 2001). Reduced S inputs from atmospheric depositions resulted in a negative S balance in agricultural soils, since crop plants have become increasingly dependent on the soil to supply the S that they need for the synthesis of proteins and a number of essential vitamins and cofactors (Kertesz and Mirleau, 2004). The agronomic consequences of insufficient S are documented with decreased yields and a substantial impact on

S content under extreme deficiency. Most of the S in soil environments (495% of total S) is bound to organic molecules, and therefore not directly plant available (Zhao *et al.,* 1999a).

Lemon balm, *Melissa officinalis* L. (Lamiaceae), a native of the northern Mediterranean region is cultivated as a medical herb (Schultze *et al*. 1993). It is listed in a number of European Pharmacopoeia for its carminative, digestive, diaphoretic and stimulant activities. Lemon balm is an herbaceous perennial in the mint family and extraction of shoots of this plant inhibited the germination and the growth of roots and shoots of cockscomb *(Amaranthus caudatus* L), cress *(Lepidium sativum* L.), crabgrass (*Digtaria sanguinalis* L.), timothy *(Phleum pratense* L.), lettuce *(Lactuca sativa* L.) and ryegrass (*Lolium multiflorum*) Lain. Its foliage has a distinctive lemony fragrance when bruised. The leaves are light green, crinkled, slightly hairy, and strongly toothed on the margins, more or less egg shaped, and about 1-3 in (2.5-7.6 cm) in length (Hisashi Kato-Noguchi. 2001).

Plant them in August or September so they can establish new growth before the first frost and then mulch heavily for the winter. Lemon balm responds to general all purpose fertilizer. Feed in the spring to encourage new growth and again after harvest to encourage additional leaf growth (Schultz *et al.,* 1993 and Patora *et al*., 2003). Over-fertilization causes excessive growth and poor flavor development. Dry leaves of lemon balm contain 0.02-0.30% essential oil. As a result of low essential oil content, lemon balm oil has a very high price level. The chemical composition of its essential oil has been extensively studied. The main compounds of the essential oil of lemon balm leaves are citral, citronellal, geraniol, linalool, caryopliyllene, caryopliyllene oxide, germacrene and ocimene. Many studies have been conducted to determine essential oil content and composition of lemon the balm (Sari and Ceylan, 2002).

MATERIAL AND METHODS

Sulphur amounts, organic material and Thiobacillus

Three amounts of Sulphur (200, 400 and 600 Kg/ha) and combination of it by *Thiobacillus* and organic material were used, inclusive 200Kg/ha Sulphur, 400Kg/ha Sulphur, 600 Kg/ha Sulphur, 200Kg/ha Sulphur+*Thiobacillus,* 400Kg/ha Sulphur+*Thiobacillus,* 600Kg/ha Sulphur+*Thiobacillus*, 200Kg/ha Sulphur+ *Thiobacillus+* Organic material*,* 400Kg/ha Sulphur+*Thiobacillus*+Organic material*,* 600Kg/ha Sulphur+ *Thiobacillus*+ Organic material and without application of Sulphur, *Thiobacillus* and organic material.

Experimental conditions

Field experiment was conducted at Shahrekord (latitude 50 ^{0} 51 $^{/}$ N, 32^{0} 17$^{/}$ E), located at about 500 Km of capital town of Iran on spring and summer 2008. The medial annual rainfall is about 337.2-mm per year. Average annual temperature is 11.2 ^{0}C. Soil texture was Loam. C, N, P and K content, EC, pH and percentage of sand, silt and clay were determined (Table 1). The experiment was arranged in a randomized complete design and three replications in pots by filed condition. Each pot has 10 Kg of soil and then combined by sulphur, Sulphur+*Thiobacillus*, Sulphur+*Thiobacillus+* organic material. whole of the exam has 120 pots. Each pot has 30Cm space of the other pots. Inoculation of *Thiobacillus* was mached by soil in 6% of weight of Sulphur that added in soil. Organic material was mached by soil in 5% of weight of Sulphur that added in soil. Each replication has 4pots. Sowing was achieved in 4May. Topsoil of the experimental plot area was kept moist throughout the growing season when necessary. The characteristics under investigation were

Table 1: Some physical and chemical properties of soil

Texture	pH	EC (dS.m^{-1})	T.N.V	O.C (%)	N_{total}	$P_{available}$ (mg.kg^{-1})	$K_{available}$	$Zn_{available}$	$Fe_{available}$	$Mn_{available}$	$Cu_{available}$
Loam	7.76	1.82	23	1.32	0.09	30.2	768	0.7	3.4	3.8	0.8

Table 2: Analysis of variance of available Cupper, Zinc, Iron and Manganese content in soil after harvesting and fresh weight, dry weight and lateral stems in plants produced by lemon balm plants that infected by various amount of sulphur that combined with organic material and *Thiobacillus*

Source of Variation	Degree of freedom	Cupper M.S	Zinc M.S	Manganese M.S	Iron M.S	Essence M.S	Fresh Weight M.S	Dry Weight M.S	Number of lateral shoot M.S
Replication	2	0.013	0.005	0.068	0.036	0.0001	25102.9	0.047	3.03
Treatment	9	0.033**	0.034**	0.722**	0.25**	0.005**	13352.13**	296.88**	56.38**
Error	18	0.006	0.004	0.081	0.056	0.0003	1125.18	15.58	3.44
C.V		8.96	8.5	4.39	4.7	10.5	13.72	10.5	11.21

ns,* and ** : Non significant, significant at the 5% and 1% levels of probability, respectively

Table 3: Average of available Cupper, Zinc, Manganese and Iron (ppm) content in soil after harvesting, Essence (CC), fresh weight (g), dry weight (g) and lateral stems in lemon balm plants that infected by various amount of sulphur that combined with organic material and *Thiobacillus*.

Mean of lateral shoot			Mean of Dry weight			Mean of fresh weight			Mean of Essence			Mean of Fe			Mean of Mn			Mean of Zn			Mean of Cu		
Amount	Group	Code	Amount	Group	Code	Amount	Group	Code	Amount	Group	Code	Amount	Group	Code	Amount	Group	Code	Amount	Group	Code	Amount	Group	Code
23.33	A	9	52.38	A	9	344.84	A	9	0.007	A	9	5.56	A	10	6.9	A	9	0.96	A	8	1.02	A	9
22.33	A	10	49.2	AB	10	319.05	AB	10	0.0068	AB	10	5.5	A	9	6.86	AB	6	0.94	A	7	0.98	AB	10
20.67	AB	8	45.2	BC	8	295.6	AB	8	0.006	BC	7	5.32	AB	8	6.77	AB	4	0.93	A	9	0.94	AB	6
18	BC	7	44.4	BC	7	295.6	AB	7	0.006	BC	8	5.13	AC	5	6.76	AB	3	0.91	A	10	0.92	AB	4
16	DC	6	40.4	C	6	261.1	B	6	0.005	C	6	4.9	BC	3	6.68	AB	5	0.88	AB	5	0.91	AB	3
14.33	DE	5	31.7	D	4	203.1	C	4	0.004	D	4	4.9	BC	1	6.57	AB	10	0.76	BC	1	0.84	BC	5
14	DE	2	30.9	D	5	199.2	C	5	0.004	D	5	4.87	BC	6	6.54	AB	8	0.73	C	6	0.84	C	2
13.33	DE	4	30.1	D	3	193.6	C	3	0.004	D	3	4.85	C	7	6.32	BC	2	0.72	C	4	0.75	C	8
12.33	E	3	26.58	D	2	172.2	C	2	0.003	D	2	4.85	C	4	5.96	C	7	0.72	C	3	0.74	C	7
11	E	1	24.6	D	1	158.7	C	1	0.003	D	1	4.83	C	2	5.33	D	1	0.71	C	2	0.71	C	1

Codes: 1= Control¡ 2=200S¡ 3=400S¡ 4=600S¡ 5= 200S+*Thiobacillus* ¡ 6= 400S+*Thiobacillus* ¡ 7= 600S+*Thiobacillus* ¡ 8= 200S+T+Organic Material¡ 9= 400S+T+ Organic Material ¡ 10= 600S+T+ Organic Material

Average of essence of shoot, fresh weight, dry weight, number of lateral shoots and mean of available Cu,Zn, Mn and Fe in soil after harvesting. At initial flowering, plants harvested upon the soil and by Clevenger, essence of dry material was measured. All data were subjected to ANOVA using the statistical computer package SAS_9 and treatment means separated using Duncan's multiple range test at $P \leq 0.05$ level.

RESULTS

The effects of treatments on the characters are given in Table 2 and Table 3. We found significant different effects induced by sulphur, Thiobacillus and organic material on growth parameters of lemon balm. Plant also showed different responses where the soil combined by organic material. *Thiobacillus* treatments in 400Kg/ha sulphur+organic and in 600Kg/ha sulphur+ organic material increased significantly essence of shoot, fresh weight, Dry weight, number of lateral shoots and mean of Cu, Zn, Mn and Fe in soil after harvesting. Treatment by *Thiobacillus* promoted the essence production over control treatment, where the highest values for essence were observed by combination of 400Kg/ha sulphur+organic+*Thiobacillus*. Similar result was obtained for measured characters in treatment of 600Kg/ha sulphur+organic material+ *Thiobacillus* but the treatment of 400Kg/ha sulphur+organic+*Thiobacillus* was the best. We observed that the more sulphur produced the more essence of shoot, fresh weight, Dry weight, lateral shoots and mean of Cu, Zn, Mn and Fe in soil after harvesting but in treatment of 600Kg/ha sulphur+organic+*Thiobacillus* there was the antagonist effect of absorption of the nutrients for plants and then more sulphur application results the less essence. Sulphur and *Thiobacillus* in soil resulted that plants had greater essence, fresh weight, Dry weight, lateral shoots and mean of Cu, Zn than no-Sulphur and no- *Thiobacillus* (control). The most essence of shoot, fresh weight, dry weight, lateral shoots and mean of Cu, Zn, Mn and Fe in soil after harvesting was achieved when Sulphur and *Thiobacillus* were combined by organic material but more consumption of sulphur made the less essence. Increased the essence by the treatment of Sulphur+*Thiobacillus*+organic material, significantly ($P \leq 0.01$) was over 100% than control treatment. In fresh weight, dry weight, lateral shoots similar results was observed. Although treatments of 400Kg/ha or 600Kg/ha sulphur made more essence of shoot, fresh weight, dry weight, lateral shoots and mean of Cu and Mn in soil after harvesting than control plants, but by means separated using Duncan's multiple range test at $P \leq 0.05$ level they had similar group with control plants.

Table 4: Results of correlations between characters in lemon balm plants were infected by various amount of sulphur that combined with organic material and *Thiobacillus*

Number of lateral shoot	Dry Weight	Fresh weight	Essence	Fe	Mn	Zn	Cu	Characters
							1	Cu
						1	0.02	Zn
					1	0.12	0.81**	Mn
				1	0.41*	0.77**	0.48**	Fe
			1	0.51**	0.31	0.62**	0.29	Essence
		1	0.85**	0.48**	0.34	0.55**	0.37*	Fresh Weight
	1	0.85**	0.99**	0.51**	0.31	0.62**	0.29	Dry Weight
1	0.83**	0.67**	0.83**	0.73**	0.35	0.74**	0.33	Number of Lateral shoot

* And **: Significant at the 5% and 1% levels of probability, respectively

Results of correlation between characters showed that the effective nutrients that affect on essence were Zn and Fe, However the dry and fresh weight of shoot and number of lateral shoot were significant effect on essence (Tables 2-4) .

DISCUSSION

This work has shown the effects of Sulphur, *Thiobacillus* and organic material on the essence production in lemon balm. However, the climate condition affect on essence and other characters in lemon balm (Sari and Ceylan, 2002). Tinmaz et al (2001) was reported that the highest essential oil's ratio (0.14%) was obtained from the plants, cut in the beginning of blooming, grown in Çanakkale ecological conditions. Essential oil obtained from a few different populations of *Melissa officinalis* L. cultivated in Poland had been investigated by Patora et al. (2003). The best amount of Sulphur for increased production under various organic material diverse climates, improved compatibility and competitiveness. Sulphur that combined by *Thiobacillus* made the more nutrients for plant. In evaluation the effect of sulphur inoculated with *Thiobacillus* on soil salinity and growth of tropical tree legumes, Stamford et al (2002) showed that sulphur inoculated with *Thiobacillus* was more efficient than gypsum in the reduction of the exchangeable sodium of the soil and promoting leaching of salts, especially sodium. Sulphur inoculated with *Thiobacillus* reduced the EC of the soil saturation extract to levels below that adopted in soil classification of sodic or saline sodic. Lipopolysaccharides (LPS) of the outer membrane of *Thiobacillus* negatively influenced the attachment of the bacteria to minerals and the bioleaching process. LPS play an important role in the attachment of the microorganisms and therefore, its presence or absence could affect the bioleaching process (Escobar *et al.,* 1997). The effects of sulphur amounts on plant growth and essence were given in Tables 2-3. We found significant different effects induced by sulphur on growth of lemon balm. Organic material also showed different responses due to various sulphur amounts.

Sulphur amounts significantly ($P \leq 0.01$) increased essence of shoot, fresh weight, Dry weight, lateral shoots and mean of Cu, Zn, Mn and Fe in soil after harvesting. Sulphur consumption promoted plant growth and development over planting without sulphur. Organic material and *Thiobacillus* had the same effect, where the highest values for essence and fresh weight, Dry weight, lateral shoots and mean of Cu, Zn, Mn and Fe in soil were observed by combination of more sulphur and organic material, however the treatment of 600Kg/ha sulphur+organic material+*Thiobacillus* made less essence than treatment of 400Kg/ha sulphur+organic material+*Thiobacillus*. Perhaps increasing of sulphur had the antagonist effect of nutrient absorption. Combination sulphur by *Thiobacillus* and organic material resulted in higher essence and other characters.

Essence in lemon balm can increase by amount of Mn and Cu concentrations in soil after harvesting but correlation between them no significant. In this research in more characters by more amount of fresh weight, dry weight, Zn and Fe in soil after harvesting more essence was achieved (Table 4) in the same research, Intodia and Sahu (2005) showed that Sulphur application significantly increased dry matter accumulation/ plant, leaf area index, and crop growth rate and leaf area duration. Chlorophyll content of leaves of opium poppy increased while leaf sap pH reduced by S application. Increasing levels of S up to 150 kg/ ha enhanced growth of crop, whereas, chlorophyll content of leaves increased up to 200 kg/ha sulphur application.

Sulphur mainly enhances the reproductive growth and the proportion of the reproductive tissues (inflorescences & pods) in total dry matter (McGrath & Zhao, 1996). Under S deficient conditions, the amount of amino acids and nitrates protein degradation within chloroplasts occurred (Dannehl *et al*., 1995). Besides, sulphur affects photosynthetic characteristics. Thus limits protein synthesis by limiting the amount of methionine and cysteine available for the assembly of new proteins (Sexton *et al*., 1997). According to Andersen *et al*. (1996) reported that seeds may be regarded as consisting of nitrogen- free structural material, stored proteins and stored oil. The proportion of structural material is expected to decrease with increasing seeds weight, while protein and oil may compete for the remaining space in seeds.

Similarly, proportion of different fatty acids may change in the oil.

Afzal and Asghari (2008) in examination of wheat reported that single and dual inoculation with fertilizer (P_2O_5) significantly increased root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content of the control crop. It is concluded that single and dual inoculation along with P fertilizer is 30-40% better than only P fertilizer for improving yield of wheat and dual inoculation without fertilizer (P) improved grain yield up to 20% as compared to P application.

In this research we observe that by more application of sulphur more residual micronutrient was made in soil, in same research Raja et al (2007) reported that the higher application of sulphur resulted the higher utilization of nitrogen and made the highest seed production in sesame varieties. Also by more consumption of *Thiobacillus* and organic material, more biomass and other characters were made. Anandham et al (2007) reported that Co inoculation with *Thiobacillus* and *Rhizobium* in Groundnut made the most plant biomass, nodule number and dry weight, and pod yield. Co-inoculation of *Thiobacillus* sp. strain LCH (applied at 60 kg ha^{-1}) with *Rhizobium* under field condition recorded significantly higher nodule number, nodule dry weight and plant biomass 136.9 $plant^{-1}$, 740.0 mg $plant^{-1}$ and 15.0 g $plant^{-1}$, respectively, on 80 days after sowing and enhanced the pod yield by 18%. These results suggested that inoculation of S-oxidizing bacteria along with rhizobia results in synergistic interactions promoting the yield and oil content of groundnut, in S-deficit soils. Genuses of *Thiobacillus* affect on nutrient adsorption and then made better biomass production and more Essence content was made. Lombardi *et al* (2002) in investigation of *Thiobacillus ferrooxidans* on Biological leaching of Mn, Al, Zn, Cu and Ti in an anaerobic sewage sludge reported that this bacteria affected the partitioning of Mn and Zn, increasing its percentage of elution in the KNO_3 fraction while reducing it in the KF, $Na_4P_2O_7$ and EDTA fractions. No significant effect was detected on the partitioning of Cu and Al. However, quantitatively the metals Mn, Zn, Cu and Al were extracted with higher efficiency after the bacterial activity. Titanium was unaffected by the bioleaching process in both qualitative and quantitative aspects.

CONCLUSION

This study showed that plant growth and essence potential increased by sulphur, *Thiobacillus* and organic material. Consumption of sulphur by the lemon balm with *Thiobacillus* and organic material resulted in more lateral shoot, dry and fresh weight of plant and thereby produced greater essence. The results indicate that in spite of the fact that sulphur application can increase the proportion of dry weight and essence in plants, application of increasing organic material for planting is needed.

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