



# Mitigating Salinity Stress and Enhancing Essential Oil Yield in *Satureja mutica* Using Nano-Selenium: Impacts on Growth, Photosynthesis, and Phytochemical Compositions

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## ABSTRACT

This study investigates the potential of nano-selenium (SeNP) to alleviate salinity stress and enhance growth, photosynthetic performance, and essential oil (EO) production in *Satureja mutica*. A factorial greenhouse experiment was conducted at the Agricultural and Natural Resources Research and Education Center, Kermanshah, Iran, in 2019. Treatments included four NaCl concentrations (0, 50, 100, and 150 mM) and two levels of SeNP application (0 and 50 mg L<sup>-1</sup>). The results revealed that 50 mg L<sup>-1</sup> SeNP significantly enhanced plant fresh weight (51.8%), total chlorophyll content (10.53%), carotenoid content (6.88%), Fv/Fm (5.48%), and chlorophyll index (8.93%), in response to NaCl. Additionally, SeNP application increased essential oil (EO) percentage (40.63%), EO yield per plant (55.55%), carvacrol content (21.43%), and  $\rho$ -cymene content (40.99%) under saline conditions. These findings suggest that applying 50 mg L<sup>-1</sup> SeNP mitigates the hostile interventions of NaCl on the photosynthetic system, physiological status and growth of *S. mutica*. It is recommended to use 50 mg L<sup>-1</sup> SeNP to alleviate salinity-induced stress and improve EO yield and carvacrol content, especially in low to moderately saline soils. This study highlights the potential of SeNP as an effective strategy to enhance the productivity and quality of *S. mutica* in saline environments.

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## 1. Introduction

Throughout history, humans have largely used scented plants in medicine and cooking. Medicinal plants have received attention from chemists, pharmacists, and botanists as potential alternatives to synthetic pharmaceuticals. Among these, the Lamiaceae family stands out as one of the most important families of medicinal plants, comprising over 6,000 species with a global distribution. Many Lamiaceae plants, including savory, are rich in thymol, carvacrol, and other phenolic monoterpenes, which are valued as antioxidants, antibacterial, antispasmodic,

and anticancer resources (Krause *et al.*, 2021). *Satureja mutica*, commonly known as forest savory, thrives on limestone soils in northern and northeastern Iran, Transcaucasia, and Turkmenistan. The valuable oil of forest savory is predominantly composed of carvacrol and thymol, along with  $\gamma$ -terpinene and  $\rho$ -cymene (Karimi *et al.*, 2016). Research efforts have been undertaken to cultivate and domesticate this plant, with results confirming the feasibility of successful rainfed cultivation (Yousefi and Safari, 2022).

Salinity is a chief ecological stressor that considerably reduces plant production. High salt levels

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in the plant disrupt the absorption and transport of nutrients and elevate reactive oxygen species (ROS), resulting in disrupting physiological, molecular, and biochemical operations (Balasubramaniam et al., 2023). Salt-induced oxidative stress activates signaling pathways involved in metabolic reprogramming. Both primary and secondary metabolites undergo significant changes in response to ion toxicity and salt-mediated oxidative and osmotic stress (Leschevin et al., 2021). The accumulations of ROSs in the cell destabilize protein-pigment complexes, activates chlorophyllase enzyme activity, and destroys chloroplast membrane (Zhao et al., 2019a), which consequently causes a decrease in photosynthetic activities and overall plant performance (Dong et al., 2017). Selenium (Se) is critical for regulating intracellular signaling and participates in the structure of selenoproteins. Although trace amounts of Se are essential, even low doses can be toxic to plants. Agricultural nanotechnology offers advanced policies for improving crop production in stress-sensitive species (Nawaz et al., 2024). The efficacy of Nano selenium on stress tolerance, photosynthesis, and plant performance has been proven (Chauhan et al., 2024; Khan et al., 2023; Kang et al., 2022).

Zhao et al. (2019b) proved that SeNPs inhibit chlorophyll degradation, restore chloroplast structure, and enhance chlorophyll biosynthesis and glucose metabolism under stressful conditions. The effects of SeNPs on essential oil content and its constituents have received relatively little attention. In a few past studies,

SeNp has pointedly improved EO efficiency (Babashpour-Asl et al., 2022; Zhang et al., 2023), which demonstrates the capability of SeNp as a valuable tool for enhancing EO biosynthesis as well as improving plant tolerance to saline conditions. The effects of SeNPs on EO production and chemical, photosynthesis, and growth of forest savory cultivated under salt stress have not yet been surveyed. Therefore, we experimented to study these aspects.

## 2. Materials and methods

### 2.1. Experimental design

The research was conducted in a greenhouse factorial experiment (CRD,  $r=3$ , 2019) at the Agricultural and Natural Resources Research and Education Center, Kermanshah, Iran (15.34°N 34.34°E 47.04°E, at an altitude of 320 meters above sea level). Forest savory (*S. mutica*) plants were subjected to NaCl (0, 50, 100, and 150 mM) (Factor A) and SeNp (0 and 50 mg L<sup>-1</sup>) (Factor B).

### 2.2. Seed cultivation

Seeds were prepared from RIFR, Iran, sown in a bed consisting of an equal ratio of coco peat and moss in a greenhouse. Plants were kept under 24±1°C and watered with a sprinkler system during experiment. At the six to eight-leaf stages, seedlings were transferred to an equal mixture of manure, sand, and field soil and irrigated every three days (250 ml) (Alagoz et al., 2022). Table 1 summarizes the characteristics of the culture medium and experimental conditions.

**Table 1. Culture bed and experimental conditions**

Soil texture	Soil electric conductivity (dS m <sup>-1</sup> )	Culture bed weight (kg)	Pots dimensions (cm)	Soil pH	Organic carbon (%)	Total phosphor (ppm)	Total nitrogen (%)	Photoperiod (h d <sup>-1</sup> )	Relative humidity (%)	Light intensity (mMOL m <sup>2</sup> S)
Clay-loam	0.70	4.5	17×22	7.03	175	138	0.28	17	50-60	300

### 2.3. Preparation and application of treatments

The 50 mg L<sup>-1</sup> SeNp was prepared from a 1000 ppm stock solution. Table 2 and Fig. 1 provide the specifications of the SeNp stock solution. The seedlings were treated with eight combined treatments, including 0, 50, 100, and 150 mM NaCl without or with 50 mg L<sup>-1</sup> SeNp (Nawaz et al., 2024). NaCl treatments were applied through irrigation and SeNp treatments as foliar spray. After four rounds of irrigation with NaCl treatments (12 days), all plants were irrigated with distilled water to remove excess salt. Selenium

nanoparticle treatments were initiated one week after the start of NaCl treatments. Plants receiving SeNp were sprayed with 100 ml of a 50 mg L<sup>-1</sup> SeNp solution once a week for eight weeks.

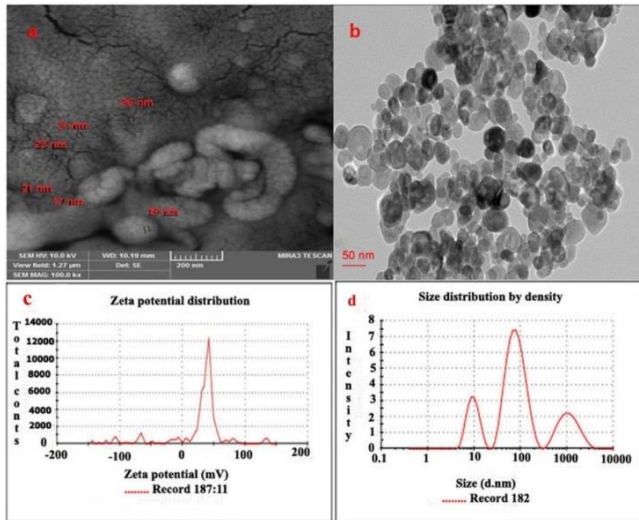
### 2.4. Measurements

#### 2.4.1. Growth traits

Plants were harvested, and both shoot fresh weight (g) and shoot dry weight were recorded. The aerial parts of plant were dried at room temperature and stored for essential oil (EO) extraction.

**Table 2. Specification of SeNp stock solution used in the experiment**

Company	Factory stock cod	SeNp stock concentration (mg L <sup>-1</sup> )	Cas number	Particle size (nm)	Particle density (g cm <sup>3</sup> )	Specific surface area (m <sup>2</sup> g <sup>-1</sup> )	Z-average (d. nm)	Zeta potential (mV)	Particle shape
Nanosany, Mashhad, Iran	Np-S-01	1000	7782-49-2	10-45	3.98	30-50	42.14	-7.98	Almost spherical



**Figure 1.** Scanning electron (a) and transmission electron (b) microscopy, zeta potential (c) and size distribution by density (d) of the SeNp stock solution used in the experiment

#### 2.4.2. Photosynthetic parameters

After dark adaptation of leaves (for 30 minutes), Fv/Fm was assessed at 695 nm by a chlorophyll fluorimeter (Hansatech Pocket PEA, UK) (Stefanov et al., 2024). SPAD was determined using a SPAD-502Plus chlorophyll meter (Minolta, Japan). The concentrations of carotenoid and chlorophyll (mg g<sup>-1</sup> FW) were evaluated by the method of Lichtenthaler and Wellburn (1985) using a BioTek microplate reader (USA) and Equations 1 and 2.

$$(1) \quad Chl \ t \ (mg \ g^{-1} \ FW) = 5.24 (A_{664.2}) + 22.24 (A_{648.6})$$

$$(2) \quad \frac{Carotenoid \ (mg \ g^{-1} \ FW)}{= \frac{[1000 (A_{470}) - 2.13 (chl \ a) - 97.64 (chl \ b)]}{209}}$$

#### 2.4.3. Essential oil

Essential oils (EOs) were drawn out with a Clevenger apparatus, following British Pharmacopoeia (1993), then were dehydrated using anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), and weighed. The EO samples were stored in aluminum foil-covered containers at 4 °C in a refrigerator for subsequent GC and GC/MS analysis. The EO percentage and Yield (W/W) were calculated using Equations 3 and 4, as described by Khademi Doozakhdarreh et al. (2022).

$$(3) \quad EO \ (%) = \left[ \frac{EO \ weight \ (g)}{sample \ dry \ weight \ (g)} \right] \times 100$$

$$(4) \quad EO \ yield \ /plant \ (g) = EO\% \times \ shoot \ dry \ weight \ (g)$$

#### 2.4.4. GC and GC/MS analysis

A Thermo-UFM gas chromatograph and a Varian 3400 GC/MS were used to identify the EO chemical. The Thermo-UFM was equipped with a Chrom-Card A/D data processor; a nonpolar capillary column Ph-5 coated with 5% dimethylphenylsiloxane was used as the stationary phase. The Varian 3400 GC/MS was equipped with a Saturn II mass spectrometer and a telephoto ion system and a DB-5 semipolar column (Yousefi and Safari, 2022). The chemicals were identified according to the method of Adams (2017).

#### 2.5. Statistical analysis

The data were analyzed using IBM SPSS Statistics 26 and Minitab (ver. 16). The graphs were plotted using Minitab software (ver. 16) and Microsoft Excel (2010).

### 3. Results and discussion

#### 3.1. Results of ANOVA

Results ANOVA (Table 3) revealed significant differences for the interaction of NaCl and SeNp for EO percentage, EO yield/plant, growth, and photosynthetic parameters at the level of 1%. Regarding EO chemicals, the main effects of NaCl or SeNp treatments were significant (p≤0.05); however, their interaction was not significant.

#### 3.2. Plant growth and photosynthetic characters

Increasing NaCl concentrations caused a significant decline in both shoot fresh and dry weight (Fig. 2a). The highest shoot fresh weight (19.92 g) was recorded in the 50 mM NaCl + 50 mg L<sup>-1</sup> SeNp treatment group (Fig. 2a). In contrast, the utmost shoot dry weight (7.93 g) was found in the non-treated plants (Fig. 2a). Foliar application of 50 mg L<sup>-1</sup> SeNp pointedly improved shoot fresh weight, with an average increase of 51.8% in NaCl-treated plants (Fig. 2a). Similarly, SeNp application enhanced shoot dry weight by 34.38% in plants subjected to 50 mM NaCl (Fig. 2a).

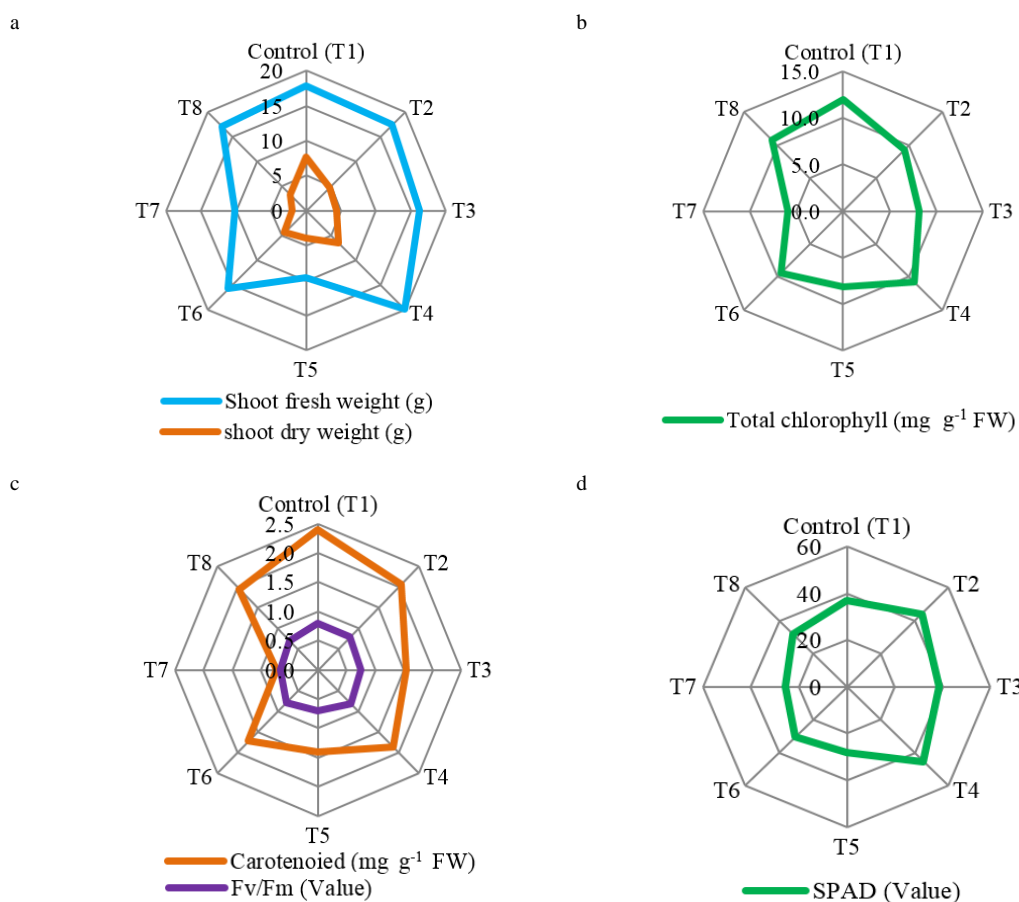
**Table 3. The results of ANOVA for growth, EO, and photosynthetic parameters in *Satureja mutica* treated with NaCl × SeNp.**

S. O. V.	d.f.	Shoot fresh weight	Shoot dry weight	EO Percent	EO yield plant <sup>-1</sup>	Thymol	Carvacrol
NaCl	3	44.86**	14.52**	3.55**	0.03**	192.4**	408.8**
SeNp	1	101.81**	0.43 <sup>ns</sup>	3.65**	0.01**	333.9**	31.65*
NaCl × SeNp	3	16.31**	7.52**	0.53**	0.01**	17.24 <sup>ns</sup>	18.55 <sup>ns</sup>
Error	6	2.41	0.97	0.07	0.001	34.67	4.82
CV (%)		10.00	21.32	11.46	27.22	12.83	18.47

S. O. V.	d.f.	y-terpinene	$\rho$ -cymene	Total chlorophyll	Carotenoid	SPAD	Fv/Fm
NaCl	3	24.69**	8.20 <sup>ns</sup>	13.09**	0.95**	298.3**	0.014**
SeNp	1	35.75**	94.37**	5.29**	0.30**	161.6**	0.014**
NaCl × SeNp	3	0.83 <sup>ns</sup>	4.38 <sup>ns</sup>	10.55**	0.72**	66.5**	0.001**
Error	6	3.24	3.145	0.006	0.01	3.97	0.0001
CV (%)		9.62	15.24	0.60	0.60	5.67	1.33

Ns= non-significant, \* and \*\* respectively significant at 0.05 and 0.01 levels.



**Figure 2. a: Shoot fresh and dry weight, b: total chlorophyll content, c: carotenoid and Fv/Fm value, and d: SPAD value. Treatments sharing similar letters are not meaningfully different. Values are presented as means  $\pm$  SDs ( $P < 0.05$ ). T1: Control; T2: 0 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T3: 50 mM NaCl; T4: 50 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T5: 100 mM NaCl; T6: 100 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T7: 150 mM NaCl; and T8: 150 mM NaCl + 50 mg L<sup>-1</sup> SeNp**

Increasing NaCl concentrations caused significant decreases in chlorophyll and carotenoid content, and Fv/Fm values (Fig. 2b and 2c). Furthermore, the high NaCl levels meaningfully condensed the SPAD value (Fig. 2d). Application of 50 mg L<sup>-1</sup> SeNp knowingly boosted the photosynthetic parameters in salt-treated plants: SeNp increased total chlorophyll content,

carotenoid content, and chlorophyll index (SPAD value) by 45.53, 68.18, and 8.93%, respectively, in response to 150 mM NaCl (Fig. 2b-2d). Additionally, SeNp application improved the Fv/Fm value by an average of 5.48% in NaCl-treated plants (Fig. 2c). Morpho-physiological and photosynthetic parameters are useful indicators for monitoring salinity stress. We

found that SeNp significantly enhances plant growth, total chlorophyll and carotenoid contents; Fv/Fm and SPAD values, in response to NaCl-induced saline stress. These conclusions line up with the results of Ghanbari et al. (2023), Shahraki et al. (2022), and Zhu et al. (2022). Kang et al. (2022) observed that exogenous SeNp application improved photosynthetic metabolites such as fructose, glucose, and various organic acids in melon cultivars.

### 3.3. Essential oil percentage and yield

Increasing NaCl concentrations significantly reduced EO yield and percentage (Fig. 3a and 3b). The interaction between Nano-selenium and NaCl was remarkable for these traits ( $P < 0.01$ ) (Table 4). The peak EO (3.61%) was detected by application of 0 mM NaCl + 50 mg L<sup>-1</sup> SeNp (T2) (Fig. 3a). The maximum EO yield/plant (0.23 g plant<sup>-1</sup>) was achieved under 50 mM NaCl + 50 mg L<sup>-1</sup>

SeNp (T4) (Fig. 3b). Essential oil percentage and EO yield/plant lesser by on average (40.63 and 55.55%, respectively) across the NaCl treatments (Fig. 3a and 3b). Foliar application of 50 mg L<sup>-1</sup> SeNp significantly enhanced these traits in plants treated with low and moderate NaCl levels. While stresses generally stimulated EO biosynthesis in oil-bearing species (Assaf et al., 2022), the results are not always this way (Neffati and Marzouk, 2010). This disparity is likely influenced by stress intensity, plant morphology, ecophysiology, and biochemical responses to stress (Stevovic et al., 2012). We found that low salinity stress increases oil production, while high salinity levels decrease it. These findings are consistent with those of Sarmoum et al. (2019), who reported that high salinity diminishes EO biosynthesis through disruption of normal metabolic processes, photosynthesis and growth, and changes in nodular growth.

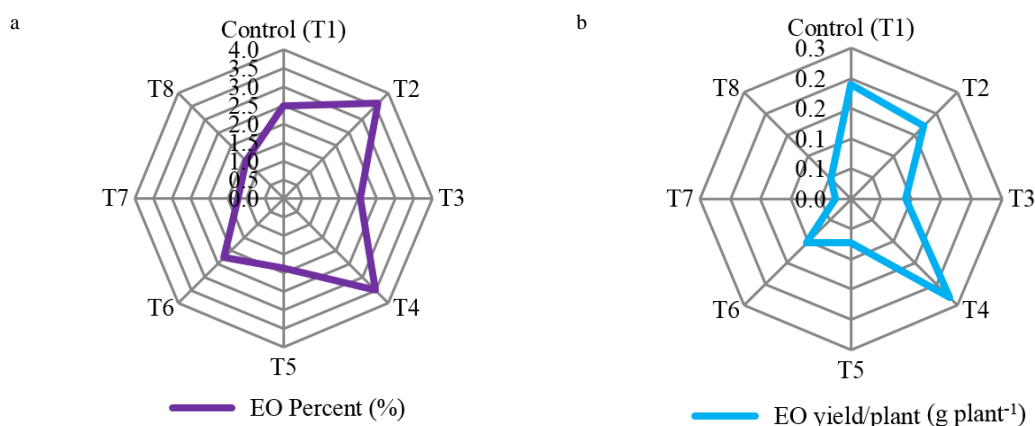


Figure 3. a: EO content (%) and b: EO yield/plant (g). Similar letters are not meaningfully different. Values are presented as means  $\pm$  SDs ( $P < 0.05$ ). EO: essential oil. T1: Control; T2: 0 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T3: 50 mM NaCl; T4: 50 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T5: 100 mM NaCl; T6: 100 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T7: 150 mM NaCl; and T8: 150 mM NaCl + 50 mg L<sup>-1</sup> SeNp

### 3.4. Essential oil compounds

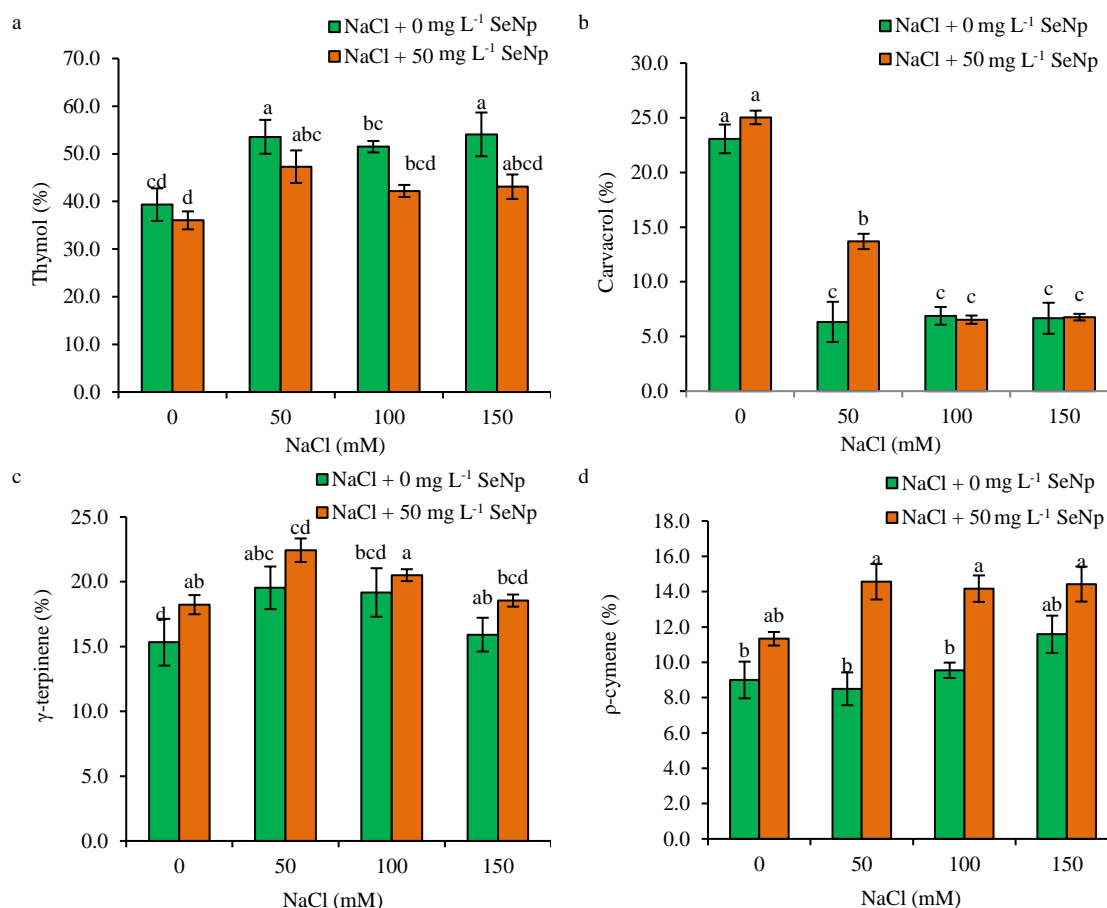
Nine monoterpene chemicals were identified in the essential oil (Table 4). NaCl and SeNp treatments solely caused significant effects on thymol,  $\gamma$ -terpinene, and carvacrol (Table 4). NaCl treatments significantly decreased carvacrol percentage. Conversely, they increased thymol percentage, with the peak value (54.08%) in the 50 mM (Fig. 4a and 4b). NaCl concentrations up to 100 mM increased  $\gamma$ -terpinene percentage; however, 150 mM NaCl decreased it (Fig. 4c). The peak  $\gamma$ -terpinene value (22.41%) was found by application of 50 mM NaCl + 50 mg L<sup>-1</sup> SeNp (Fig. 4c). Foliar application of 50 mg L<sup>-1</sup> SeNp had the following significant effects: Carvacrol increased by 21.43% by application of 50

mM NaCl (Fig. 4b). The  $p$ -cymene content was enhanced by an average of 40.99% across NaCl-treated plants (Fig. 4d). These results demonstrate that both NaCl and SeNp treatments can influence the chemicals of *S. mutica*. In one of the savory species, NaCl reduced carvacrol while increasing other major essential oil compounds (Zaremanesh et al., 2021), which confirms the present results in forest savory. Salinity has also caused an increase in thymol in the *Plectranthus amboinicus* (Sany et al., 2020), which is in line with our results. Samynathan et al. (2023) believe that stressful factors induce production of secondary metabolites, while the application of stress-modifier agents drives the plant more towards primary metabolism.

**Table 4. The EO chemical identified in *S. mutica* treated with NaCl × SeNp**

Chemical compounds	Classification	RT	RI	CAS number	Formula	Range (%)
Carvacrol	Phenolic monoterpenoids	4.07	1289.96	000499-5-2	C <sub>10</sub> H <sub>14</sub> O	6.71-24.05
Thymol		3.96	1281.22	000089-83-8	C <sub>10</sub> H <sub>14</sub> O	37.68-50.43
α-pinene	Bicyclic monoterpenes	0.87	931.13	000080-56-8	C <sub>10</sub> H <sub>16</sub>	0.22-1.35
β-pinene		0.87	931.13	000127-91-3	C <sub>10</sub> H <sub>16</sub>	1.02-2.18
Trans-caryophyllene		5.33	1376.02	000087-44-5	C <sub>15</sub> H <sub>24</sub>	0.86-1.41
Camphene		1.16	953.1	000565-00-4	C <sub>10</sub> H <sub>16</sub>	0.05-1.06
γ-terpinene	Isometric monoterpenes	1.63	1051.59	000099-85-4	C <sub>10</sub> H <sub>16</sub>	16.78-20.98
α-terpinene		1.63	1051.21	000099-35-3	C <sub>10</sub> H <sub>16</sub>	1.09-2.47
ρ-cymene	Monocyclic monoterpenes	1.36	1014.15	000099-87-6	C <sub>10</sub> H <sub>14</sub>	10.17-13.01

RI: Retention index, RT: Retention time



**Figure 4. a: thymol, b: carvacrol, c: γ-terpinene, and d: ρ-cymene content. Similar letters are not meaningfully different. Values are presented as means ± SDs (P<0.05).**

As noted by Khan et al. (2014), salt stress causes ionic imbalances in the root environment, which affects nutrient absorption and disrupts enzyme activities involved in EO biosynthesis (Seif Sahandi et al., 2019). For instance, nitrogen availability has been shown to promote terpenoid synthesis (Nejatzadeh-Barandozi, 2014) while limiting sesquiterpene formation (Sany et al., 2020). The variations in EO chemical composition under salt stress are influenced by the intensity of stress and the plant's genetic factors, as advocated by Tsusaka et al. (2019). SeNp application significantly enhanced EO percentage and yield in NaCl-treated plants, which is similar to the results obtained by Ghanbari et al.

(2023) and Nazari et al. (2022). SeNp caused a noteworthy upgrading in the carvacrol and ρ-cymene contents in response to NaCl; however, it did not have significant effects on thymol or γ-terpinene levels. Previous research supports the confident role of SeNp in enhancing secondary metabolite levels (Zhang et al., 2023; Nazari et al., 2022). The specific role of SeNp in biosynthesis of terpenoid compounds is not studied. Zhang et al. (2023) and Al-Deriny et al. (2020) proved that SeNp can stimulate invention of plant defense metabolites and nutrient absorption by activating methyl jasmonate (JA) and salicylic acid (SA) signaling pathways. Specifically, SeNp regulates

phenylalanine ammonia lyase and isochorismate synthase, resulting induces salicylic acid and terpenoids (Fan et al., 2022; Shine et al., 2016). Zhang et al. (2023) and Es-sbihi et al. (2020) believe that SeNp influence the production of thymol, carvacrol and other phenolic compounds by modulating endogenous SA levels. To better know the correlation of key chemicals of *S. mutica* in present study, the counter diagrams between the thymol and carvacrol with the gamma-terpinene and para-cymene were drawn. Fig. 5 depicting the relationship between carvacrol,  $\gamma$ -terpinene, and thymol provides valuable insights into the interactive effects of these compounds on thymol accumulation. Thymol and carvacrol are biosynthesized in two parallel and separate pathways from the precursor gamma-terpinene (Fig. 6). These results demonstrate that thymol content is strongly influenced by both carvacrol and  $\gamma$ -terpinene levels, with a notable synergistic effect observed at specific concentration ranges. Maximum thymol levels (>54%) were achieved when carvacrol concentrations were relatively low (10–15%) and  $\gamma$ -terpinene was maintained at intermediate levels (approximately 18–20%). This suggests that while  $\gamma$ -terpinene plays a

promotive role in thymol and carvacrol biosynthesis, the concentration of either of these two products (thymol or carvacrol) also affects the biosynthesis of the other due to substrate competition (Abbasi et al., 2024; Krause et al., 2021). Interestingly, when carvacrol concentrations increased beyond 20%, thymol levels declined sharply, regardless of  $\gamma$ -terpinene concentration.

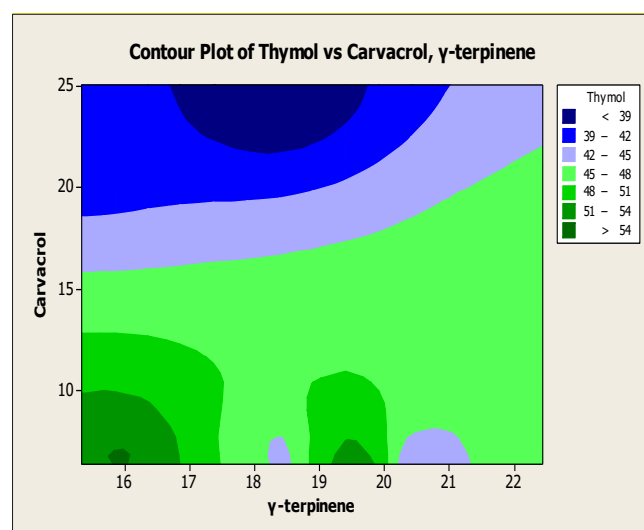


Figure 5. Counter diagram: relationship between thymol, carvacrol, and gamma-terpinene in the *S. mutica* essential oil

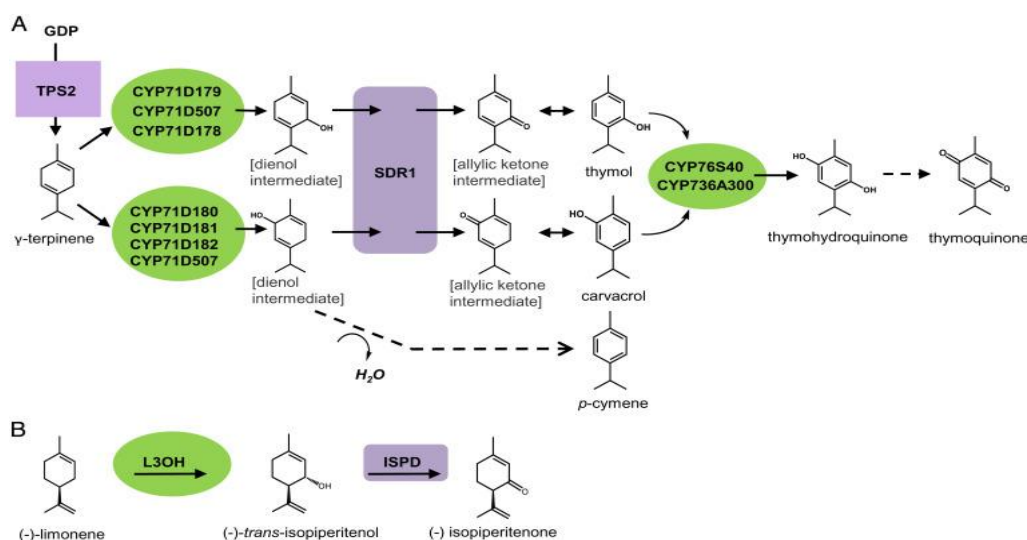


Figure 6. The schematic diagram of the pathway for the biosynthesis of thymol and carvacrol from gamma-terpinene (enzymes and genes involved) in Lamiaceae plants (Krause et al., 2021).

Fig. 7 showed thymol levels are strongly influenced by the combined concentrations of carvacrol and  $\rho$ -cymene, with distinct zones of high and low accumulation. Maximum thymol concentrations (>54%) were observed when carvacrol levels were relatively low (approximately 10–15%) and  $\rho$ -cymene concentrations ranged between 11 and 13%, as indicated by the light green zones. This advises that

increased  $\rho$ -cymene and carvacrol contents are associated with decreased thymol biosynthesis. Interestingly, increasing carvacrol levels beyond 20% led to a sharp reduction in thymol levels across all  $\rho$ -cymene concentrations, reflected by the dominance of blue and purple zones (<42%). Similarly, both low (<10%) and high (>14%) concentrations of  $\rho$ -cymene were associated with reduced thymol accumulation. *P*-

cymene is first produced from  $\gamma$ -terpinene and then catalyzed to thymol and carvacrol (Fig. 6).

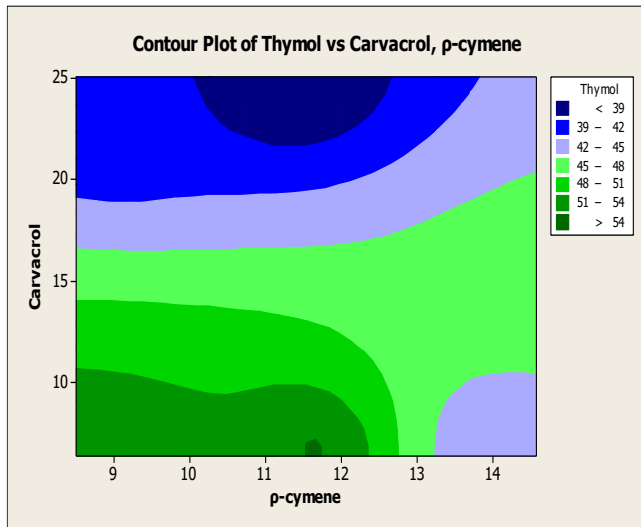


Figure 7. Contour diagram: relationship between thymol, carvacrol, and Para-cymene in the *S. mutica* essential oil

### 3.5. Principal component analysis

PCA (Fig. 8) accounted for 78% of the variance between treatments; the first component elucidated 61% and the second component 17% of variance. PC1 (61%) was positively associated with shoot dry weight, chlorophyll content, carotenoid content, and carvacrol percentage, but inversely correlated with thymol content, following a Q1 trend. PC2 (17%) correlated positively with shoot fresh weight, Fv/Fm, SPAD,  $\gamma$ -terpinene, and  $\rho$ -cymene (Q4).

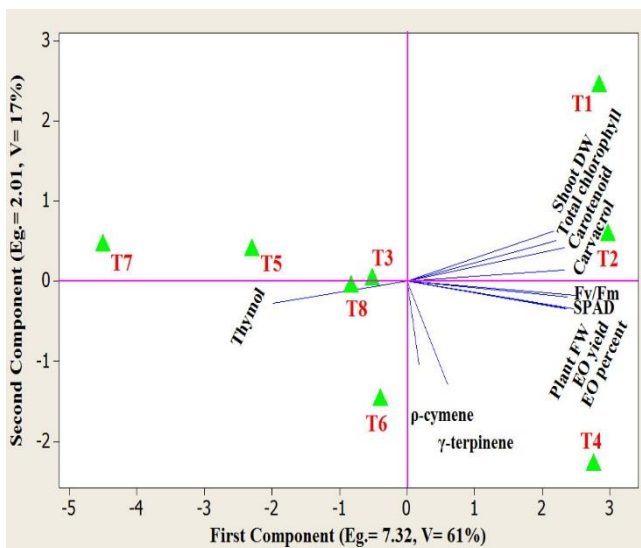


Figure 8. Diagram of biplot for growth, photosynthetic, and essential oil characters of *S. mutica* in response to different NaCl  $\times$  SeNp levels. T1: control; T2: 0 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T3: 50 mM NaCl; T4: 50 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T5: 100 mM NaCl; T6: 100 mM NaCl + 50 mg L<sup>-1</sup> SeNp; T7: 150 mM NaCl; and T8: 150 mM NaCl + 50 mg L<sup>-1</sup> SeNp. DW: dry weight, EO: essential oil

Results showed that growth, photosynthetic traits and EO production were associated with SeNp application, so that 50 mg L<sup>-1</sup> SeNp increased plant fresh and dry weight, total chlorophyll, carotenoid, Fv/Fm value, SPAD value, and EO percent and yield. Similar to the present findings, a PCA analysis has shown that 40 and 50 mg L<sup>-1</sup> SeNP increase the total chlorophyll content in sesame, as 40 mg L<sup>-1</sup> has augmented it by 61-94.84% in different varieties compared to control (Ahmad et al., 2024). Nawaz et al. (2024) suggest that 50 mg L<sup>-1</sup> SeNP has meaningfully amended growth and photosynthesis in lime seedlings in response to 200 mM NaCl. Ahmad et al. (2024) confirmed that 40 mg L<sup>-1</sup> SeNP significantly enhanced oil percent in *Sesamum indicum*. Babashpour-Asl et al. (2022) said that 40 and 60 mg L<sup>-1</sup> SeNPs have significantly boosted the EO content of coriander in response to Cd stress.

The thymol content exhibited a moderate increase under low NaCl concentration, whereas a marked enhancement was observed at higher salinity levels (Fig. 8). In experiment of Wang et al. (2020), the accumulation of phenolic compounds, gallic acid, protocatechuic, fisetin, myricetin and quercetin in barley plants occurred under NaCl levels. Hawrylak-Nowak et al. (2021) mentioned that 50 and 100 mM NaCl hinder the growth of *Melissa officinalis*, while concurrently enhancing the phenolic compounds.

Carvacrol content was improved with 50 mg L<sup>-1</sup> SeNp in control and low-NaCl treatments (T1 and T2). Similarly,  $\gamma$ -terpinene and  $\rho$ -cymene levels increased with 50 mg L<sup>-1</sup> SeNp across both control and NaCl-treated plants. However, SeNp application consistently reduced thymol content, aligning with the Q1 trend. In conclusion, the PCA highlights the distinct effects of NaCl and SeNp treatments. While SeNp enhanced carvacrol,  $\gamma$ -terpinene, and  $\rho$ -cymene levels, it simultaneously suppressed thymol content in both control and NaCl-treated plants. Babashpour-Asl et al. (2022) stated that the 40 mg L<sup>-1</sup> SeNp improved the n-decanol percent in coriander essential oil by 28.7% in response to cadmium stress, while decreasing the 2E-decanol content. However, selenium nanoparticles had no significant effect on n-nonane. Nanoparticles – mediated increase of essential oil production associated with the role of nanoparticles in improving root growth, increasing nutrient absorption, and photosynthesis, which leads to an increase in precursors and

intermediates involved in essential oil biosynthesis (Asghari et al., 2023). The influence of SeNp on the EO chemicals has not been well studied; therefore, further studies are recommended.

#### 4. Conclusion

SeNp 50 mg L<sup>-1</sup> lightened the contrary effects of NaCl-induced stress on growth traits and photosynthetic performance in *S. mutica* plants. Moreover, the application of SeNp boosted essential oil and some monoterpenes production in response to NaCl. Hence, we recommend 50 mg L<sup>-1</sup> SeNp as an effective treatment for the reduction of hostile possessions of salinity and amplification of EO production in *S. mutica*. Also, applying other concentrations of SeNp in future studies is recommended to achieve more effective results and optimize the appropriate formulation of Nano-selenium.

#### Abbreviation

C: Control; Car: carotenoid; Chl.: Chlorophyll; DW: dry weight; FW: fresh weight; TI: Retention index; ROS: reactive oxygen species; RT: Retention temperature; SeNp: Nano-selenium; EO: Essential oil; SA: Salicylic acid; FW: Fresh weight; SPAD: Soil Plant Analysis Development; Fv/Fm: Maximum quantum efficiency of photosystem II; ROS: Reactive oxygen species

#### Conflict of interests

The authors declared no potential conflicts of interest regarding the research, authorship and publication of this article.

#### Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

#### Consent for publications

The authors approved the final version of the manuscript for publication.

#### Availability of data and material

All data from this study are devoted to this manuscript.

#### Authors' contributions

The authors collaboratively conducted the research and jointly prepared the manuscript.

#### Informed consent

The authors declare not to use any patients in this research.

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