Salt stress changes biochemical, physiological and photosynthetic attributes of *Satureja spicigera*

Hoshang Rahmati[*1] and Borzou Yousefi[2]

1.Department of Agriculture, Technical and Engineering Faculty, Payam Noor University, Tehran, Iran,
2.Kermanshah Agricultural and Natural Resources Research and Education Center, Kermanshah, Iran

Abstract

We investigated the effect of salt stress on photosynthetic, physiological, and biochemical traits of *Satureja spicigera* (C. Koch) Boiss., a medicinal plant used in edible products and healthcare industries. The experiment was designed in a randomized complete block design (RCB) with three replications in a greenhouse. The salt treatments included four levels of NaCl (0, 50, 100, and 150 mM). Results showed that salinity levels caused a significant reduction in some photosynthetic, morpho-physiological, physiological, and biochemical characteristics; however, it boosted antioxidant activity. Salinity levels significantly reduced leaf fresh weight (12.56%), leaf dry weight (18.53%), relative water content (11.94%), chlorophyll a (33.33%), chlorophyll b (15.62%), chlorophyll a+b (29.24%), and carotenoid content (42.46%). However, salinity significantly boosted the antioxidant activity of superoxide dismutase (236.50%), peroxidase (85.67%), and catalase (82.78%) on average. Salt stress also significantly increased proline content (373.33%), protein content (84.49%), and leaf electrical conductivity (333.26%) on average. Results confirm that *S. spicigera* tolerates NaCl concentrations below 100 mM; however, it is highly sensitive to NaCl concentrations above 100 mM, so that a salinity of 150 mM causes a dramatic decrease in photosynthesis and growth. Therefore, we do not recommend the cultivation of this plant in highly saline and semi-saline soils.

Keywords: Antioxidant activity, Medicinal plants, Photosynthesis, Salt stress.

Abbreviation: C: control; Car: carotenoid; CAT: catalase; Chl: chlorophyll; LFW: leaf fresh weight; LDW: leaf dry weight; LTW: Leaf turgor weight; OD: optical density; POD: peroxidase; Pro: proline; ROS: reactive oxygen species; RWC: relative water content; SOD: superoxide dismutase.

Rahmati, H. and B. Yousefi, 2024**.** Salt stress changes biochemical, physiological and photosynthetic attributes of *Satureja spicigera*. *Iranian Journal of Plant Physiology 14* (4), 5257- 5264.

Introduction

Abiotic stresses affect the biosynthesis of primary and secondary metabolites in plants (Jan et al., 2021). Salinity stress interrupts the growth and development of crops by reducing water absorption, causing ionic imbalance, and leading to membrane and photosynthetic damage through excessive ROS production (Balasubramaniam et al., 2023), which negatively impacts food security. Under salinity stress, the production of large amounts of ROS causes oxidative stress, which harms cell membranes and cellular function. The enzymatic antioxidant

* *Corresponding Author*
E-mail Address hoshang.rahmatipnu@pnu.ac.ir
Received: May, 2024
Accepted: August, 2024

defense scavenges ROS by converting superoxide into H2O, which helps plants tolerate oxidative stress (Afridi et al., 2019). Salt stress also adversely affects photosynthetic pigments (Mohammadi et al., 2019; Zarei et al., 2019) and osmotic adjustment (Zhang et al., 2013). Proline, as a ROS scavenger (Kumar et al., 2017), and salt stress proteins (Athar et al., 2022) have a prominent effect on cell osmotic adjustment in response to salinity stress. Plant growth and development usually decrease due to the adverse effects of salinity on photosynthesis and related processes (Saadatfar and Jafari, 2024; Zarei et al., 2019). Throughout history, humankind has used aromatic plants in medicine and cooking. Recently, medicinal plants have attracted the attention of chemists, pharmacists, and botanists for their potential roles as alternatives to synthetic pharmaceuticals(Ahad et al., 2021; Kulak, 2020). The Lamiaceae family is one of the most important families of medicinal plants (Kulak, 2020), containing more than 6000 species with a global distribution. *Satureja spicigera* is a perennial medicinal plant belonging to the Lamiaceae family that grows in rocky places in northern and north-western Iran. Native people use this plant as a food additive and vegetable. This plant is also used in food, pharmaceutical, and health industries. There is limited data on the effect of salinity stress on this plant. This experiment was carried out to explore the possibility of growing this plant in low and semi-saline soils.

Materials and Methods

Experimental Design and Treatments

A greenhouse experiment with three replications, based on a randomized complete block design (RCB), was carried out at the Research Center for Agricultural and Natural Resources in Kermanshah, Iran. Seeds were disinfected with 0.5% sodium hypochlorite, washed, and dried. They were planted in a peat moss bed and watered by sprinkling. The seedlings were transferred to plastic pots (one seedling per pot), filled with a mixture of farm soil, sand, and composted cow manure. The plants were maintained under a 17-hour light photoperiod with 300 µmol/m²s (equivalent to 110 lux of light intensity), followed by 7 hours of darkness (Hernández-Adasme et al., 2023), with a relative humidity of 50-60%. Each pot was irrigated twice a week with 2500 ml of well water. Four treatments were implemented, involving irrigation (250 ml per pot, twice a week) with 0, 50, 100, and 150 mM NaCl (Kumar et al., 2022). We removed the accumulated salts from the pots with distilled water after every four irrigations with the NaCl treatments.

Morpho-physiological Measurements

We measured leaf fresh weight (LFW), leaf turgor weight (LTW), and leaf dry weight (LDW) using 30 young leaves from each plant. These young leaves were immediately weighed with precision (0.0001 g) (LFW). The leaves were immersed in double-distilled water for 18 hours to reach full hydration (22 °C). After drying the leaf surfaces, they were weighed again (LTW). The leaves were then placed in an oven (70 °C, 48 h), and the leaf dry weight (LDW) was measured. The means of LFW and LDW were calculated in grams (g). RWC was calculated using the following formula (Bian and Jiang, 2009):

$$RWC\ (\%) = (LFW–LDW)/(LTW–LDW)100$$

Photosynthetic Assays

Chlorophyll a, b, and carotenoid content were measured. The samples were centrifuged for 10 minutes (10000 rpm, 4 °C), and the supernatant was measured at 663, 646, and 470 nm using a microplate reader. The content of photosynthetic pigments (mg g^{-1} FW) was calculated using the following formulas (Lichtenthaler and Wellburn, 1983):

$$\text{Chl a} = 12.21\ (\text{A663}) - 2.81\ (\text{A646})$$

$$\text{Chl b} = 20.13\ (\text{A646}) - 5.1\ (\text{A663})$$

$$\text{Chl T} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenoid} = (1000\ \text{A470} - 3.27\ [\text{Chl a}] - 104\ [\text{Chl b}]/227)$$

Biochemical Assays

The extraction buffer and crude leaf extract were prepared (Reddy et al., 2004). The enzymatic

Table 1

Analysis of variance of photosynthetic pigments, proline content, protein content, RWC, LFW, LDW, and SOD, POD, and CAT activities in S. spicigera under different NaCl or, and NSe treatment.

S.O.V.	df	Chl a	Chl b	Carotenoid	Chl a+b	Proline	
Salt	3	34.99**	1.70 **	2.82 **	51.50 **	0.07**	
Error	6	0.28	0.08	0.05	0.58	0.02	
CV (%)		6.07	8.52	8.78	5.68	17.74	
S.O.V.	df	Soluble protein		Relative water content	SOD	POD	CAT
Salt	3	2384.00**		114.3 **	5.79 **	16.94**	7.39**
Error	6	1.22		16.5	0.02	0.1	0.013
CV (%)		0.17		4.79	7.38	12.54	2.95

* and **= significant differences at the level of 0.05 and 0.01, respectively and Ns = no significant difference

activity rate of superoxide dismutase (SOD, EC 1.15.1.1) was measured based on the ability of SOD to stop the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals in the presence of riboflavin under light conditions (Beauchamp and Fridovich, 1971). The optical absorbance was read at 560 nm (one enzymatic unit equals 50% inhibition) using a microplate reader. The activity of SOD was calculated using the following formula:

$$\mathrm{SOD}(\mu\mathrm{mol\ g^{-1}\ FW}) = \frac{100 - \left[\frac{(\mathrm{OD\ control} - \mathrm{OD\ sample})}{\mathrm{OD\ control}}\right] \times 100}{50}$$

where:

- **OD control**: absorbance of control at 560 nm
- **OD sample**: absorbance of samples at 560 nm

The peroxidase (POD; E.C. 1.11.1.7) activity was measured using a microplate reader and expressed in terms of H_2O_2 consumption (µmol min^{-1} mg^{-1} of soluble protein) (Chance and Maehly, 1955). The optical absorbance was recorded for 15 minutes at 30-second intervals at a wavelength of 470 nm, and calculated using the Beer-Lambert law (0.0266 $M^{-1}cm^{-1}$). The activity of catalase (CAT; E.C. 1.11.1.6) was measured (Sinha, 1972) with some modifications, using the Beer-Lambert law (extinction coefficient of 0.0394 $M^{-1}cm^{-1}$). Proline content was measured (Bates et al., 1973) as well as soluble protein concentration (mg g^{-1} FW) (Bradford, 1976).

Statistical Analysis

Analysis of variance and Duncan's Test ($p < 0.05$) were performed using IBM SPSS Statistics 26 software. The charts were created using Excel software.

Results

The results of ANOVA revealed significant differences ($p < 0.01$) for chlorophyll a, b, carotenoid, chlorophyll a + b, proline, soluble protein, relative water content, and SOD, POD, and CAT activities (Table 1).

Photosynthetic Pigments

The highest chlorophyll a (12.90 mg g^{-1} FW), b (3.67 mg g^{-1} FW), a + b (15.86 mg g^{-1} FW), and carotenoid (3.87 mg g^{-1} FW) were observed in the non-NaCl-treated plants. Salinity treatments significantly decreased, on average, the amounts of chlorophyll a, b, carotenoid, and chlorophyll a + b by 33.33%, 15.62%, 42.46%, and 29.24%, respectively (Fig. I). The highest photosynthetic performance index (2.86) was observed in the control plants. Salt treatments significantly reduced this trait by an average of 35.31% (Fig. II).

Antioxidant Activity

The highest SOD (3.64 µmol min^{-1} mg^{-1} protein), POD (3.05 µmol min^{-1} mg^{-1} protein), and catalase

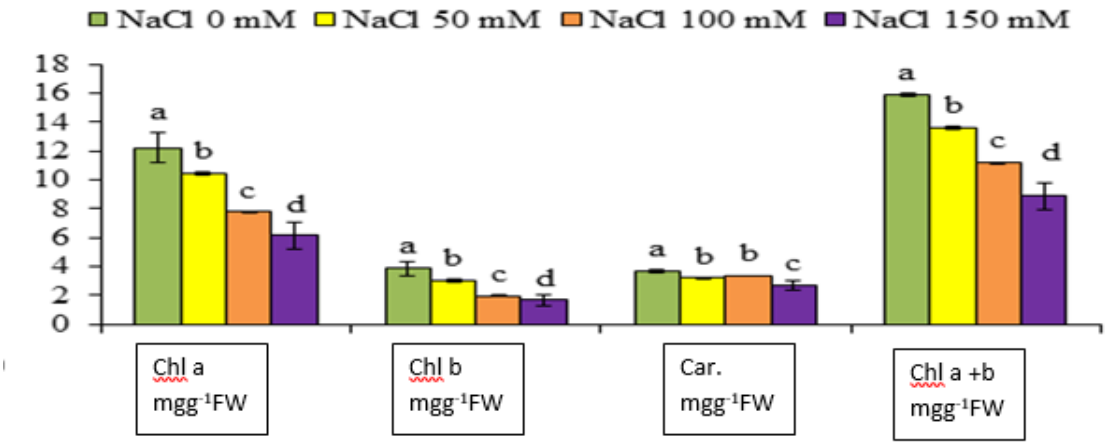

Fig I. Means comparison of chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll (a + b) content in *Satureja spicigera* plants in response to different NaCl treatments. Columns with the same letters are not significantly different based on LSD test (Mean ± SD, p = 0.05).

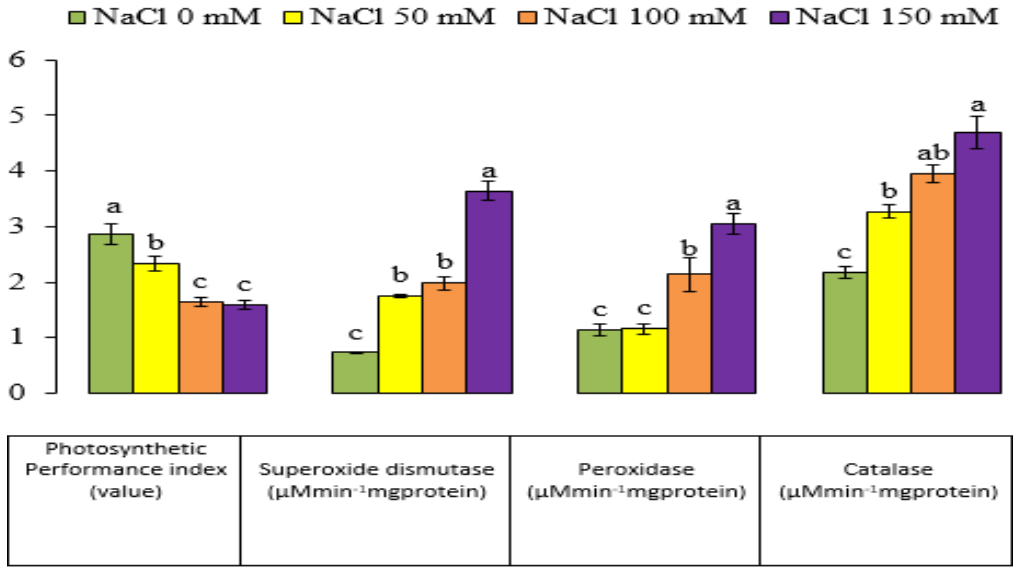

Fig.II. Means comparison (LSD Means ±SD) for photosynthetic performance index, superoxide dismutase, peroxidase, and catalase activity of *Satureja spicigera* plants in response to different NaCl treatments. The same letters are not show significant difference (P= 0.05).

activity (4.69 µmol min^{-1} mg^{-1} protein) were observed in plants treated with 150 mM NaCl (Fig. II). Salinity levels significantly increased SOD, POD, and catalase activity, on average, by 236.50%, 85.67%, and 82.78%, respectively, compared to the control plants (Fig. II).

The highest leaf fresh weight (12.31 mg) and leaf dry weight (2.45 mg) were observed in the plants treated with 50 mM NaCl (Fig. III a). The highest RWC (89.13%) was observed in the non-NaCl-treated plants; however, the highest LEC (378.23 µS cm^{-1}) was observed in the plants treated with 150 mM NaCl (Fig. III b). Salinity significantly decreased the leaf fresh weight by 12.56%, leaf dry weight by 18.53%, and leaf relative water content by 11.94% in the salt-treated plants compared to the control (Fig. III a, b). Salinity significantly increased LEC by 333.26% in the salt-treated plants compared to the control (Fig. III b).

Morpho-Physiological and Physiological Attributes

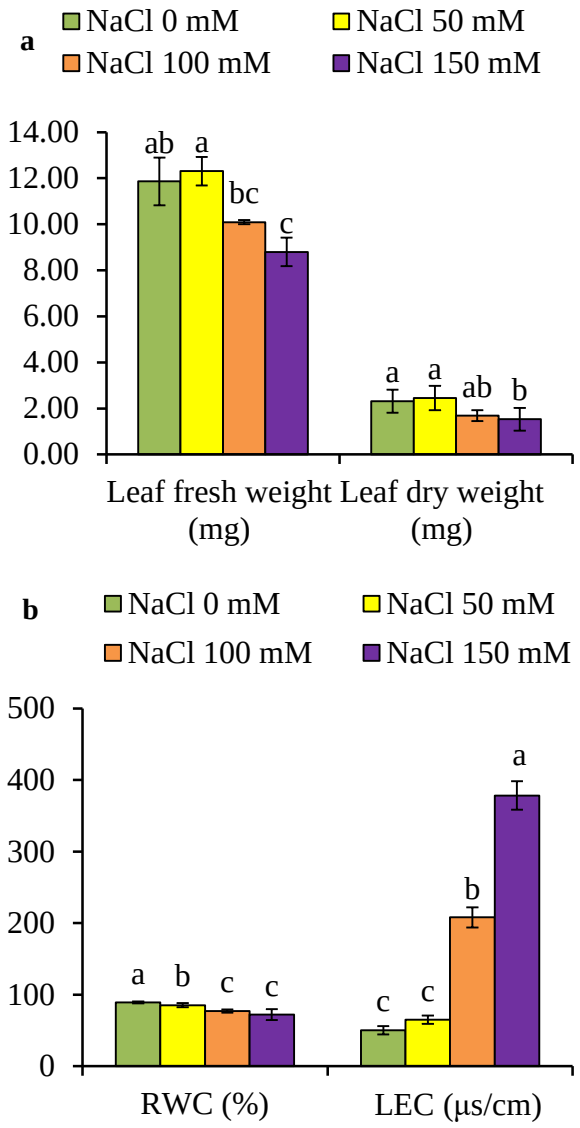

Fig III. Means comparison (LSD Mean's ± SD): (a) leaf fresh weight and leaf dry weight, (b) relative water content (RWC) and leaf electrical conductivity (LEC) of *Satureja spicigera* plants in response to different NaCl treatments. The same letters are not show significant difference (P= 0.05).

The relationship between the leaf relative water content and the electrical conductivity is inverse. Its trend can be seen in Fig. IV. This relationship shows that under salinity stress greater than 50 mM, the relative water content decreases with a gentle and uniform slope, but the electrical conductivity increases with a very steep slope.

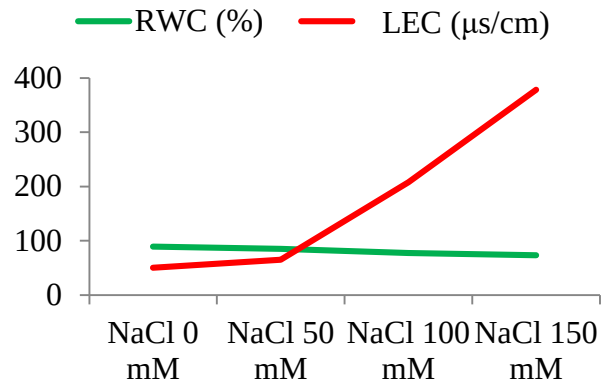

Fig IV. Relationship between relative water content (RWC) and leaf electrical conductivity (LEC) of *Satureja spicigera* plants during enhancement NaCl concentration.

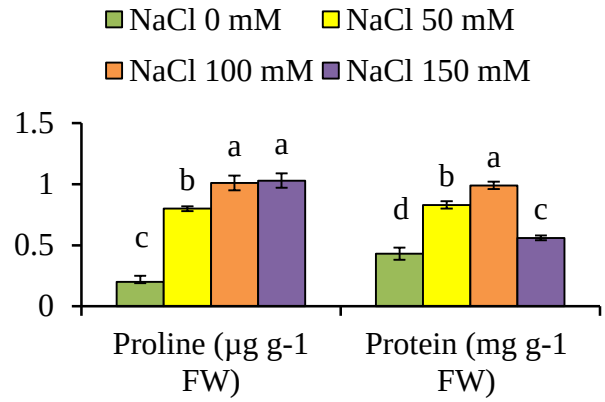

Fig. V. Means comparison (LSD Mean's ± SD) leaf proline content and leaf protein content of *Satureja spicigera* plants in response to different NaCl treatments. The same letters are not show significant difference (P= 0.05).

Biochemical Traits

Salt levels severely increased proline content. The highest proline (1.08 µg g^{-1} FW) was observed in 150 mM NaCl (Fig. V). Salt levels significantly increased proline content by 373.33% on average. The 50 and 100 mM NaCl treatments caused a severe rise in protein content (112% on average) compared to the control; however, 150 mM NaCl

improved the protein content by 30.23% compared to the control (Fig. V).

Discussion

In salt-sensitive plants, chlorophyll concentration decreases significantly at high levels of NaCl; however, in salt-resistant plants, the concentration of chlorophyll is less affected by salt stress(Srivastava and Sharma, 2021). In the present study, different NaCl levels caused a significant decrease in Chl a, Chl b, total Chl, carotenoid content, and the performance index of photosynthesis. Similar to our findings, salinity significantly decreased chlorophyll a and b in *Satureja hortensis* (Mohammadi et al., 2019)and *Satureja khuzestanica* (Saadatfar and Jafari, 2024), as well as carotenoid content in *Satureja hortensis* (FABRIKI and MEHRABAD, 2016).

Under oxidative stress, enzymatic antioxidant activity increases and scavenges excessive ROS. These enhanced enzymatic activities ameliorate oxidative damage caused by stressful agents (Garcia-Caparros et al., 2021). In the present research, the antioxidant activity of SOD, POD, and CAT significantly increased in response to NaCl concentrations. In *S. khuzestanica*, different NaCl levels significantly increased the activity of SOD, POD, and CAT (Saadatfar and Jafari, 2024). These results confirm our findings.

In plants under osmotic stress, the accumulation of proline and other osmoprotectants increases. In the present research, the proline content significantly augmented in response to salt levels. Similar to our findings, salinity levels significantly increased the proline content in *Satureja hortensis* (Mohammadi et al., 2019), *Satureja khuzestanica* (Saadatfar and Jafari, 2024), and *Thymus danensis* (Harati et al., 2015).

Low salt levels stimulate plant growth and cause the synthesis of de novo proteins; however, high salinity levels reduce the synthesis of proteins (Zhang et al., 2013). Also, a part of the proteins is decomposed in response to high salt (Hao et al., 2021). Different salt levels caused a significant increase in the protein content; however, this increase was limited at 150 mM NaCl compared to the other NaCl treatments. Similar to these results, low salinity treatments increased soluble protein, while high levels of NaCl significantly declined it in *Thymus vulgaris* (Harati et al., 2015).

Salinity reduces turgor pressure and interrupts the ionic balance between the plant and soil (Wang et al., 2023). Therefore, salinity strongly affects the plant's water status. The relative water content significantly decreased in response to different levels of NaCl; however, leaf electrical conductivity significantly increased. Similar to these findings, RWC significantly decreased in response to different salt concentrations in *Lemon verbena* (Ghanbari et al., 2023)and *Oryza sativa* (Jini and Joseph, 2017).

In the present study, NaCl caused a significant decrease in leaf fresh weight and leaf dry weight. A high salt concentration in the root zone reduces the osmotic potential of water in the surrounding roots. Therefore, the plant's access to water is reduced. A lack of water in the plant causes partial closure of the stomata, thus reducing the entry of CO_2 into the plant (Shanker et al., 2022). Reducing the absorption of CO_2 in plants diminishes photosynthesis and growth. Salinity causes ion toxicity (in the form of Na^+ and Cl^-). A high concentration of Na^+ decreases nitrate reductase activity and inhibits the functioning of photosystem II ((Sheldon et al., 2017), which leads to a decrease in plant growth. Additionally, salinity causes nutrient deficiency (N, P, K, Zn, and Fe), which decreases plant growth and development (Sheldon et al., 2017).

The destruction of cell membranes due to salt is associated with a diminution in the absorption and transfer of nutrients through the roots, a decrease in the biosynthesis of chlorophyll, and an increase in the destruction of chlorophyllase due to salinity, which causes a decrease in plant growth and development.

Conclusion

The present study confirms that *S. spicigera*, to some extent, tolerates concentrations of less than 100 mM NaCl, but it is highly sensitive to concentrations greater than 100 mM NaCl, such

that a salinity of 150 mM causes a significant decrease in photosynthesis and growth. Therefore, we do not recommend the cultivation of this plant in saline or semi-saline soil.

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