International Journal on Advanced Science Engineering Information Technology

Vol.8 (2018) No. 4
ISSN: 2088-5334

Effect of Vermicompost Supplemented by Foliar Application of Silicate on Marjoram Plants Grown in Saline Soil

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***Abstract*— This study aimed to reduce the negative effects of soil salinity on marjoram plants by adding vermicompost to the saline soil complemented by foliar application of silicate (Si^{+2}) to their leaves. To achieve this purpose in field, the experiment was arranged as a factorial experiment. (Two way) Based on randomized complete block design with four replications. The first factor was vermicompost which was added at four rates (0, 3, 4 and 5 ton fed^{-1}). The second factor was silicon which was used at four rates (0, 2.5, 3.75 and 5 mM). Results revealed that the single application of either vermicompost or silicon reduced Na and proline concentration in plant shoot and enhanced plant growth, nutrient content, carbohydrate percentage and essential oil yield in plant shoots. This increase remained constant and not correlated with the rates of application. The combined application of vermicompost and foliar spraying of silicon resulted in noticeable increase in plant growth, nutrient content (N, P, K and Ca), carbohydrate percentage and essential oil yield in marjoram plants; the highest values of all parameters were recorded by using vermicompost at rate 3 or 4-ton fed^{-1} coupled with spraying 3.75 mM of silicon solution on plant leaves. These results proved that amendment of saline soil by adding vermicompost and spraying silicon solution on plant leaves directly is a good strategy for the reclamation of saline soil and mitigation of salt stress within plant tissues.**

***Keywords*— salt stress mitigation; silicon; marjoram plant; vermicompost.**

I. INTRODUCTION

Soil salinity negatively influences growth and productivity of crops. Therefore, cultivation in saline soil is a big challenge for those engaged in agriculture. The physiological and biochemical processes in the plant are disrupted when plant exposes to salt stress [1], [2]. Two characteristics distinguish these effects on crop growth: (1) high osmotic pressure of soil solution restricts water absorption by plants. (2) Specific impacts of some elements (Na, Cl, B) when present in high concentrations [3,4]. The high osmotic potential of the soil solution, nutrient imbalance, and specific ion toxicity are the salinity consequences that have a detrimental effect on plant growth [5]. Furthermore, limited nutrient supply in saline soils is a significant problem that should be resolved by suitable means [6], [7] Finding suitable mediators or stress relievers is one of the responsibilities of researchers [1]. Due to the high cost and the harmful impact of inorganic fertilizers, using organic material is developing. The use of organic sources ameliorates soil structure and increase nutrient availability by sustaining the balance of organic matter, thereby helping to improve physical, chemical, and biological properties of the soil [8], [9] Vermicompost, which is organic wastes digested by earthworms, contains nutrients in a form that is easily absorbed by the plant.

Vermicompost has a very positive influence on soil and plant [7]. Previous reports have shown that vermicompost moderates the detrimental effects of salinity stress on several crops 9-16]. Silicon (Si) is the second most abundant element in soils; it is mostly inert and only slightly soluble. Although Si is not considered an essential nutrient for plants [17], this element is valuable for higher plants, particularly under stressful environments [18]. Silicon has a role in improving crops' abilities to cope with biotic and abiotic stresses, such as disease and pest resistance, alleviation of heavy metal (Al, Mn, and Fe) toxicities, tolerance of drought stress, and moderation of frost and salinity stress [19]. Silicon can increase water use efficiency by diminishing leaf transpiration and water flow rate in the xylem vessels [20].

Marjoram (*Origanum Majorana*) is an aromatic plant of the mint family which originated in Egypt. Marjoram plants produce essential and fixed oil, from their leaves and seeds respectively. The essential oil has many uses. As a cooking additive; it is commonly used with meat dishes, salads, sauces and flavor soups. Cosmetically, marjoram plants are used as a cream for skin, shaving gel, bath soaps and body lotion. Marjoram has several uses with abundant health benefits whether used as an essential oil, powder, fresh or dried leaves. Marjoram plants are considered sensitive to salinity at seed germination, seedling growth [21], and vegetative stage [22]. Recently, mitigation of soil salinity, when cultivating marjoram plants, by applying different combinations of vermicompost and calcium silicate to the soil indicated a good plant response [23].

In our study, silicon was applied as a foliar application to avoid its interaction with the soil components. Also, foliar application of Si facilitates its absorption within the plant that in turn alleviate salt stress in plant tissues directly [24]. This work is, therefore, aimed to assess the efficiency of vermicompost along with the foliar application of silicon to alleviate the negative impacts of salinity on growth, essential oil yield and nutrient composition of marjoram shoots.

II. Materials and Method

A field experiment was executed on the saline soil in a private farm - El- Kassasin Elgadima, Ismailia Governorate. Seeds of sweet marjoram plants were germinated in the arboretum on July 15, 2017. Four months old Seedlings were transplanted into the field. Treatments started one week later. A field experiment was arranged as a factorial experiment (two way) based on randomized complete block design with four repetitions. The first factor was rates of vermicompost (0, 3, 4 and 5 ton fed^{-1}). The second factor was rates of foliar application of silicon as potassium silicate (0, 2.5, 3.75 and 5 mM). The vermicompost used in this experiment was made of animal wastes and plant residues, which were digested by local species of earthworms such as *Eisenia Andrei* (Oligochaete) and *Eisenia fetida*. Potassium silicate (K_2SiO_4) was purchased from AlAhram Mining Co. Egypt. Some chemical properties of saline soil and vermicompost were determined (Table 1) using the standard procedures outlined by Cottenie [25].

TABLE I
Some chemical properties of saline soil and vermicompost.

Saline soil		Vermicompost	
pH	8.10	pH	7.3
EC (dS m^{-1})	11.7	EC (dS m^{-1})	2.4
Anions	[meq l^{-1}] *	Moisture content	25%
CO_3	-	Organic matter	32.1 %
HCO_3	7.2	Organic carbon	55.2 %
Cl	108	C/N ratio	29.1
SO_4	30.9	N	1.9 %
Cations	[meq l^{-1}] *	P	0.6%
Na	115	K	1.3%
K	1.35	Fe	1299 ppm
Ca	14.3	Zn	30 ppm
Mg	15.6	Mn	120 ppm

*milliequivalent per liter

Plants were harvested at vegetative stage, where essential oil was extracted from fresh plant shoots. Plant shoot samples were dried at 65 °C for 48 hrs to be the ground and wet digested using H_2SO_4: H_2O_2 method described by Cottenie [25]. The digests were then subjected to measurement of N using the micro Kjeldahl method; P was assayed using ammonium molybdate blue method while flame photometer, [26] measured k and Na. Total carbohydrates in herb were assayed according to A.O.A.C [27]. Proline was determined calorimetrically using ninhydrin reagent according to Bates [28].

III. Results and Discussion

Mitigation of the negative effects of soil salinity is a big challenge facing plant nutrition scientists; they always attempt to subject the plant to materials, compounds or elements whether added into the soil or foliar application, to help the plant to cope with salt stress. Soil salinity stresses plants in two ways: (1) High concentrations of salts into the soil lead to plant roots unable to extract water. (2) When a plant absorbs saline water, high concentrations of salts within the plant could be toxic [29].

A factorial experiment was conducted on saline soil and aimed to reduce undesirable effects of salinity on marjoram plants. The first factor was vermicompost, which is expected to reduce salinity effects through improving physical, chemical and biological soil properties and the second factor is the foliar application of silicon which might directly enhance plant tolerance to cope with salt stress. Results indicated that vermicompost and foliar application of silicon and their combination had a significant effect on Na and proline in shoots of marjoram plants (Fig. 1 a, b).

In comparison to control, vermicompost reduced Na and proline concentrations in plant shoots by about 50 and 20% respectively. Moreover, by increasing the rate of application of vermicompost from 3 up to 5-ton fed^{-1}, the concentrations of proline and Na remained almost constant. (Fig. 1 a, b). Foliar application of Si severely declined Na concentration in plant tissues (about 50 % of control); but had no significant effect on proline content in plant tissues (Fig. 1 a, b). This finding implies that silicon might have a specific effect on Na and other salts may still be present in high concentration. As in the case of vermicompost, despite the increase in the rate of Si supply from 2.5 up to 5 mM, Na concentration remained almost constant (Fig. 1 a, b).

Concerning the interaction between the two factors (vermicompost and Si application), both factors affected each other. Vermicompost showed the effect on Si treatments where using 4 or 5-ton fed^{-1} vermicompost where Na and proline concentration declined in the shoot of Si-supplied plants from 0.8 down to 0.3 mg Na g^{-1} dry shoot and from 9 down to 2 mg proline g^{-1} dry shoot mainly that received 3.75 mM silicon which recorded the lowest values. Whereas, the high level of Si application (5 mM Si) had the reverse effect when applied with 4 or 5 ton (Fig. a, b). Silicon also influenced vermicompost, where, using 3.75 mM Si reduced Na and proline in the shoot of fertilized plants with vermicompost at rate 4 or 5-ton fed^{-1}, from 0.7 down to 0.3 mg Na g^{-1} dry shoot and from 7 down to 2 mg proline g^{-1} dry shoot (Fig. 1 a, b). It is also observed that the highest rate of Si (5 mM) increased Na and proline when increasing the rate of vermicompost up to 4 tons (Fig. 1 a, b). The obtained results emphasized that vermicompost and silicon, mainly when used together succeeded in diminishing Na concentration. Reduced proline concentration in plant shoots was evidence that salt stress was minimized.

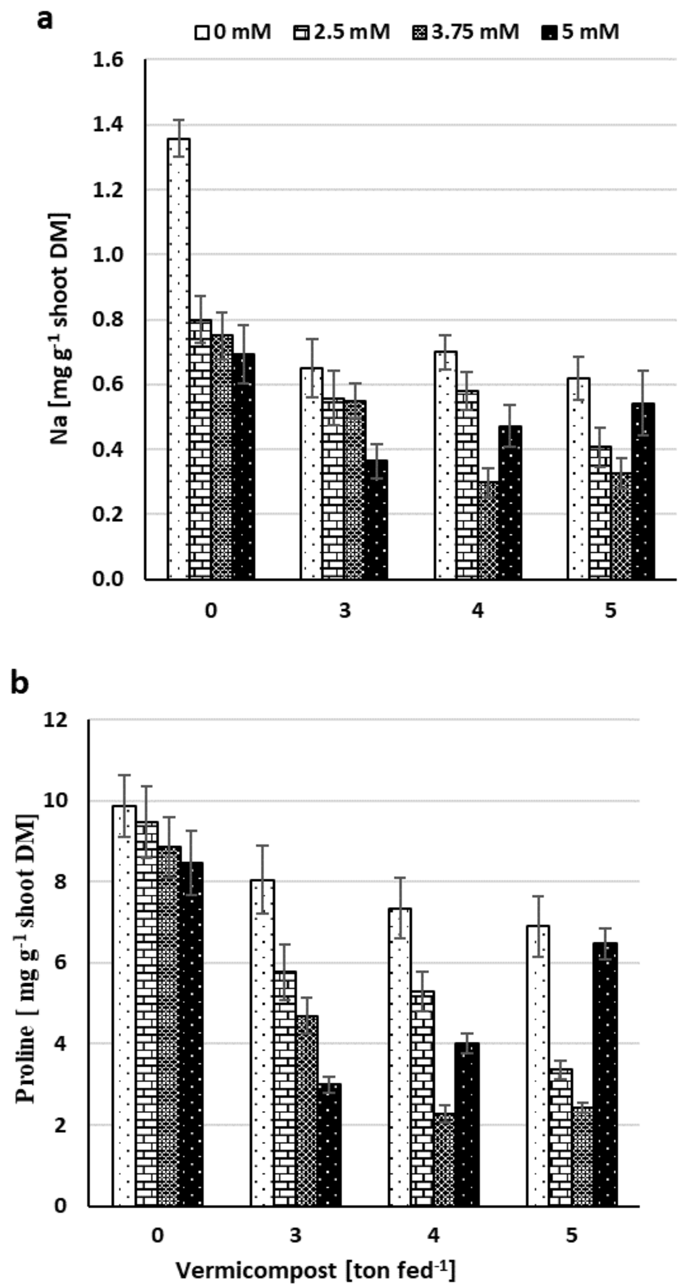

Fig. 1 Effect of vermicompost and foliar application of silicon on Na (a) and proline (b) concentration in the shoot of marjoram plants. Vertical lines indicate standard errors of means (n=4).

Addition of vermicompost to the soil supplemented by foliar application of silicon had a significant effect on sweet marjoram plants. Vermicompost and silicon increased growth parameters of marjoram plants. (Table 2). Adding vermicompost resulted in increased plant length, a number of branches and dry mass of marjoram plants by 50, 30 and 100% of control plants respectively. Furthermore, all parameters (plant length, number of branches and dry mass) were constant at different rates of vermicompost (3, 4 or 5 ton fed^{-1}). Foliar application of Si, independently, did not affect growth parameters, except for the application of 5 mM of Si which slightly increased growth parameters.

TABLE III

Effect of vermicomposting and foliar application of Si on growth parameters, (plant length, AND NUMBER of branches and dry mass of marjoram plants. Different letters within a column indicate significant (Tukey-Kramer's test, P<0.05) differences among nutrient treatments.

Vermicompost	Silicon [mM]	Length cm	Branches No.	Plant DM (g)
0-ton fed^{-1}	0.00	18 m	10 l	04.4 l
	2.50	20 lm	10 l	05.4 kl
	3.75	21klm	11 jkl	06.3 jkl
	5.00	32 kl	11 jkl	07.0 ijk
3-ton fed^{-1}	0.00	24 jkl	12 ijk	07.7 hijk
	2.50	33 gh	14 fg	10.7 efg
	3.75	39 ef	15 de	12.5 cde
	5.00	47 cd	16 c	15.3 ab
4-ton fed^{-1}	0.00	26 ijk	13 hij	08.1 hij
	2.50	37 fg	15 ef	11.4 def
	3.75	52 a	18 a	16.3 a
	5.00	41 de	16 cd	13.3 bcd
5-ton fed^{-1}	0.00	28 ij	13 ghi	09.0 ghi
	2.50	44 cd	17 bc	14.4 abc
	3.75	49 ab	18 a	16.6 a
	5.00	30 hi	14 fgh	09.9 fgh

Regarding the interaction between vermicompost and Si application, both factors affected each other. Vermicompost showed the effect on Si treatments where using 4 or 5-ton fed^{-1} vermicompost increased length and number of branches and dry mass of Si-supplied plants particularly that received 3.75 mM silicon which recorded the highest values. Whereas, the high level of Si application (5 mM Si) had the reverse effect when used with 4 or 5-ton vermicompost application (Table 2).

Silicon also influenced vermicompost, where, using 3.75 mM Si increased plant length, number of branches and dry mass of fertilized plants with vermicompost at rate 4 or 5-ton fed^{-1} the increment represented 100, 75 and 100% of non-Si-supplied plants respectively. It is also observed that the highest rate of Si (5 mM) reduced growth parameters when increasing the rate of vermicompost up to 4 tons (Table 2).

Vermicompost, foliar application of silicon and their combination had a significant effect on N, P, K and Ca concentration in the shoot of marjoram plants (Fig. 2 a, b; Fig. 3 a, b).

Using vermicompost only resulted in increased N, and Ca concentration in plant shoots. Nitrogen concentration tripled due to vermicompost addition. The concentration of Ca increased 50 % more than in control plants. This increment remained almost constant despite raising Si supply from 2.5 up to 5 mM. Also, foliar Si supply increased N and Ca concentration in plant shoots; whereas, there were no significant differences among the three rates of Si application (2.5, 3.75 and 5 mM Si).

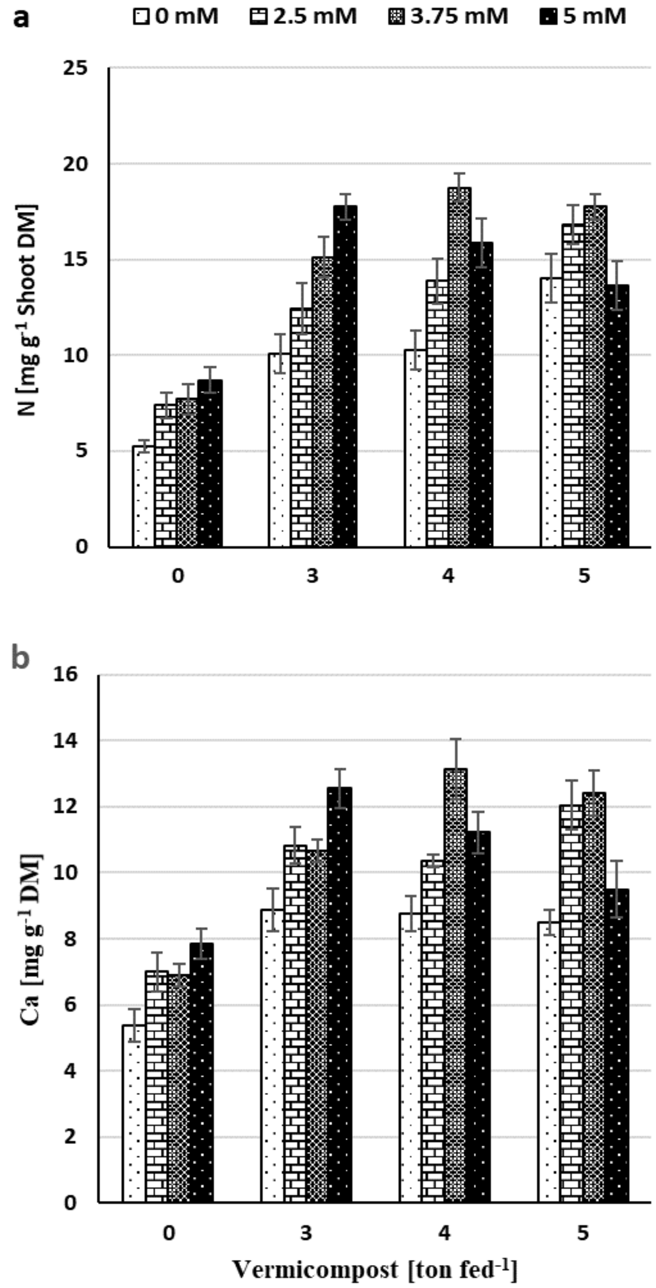

Fig. 2 Effect of vermicompost and foliar application of silicon on N (a) and Ca (b) concentration in the shoot of marjoram plants. Vertical lines indicate standard errors of means (n=4).

Regarding the interaction between the factors (vermicompost and Si application), both factors affected each other. Adding 4 or 5-ton fed^{-1} vermicompost increased N and Ca concentration in the shoot of Si-supplied plants from 7.5 up to 19 mg N g^{-1} dry shoot and from 7 up to 13 mg Ca g^{-1} dry shoot mainly that received 3.75 mM silicate which recorded the highest values. Whereas, the high level of Si application (5 mM Si) had the reverse effect when used with 4 or 5-ton vermicompost application (Fig. 2 a, b). Silicon also influenced vermicompost, where, applying 3.75 mM Si raised N and Ca in the shoot of fertilized plants, with vermicompost at rate 4 or 5-ton fed^{-1}, from 10 up to 19 mg N g^{-1} dry shoot and from 9 up to 13 mg Ca g^{-1} dry shoot (Fig. 2 a, b).

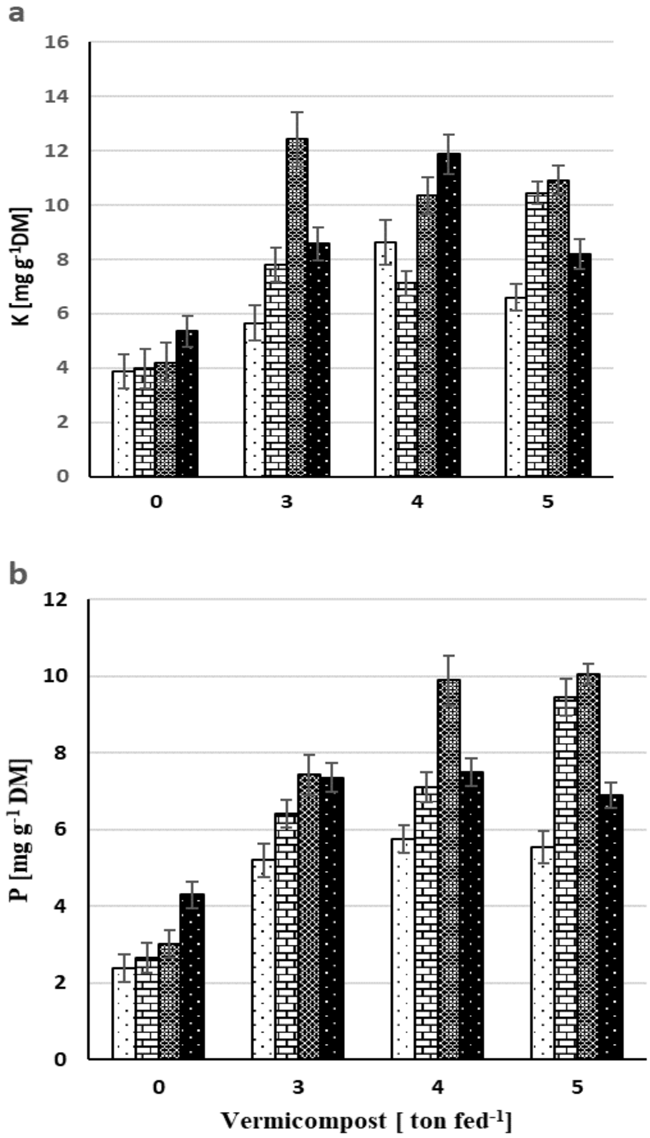

Fig. 3 Effect of vermicompost and foliar application of silicon on K (a) and P (b) concentration in the shoot of marjoram plants. Vertical lines indicate standard errors of means (n=4).

It is also observed that the highest rate of Si (5 mM) reduced N and Ca concentration in shoots when used with a high rate of vermicompost (more than 3 tons) (Fig. 2 a, b). The obtained results emphasized that vermicompost and silicon, notably when used together succeeded in raising N concentration 5 times compared with control plants. Ca concentration in plant shoots was increased more than twice as compared with its concentration in control plants.

Using vermicompost independently resulted in increased K and P concentration in plant shoots, the increment of K ranged from 4 up to 9 mg K g^{-1} DM shoot according to the rate of vermicompost addition. The concentration of P was increased from 2.5 to 6 mg g^{-1} DM shoot. This increment remained almost constant despite any increase in Si supply up to 5 mM. Also, foliar Si supply had no effect on K and P concentration in plant shoots except for the highest level of Si (5 mM) which increased P concentration in plant shoots (Fig. 3 a, b). Even though Si was supplied in the form of potassium silicate (K_2SiO_4), it did not affect K concentration in plant shoot. This finding supports the assumption that the reduction in Na concentration (which was due to foliar application of Si) was compensated for by Ca rather than K.

The interaction between the factors (vermicompost and Si application), they seem to have affected each other. Vermicompost showed the effect on Si treatments where adding 3 or 4-ton fed^{-1} vermicompost increased K and P concentration in the shoot of Si-supplied plants from 4 up to 12 mg K g^{-1} dry shoot and from 3 up to 10 mg P g^{-1} dry shoot mainly that received 3.75 mM silicate which recorded the highest values. Whereas, the high level of Si application (5 mM Si) had contrary effect when used with 4 or 5-ton vermicompost application (Fig. 3 a, b). Silicon also influenced vermicompost, where, applying 3.75 mM Si raised K and P in the shoot of fertilized plants, with vermicompost at rate 4 or 5-ton fed^{-1}, from 6 up to 12 mg K g^{-1} dry shoot and from 6 up to 10 mg P g^{-1} dry shoot (Fig. 3 a, b).

It is also observed that P concentration in the shoot of the supplied plant with 5 mM Si was remained constant at different rates of vermicompost application (3. 4 and 5-ton fed^{-1}) (Fig. 3 b). The obtained results emphasized that vermicompost and silicon, mainly when used together succeeded in increasing K and P concentration 3 times as compared with their concentration in control plants.

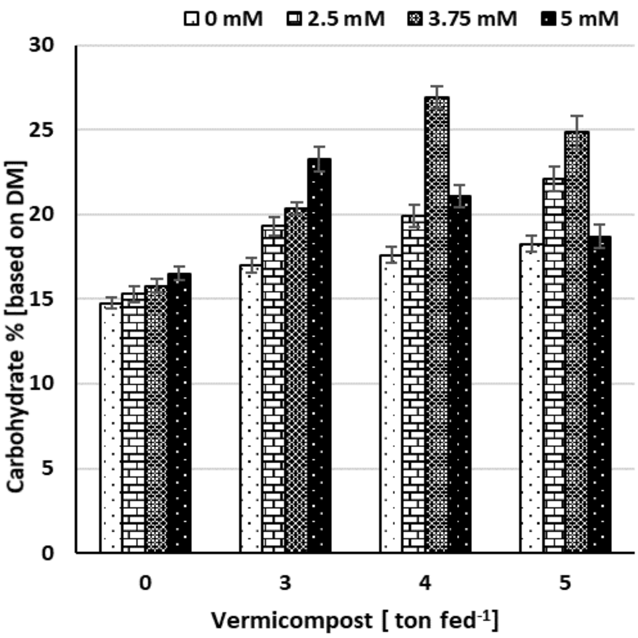

Fig. 4 Effect of vermicompost and foliar application of silicon on carbohydrates percentage in shoots dry mass (DM) of marjoram plants. Vertical lines indicate standard errors of means (n=4).

Both Vermicompost and foliar application of silicon had a significant effect on carbohydrates percentage in plant shoots (Fig. 4). In comparison to control, vermicompost increased carbohydrate percentage from 15 % to 17.5 % in dry shoots of marjoram plants. This increment remained constant at different rates of vermicompost. Foliar application of Si alone had a significant effect on carbohydrate percentage in dry shoots. Concerning the interaction between the factors (vermicompost and Si application), both factors affected each other. Vermicompost showed the effect on Si treatments where foliar Si supply resulted in increased carbohydrate percentage which reached 27% at rate of 3.75 mM Si and 4 tons vermicompost. Nevertheless, the high level of Si application (5 mM) had negative effect when used with 4 or 5-ton vermicompost application. It is also observed that the highest rate of Si (5 mM) reduced carbohydrate % with increasing the vermicompost rate higher than 3 tons fed^{-1}(Fig. 4).

Marjoram plants are cultivated with the aim of extracting essential oil from their leaves, which is considered the maximum yield of these plants. Single or combined application of vermicompost and silicon had significant effects on the quantity of essential and total oil yield in shoots of marjoram plants. However, essential oil concentration in plant shoots was not significantly affected by vermicompost, silicon of their interaction (Fig 5 a, b).

In comparison to control, vermicompost independently increased essential oil yield from 2 to 4 L fed^{-1}. However, this increment remained constant using different rates of vermicompost. Similar to vermicompost, foliar application of silicon gradually increased essential oil yield from 2 to 4 L fed^{-1} according to the rate of Si supply.

Concerning the interaction effect, adding 4 or 5 ton fed^{-1} vermicompost increased total essential oil in shoots of Si-supplied plants from 3 up to 11 L fed^{-1}; treatment which received 3.75 mM silicate recorded the highest essential oil yield. Whereas, the high level of Si application (5 mM Si) had negative effect when used with 4 or 5-ton vermicompost application (Fig. 5 a, b). Silicon also influenced vermicompost, where, applying 3.75 mM Si raised total essential oil in shoots of plants, fertilized with vermicompost at rate 4 or 5-ton fed^{-1}, from 4 up to 11 L fed^{-1} (Fig. 5 a, b). It is also observed that the highest rate of Si (5 mM) reduced essential oil yield when increasing the rate of vermicompost more than 3 tons fed^{-1}.

In this study, the addition of vermicompost to the soil supplemented by foliar application of silicon had a significant effect on marjoram plants. Both factors (vermicompost and silicon) independently increased growth parameters (Table 2), nutrient content (Fig. 2 a, b; Fig. 3 a, b), carbohydrate percentage (Fig. 4) and oil content (Fig. 5 b). Vermicompost as several organic materials can be used as an amendment to promote and sustain soil fertility or to remediate saline soil [7, 30]. In comparison to other organic material, vermicompost is associated with higher nutrients available for plants [7], [31].

Therefore, using vermicompost only reduced Na concentration in plant shoot thus increasing the concentration of other nutrients, since it is rich in humus, P, K, Ca and micronutrients [31]. Consequently, salt stress within plant shoot was reduced, the reduction of proline emphasized that assumption (Fig. 1 b). Previous studies reported several strategies for using silicon to promote abiotic stress tolerance of higher plants [32], [33]. Also, Si application to mitigate salt stress showed satisfactory results in barley [34], rice [35], wheat [36] and marjoram [23].

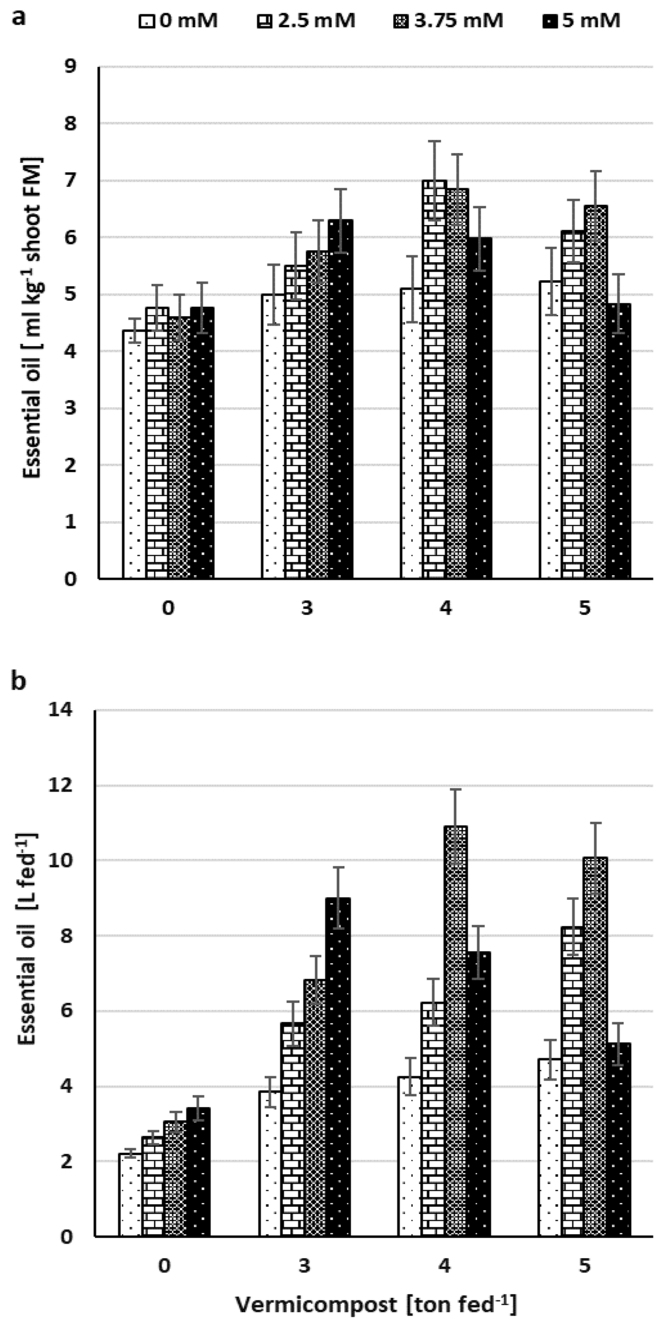

Fig. 5 Effect of vermicompost and foliar application of silicon on essential oil concentration (a) and total oil yield (b) in the shoot of marjoram plants. Vertical lines indicate standard errors of means (n=4).

The results revealed that foliar application of Si succeeded in reducing Na and proline concentration in plant shoots. (Fig. 1 a, b). Therefore, the highest level of Si application with high rates of vermicompost 4 and 5-ton fed^{-1} reduced growth parameters, nutrient, carbohydrate contents and essential oil yield in plant shoots compared to using 3-ton fed^{-1} (Table 2: Fig. 2 a, b; Fig. 3 a, b; Fig. 4 and Fig. 5 b). This may be due to that high rates of vermicompost and potassium may introduce more available minerals into the soil and thus inducing salt stress.

The role of silicon is confined to alleviate salt stress within plant tissues, probable explanations proposed for these results include limiting the transpiration by the accumulation of Si in leaves, the formation of complexes with Na in roots, protection of cell membranes and increased activity of antioxidative enzymes [34-37]

The increment of growth, nutrient content, carbohydrate and essential oil yield remained constant despite increased vermicompost or silicon intensity (Table 2; Fig. 2 a, b, Fig. 3 a, b; Fig. 4, and Fig. 5 b). Previous studies indicated that moderate rates of vermicompost reduced soil exchangeable Na. Higher rates of vermicompost, however, did not further decrease exchangeable Na with significant amount [30]. High rates of vermicompost did not affect growth parameters when used with high rates of silicate salts, the effect on growth is significant under low rates of silicate salts [38]. Also, previous studies confirmed the role of silicon in mitigating salt stress within plant [19], [20], [23]. That elucidates why the increment in growth, nutrient and oil yield in plants remained constant even though the vermicompost rate increased. However, salt stress was mitigated by using silicon increasing vermicompost supply extremely enhanced most of parameters.

Mohsen et al. [23] used vermicompost and calcium silicate on the same plant (marjoram plants) they added both to the soil, in our approach instead of adding silicon to the soil, Silicon was sprayed directly on plant leaves subsequently Si accumulates in leaves causing limitation of transpiration [35], protect cell membranes [34] or increase activity of antioxidative enzymes [37]. The amount of Si, which drop on the ground, can form a complex with Na in roots [36]. Therefore, vermicompost enhances soil fertility. Meanwhile, silicon mitigates salt stress within plant tissues. Soil addition of vermicompost complemented by foliar application of silicon had pronounced effect on marjoram plant; where both significantly increased plant growth, nutrients content and oil yield in marjoram plants. Results illustrated that 3-ton fed^{-1} was sufficient to obtain the highest values of plant growth parameters (Table 2), nutrient content (Fig. 2 a, b Fig. 3 a, b), carbohydrate percentage (Fig. 4) and essential oil yield (Fig. 5 a,b). Using 3.75 mM of silicon was sufficient to obtain positive impact; whereas, higher rates (5 mM Si) gave negative effect.

IV. Conclusions

Based on the results of this study, it could be concluded that under salt stress conditions, a single application of low rates of either vermicompost or silicon enhanced growth parameters, nutrient content, carbohydrate percentage and essential oil yield in marjoram plants. This increase remained constant with higher rates of application Whereas, adding vermicompost combined with foliar application of silicon profoundly increased plant growth, nutrients content and essential oil yield in marjoram plants, the highest values of all parameters were recorded by using vermicompost at rate 3 or 4-ton fed^{-1} complemented by spraying 3.75 mM of silicon on plant leaves.

Acknowledgment

This research is extracted from Project No. 11030149 financed by the Research Projects Sector - National Research Centre; to whom the research team would like to express thanks and gratitude.

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