

# The Effect of NaCl Stress on Biochemical, Physiological, Photosynthetic and Morpho-physiological Indicators of the Medicinal Plant Forest Savory

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## ABSTRACT

*Satureja mutica* Fisch. & C. A. Mey (Forest Savory) is a valuable wild plant species widely used in the medicinal, health, and food industries. In this study, we investigated the effects of 0, 50, 100, and 150 mM NaCl on various physio-biochemical, morpho-physiological, and photosynthetic parameters of this plant through a greenhouse experiment. The experiment was conducted using a randomized complete block design (RCBD,  $r = 3$ ) at the Agricultural and Natural Resources Research Center, Kermanshah, Iran. The highest shoot fresh weight (17.80 g) and shoot dry weight (6.73 g) were observed in the control plants. The highest essential oil content (EO) percentage (3.51%) was recorded in plants treated with 100 mM NaCl. The results showed that NaCl concentrations of 100 and 150 mM significantly reduced the leaf dry weight (by 37.13% and 41.86%), the shoot dry weight (by 51.54% and 82.74%), root fresh weight (by 77.92% and 82.74%), and the root dry weight (by 70.79% and 78.97%). Additionally, 100 and 150 mM NaCl significantly decreased leaf areas (by 23.55% and 28.01%), leaf relative water content (by 25.66% and 28.53%), SPAD values (by 6.36% and 41.35%), and the Fv/Fm ratio (by 10.21% and 16.40%). Furthermore, 150 mM NaCl resulted in a 44.81% reduction in the photosynthetic index (PI). The 50 and 100 mM NaCl treatments significantly increased leaf protein content by 50.67% and 82.22%, respectively, whereas 150 mM NaCl significantly decreased it. All salinity treatments caused a sharp increase in leaf proline content. In conclusion, the results confirmed that *S. mutica* is sensitive to salinity concentrations of 100 mM and higher, and thus, cultivating this plant in semi-saline or saline soils is not recommended.

**Keywords:** Forest savory, Medicinal plants, Photosynthesis, Salt stress

### Abbreviation

C: control; EO: essential oil, Fv/Fm: maximum quantum yield of photosystem II, LFW: leaf fresh weight; LDW: leaf dry weight; OD: optical density; PI: photosynthetic index; PROT: protein; PROL: proline; RWC: relative water content; SFW: shoot fresh weight, SDW: shoot dry weight, SPAD: chlorophyll index; RFW: root fresh weight, RDW: root dry weight.

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## INTRODUCTION

Humankind has used aromatic plants in medicine and cooking throughout history. Recently, medicinal plants have increasingly attracted the attention of chemists, pharmacists, and botanists as alternatives to synthetic pharmaceuticals [1]. The Lamiaceae family is one of the most important groups of medicinal plants [1], comprising more than 6,000 species with a cosmopolitan distribution. Forest savory, a medicinal species in the mint family, is a highly aromatic plant native to northern and northeastern Iran, Transcaucasia, and Turkmenistan [2]. It has been traditionally used to treat rheumatic pain, migraines, toothaches, and diarrhea [3]. Additionally, it is utilized in modern pharmaceutical, hygiene, and food industries. Recently, the cultivation of this plant has gained attention from experts in agriculture, horticulture, and medicinal plant sciences. Salinity reduces carbon dioxide absorption, disrupts cellular and photosynthetic membranes, and causes ionic imbalances [4]. It also decreases water and nutrient uptake and

transport [5], significantly impairing plant growth and development [6]. Photosynthetic indices are important indicators of plant tolerance to salt stress [7]. High salt concentrations destabilize protein-pigment complexes, stimulate chlorophyllase enzyme activity, and increase the production of reactive oxygen species (ROS) [7]. These responses reduce the levels and efficiency of photosynthetic pigment [8], ultimately leading to lower plant biomass. Plant growth and development generally decline due to the adverse effects of salinity on photosynthesis and its associated processes [9,10].

Relative water content, leaf proline levels, and soluble proteins are key determinants of leaf survival and overall plant metabolic activity under salinity stress conditions. High salt concentrations (lower osmotic potential) in the soil reduce water potential ( $\psi$ ), making water uptake more difficult for plants [6,11], leading to a significant decrease in leaf water content [12]. Under osmotic stress conditions, proline accumulation helps plants maintain osmotic

balance [13]. Furthermore, proline functions as a non-enzymatic cellular antioxidant, preventing ROS buildup and protecting plants against abiotic stresses [14]. Soluble proteins in leaves accumulate under mild salt stress through de novo biosynthesis of stress-related proteins [15]. However, severe salt stress inhibits de novo protein biosynthesis, including those associated with the photosystem [16]. Additionally, under high salt stress, some leaf proteins degrade into amino acids that function as compatible solutes (osmolytes) [17]. Proline, as both a ROS scavenger and a salt stress-responsive protein [18,15], plays a crucial role in cellular osmotic adjustment under salinity stress. This experiment was conducted to evaluate the feasibility of cultivating forest savory in saline soils.

## MATERIALS AND METHODS

### Experimental Design and Treatments

A greenhouse experiment (three replications) based on a randomized complete block design (RCBD), was carried out in the research center of Agricultural and Natural Resources, Kermanshah, Iran. Seeds were disinfected with 0.5% sodium hypochlorite, washed, and dried. Seeds 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 1:1:1 mixture of farm soil, sand, and decomposed cow manure. The plants were maintained under a 17-hour light/7-hour dark photoperiod, with a light intensity of 300  $\mu\text{mol}/\text{m}^2/\text{s}$  (equivalent to 110 lux) [19], and a relative humidity of 50–60%. For two weeks prior to the initiation of NaCl treatments, each pot was irrigated twice a week with 2500 mL of well water. Subsequently, four irrigation treatments (250 mL per pot, twice a week) with 0, 50, 100, and 150 mM NaCl solutions were applied [20]. After every four NaCl irrigations, the accumulated salts in the pots were leached by irrigating with distilled water.

### Measurements of Studied Variables

#### Morpho-physiological Variables

We measured leaf fresh weight (LFW), and leaf dry weight (LDW) using the 30 young leaves from each plant. These young leaves were immediately weighed precisely meticulously (0.0001 g) (LFW). The leaves were immersed in double distilled water for 18 hours to complete dehydration (22 °C). Then we dried the surface of the leaves and weighed them immediately (LTW). The leaves were placed in an oven (70 °C, 48 h) and the leaf dry weight (LDW) was measured. The means of LFW and LDW were calculated (g). After plant harvesting, we measured shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), and root dry weight (g). The shoots and roots, separately were placed in an oven (75 °C, 72 h) and the shoot dry weight (SDW) and root dry weight (RDW) were measured.

#### Physiological Variables

Relative water content (RWC) was calculated from the following formula [21]:

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

### Leaf Electrical Conductivity (LEC)

The thirty leaves from each plant were separated and washed with distilled water and they were immersed in 25 ml of double-distilled water (22 °C and 24 h). Then the leaf EC (mS/cm) was measured with EC COND 3110, WTW, Germany [22].

### Biochemical Assays

#### Preparation of Extraction

The extraction buffer (200 ml) was prepared based on the method of Ramachandra *et al.* 2014 [23]. The 2.428 g of Tris with 0.2 g PVP dissolved well in 40 ml of DDW (pH= 8), final volume reached 200 ml, the containers were covered with aluminum foil and kept in the refrigerator (4 °C).

#### Evaluation of Proline Content

Proline content (PROL) was evaluated according to Bates' method [24]. The ODs of proline samples were read at 520 nm using a Bio Tek XS2 Microplate Reader, USA. ( $\mu\text{mol}/\text{g}$  FW)

#### Measurement of leaf Soluble Proteins

Leaf soluble proteins (PROT) were measured by the Bradford method (1976). The 1  $\mu\text{l}$  of the crude leaf extract was added to 200  $\mu\text{l}$  of Coomassie Brilliant Blue. After 15 minutes, the OD of samples was read at 595 nm by a Bio Tek XS2 Microplate Reader, USA. The concentration of soluble protein was obtained according to the absorption of the samples and using the Bovine Serum Albumin (BSA) standard curve [25]. Soluble protein concentration was expressed as mg/g FW.

#### Photosynthetic Variables

For measurement of Fv/Fm value and Photosynthetic index (PI), 30 leaves from each repeat were covered with aluminum foil and were adapted in the dark for 30 minutes. Fv/Fm and PI were estimated using a chlorophyll fluorimeter (Hansatech Pocket PEA, UK) at 695 nm. The Photon flux density (PFD) was 400  $\mu\text{mol}/\text{m}^2/\text{s}$  and the light duration was 5 seconds. Leaf chlorophyll index (SPAD) was measured using a SPAD-502Plus, Minolta, Japan (30 leaves of each plant).

#### Statistical Analysis

Analysis of variance, Duncan's test ( $p < 0.05$ ), and Pearson's correlation estimation were performed using IBM SPSS Statistics 26 software. The charts were drawn using Excel software. Principal component analysis was done using Minitab software (ver.16).

## RESULTS

The effect of NaCl treatments was significant for all studied morpho-physiological, physiological, photosynthetic, and yield traits ( $p \leq 0.01$ ) (Table 1) except for leaf fresh weight (LFW).

**Table 1** Results of ANOVA for studied morpho-physiological, photosynthetic and physiological traits of *S. mutica* under effect of 0, 50, 100 and 150 mM NaCl treatments

S.O.V	df	LFW	LDW	SFW	SDW	RFW	RDW	Plant height	RWC
NaCl	3	1.94 ns	0.58 **	52.36 **	11.74 **	92.24 **	20.31 **	279.86 **	617.96 **
Error	6	0.84	0.24	3.45	0.43	4.49	0.01	43.53	9.24
CV		9.32	22.00	13.81	16.48	23.04	9.10	11.04	3.84
S.O.V	df	SPAD	PI	Fv/Fm	Leaf area	Protein	Proline	EO percent	LEC
NaCl	3	316.98 **	1.26 **	0.01 **	0.03 **	3202.2 **	0.003 **	0.63 **	49012.0 **
Error	6	3.48	0.28	4.30	0.00	3696.1	0.002	0.05	68.51
CV		5.21	21.81	28.56	9.35	7.08	23.17	7.74	6.04

\* and \*\* respectively refer to significant at 0.05 and 0.01 levels, ns: non-significant

### Morpho-physiological and Yield Traits

The highest leaf fresh weight (LFW: 10.43 mg) was observed in the control plants; however, the maximum leaf dry weight (LDW: 1.92 mg) was obtained in the plants treated with 50 mM NaCl (Fig. 1). 50 and 100 mM NaCl had no significant effects on leaf fresh weight; nevertheless, 150 mM NaCl significantly decreased it (as much as 16.84% compared to control). Also, 100 and 150 mM NaCl, respectively reduced the leaf dry weight by 37.13% and 41.86% compared to control (Fig. 1).

The highest shoot fresh weight (SFW: 17.80 g) and shoot dry weight (SDW: 6.73 g) were observed in the control plants (Fig. 1). We observed that the shoot fresh weight decreased considerably by 46.26% and 42.74%, respectively in the *S. mutica* plants treated with 100 and 150 mM NaCl compared to control (Fig.1). In addition, the shoot dry weight was significantly reduced by 42.57, 51.54 and 82.74% in the plants treated with 50, 100 and 150 mM NaCl, respectively (Fig.1).

The highest root fresh weight (RFW: 14.52 g) and root dry weight (RDW: 7.70 g) were recorded in the control plants (Fig. 1). Although the 50 mM NaCl did not have an adverse effect on the fresh and dry weights of leaf and shoot (it induced leaf and shoot growth), however, it significantly was reduced root fresh and dry weight. All NaCl treatments negatively and significantly affected the root fresh and root dry weights, so that, the root fresh weight reduced by 62.65, 77.92 and 82.74%, respectively in the plants treated with 50, 100 and 150 mM NaCl. Additionally, root dry weight (RDW) decreased by 62.62, 70.79, and 78.97% in the plants treated, respectively (Fig. 1).

The highest plant height (PLH: 66.67 cm) and relative water content (RWC: 91.66%) were observed in the control plants, however, the maximum chlorophyll index (SPAD: 45.27 value) was measured in the plants treated with 50 mM NaCl (Fig. 2). Plant height was significantly reduced by 31.50% in the plants treated with 150 mM NaCl compared to the control (Fig. 2). 100 and 150 mM NaCl treatments caused a significant decline in the relative water content as much as 25.66 and 28.53% compared to the control, respectively (Fig. 2). SPAD values were also diminished by 36.36 and 41.35% in the plants treated with 100 and 150 mM NaCl, respectively (Fig. 2).

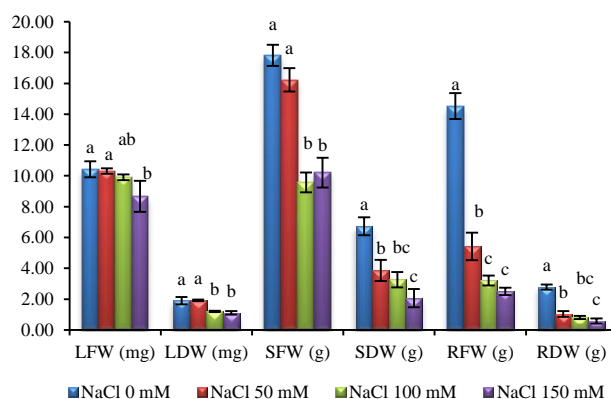
### Photosynthetic Traits

The highest Photosynthetic index (PI: 2.99 value) was observed in the 50 mM NaCl-treated plants, but the maximum quantum yield of PSII (Fv/Fm: 0.79 value) was detected in the control plants. The photosynthetic index was significantly affected only through 150 mM NaCl, while the maximum quantum yield of PSII was significantly affected by 100 and 150 mM NaCl. We observed that PI significantly decreased by 44.81% in the plants treated with 150 mM NaCl, and Fv/Fm meaningfully declined by 10.21 and 16.40% in plants treated with 100 and 150 mM NaCl, respectively, compared with control (Fig. 3). The highest leaf area (LA: 0.67 cm<sup>2</sup>) was observed in the control and plants treated with 50 mM NaCl (equally). This trait decreased significantly by 23.55 and 28.01% in the plants treated with 100 and 150 mM NaCl, respectively, compared to control (Fig. 3).

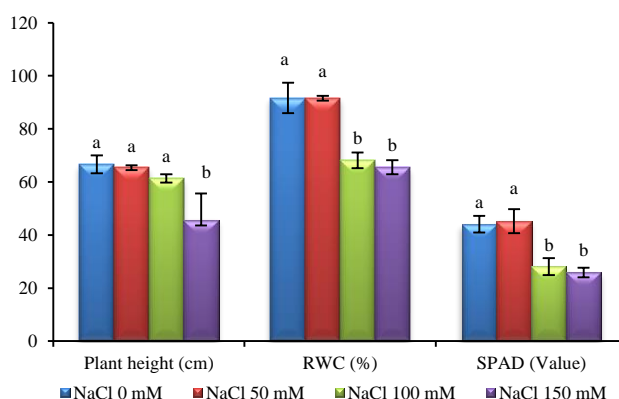
### Bio-physiological and Essential Oil Traits

50 and 100 mM NaCl caused increases of 50.67 and 82.22% in the leaf protein content, respectively, compared to the control treatment (0 mM NaCl); however, 150 mM NaCl significantly decreased it by 24.56% (Fig. 3). The trend of the effect of NaCl on leaf proline content difference from that of leaf protein content,

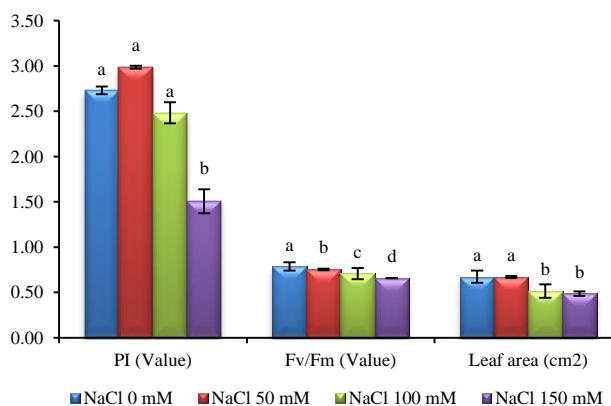
such that all three NaCl treatments significantly increased leaf proline content. 50, 100 and 150 mM NaCl caused drastic increases in the leaf proline content by 461.54, 976.62 and 976.16%, respectively (Fig. 3). The essential oil content increased significantly by 9.17 and 31.39% in the plants treated with 50 and 100 mM NaCl, respectively; however, 150 mM NaCl reduced it by approximately 8.47% compared to the control (Fig. 3). Finally, we observed that all NaCl treatments significantly increased leaf electrical conductivity (LEC), such that LEC intensified by 55.86, 140.16 and 621.10% in plants treated with 50, 100, and 150 mM NaCl, respectively compared to control (Fig. 5).



**Fig. 1** Means comparison (Duncan test,  $p \leq 0.05$ ,  $r=3$ ,  $n=30$ ) for leaf fresh weight (LFW), leaf dry weight (LDW), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) of *S. mutica* plants treated with 0, 50, 100, and 150 mM NaCl.

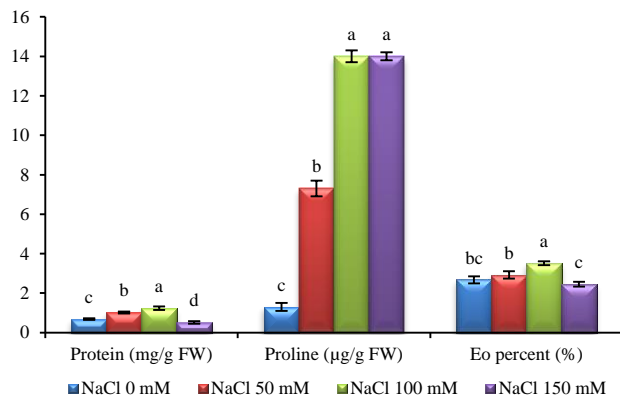


**Fig. 2** Means comparison (Duncan test,  $p \leq 0.05$ ,  $r=3$ ,  $n=30$ ) for plant height (PLH), relative water content (RWC) and photosynthetic index (SPAD value) of *S. mutica* plants treated with 0, 50, 100, and 150 mM NaCl.

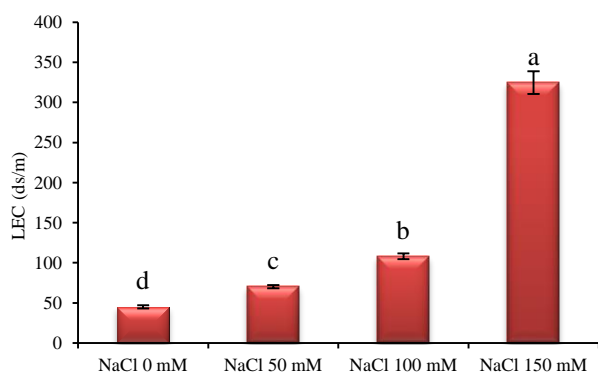


**Fig. 3** Means comparison (Duncan test,  $p \leq 0.05$ ,  $r=3$ ,  $n=30$ ) for photosynthetic index (PI), maximum quantum yield of PSII (Fv/Fm) and

leaf area index (LA) of *S. mutica* plants treated with 0, 50, 100, and 150 mM NaCl.



**Fig. 4** Means comparison (Duncan test,  $p \leq 0.05$ ,  $r=3$ ,  $n=9$ ) for leave protein content (PROT), proline content (PROL) and essential oil content (EO) of *S. mutica* plants treated with 0, 50, 100, and 150 mM NaCl.



**Fig. 5** Means comparison (Duncan test,  $p \leq 0.05$ ,  $r=3$ ,  $n=30$ ) for leaf electrical conductivity (LEC) of *S. mutica* plants treated with 0, 50, 100, and 150 mM NaCl.

### Principal Components Analysis

The results of principal components analysis revealed that the first two components explained 94% of the total variance (Table 2). Morpho-physiological, physiological, yield, and photosynthetic

traits showed a high correlation with the control and low salinity (50 mM NaCl) treatments. According to Figure 6, the variables of leaf area (LA), fresh and dry weight of shoot (SFW and SDW), fresh and dry weight of root (RFW and RDW), dry weight of leaf (LDW), relative water content (RWC), and chlorophyll index (SPADS) had similar trends and showed the strongest associations with the control treatment (Fig. 6).

**Table 2** Variance contribution of different variables in components (PC1 and PC2)

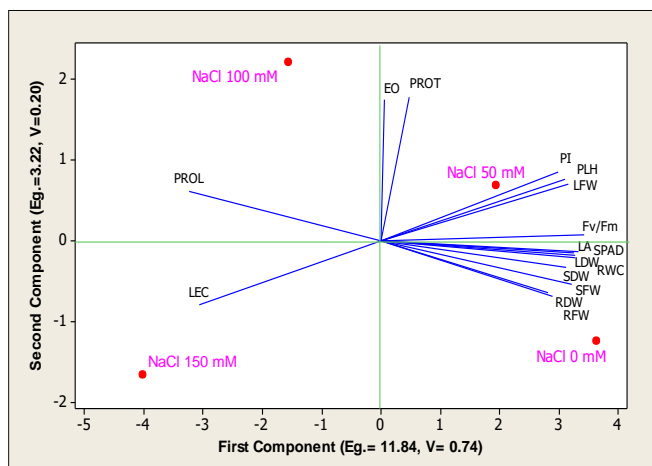
Variable	PC1	PC2	Variable	PC1	PC2
LFW	0.268	0.216	SPAD	0.275	-
LDW	0.277	-0.056	PI	0.252	0.046
SFW	0.272	-0.167	Fv/Fm	0.290	0.024
SDW	0.264	-0.101	LA	0.281	-
RFW	0.245	-0.213	PROT	0.040	0.044
RDW	0.239	-0.198	PROL	-0.272	0.190
PLH	0.263	0.235	EO	0.005	0.541
RWC	0.278	-0.064	LEC	-0.258	-
Eg.	11.84	3.22	Eg.	11.84	3.22
V%	0.74	0.20	V%	0.74	0.20

The highest values of these variables were observed in the control group, indicating that is, salinity caused a significant reduction in them. Plant height (PLH), leaf fresh weight (LFW), and photosynthetic traits (PI and Fv/Fm) showed similar patterns and were strongly associated with 50 mM NaCl. It can be concluded that low salinity (50 mM NaCl) stimulated the photosynthetic system and improved plant height. Leaf soluble protein and the percentage of essential oil content followed a similar trend with a closer relationship to the 50 mM NaCl and a lesser extent 100 mM NaCl. Proline content had a unique pattern, exhibiting the strongest association with 100 mM NaCl. On the other hand, the leaf electrical conductivity LEC exhibited the highest correlation with 150 mM NaCl (Fig. 6).

**Table 3** Pearson's correlation estimation between studied morpho-physiological, photosynthetic and physiological traits of *S. mutica* under effect of 0, 50, 100 and 150 mM NaCl treatments

Traits	LFW	LDW	SFW	SDW	RFW	RDW	Plant height	RWC
LFW	1.00	-	-	-	-	-	-	-
LDW	0.83	1.00	-	-	-	-	-	-
SFW	0.74	0.98 *	1.00	-	-	-	-	-
SDW	0.79	0.78	0.83	1.00	-	-	-	-
RFW	0.65	0.73	0.83	0.98 *	1.00	-	-	-
RDW	0.64	0.69	0.79	0.98 *	0.99 **	1.00	-	-
Plant height	0.99 **	0.80	0.71	0.77	0.63	0.62	1.00	-
RWC	0.82	0.99 **	0.98 *	0.79	0.74	0.71	0.80	1.00
SPAD	0.82	0.99 **	0.97 *	0.76	0.71	0.67	0.80	0.99 **
PI	0.98 *	0.82	0.70	0.65	0.49	0.48	0.98 *	0.81
Fv/Fm	0.94	0.93	0.91	0.92	0.85	0.83	0.92	0.93
Leaf area	0.85	0.98 *	0.97	0.80	0.75	0.72	0.82	0.99 **
Protein	0.51	0.04	-0.16	-0.06	-0.27	-0.25	0.54	0.03
Proline	-0.73	-0.91	-0.97 *	-0.94	-0.95	-0.92	-0.71	-0.92
EO percent	0.40	-0.15	-0.32	-0.07	-0.27	-0.23	0.44	-0.16
LEC	-0.99 **	-0.77	-0.68	-0.77	-0.62	-0.62	-0.99 **	-0.77
Traits	SPAD	PI	Fv/Fm	Leaf area	Protein	Proline	EO percent	LEC
SPAD	1.00	-	-	-	-	-	-	-
PI	0.83	1.00	-	-	-	-	-	-
Fv/Fm	0.92	0.88	1.00	-	-	-	-	-
Leaf area	0.99 **	0.84	0.95	1.00	-	-	-	-
Protein	0.06	0.59	0.18	0.06	1.00	-	-	-
Proline	-0.89	-0.64	-0.92	-0.92	0.21	-	-	-
EO percent	-0.14	0.44	0.07	-0.12	0.96	0.30	1.00	-
LEC	-0.77	-0.97 *	-0.91	-0.80	-0.56	0.69	-0.47	1.00

\* and \*\* respectively refer to significant correlations at 0.05 and 0.01



**Fig. 6** Diagram of principal components analysis for the first and second components ( $V\% = 0.94$ )

### Pearson's Correlation Estimation

The results of Pearson's correlation analysis (Table 3) for the studied traits revealed a positive correlation between leaf fresh weight (LFW), plant height (PLH), and leaf area (LA) ( $r = 0.01$ ). Additionally, LFW exhibited a significant positive correlation with the photosynthetic index (PI) ( $r = 0.05$ ). In contrast, the correlation between leaf fresh weight and leaf electrical conductivity (LEC) was negative ( $r = 0.01$ ).

Leaf dry weight (LDW) showed positive correlations with leaf fresh weight ( $r = 0.05$ ), leaf area ( $r = 0.05$ ), relative water content (RWC) ( $r = 0.01$ ), and chlorophyll index (SPAD) ( $r = 0.01$ ). Shoot fresh weight (SFW) had a significant positive correlation ( $r = 0.05$ ) with the chlorophyll index, relative water content, and proline (PROL) content. Similarly, shoot dry weight exhibited a significant correlation ( $r = 0.05$ ) with both root fresh weight and root dry weight. A strong positive correlation was observed between root fresh weight and root dry weight at the 1% significance level. Plant height showed a significant positive correlation with the photosynthetic index ( $r = 0.05$ ) but a significant negative correlation with leaf electrical conductivity (LEC) ( $r = 0.01$ ). Additionally, relative water content displayed a significant positive correlation with both leaf area ( $r = 0.01$ ) and the chlorophyll index ( $r = 0.01$ ). A similar significant positive correlation was found between the chlorophyll index and leaf area ( $r = 0.01$ ). Finally, a significant negative correlation was observed between the photosynthetic index and leaf electrical conductivity ( $r = 0.05$ ).

### DISCUSSION

All studied morpho-physiological variables of *Satureja mutica* were affected by salt stress. NaCl concentrations of 100 and 150 mM resulted in a decrease in plant height, leaf area, leaf fresh and dry weight, shoot fresh and dry weight, as well as root fresh and dry weight. In line with these findings, salinity stress has been reported to reduce plant height in *Satureja khuzestanica* [26], leaf area in *Thymus vulgaris* [27], and leaf fresh and dry weights in *Pyrus betulifolia* Bunge [28]. Similarly, in *Amaranthus cruentus*, NaCl concentrations of 50, 75, and 100 mM have been shown to diminish shoot dry weight, root fresh weight, and root dry weight [29]. Su *et al.* (2013) stated that salt stress negatively affects the morpho-physiological characteristics of plants, with leaf area and plant height being among the first traits to exhibit these effects [30].

Photosynthetic performance, Fv/Fm, and SPAD values are typically reduced under stress conditions [31]. The chlorophyll index is an appropriate indicator for evaluating a plant's photosynthetic potential [32]. Shah *et al.* (2017) demonstrated that SPAD-502 readings and plant photosynthetic pigment content are profoundly affected by salinity [33].

In this study, we observed a significant decrease in the chlorophyll index (SPAD) in plants treated with high NaCl concentrations. Similar findings have been reported, where salt stress led to a decrease in SPAD values in citrus [34], *Triticum aestivum* [33], and tomato [35]. Chlorophyll fluorescence provides valuable information for assessing plant physiological changes under stress. The maximum quantum yield of PSII (Fv/Fm) is a sensitive indicator for the early detection of plant responses to environmental stressors [36]. Salinity stress disrupts chlorophyll structure, causing fluctuations and disturbances in chlorophyll fluorescence. In the present study, the Fv/Fm value declined in NaCl-treated plants compared to the control group. Similarly, high salinity levels have been shown to decrease Fv/Fm in *Hypericum perforatum* [37] and *Nitraria schoberi* L. (Nitre Bush) [38].

We observed that NaCl treatment significantly decreased leaf relative water content (RWC), while it critically increased leaf electrical conductivity. Consistent with these findings, salinity significantly reduced RWC in *Trigonella foenum-graecum* L. [39] and increased relative leaf electrical conductivity (REC) in *Dianthus superbus* [40].

Plants regulate their osmotic potential by accumulating osmolytes to prevent dehydration during water stress [41]. In the present study, proline content significantly increased in plants treated with NaCl. Similarly, a meaningful increase in proline content has been observed with increasing salt levels in *Satureja khuzestanica* [9]. Leaf soluble protein levels increased with salinity up to 100 mM NaCl but decreased at 150 mM NaCl. This pattern aligns precisely with findings in *Thymus vulgaris*, where protein content increased under mild salt stress but declined at higher salt concentrations [27]. Athar *et al.* (2022) noted that mild salinity stimulates the biosynthesis of stress and structural proteins, whereas severe salinity stress leads to the breakdown of storage and structural proteins into their basic units (amino acids) [15]. Additionally, under extreme salinity stress, reduced photosynthesis, limited nutrient availability, and cellular disruption result in fewer resources for protein biosynthesis. Thus, while mild salt stress enhances leaf-soluble proteins, severe salt stress ultimately causes a decline in total leaf-soluble protein content.

### CONCLUSION

We concluded that mild and high salt concentrations negatively affected the studied biochemical, physiological, morpho-physiological and photosynthetic traits. These adverse effects reduced the growth and yield of the *Satureja mutica* plants. We found that the tolerance threshold of *Satureja mutica* to salinity was below 100 mM. Our results confirm that applying salinity treatment at 50 mM or lower enhances essential oil (EO) production in forest savory plants. Based on the observations, we do not recommend the cultivation of this plant in semi-saline and saline soils, unless remedial methods are used to reduce soil salinity or modifying methods (genetic modification) and/or modifying materials (plant hormones, nanomaterial, etc) are applied to increase salt tolerance.



### Authorship Contribution Statement

Hooshang Rahmati performed the experiments and Amir Reza Yousefi analyzed data and wrote the manuscript.

### Competing Interests

The authors have no competing interests to declare that they are relevant to the content of this article.

### Data Availability

All data generated during this study are included in this article.

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