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The Effect of Salt Stress and Selenium on Some Morphological and Flowering Traits of Petunia Plants (*Petunia hybrida* L.)

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Abstract

This study carried out in one of the nurseries of Kufa city/Iraq during the winter season of 2024 to study the effect of salt stress caused by sodium chloride at concentrations (0, 25, 50) mmol and foliar spraying with selenium at concentrations (0, 10, 20) mg.L^{-1} and the effect of the study factors on some growth and flowering traits and the percentage of nitrogen, phosphorus and potassium as well as the interaction between them. The results indicated a significant decrease caused by the 50 mmol salt concentration in plant height, number, leaf area, flowers number, and flower diameter, while the concentration of 25 mmol of NaCl also had a significant effect on plant height, number of flowers, and their diameters. Spraying plants with Se at concentrations of 10 and 20 mg/L^{-1} caused a significant increase in plant growth traits, as it plays a role in reducing the effect of salt stress on the studied plants. The combination (25 mmol NaCl + 20 mg.L^{-1} Se) caused the highest rates of growth traits studied. Foliar spraying with NaCl at 25 and 50 mmol caused a significant decline in the percentage of N, P, and K in the leaves at both concentrations. Spraying plants with Se at concentrations of 10 and 20 mg.L^{-1} resulted in a significant increase in these percentages.

Keywords: *Petunia* plants, selenium, salts, nitrogen, sodium

INTRODUCTION

Petunia hybrida L. belongs to the *Solanaceae* family. It is an annual winter ornamental plant characterized by its single, multi-colored flowers. It grows in both shady and sunny places. The genus *Petunia* contains approximately 25 species spread across South America. The current *Petunia* species are hybrids resulting from the hybridization between (*P. violacea* and *P. axilbris*) [1]. *Petunias* are found in all the colors of the rainbow, and may be solid colors, variegated waves, vines, or star patterns, and the flowers may be large, small, ruffled, fringed, or double [2]. *Petunia* is suitable for planting in panels and in basins to decorate gardens and public places, as its flowers are large, trumpet-shaped, and have many colors, including white, red, and pink [3, 4]. Ornamental plants are grown to decorate and beautify domestic and public environments, such as public gardens, parks, and streets, and improve the aesthetic appearance of the surrounding environment. These plants are distinguished by their flowers with beautiful, wonderful colors, which greatly improve the quality of life, whether in the countryside or in the city [5].

The presence of many activities caused by humans or nature has led to the accumulation of salts in the environment, which has affected the metabolic activities of plants, generated excessive stress, disturbance in ions, and the production of some metabolic products or proteins to combat stress and mitigate its effects [6]. It is a well-known fact that stresses, whether biotic or abiotic, cause oxidative stress in plants to varying degrees, and

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that there is a close relationship between a plant's ability to control oxidative stress levels and stress tolerance. The role of selenium as an antioxidant has been widely studied [7]. Selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}) are the main chemical compounds available in soil and water-soluble hexagonal fertilizers [8], especially sodium selenate Na_2SeO_4, which is an effective micronutrient in reducing abiotic stresses such as salinity. Foliar sprays often have a higher biological benefit than fertilizers added to the soil [9]. A study by [10] showed that the N, P, and K content in eggplant leaves and fruits irrigated with different salinity levels increased with increasing treatment with selenium up to 20 µL. Under salt stress conditions in some ornamental plants, such as *Rosa*, the use of salinity caused a significant decrease in growth indicators [11]. The study of Naqi et al. (2025) [12] showed that foliar application of selenium caused an improvement in the mineral content of leaves in grapevines treated with 75 µL sodium chloride. In general, foliar application of selenium (at 5 or 10 mg/L) caused an increase in salt tolerance by improving the nutritional balance and the enzymatic and non-enzymatic antioxidant capacity in grape leaves. El-Missiry (2012) [13] showed that irrigating Vinca plants with saline water at levels (4.5 and 9 $dS.m^{-1}$) caused a significant decrease in plant height, leaf area, number of plant branches, number of leaves, number of flowers, flower diameter, and leaf nutrient content (nitrogen, selenium, potassium). Spraying plants with selenium improved these traits. Therefore, through this study, we will attempt to examine the negative impact of salt stress and the role of selenium in reducing this effect on all studied traits.

MATERIALS AND METHODS

Experimental Sit

A field experiment was conducted in one of the nurseries of Kufa city in Najaf Governorate during the winter season of 2023–2024 to study the effect of selenium on the growth and flowering of petunia plants growing under salt stress and to estimate some of their nutrients. The experiment used hybrid petunia seeds planted in small anvils with dimensions of (15 x 20) cm filled with a mixture of soil and peat moss (1:1). The experiment included 9 treatments with three replicates in each experimental unit (treatment) of three anvils. The field experiment was designed using a Completely Random Block Design (CRBD). The results were analyzed using the statistical program Genstat, version 2012. All experimental data were subjected to statistical analysis (ANOVA) and the averages of the treatments were compared according to Duncan's Multiple Range Test at a probability level of 0.05.

Preparation of the Salt and Selenium Treatments Used in the Experiment

The salt concentrations of NaCl were prepared by dissolving 1 gram molecular weight of NaCl (58.5 g and completing it to one liter of distilled water to obtain a solution with a concentration of 1 mole representing the stock, then dilutions were prepared of the stock solution at concentrations of 25 and 50 mmol. The concentrations of 10 and 20 mg/liter of selenium were prepared by dissolving 10 and 20 g of Na_2SeO_4, respectively, in a little distilled water and completing the volume to one liter. The treatment of the plants began after the fourth true leaf was fully grown. Selenium was sprayed twice during the experiment period, with 21 days between each spray. The plants were sprayed until completely wet using a two-liter hand sprayer, while the control treatment was sprayed with distilled water.

The Vegetative Growth Indicators Estimation

Three plants were selected from each treatment after the end of the experiment and the vegetative growth measurements were taken, which included: the plant height (cm) calculated using a measuring tape starting from the soil surface to the top of the plant. After that, the height rate of these plants was taken, the number of branches in the stem (branch. plant-1) which was calculated on the main stem of each plant, the number of leaves for each plant, The leaf area (cm2) was calculated according to the method [14, 15] by drawing the taken plant leaf on graph papers. After that, the number of complete and missing squares occupied by the leaf image in calculating its area was calculated using the following law:

$$\text{Leaf area cm}^2 = \text{Number of complete squares} + \frac{\text{Area of one square} \times \text{Number of missing squares}}{2}$$

The Flower Traits

The total number of flowers (number of flowers throughout the plant growth period until the end of the experiment) was calculated by selecting five random plants from each treatment, and the flower diameter (mm) was measured using a digital vernier caliper device. Then the average diameter was extracted Flower for each treatment.

Estimation of Nutrients in Plant Leaves

0.5 grams of dried leaves were taken, and wet digestion was carried out according to the method of Fleeha and Gamil (1987) and Francini et al. (2022) [16, 17]. The samples were placed in flasks, and 5 ml of concentrated sulfuric acid was added to them with 5 ml of HClO, and the mixture was heated quietly for half an hour with continuous shaking and stirring and gradually raising the temperature. After the vapors rose and the color of the samples changed gradually, a clear, colorless solution was obtained. It was then cooled to cool at laboratory temperature, and the volume was completed with distilled water to 100 ml in a volumetric flask and stored in opaque plastic containers.

Estimation of the Nitrogen Content of Leaves (N) (%)

It was estimated by distillation using the Kjeldahl method through oxidation of the organic matter according to what was stated in Habas et al. (2019) [18], where a mixture of boric acid (2%) was placed with the two dyes methyl red and bromcresol green dissolved in ethanol (20 ml) in a 100 ml conical flask, then 10 cubic centimeters of the digested sample and 10 cubic centimeters of 10-molar sodium hydroxide (40% concentration) were added to the mixture. The distillation process was carried out, and NH_4^+ was released until the color of the mixture changed from red to green. Then, the titration was carried out with sulfuric acid (0.04 molar) until reaching the light purple equivalence point, and according to the volume of acid consumed in the titration, the percentage of N was calculated.

Estimation of Leaf P Content (P%)

Phosphorus was determined according to the method of Hassan (2022) and Hassanuzzaman and Fujita (2010) [19, 20] where 10 ml of the digested sample solution was taken from each replicate and diluted in 40 ml of distilled water, then 10 ml of the solution was taken and 0.1 g of ascorbic acid and 4 ml of NH_4^+ molybdate solution were added to it and the volume was completed in a flask to 100 ml with distilled water and heated in a conical flask on a hot plate for one minute until the solution turned blue, then it was transferred to a 100 ml flask and the volume was completed with distilled water. The optical absorption reading of the solutions was taken by a spectrophotometer at a wavelength of 620 nm, and the readings were plotted on the standard curve for P. The percentage of P was calculated. Figure 1 shows the phosphorus standard curve.

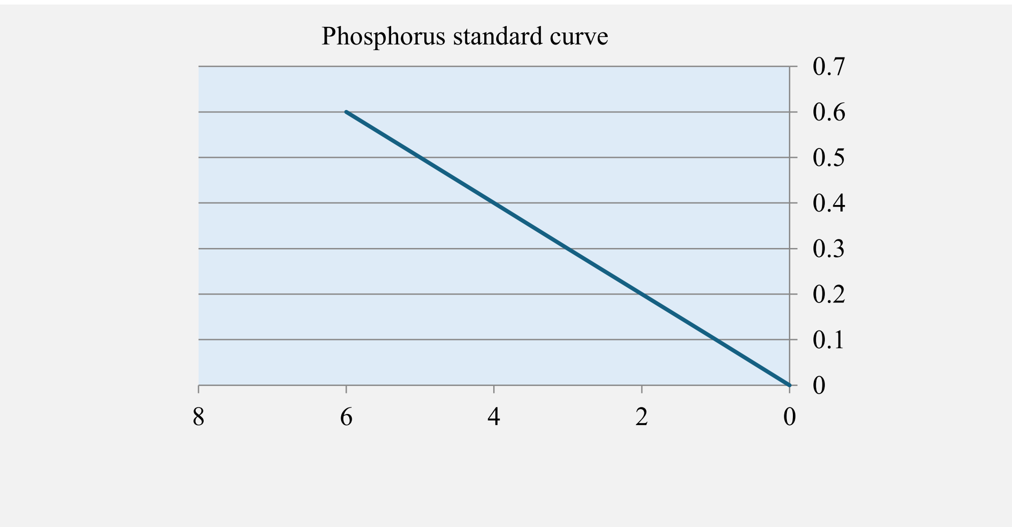

Figure 1. Phosphorus standard curve.

Determination of Leaf Potassium Content (K) (%)

The analysis was carried out using a flame photometer according to the method described in Hawrylak-Nowak et al. (2019) and Javadi et al. (2020) [21, 22] 0.5 g of dried plant material was taken in a conical flask and digested in a ternary acid mixture (H_2SO_4 + HNO_3 + $HC1O_4$ in a ratio of 1:3:9). The colorless digested material was filtered through Whatman filter paper into a 100 ml volumetric flask to make a total volume of 25 ml. Then 5 ml was taken and made up to 25 ml with distilled water, and read directly on a digital flame photometer, using K filters separately, and the percentage of K was determined using standard solutions.

RESULTS AND DISCUSSION

The Effect of Salt Stress and Selenium on Vegetative Growth Indicators

Table 1 shows the effect of NaCl and Se foliar application on plant height. Concentrations of 25 and 50 mM showed a significant decrease in this trait, with values of 7.14 and 6.49 cm, respectively, compared to the control, which was 7.39 cm. Se also caused a significant decrease in plant height at both concentrations, with the highest average reaching 7.90 cm at the control concentration. When the two factors were combined, plant height decreased significantly compared to the control plants.

Table 2 shows the effect of irrigating plants with saline water and spraying selenium on the number. The number decreased significantly, reaching 105.10 in plants irrigated with a 50 mmol salt concentration, compared to 115.10 leaves in control. The addition of Se caused a significant increase in the number at both concentrations used, with the highest value of 117.80 at a concentration of 20 mg. L^{-1}.

Similarly, Table 3 explained a significant decrease in leaf area due to salt stress. The 50 mmol salt concentration produced the lowest significant value in leaf area, 48.47 cm^2, compared to the control, which was 51.79 cm^2.

Meanwhile, foliar spraying with selenium had a positive effect in increasing leaf area at both concentrations. The highest value was recorded at the 20 mg L^{-1} concentration, reaching 55.61 cm^2. The selenium spraying mitigated the effects of salinity in all interactions. The treatment (25 mmol sodium chloride + 20 mg L^{-1} selenium) showed the highest values, 57.87 cm^2, compared to the control, which was 47.90 cm^2.

Table1. Effect of sodium chloride, selenium, and their interaction on plant height (cm).

NaCl mmol	Se mg/L			The Effect of NaCl
	0	*10*	*20*	
0	8.80a	7.23c	6.13f	7.39a
25	8.30 b	6.70 d	6.43 def	7.14 b
50	6.60 de	6.57 de	6.30 ef	6.49 c
The effect of Se	7.90 a	6.83 b	6.29 c	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 2. Effect of sodium chloride, selenium, and their interaction in leaves No.

NaCl mmol	Se mg/L			The Effect of NaCl
	0	*10*	*20*	
0	111.30 c	114.70 bc	117.30 ab	115.10 a
25	111.70 c	113.70 bc	120.00 a	114.40 a
50	94.00 e	105.30 d	116.00 b	105.10 b
The effect of Se	105.70 c	111.2 b	117.80 a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 3. Effect of sodium chloride, selenium, and their interaction on leaf area (cm^2).

NaCl mmol	Se mg/L			The Effect of NaCl
	0	10	20	
0	47.90 f	51.77 d	55.57 b	51.79 a
25	46.50 g	51.00 d	57.87 a	51.74 a
50	42.33 h	49.67 e	53.40 c	48.47 b
The effect of Se	45.58 c	50.81 b	55.61 a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

From the Table 4, we find that irrigating plants with saline water caused clear damage to plant growth, represented by a decrease in plant height, leaf area, and the number of leaves in general. These results were compatible with those of Karimi et al. (2020) [23] on some ornamental plants and [23] on the *Vinca* plant. This is since the increase in salt concentration hinders water absorption by primarily affecting the decrease in water potential in the soil solution, thus limiting the amount of water available to the plant. This causes disturbances in key vital processes such as photosynthesis, respiration, and osmoregulation [24]. Consequently, cell filling decreases, cell elongation decreases, and mitotic division is disrupted, limiting cell division. All these causes lead to a general weakness in plant growth [25]. The decrease in leaf area resulting from a decrease in leaf expansion is due to inhibition of cell filling and the acceleration of leaf senescence due to Free radicals that are formed under stress [26]. We observe the positive role of selenium through the increased number of branches, leaf area, and number of leaves, except for plant height, which decreased due to selenium. This may be due to the plant's tendency to develop increased branching rather than elongation. One of the best biological mechanisms that plants resort to in the presence of selenium is the binding of selenium to amino acids, especially methionine and cysteine, where the sulfur atom is replaced by selenium and the formation of selenium proteins (Selenoproteotines), these proteins are characterized by their high ability to make cell membranes tolerate water deficiency and prevent the demolition of protein metabolism compounds [27]. Therefore, adding selenium in appropriate concentrations can enhance the maintenance of the plasma membrane and some cell organelles (mitochondria, chloroplasts) in leaf and root tissues, which helps them overcome the damage of salt ions [28], thus improving plant growth in general.

Table 4. Effect of sodium chloride, selenium, and their interaction on the number of flowers.

NaCl mmol	Se mg/L			The Effect of NaCl
	0	10	20	
0	19.17 de	29.00 a	26.33 b	24.83 a
25	17.50 e	26.33 b	23.67 c	22.50 b
50	13.50 f	20.67 d	18.33 e	17.50 c
The effect of Se	16.72 c	25.33 a	22.78 b	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 5. Effect of sodium chloride, selenium, and their interaction in flower diameter (mm).

NaCl mmol	Se mg/L			The Effect of NaCl
	0	10	20	
0	69.18 b	70.27 b	72.93 a	70.79 a
25	67.63 c	67.63 c	70.20 b	68.02 b
50	53.28 f	64.67 e	67.67 c	61.87 c
The effect of Se	62.89 c	67.52 b	70.27 a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

The Effect of Salt Stress and Selenium on Floral Traits

Tables 4 and 5 indicate the significant decrease caused by NaCl in the number and diameter of flowers per plant. The 50 mM NaCl concentration produced the lowest values for flower number and diameter, reaching 17.50 and 61.87 mm, respectively, compared to the control, which produced the highest values for flower number and diameter, at 24.83 and 70.79 mm. While selenium had a positive effect on increasing flower number and diameter at both concentrations, the 10 mg L^{-1} concentration recorded the highest value in flower number, 25.33 flowers per plant, while the 20 mg L^{-1} concentration recorded the highest value in flower diameter, at 70.27 mm. Regarding the interaction between the two factors, selenium mitigated the effects of salinity in all interactions and produced the highest value for flower number, 29.00 flowers, when plants were treated with 10 mg L^{-1} selenium and irrigated with 25 mM NaCl. The interaction of the two factors mitigated the effects of salinity and significantly increased flower diameter. Selenium treatment at a concentration of 20 mg/L and irrigated with 50 mmol sodium chloride yielded the highest value, reaching 67.67 mm, compared to 53.28 mm when plants were irrigated with 50 mmol sodium chloride and without selenium.

The Effect of Salt Stress and Selenium on the Nutrients in Plant Leaves (NPK)

The results of Tables 6–8 show the effect of treating plants with water with salt concentrations of 25 and 50 mmol of sodium chloride and adding selenium on a significant change in the percentage content of nitrogen, phosphorus, and potassium in the leaves. The percentage of nutrients decreased to 1.72%, 0.29%, and 0.88% for nitrogen, phosphorus, and potassium, respectively, at a salt concentration of 50 mmol, compared to the control, which was 1.89%, 0.34%, and 1.18%, respectively. The addition of selenium caused an improvement in these percentages and produced significant differences in these percentages, which rose to 1.88%, 0.34%, and 1.17% for nitrogen, phosphorus, and potassium, respectively, at a concentration of 20 mg.L^{-1}. The interaction of the two experimental factors resulted in a significant improvement in the percentages of nitrogen and phosphorus and a mitigation of the effects of salt stress when spraying selenium. The percentages of nitrogen and phosphorus increased to 1.79% and 0.35%, respectively, in the treatment (50 mM sodium chloride + 20 mg L^{-1} selenium), after having been 1.62% and 0.24%, respectively, in the treatment (50 mM sodium chloride + 0 mg L^{-1} selenium). The interaction between salt and selenium on the percentage of potassium also showed that selenium did not reduce the salt concentration, as it caused a significant increase in this percentage for plants treated with a concentration of 20 mg L^{-1} selenium, and those not treated with the various salt concentrations. The highest value for the percentage of potassium was recorded at 1.38%. In the above-mentioned tables, we find that salt stress led to a decrease in the percentage of nitrogen, phosphorus, and potassium.

Table 6. Effect of sodium chloride, selenium, and their interaction on nitrogen content of leaves (%).

NaCl mmol	**Se mg/L**			**The Effect of NaCl**
	0	***10***	***20***	
0	1.79c	1.89b	1.96a	1.89a
25	1.70d	1.87b	1.89ab	1.82b
50	1.62e	1.75cd	1.79 c	1.72c
The effect of Se	1.70c	1.83b	1.88a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 7. Effect of NaCl, Se, and their interaction on leaf phosphorus content (%).

NaCl mmol	**Se mg/L**			**The Effect of NaCl**
	0	***10***	***20***	
0	0.31 c	0.32 b	0.35 a	0.34 a
25	0.27 e	0.29 d	0.32 bc	0.29 b
50	0.24 f	0.31 bc	0.35 a	0.29 b
The effect of Se	0.27 c	0.31 b	0.34 a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 8. Effect of NaCl, Se, and their interaction in content of leaves (%).

NaCl mmol	Se mg/L			The Effect of NaCl
	0	*10*	*20*	
0	0.90 e	1.27 b	1.38 a	1.18 a
25	0.89 e	0.98 d	1.15 c	1.01 b
50	0.78 g	0.86 f	0.99 d	0.88 c
The effect of Se	0.86 c	1.04 b	1.17 a	

Note: Rates with similar letters are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Competition between elements plays a complex role in determining the scarcity or abundance of mineral elements in plants [25]. A decrease in nitrogen percentage was observed in plants under salt stress due to the antagonism between chloride ions (Cl^-) and nitrate ions (NO^{3-}) (Munns and Gilliham, 2015). The decrease in potassium content results from the competitive effect of sodium and potassium ions on uptake sites in the roots and on transport proteins. These proteins, as well as potassium transport channels (K±channels), work to transport sodium ions instead of potassium when sodium ions are present in high concentrations, leading to a decrease in potassium concentration in the leaves [26].

These results are consistent with those of Munns and Gilliham (2015) and Negrão et al. (2017) [27, 28] in the Ain al-Buzun plant and [29] in the Ain al-Buzun plant. In these tables, we find that selenium had a positive effect on improving the percentage of the aforementioned elements and reducing the negative impact of salts. This was explained by selenium's ability to prevent lipid oxidation, increase nutrient absorption, and reduce sodium and chloride levels because of improving cell membrane permeability and increasing the concentration of proteins that protect cell membranes and membrane-associated enzymes [30–32]. It is also believed that the increased absorption of elements in the presence of selenium is due to its role as an antioxidant for cytochromes, which are important in the cytochrome pump for salt absorption [33]. These results were consistent with Singh et al. (2019) and Tavakoli et al. (2020) [34] in ornamental plants [35, 36].

CONCLUSIONS

Our current study revealed that irrigating plants with saline water negatively affected the vegetative and reproductive growth of plants and leaf element content, especially at high saline concentrations of 50 mM NaCl. Selenium played an important role in improving the studied vegetative and reproductive growth traits, as well as improving the plant's ability to withstand stress. Therefore, we recommend planting petunia in soils with low saline concentrations and benefiting from it as an ornamental plant in abandoned urban areas to decorate them with the beauty of its flowers. It is also possible to spray selenium at low concentrations on ornamental plants to increase the number and diameter of flowers to enhance the beauty of these plants.

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