Journal of Medicinal Plants and By-Products (2024) 4: 971– 981
https://doi.org/10.22034/jmpb.2023.363478.1603

Original Article

Nano-selenium Reduced the Adverse Effects of Salinity Stress on *Satureja spicigera* (C. Koch) Boiss.

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Article History

Received: 09 September 2023
Accepted: 10 October 2023

Keywords
Antioxidant activity
Nanoparticle
Photosynthesis
Salt stress
Savory

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ABSTRACT

Creeping savory [*Satureja spicigera* (C. Koch) Boiss.)] is a wild edible and medicinal plant that is use in food industry and preparation of herbal drugs. To investigate the effects of Nano-selenium on photosynthetic pigments, antioxidant enzymes, osmolytes, and relative water contents in *S. spicigera* under NaCl stress conditions, a factorial experiment was conducted at the greenhouse of the Kermanshah Research Center of Agricultural and Natural Resources, Iran, based on a Completely Randomized Design (CRD). The experiment was conducted in 2019 and each experimental unit had 3 replications in one treatment. Factor A included four levels of NaCl (0-50-100-150 mM NaCl) and Factor B included two levels of Nano-selenium (control and 50 PPM). The results showed that there are significant differences between the different NaCl treatments for all studied traits ($P< 0.01$). Also significant differences were observed between Nse treatments for SOD enzymatic activity and RWC ($P< 0.01$) and for protein content, Chl b and enzymatic activities of POD and CAT ($P< 0.01$). The interaction effect of salinity × NSe was significant for all studied traits except Chl b. Increasing salinity levels caused a significant reduction in relative water content, chlorophyll a, b, total chlorophyll, and carotenoid content. Salinity drastically enhanced the antioxidant activities of superoxide dismutase, peroxidase, and catalase. Also, salinity increased cell proline content. Foliar application of NSe (50 PPM) decreased chlorophyll b, proline content, and antioxidant activity of super oxide dismutase but improved antioxidant activities of peroxidase and catalase and enhanced chlorophyll an and carotenoid content under salt stress conditions. Also, NSe decreased protein content in moderate salt stress conditions (50 to 100 mM NaCl) but increased it under sever salinity stress (150 mM NaCl). NSe reduced the destructive effects of salinity on physiological and biochemical characteristics in creeping savory. 50 PPm Nse, has negative effects on the Chl a, carotenoid, total chlorophyll, soluble proteins, and RWC at the low NaCl stress conditions but it improved these traits in high NaCl stress conditions. Appropriate concentrations of Nano-selenium promote plant growth and help plant resistance to stresses, but high concentrations may induce oxidative stress and reduce plant growth.

INTRODUCTION

Satureja Spicigera is a procumbent perennial plant that grows in eroded banks of the rivers and rocky places at 20-1500 m altitude [1]. This species is a medicinal oil-bearing plant, and its important essential oil compounds are thymol and carvacrol. It grows in the north and north-west of Iran, and is somewhat drought tolerant [2]. This wild plant has recently attracted the attention of Iranian herbalists for domestication and cultivation.

Salinity stress is an essential factor in limiting plant production. Salinity stress outstandingly declines the crop's growth by reducing water absorption and accumulation of reactive oxygen species (ROS) [3]. Recently, nanoparticles of essential trace elements have been increasingly considered in agriculture as growth stimulators increasing adaptive potential of crops [4]. Applying diverse NPs and nanomaterials (NMs) effectively improved crop plant nutrition compared to standard fertilizers [5]. Applying nanoparticles (NPs) alters ROS-dependent signaling pathways, which control plant growth by increasing antioxidant activity and chloroplast function [6]. Selenium (Se) is an essential micronutrient that protects plants against various abiotic stresses as a dose-dependent antioxidant or stimulant [7].

Although Se in high doses is toxic, its low doses have many positive effects on plant growth and development [8].

Nano selenium (NSe or SeNP), is very mobile in the plant, is more bioactive, and is less toxic than selenium [9, 10]. NSe improves plant tolerance to abiotic stress [11]. NSe reduces adverse salinity effects on plants by osmotic regulation, inducing the antioxidant defense system, and enhancing photosynthetic efficiency [7, 12]. NSe, under salinity stress conditions, has increased superoxide dismutase (SOD) and peroxidase (POD) activities in Strawberry [10] and Capsicum annum [13], proline (Pro) concentration and relative water content (RWC) in Lemon verbena [14], and *Momordica charantia* [15], and *Triticum asetivum* [16]. Also, NSe has improved yield of *Cyamopsis tetragonoloba* under salt stress conditions [17], and has increased the soluble protein (SP) content in sorghum plants under high-temperature stress [18].

So far, there has been no report on the application of NSe under salinity stress in the *S. spicigera* plants. Therefore, we investigated the effect of NSe on *S. spicigera* under salt stress conditions

MATERIALS AND METHODS

Plant Materials

In this experiment, we studied *S. spicigera* (C. Koch) Boiss. [Flora Orientalis, 4, 566 (1879)].

Experimental Design and Treatments

A greenhouse factorial experiment based on a Completely Randomized Design (CRD), with four levels of NaCl (0-50-100-150 mM), two levels of NSe (0 and 50 PPM), and three replications was carried out in a controlled environment (greenhouse) at the research center of agricultural and natural resources (47°, 04" E; 34°, 15" N), Kermanshah, Iran, in 2019.

Seeds Cultivation

The seeds of the creeping savory were obtained from the Research Institute of Forests and Ranges of Iran (RIFR). Seeds were disinfected with 0.5% sodium hypochlorite, washed with distilled water, and dried with blotting paper. Seeds planted in a tray, in a mixed soft bed of coco peat and peat moss (1:1). Seeds were watered by sprinkling method every day during germination. The seedlings were watered every two days until reaching the 6-leaf stage. The unique healthy seedlings, with similar size (6 to 8 leaves plants), were transferred to the plastic pots (one seedling per pot). The pots filled with a 1:1:1 mixture of farm soil, sand, and rotten cow manure (soil weight = 4.5 kg, P^H = 7.03, soil texture= clay-loam, EC= 0.70 dS/m, P= 138 ppm, O.C. = 1.75% and Total N= 0.28%). The plants were kept under 17 h/d light photoperiod by 1100 lux of light intensity, seven hours of darkness, a relative humidity of 50-60%, and 24±1-18±1 ºC [19]. The plants were irrigated two weeks (once every three days) with 2500 ml of farm well water before the implementation of salt treatments.

Preparation of NaCl treatments

Merck NaCl (CAS #: 7647-14-5, EC Number: 231-598-3, Molar Mass: 58.44 g/mol) was used to prepare NaCl treatments after modifying purity. The concentrations of 0, 50, 100, and 150 mM NaCl (2.2, 6.5, 9.1, and 13 dS/m) were prepared by adding double distilled water (DDW).

Preparation of NSe Treatments

In this study, the stock solution (Stok#Np-S-01) of NSe 1000 mg/liter (1000 PPM) prepared from Nanosany company, Mashhad, Iran (Cas number: 7782-49-2). The nanoparticle size was 45-10 nm, the specific surface area was 30-50 m^2/g, its particle density was 3.98 g/cm^3, and it was almost spherical. The images of NSe Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Zeta potential distribution diagram were presented in Figures 1 to 3. The degree of purity of the solution was 99.95%, and the pH of the solution was 3-5. We prepared the 50 ppm NSe solution from this colloidal stock solution [20]. NSe solution was covered by aluminum foil, and kept in the refrigerator (4 ºC).

Implementation of Treatments

Eight treatments consisting of irrigation (250 ml to each pot, once every three days, twelve times) by 0, 50, 100, and 150 mM NaCl concentrations [20] and two foliar spraying with 0 and 50 PPM NSe were implemented [21]. Foliar spraying was done once every seven days (8 times) with 100 ml of 50 PPM NSe twelve days after the start of salinity treatments [22]. The Non-NSe-treated plants were sprayed with 100 ml DDW. In order to adapt the plants to salinity and to avoid osmotic shock, in two steps (one week), the pots (except plants treated with 0 mM NaCl that were irrigated by DDW) were irrigated with 20 mM NaCl (250 ml) and then salt treatments

were performed. Salt treatments were performed every three days and NSe treatments were performed every seven days for eight weeks.

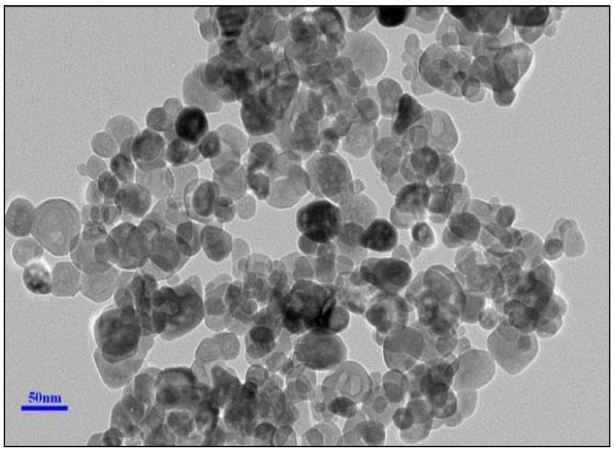


Fig. 1 The image of transmission electron microscopy (TEM) of NSe stock solution

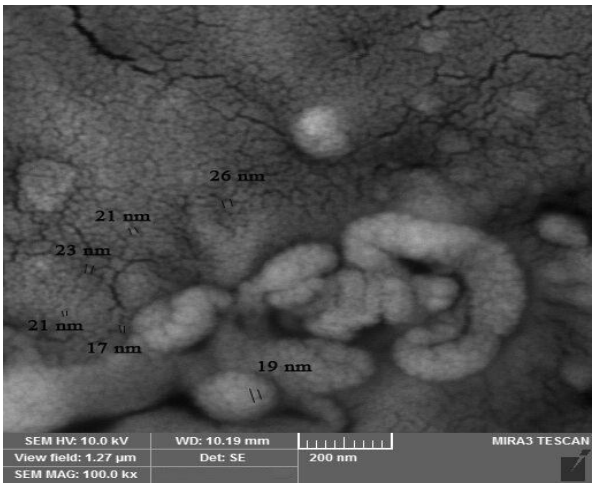


Fig. 2 The image of scanning electron microscopy (SEM) of NSe stock solution

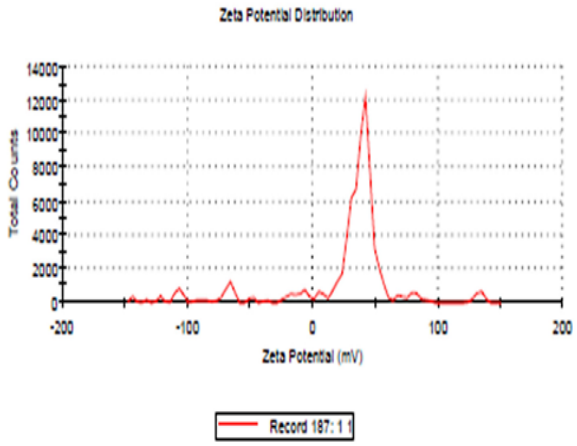


Fig. 3 Diagram of Zeta potential distribution of NSe stock solution

The plants were watered once, after every 4 NaCl treatments (12 days), with distilled water to remove the accumulated salts in the pots.

Studied Traits

Some morphological, physiological, photosynthetic, and biochemical traits were studied include: leaf fresh weight (LFW), leaf dry weight (LDW), relative water content (RWC), Proline content (Pro), soluble protein content, chlorophyll (Chl) a, Chl b, total chlorophyll (Chl t), carotenoid (Car) content, and the enzymatic activities of super oxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

Collection of Samples and Measurement of Traits

In order to the measurement of photosynthetic pigments, Pro, protein, and the activity of antioxidant enzymes, the healthy and active leaves of the plant separated, and, after freezing in liquid nitrogen, they were stored at -20 °C.

Measurement of Relative Water Content

To determine the RWC (%), the 30 young leaves were selected from each plant, separated, and immediately weighed (LFW) in the laboratory with a weighing scale (Sartorius BP210D, Germany; 0.0001 g); then they were placed in DDW 16 to 18 hours (for complete dehydration) in the 22 °C. The leaf samples were dried with filter paper and the samples were reweighed (LTW). The leaves were placed in an oven at 70 °C for 48 hours, and the LDW was measured. The means of LFW and LDW were calculated (g). RWC of the leaf calculated from the following formula [23]. The means of LFW and LDW were calculated (g).

RWC (%) = (FW–DW) / (TW–DW) 100

FW: fresh weight, DW: dry weight, TW: turgor weight

Measurement of Chlorophyll and Carotenoid Contents

Chlorophyll a, Chl b, and carotenoid content were measured by the standard method of Lichtenthaler and Welburn [24]. The 25 mg of fresh leaves were powdered in a Chinese mortar with liquid nitrogen and then wholly homogenized with 2 ml of 96% ethanol in the dark condition. Samples were shaken well and centrifuged for 10 minutes (10000 rpm, 4 °C). Supernatant was transferred into the micro tubes and its optical absorption was read by a Bio Tek Power-Wave (XS2) micro plate spectrophotometer, USA device at 663, 646, and 470 nm. Amounts of chlorophyll a, chlorophyll b,

total chlorophyll, and carotenoids content (mg g^{-1} FW) were calculated by following formulas:

Chl a = 12.21 ($A_{664.2}$) – 2.81 ($A_{646.8}$); Chl b = 20.13 ($A_{646.8}$) – 5.1 ($A_{664.2}$); Chl T = Chl a + Chl b

Car = (1000 A_{470} – 2.13 [Chl a] – 97.64 [Chl b]/227

Preparation of Extraction Buffer

We prepared the extraction buffer (200 ml) according to the methods of Ramachandra *et al*. [23]. The 2.428 g of Tris with 0.2 g PVP dissolved well in 40 ml of DDW (pH= 8) and final volume reached 200 ml. The container of solution covered with aluminum foil and it was kept in the refrigerator (4 °C).

Preparation of Crude Leaf Extract

Based on the methods of Ramachandra *et al*. [25], leaf samples were crushed entirely in liquid nitrogen. The 250 mg of crushed leaves were transferred in a 2 ml micro tube, and then 1 ml of extraction buffer was added. Samples were mixed by vortex (twice, 30 seconds, in 2 hour intervals, whereas the samples were kept in the refrigerator between each step), then the samples were kept in the refrigerator for 12 hours and again were mixed (30 seconds). Mixtures were centrifuged (15 min., 4°C, and 13,000 rpm), and then the supernatant phase was separated and kept at -20 °C.

Measurement of Enzymatic Activity

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 (µ mole min-1 mg soluble protein) [26]. The samples were transferred to the Bio Tek Power-Wave XS2 micro plate spectrophotometer, USA, after the completion of the reactions, and its optical absorbance was read at 560 nm wavelength (enzymatic unit equivalent to 50% inhibition) by Bio Tek Gen 5 software. The rate of enzymatic activity was calculated using the following formula:

$$SOD\,(\mu mo\,\mathrm{lg}^{-1}\,FW) = \frac{100 - \left[\frac{(OD_{cont} - OD_{sample})}{OD_{cont}} \times 100\right]}{50}$$

OD_{cont}: absorbance of control at 560 nm

OD_{sample}: absorbance of samples at 560 nm

Enzymatic activity of peroxidase (POD; E.C. 1.11.1.7) was measured based on Maehly and Chance with modifications [27]. The optical absorbance of the extracts was read for 15 min at 30 s intervals at a wavelength of 470 nm by Bio Tek Gen 5 software in a Bio Tek Power-Wave XS2 micro plate spectrophotometer, USA. Rate of POD enzymatic activity was calculated using the Beer-Lambert law (0.0266 Mcm^{-1}) and was expressed in terms of H_2O_2 consumption (µ mole min^{-1} mg of soluble protein).

We measured the enzymatic activity of catalase (CAT; E.C. 1.11.1.6) by method of Sinha with some modifications [28]. OD of the samples was read by Bio Tek Power-Wave XS2 micro plate spectrophotometer, USA, at 570 nm after completion of the reactions. The rate of CAT enzymatic activity (H_2O_2 consumption) was calculated using the Beer-Lambert law (0.0394 Mcm^{-1} extinction coefficient).

Measurement of Soluble Proteins

Soluble protein concentration (mg/g FW) was measured based on the method of Bradford [29]. We added the 1 µl of the crude leaf extract in 200 µl of Coomassie Brilliant Blue. After fifteen minutes, The OD of samples was read at 595 nm by Bio Tek Gen 5 software in a Bio Tek Power-Wave XS2 micro plate spectrophotometer, USA. Soluble protein concentration was obtained according to the absorption of the samples and using standard curve of the Bovine Serum Albumin (BSA).

Estimation of free Proline Content

Proline content was measured based on Bates *et al.* [30]. The ODs of samples and proline standard were read at 520 nm by Bio Tek Gen 5 software in a Bio Tek Power-Wave XS2 micro plate spectrophotometer, USA. Then the OD of each sample was put into the standard equation and was reported as µmol/g FW.

Statistical Analysis

Analysis of variance (factorial) and mean comparison were performed using IBM SPSS Statistics 26 software. The means (±SD) were compared using Duncan's Test ($p< 0.05$). The charts were drawn using Excel software.

RESULTS

Results of ANOVA

Significant differences were observed between different NaCl treatments for the chlorophyll a, b, carotenoid, Total chlorophyll, soluble protein, RWC, and the activity of SOD, POD, and CAT (P<

0.01). Also significant differences were observed between Nse treatments for SOD enzymatic activity and RWC (P< 0.01) and for protein content, Chl b and enzymatic activities of POD and CAT (P< 0.05). The interaction effect of salinity × NSe (Table 1) was significant for proline content, soluble protein, RWC, and the enzymatic activities of SOS, POD, and CAT (P< 0.01) and for the Chl a, carotenoid, and total Chl (P< 0.01).

Table 1 Analysis of variance of photosynthetic pigments, proline content, protein content, relative water content, leaf fresh weight, leaf dry weight, and SOD, POD, and CAT activities in *S. spicigera* under different NaCl and NSe treatments

S. V.	df	Chl a	Chl b	Car	Chl t	Proline	
Salt	3	34.99 **	1.70 **	2.82 **	51.50 **	0.07 **	
NSe	1	0.07 ns	0.70 *	0.09 ns	1.23 ns	0.01 ns	
Salt×NSe	3	2.52 *	0.18 ns	0.24 *	3.20 *	0.04 **	
Error	6	0.28	0.08	0.05	0.58	0.02	
CV (%)		6.07	8.52	8.78	5.68	17.74	
S. V.	df	Soluble protein		RWC	Enzymatic activity		
					SOD	POD	CAT
Salt	3	238400.0 **		114.3 **	5.79 **	16.94 **	7.39 **
NSe	1	90770.0 *		1255.31 **	1.61 **	10.19 *	2.99 *
Salt×NSe	3	75700.0 **		334.30 **	0.36 **	7.72 **	0.64 **
Error	6	1.22		16.5	0.02	0.1	0.013
CV (%)		0.17		4.79	7.38	12.54	2.95

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

RESULTS

Chlorophyll a

By increasing the intensity of salinity stress, the amount of chlorophyll a decreased. NSe decreased chlorophyll a by 13.72% and 41.41% respectively in the 0 and 50 mM NaCl treatments but increased it by 10.67% and 16.05%, respectively in the 100 and 150 mM NaCl treatments compared to Non-NSe-treated plants (Fig. 1A).

Chlorophyll b

Salinity stress decreased the amounts of chlorophyll b. The highest amount of chlorophyll b (4.28 mg/g FW) was observed in the salt control (distilled water). The lowest chlorophyll b (2.62 mg/g FW) was observed in the NaCl 150 mM + NSe 50 ppm. NSe decreased the amounts of chlorophyll b by 15.89, 17.46, and 3.32% in salinity conditions of 0, 100, and 150 mM NaCl, respectively, but it did not affect the amounts of chlorophyll b in the 50 mM NaCl treatment compared to Non-NSe-treated plants (Fig. 1B).

Carotenoid

The highest level of carotenoid (3.58 mg/g FW) was observed in the salt control (distilled water). The lowest carotenoid (1.68 mg/g FW) observed in the 150 mM NaCl. NSe decreased the amounts of carotenoids by 7.82 and 5.32% in 0 and 50 mM NaCl, respectively, but increased the amount of carotenoids by 20.60 and 30.95% in the treatments of 100 and 150 mM NaCl, respectively compared to Non-NSe-treated plants (Fig. 1C).

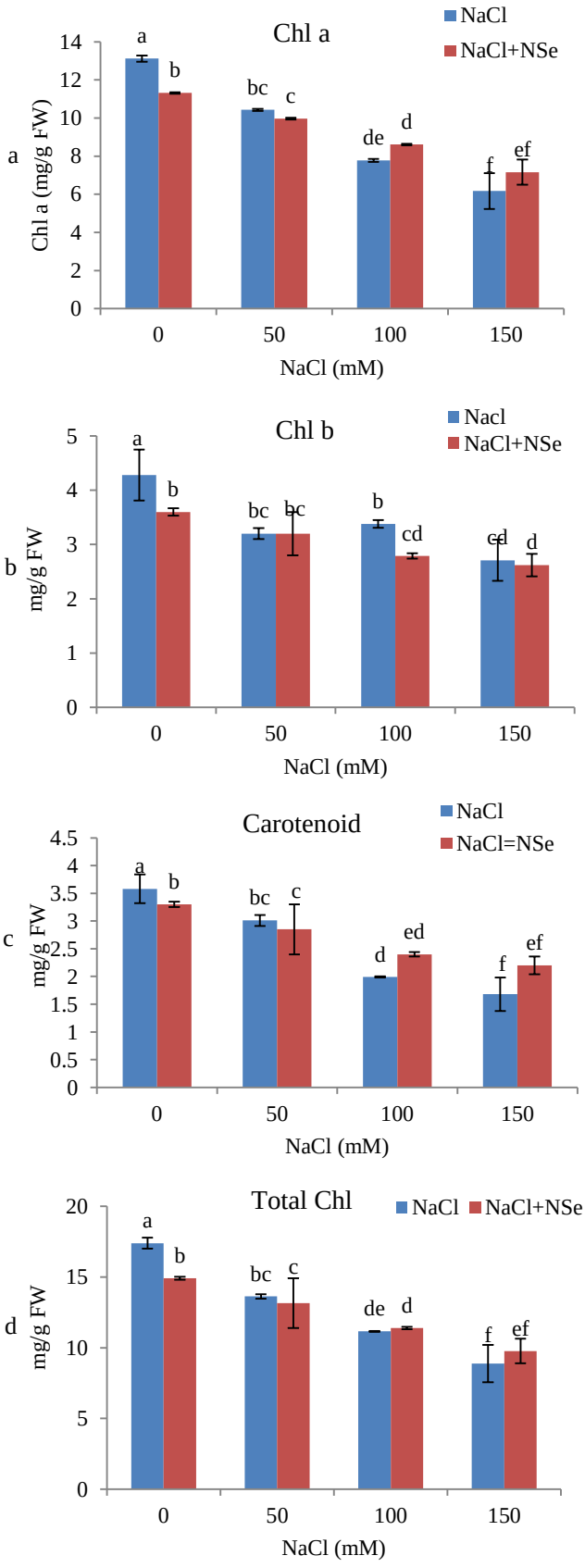
Total Chlorophyll

The highest amount of total chlorophyll (17.40 mg/g FW0 was observed in the salt control treatment and its lowest amount (8.88 mg/g FW) was observed at 150 mM NaCl. Application of 50 PPM NSe were decreased total chlorophyll by 25.14 and 3.45% in the 0 and 50 mM NaCl treatments, respectively compared to the Non-NSe-treated plants. Nano-selenium increased total chlorophyll by 2.15 and 10.02% in the 100 and 150 mM NaCl treatments, respectively compared to the Non-NSe-treated plants (Fig 1D).

Proline Content

Increasing the salinity levels caused an increase in the proline content. The highest amount of proline (12.02 µmol/g FW) was observed at 150 mM salinity stress condition. The lowest proline content (2.34 µmol/g FW) was observed at 0 mM NaCl treatment. Nano-selenium increased the proline content (260.9%) in the control salt treatment, while it decreased the proline contents by 52.30 and 26.88%, respectively in the 50 and 100 mM NaCl concentrations compared to the Non-NSe-treated

plants. Nano-selenium had no significant effect on proline content in the 150 mM salinity treatment (Fig. 1E).



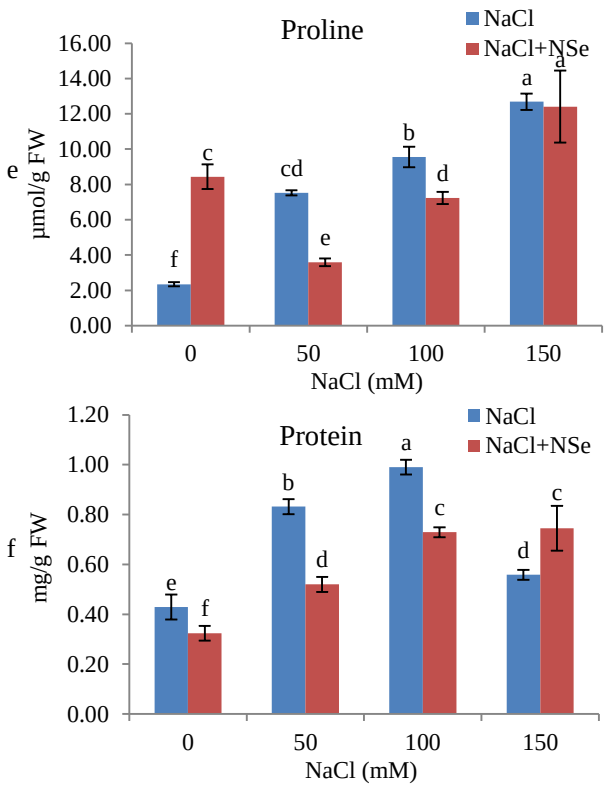


Fig. 1 The effect of NSe on Chl a (a), Chl b (b), carotenoid (c), total chlorophyll (d), proline content (e), and soluble protein content (f) in *S. spicigera* plants under different salinity treatments.
Means of common followed by similar letters indicate no significant differences using DMRT (α=0.05).

Soluble Protein

The amounts of leaf-soluble protein were increased in all salinity treatments compared to the salt control. The highest amount of leaf soluble protein (0.99 mg/g FW) observed in 100 mM salt treatment. The lowest soluble protein (0.32 mg/g FW) observed in the treatment of 0 mM NaCl+ 50 ppm NSe. NSe decreased the amounts of soluble protein by 24.55, 37.54, and 26.39% in the plants treated by 0, 50, and 100 mM NaCl, respectively compared to the Non-NSe-treated plants but increased it (33.49%) in the 150 mM NaCl treatment compared to the Non-NSe-treated plants (Fig. 1F)

RWC

RWC decreased in all NaCl treatments compared to the control. The highest relative water content (91.70%) observed in the treatment of 0 mM NaCl (control). The lowest RWC (66.08%) observed in the 150 mM NaCl. NSe reduced the RWC by 65.38% in control NaCl plants but it increased RWC by 15.18, 35.52, and 39.47%, respectively in the 50,

100, and 150 mM NaCl treatments compared to the Non-NSe-treated plants (Fig. 2A).

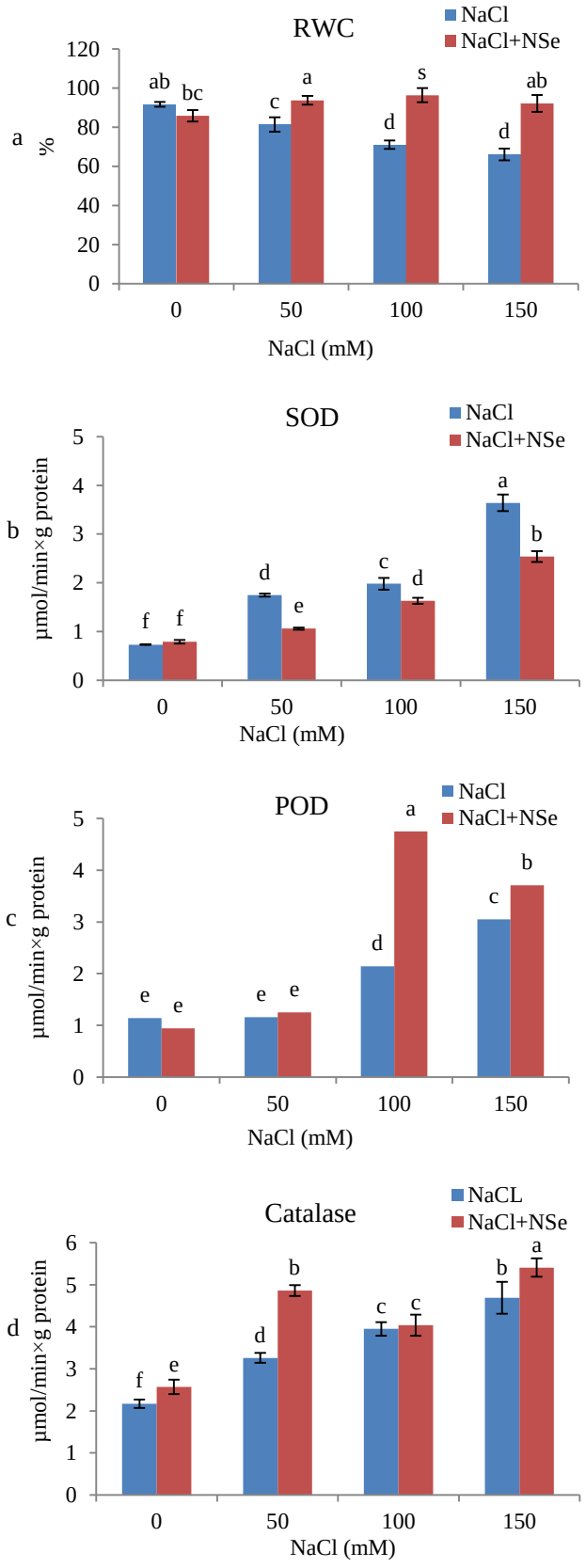


Fig. 2 The effect of NSe on leaf relative water content (a) and the enzymatic activities of super oxide dismutase (b), peroxidase (c), and Catalase (d) in *S. spicigera* plants under different salinity treatments.

Means of common followed by similar letters indicate no significant differences using DMRT (α=0.05).

SOD Enzymatic Activity

By increasing salinity, the activity of superoxide dismutase increased. The highest activity of SOD (3.64 µmol/min× mg protein) observed in 150 mM salt treatment, and the lowest activity of SOD (0.73 µmol/min× mg protein) observed in 0 mM NaCl treatment. Foliar spraying with 50 PPM NSe caused an increase of SOD activity (8.22%) in control NaCl treatment. Of course, the SOD activity decreased in NSe-treated plants by 39.43, 17.68, and 30.22%, respectively in the treatments of 50, 100, and 150 mM NaCl compared to the Non-NSe-treated plants (Fig. 2B).

POD Enzymatic Activity

Peroxidase activity was increased in all NaCl treatments compared to the control. The highest activity of POD (6.80 µmol/min× mg protein) observed in the treatment of 100 mM NaCl + 50 PPM NSe, and the lowest POD activity (0.94 µmol/min× mg protein) was observed in the 0 mM NaCl + 50 PPM NSe. Foliar spraying with 50 PPM NSe reduced POD activity (17.54%) in the control NaCl plants (0 mM). However, NSe improved the POD activities by 7.76, 217.76, and 21.64% respectively, in the 50, 100, and 150 mM NaCl treatments compared to the Non-NSe-treated plants (Fig. 2C).

Catalase Enzymatic Activity

Increasing salinity caused an increase in catalase activity. The highest activity of catalase (5.41 µmol/min× mg protein) observed in the treatment of 150 mM NaCl+50 PPM NSe, and the lowest activity (2.17 µmol/min× mg protein) observed in the treatment of 0 mM NaCl. NSe improved the CAT enzymatic activities by 18.43, 49.08, 2.28, and 15.35%, respectively in the treatments of 0, 50, 100, and 150 mM NaCl compared to Non-NSe-treated plants (Fig. 2D).

DISCUSSION

Chlorophyll concentration, under salt stress conditions, is an index of plant tolerance to salinity. In the present study, the increase in salinity caused a significant decrease in Chl a, Chl b, and total Chl. Similar to our finding, salinity has caused a significant decrease in photosynthetic pigments in *S.*

hortensis, *S. khuzestanica*, *Lantana camara*, *Linum usitatissimum*, and *Nigella sativa* L. [31-35].

NSe 50 PPM caused a significant decrease in chlorophyll a and Chl t in control and 50 mM NaCl treatments but increased them in 100 and 150 mM NaCl treatments. NSe decreased Chl b in the control and salinity treatments. Nanoparticles, depending on their type, particle size, and concentration, have different and sometimes opposite effects on the plant, so that they may cause a decrease or increase in photosynthetic pigments and as result, decrease or increase the rate of photosynthesis [36]. The harmful effects of nanoparticles on photosynthesis are due to their accumulation in chloroplast and destruction of the photosynthesis apparatus [37, 38]. In several reports, the positive effects of NSe on photosynthetic pigments under severe stress have been presented. Consistent with our results, NSe significantly increased Chl a and total Chl in *Lemon verbena* plants and *Brassica chinensis* under metal stress [14, 39]. Nse increased the chlorophyll a in *Solanum lycopersicum* L. compared to the control, but it did not effects on the chlorophyll b in this plant [40].

Carotenoids are essential in reducing oxidative stress and regulating ROS cellular homeostasis in plants [41]. In this study, the increase in salinity caused a decrease in the carotenoid content. Similar to our results, carotenoid content was decreased under salinity stress in *capsicum annuum*, *Nigella sativa* L., and *S. hortensis* [21,35,42]. In the present study, NSe increased carotenoid content in high salinity conditions. In line with our findings, NSe significantly increased photosynthetic pigments in *Lemon verbena* plants under salt stress [14]. Also, bulk selenium has increased the carotenoid content in *Allium sativum* plants [43].

In our study, the proline content was augmented significantly by increasing salinity stress. Similar to our results, the proline content was increased significantly by salinity levels in *S. hortensis*, *S. khuzestanica*, and *Thymus vulgaris* [31, 44, 45].

Nano-selenium increased the proline content in the NaCl control treatment, while decreased it in the 50 and 100 mM NaCl. NSe had no significant effect on the proline content in the 150 mM NaCl treatment. NSe has different effects on proline in plants depending on the concentration, environmental conditions, and tolerance of plant species to selenium. Unlike to our results, Nano-selenium increased proline concentration in *Lemon verbena* and bitter melon under salt stress conditions [14, 15].

By increasing salinity up to 100 mM, the soluble protein increased significantly, but in the treatment of 150 mM NaCl, it showed a decreasing trend. Similar to our results, the protein content increased at low NaCl treatments and declined at severe NaCl stress conditions in *Thymus vulgaris* [45].

NSe decreased the content of leaf soluble protein in the treatments of 0, 50, and 100 mM NaCl but increased it in more severe salinity stress conditions (150 mM NaCl). In line with our results, NSe increased total protein under severe salt stress in *Lemon verbena* [14] and under high-temperature stress in sorghum plants [18]. These results are consistent with our findings.

RWC is a valuable trait for investigating plant water status. In our study, the RWC decreased significantly with increasing salinity intensity. Similar to our results, RWC has decreased under different salt concentrations in some medicinal plants or crops such as *Lemon verbena* and *Oryza sativa* [14, 37]. Application of NSe caused a significant increase in the relative water content in the salt stress conditions. Like our results, NSe considerably increased the RWC in *Lemon verbena* and bitter melon under salinity stress conditions [14, 15].

The increased antioxidant activity enables plants to resist potential oxidative damage caused by NaCl salinity [46]. In the present research, increasing the NaCl up to 150 mM significantly enhanced the activity of the SOD, POD, and CAT. In line with our finding, NaCl has increased the activities of SOD, POD, and CAT in many medicinal and agricultural plants, such as *S. khuzestanica* [32]. Also, NaCl stress has enhanced POD and, or CAT activities in some plants, such as *Capsicum annuum* [21].

Nano selenium had no significant effect on the activity of superoxide dismutase in the 0 mM NaCl (control), but it decreased the activity of the SOD in salt stress conditions. Similar to our results, the application of NSe reduced SOD activity in *Ocimum basilicum* under salinity stress [47] but contrary to our results, NSe was increased SOD activity in Strawberry plants [10]. In our study, NSe had no significant effect on peroxidase activity in Non-NaCl-treated plants, but it increased the peroxidase

activity in salt stress conditions. Like our result, NSe increased peroxidase activity in Strawberry [10] and *Capsicum annum* under salt stress conditions [13].

In the present study, NSe increased catalase activity under salt stress conditions. In line with our results, NSe increased catalase activity in *Capsicum annum* under salt stress conditions [14]. Some studies have reported the ability of NSe to alleviate salt stress by increasing antioxidant activity in different plants, such as *Lemon verbena* and bitter melon [14, 15]. These reports confirm the validity of our findings.

Depends on the type of nanoparticle, particle concentration and size, and plant species, NPs may reduce or induce oxidative stress in plants [38]. Therefore, different results in antioxidant enzymatic activities are documented across different plant species as typical responses to NPs. In our study, Nano-selenium (50 PPM) has negative effect on Chl a, carotenoid, total chlorophyll, soluble proteins, and RWC in the salt control plants and plants treated with low NaCl concentrations (low salt stress conditions), but in the high NaCl concentrations, it improved these traits. Nano-selenium has a dual effect (beneficial/adverse) on the plants depending on the particle size and the ratio of surface area to volume [9, 18]. Application of NSe in the right time and appropriate concentrations promote plant growth and help plant resistance to stresses, but high concentrations may induce or intensify oxidative stress and reduce plant growth.

CONCLUSION

Salt stress declined leaf RWC, photosynthesis, and subsequently, growth in *S. spicigera*. Salt stress enhanced leaf proline and protein content and antioxidant activity. The exogenous application of 50 PPM NSe increased RWC and antioxidant activity under salt stress conditions and subsequently, reduced salt-mediated-oxidative damage. In the creeping savory plants, the 50 PPM NSe increased ROS levels at chloroplasts, declined photosynthetic pigments, and reduced photosynthesis in the low and non-salt-threated plants, but improved photosynthesis and growth in the high-salt-treated-plants.

HIGHLIGHTS

- This article is the first report on the effect of Nano selenium on the *S. spicigera* plants under salt stress conditions
- *S. spicigera* plants can tolerate up to 100 mM salinity
- 100 and 150 mM NaCl significantly induced oxidative stress in *S. spicigera* plants
- 100 and 150 mM NaCl significantly reduced photosynthesis and plant growth in *S. spicigera* plants
- Foliar application of 50 PPM NSe reduced proline content and SOD activity but increased POD and CAT activities of *S. spicigera* plants under salt stress conditions

Conflict of Interests

The authors declared that there is no conflict of interest.

Acknowledgment

We would like to definite our thanks to the colleagues in the tissue culture laboratory of Kermanshah Agriculture and Natural Resources Research and Education Center and the laboratory of the Faculty of Agriculture and Natural Resources of Razi University, who cooperated in the implementation of this research.

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