Trends Phytochem. Res. 8(4) 2024 248-260

Trends in Phytochemical Research (TPR)

Journal Homepage: https://sanad.iau.ir/journal/tpr

Original Research Article

Salt stress alters phytochemical, physio-biochemical, photosynthetic and antioxidant attributes of *Satureja mutica*

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ABSTRACT

Forest savory (*Satureja mutica* Fisch. & C. A. Mey) is known as an oil-bearing plant which is used in the pharmaceutical, health and food industries. We studied the effect of 0, 50, 100 and150 mM NaCl on some physio-biochemical, photosynthetic and antioxidant characteristics in a greenhouse completely randomized design experiment (CRD, r = 3). Results showed that NaCl levels on average reduced the shoot dry weight, EO yield/plant, chlorophyll a, b, a+b, carotenoied and carvacrol content respectively by 40.90, 38.65, 38.39, 24.41, 34.92,444, and 63.90%. However NaCl levels on average increased leaf proline content (805.13%), leaf protein content (36.06%), SOD activity (392.98%), POD (115.52%), catalase activity (704.60%), EO percent (10.70%), *p*-cymene (58.06%), and thymol content (22.66%). Results confirmed that *S. mutica* tolerates salt less than 100 mM. Therefore, plantation of this species don't recommend in the salty soils.

ARTICLE HISTORY

Received: 22 September 2024
Revised: 21 December 2024
Accepted: 25 December 2024
ePublished: 30 December 2024

KEYWORDS

Antioxidant activity
Essential oil
Medicinal plants
Photosynthesis
Phytochemicals
Thymol

1. Introduction

From ancient times until now, humans have used medicinal plants to treat diseases (Dabaghian et al., 2024). In recent times, plants have attracted the attention of pharmacists as important sources of effective medicinal compounds with low side effects and compatible with the physiological structure of the body (Dahanayake et al., 2020; Mohammadhosseini et al., 2021; Kazeminia et al., 2022; Mohammadhosseini et al., 2022; Nalawade et al., 2022). Species of mint family are important sources of medicinal constituents that are used in traditional medicine, modern medicine, and pharmaceutical, health and food industries (Zebib and Merah, 2017). Some of these species are rich in phenolic monoterpenes such thymol and carvacrol. These chemicals are used in medicine and pharmaceutical sciences as antispasmodic, antibacterial, antioxidant, and anticancer agents (Kulak, 2020). They are also used in cosmetics, food products and aromatherapy (Nieto, 2020). Saturja plant is one of the important sources of phenolic monoterpenes constituents. Forest savory (*Satureja mutica* Fisch. & C. A. Mey) growing in north and northeastern Iran, Transcaucasus, and Turkmenistan (Jamzad, 2012). It is used in traditional medicine to treat rheumatic pain, migraine, toothache and diarrhea (Mazandarani and Monfaredi, 2017). In addition, in recent years, this plant used in food industries, pharmaceutical and hygiene industries (Rahimi et al., 2016).

Salt stress (SS) relatively closes stomata and reduces carbon dioxide absorption. Also, SS disrupts cell and photosynthetic membrane, reduces water and nutrients absorption and transfer in plants and causes ionic imbalance (Sarker and Oba, 2020). In addition, SS causes abiotic stress, which shake molecular, biochemical,

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and physiological functions in plants, resulting severely decrease plant growth and development (Balasubramaniam et al., 2023).

The photosynthetic factors are the most important factors mentioned as an indicator of plant tolerance to salt stress (Zhao et al., 2018). It has been documented that SS adversely affects photosynthetic pigments (Zarei et al., 2019; Mohammadi et al., 2023). High salt concentration disturbs the absorption and metabolism of essential elements and induces reactive oxygen species (ROS) production in plants. High ROS levels destabilize protein-pigment complexes and stimulate chlorophyllase enzyme activity, resulting degrade chlorophyll molecules (Zhao et al., 2019). These responses can diminish the photosynthetic pigments and photosynthetic efficiency (Dong et al., 2017), and subsequently lessen plant biomass. In recent publications, salinity significantly has decreased chlorophyll content in *Satureja hortensis* (Mohammadi et al., 2023), *Hordeum vulgare* L. (Akhter et al., 2021), *Triticum asetivum* (Wang et al., 2017) and Pistachio seedlings (Alipour, 2018).

Reactive oxygen species (in regulated concentrations) plays an important signaling role in controlling plant process in response to environmental stresses (Sahu et al., 2022). However, an excess concentration of these ROS causes a variety of harmful responses such DNA destruction, protein denaturation, chlorophyll structure dissociation, and cell membrane demolition (Yang and Guo, 2018; Sachdev et al., 2021). Under severe salt stress, a large amount of reactive oxygen species such superoxide and single oxygen is produced in plants (Singh et al., 2023). ROS induced enhanced accumulation of compatible solutes (non-enzymatic cellular antioxidants) and enzymatic antioxidant activity (Muchate et al., 2019). This enzymatic antioxidant defense detoxifies superoxide (O_2^-) to H_2O (Ugalde et al., 2021), resulting helps plant against adverse effects of oxidative stress. Proline and salt stress proteins have a prominent effect on cell osmotic adjustment in response to salinity stress (Shen et al., 2022). Also, proline as a non-enzymatic cellular antioxidant prevents the buildup of ROS and protects plants against abiotic stresses (Kaur and Asthir, 2015). In response to stress, some stress-proteins are biosynthesized and accumulate in leaves (Athar et al., 2022). However, high salt stress inhibits the de novo biosynthesis of proteins, including stress-protein and photosystem-related proteins (Yang et al., 2020). In additions, under high salt stress some leaf proteins breakdown to osmoprotectants amino acids (Abdelkader et al., 2023).

Phytochemical compounds will be changed under stress conditions. Abiotic stresses affect the biosynthesis of primary and secondary metabolites in plants (Zhu, 2016). Salt-mediated oxidative stress triggers signaling pathways involved in metabolic reprogramming. It is proven that the levels of primary metabolites (Leschevin et al., 2021) and secondary metabolites (Akula and Ravishankar, 2011) change in response to salt-mediated osmotic/oxidative stress and/or ion toxicity. Furthermore, ROS disrupt the integrity of cell membranes, impair the absorption and metabolism of essential elements (Sachdev et al., 2021), destabilize protein-pigment complexes, and enhance the activity of the chlorophyllase enzyme (Zhao et al., 2019). These responses ultimately can diminish the photosynthetic pigments, photosynthetic efficiency and plant production.

The effects of salt stress on EO yield and/or EO compounds in several Lamiaceae medicinal plants, such as *Satureja khozestanica* Jamzad (Zaremanesh et al., 2021), *Mentha spicata* (Ounoki et al., 2021), *Mentha suaveolens* (Kiumarzi et al., 2022), *Salvia officinalis* L. (Kulak, 2020), *Rosmarinus officinalis* (Dehghani Bidgoli et al., 2019), *Ocimum basilicum* L. (Oprică et al., 2019), *Thymus vulgaris* and *Thymus danensis* (Bistgani et al., 2019), *Salvia mirzayanii* (Valifard et al., 2014) and *Mentha x piperita* L., *Mentha pulegium* L. and *Mentha suaveolens* Ehrh (Aziz et al., 2008) are well documented. However, there is a lack of knowledge about the antioxidant activity, EO chemical and photosynthetic traits of *S. mutica* in response to salt stress.

Therefore, we investigated the effects of salinity stress on photosynthesis, antioxidant activity, and the quantity and quality of essential oil in forest savory. Additionally, we assessed the feasibility of cultivating this plant in saline soils.

2. Experimental

2.1. Experimental design, conditions and treatments

A completely randomized design (CRD) (greenhouse experiment, r = 3) was carried out in the research center of agricultural and natural resources, Kermanshah, Iran. Seeds were disinfected with sodium hypochlorite (0.5%), washed, and dried. Seeds were planted in a peat moss bed and watered by sprinkling. The seedlings transferred to the plastic pots (one seedling per pot), which filled with a mixture of farm soil, sand, and rotten cow manure (Table 1). The plants kept under 17 h/d light photoperiod by 300 mMOL/m^2×s (110 lux of light intensity), and 7 hours of darkness (Hernández-Adasme et al., 2023), and relative humidity of 50-60%. Four irrigation treatments (250 mL to each pot, tow times a week) consisting of 0, 50, 100, and 150 mM NaCl were implemented (Kumar et al., 2022). After 4 times irrigation with NaCl treatment, we removed the accumulated salts in the pots with irrigation by distilled water.

2.2. Photosynthetic assays

To measure the amount of photosynthetic pigments (Lichtenthaler and Wellburn, 1985), the samples centrifuged for 10 minutes (10000 rpm, 4°C) and the supernatant was read at 663, 646, and 470 nm by a Microplate reader. The content of photosynthetic pigments (mg g^{-1} FW) calculated using equations 1 to 4 (Lichtenthaler and Wellburn, 1985).

Chl a = 12.21 (A663) - 2.81 (A646) (Eqn.1)

Chl b = 20.13 (A646) - 5.1 (A663) (Eqn.2)

Chl T = Chl a + Chl b (Eqn. 3)
Carotenoid = (1000 A470 - 3.27 [Chl a] - 104 [Chl b]/227 (Eqn. 4)

2.3. Biochemical assays

The extraction buffer and crude leaf extract prepared (Ramachandra Reddy et al., 2004). The enzymatic activity rate of superoxide dismutase (SOD, EC 1.15.1.1) was measured based on the ability of SOD to stop the photochemical regeneration of Nitrotetrazolium Blue chloride (NBT) by superoxide radicals in the presence of riboflavin at light condition (Beauchamp and Fridovich, 1971). The optical absorbance was read at 560 nm (enzymatic unit equivalent to 50% inhibition) by a microplate reader. The activity of SOD calculated using the following formula (Eqn. 5).
SOD(µmol g^{-1}) FW) = (100-[((ODcontrol - ODsample))/(ODcontrol)] × 100)/50 (Eqn.5)
In this equation, the terms $OD_{control}$ and OD_{sample} respectively count for the absorbance of control at 560 nm and the absorbance of samples at 560 nm.
The peroxidase (POD; E.C. 1.11.1.7) activity was measured using microplate reader and expressed in terms of H_2O_2 consumption (µ mole min^{-1} mg of soluble protein) (Chance and Maehly, 1955). The optical absorbance was read for 15 min at 30 s intervals at a wavelength of 470 nm and calculated using the Beer-Lambert law (0.0266 Mcm^{-1}). The activity of catalase (CAT; E.C. 1.11.1.6) was measured (Sinha, 1972) with some modifications using the Beer-Lambert law (0.0394 Mcm^{-1} extinction coefficient). Proline content (Bates et al., 1973) and soluble protein concentration (mg g^{-1}FW) (Bradford, 1976) were measured.

2.4. Morpho-physiological measurements

At the end of experiment, the plants were harvested and the shoot fresh weight (SFW) (0.0001 g) was subsequently measured. The plant shoots were dried in shadow and room temperature and the shoot dry weight (SDW) measured. The dried plants were kept and used to EO extraction.

2.5. EO extraction and EO profile identification

2.5.1. Essential oil extraction

We extracted the essential oils (EOs) of all the aerial parts of the plants by the Clevenger system and water distillation for 3 hours (British Pharmacopoeia, 1993). The EO samples were dehydrated with anhydrous sodium sulfate (Na_2SO_4). The net weight of the EOs was measured. The EO samples were covered with aluminum foil and kept in a refrigerator (4 °C) for GC and GC/MS analysis.

2.5.2. EO percentage and EO yield calculation

The EO percentage (*W/W*) and EO yield were calculated using Eqn. 6 and Eqn. 7, respectively (Khademi Doozakhdarreh et al., 2022).
EO (%) = [(EO weight (g))/(sample dry weight (g))] × 100 (Eqn. 6)
EO yield (g) = EO% × shoot dry weight (g) (Eqn. 7)

2.5.3. GC analysis

We analyzed the EO samples by a Thermo-UFM gas chromatograph (ultrafast model) with a Chrom-Card A/D data processor. A Ph-5 cap column (nonpolar) was used (length = 10 m, and inner diameter thickness = 0.1 and 0.4 µm). The inner surface of the device was coated with 5.0% dimethyl siloxane phenyl (Stationary phase) (Thermo Fisher Scientific, Italy). The column temperature program was as follows: Initial temperature of 60 °C, starting at a final temperature of 285 °C, at which point 80 °C was added every minute, after which the reaction was stopped at this temperature for 3 minutes. The detector type was an FID at 290 °C. The temperature of the injection chamber was 280 °C. The carrier gas was helium. The inlet pressure to the column was set at 0.5 $kgcm^{-2}$.

2.5.4. GC/MS analysis

We analyzed the EO samples by a Varian 3400 GC/MS connected to a Saturn II mass spectrometer and ion telephoto system (with ionization energy of 70 electron volts). The column was a semipolar DB-5 column (30 m in length, 0.25 mm in inner diameter, and 0.25 µm in stationary phase thickness). The pressure of the column head gas was set at 35 PSI and 40 to 250°C. The rate of temperature increase was 4 °C min^{-1}. The injection chamber temperature was 260 °C, and the line transfer temperature was 270 °C. The retention indices were calculated by injection of normal hydrocarbons (C_7-C_{25}). Finally, the EO chemicals were identified by comparison of spectra with different libraries (Adams, 2017).

2.6. Statistical analysis

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

3. Results and Discussion

3.1. Results of GC and GC/MS

We identified a total of nine monoterpene compounds in the EO samples. The EO chemicals and their specifications presented in Table 2. Thymol, *p*-cymene, carvacrol, and γ-terpinene were the main EO components.

3.2. Results of ANOVA

Results of ANOVA revealed that the effects of NaCl levels on plant dry weight, EO percent, EO yield, proline

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	0.7	7.03	175	138	0.28
Culture bed weight (kg)	**Pots dimensions (cm)**	**Photoperiod (h d^{-1})**	**Relative humidity (%)**	**Light intensity (mmol m^2s)**	
4.5	17×22	17	50-60	300	

Table 2
The EO chemical identified in *S. mutica* under NaCl× SeNp treatments.

Chemical compounds	Classification	RT(min.)	RI	CAS number	Formula	Range (%)
Carvacrol	Phenolic monoterpenoids	4.07	1289.96	000499-5-2	$C_{10}H_{14}O$	6.71-24.05
Thymol		3.96	1281.22	000089-83-8	$C_{10}H_{14}O$	37.68-50.43
α-Pinene	Bicyclic monoterpenes	0.87	931.13	000080-56-8	$C_{10}H_{16}$	0.22-1.35
β-Pinene		0.87	931.13	000127-91-3	$C_{10}H_{16}$	1.02-2.18
trans-Caryophyllene		5.33	1376.02	000087-44-5	$C_{15}H_{24}$	0.86-1.41
Camphene		1.16	953.1	000565-00-4.	$C_{10}H_{16}$	0.05-1.06
γ-Terpinene	Isometric monoterpenes	1.63	1051.59	000099-85-4	$C_{10}H_{16}$	16.78-20.98
α-Terpinene		1.63	1051.21	000099-35-3	$C_{10}H_{16}$	1.09-2.47
p-Cymene	Monocyclic monoterpenes	1.36	1014.15	000099-87-6	$C_{10}H_{14}$	10.17-13.01

RI: Retention index, RT: Retention time.

content, soluble protein content; superoxide dismutase, peroxidase and catalase activities; chlorophyll a, b, a+b and carotenoid contents, and carvacrol, γ-terpinene and *p*-cymene contents were significant ($p \leq 0.01$), however the effect of NaCl treatments was not significant for thymol content (Table 3).

3.3. Means comparison

3.3.1. Plant Yield and EO yield traits

The high NaCl levels (100 and 150 mM) significantly reduced plant dry weight compared to control plants (Fig. 1a). 50 mM NaCl reduced plant dry weight, but this reduction was not significant compare to its value in the control plants. The highest plant dry weight (7.6 g) was obtained in the control plants and the minimum value (2.9 g) was obtained in the plants treated with 150 mM NaCl (Fig. 1a).

100 mM NaCl significantly enhanced essential oil percent compared to the control and plants treated with 50 mM NaCl. However 150 mM NaCl significantly diminished EO percent. The highest EO percent (3.5%) was obtained in the plants treated with100 mM NaCl and the lowest EO percent (2.5%) was observed in the 150 mM NaCl- treated plants (Fig. 1b).

The different NaCl levels significantly and nearly linearly decreased the essential oil yield. The maximum EO yield (0.2 g/plant) was obtained in the control plants, while the lowest its value (0.08 g/plant) was observed in the plants treated with 150 mM NaCl (Fig. 1c).

In the present study, the high NaCl levels (100 and 150 mM) significantly reduced shoot dry weight. In line with this result NaCl has reduced the shoot fresh dry weight in *S. khuzestanica* (Saadatfar and Hossein Jafari, 2023), Salinity strongly affects the plant water status. It reduces the turgor pressure and interrupts the ionic balance between plant and soil (Wang et al., 2023). High salt concentration in the root zone reduces the osmotic potential of water in the surrounding roots so; the plant's access to water is reduced. This water deficiency causes partial closure of the stomata, thus reducing the entry of CO_2 and leaf CO_2 concentration (Parida et al., 2005). The reduced leaf CO_2 concentration diminishes photosynthesis and growth. In addition, Salinity causes ion toxicity (in the form of Na^+ and Cl^-) which leads to nutrient deficiency (N, P, K, Zn, and Fe). In this situation, nitrate reductase activity, photosystem II activity and plant growth decrease (Sheldon et al., 2017).

Biotic and abiotic stresses strongly affect the production of secondary metabolites (Yang et al., 2018). Our results showed that 100 and 150 mM NaCl treatments (especialy 100 mM NaCl) significantly enhanced essential oil percent.

The response of plants to stress varies depending on their morphology, ecophysiology, and biochemical characteristics (Stevović et al., 2010). Some studies have confirmed that salinity induces EO biosynthesis (Assaf et al., 2022). Nevertheless, some research emphasize that this effect is unstable and dependes on other environment conditions, salt concentration and plant genetics (species). In such a way, it is possible that salt stress has an inhibitory effect on EO biosynthesis (Neffati and Marzouk, 2010). In line with present results,

Table 3
Analysis of variance of the effect of NaCl levels on yield, biochemical, antioxidant, photosynthetic and phytochemical traits of *S. mutica* plants.

S.O.V.	df	Yield traits			Biochemical traits		Antioxidant activity traits		
		Plant dry weight	EO percent	EO yield	Proline	Soluble protein	SOD	POD	CAT
Salt	3	12.10**	0.63**	0.001**	0.03**	320.20**	1.26**	0.46**	5.93**
Error	6	0.77	0.05	0.000	0.01	3.7	0.001	0.008	0.013
CV (%)		18.44	7.74	20	33.17	7.08	0.000	8.28	6.26
S.O.V.	**df**	**Photosynthetic traits (pigments content)**				**Phytochemical traits (EO main compounds)**			
		Chl a	Chl b	Chl a+b	Carotenoid	Thymol	Carvacrol	γ-Terpinene	*p*-Cymene
Salt	3	13.63**	0.45**	18.77**	1.42**	64.84ns	224.55**	34.23**	105.03**
Error	6	0.009	0.014	0.004	0.009	60.36	16.97	4.18	9.831
CV (%)		1.49	4.85	0.72	5.93	18.43	31.59	11.05	19.27

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

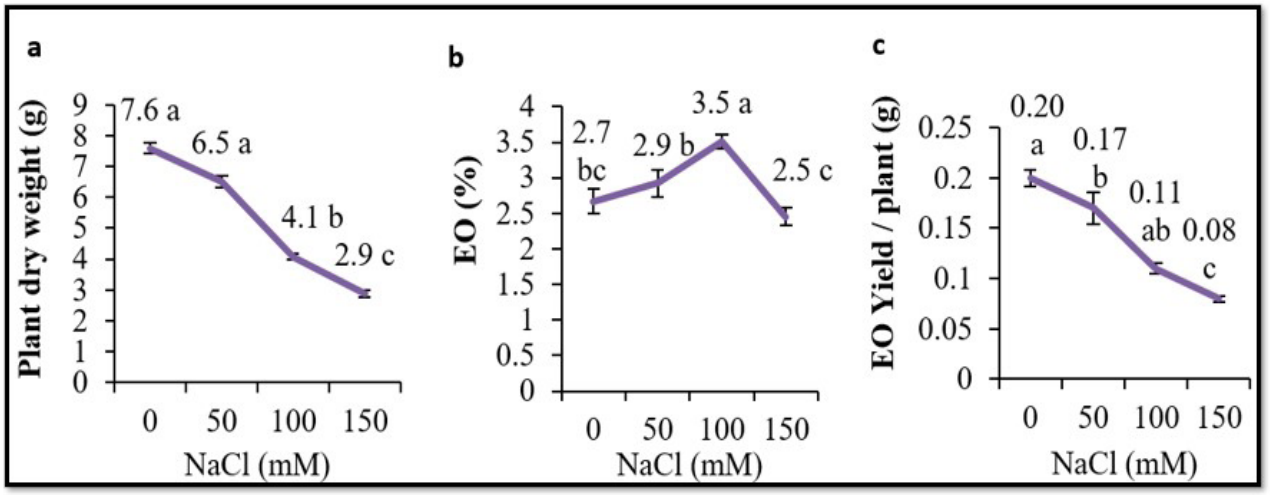

Fig. 1. Means comparison (Means ± SD) Plant dry weight (**a**), EO percent (**b**) and EO yield (**c**) of *S. mutica* plants in response to different NaCl levels, columns with the same letters are not significantly different (Duncan test, $p = 0.05$).

100 and 150 mM NaCl has increased EO content of *Origanum onites* (Stefanakis et al., 2024). Also, 15 dS/m NaCl has increased EO content of *Achillea millefolium* L. by 18.75% compared to control treatment (Dehghan and Rahimmalek, 2018). Hosseini et al. (2023) mentioned that salinity has induced essential oil present in some Mentha genus. Contrary to these results, in *Pelargonium graveleons* 100, 150, and 200 mM NaCl significantly has reduced the oil content (Sarmoum et al., 2019). Dehghani Bidgoli et al. (2019) concluded that In *Rosmarinus officinalis* L. 10.0 g NaCl per liter has enhanced EO percent, while 12.5 g NaCl per liter reduced EO percent compare to control plants.
Our observation confirmed that, although salinity stress increased the percentage of essential oil, but due to the decrease in the weight of the plant, it overall reduced the yield/plant of essential oil in the forest savory (especially100 and 150 mM NaCl significantly declined the essential oil yield/plant). In line with this, SS caused a reduction in the essential oil yields/plant in three species of Lamiaceae including *Mentha x piperita* L., *Mentha pulegium* L. and *Mentha suaveolens* Ehrh (Aziz et al., 2008). Also, in another study, saline treatment has induced lower EO yield of *Rosmarinus officinalis* L. (Swamy Gowda et al., 2022).

3.3.2. Bio-physiological traits

50 and 100 mM NaCl significantly and linearly enhanced leaf proline and protein contents. However, 150 mM NaCl had not significant changes in the proline content compared to 100 mM NaCl (Fig. 2a), while it significantly reduced leaf protein content compared to 100 mM NaCl treatment (Fig. 2b).
Under osmotic stress, the accumulation of proline and other osmoprotectant increases. In present research, the proline content significantly augmented in response to salt levels. Similar to this finding, salinity levels significantly increased the proline content in *Satureja hortensis* (Mohammadi et al., 2023), *Satureja*

khuzestanica (Saadatfar and Hossein Jafari, 2023), and *Thymus danensis*t (Harati et al., 2015). Also, in some Mentha species salinity induced proline content (Hosseini et al., 2023). Present findings revealed that 50 and 100 mM NaCl significantly augmented protein content in *S. mutica* plant; however this accumulation decreased at 150 mM NaCl. Similar to these results, low salinity treatments has increased soluble protein in *Thymus vulgaris*, while high NaCl levels significantly declined it (Harati et al., 2015).

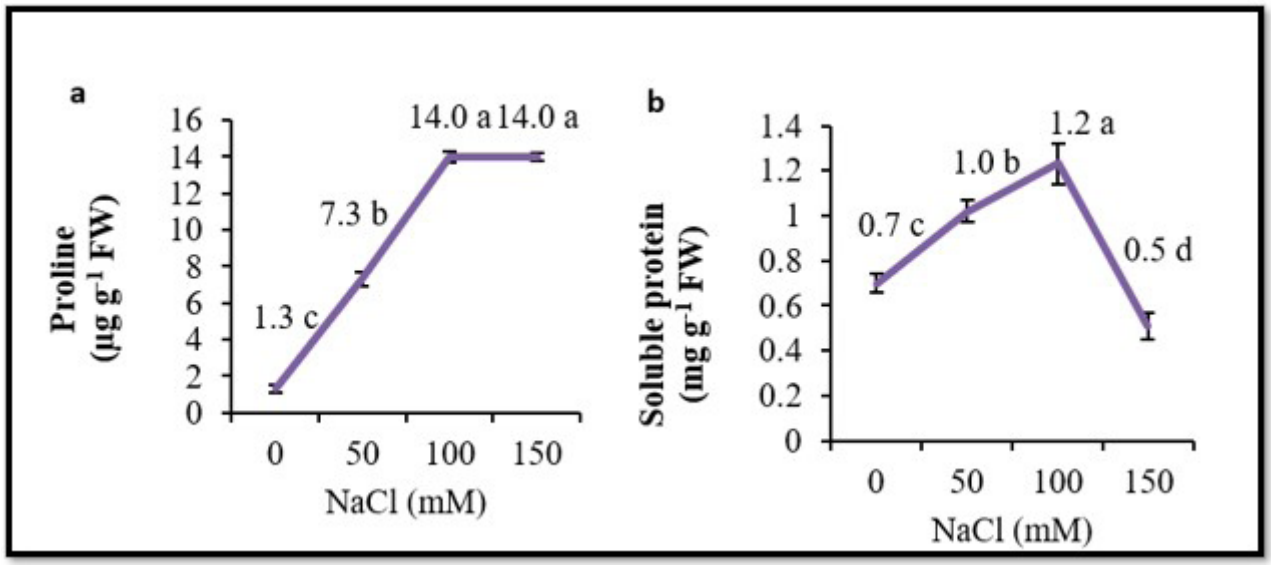

Fig. 2. Means comparison (Means ± SD): leaf proline content (**a**) and leaf soluble content (**b**) of *S. mutica* plants in response to different NaCl levels, the same letters are not show significant difference (Duncan test, $p = 0.05$).

3.3.3. Biochemical traits (Antioxidant activity)

As exhibited in the Fig. 3a-c, application of different NaCl levels (50, 100, and 150 mM) significantly boosted antioxidant activities of SOD, POD and catalase compared to control treatment. The lowest SOD activity (0.2 mg min^{-1} g protein), POD (06 mg min^{-1} g protein) and catalase activity (0.3 mg min^{-1} g protein) were observed in the control plants (Fig. 3a-c). 50, and 100 mM NaCl significantly and linearly (approximately) boosted the SOD, POD and catalase activities. 150 mM NaCl linearly enhanced the antioxidant activities of POD and catalase, while 150 mM NaCl sharply enhanced the SOD activity. The highest SOD activity (1.7 mg min^{-1} g protein), POD activity (1.5 mg min^{-1} g protein) and catalase activity (3.5 mg min^{-1} g protein) were observed in the plants treated with150 mM NaCl (Fig. 3a-c).

Oxidative stress releases ROS. Enhanced enzymatic antioxidant activities scavenge excessive ROS and ameliorate oxidative damages caused by stressfully agents (Ahmad et al., 2011). In the present research, the antioxidant activity of the SOD, POD, and CAT significantly increased in response to NaCl concentrations. In *S. khuzestanica* different NaCl levels significantly has increased the activity of SOD, POD, and CAT (Saadatfar and Hossein Jafari, 2023). NaCl have increased the activities of POD and, or CAT activities in *C. annuum* (Kumar et al., 2022) and *Amarantus tricolor* (Sarker and Oba, 2020), SOD, POD, and CAT in *B. carinata* (Husen et al., 2018). These results confirm our findings.

3.3.4. Photosynthetic pigments

The different NaCl levels significantly and linearly decreased chl. a, b. chl. a + b and carotenoid contents (Fig. 4a-d). The maximum chl. a (8.9 mg g^{-1} FW) was observed in the control plants, however the lowest its value (3.8 mg g^{-1} FW) was observed in the plants treated with 150 mM NaCl (Fig. 4a).We observed the highest chl. b (3.0 mg g^{-1} FW) in the control plants, however the lowest chl. b (2.1 mg g^{-1} FW) was observed in the plants treated with 150 mM NaCl (Fig. 4b). The maximum chl. a + b (11.9 mg g^{-1} FW) was observed in the control plants and the lowest it value (5.9 mg g^{-1} FW) was observed in the plants treated with 150 mM NaCl (Fig. 4c). Also, the highest carotenoid content (2.4 mg g^{-1} FW) and the lowest carotenoid content (0.7 mg g^{-1} FW), respectively were observed in the control plants and plants treated with150 mM NaCl (Fig. 4d).

Photosynthesis parameters are beneficial indicators for assessing the effects of salt stress. In salt sensitive plants chlorophyll concentration is decrease significantly in response to high levels of NaCl, however in salt resistant plants, the concentration of chlorophyll is less affected by salt stress. In the present study, 100 and 150 mM

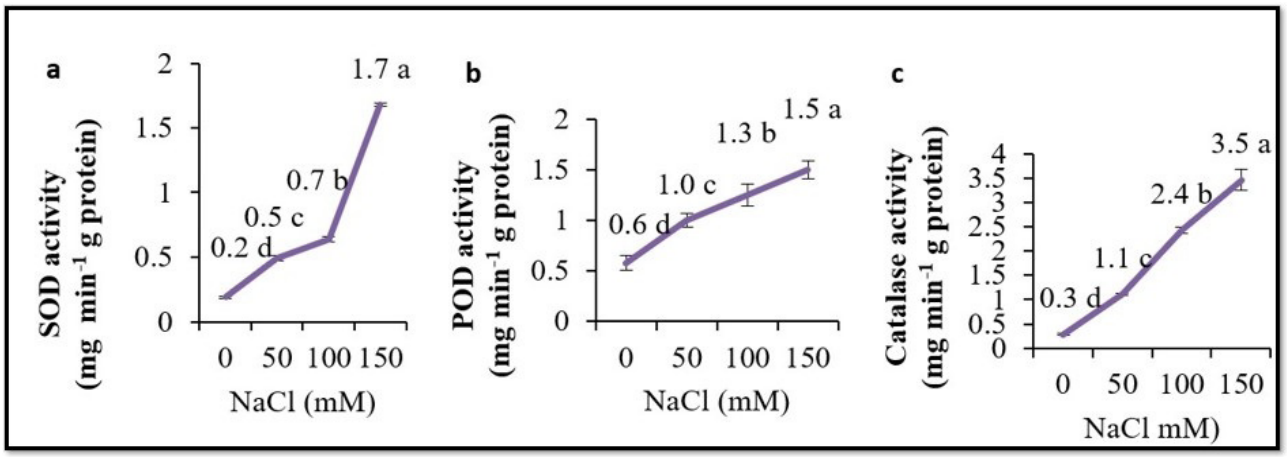

Fig. 3. Means comparison (Means ± SD) superoxide dismutase (**a**), peroxidase (**b**), and catalase activity (**c**) of *S. mutica* plants in response to different NaCl levels. The same letters are not show significant difference (Duncan test, $p = 0.05$).

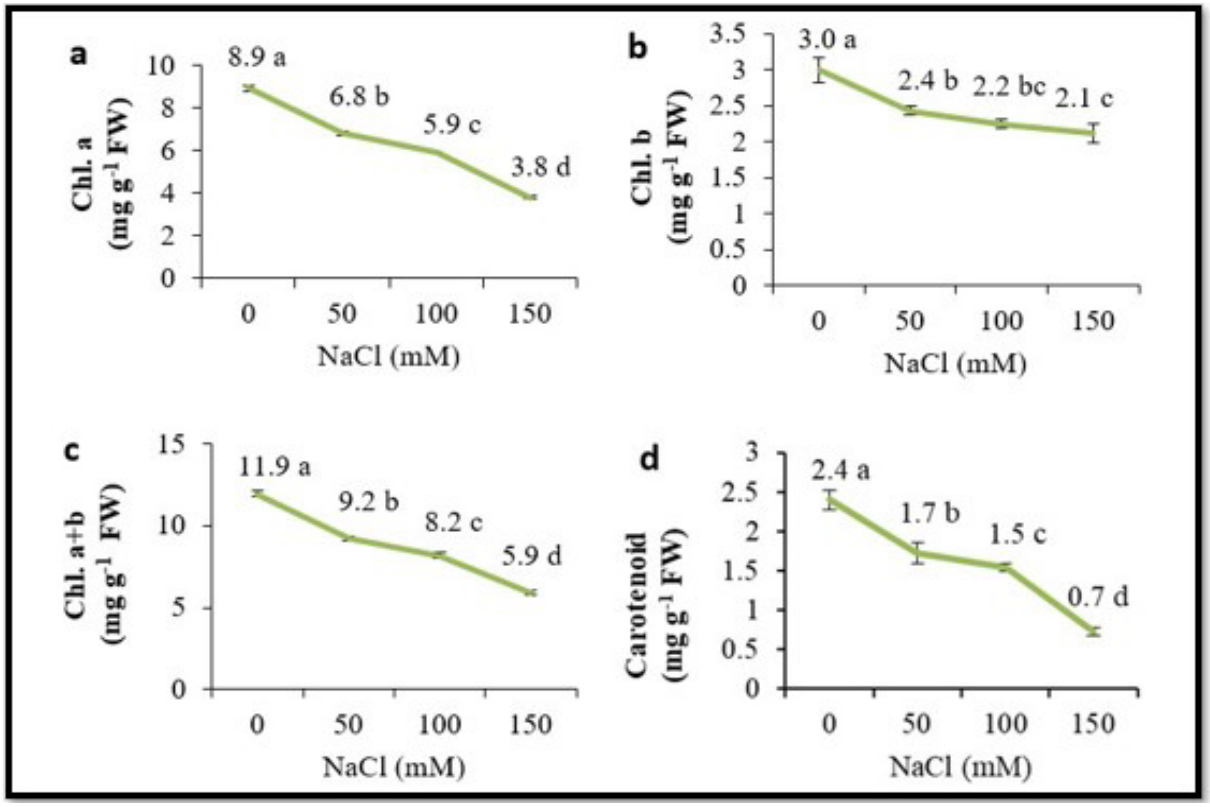

Fig. 4. Means comparison (Means ± SD): chlorophyll a, b, a+b, and carotenoid (**a**, **b**, **c** and **d**, respectively) of *S. mutica* plants in response to different NaCl levels. Columns with the same letters are not significantly different (Duncan test, $p = 0.05$).

NaCl sigificatly reduced chl a, b, a+b and carotenoid contents. Consistent with these finding, 100 and 150 mM NaCl significantly has reduced total chlorophyll concentration in *Mentha spicata* plants (Stefanakis et al., 2024). The most reports demonstrated that salt stress lessens chlorophyll contents in the most species of the mint family. For ex: salinity significantly decreased chlorophyll a and b in *Satureja hortensis* (Mohammadi et al., 2023) and *Satureja khuzestanica* (Saadatfar and Hossein Jafari, 2023); and carotenoid content in *Satureja hortensis* (Fabriki ourang and Mehrabad-Pourbenab, 2016). Also, as examples in some other medicinal

species, salinity has caused a significant decrease in photosynthetic pigments in *Lantana camara* (Dehestani Ardakani et al., 2021), *Linum usitatissimum* L. (Dubey et al., 2020), and *Nigella sativa* L. (Fazeli et al., 2017).
The salt-mediated water and nutrients reduction lessen the biosynthesis of chlorophyll. In addition, high salt concentrations induced chlorophyllase, which converts chlorophyll to chlorophyllide (Chlide) by removing the phytol side chain (Taïbi et al., 2016). Important ultrastructure damages such as disorganization, have observed by transmission electron microscopy in leaf chloroplasts of *Mentha spicata* L. treated with 150 mM NaCl (Ounoki et al., 2021). This ultrastructure disassociation has decreased the maximal and actual quantum efficiency of photosystem II and relative chlorophyll content (Ounoki et al., 2021).

3.3.5. EO phytochemical compounds

Different NaCl levels had not significant effects on the thymol content; however these treatments significantly changed the contents of carvacrol, γ-terpinene and *p*-cymene (Fig. 5 a-d). Carvacrol content significantly reduced by 72, 72 and 45%, respectively in the plants treated with 50, 100, and 150 mM NaCl compared to control (Fig. 5b). γ-Terpinene content significantly enhanced by 23% in the plants treated with 50 mM NaCl (Fig. 5c) and p-cymene content significantly raised up by 28% in the plants treated with 100 mM NaCl (Fig. 5d).
Salt stress alters extensively terpenoids metabolism. Resulting alteration in EO compositions depend on the intensity of the stress and the plant genetic (Tsusaka et al., 2019). In the present study, carvacrol content significantly reduced in response to NaCl, while γ-terpinene and *p*-cymene content significantly enhanced in the plants treated with NaCl. Well-matched with these results, NaCl significantly has increased γ-terpinene and *p*-cymene in *S. khozestanica* Jamzad (Zaremanesh et al., 2021) and thymol in *Plectranthus amboinicus* (Sany et al., 2020). However, in contrast to our finding, NaCl has diminished γ-terpinene and *p*-cymene in *P. amboinicus* (Sany et al., 2020). Also, the essential oil composition of spearmint has unaffected by 150 mM NaCl (Ounoki et al., 2021). Kasrati et al. (2017) noted that NaCl stress induces an interruption in normal metabolism, e.g., photosynthesis and growth and development. This abnormality development transforms glandular hairs, and limits EO biosynthesis (Sarmoum et al., 2019). Also, salt stress leads to ionic imbalance in the roots surroundings (Khan et al., 2014). Seif Sahandi et al. (2019) mentioned that ionic imbalance causes inadequate absorption of nutrients, which affects the activities of enzymes involved in EO biosynthesis. For example, sufficient nitrogen increases terpenoid synthesis (Nejatzadeh, 2021), however it caused reduce in the formation of sesquiterpenes (Sany et al., 2020). In a previous study, the relative level of various constituents of essential oil in response to NaCl has increased, decreased, or did not change in three different species of peppermint (*Mentha x piperita* L., *Mentha pulegium* L. and *Mentha suaveolens* Ehrh.) (Aziz et al., 2008). In *Rosmarinus officinalis* L., salt stress enhanced monoterpens, oxygenated monoteroens and oxygenated sesquieterpenes, however it reduced sesquieterpenes and Ketons (Swamy Gowda et al., 2022). In this plant species, saline treatment resulted in increase of D-verbenone, camphene, caryophyllene oxide and α-pinene (Swamy Gowda et al., 2022). Unlike with our results, in a recent publication, the essential oil composition of spearmint has unaffected by 150 mM NaCl (Ounoki et al., 2021).

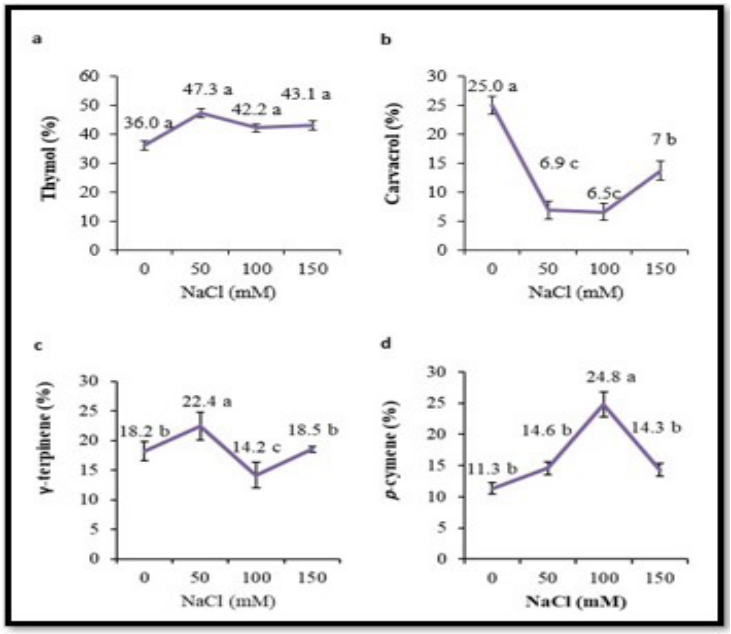

Fig. 5. Means comparison (Means ± SD) thymol (**a**), carvacrol (**b**), γ-terpinene (**C**) and *p*-cymene (**d**) contents in the EO of *S. mutica* plants in response to different NaCl levels, columns with the same letters are not significantly different (Duncan test, $p = 0.05$).

3.3.6. Principal component analysis

In the principal components analysis, that the first 3 components had eigenvalues higher than 1 (Table 4, Fig. 6). The eigenvalue of the first component was 10.2 values and the second component was 3.86 values. The first 2 components (Table 4) revealed 88% of the variance (64% through the first component and 0.24 via the second component). The variables of chlorophyll b, a+b, a, carotenoid, plant dry weight, essential oil yield, and carvacrol content (respectively) had the highest positive contribution in the first component. On the other hand, the variables of peroxidase activity, proline content, catalase activity, superoxide dismutase activity, and thymol content, respectively, had the highest negative contribution in the first component (Table 4). The variables of carvacrol content, gamma-terpinene content and superoxide dismutase activity (respectively) had the most positive contribution in the second component, While, the variables of essential oil percentage, soluble protein, *p*-cymene content and essential oil yield (respectively) had the most negative contribution in the second component (Table 4).

Based on the PCA diagram (Fig. 6), the variables of carvacrol, gamma-terpinen and chlorophyll b content showed the same trend and the highest amount of these traits was observed in the control treatment. The variables of superoxide dismutase and catalase activities showed the same trend and had the highest correlation with the treatment of 150 mM NaCl. The highest values of these traits were observed in the mentioned treatment. The variables of essential oil percentage and para-cymene content and to some extent the variable of leaf soluble protein showed a similar trend and the highest amount of these traits was observed in the treatment of 100 mM NaCl. In addition, the variables of peroxidase activity, thymol and proline content showed the same trend and had a relative-high correlation with the treatment of 100 mM NaCl. Yield variables including plant dry weight and essential oil yield, as well as photosynthetic pigment traits, including chlorophyll a, a+b, and carotenoid, showed similar characteristics. The highest amount of these traits was observed in the 50 mM NaCl. Also, the content of soluble protein showed relative correlation with 50 mM NaCl treatment (Fig. 6).

Table 4
Eigenvalue, proportion and cumulative variance of components and the variance proportion of variables in first to third components.

Variable	PC1	PC2	PC3
Plant dry weight	0.302	-0.029	0.181
EO percent	-0.016	-0.509	0.001
EO yield	0.277	-0.203	0.172
p-Cymene	-0.138	-0.442	0.162
γ-Terpinene	0.039	0.275	-0.598
Thymol	-0.204	-0.073	-0.535
Carvacrol	0.213	0.289	0.331
SOD	-0.27	0.231	0.163
POD	-0.311	0	0.086
CAT	-0.296	0.033	0.227
Chl. a	0.308	-0.078	-0.078
Chl.b	0.31	0.07	0.025
Carotenoied	0.303	-0.119	-0.067
Chl a+b	0.31	-0.057	-0.063
Protein	0.004	-0.49	-0.196
Proline	-0.297	-0.13	0.14
Eigenvalue	10.2	3.86	1.94
Proportion	0.64	0.24	0.12
Cumulative	0.64	0.88	1

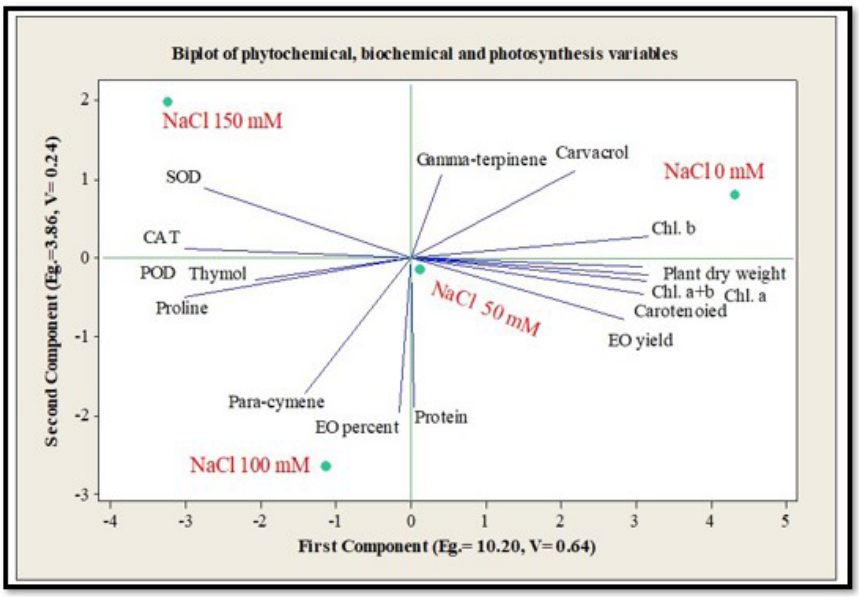

Fig. 6. PCA diagram for phytochemical, physio-biochemical, photosynthetic and antioxidant traits. PC1 (Eg. = 10.2, V = 0.24) and PC2 (Eg. = 3.86 and V = 0.64).

4. Concluding remarks

Results demonstrated that increasing NaCl levels negatively impacted shoot dry weight, essential oil yield, chlorophyll content, carotenoids, and carvacrol levels, while significantly enhancing leaf proline and protein content, enzymatic activities (SOD, POD, CAT), essential oil percentage, p-cymene, and thymol content. The findings suggest that *S. mutica* exhibits moderate salt tolerance, with thresholds below 100 mM NaCl. Consequently, its cultivation is not recommended in saline soils to maintain optimal growth and yield. New insights were obtained regarding the effects of salinity stress on the content and chemical composition of the essential oil of forest savory. Although salinity increased the percentage of essential oil in the plant, it reduced the plant's biomass, thereby decreasing the overall essential oil yield. Future studies are recommended to focus on investigating genes associated with salt stress responses and identifying molecular pathways involved in regulating salt tolerance. These studies could facilitate genetic modification and improve plant performance under salt stress conditions. Additionally, a more detailed analysis of changes in active compounds such as thymol, carvacrol, and other essential oil components under varying salt conditions, along with their impact on the quality of pharmaceutical and food applications, is advised. Management practices, including the use of biofertilizers and the application of nanomaterials, should also be considered to mitigate the adverse effects of salinity and enhance nutrient uptake by the plant.

Authorship contribution statement

Hooshang Rahmati and Borzou Yousefi performed the experiments 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; **Car:** Carotenoid; **CAT:** Catalase; **Chl:** Chlorophyll; **CRD:** Completely Randomized Design; **OD:** Optical Density; **POD:** Peroxidase; **ROS:** Reactive Oxygen Species; **SDW:** Shoot Dry Weight; **SFW:** Shoot Fresh Weight; **SOD:** Superoxide Dismutase, **RT:** Retention Temperature; **RI:** Retention Index.

Conflict of interest

The authors declare that there is no conflict of interest.

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