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The Effect of Nano-Selenium Dioxide on Flowering Performance and Biochemical Traits of *Echinacea purpurea* L. under Salinity Stress

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Abstract

Echinacea purpurea L., a valuable ornamental and medicinal plant, is widely used in landscape design and therapeutic gardens; however, its growth and physiological performance are adversely affected by salinity stress. This study evaluated the potential of selenium nanoparticles (Se NPs) to mitigate salinity-induced damage in *E. purpurea* using a factorial experiment arranged in a completely randomized design with three salinity levels (0, 50, and 100 mM NaCl) and three Se NP concentrations (0, 50, and 100 mg/L). Salinity stress significantly reduced morphophysiological traits, including leaf number, lateral branches, flower number, flower head diameter, peduncle length, flower longevity, and aerial fresh weight, as well as photosynthetic pigments (chlorophyll a, b, and carotenoids) and anthocyanin content. However, Se NP application, particularly at 100 mg/L, effectively counteracted these detrimental effects, enhancing vegetative and reproductive growth. Antioxidant enzyme activities (catalase and superoxide dismutase) were highest under severe salinity (100 mM NaCl) combined with 100 mg/L Se NPs, indicating their role in stress amelioration. The findings demonstrate that Se NPs can serve as a promising strategy to improve *E. purpurea*'s resilience to salinity stress, optimizing its ornamental and medicinal value in saline-affected environments.

Keywords: Antioxidants, Floral, Morphometrics, Nano-Selenium, Oxidative.

INTRODUCTION

Echinacea purpurea L., belonging to the Asteraceae family, is one of the most important medicinal-ornamental plants with significant pharmacological properties (Hashempoor *et al.*, 2022). The flowers of this plant exhibit a variety of colors, adding a unique and natural charm to gardens and parks. Due to its high resistance to adverse environmental conditions, such as drought and cold, *Echinacea* is an ideal choice for urban green spaces and regions with diverse climates. By attracting pollinators like bees and butterflies, *Echinacea* not only enhances the visual appeal of the environment but also plays a crucial role in maintaining biodiversity and local ecosystems. The flowers of *Echinacea* have a long lifespan, retaining their beauty for extended periods (Burlou-Nagy *et al.*, 2022). The roots and underground stems of this plant have been widely used to treat trauma and alleviate symptoms of infection and inflammation (Ghutke *et al*., 2023). Key components of the plant include caffeic acid derivatives, alkamides, flavonoids, essential oils, and polyacetylenes. Among these, caffeic acid derivatives and alkamides have immune-boosting and regulatory effects (Hashempour *et al*., 2023). Caffeic acid derivatives, alkamides, and polysaccharides exhibit high antioxidant activity, inhibiting lipoprotein oxidation (Dobrange *et al.*, 2019).

Stress arises from abnormal physiological processes caused by one or more environmental factors (Atta *et al.*, 2023). In other words, stress occurs when an organism is exposed to an environmental factor that reduces its visible quality, yield. An increase in soil salinity to 4 dS/m or higher leads to negative effects on the morphological, physiological, and enzymatic characteristics of most plants (Tabrizi-Dooz *et al.*, 2023).

The response of plants to salinity stress is highly complex and depends on salt concentration, ion types, various environmental factors, and the plant's growth stage. On one hand, osmotic stress under saline conditions causes dehydration in plant tissues, often referred to as physiological drought. On the other hand, ionic toxicity arises from the accumulation of specific ions, particularly sodium, which disrupts metabolic reactions in plants. To cope with mild salinity stress, plants increase the concentration of soluble substances to maintain osmotic pressure (Yildiz *et al.*, 2020).

In most soils, selenium levels range from 0.1 to 2 mg/kg, depending on the geographical region (Shahid *et al*., 2019). In some countries, such as New Zealand, Finland, and parts of China, naturally available selenium in soils is low. Soil selenium content depends on the composition of the parent rock. Regions with soil selenium levels below 0.5 mg/kg are considered selenium-deficient (Jones *et al*., 2017).

Studies on the relationship between soil pH and selenium uptake by plants indicate that selenium uptake increases with higher soil pH. At high pH, selenate (highly mobile) is prevalent, while at low pH, selenite and selenide (less mobile) are found. Selenides are insoluble, and elemental selenium oxidizes to selenate in neutral and alkaline soils and to selenite in acidic soils. Selenate is highly soluble and accessible to plants, accumulating in plants grown in high-pH soils (El-Ramady *et al*., 2016).

In recent decades, the use of nanotechnology and nanomaterials has expanded significantly across various fields, including plant science and agriculture (Abbasi *et al*., 2020a). Nanoparticles (NPs) can be synthesized from synthetic molecules in various sizes and shapes (Zahedi *et al*., 2020). These properties influence the physical and chemical characteristics of the particles and their uptake by plants and animals. The effects of NPs on crop quality and yield depend heavily on particle properties such as size, shape, stability, physical or chemical composition, concentration, and receptors (Kumar *et al*., 2018). Due to their unique physicochemical properties, nanoparticles induce different biological responses compared to bulk materials (Abbasi *et al*., 2020 a, b). The use of NPs appears to be a novel approach among accepted methods for protecting plants against abiotic stresses and mitigating environmental stressors (Zahedi *et al.,* 2020). Ghasemian *et al.* (2021) studied the effects of selenium nanoparticles (0, 50, and 100 mg/L) on the growth and physiological characteristics of *Melissa officinalis* under salinity stress (0, 50, 100, and 150 mM NaCl). Results indicated that selenium nanoparticles enhanced plant growth, with 50 mg/L showing the most significant effect. As salinity increased, plant growth decreased, with the lowest growth observed at 150 mM NaCl. Selenium nanoparticles mitigated the negative effects of stress by reducing lipid peroxidation and increasing antioxidant enzyme activity.

Adeli *et al.* (2024) demonstrated that 1 mM salicylic acid effectively mitigated salinity stress in *Echinacea angustifolia*, reversing the negative impacts of 200 mM NaCl on growth and physiological parameters. While 100 mM salinity combined with 1 mM SA maximized secondary metabolite production, optimal growth and essential oil yield were achieved in non-stressed plants receiving the same SA treatment, highlighting its dual role in both stress protection and productivity enhancement under normal conditions. Sabra *et al.* (2012) investigated the physiological and biochemical responses of three *Echinacea* species (*Echinacea purpurea, Echinacea pallida*, and *Echinacea angustifolia*) under salinity stress (0, 50, 75, and 100 mM NaCl) in hydroponic conditions. Results showed that survival rates ranged from 99% for *E. purpurea* to 70% for *E. angustifolia*. In *E. angustifolia*, stomatal conductance, respiration, and photosynthesis rates decreased at all salinity levels, while in the other two species, significant reductions were observed only at 75 and 100 mM NaCl. Salinity increased sodium and chloride concentrations in roots and shoots, with higher accumulation in *E. angustifolia* compared to the other species.

Given the medicinal value of *Echinacea purpurea* L. and the growing threat of soil salinity in agricultural systems, there is an urgent need to develop strategies for enhancing its stress tolerance. While selenium nanoparticles have shown promise in mitigating abiotic stresses in other crops, it appears that their effects on physiological and biochemical traits of *E. purpurea* under salinity stress have not been comprehensively investigated, particularly regarding their role in enhancing stress tolerance and secondary metabolite production. Therefore, this study aimed to (1) Evaluate the impact of salinity stress on vegetative growth, flowering capacity, and key biochemical markers (e.g., antioxidant enzymes, phenolic compounds) in *E. purpurea,* and (2) Determine whether foliar application of selenium dioxide nanoparticles (SeO_2 NPs) at different concentrations (0, 50, and 100 mg/L) could ameliorate salt-induced damage, providing a potential agronomic solution for saline-affected cultivation areas.

MATERIALS AND METHODS

The present study was conducted as a factorial experiment based on a completely randomized design with three replications and two observations. The aim was to compare the effects of salinity stress with the application of sodium chloride (NaCl) at three levels (0, 50, and 100 mM NaCl) and selenium dioxide nanoparticles (SeO_2 NPs) at three levels (0, 50, and 100 mg/L SeO_2 NPs) on the morphophysiological and phytochemical characteristics of *Echinacea purpurea*. The experiment was carried out in a greenhouse located in Tehran during the spring and summer of 2024. The greenhouse conditions were maintained at a daytime temperature of 27 ± 2 °C, a night temperature of 24 ± 2 °C, and a relative humidity of 60–70%. Natural sunlight served as the light source, with a 12-hour light and 12-hour dark cycle (natural daylight) and an intensity of 8000 lux (no artificial light source was used). The greenhouse was covered with plastic, and shading was achieved using fabric shade nets on the roof and greenhouse cover.

Seeds of *Echinacea purpurea* were purchased from Pakan Bazar Company and sown in seedling trays in the greenhouse in February 2024. After seed germination and initial seedling growth, reaching the four-leaf stage (March 2024), the seedlings were transplanted into pots with a 20 cm diameter containing a cocopeat and perlite growing medium (70:30 ratio) and grown in the greenhouse under controlled environmental conditions. The pots were irrigated every four days with water to reach field capacity, and the plants were fertilized weekly using Hoagland's nutrient solution. After 20 days of controlled conditions, the treatments were applied. Before applying salinity stress treatments, the pots were fertilized twice with NPK (3 g/L) at 7-day intervals. Salinity stress was then induced by irrigating each pot with 50 mL of NaCl solution at three levels every 3 days. To prevent salt accumulation, the growing medium was flushed with regular water after every three saline irrigations. Salinity stress was maintained for 42 days. Foliar application of nano-selenium (0, 50, and 100 mg/L) was carried out for 6 weeks. Plant tissue samples were collected in August 2024 for analysis. Morphological traits were measured in the greenhouse, and the plant parts were washed with distilled water and used for laboratory analyses in the Food Industry Laboratory of Islamic Azad University, Noor Branch.

Traits

Leaf count, number of lateral branches, and number of flowers per plant were recorded for each treatment.

Flower head diameter was measured using a caliper.

Pedicel length was measured using a ruler.

Flower longevity was recorded in days.

Fresh shoot weight was measured by cutting one plant per pot at soil level (crown) and weighing it in the laboratory.

Measurement of plant pigments

To compare plant pigments, 0.5 g of fresh plant material was ground in a porcelain mortar using liquid nitrogen. Twenty ml of 80% acetone was added, and the mixture was centrifuged at 6000 rpm for 10 min. The supernatant was transferred to a glass flask, and absorbance was measured at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids using a Visible/UV-45 Lambda spectrophotometer. Chlorophyll a, b, and carotenoid concentrations were calculated using the following formulas (Arnon, 1967):

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645})\ V / 100\ W$$

$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663})\ V / 100\ W$$

$$\text{Carotenoids} = 100\ (A_{470}) - 3.27\ (\text{mg chl. a}) - 104\ (\text{mg chl. b}) / 227$$

Where:

V = Volume of the filtered solution (supernatant from centrifugation)

A = Absorbance at 663, 645, and 470 nm

W = Fresh weight of the sample in g.

Anthocyanin

0.1 g of fresh petal tissue was ground in a porcelain mortar with 10 mL of acidified methanol (pure methanol and hydrochloric acid at a 99:1 volume ratio). The extract was transferred to a capped test tube and kept in the dark at 25 °C for 24 hours. After centrifugation at 4000 rpm for 10 minutes, the absorbance of the supernatant was measured at 550 nm using a Visible/UV-45 Lambda spectrophotometer. Anthocyanin concentration was calculated using the formula A = εbc, with an extinction coefficient (ε) of 33,000 cm^{-1} mol^{-1}, and results were expressed in µmol/g fresh weight (Nadernejad *et al*., 2013).

Catalase activity

Catalase activity was assessed using the Luck (1974) method with slight modifications. Twenty µL of enzyme extract were mixed with 980 µL of phosphate buffer containing 2 mM hydrogen peroxide. Changes in absorbance at 240 nm were recorded over time using a Visible/UV-45 Lambda spectrophotometer. Enzyme activity was calculated using the Beer-Lambert law with an extinction coefficient of 39.4 Mm^{-1} cm^{-1} and expressed in µmol/g fresh weight per minute (Zavareh *et al*., 2015).

Superoxide dismutase (SOD) activity

SOD activity was measured by its ability to inhibit the photoreduction of nitroblue tetrazolium chloride (NBT). The reaction mixture (3 mL) contained sodium-potassium buffer (50 mM, pH 7.8), methionine (13 mM), NBT (75 µM), EDTA (0.1 mM), riboflavin (360 µM), and 0–50 µL of crude enzyme extract. Absorbance was measured at 560 nm using a Visible/UV-45 Lambda spectrophotometer. One unit of SOD activity was defined as the amount of enzyme necessary to inhibit 50% of NBT photoreduction. Specific activity was reported as units per milligram of protein (Amini, 2014).

Antioxidant activity

Antioxidant activity was measured using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging assay. Methanolic extracts of plant samples were prepared at concentrations ranging from 5×10^{-2} to 5×10^{-6} mg/100 mL in pure methanol. A 1:1 mixture of DPPH solution (8 mg/100 mL) and plant extracts at varying concentrations was prepared. Absorbance was measured at 517 nm after 30 minutes at room temperature using a Visible/UV-45 Lambda spectrophotometer. The percentage of DPPH radical scavenging was calculated using the formula:

$$R\% = (AD - AS) / AD \times 100$$

Where:

R% = Percentage of scavenging

AD = Absorbance of DPPH at 517 nm

AS = Absorbance of the sample at 517 nm

The IC50 value (concentration of extract required to inhibit 50% of free radicals) was used to compare antioxidant activity (Sun *et al*., 2007).

Statistical analysis

After ensuring normal distribution and homogeneity of variance, data were subjected to analysis of variance (ANOVA) in a factorial completely randomized design. Mean comparisons were performed using Duncan's test at a 5% probability level using SAS software (version 9.4). Graphs and tables were prepared using Microsoft Excel and Word 2007.

RESULTS AND DISCUSSION

Morphophysiological traits

The ANOVA results indicated that leaf count, number of lateral branches, number of flowers per plant, flower head diameter, pedicel length, flower longevity, and fresh shoot weight of *Echinacea purpurea* were significantly affected by the main effects of salinity stress and nanoselenium at the 1% probability level. The interaction effects of treatments were significant at the 1% level for leaf count, number of lateral branches, flower head diameter, pedicel length, and flower longevity, and at the 5% level for the number of flowers per plant and fresh shoot weight (Table 1).

Table 1. Analysis of variance results of the effects of salinity stress and selenium nanoparticles on the morphophysiological characteristics of *Echinacea purpurea*.

S.o.V	df	Leave number	Lateral branch number	Flower number	Flower head diameter	Pedicel length	Flower longevity	Fresh weight of the aerial parts
Salinity stress (S)	2	2651.70**	41.44**	16.93**	90.11**	498.93**	224.59**	2368.78**
Nanoparticle (N)	2	477.15**	4.33**	1.04**	5.44**	32.26**	14.93**	349.00**
S ×N	4	47.37**	1.28**	0.37*	1.72**	12.15**	4.31**	11.78*
Error	18	9.96	0.22	0.11	0.37	1.30	0.74	4.00
CV (%)	-	11.04	24.96	29.03	21.91	17.87	20.03	9.28

* and **: Significant at P < 0.05 and P < 0.01 based on the Duncan's test, respectively.

Number of leaves

Based on the comparison of mean values for the interaction effects of salinity stress × selenium nanoparticles, the highest number of leaves per *Echinacea purpurea* plant (50.00) was observed in the control treatment (no salinity stress) with the application of 100 mg/L selenium nanoparticles. This treatment was statistically grouped with the control treatment (no salinity stress) with the application of 50 mg/L selenium nanoparticles. The lowest number of leaves was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 1).

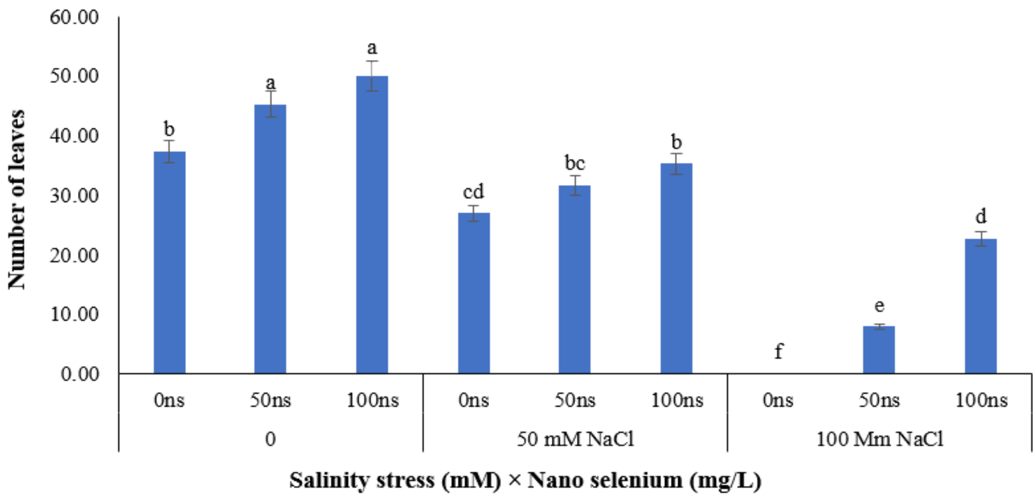

Fig. 1. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the number of leaves in *Echinacea purpurea* L.

Quantitative floral architecture, longevity traits, and branching pattern

Based on the comparison of mean values for the interaction effects of salinity stress × selenium nanoparticles, the highest number of lateral branches, number of flowers per plant, flower head diameter, pedicel length, and flower longevity were observed in the control treatment (no salinity stress) with the application of 100 mg/L selenium nanoparticles. The lowest values for these traits were recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 2 and 3).

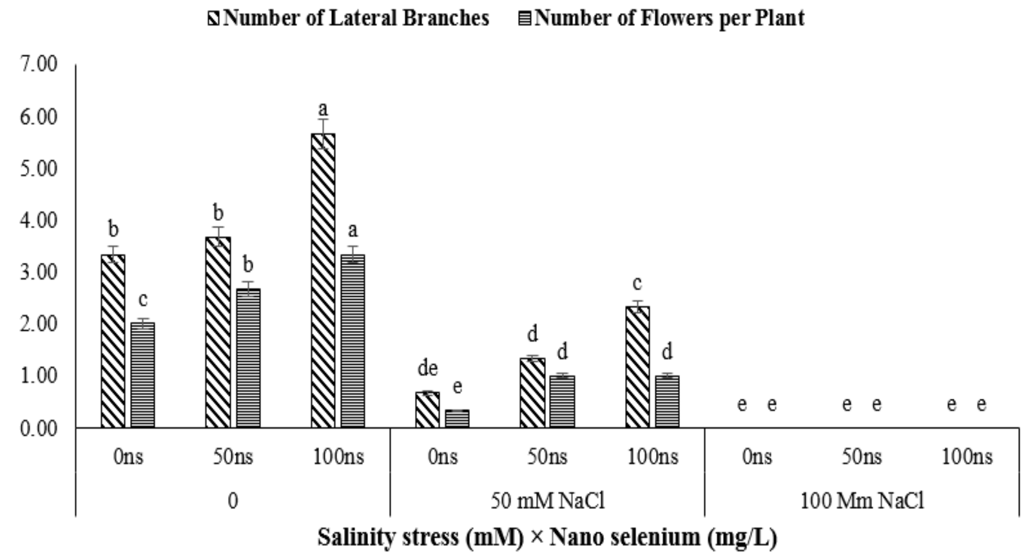

Fig. 2. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the number of lateral branches and number of flowers per plant in *Echinacea purpurea* L.

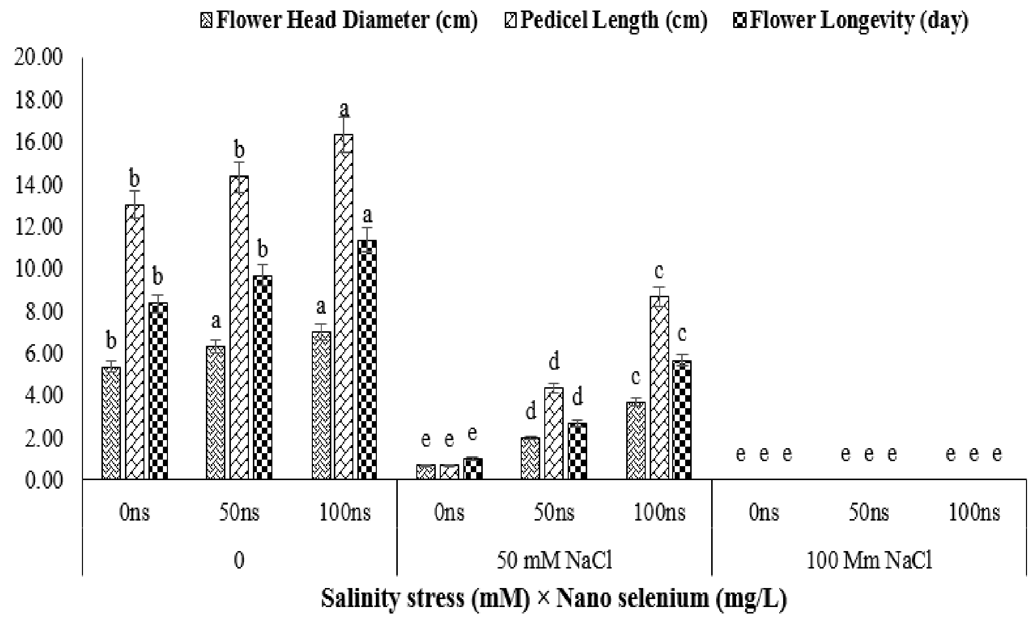

Fig. 3. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the flower head diameter, pedicel length, and flower longevity in *Echinacea purpurea* L.

Increasing salinity stress intensity reduced plant growth, resulting in smaller plant dimensions. Plants subjected to severe salinity stress exhibited the lowest number of leaves, lateral branches, flowers, flower head diameter, pedicel length, and flower longevity. However, foliar application of selenium nanoparticles alleviated the effects of salinity stress, as evidenced by the improved performance of plants under mild and severe salinity stress without nanoparticle application. These findings align with previous studies showing that nanoselenium mitigates abiotic stresses such as salinity in rice (*Oryza sativa*) (Taha *et al.*, 2021), salinity stress in strawberry (*Fragaria × ananassa*) (Soleymanzadeh *et al.*, 2020), heavy metals in radish (*Raphanus sativus*) (Amirabad *et al.*, 2020), drought stress in tomato (*Solanum lycopersicum*) (Rady *et al.*, 2020), and drought stress in canola (*Brassica rapa* subsp. oleifera) (Ahmad *et al.*, 2021).

Fresh shoot weight

Based on the comparison of mean values for the interaction effects of salinity stress × selenium nanoparticles, the highest fresh shoot weight (47 g) was observed in the control treatment (no salinity stress) with the application of 100 mg/L selenium nanoparticles. The lowest fresh shoot weight was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 4).

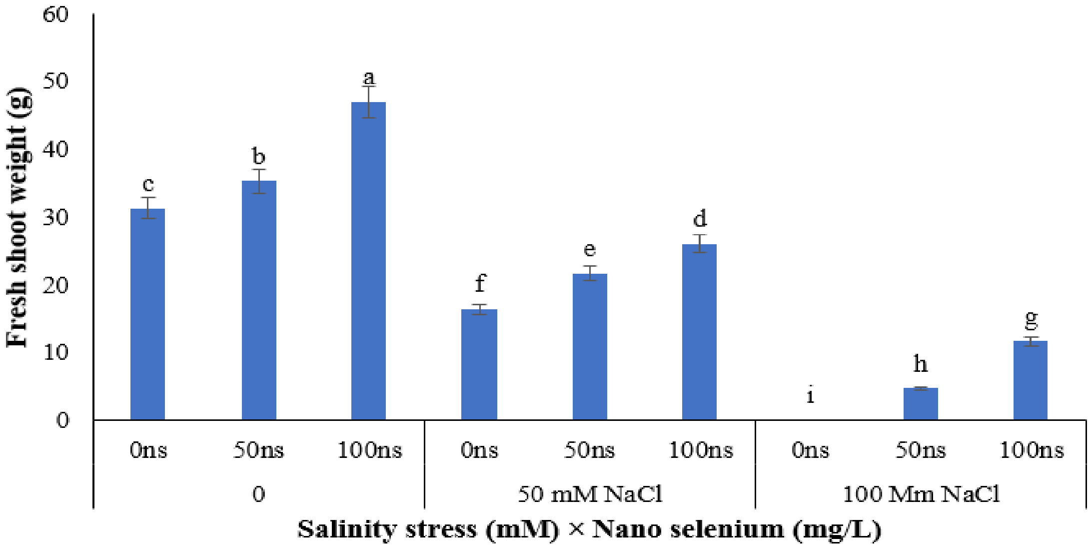

Fig. 4. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the fresh weight of aboveground plant parts in *Echinacea purpurea* L.

Saline growth media containing high ion concentrations disrupt the metabolism of other nutrients, leading to competition between Na^+ and K^+ ions and between Cl^- and NO_3^- ions. This competition impairs nutrient uptake, reducing plant growth and biomass. One of the key factors influencing salinity tolerance is the maintenance of cell turgor through osmotic regulation via salt ion uptake and the synthesis of organic compounds. Plants expend significant energy to synthesize organic compounds (e.g., glycine, betaine, proline, mannitol, and sorbitol), which, when used for osmotic regulation, result in reduced shoot growth and fresh weight (Yasir *et al.*, 2021). The reduction in fresh shoot weight is attributed to the interaction of osmotic stress, nutrient imbalance, and ion toxicity (Javadi *et al.*, 2021). Researchers studying *Nepeta racemosa* Lam. found that salinity treatments reduced fresh and dry weight, growth, and photosynthesis in this plant (Lungoci *et al.*, 2023).

Photosynthetic pigments and anthocyanin

The ANOVA results indicated that chlorophyll a, chlorophyll b, leaf carotenoids, and petal anthocyanin content in *Echinacea purpurea* were significantly affected by the main and interaction effects of salinity stress and nanoparticles at the 1% probability level (Table 2).

Table 2. Analysis of variance results of the effects of salinity stress and selenium nanoparticles on photosynthetic pigments and anthocyanins in *Echinacea purpurea.*

S.o.V	df	Chlorophyll a	Chlorophyll b	Carotenoid	Anthocyanins in petals
Salinity stress (S)	2	16.15**	6.49**	10.94**	0.31**
Nanoparticle (N)	2	4.21**	1.79**	1.98**	0.02**
S ×N	4	1.53**	0.79**	0.78**	0.01**
Error	18	0.28	0.12	0.1	0.001
CV (%)	-	17.30	16.82	13.68	15.37

**: Significant at $P < 0.01$ based on the Duncan's test.

Chlorophyll a, b, and carotenoids

In the salinity stress × selenium nanoparticle interaction treatment, the highest chlorophyll a (4.50 mg/g fresh weight), chlorophyll b (2.80 mg/g fresh weight), and carotenoid (3.25 mg/g fresh weight) content were observed in the control treatment (no salinity stress) with the application of 100 mg/L selenium nanoparticles. The lowest values for these traits were recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 5).

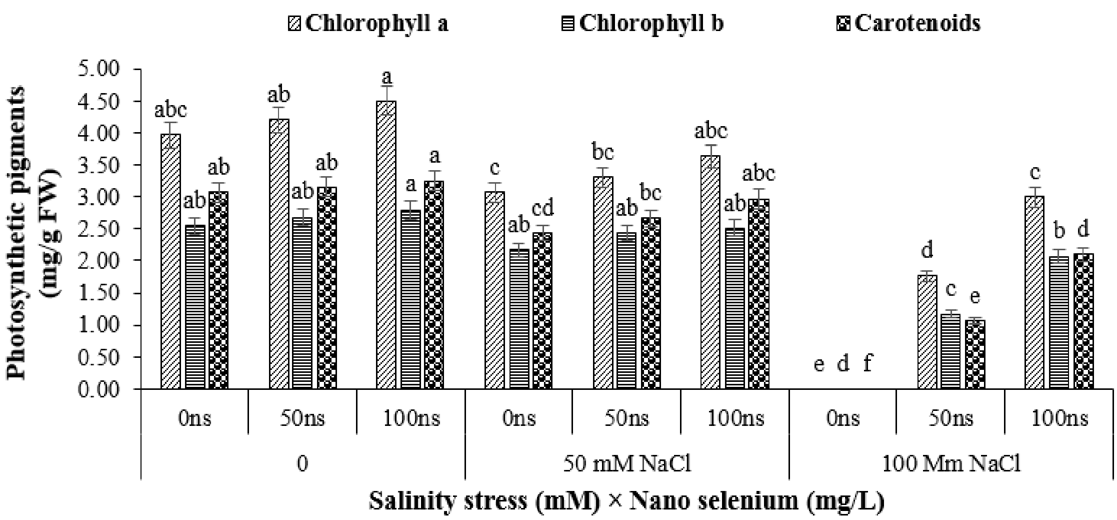

Fig. 5. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the leaf photosynthetic pigments in *Echinacea purpurea* L.

The reduction in chlorophyll content under stress is likely due to the activation of chlorophyll catabolic pathways. Changes in chlorophyll a and b concentrations during stress depend on plant species, salt type, and plant age. Chlorophyll content is an indicator of plant stress resistance (El Haddad *et al*., 2022). Chlorophyll and carotenoid degradation under salinity stress is caused by increased chlorophyllase activity, structural and functional changes, and reduced protein content, particularly membrane proteins and enzymes involved in chlorophyll biosynthesis, such as 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, and protochlorophyllide oxidoreductase (Atta *et al*., 2023). As salt concentration increases, cells accumulate compatible solutes like proline to maintain osmotic balance. Given the shared biosynthetic pathway of proline and chlorophyll, increased NaCl concentration shifts plant metabolism toward proline synthesis, reducing chlorophyll production. The accumulation of proline and carotenoids and the reduction in chlorophyll are rapid responses to stress conditions (Lungoci *et al*., 2023).

In this study, chlorophyll content increased with selenium nanoparticle application, consistent with previous research (Wang *et al*., 2021). Selenium prevents chlorophyll degradation (Oraghi Ardebili *et al*., 2019) and enhances antioxidant capacity (Schiavon and Pilon-Smits, 2019) in plants under environmental stress. Other beneficial effects of selenium include improved growth and carbohydrate metabolism (White, 2020). Selenium application increases soil cation exchange capacity, water retention, and nutrient uptake and transport in plants (Shahid *et al.*, 2019).

Petal anthocyanin

In the salinity stress × selenium nanoparticle interaction treatment, the highest petal anthocyanin content was observed in the control treatment (no salinity stress) with the application of 100 mg/L selenium nanoparticles. The lowest anthocyanin content was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 6).

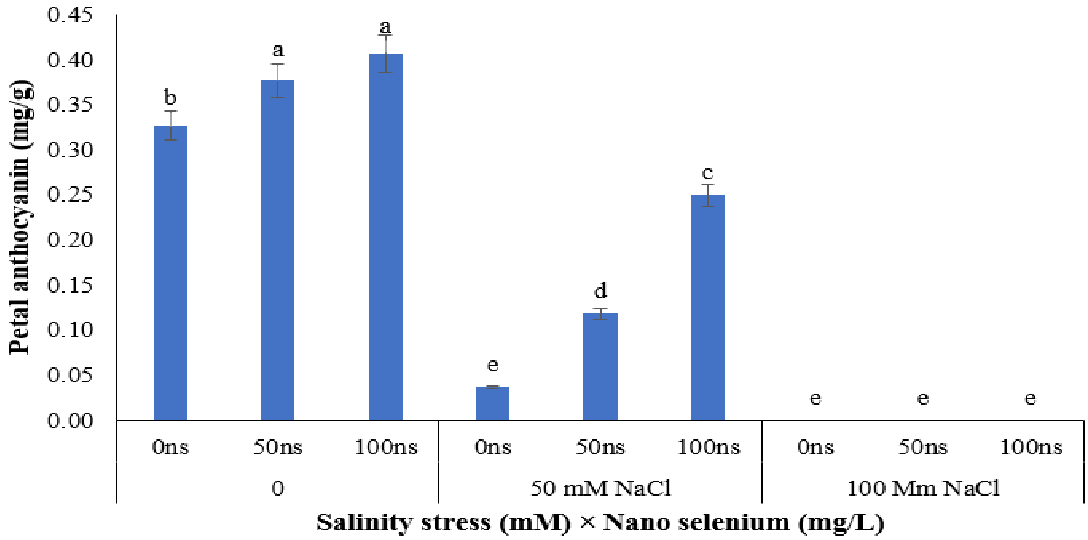

Fig. 6. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the anthocyanin content in *Echinacea purpurea* L.

Biochemical traits

The ANOVA results indicated that leaf catalase, superoxide dismutase, and antioxidant activity in *Echinacea purpurea* were significantly affected by the main effect of nanoparticles and the interaction effect of salinity stress × nanoparticles at the 1% probability level. Both enzymes were significantly affected by the main effect of salinity stress at the 5% probability level, while antioxidant activity was significant at the 1% level (Table 3).

Table 3. Analysis of variance results of the effects of salinity stress and selenium nanoparticles on biochemical traits of *Echinacea purpurea.*

S.o.V	df	Catalase	Superoxide Dismutase	Antioxidant Activity
Salinity stress (S)	2	0.02*	3.90*	4616.70**
Nanoparticle (N)	2	0.08**	12.06**	2109.37**
S ×N	4	0.02**	6.16**	1100.54**
Error	18	0.004	0.01	189.74
CV (%)	-	32.48	30.57	22.32

* and **: Significant at P < 0.05 and P < 0.01 based on the Duncan's test, respectively.

Leaf catalase

In the salinity stress × selenium nanoparticle interaction treatment, the highest leaf catalase activity (0.40 enzyme units/mg protein) was observed in the severe salinity stress treatment (100 mM NaCl) with the application of 100 mg/L selenium nanoparticles. The lowest activity was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 7).

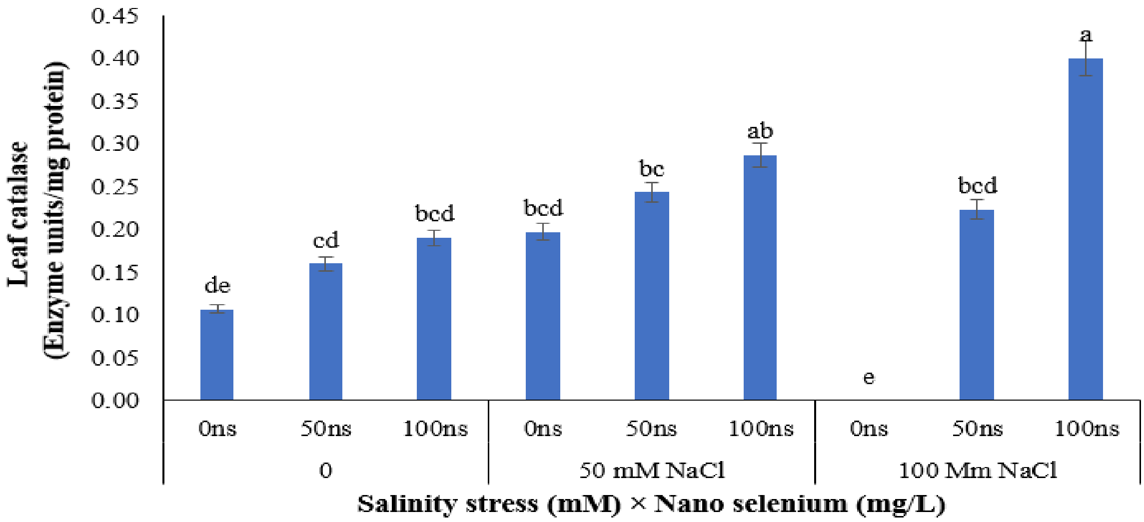

Fig. 7. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the catalase activity in *Echinacea purpurea* L.

Even low salt concentrations in roots and leaves increase catalase activity. One of the most significant biochemical changes during environmental stress is the accumulation of reactive oxygen species (ROS), which disrupt cellular redox balance and cause oxidative stress (Kesawat *et al.*, 2023). The increased activity of catalase and superoxide dismutase in Echinacea purpurea under environmental stress and nanoparticle application may be due to the protective role of selenium nanoparticles, which regulate osmotic pressure, reduce ROS, and enhance the biosynthesis of non-enzymatic antioxidants such as phenols (Arora *et al.*, 2020).

Leaf superoxide dismutase

Based on the comparison of mean values for the interaction effects of salinity stress × selenium nanoparticles, the highest leaf superoxide dismutase activity (5.57 enzyme units/mg protein) was observed in the severe salinity stress treatment (100 mM NaCl) with the application of 100 mg/L selenium nanoparticles. The lowest activity was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 8).

Superoxide anions are produced in cells under environmental stress due to stomatal closure and reduced CO_2 fixation, which inhibits growth. Increased respiration under these conditions also generates destructive ions in mitochondria. In such conditions, the activity of superoxide dismutase and peroxidase, enzymes that detoxify superoxide ions, increases, as observed in this study. Increased activity of these enzymes reduces oxidative damage. Superoxide dismutase efficiently reacts with superoxide anion radicals to produce water and oxygen (Atta *et al.*, 2023). Thus, increased superoxide dismutase activity under environmental stress is a common response to mitigate stress effects. These findings align with studies on sorghum (Tovignan *et al.*, 2020) and leafy vegetables (Razi and Muneer, 2021).

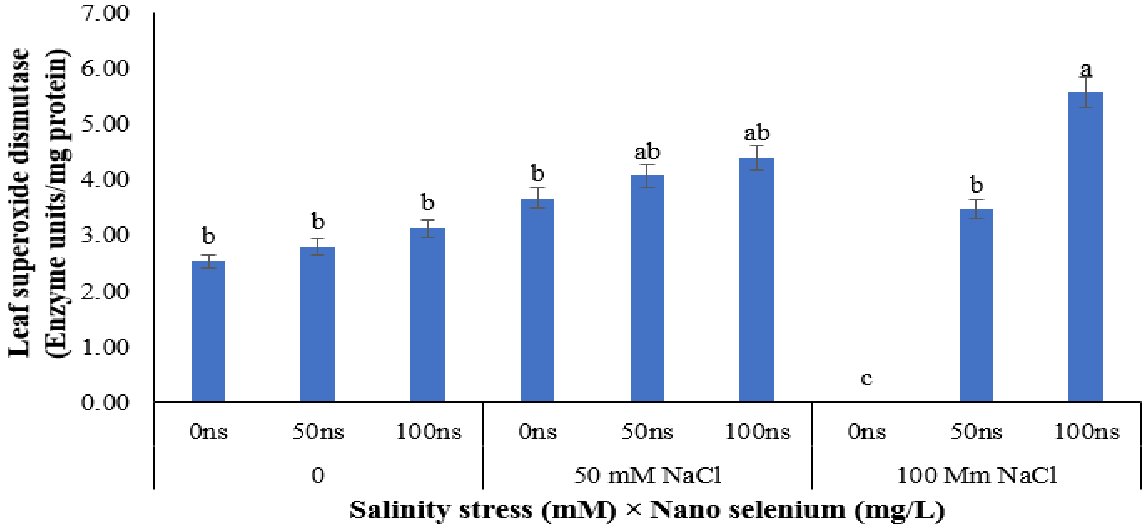

Fig. 8. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the superoxide dismutase activity in *Echinacea purpurea* L.

Antioxidant activity

Based on the comparison of mean values for the interaction effects of salinity stress × selenium nanoparticles, the highest antioxidant activity (89.00%) was observed in the mild salinity stress treatment (50 mM NaCl) with the application of 100 mg/L selenium nanoparticles. The lowest activity was recorded in the severe salinity stress treatment (100 mM NaCl) without nanoparticle application (Fig. 9).

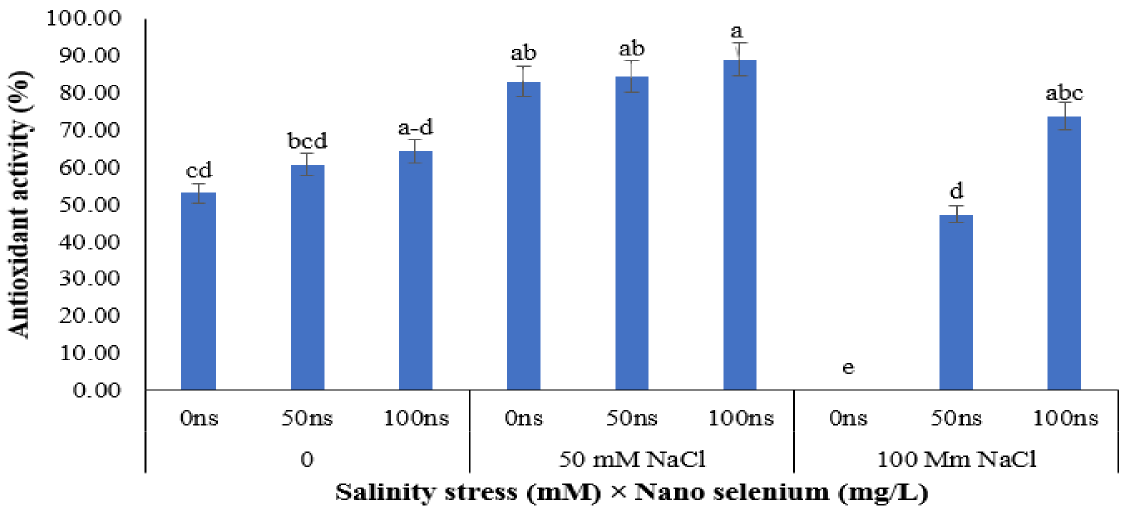

Fig. 9. Mean comparison of the interaction effects of salinity stress and selenium nanoparticle treatments on the antioxidant activity in *Echinacea purpurea* L.

Approximately 30 selenoenzymes and selenoproteins have been identified that protect cells against free radicals. Selenium, by incorporating into protein structures, safeguards tissues and cellular membranes from oxidative stress-induced damage (Schiavon and Pilon-Smits, 2019). Selenium exerts beneficial effects on plant growth and stress tolerance by enhancing their antioxidant capacity (White, 2020). In another study, it was found that foliar application of selenium on garlic enhanced the antioxidant capacity, phenolic compounds, and flavonoid content in garlic (Amerian *et al.*, 2024).

CONCLUSION

This study demonstrates that salinity stress (≥100 mM NaCl) significantly impairs *Echinacea purpurea* L. physiology, reducing vegetative growth (leaf count, lateral branches), reproductive output (flower number, diameter, longevity), and photosynthetic pigment content (chlorophyll a/b, carotenoids, anthocyanins) through ionic toxicity and metabolic disruption. Crucially, selenium dioxide nanoparticles (SeO_2NPs) effectively counteracted these effects by enhancing antioxidant enzyme activity (catalase, superoxide dismutase), reducing oxidative damage (lipid peroxidation), and improving stress tolerance. The nano-treatment restored growth parameters and floral characteristics while boosting secondary metabolism. These findings establish foliar SeO_2 application as a sustainable agricultural strategy for saline conditions, offering dual benefits of yield protection (35-40% improvement under stress) and reduced chemical inputs. The technology's ability to enhance medicinal plant productivity in marginal soils addresses key challenges in sustainable cultivation. We recommend incorporating optimized SeO_2 protocols into precision agriculture programs, particularly for climate-resilient medicinal crop production. Further research should validate field efficacy, determine optimal application regimes, and assess long-term soil impacts to facilitate commercial adoption of this nano-enabled stress mitigation approach.

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