# Effects of NaCl stress on growth, photosynthesis, and phytochemical composition of creeping savory (*Satureja spicigera* (C. Koch) Boiss.)

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## Abstract

The study examined the effects of NaCl treatments (50, 100, and 150 mM) on various morpho-physiological, photosynthetic and phytochemical traits of creeping savory, a wild medicinal plant, in a greenhouse experiment based on a completely randomized design (CRD) with three replications. Increasing NaCl concentration led to a linear decline in growth parameters, with the highest reductions at 150 mM. Leaf, shoot, and root fresh and dry weights decreased significantly under all NaCl levels. Photosynthetic index improved at 50 mM but declined at 150 mM NaCl. Chlorophyll index also decreased significantly at 150 mM. Essential oil percentage increased under 50 and 100 mM NaCl, however dropped at 150 mM. While α-pinene, β-bisabolene, terpinene-4-ol, methyl ether thymol, methyl ether carvacrol, *trans*-caryophyllene, *p*-cymene, and γ-terpinene were decreased in plants treated with NaCl, the percentages of thymol and carvacrol were increased.

## Key words:

Essential oil; Medicinal plants; Photosynthetic indices; Phytochemicals; Salt stress; *Satureja spicigera* (C. Koch) Boiss.

### 1. Introduction

Humans have utilized medicinal plants to treat diseases since ancient times. Recently, these plants have attracted considerable attention from pharmacists as valuable sources of effective medicinal compounds with minimal side effects (Dahanayake et al., 2020; Kazeminia et al., 2022; Mohammadhosseini et al., 2022; Nalawade et al., 2022). Species from the mint family including creeping savory (*Satureja spicigera* (C. Koch) Boiss.) are key sources of thymol, carvacrol, γ-terpinene, and *p*-cymene (phenolic monoterpenes), which are used in traditional and modern medicine, pharmaceutical, aromatherapy, as well as health and food industries (Kulak, 2020; Nieto, 2020). Creeping savory is native to the northern and northeastern regions of Iran (Jamzad, 2012). In recent years, research has been done to cultivate and domesticate this herbal plant. The results confirm the possibility of its successful rainfed cultivation in areas with an annual rainfall of more than 450 mm (Yousefi et al., 2023).

Salt stress (SS) induces both osmotic stress (resulting in reduced water and nutrient absorption and ionic imbalance) and oxidative stress (causing a sharp increase in reactive oxygen species (ROSs)) (Sarker and Oba, 2020), which disrupt normal physiological, molecular, and biochemical functions (Balasubramaniam et al., 2023). Photosynthetic indices are critical indicators of plant tolerance to salt stress (Zhao et al., 2018). ROS induced by SS interfere with the absorption and metabolism of essential nutrients (Sachdev et al., 2021), destabilize protein-pigment complexes, and degrade chlorophyll molecules (Zhao et al., 2019), leading to decreased plant growth and productivity. Recent studies have shown that SS significantly reduces growth and photosynthetic characteristics in several medicinal plants, including *Satureja mutica* (Yousefi and Karamian, 2025a, 2025b), *Satureja hortensis* (Mohammadi et al., 2023), *Satureja khuzestanica* (Saadatfar and Hossein Jafari, 2022), as well as *Pimpinella anisum* L., *Foeniculum vulgare* Mill., and *Trachyspermum ammi* L. (Nouripour-Sisakht et al., 2022).

Phytochemicals play an important role in plant stress tolerance and under abiotic stresses, the biosynthesis of secondary metabolites undergoes various changes (Zhu, 2016). Salt-induced oxidative stress activates signaling

pathways involved in metabolic reprogramming, which results in either an increase or decrease in the levels of secondary metabolites (Ramakrishna et al., 2011).

The effects of salt stress on the quantity and quality of essential oils (EOs) in several mint family species, including *Satureja mutica* (Rahmati and Yousefi, 2024), *Mentha suaveolens* (Kiumarzi et al., 2022), *Mentha spicata* (Ounoki et al., 2021), *Salvia officinalis* L. (Kulak, 2020), *Thymus vulgaris* and *Thymus danensis* (Bistgani et al., 2019), and *Rosmarinus officinalis* (Dehghani Bidgoli et al., 2019) have been well documented. However, to the best of our knowledge, the effects of salt stress on the essential oil content and composition, as well as the morpho-physiological traits and photosynthetic features in creeping savory, have not been studied. Therefore, this study was conducted to investigate the effects of different salt levels on these attributes in creeping savory.

## 2. Experimental

### 2.1 Experimental design, conditions, and treatments

A completely randomized design (CRD) (greenhouse experiment, n = 3) was conducted at the Research Center for Agriculture and Natural Resources, Kermanshah, Iran. Seeds were disinfected with a 0.5% (*w/v*) sodium hypochlorite solution, washed, and dried. They were then planted in a peat moss bed and watered by sprinkling. The seedlings were transferred to plastic pots (one seedling per pot), which were filled with a mixture of farm soil, sand, and decomposed cow manure (Table 1). The plants were kept under a 17-hour light photoperiod with a light intensity of 300 µmol/m²·s (approximately 110 lux) and 7 hours of darkness (Hernández-Adasme et al., 2023), with a relative humidity of 50-60%. Four irrigation treatments (250 mL per pot, twice a week) were applied, consisting of 0, 50, 100, and 150 mM NaCl (Kumar et al., 2022). After four irrigation events with NaCl treatments, accumulated salts in the pots were leached by irrigating with distilled water.

**Table 1.** Culture bed specifications and experiment conditions.

| Soil texture | Soil electric conductivity (dS $m^{-1}$) | Soil pH | Organic carbon (%) | Total Phosphor (ppm) | Total nitrogen (%) |
|---|---|---|---|---|---|
| Clay-loam | 0.70 | 7.03 | 175 | 138 | 0.28 |
| **Culture bed weight (kg)** | **Pots dimensions (cm)** | **Photoperiod (h $d^{-1}$)** | **Relative Humidity (%)** | **Light intensity (mMOL $m^2$ S)** | |
| 4.5 | 17×22 | 17 | 50-60 | 300 | |

### 2.2 Morpho-physiological measurements

Leaf fresh weight (LFW) and leaf dry weight (LDW) were measured using 30 young leaves from each plant. Fresh leaves were immediately weighed with precision of 0.0001 g to determine LFW. The leaves were immersed in double-distilled water for 18 hours at 22 °C to achieve full turgor. After blotting their surfaces dry, the leaves were weighed again to measure leaf turgor weight (LTW). Subsequently, the leaves were dried in an oven at 70 °C for 48 hours to determine LDW. Mean values for LFW and LDW were calculated.
After harvesting, shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) were measured. In this relation, shoots and roots were separately oven-dried at 75 °C for 72 hours before recording SDW and RDW.

### 2.3 Photosynthetic indices

Thirty leaves from each plant were covered with an aluminum foil and dark-adapted for 30 minutes. The maximum quantum yield of PSII (Fv/Fm) and photosynthetic performance index (PI) were measured using a Hansatech Pocket PEA (UK) at 695 nm. The photon flux density (PFD) was set at 400 µmol $m^2 \cdot s^{-1}$, and measurements were taken with a light duration of 5 seconds. The leaf chlorophyll index (SPAD) was determined using a SPAD-502Plus chlorophyll meter (Minolta, Japan).

### 2.4 Essential oil extraction and profiling

#### 2.4.1 EO extraction

EOs were extracted from the aerial parts of the plants using a Clevenger-type apparatus through water distillation for 3 hours, following the British Pharmacopoeia (1993). The EO samples were dehydrated using anhydrous sodium sulfate ($Na_2SO_4$), and the weight of the extracted EOs was recorded. The EO samples were stored at 4 °C in the refrigerator, protected from light with an aluminum foil, until further analysis via gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS).

#### 2.4.2 EO Percentage and yield calculation

EO percentage and yield were calculated based on the net weight of the extracted oils relative to the dry weight of the plant material (Eq. 1 and Eq. 2, respectively).

EO (%) = [(EO weight (g))/(sample dry weight (g))] × 100 (1)

EO yield (g) = EO% × shoot dry weight (g) (2)

### 2.4.3 GC analysis

EO samples were analyzed using a Thermo-UFM (ultra-fast model) gas chromatograph equipped with a Chrom-Card A/D data processor. A non-polar HP-5 (5%-phenyl)-methylpolysiloxane column (Thermo Fisher Scientific, Italy) with a length of 10 m and an internal diameter of 0.1-0.4 µm was used.
The temperature program began at 60 °C, ramping up to a final temperature of 285 °C at a rate of 80 °C per minute. This final temperature was held for 3 minutes. The detector was a flame ionization detector (FID) set at 290 °C, and the injection chamber temperature was maintained at 280 °C. Helium was used as the carrier gas with an inlet pressure of 0.5 kg $cm^{-2}$.

### 2.4.4 GC/MS analysis

EO samples were further analyzed using a Varian 3400 GC/MS system, coupled with a Saturn II mass spectrometer and an ion telephoto system, operated at 70 eV ionization energy. The column used was a semi-polar DB-5 column with a length of 30 m, an inner diameter of 0.25 mm, and a stationary phase thickness of 0.25 µm. The head gas pressure was set at 35 pounds per square inch gauge (PSIG), with the column temperature programmed to increase from 40 °C to 250 °C at a rate of 4 °C per minute. The injection chamber temperature was set to 260 °C, and the transfer line was maintained at 270 °C. Retention indices were calculated by injecting a series of normal hydrocarbons ($C_7$-$C_{25}$), and EO compounds were identified by comparing their spectra with established libraries (Adams, 2017).

### 2.5 Statistical analysis

Data analysis was conducted using IBM SPSS Statistics 26 software. Analysis of variance (ANOVA) and Duncan's test were performed at a significance level of $p < 0.05$. All graphical representations were generated using Microsoft Excel.

## 3. Results and discussion

### 3.1 Morpho-physiological traits

Significant differences were observed among the NaCl treatments at the 1% significance level for plant height, leaf area, fresh and dry weights of leaves, shoots, and roots (Table 2). As the NaCl concentration increased, all the studied morpho-physiological traits exhibited a linear decline, with either a steep or moderate slope (Fig. 1).
NaCl treatments of 100 and 150 mM resulted in a significant reduction in plant height compared to the control. The tallest plants were recorded in the control group (86.5 cm), while the shortest plants (65.0 cm) were observed in the 100 and 150 mM NaCl treatments (Fig. 2).
Furthermore, the 100 and 150 mM NaCl treatments significantly reduced leaf fresh and dry weight compared to the control. The highest leaf fresh weight (11.86 g) was recorded in the control group, while the lowest (8.80 g) was observed in plants treated with 150 mM NaCl (Fig. 2). The maximum leaf dry weight (2.32 g) was observed in the control, while the minimum (1.53 g) occurred under 150 mM NaCl treatment (Fig. 3).
According to our findings, the shoot fresh weight was significantly reduced by 100 and 150 mM NaCl treatments. The highest shoot fresh weight (22.34 g) was recorded in the control group, while the lowest (11.36 g) was noted in plants subjected to 150 mM NaCl (Fig. 2). The treatments of 50, 100, and 150 mM NaCl also caused significant reduction in shoot dry weight. The maximum shoot dry weight (7.52 g) was recorded in the control, while the minimum (3.56 g) was seen in the plants treated with 150 mM NaCl (Fig. 3).
The treatments of 50, 100, and 150 mM NaCl caused significant reductions in root fresh and dry weights. The highest root fresh weight (22.50 g) was recorded in the control group, while the lowest (7.26 g) was observed under 150 mM NaCl (Fig. 2). For root dry weight, the control group exhibited the maximum value (3.41 g), whereas the minimum (0.92 g) was recorded in the 150 mM NaCl treatment (Fig. 3).

The leaf area decreased significantly in the plants treated with 100 and 150 mM NaCl treatments compared to control. The maximum leaf area (0.72 $cm^2$) was recorded in the control group, while the minimum (0.52 $cm^2$) was observed in plants subjected to 150 mM NaCl (Fig. 4).

Saline conditions significantly impact the morpho-physiological characteristics of plants, particularly in salt-sensitive species (Pandit et al., 2024). These reductions are attributed to osmotic stress, nutrient imbalance, and oxidative stress caused by high salinity levels (Atta et al., 2023). In the present study, 50, 100, and 150 mM NaCl treatments significantly reduced the fresh and dry weights of leaves, shoots, and roots. These findings are consistent with the previous studies demonstrating that salinity negatively affects plant growth traits, including reductions in plant height, leaf area, and shoot and root biomass in various plant species such as *S. mutica* (Yousefi and Karamiian, 2025b), *Pimpinella anisum* L., *Foeniculum vulgare* Mill., and *Trachyspermum ammi* L. (Nouripour-Sisakht et al., 2022), and broccoli (Ali et al., 2022). Similar observations have been made in *Satureja khuzestanica* (Saadatfar and Jafari, 2022), and *Amaranthus cruentus* (Menezes et al., 2017).

**Table 2.** The results of ANOVA for studied morpho-physiological, photosynthetic and phytochemical traits of *S. spicigera* (C. Koch) Boiss. in the different NaCl treatments.

| **Source** | **df** | **PLH** | **LEA** | **LFW** | **LDW** | **SFW** | **SDW** |
|---|---|---|---|---|---|---|---|
| NaCl | 3 | 654.93$^{**}$ | 0.045$^{**}$ | 15.76$^{**}$ | 1.23$^{*}$ | 140.76$^{**}$ | 16.39$^{**}$ |
| Error | 6 | 39.17 | 0.001 | 2.886 | 0.34 | 1.09 | 0.36 |
| CV% | | 8.82 | 4.93 | 15.78 | 29.23 | 6.39 | 11.09 |
| Source | df | RFW | RDW | Fv/Fm | PI | SPAD | |
| NaCl | 3 | 235.09$^{**}$ | 6.94$^{**}$ | 0.001$^{ns}$ | 0.39$^{*}$ | 15.76$^{*}$ | |
| Error | 6 | 2.39 | 0.111 | 0.0001 | 0.182 | 2.89 | |
| CV% | | 10.12 | 14.08 | 0.13 | 16.93 | 5.58 | |

* and ** Significant differences at the level of 0.05 and 0.01, respectively; Ns: No significant difference.

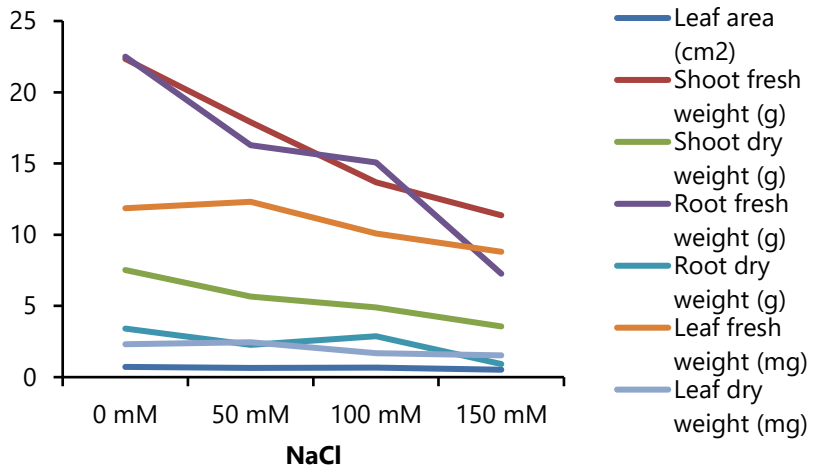


**Figure 1.** Trend of changes in the studied morpho-physiological traits in response to different salt concentrations.

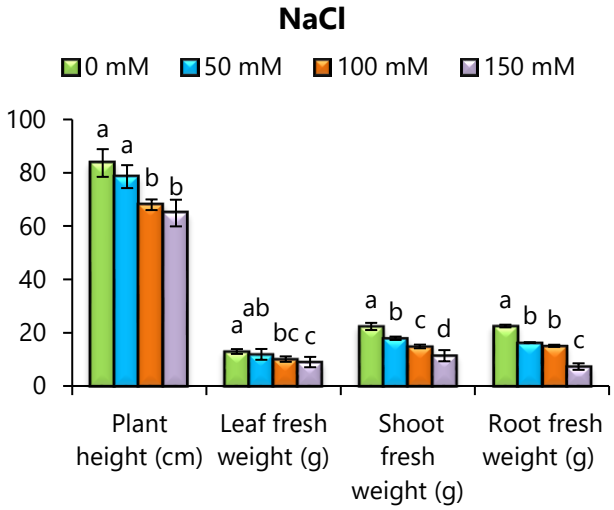


**Figure 2.** Means comparison (Means ± SD): Plant height, leaf fresh weight, shoot fresh weight and root fresh weight of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

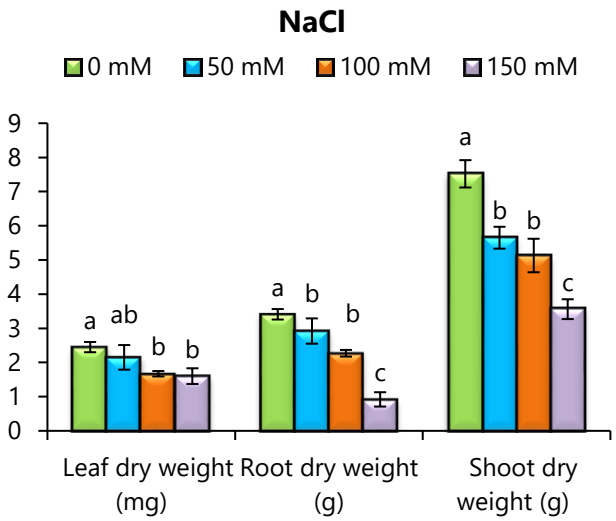


**Figure 3.** Means comparison (Means ± SD): Leaf dry weight, root dry weight and shoot dry weight of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

### 3.2 Photosynthetic traits

Significant differences were observed among the NaCl treatments at the 5% of significance level for photosynthetic index and chlorophyll index (Table 2). Accordingly, it was concluded that Fv/Fm significantly not affected by any of the NaCl treatments (Table 2, Fig. 4).

Our observations revealed that the 50 mM NaCl treatment significantly increased the photosynthetic index (PI) compared to the control, whereas the 150 mM NaCl treatment caused a significant reduction (Fig. 4). The highest PI value (3.18) was recorded under the 50 mM NaCl treatment, while the lowest PI value (2.47) was observed in plants exposed to 150 mM NaCl (Fig. 4). Additionally, the 150 mM NaCl treatment resulted in a significant decrease in the chlorophyll index (SPAD) compared to the control. The maximum chlorophyll index (33.67) was recorded in the control group, whereas the minimum (29.31) was achieved under the 150 mM NaCl treatment (Fig. 5).

A decline in chlorophyll content under saline conditions is a well-documented response to salt stress. This adverse effect is primarily attributed to oxidative stress induced by ROS, which damage chloroplasts and disrupt chlorophyll biosynthesis (Li et al., 2024; Wang et al., 2024). ROS also cause osmotic imbalances, reduce cellular $CO_2$ levels, and disrupt photosystem II (PSII), leading to diminished photosynthetic efficiency (Sachdev et al., 2021; Hameed et al., 2021; Wang et al., 2024).

In the present study, both SPAD value and photosynthetic index showed a limited increase in plants-treated with 50 mM NaCl, however they were significantly reduced by 150 mM NaCl. Hussain et al. (2020) stated that moderate salinity improved photosynthetic performance due to stress-resistance mechanisms such as osmotic adjustment and activation of antioxidant enzymes, while high salinity sharply reduced it. Similar effects of salinity on photosynthetic traits have been reported in *S. mutica* (Yousefi and Karamiian, 2025a), citrus (Othman et al., 2023), wheat (Shah et al., 2017), and tomato (Zushi and Matsuzoe, 2017).

In addition, the Fv/Fm ratio, a key indicator of PSII efficiency, increased non-significantly in *S. spicigera* plants treated with 50 mM NaCl; however, it declined non-significantly in plants treated with 100 and 150 mM NaCl. This result is consistent with the report of Hnilickova et al. (2021), which showed that in purslane plants treated with 100 mM NaCl, the maximum quantum efficiency of photosystem II (Fv/Fm) remained unchanged compared to the control, whereas plants exposed to 300 mM NaCl exhibited a significant decrease in Fv/Fm.

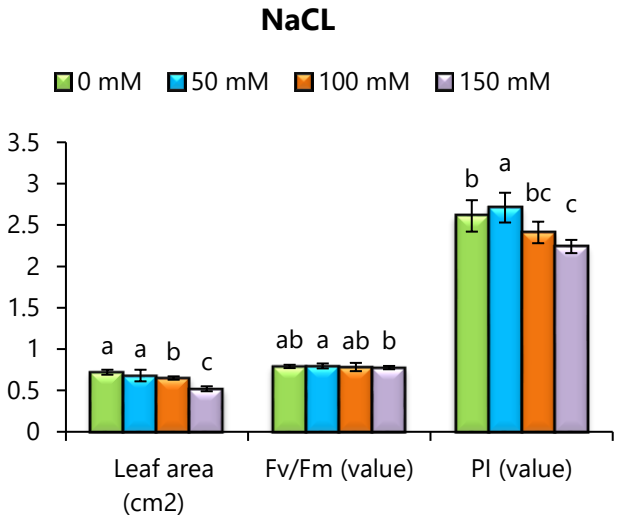


**Figure 4.** Means comparison (Means ± SD): Leaf area, maximum quantum yield of PSП (Fv/Fm) and photosynthetic index (PI) *of S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

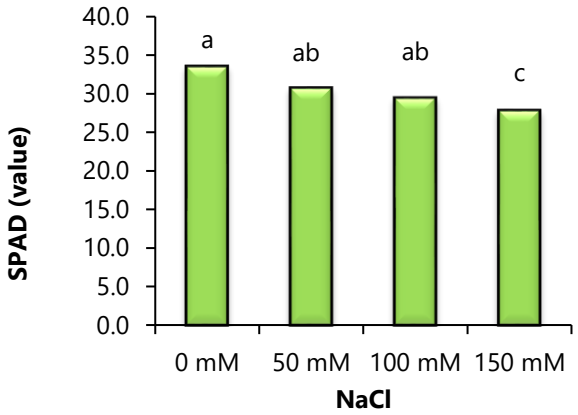


**Figure 5.** Means comparison (Means ± SD): Chlorophyll index (SPAD value) of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

### 3.3 Essential oil content and profile

#### 3.3.1 Essential oil percentage

The EO percentage showed a significant difference between NaCl treatments (Table 4). As shown in Fig. 6, 50 and 100 mM NaCl treatments significantly increased the essential oil percentage compared to the control, while the 150 mM NaCl treatment caused a significant reduction. The highest essential oil percentage (3.75%) was obtained under the 100 mM NaCl treatment; however, the lowest (2.11%) was recorded in plants treated with 150 mM NaCl (Fig. 6). The results demonstrated that moderate salinity (50-100 mM NaCl) increased essential oil yield in *S. spicigera*, whereas high salinity (150 mM NaCl) significantly reduced it. This aligns with findings from other

studies showing that moderate salinity enhances EO biosynthesis in aromatic plants, including *S. mutica* (Rahmati and Yousefi, 2024), *Origanum onites* (Stefanakis et al., 2024), and *Achillea millefolium* L. (Dehghan and Rahimmalek, 2018). High salinity (≥150 mM NaCl) disrupts glandular hair development and diverts metabolic resources from secondary metabolite production to stress management pathways (Sarmoum et al., 2019; Assaf et al., 2022). These effects are particularly evident in the Lamiaceae family, where high NaCl levels decline EO production (Stefanakis et al., 2024). It should be noted that the effect of salt on yield and essential oil chemicals is highly dependent on the plant species. According to Karim et al. (2025), the essential oil content of thyme (*Thymus* × *citriodorus*) peaked in unstressed plants (0.45 mL/100 g dry weight), while its levels decreased in response to mild and severe salinity stress.

### 3.3.2 Essential oil profile

The EO chemical composition identified by GC and GC/MS has been presented in Table 4. The main EO compounds were γ-Terpinene, p-cymene, thymol and carvacrol (Fig. 6). As seen, significant differences were observed among NaCl treatments at 1% level of significant for essential oil percentage, and the concentrations of metyle ether thymol, additionally significant differences were seen at 5% significant level for α-pinene, β-bisabolene, *trans*-caryophyllene, terpinolene, *p*-cymene, thymol and carvacrol (Table 5).

**Table 4.** EO profile of *S. spicigera* (C. Koch) Boiss. planted under different NaCl treatments.

| Chemical classification | Chemical name | RT | RI | Formula | Range (%) | |
|---|---|---|---|---|---|---|
| | | | | | Min. | Max. |
| Monoterpene | Terpinene-4-ol | 2.58 | 1146.53 | $C_{10}H_{18}O$ | 0.12 | 0.75 |
| | Myrcene | 1.16 | 986.46 | $C_{10}H_{16}$ | 1.38 | 2.00 |
| | α-Terpinolene | 1.65 | 1054.11 | $C_{10}H_{16}$ | 0.11 | 0.54 |
| Phenol monoterpenoids | Carvacrol | 4.07 | 1289.96 | $C_{10}H_{14}O$ | 19.65 | 29.11 |
| | Thymol | 3.96 | 1281.22 | $C_{10}H_{14}O$ | 23.66 | 36.69 |
| | Methyl ether thymol | 3.32 | 1224.97 | $C_{11}H_{16}O$ | 0.48 | 1.55 |
| | Methyl ether carvacrol | 3.60 | 1250.81 | $C_{11}H_{16}O$ | 0.42 | 0.86 |
| Bicyclic monoterpenes | α-Pinene | 0.87 | 931.13 | $C_{10}H_{16}$ | 0.47 | 1.20 |
| | *trans*-Caryophyllene | 5.33 | 1376.02 | $C_{15}H_{24}$ | 0.11 | 1.52 |
| Ketonic monoterpene | α-Thujone | 0.84 | 924.38 | $C_{10}H_{16}O$ | 0.34 | 1.09 |
| Isometric monoterpene | γ-Terpinene | 1.63 | 1051.59 | $C_{10}H_{16}$ | 10.39 | 17.68 |
| Sesquiterpenes | β-Bisabolene | 6.42 | 1493.57 | $C_{15}H_{24}$ | 0.09 | 0.29 |
| Benzene alkyl | *ρ*-Cymene | 1.36 | 1014.15 | $C_{10}H_{14}$ | 13.48 | 27.47 |

* RT: Retention Time; RI: Retention Index.

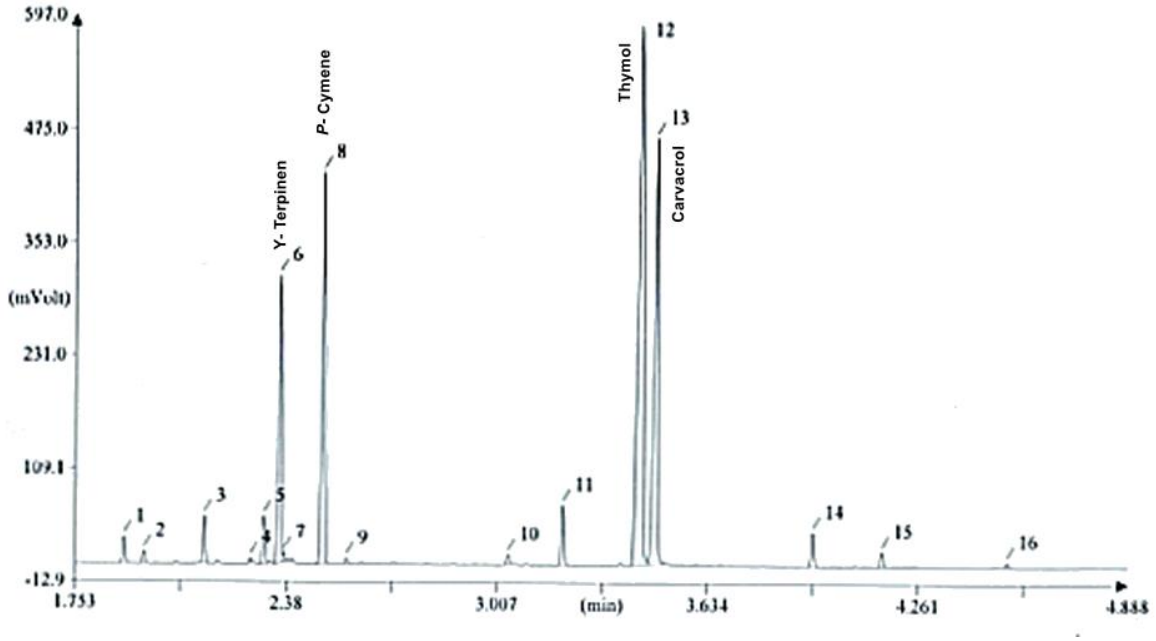


**Figure 6.** The chromatogram of EO compounds of *S. spicigera* (C. Koch) Boiss.

The compounds of α-pinene, β-bisabolene, terpinene-4-ol, methyl ether thymol, methyl ether carvacrol and *trans*-caryophyllene showed a significant decrease in plants treated with 150 mM NaCl compared to control. α-Terpinolene showed a significantly decrease only in plants treated with 150 mM NaCl (Table 5).

**Table 5.** The results of ANOVA for EO percent and EO profile of *S. spicigera* (C. Koch) Boiss. planted under different NaCl treatments.

| S.O.V. | df | EO percent | α-Thujone | α-Pinene | Myrcene | β-Bisabolene | Terpinene-4-ol | Metyl ether thymol |
|---|---|---|---|---|---|---|---|---|
| NaCl | 3 | 1.07** | 0.05ns | 0.105* | 0.05ns | 0.01* | 0.05ns | 0.47** |
| Error | 8 | 0.035 | 0.034 | 0.025 | 0.025 | 0.001 | 0.034 | 0.023 |
| CV | | 1.15 | 5.99 | 3.12 | 1.40 | 0.51 | 8.58 | 2.41 |
| S.O.V. | df | Methyl ether carvacrol | *trans*-Caryophyllene | α-Terpinolene | *p*-Cymene | γ-Terpinene | Thymol | Carvacrol |
| NaCl | 3 | 0.035ns | 0.36* | 0.05* | 47.49* | 7.18 ns | 43.77* | 13.15* |
| Error | 8 | 0.012 | 0.074 | 0.01 | 5.626 | 2.739 | 7.047 | 4.377 |
| CV | | 1.85 | 8.41 | 2.92 | 27.46 | 16.52 | 24.26 | 17.96 |

* and ** Significant differences at the level of 0.05 and 0.01, respectively and Ns: No significant difference.

The maximum percentage of α-thujone (0.73%), α-pinene (1.07%), terpinene-4-ol (0.59%), methyl ether thymol (1.36%), methyl ether carvacrol (0.79%) and *trans*-caryophyllene (0.42%) was observed in the control (Fig. 7-Fig. 9). while the maximum myrcene percentage (1.88%) was recorded in in plants treated with 100 mM NaCl and the maximum β-bisabolene (0.25%) in plants treated with 50 mM NaCl (Fig. 7, Fig. 8 and Fig. 9).

Thymol levels significantly and carvacrol non-significantly were increased in the plants treated with 50, 100, and 150 mM NaCl. However, *p*-cymene percentage (significantly) and γ-terpinene percentage (non-significantly) were decreased in the NaCl-treated plants compared to control (Fig. 10). The highest thymol percentage (34.81%) was observed under the 150 mM NaCl treatment, while the lowest (23.83%) was recorded in the control (Fig. 10). The lowest carvacrol percentage (23.83%) was observed in plants treated with 150 mM NaCl, while the highest (27.99%) was recorded in the control (Fig. 10). *p*-Cymene levels significantly and γ-terpinene (non-significantly) were reduced in plants treated with 100 and 150 mM NaCl compared to the control (Fig. 10). The highest percentage of *p*-cymene (22.63%) was obtained in control and the highest γ-terpinene (15.58%) was observed in plants treated with 50 mM NaCl (Fig. 10).

Consistent with the results of Karim et al. (2025) that reported fluctuations in EO compounds in *Thymus* × *citriodorus*, EO compounds in *Satureja spicigera,* also showed significant fluctuations in response to salinity, indicating stress-induced adjustments in secondary metabolism related to their defense roles. These findings provide comprehensive insights into how *Satureja spicigera* modulates growth and biochemical pathways under abiotic stress.

The production of specific EO components varies under salinity stress. In the present study, thymol increased significantly with different salinity levels, so that the maximum thymol content was achieved at 150 mM NaCl. This is consistent with findings in *Plectranthus amboinicus* (Sany et al., 2020) and *Thymus pannonicus* (Etri et al., 2024). No significant change was observed for γ-terpenine and carvacrol percentage due to different salinity treatments, as observed in *Origanum vulgare* (Azimzadeh et al., 2023) and *Thymus pannonicus* (Etri et al., 2024). *p*-Cymene content declined significantly in the plants treated with 100 and 150 mM NaCl concentrations, corroborating results in *Plectranthus amboinicus* (Azimzadeh et al., 2023) and *Satureja hortensis* L. (Najafi et al., 2010).

The effect of salinity on specific volatile terpenoid compounds, such as thymol and carvacrol, varies depending on the plant species and the concentration of salt (Tsusaka et al., 2019). Stefanakis et al. (2024) confirmed that 150 mM NaCl significantly alters the composition of EOs in various species of the Lamiaceae family. However, Ounoki et al. (2021) reported that the EO composition of spearmint remained unaffected under 150 mM NaCl stress.

The biosynthesis of the terpenoid compounds like carvacrol and thymol is influenced by the plant's genetics and environmental conditions (Hu et al., 2024). Carvacrol and thymol help the plant cope with stressors such as

drought, salinity, and high temperatures (Cheng et al., 2020). The biosynthesis of carvacrol and thymol (two important phenolic compounds with antimicrobial and antioxidant properties) in plants primarily occurs through the phenylpropanoid and terpenoid pathways. Geranyl pyrophosphate is converted to γ-terpinene by the enzyme monoterpene synthase. γ-Terpinene is then converted to *p*-cymene by the enzyme γ-terpinene dehydrogenase. *p*-Cymene is hydroxylated by a cytochrome P450 oxidase enzyme, such as CYP71D178, and is transformed into carvacrol. Carvacrol is then converted into thymol by the enzyme carvacrol methyltransferase (Crocoll, 2011).
Salinity stress triggers gene expression changes in terpenoid biosynthesis pathways, including the methylerythritol phosphate (MEP) pathway, which produces phenolic monoterpenes like thymol and carvacrol (Crocoll., 2011; Morshedloo et al., 2017).
The report of Azimzadeh et al. (2023) which shows that the key enzymes such as γ-terpinene synthase (Ovtps2) and cytochrome P450 monooxygenases (*e.g.,* CYP71D180), involved in thymol synthesis, are upregulated under moderate salinity, support the present results regarding the increase of γ-terpinene and thymol in *S. spicigera* under saline conditions. While the amount of carvacrol increased non-significantly or remained constant, the amount of thymol showed a prominent increase.

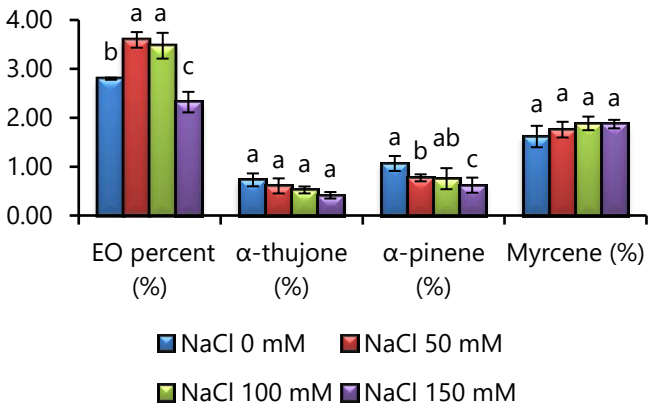


**Figure 7.** Means comparison (Means ± SD): Essential oil, α-thujone, α-pinene, and myrcene content of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

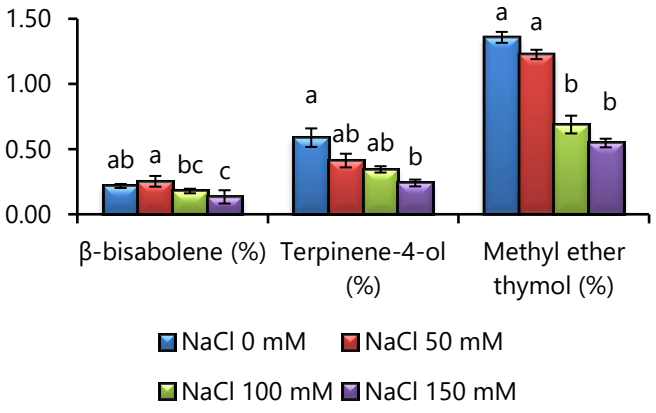


**Figure 8.** Means comparison (Means ± SD): β-bisabolene, terpinen-4-ol, and methyl ether thymol content of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p < 0.05$).

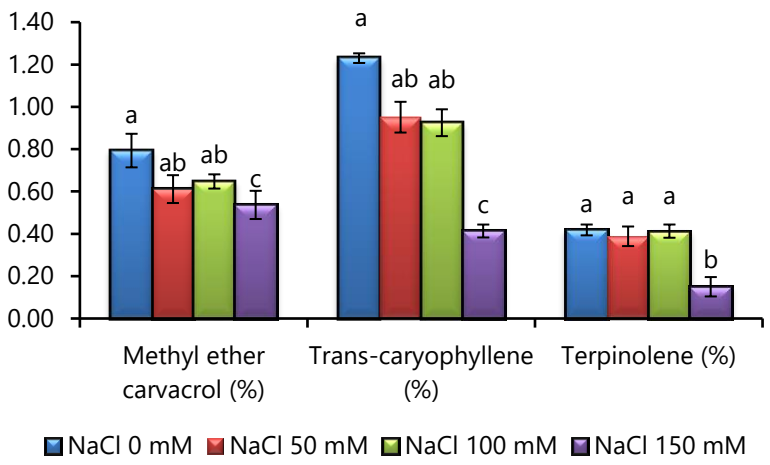


**Figure 9.** Means comparison (Means ± SD): Methyl ether carvacrol, *trans*-caryophyllene, and terpinolene content of *S. spicigera* (C. Koch) Boiss. in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p$ <0.05).

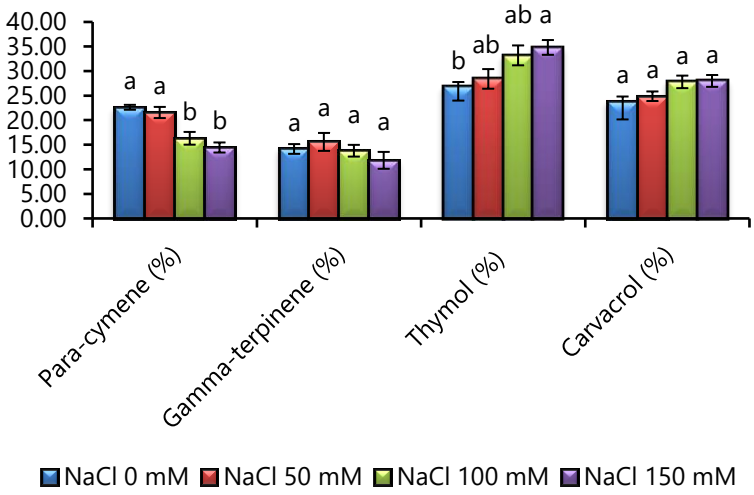


**Figure 10.** Means comparison (Means ± SD): *p*-Cymene, γ-terpinene, thymol and carvacrol content of *S. spicigera* (C. Koch) Boiss. plants in response to different NaCl concentrations, the same letters do not show significant difference (Duncan test, $p$ < 0.05).

## 4. Concluding remarks

This study highlights the dual effects of salinity on *S. spicigera*. Moderate salinity (50-100 mM NaCl) can enhance EO yield and promote the biosynthesis of key compounds like thymol and carvacrol, while high salinity (150 mM NaCl) disrupts these processes. The findings underscore the potential of controlled salinity to optimize EO production in aromatic plants. In addition, the results highlight the complex and concentration-dependent effects of NaCl on both the physiological and biochemical responses of *S. spicigera*. Moderate salinity (50 mM) appears to stimulate photosynthetic activity, while higher salinity (150 mM) adversely affects these parameters. Furthermore, the salinity treatments significantly influenced the composition of secondary metabolites, with varying impacts on terpenoid compounds such as thymol, carvacrol, and β-pinene. Moreover, the increase in essential oil production at moderate salinity levels and the shift in terpenoid composition under high salinity suggest that plants can adapt to salt stress by altering their metabolic pathways, which may offer potential for

enhancing essential oil production in saline environments. These findings are the first report on the effect of salinity stress on the essential oil chemicals of creeping savory. Future studies need to optimize salt concentration to increase thymol levels in this plant, without reducing plant biomass and essential oil yield.

**Authorship contribution statement**

Borzou Yousefi performed the experiments. Borzou Yousefi and Hooshang Rahmati analyzed data and wrote the manuscript in cooperation. Hooshang Rahmati and Borzou Yousefi critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

**Data availability**

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

**Abbreviations**

**C:** Control; **EO:** Essential Oil; **LFW**: Leaf Fresh Weight; **LDW:** Leaf Dry Weight; **OD:** Optical Density; **PI:** Photosynthetic Index; **RFW:** Root Fresh Weight; **RDW:** Root Dry Weight**;** Fresh Weight; **RI:** Retention Index; **RT:** Retention Time; **SDW:** Shoot Dry Weight; **SFW:** Shoot; **SS:** Salt Stress.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**

Adams, R.P. (2017) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 5 Online Ed, Allured Pub Corp, Illinois, USA.

Ali, L., Shaheen, M.R., Ihsan, M.Z., Masood, S., Zubair, M., Shehzad, F., Khalid, A.-U.-H. (2022) Growth, photosynthesis and antioxidant enzyme modulations in broccoli (*Brassica oleracea* L.) under salinity stress. *South Afri. J. Bot.* 148:104–111. DOI: http://dx.doi.org/10.1016/j.sajb.2022.03.050

Assaf, M., Korkmaz, A., Karaman, Ş., Kulak, M. (2022) Effect of plant growth regulators and salt stress on secondary metabolite composition in Lamiaceae species. *South Afri. J. Bot.* 144:480–493. DOI: https://doi.org/10.1016/j.sajb.2021.10.030

Atta, K., Mondal, S., Gorai, S., Singh, A.P., Kumari, A., Ghosh, T., Roy, A., Hembram, S., Gaikwad, D.J., Mondal, S., Bhattacharya, S., Jha, U.C., Jespersen, D. (2023) Impacts of salinity stress on crop plants: Improving salt tolerance through genetic and molecular dissection. *Front. Plant Sci.* 14:1241736. DOI: https://doi.org/10.3389/fpls.2023.1241736.

Azimzadeh, Z., Hassani, A., Mandoulakani, B.A., Sepehr, E., Morshedloo, M.R. (2023) Intraspecific divergence in essential oil content, composition and genes expression patterns of monoterpene synthesis in *Origanum vulgare* subsp. *vulgare* and subsp. *gracile* under salinity stress. *BMC Plant Biol.* 23(1):380. DOI: https://doi.org/10.1186/s12870-023-04387-5.

Balasubramaniam, T., Shen, G., Esmaeili, N., Zhang, H. (2023) Plants' response mechanisms to salinity stress. *Plants (Basel)* 12(12):2253. DOI: https://doi.org/10.3390/plants12122253.

British Pharmacopoeia (1993) Vol. 1. British Pharmacopoeia Commission. H.M. Stationery Office, Great Britain, p. 714.

Bistgani, Z. E., Hashemi, M., Dacosta, M., Craker, L., Maggi, F., Morshedloo, M.R. (2019) Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. *Ind. Crops Prod.* 135:311–320. DOI: https://doi.org/10.1016/j.indcrop.2019.04.055.

Cheng, Y.-W., Kong, X.-W., Wang, N., Wang, T.-T., Chen, J., Shi, Z.Q. (2020) Thymol confers tolerance to salt stress by activating anti-oxidative defense and modulating $Na^+$ homeostasis in rice root. *Ecotoxicol. Environ. Saf.* 188:109894. DOI: https://doi.org/10.1016/j.ecoenv.2019.109894.
Crocoll, C. (2011) Biosynthesis of the Phenolic Monoterpenes, Thymol and Carvacrol, by Terpene Synthases and Cytochrome P450s in Oregano and Thyme. Germany: Academic Dissertation: der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena. DOI: https://www.db-thueringen.de/receive/dbt_mods_00018555.
Dahanayake, J.M., Perera, P.K., Galappaththy, P., Arawwawala, M. (2020) A mini review on therapeutic potentials of *Phyllanthus niruri* L. *Trends Phytochem. Res.* 4(3):101–108. DOI: https://oiccpress.com/tpr/article/view/11777.
Dehghani Bidgoli, R., Azarnezhad, N., Akhbari, M., Ghorbani, M. (2019) Salinity stress and PGPR effects on essential oil changes in *Rosmarinus officinalis* L. *Agric. Food Secur.* 8(2):2019.
DOI: https://doi.org/10.1186/s40066-018-0246-5.
Etri, K., Gosztola, B., Végvári, G., Ficzek, G., Radácsi, P., Simon, G., Pluhár, Z. (2024) Unravelling the impact of drought and salt stresses on *Thymus pannonicus*: Morpho-physiological and biochemical insights. *Plant Stress* 13:100557. DOI: https://doi.org/10.1016/j.stress.2024.100557.
Hameed, A., Ahmed, M.Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B., Nielsen, B.L. (2021) Effects of salinity stress on chloroplast structure and function. *Cells* 10(8):2023. DOI: https://doi.org/10.3390/cells10082023.
Hernández-Adasme, C., Palma-Dias, R., Escalona, V.H. (2023) The effect of light intensity and photoperiod on the yield and antioxidant activity of beet microgreens produced in an indoor system. *Horticulturae* 9:493.
DOI: https://doi.org/10.3390/horticulturae9040493.
Hnilickova, H., Kraus, K., Vachova, P., Hnilicka, F. (2021) Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. *Plants (Basel)* 10(5):845.
DOI: https://doi.org/10.3390/plants10050845.
Hu, Y., Zheng, T., Dong, J., Li, W., Ma, X., Li, J., Fang, Y., Chen, K., Zhang, K. (2024) Regulation of the main terpenoids biosynthesis and accumulation in fruit trees. *Hort. Plant* 11(5):1761–1776.
DOI: https://doi.org/10.1016/j.hpj.2024.08.002.
Hussain, M., Ali, I., Jan, F., (2020) Impact of salinity stress on chlorophyll content and photosynthetic efficiency in plants. *Environ. Exp. Bot.* 178:104–115. DOI: https://doi.org/10.3389/fpls.2020.00481.
Jamzad, Z. (2012) Flora of Iran, Research Institute of Forests and Rangelands Publication, Tehran, Iran.
Karim, E., Beáta, G., Zsuzsanna, P. (2025) Essential oils under stress: How drought and salinity shape the physiological and biochemical profile of *Thymus ×citriodorus*. *Ind. Crops Prod.* 233:121368.
DOI: https://doi.org/10.1016/j.indcrop.2025.121368.
Kazeminia, M., Mehrabi, A., Mahmoudi, R. (2022) Chemical composition, biological activities, and nutritional application of Asteraceae family herbs: A systematic review. *Trends Phytochem. Res.* 6(3):187–213.
DOI: https://doi.org/10.30495/tpr.2022.1954612.1248.
Kiumarzi, F., Morshedloo, M.R., Zahedi, S.M., Mumivand, H., Behtash, F., Hano, C., Chen, J.T., Lorenzo, J.M. (2022) Selenium nanoparticles (Se-NPs) alleviate salinity damages and improves phytochemical characteristics of pineapple mint (*Mentha suaveolens* Ehrh.). *Plants (Basel)* 11:1384. DOI: https://doi.org/10.3390/plants11101384.
Kulak, M. (2020) Recurrent drought stress effects on essential oil profile of Lamiaceae plants: An approach regarding stress memory. *Ind. Crops Prod.* 154:112695. DOI: https://doi.org/10.1016/j.indcrop.2020.112695.
Kumar, S., Abass Ahanger, M., Alshaya, H., Latief Jan, B., Yerramilli, V. (2022) Salicylic acid mitigates salt induced toxicity through the modifications of biochemical attributes and some key antioxidants in *capsicum annuum*. *Saudi J. Biol. Sci.* 29:1337–1347. DOI: https://doi.org/10.1016/j.sjbs.2022.01.028.
Li, X., Zhang, W., Niu, D., Liu, X. (2024) Effects of abiotic stress on chlorophyll metabolism. *Plant Sci.* 342:112030.
DOI: https://doi.org/10.1016/j.plantsci.2024.112030.
Menezes, R.V., Azevedo Neto, A.D., Oliveira Ribeiro, M., Watanabe Cova, A.M. (2017) Growth and contents of organic and inorganic solutes in amaranth under salt stress. *Pesqui. Agropecu. Trop.* 47:22–30.
DOI: http://dx.doi.org/10.1590/1983-40632016v4742580.
Mohammadhosseini, M., Frezza, C., Venditti, A., Sarker, S. (2021) A systematic review on phytochemistry, ethnobotany and biological activities of the genus *Bunium* L. *Chem. Biodivers.* 18(11):e2100317.
DOI: https://doi.org/10.1002/cbdv.202100317.

Mohammadi, H., Hazrati, S., Parviz, L. (2023) Morphophysiological and biochemical response of savory medicinal plant using silicon under salt stress. *Ann. Univ. Mariae Curie-Skłodowska, C Biol.* 2:29–40.
DOI: http://dx.doi.org/10.17951/c.2017.72.2.29-40.
Morshedloo, M.R., Craker, L.E., Salami, A., Nazeri, V., Sang, H., Maggi, F. (2017) Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono-and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. *Plant Physiol. Biochem.* 111:119–128.
DOI: https://doi.org/10.1016/j.plaphy.2016.11.023.
Najafi, F., Khavari-Nejad, R.A., Siah Ali, M. (2010) The effects of salt stress on certain physiological parameters in summer savory (*Satureja hortensis* L.) plants. *Stress Physiol. Biochem.* 6:13–21.
Nalawade, A.S., Gurav, R.V., Patil, A.R., Patwekar, M., Patwekar, F. (2022) A comprehensive review on morphological, genetic and phytochemical diversity, breeding and bioprospecting studies of genus *Chlorophytum* Ker Gawl. from India. *Trends Phytochem. Res.* 6(1):19–45.
DOI: https://doi.org/10.30495/tpr.2022.1949493.1238.
Nieto, G. (2020) A review on applications and uses of thymus in the food industry. *Plants (Basel)* 9(8):961.
DOI: https://doi.org/10.3390/plants9080961.
Nouripour-Sisakht, J., Ehsanzadeh, P., Ehtemam, M.H. (2022) Fennel outperforms ajwain and anise in the saline environment: Physiological response mechanisms in germinating seeds and mature plants. *Ital. J. Agron.* 17(3):2096. DOI: http://dx.doi.org/10.4081/ija.2022.2096.
Othman, Y.A., Hani, M.B., Ayad, J.Y., St Hilaire, R. (2023) Salinity level influenced morpho-physiology and nutrient uptake of young citrus rootstocks. *Heliyon* 9(2):e13336. DOI: https://doi.org/10.1016/j.heliyon.2023.e13336.
Ounoki, R., Ágh, F., Hembrom, R., Ünnep, R., Szögi-Tatár, B., Böszörményi, A., Solymosi, K. (2021) Salt stress affects plastid ultrastructure and photosynthetic activity but not the essential oil composition in spearmint (*Mentha spicata* L. var. crispa "Moroccan"). *Front. Plant Sci.* 12:739467. DOI: https://doi.org/10.3389/fpls.2021.739467.
Pandit, K., Chandni, Kaur, S., Kumar, M., Bhardwaj, R., Kaur, S. (2024) Chapter Six -Salinity Stress: Impact on Plant Growth. In: Sharma, A., Kumar, M., Sharma, P. (Eds.), Advances in Food Security and Sustainability. Elsevier, pp. 145–160. DOI: http://dx.doi.org/10.1016/bs.af2s.2024.07.002.
Rahmati, H., Yousefi, B. (2024) Salt stress alters phytochemical, physio-biochemical, photosynthetic and antioxidant attributes of *Satureja mutica*. *Trends Phytochem. Res.* 8(4):248–260.
DOI: https://doi.org/10.71596/tpr.2024.1184720.
Ramakrishna A., Ravishankar, G.A. (2011) Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav.* 6(11):1720–1731. DOI: https://doi.org/10.4161/psb.6.11.17613.
Saadatfar, A., Hossein Jafari, S. (2023) Application of 24-epibrassinolide as an environmentally friendly strategy alleviates negative effects of salinity stress in *Satureja khuzistanica* Jamzad. *JRS* 14(3):1–9.
DOI: https://doi.org/10.57647/j.jrs.2024.1403.25.
Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M., Hasanuzzaman, M. (2021) Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. *Antioxidants* 10(2):277.
DOI: https://doi.org/10.3390/antiox10020277.
Sarker, U., Oba, S. (2020) The response of salinity stress-induced *A. tricolor* to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. *Front. Plant Sci.* 11:559876. DOI: https://doi.org/10.3389/fpls.2020.559876.
Sarmoum, R., Haid, S., Biche, M., Djazouli, Z., Zebib, B., Merah, O. (2019) Effect of salinity and water stress on the essential oil components of Rosemary (*Rosmarinus officinalis* L.). *Agronomy* 9:214.
DOI: https://doi.org/10.3390/agronomy9050214.
Shah, S.H., Houborg, R., McCabe, M.F. (2017) Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* 7(3):61.
DOI: https://doi.org/10.3390/agronomy7030061.
Stefanakis, M.K., Giannakoula, A.E., Ouzounidou, G., Papaioannou, C., Lianopoulou, V., Philotheou-Panou, E. (2024) The effect of salinity and drought on the essential oil yield and quality of various plant species of the Lamiaceae family (*Mentha spicata* L., *Origanum dictamnus* L., *Origanum onites* L.). *Horticulture* 10(3):265.
DOI: https://doi.org/10.3390/horticulturae10030265.
Tsusaka, T., Makino, B., Ohsawa, R., Ezura, H. (2019) Genetic and environmental factors influencing the contents of essential oil compounds in *Atractylodes lancea*. *PLoS One* 14:e0217522.

DOI: https://doi.org/10.1371/journal.pone.0217522.
Wang, X., Chen, Z., Sui, N. (2024) Sensitivity and responses of chloroplasts to salt stress in plants. *Front. Plant Sci.* 15:2024. DOI: https://doi.org/10.3389/fpls.2024.1374086.
Yousefi, B., Karamian, R. (2025a) Effects of salicylic acid on photosynthetic pigments, osmolytes, and antioxidant enzyme activities in white savory (*Satureja mutica* Fisch.) exposed to various salt levels. *IJHST* 12(1):69–82.
DOI: https://doi.org/10.22059/ijhst.2024.365136.693.
Yousefi, B., Karamian, R. (2025b) Effect of salinity stress and salicylic acid on morpho-physiological and growth characteristics *Satureja mutica* Fisch. & C. A. Mey. *JRS* 15(1):1–9. DOI: https://doi.org/10.57647/j.jrs.2025.1501.04.
Yousefi, B., Lebaschy, M., Sefidkon,F., Safari, H. (2023) Effects of different planting densities and organic fertilizers on yield characteristics of *Satureja spicigera* (K.Koch) Boiss. under rainfed conditions. *IJMAPER* 39(1):69–81.
DOI: https://doi.org/10.22092/ijmapr.2021.353696.2947.
Zhao, H., Liang, H., Chu, Y., Sun, C., Wei, N., Yang, M., Zheng, C. (2019) Effects of salt stress on chlorophyll fluorescence and the antioxidant system in *Ginkgo biloba* L. seedlings. *Hortscience* 54(12):2125–2133.
DOI: https://doi.org/10.21273/HORTSCI14432-19.
Zhao, H.Y., Wei, N., Sun, C.C., Bai, Y.L., Zheng, C.X. (2018) Effects of salt stress on anatomic structure of tissue and photosynthesis in *Ginkgo biloba* seedlings. *J. Beijing For. Univ.* 40:28–41.
DOI: http://dx.doi.org/10.13332/j.1000-1522.20180258.
Zhu, J.K. (2016) Abiotic stress signaling and responses in plants. *Cell* 167(2):313–324.
DOI: https://doi.org/10.1016/j.cell.2016.08.029.
Zushi, K., Matsuzoe, N. (2017) Using of chlorophyll a fluorescence OJIP transients for sensing salt stress in the leaves and fruits of tomato. *Sci. Hortic.* 219:216–221. DOI: https://doi.org/10.1016/j.scienta.2017.03.016.