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# Morphophysiological and biochemical response of savory medicinal plant using silicon under salt stress MID MOHAMMADI<sup>\*</sup>, SAEID HAZRATI, LALEH PARVI<br>
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#### ABSTRACT

Salt stress is one of the most important factors limiting the growth and yield of plants around the world. however, silicon can reduce the harmful effects of salt stress on plants. For this purpose, an experiment was conducted in a factorial arrangement on randomized complete block design with three replications in a research greenhouse on the *Satureja hortensis* medicinal plant. Experimental treatments consisted of two salinity levels (control and 100 mM) and potassium silicate (Si) at three levels (0, 1, and 2 mM). The results showed that salinity reduced shoot dry weight, photosynthetic pigments and potassium content of shoot. However, sodium, proline, MDA, and  $H_2O_2$  contents in shoot increased. The highest shoot dry weight, photosynthetic pigment content, proline, RWC, and the lowest content of MDA and  $H_2O_2$  of the shoot were observed with Si application under salt stress and non-salt stress conditions. The highest yield of essential oil was also observed with Si application under salt stress and non-salt stress conditions. Therefore, the use of silicon in salt stress condition not only minimizes the harmful effects of salt stress by increasing the K+/Na+ ratio and improving the morphological and physiological traits of the *Satureja hortensis* medicinal plant but also improves the essential oil yield of this medicinal plant in salt stress and non-salt stress conditions.

**Keywords:** salinity stress tolerance, *Satureja hortensis*, ion status, silicon

#### INTRODUCTION

Salinity is one of the environmental factors limiting crop and medicinal plants yield in arid and semi-arid regions that disrupt the natural growth and development of plants in vast areas of the earth's surface (46). Increasing soil salinity results in high osmotic potential of the soil, resulting in

#### hAMID MOhAMMADI, SAEID hAZRATI, LALEh PARVIZ

water loss in plants. In addition, high concentrations of sodium and chlorine ions cause ion imbalance, resulting in increased production of reactive oxygen species (ROS). The ROS such as radical superoxide, hydrogen peroxide, and radical hydroxyl cause lipid peroxidation (30), membrane damage, loss of growth and plant biomass (60), and ultimately cell death (33, 58). When exposed to soil salinity, plants accumulate toxic concentrations of sodium ion in the leaves, which, by reducing the photosynthesis of tissues, cause additional growth restriction (46). Control of sodium ion transfer and its effective exclusion from mesophyll cells of leaves is an important requirement for salinity tolerance. Studies show that the main components that regulate salinity tolerance include reduced salt uptake or salt exclusion, increased potassium ion/ sodium ion ratio, tissue tolerance, stomatal closure, up-regulation of antioxidant system to protect against ROS, osmolyte synthesis, high water use eficiency, early lowering and further growth to dilute the concentration of salt in plant tissues (14, 31). In the study of salt stress on morphological and physiological characteristics of ive summer *Satureja hortensis* populations, it was determined that different levels of salt stress (0, 25, 50, 100 mM) had a signiicant effect on morpho-physiological characteristics and yield of this plant (49). Majnoon hosseini and Davazdahemami (42) reported that salinity had no signiicant effect on seed essential oil content and plant height in spring planting, but its effect on biological yield reduction, seed yield and essential oil yield of the aerial organ was significant and it also reduced the plant germination by 30%. In another study, Davazdah Emami and Mazaheri (15) reported that with increasing salinity levels, the percentage of essential oil of seeds and vegetative parts of Ajwain plant signiicantly decreased. The results of Piri et al. (48) studies showed that salt stress reduced dry weight, fresh weight of leaf, leaf to stem ratio and essential oil percentage of Rosemary plant. ants accumulate toxic concentrations of sodium ion in the leaves, which, by reductions and saccumulate toxic concentrations of sodium ion in the leaves, which, by reductions cectusion from mesophyl cells of leaves is an im

Several strategies have been used to reduce the effects of salinity and obtain an acceptable yield of plants, including the application of silicon (36, 60). Several reports show that silicone application can increase tolerance to salt stress (23, 38). The beneicial effects of Si on growth, biomass and photosynthetic pigments in rice (23) and *Spartia densilora* (45) under salt stress have been reported. Also, studies have shown that the application of Si in plants under salt stress reduces the harmful effects of stress, including reducing sodium and chlorine uptake (53), increasing mineral absorption (28), solute biosynthesis (61), and improving the antioxidant system of the plant (59).

Summer savory (*Satureja hortensis* L.), a herbaceous plant belonging to the family Lamiaceae, is used as a spice and traditional herb in Iran (62). The antispasmodic, antidiarrheal, antioxidant, sedative and antimicrobial properties of summer savory oils and extracts resulted in their extensive application in the food and pharmaceutical industries (24, 55). Essential oil (EO) of summer savory has a high percentage of Carvacrol, which is mainly responsible for biological activities, including antimicrobial, antioxidant, anti-diabetic, anti-hyperlipidemic, antispasmodic, anti-nociceptive, antiinflammatory, antiproliferative, sedative, and reproduction stimulatory (3, 44).

By considering the positive effects of Si as an essential element on plants, the use of Si in agriculture systems can noticeably improve plant growth and productivity, especially under biotic and abiotic stresses conditions. In addition, Si application may alleviate salinity stress in medicinal plants to increase plant secondary metabolites.

The main goals of this study were to evaluate the inluences of Si application on some morphological, physiological characteristics and essential oil yield in summer savory plant under salinity stress conditions.

#### MATERIALS AND METhODS

In order to evaluate whether Si application favours morphological, physiological traits and essential oils yield in the savory plant *Satureja hortensis*, the effects of Si application and salinity stress were carried out as a factorial experiment based on randomized complete block design with MORPhOPhySIOLOGICAL AND BIOChEMICAL RESPONSE OF SAVORy MEDICINAL PLANT...

three replications in greenhouse. Experimental treatments consisted of two salinity levels (0 and 100 mM) and potassium silicate at three levels (0, 1 and 2 mM).

Plants were grown in pots, each containing approximately 4 kg of soil comprised of a mixture of sand, silt and clay at a ratio of 2, 2 and 1, respectively with electric conductivity of 0.84 dS.m-1 (control), and ph 7.54. Ten seeds were sown in each pot. Following germination, the seedlings were thinned to four per pot. Supplementary light was provided in the greenhouse for 16 h per day. The daytime and nighttime temperatures of the greenhouse were 27.5 and 18˚C, respectively. The salt treatments began during initial lowering (75 days after planting during 6 weeks). Treatments were started with saline water of 20 mM, and increased gradually up to 100 mM over 10 days. Plants were harvested at the flowering stage. After the experiment period, shoot dry weight was characterized, and then sodium and potassium ion measurements were taken from the 2 N chloride acid extract of the samples that had been burned at  $600^{\circ}$ C for 4 h, using a flame photometer (PF5 Carl Ziess Germany model). Part of samples was shade dried for a week and used for extraction of essential oils. The aerial parts of each plant had their oil yields (w/w) and components extracted by hydrodistillation in a Clevenger type apparatus for 3-4 h, dried over anhydrous sodium sulfate (20). RWC was determined by using fully developed young leaf (10). Also, harvested plants were immediately frozen in liquid nitrogen for 2 minutes and then stored at  $-70^{\circ}$ C for the physiological measurements such as total chlorophyll and carotenoids (39), malondialdehyde (MDA) (27),  $H_2O_2(57)$  and proline contents (11). and pH 7.54. Ten seeds were sown in each pot. Following germination, the seedlif<br>our per pot. Supplementary light was provided in the greenhouse for 16 h per of<br>four per pot. Supplementary light was provided in the greenh

#### **StatiStical analySiS**

The data were analyzed through SAS statistical package and the mean comparisons were made following Duncan's Multiple Range Test at  $P = 0.05$  through MSTATC (version 2.10, Inc, Michigan State University).

#### RESULTS AND DISCUSSION

### **Growth parameterS**

The results of the statistical analysis indicated that shoot dry weight decreased with increasing salinity level (Table 1). Reduction in dry weight is probably due to differences in the inhibition of photosynthesis by salinity among species, or

Table 1. Analysis of variance (ANOVA) for studied traits in savory plants with Si application under salinity stress



\*, \*\*: significantly different at the 5% and 1% probability level, respectively, ns: non-significant.

#### hAMID MOhAMMADI, SAEID hAZRATI, LALEh PARVIZ

the decrease in RUBP carboxylase activity, or the reduction of regeneration of RUBP, or the photosystem II sensitivity to sodium chloride, which leads to a decrease in dry weight (9). Also, Si had a significant effect on plant dry weight, so that, with using Si, plant dry weight improved under salt stress (Table 2). Several studies have reported growth and plant biomass increase in Si application conditions, which is attributed to increased photosynthetic pigment concentrations and improved photosynthetic system (2, 22). In our present study, plant exposed to Si application resulted in an improvement of photosynthetic pigments content under salinity stress and non-stress conditions.

Table 2. Mean comparison of physio-morphological and biochemical traits in savory plants with Si application under salinity stress



\*: Means followed by the same letter(s) in each column are not signiicantly different based on Duncan's Multiple Range Test (n=3).

#### **photoSynthetic piGmentS**

The results show that salinity and Si effects on carotenoid and total chlorophyll content are signiicant. Comparison of means shows that salinity level of 100 mM leads to decrease of total chlorophyll content and increase of carotenoid content (Table 2). The results of this study are consistent with the results of Maiti et al. (41), who reported that the reduction of chlorophyll content in salt stress condition is due to the activity of the chlorophyllase enzyme or because of the alteration of nitrogen metabolism in the production of compounds such as proline that is used in osmotic adjustment. Also, Khan et al. (35) in their study on alfalfa reported that the levels of chlorophyll a and b and total chlorophyll decrease with increasing salinity in alfalfa cultivars. Strogonov et al. (54) stated that the inhibition effects of salt on chlorophyll could be due to the prevention of the activity of specific enzymes responsible for the synthesis of green pigments. They stated that reducing chlorophyll content in sensitive plants is probably due to the chlorophyll destruction under salinity stress. While in salt tolerant plants, increasing chlorophyll content per leaf area is probably due to the improvement of photosynthetic apparatus. Also, the results showed that Si increased the content of photosynthetic pigments under salt stress (Table 2). In several studies, Si application has been reported to increase the concentration of pigments (2, 22).

# **relative water content (rwc)**

The interaction between salt stress and Si application on RWC was significant (Table 1). Comparison of mean values showed that Si application increased RWC under salt stress (Table 2). Under salt stress, RWC reduction is a common reaction, indicating that the plants are under osmotic stress (19). Si application has been reported to reduce osmotic potential in leaves of a number of plants under salt stress (13, 34, and 40). Possibly, application of Si increases the tolerance of plants to salinity by decreasing salt induced osmotic stress.

# **malondialdehyde (mda) and hydroGen peroxide (h2o<sup>2</sup> )**

In this study, the content of  $H_2O_2$  and MDA of shoot was influenced by the interaction between salt stress and Si application (Table 1). Salt stress increased  $H_2O_2$  and MDA contents in shoot, but the application of Si reduced the  $H_2O_2$  and MDA levels of shoot and improved the dry weight of the plants under salt stress (Table 2). The use of silicon probably reduces ROS production and increases the activity of antioxidant enzymes such as SOD, POD, CAT, and APX under salt stress. Abbas et al. (1) reported that Si application on leaves reduced lipid peroxidation and increased antioxidant enzymes activity. Also, in tomato seedlings under salt stress, it was determined that salinity increased the content of  $H_2O_2$  and MDA, resulting in oxidative stress, and the application of Si by increasing the activity of antioxidant enzymes and reducing oxidative stress in tomato plants under salt stress improved tolerance to salt stress (37). According to our data,  $H_2O_2$  and MDA contents in the shoots of savory plants significantly increased in salinity stress conditions, although the  $H_2O_2$  and MDA contents were very low in plants treated with Si application. **ATIVE WATER CONTENT (RWC)**<br>interaction between salt stress and Si application on RWC was sign<br>). Comparison of mean values showed that Si application increased<br>It stress (Table 2). Under salt stress, RWC reduction is a c

## **ionS accumulation**

Salinity had a significant effect on shoot sodium and potassium contents (Table 1). Increasing sodium chloride in the growth medium increased sodium accumulation and decreased potassium in shoot. Stress tolerance is probably the mechanism that focuses on the shoots. The decrease in potassium concentration is probably due to sodium antagonistic effects on potassium absorption sits in the roots, or the effect of sodium on potassium transfer in the plant's xylem vessels (43) or disturbance in the potassium uptake processes by this toxic ion (12). Maintaining of high levels of potassium in salt tolerant genotypes is one of the important mechanisms of salinity tolerance in plants (17). Also, the relative deiciency and inadequate potassium re-translocation is an effective factor in salinity. Under

#### hAMID MOhAMMADI, SAEID hAZRATI, LALEh PARVIZ

salinity conditions, the potassium transfer to growing tissues decreased, and as growing tissues accessed their requirement from the phloem vessel (which even under salinity conditions, potassium is predominant cation in phloem sap), potassium access reduction in salinity condition is the result of a reduction in phloem transfer cells. Since it is thought that under salt stress conditions, photosynthesis is not an inhibitor of growth, it seems that the basis of reduction in phloem transfer cells should be the limitation of loading in the phloem vessel, which can be due to low potassium or high sodium content in apoplast or due to inhibition by ABA, because ABA levels increase under salinity conditions (32). Also, the results showed that application of Si reduced the sodium content in shoot and increased K<sup>+</sup> /Na<sup>+</sup> ratio (Table 2). One of the most important mechanisms for plant tolerance to salt stress is the reduction of sodium absorption and accumulation by plants. Application of Si under salt stress in grapes (53) and wheat (4, 23) also reduced the transfer of sodium to aerial organ and increased potassium concentration. ress reduction in salinity condition is the result of a reduction in p<br>cells. Since it is thought that under salt stress conditions, photosynth<br>hibitor of growth, it seems that the basis of reduction in phloem to<br>hibitor o

## **proline content**

The loss of intracellular water, due to salinity, drought, and cold, leads to cellular dehydration. To prevent this and protect cellular proteins, plants accumulate many organic compounds, such as amino acids (proline) (56). These metabolites with their osmotic function have also been recognized as a compatible osmolytes or osmotic protector and may accumulate at high levels without interrupting intracellular biochemical reactions (21). By reducing the potential of water inside the cell, water loss is prevented and osmotic adjustment is facilitated (16). In our study, Si application increased the shoot proline content in *Satureja hortensis* plant under salt stress (Table 2). It has been reported that Si application may increase plant tolerance to salt stress by adjusting the levels of solutes and plant hormones. For example, silicon resulted in increased proline content, glycine betaine and free amino acids in okra (1) and tobacco (26) under salt stress.



Continued Table 2. Mean comparison of some traits

\*: Means followed by the same letter(s) in each column are not signiicantly different based on Duncan's Multiple Range Test (n=3).

35 MORPhOPhySIOLOGICAL AND BIOChEMICAL RESPONSE OF SAVORy MEDICINAL PLANT...

Mean squares						Source of
$K + Na +$	$K$ + content	$Na+ content$	Essential	Proline	df	variation
			oil yield	content		(S.O.V)
$4.592*$	$1.085*$	$0.011*$	$0.053*$	1127.254*	$\overline{2}$	<b>Block</b>
596.621**	85.805**	$1.901**$	$0.271**$	2318.646**		Salinity (S)
84.146**	$55.455**$	$0.168**$	$0.581**$	628.738**	$\overline{2}$	Si
$1.410**$	$2.752**$	$0.082**$	$0.002*$	211.180**	$\overline{2}$	$S*Si$
0.253	0.254	0.001	0.010	54.829	10	Error
2.58	2.00	2.28	9.01	17.42		CV

Continued Table 1. Analysis of variance (ANOVA) for studied traits

\*, \*\*: signiicantly different at the 5% and 1% probability level, respectively, ns: non-signiicant.

# **eSSential oil yield**

Essential oil yields differed in our study from 0.68% to 1.52% (Fig. 1). Si application increased their percentage of essential oils under no-stress and salinity stress conditions (Fig. 1). Other studies have showed that the oil contents of different Iranian accessions of *S. hortensis* are between 0.5% and 2.9% (25, 51), and can accumulate under severe stress at the lowering stage (8). however, when the plants were exposed to stress, they could probably accumulate a higher concentration of secondary metabolites which are the backbone of their essential oils (52). Studies showed that salinity stress reduces essential oil contents of some plant species such as mint species (6) and basil (5). however, in some other species such as marjoram, salinity stress increases the amount of several essential oil compounds (7). Moreover, it is observed that salinity stress increases the percentage of thymes, basil, and *Salvia oficinalis* essential oils (8, 18 and 29). But the results indicated different essential oil compounds. For example, in coriander roots, salinity stress increases the amount of carvacrol, but decreases the amount of  $\gamma$ -trepine (47). Studies indicated that an increase in oil gland density along with more gland production during the stress can be a reason for essential oil accumulation in some plant species. Another reason can be net assimilation or assimilation distribution during growth and differentiation processes. Sometimes, reduction of primary metabolism during stress causes the accumulation of special interface products; these interface products can shift toward secondary metabolites synthesis, such as essential oil. Secondary metabolites levels are reduced during stress, which is related to the general anabolism. Anabolism is prevented in salinity stress conditions (50). <sup>+</sup> K+ content Na+ content oil yield content dt (S.O.<br>  $^{18}$   $^{1085}$   $^{101}$   $^{0.65}$   $^{1127.254}$   $^{2}$  Bloc<br>  $^{18}$   $^{85.805**}$   $^{1901}$   $^{100**}$   $^{0.271**}$   $^{2118.646*}$   $^{11}$  Salinity<br>  $^{275.27*}$   $^{0.062**}$   $^{0.06$ 

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Fig. 1. Effects of Si application on essential oil yield of summer savory plant under salinity stress.

#### **CONCLUSION**

The results of this study showed that salt stress decreased plant dry weight, photosynthetic pigment content,  $K^{\dagger}/Na^{\dagger}$  ratio, increasing  $H_2O_2$  and MDA content, as oxidants, and proline accumulation as an osmosis adjuster with energy consumption. Application of Si by increasing of photosynthetic pigments contents probably improved the photosynthetic capacity and shoot dry weight. Also, the application of Si led to an increase in salt stress tolerance in *Satureja hortensis* plant by increasing the  $K^{\dagger}/Na^{\dagger}$  ratio, reducing the oxidants and possibly increasing the activity of the enzymatic or non-enzymatic antioxidant system, which provides significant protection for membranes against ROSs damage inside the tissues. Silicon has been able to increase the essential oil yield of *Satureja hortensis* medicinal plant under salt stress and non-stress conditions. Therefore, subsequent applied studies will create great hope in the near future, using silicon as a management tool to increase plant production capability and plant protection against the limitations of abiotic stresses and increasing the yield potential and the secondary metabolites of medicinal plants.

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39 MORPhOPhySIOLOGICAL AND BIOChEMICAL RESPONSE OF SAVORy MEDICINAL PLANT...

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