



Mitigation of the Salinity Stress in *Vicia Faba* (L.) Seedlings Using Green-Synthesized Selenium Nanoparticles

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Abstract

This study explores the potential of green-synthesized selenium nanoparticles (SeNPs), prepared from *Trigonella foenum-graecum* (fenugreek) seed extract, to alleviate salinity stress in *Vicia faba* (L.) seedlings. It was hypothesized that selenium nanoparticles (SeNPs) could enhance salinity tolerance by improving physiological performance and activating antioxidant defenses. Selenium nanoparticles (SeNPs) were synthesized using a green approach and characterized by ultraviolet–visible (UV–Vis) spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, and scanning electron microscopy (SEM). Seed priming with SeNPs was performed prior to exposure to 150 mM sodium chloride (NaCl). Both control (non-salinized) and salt-stressed seedlings, with and without SeNPs, were evaluated for growth, physiological, and biochemical traits. Salinity stress markedly inhibited seed germination, growth, and photosynthetic pigments, while promoting oxidative damage. In contrast, SeNP-primed seedlings showed improved germination and growth performance, maintained chlorophyll content, and exhibited reduced lipid peroxidation and electrolyte leakage. SeNPs also modulated osmolyte accumulation and stabilized protein and carbohydrate metabolism under stress. Green-synthesized selenium nanoparticles (SeNPs) mitigate the adverse effects of salinity in *Vicia faba* by enhancing antioxidant activity, osmotic adjustment, and photosynthetic efficiency. This eco-friendly nanobiotechnological approach represents a promising strategy to improve crop resilience and support sustainable production in saline environments.

Keywords Selenium nanoparticles · Fenugreek · Salinity stress · Antioxidants · *Vicia faba*

1 Introduction

The faba bean (*Vicia faba* L.) is a vital legume crop in Egypt, valued for its high protein content (20–30%) and nutritional importance for both human and animal consumption (Abo-Shanab et al. 2025). Ranking second only to soybean in protein accumulation, *Vicia faba* is increasingly recognized for its adaptability and nutritional benefits (Warsame et al. 2022). Beyond its significance as a staple food in Asia and Africa and a protein-rich feed source in Europe, faba bean contributes to soil fertility through its nitrogen-fixing ability, thereby reducing the need for synthetic fertilizers (Lime-nie 2024). Despite these benefits, *Vicia faba* cultivation faces mounting challenges from environmental stressors,

particularly soil salinity (Nasrallah et al. 2022; Abo-Shanab and Diab 2024).

Salinity is a major global constraint to agricultural productivity, especially in salt-sensitive legumes such as faba bean (Machado and Serralheiro 2017; Abo-Shanab et al. 2025). High salinity induces osmotic stress, disrupts nutrient balance, and causes toxic ion accumulation, leading to oxidative stress and metabolic dysfunction (Gupta et al. 2021). The progressive expansion of soil salinization impairs key physiological processes, reduces photosynthetic efficiency, and triggers water deficits and ion imbalances due to excessive sodium and chloride ions (Abo-Shanab et al. 2025). Given *Vicia faba*'s agronomic importance and its vulnerability to salinity, novel and sustainable strategies are urgently required to enhance its resilience and secure productivity under changing environmental conditions.

Nanotechnology offers innovative solutions to these challenges. Nanoparticles (NPs) have wide-ranging applications in agriculture, medicine, and industry owing to their unique physicochemical properties (Shafey

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2020; Elsilk et al. 2024). In crop production, nano-fertilizers improve nutrient uptake, reduce fertilizer loss, and mitigate environmental contamination while enhancing plant tolerance to abiotic stress (Elsilk et al. 2024). The green synthesis of nanoparticles using plant-derived macromolecules, particularly polysaccharides, represents an environmentally safe and sustainable approach (Shaikh and Shaikh 2024). Among various nanomaterials, selenium nanoparticles (SeNPs) have gained considerable attention for their role in improving plant stress tolerance (Ahmed et al. 2024). Selenium is an essential trace element that enhances antioxidant capacity and protects plants from oxidative damage. SeNPs can be synthesized through chemical, physical, or biological methods (Xiao et al. 2023), with green synthesis emerging as a safer and more biocompatible alternative. Exogenous application of SeNPs has been reported to alleviate salt-induced stress by lowering malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) accumulation, thus reducing lipid peroxidation and membrane damage (Chattha et al. 2022). Recent studies have highlighted the growing importance of green-synthesized SeNPs in enhancing crop resilience under salinity by improving ionic homeostasis, osmotic adjustment, and antioxidant enzyme activity (Hasanuzzaman et al. 2011). However, most existing research has focused on chemically synthesized SeNPs, leaving a knowledge gap regarding the effectiveness and physiological mechanisms of biologically synthesized SeNPs, especially in legume crops such as *Vicia faba*.

Fenugreek (*Trigonella foenum-graecum*) seed extract serves as an excellent reducing and stabilizing agent for SeNP synthesis due to its rich content of galactomannan, saponins, alkaloids, flavonoids, amino acids, and other bioactive compounds (Ramamurthy et al. 2013; Niknam et al. 2021). These phytochemicals not only support nanoparticle stabilization but also enhance plant growth by promoting Rubisco activity and photosynthetic pigment production in *Vicia faba* (Babaei et al. 2025). Thus, fenugreek-mediated SeNPs offer a sustainable and biologically active nanomaterial for improving plant stress tolerance. Previous studies have demonstrated that SeNPs can alleviate salinity stress by enhancing potassium uptake, reducing sodium toxicity, and activating antioxidant defense mechanisms (El-Badri et al. 2022). In *Vicia faba*, SeNPs have been shown to improve photosynthetic pigments, metabolic performance, and antioxidant enzyme activities (El-Saber 2021). Similarly, in strawberry plants, SeNPs promote photosynthesis, maintain ionic balance, and mitigate oxidative damage caused by reactive oxygen species (ROS) (Liu et al. 2024).

In this context, the present study introduces a novel and eco-friendly approach by using fenugreek extract to

biosynthesize selenium nanoparticles (SeNPs) and evaluates their role in mitigating salt stress in *Vicia faba* seedlings. The innovative aspect of this work lies in employing fenugreek-mediated SeNPs as a dual-function agent both as a nanonutrient and a natural biostimulant to improve photosynthetic efficiency and antioxidant metabolism under salinity stress. We hypothesize that fenugreek-derived selenium nanoparticles enhance salt stress tolerance in *Vicia faba* by modulating antioxidant defense systems, improving osmolyte accumulation, and maintaining ionic balance. This study aims to characterize the biosynthesized SeNPs, assess their impact on growth, physiological, and biochemical traits under saline and non-saline conditions, and elucidate their potential role as sustainable nanobiostimulants in crop salinity management.

2 Material and Methods

2.1 Preparation of Fenugreek Extract

Fenugreek (*Trigonella foenum-graecum* L. cv. Giza 2) seeds were obtained from El-Gimiza Agricultural Research Station, El-Gharbia Governorate, Egypt. To prepare the aqueous extract, 1 g of ground fenugreek seeds was added to 100 ml of boiling distilled water and stirred continuously for 15 min to ensure uniform extraction. The mixture was then filtered through Whatman No. 1 filter paper to obtain a homogeneous solution. The filtrate represented a 1% (w/v) fenugreek extract, which was centrifuged at 5000 rpm for 20 min to remove any insoluble residues. The resulting clear supernatant (1% fenugreek extract) was subsequently used as the reducing and stabilizing agent in the green synthesis of selenium nanoparticles (SeNPs).

2.2 Green-synthesis of Selenium Nanoparticles (SeNPs) from Fenugreek Extract

Selenium nanoparticles (SeNPs) were synthesized according to Ramamurthy et al. (2013). A total of 100 mL of fenugreek extract (1%) was mixed with 10 mL of sodium selenate (0.1 M) and stirred magnetically for 5 min after adding 1 mL of a 0.2 M aqueous ascorbic acid solution to initiate the reduction reaction. The mixture was then stirred overnight at room temperature (25 °C). Thereafter, it was centrifuged at 4 °C for 30 min at 10,000 rpm. The resulting pellet was washed with ethanol and distilled water to remove impurities and subsequently dried in a hot air oven at 45–50 °C. The visible color change (Fig.S1) confirmed the successful biosynthesis of SeNPs. The dried SeNP powder was then used for further analyses.

2.3 Characterization of Selenium Nanoparticles (SeNPs)

A cell-free supernatant without sodium selenate was utilized as a control throughout the experiment, and purified SeNPs were subjected to UV-Vis spectra measurement (JENWAY-6305 Spectrophotometer) at a resolution of 1 nm and frequencies ranging from 200 to 800 nm. The size and surface morphology of the produced SeNPs were detected using scanning electron microscopy (SEM, ZEISS, EVO-MA 10, Oberkochen, Germany). The EDX-BRUKER, Nano-GmbH (D-12489, M-410, Germany) was used to study the elemental composition, purity, and dispersion of the components that formed the nanoparticles. Additionally, the Spectrum of two-IR Spectrometer (Perkin-Elmer Inc., Shelton, CT, USA) was used to study the unique functional groups present in the SeNPs molecules that were synthesized. Prior to being placed onto high-pressure discs, samples were treated with potassium bromide. These discs were identified in the 400–4000 cm^{-1} region in order to generate FTIR spectra. All spectra were acquired at a 4 cm^{-1} resolution by means of 32 scans in order to get an adequate signal quality. A transmission electron microscope (JEOL JEM 100 SX) was used to analyze the size and shape of SeNPs. To ensure even dispersion in the water, the powdered extract was sonicated. Thereafter, a drop of the dispersed solution was placed on a carbon-coated copper TEM grid to prepare the selenium nanoparticles sample.

2.4 Plant material, Growth conditions, and Phytochemical Analyses of *Vicia Faba* (L.)

Seeds of *Vicia faba* (L.) (cv. Giza 3) were obtained from the Agricultural Research Center in Giza, Egypt, were selected based on their apparent uniformity in size and shape. For two minutes seeds were immersed in NaClO (1%) then in distilled water to clean the seeds surface. The initial experiment was conducted using various concentrations of NaCl (0, 50, 100, 150, 200, and 250 mM) to determine the lethal and sub-lethal doses of NaCl. Results concluded that the sub-lethal dosage of 150 mM NaCl had the most harmful effects based on the findings of initial screening before symptoms and spots of necrosis on the leaves occurred, plants displayed evidence of chlorosis and plant death and total growth suppression. For the main experiment, two sets of faba bean seeds were divided. For ten hours, as a control, the first group was immersed in distilled water, while the second group was submerged in SeNPs (40 mg L^{-1}) solution for the same duration. Each treatment's six prepped seeds were placed in plastic pots along with five kilogram of clay-sandy soil (2:1 by weight) and irrigated daily with modified half-strength Hoagland's solution (Awad-Allah et

al. 2023). There were three pots used as replicas for each treatment. Four groups were irrigated once with water until the germination, thereafter the second and fourth group was irrigated with the stress treatment (150 mM NaCl) until the field capacity reached 80% on the 7th and 14th days. Plants were grown under greenhouse conditions with 11 h of light at $28\text{ }^{\circ}\text{C} \pm 2$ and 13 h of dark at $16\text{ }^{\circ}\text{C} \pm 2$, maintaining 62% relative humidity (Benmoussa et al. 2022). Then, all roots, shoots, and leaves samples of *Vicia faba* (L.) seedlings were harvested after 21 days, washed to remove soil particles then separated. The lengths of roots and shoots length as well as leaf area of samples were determined. Leaf area was determined manually by tracing fresh leaves on graph paper and calculating the total area based on the measured dimensions and corresponding leaf weight to ensure accuracy. Sample's fresh weight was determined then the dry weight was determined after the tissue samples were dried in an oven at $50\text{ }^{\circ}\text{C}$ for at least 72 h.

2.4.1 Evaluation of Photosynthetic Efficiency (F_v/F_m) and Pigments Content of *Vicia Faba* (L.) Seedlings

An OS-30 p chlorophyll fluorometer (Hudson, NH03051 USA) was used to quantify the photosynthesis rate as the fluorescence kinetic (F_v/F_m) of dark-adapted seedling leaves (Senousy et al. 2023). Following the method of Metzner et al. (1965), the content of chlorophyll a, chlorophyll b, and carotenoids in the fresh leaves of seedlings were quantified and represented as mg g^{-1} Fresh weight.

2.4.2 Determination of Malondialdehyde and Electrolyte Leakage of *Vicia Faba* (L.) Seedlings

Heath, and Parker, (1968) approach was used to assess MDA. The fresh leaves were extracted using 0.5 gram of trichloroacetic acid (5% w/v). For 10 min at 4000 rpm, the homogenate was spun in a centrifuge. 2 ml of the liquid remaining after boiling in $100\text{ }^{\circ}\text{C}$ water were mixed with 2 ml of thiobarbituric acid (0.67% w/v). Then the absorbance was measured at 532 nm and 600 nm. The attenuation coefficient ($155\text{ mM}^{-1}\text{ cm}^{-1}$) was used to obtain the MDA concentration, which is shown as ($\mu\text{M g}^{-1}\text{ FW}$). On the other hand, Sairam et al. (2005) detected the electrolyte leakage. The fresh collected leaves were cut into little pieces. After soaking 0.5 g of leaf pieces in 20 ml of distilled water for 24 h, the electrical conductivity was measured using an EC meter at $26\text{ }^{\circ}\text{C}$.

2.4.3 Evaluation of Antioxidant Enzymes' Activity and osmo-regulatory Compounds of *Vicia Faba* (L.) Seedlings

0.5 g of fresh plant material was homogenized in 8 ml of 50 mM cold phosphate buffer at pH 7.0 after freezing

according to Beauchamp and Fridovich (1971). The homogenates were centrifuged at 4000 rpm for 20 min. The supernatant used for antioxidants assay. The activity of catalase (CAT, EC: 1.11.1.6) was assayed at 240 nm according to Havir and McHale (1987). The guaiacol peroxidase (POX, EC: 1.11.1.7) activity was determined by utilizing the method developed by Kato and Shimizu (1987). The activity of superoxide dismutase (SOD, EC: 1.15.1.1) activity was assayed at 560 nm according to van Rossum et al. (1997). The content of total amino acid was estimated according to Lee and Takahashi (1966). A total of 0.5 g of dried plant sample was homogenized using 80% ethanol. The extract was then centrifuged for 15 min at 800 rpm, neutralized with 0.1 N NaOH using methyl red as an indicator, and ninhydrin reagent was added. The content was heated for 20 min in a boiling water bath and then diluted with distilled water. The substance was detected at 570 nm. The Proline content was estimated according to Bates et al. (1973). Using 0.5 g of dried plant sample was homogenized in 3% aqueous sulfosalicylic acid then the homogenate was centrifuged for 15 min at 10,000 rpm. After adding 2 ml of the supernatant, 2 ml of acid ninhydrin, and 2 ml of glacial acetic acid, the mixture was boiled at 100 °C for 1 h. Following the reaction's termination in an ice bath, 4 ml of toluene was used to extract the reaction mixture, and the absorbance was measured at 520 nm. The amount of total soluble protein was calculated using Bradford's methodology (Bradford 1976). 3 ml of Coomassie Brilliant Blue (CBB) reagent (100 mg of CBB was dissolved in 50 ml of 95% ethanol and 100 ml of 85% phosphoric acid) was well mixed with precisely 0.1 ml of borate buffer extract before being added to one liter of distilled water. The absorbance at 595 nm was measured after two minutes. Using a calibration curve of bovine serum albumin as a standard protein. The phenol-sulfuric acid method was used to measure the total amount of soluble sugars (Dubois et al. 1956). 5 mL of borate buffer was mixed with 0.1 g of fine plant powder. After a 24-hour rest period, the mixture was centrifuged for 15 min at 3000 rpm. The mixture is then quickly mixed with a solution that contains 0.1 mL of borate buffer, 1 mL of phenol (5%), and 5 mL of strong sulfuric acid (95%). The test tubes are vortexed for 30 s after standing for 10 min, and then they are left in a water bath at room temperature for 20 min to develop their color. A spectrophotometer is then used to record the light absorption at 490 nm. All concentrations were expressed as mg g^{-1} DW.

2.5 Statistical Analysis

All tests were repeated three times, and the average value plus or minus the standard deviation (SD) represents the data. Duncan's multiple range tests and one-way analysis of

variance (ANOVA) was evaluated to compare the treatment groups post hoc. Statistical differences were identified using a significance threshold of 0.05. Using the SPSS program (version 23) to compute.

3 Results

3.1 Characterization of Selenium Nanoparticles (SeNPs)

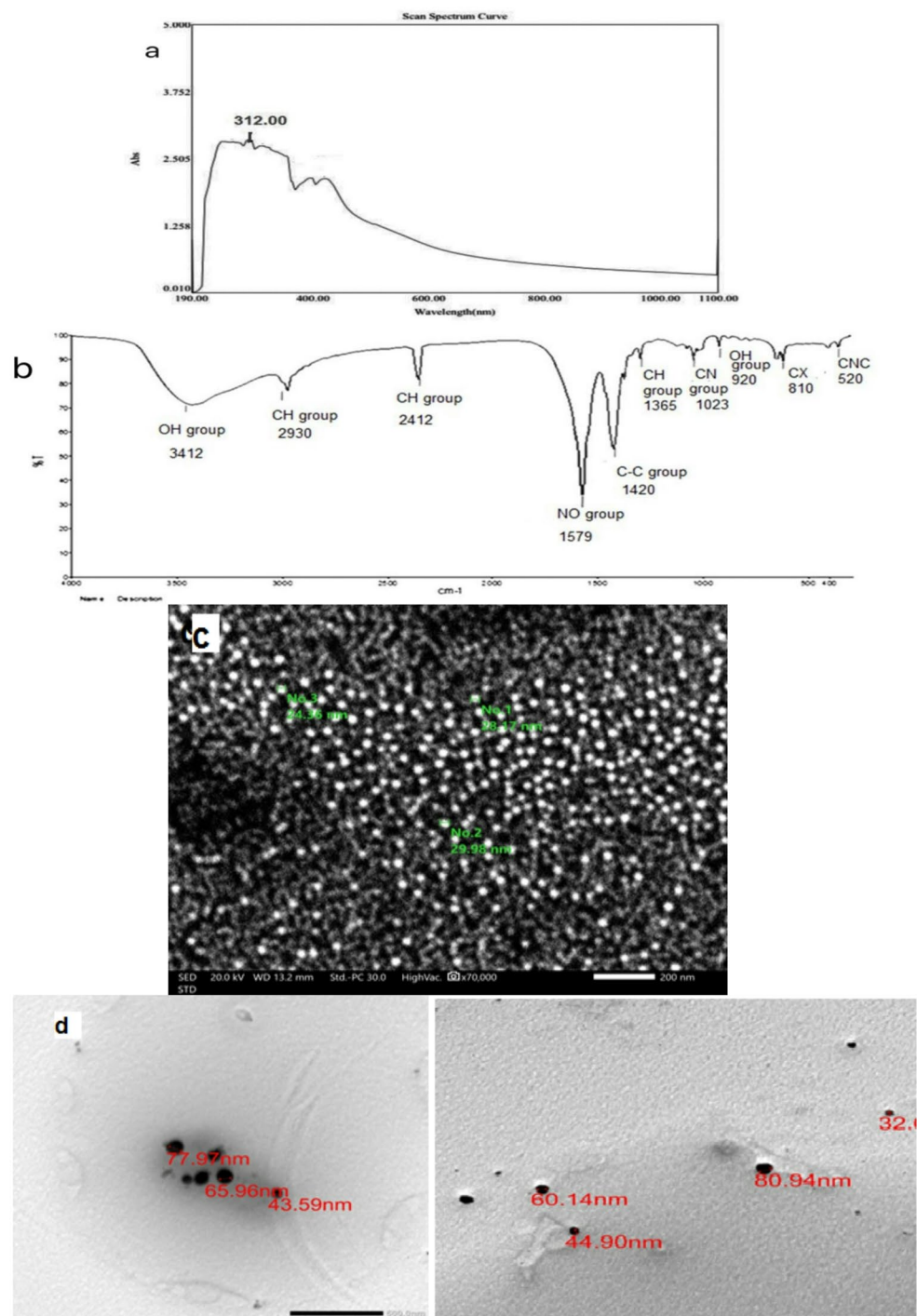
The green synthesis of selenium nanoparticles (SeNPs) using fenugreek seeds extract resulted in a yellowish-white powder, and their formation was monitored and confirmed using UV-visible spectroscopy, as illustrated in Fig. 1a. The UV-visible spectrum of SeNPs, recorded within the wavelength range of 300–1100 nm, exhibited a distinct absorption peak at 312 nm, indicating the successful synthesis of SeNPs. Fourier-transform infrared (FT-IR) spectroscopy was performed to identify the functional biomolecules involved in SeNPs' synthesis and stabilization. The FT-IR spectrum (Fig. 1b) displayed characteristic absorption bands at 3412, 2930, 2412, 1579, 1420, 1365, 1023, 920, 810, and 520 cm^{-1} , confirming the presence of various functional groups. Scanning electron microscopy (SEM) images revealed that the synthesized SeNPs were predominantly oval-shaped, with a smooth surface and sizes reaching up to 24.36 nm (Fig. 1c). Additionally, transmission electron microscopy (TEM) analysis provided further insights into the morphology of SeNPs, indicating a hexagonal shape with particle sizes ranging from 32 to 80.94 nm, with an average size of 45 nm (Fig. 1d).

3.2 Effect of Selenium Nanoparticles (SeNPs) On the Growth of Salt-stressed *Vicia Faba* (L.) Seedlings

A preliminary experiment was conducted to determine the lethal and sub-lethal concentrations of NaCl. As shown in Fig. S2, the germination percentage of *Vicia faba* (L.) seedlings significantly declined with increasing NaCl concentrations compared to the control. A significant reduction in the germination percentage was observed at 150 mM NaCl by 54%, while germination was completely inhibited at higher concentrations of 200 and 250 mM.

Table 1 and Fig. S3 presents the effects of selenium nanoparticle (SeNPs) priming on germination percentage and growth parameters of *Vicia faba* seedlings under salt stress conditions. The data in Table 1 highlight the protective role of SeNPs in mitigating the adverse effects of salinity. Salt stress (150 mM NaCl) significantly reduced germination by 54% compared to the control. SeNPs alone maintained a 100% germination rate, similar to the control.

Fig. 1 Ultraviolet–visible (UV–Vis) absorption spectrum (a), Fourier-transform infrared (FT-IR) spectra (b), scanning electron microscopy (SEM) images (c), and transmission electron microscopy (TEM) images (d) of selenium nanoparticles (SeNPs) biosynthesized using fenugreek (*Trigonella foenum-graecum*) seed extract



SeNPs priming under salt stress improved germination by 73.9% compared to salt-stressed seedlings, demonstrating its beneficial effect on seed viability. Moreover, salt stress reduced shoot length by 34% compared to the control. SeNPs treatment alone increased shoot length by 19.1% over the control. Under salt stress, SeNPs priming led to a 36.6% increase in shoot length compared to salt stress alone, indicating enhanced growth recovery. Also, salt stress caused

a 49.5% reduction in root length compared to the control. SeNPs alone increased root length by 12.7% over the control. SeNPs priming in salt-stressed seedlings increased root length by 35.2% compared to salt stress alone, suggesting improved root development under stress conditions. Salt stress decreased leaf area by 48.3% compared to the control. SeNPs treatment alone increased leaf area by 9.7%, while SeNPs priming under salt stress resulted in a 37.4%

Table 1 Effect of selenium nanoparticles (SeNPs; 40 mg L⁻¹) on germination percentage, shoot length, root length, leaf area, fresh weight of shoot, dry weight of shoot, fresh weight of root, and dry weight of root in 21-day-old salt-stressed *Vicia faba* (L.) seedlings. Sodium chloride (NaCl, 150 mM) was used to induce salinity stress. Values represent the mean of three replicates $\pm$ standard deviation (SD). Different lowercase letters within the same column indicate significant differences among treatments according to Duncan's multiple range test ($P \leq 0.001$). R² represents the coefficient of determination.

Treatments	Germination percentage (%)	Shoot length (cm)	Root length (cm)	Leaf area (cm ²)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Control	100 $\pm$ 0.0 ^a	15.67 $\pm$ 0.47 ^b	24.41 $\pm$ 0.82 ^b	27.23 $\pm$ 0.45 ^b	3.42 $\pm$ 0.14 ^b	1.47 $\pm$ 0.005 ^b	2.12 $\pm$ 0.0082 ^b	1.09 $\pm$ 0.064 ^b
150 mM NaCl	46 $\pm$ 0.47 ^c	10.33 $\pm$ 0.47 ^d	12.33 $\pm$ 0.47 ^d	14.07 $\pm$ 0.33 ^d	1.93 $\pm$ 0.17 ^d	0.85 $\pm$ 0.04 ^d	1.047 $\pm$ 0.005 ^d	0.33 $\pm$ 0.05 ^d
SeNPs (40 mg L ⁻¹)	100 $\pm$ 0.00 ^a	18.67 $\pm$ 0.47 ^a	27.51 $\pm$ 0.41 ^a	29.87 $\pm$ 0.61 ^a	4.41 $\pm$ 0.22 ^a	2.42 $\pm$ 0.02 ^a	2.963 $\pm$ 0.04 ^a	1.50 $\pm$ 0.005 ^a
SeNPs (40 mg L ⁻¹) +150 mM NaCl	80 $\pm$ 0.32 ^b	14.11 $\pm$ 0.71 ^c	16.67 $\pm$ 0.62 ^c	19.33 $\pm$ 0.47 ^c	2.97 $\pm$ 0.21 ^c	1.17 $\pm$ 0.04 ^c	1.967 $\pm$ 0.005 ^c	1.01 $\pm$ 0.05 ^c
R ²	0.9867	0.9137	0.9239	0.9265	0.9645	0.9654	0.9228	0.9954

increase compared to salt-stressed plants, demonstrating an improved ability to maintain leaf expansion.

However, salt stress reduced fresh and dry shoot weight by 43.5% and 42.2%, respectively, compared to the control. SeNPs treatment alone increased fresh and dry shoot weight by 28.9% and 64.6%, respectively. Under salt stress, SeNPs priming improved shoot fresh and dry weight by 53.9% and 37.6%, respectively, compared to salt-stressed plants, suggesting enhanced biomass. Also, salt stress led to 50.6% and 69.7% reductions in fresh and dry root weight, respectively. SeNPs treatment alone increased root fresh and dry weight by 39.8% and 37.6%, respectively. Under salt stress, SeNPs priming increased root fresh weight by 87.9% and root dry weight by 206% compared to salt stress alone, emphasizing the role of SeNPs in promoting root resilience under stress.

The high R² values (≥ 0.9137) and significant P-values ($P \leq 0.001$) indicate strong statistical reliability. These findings confirm that SeNPs priming enhances *Vicia faba* (L.) growth under salt stress by improving germination, shoot and root development, and biomass.

3.3 Role of Selenium Nanoparticles (SeNPs) On the Photosynthetic Efficiency, Chlorophyll and Carotenoids Contents of Salt-stressed *Vicia Faba* (L.) Seedlings

Data represented in Fig. 2 show that salinity stress (150 mM NaCl) significantly decreased the maximum potential efficiency of PSII (Fv/Fm) by 41.9% compared with the control, although this decrease was counteracted by the pretreatment of salt-stressed seedlings with SeNPs (40 mg L⁻¹) by 21.5% compared with untreated salt-stressed seedlings (Fig. 2a). Results represented in Fig. 2b, c, d show that salinity stress (150 mM NaCl) markedly decreased the content of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Carot.) by 50.2%, 47.1% and 33.8%, respectively compared with control. While, pretreatment of salt-stressed seedlings with SeNPs (40 mg L⁻¹) ameliorated the negative

effects induced by salinity stress on the content of the photosynthetic pigments in salt-stressed seedlings whereas, the content of Chl a, Chl b and carotenoid (Carot.) increased by 47.2%, 15.1% and 24.3% respectively compared with salt-stressed seedlings (Fig. 2b, c, d).

3.4 Improvement of Antioxidant System in Selenium Nanoparticles (SeNPs) Treated Salt-stressed *Vicia Faba* (L.) Seedlings

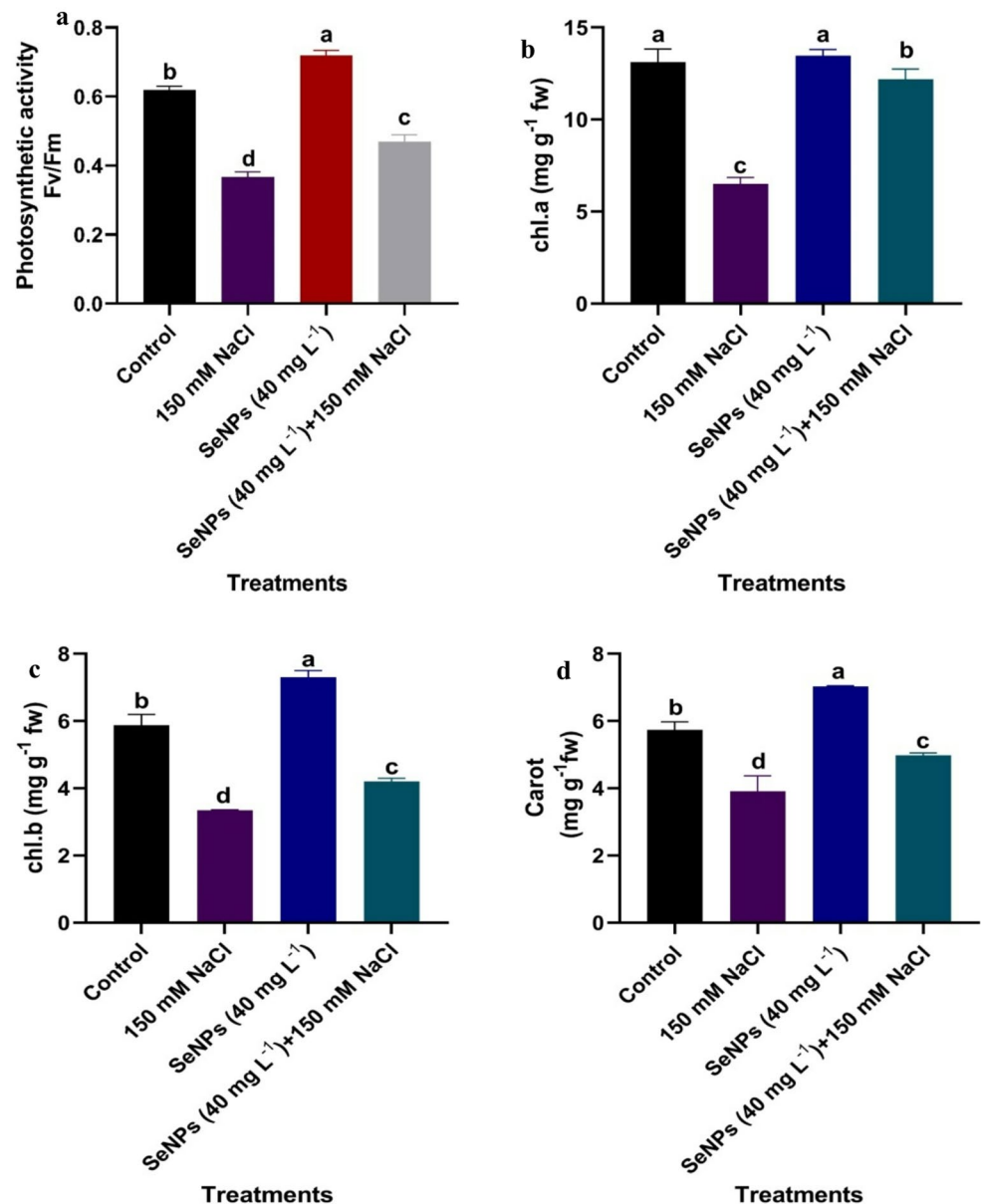
As illustrated in Fig. 3, salt stress (150 mM NaCl) significantly increased the content of MDA and the electrolyte leakage of shoot of salt-stressed faba bean seedlings by 173.3% and 28.4% respectively compared to control. While the effect of pretreatment of salt-stressed seedlings with SeNPs (40 mg L⁻¹) whereas, the content of MDA and electrolyte leakage of *Vicia faba* (L.) seedlings were significantly decreased by 28.5% and 51.3%, respectively compared with salt-stressed seedlings (Fig. 3).

Antioxidant enzymes activity is a good indicator to determine the harmful effect of salt stress on physiological activities within the cells that couldn't be measured with different treatments. Data represented in Fig. 4 indicate a significant increase in the activity of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) enzymes in response to salinity stress (150 mM NaCl), where their activity reached 41.6%, 44.4% and 130.7%, respectively relative to the control. On the other hand, pretreatment with SeNPs (40 mg L⁻¹) led to noticeable decrease in the activity of CAT, POX and SOD by 16.6%, 11.5% and 13.3%, respectively compared to untreated salt-stressed seedlings (Fig. 4).

3.5 Role of Selenium Nanoparticles (SeNPs) On the Contents of Compatible Osmolytes in Salt-stressed *Vicia Faba* (L.) Seedlings

Table 2 illustrates the biochemical responses of *Vicia faba* (L.) seedlings to salt stress and selenium nanoparticle

Fig. 2 Effect of selenium nanoparticles (SeNPs; 40 mg L⁻¹) on photosynthetic efficiency expressed as the maximum quantum yield of photosystem II (Fv/Fm) (a), chlorophyll a content (Chl a) (b), chlorophyll b content (Chl b) (c), and carotenoid content (Carotenoids) (d) in 21-day-old salt-stressed *Vicia faba* (L.) seedlings. Values represent the mean of three replicates $\pm$ standard deviation. Different lower-case letters indicate significant differences among treatments according to Duncan's multiple range test ($P \leq 0.001$)



(SeNPs) priming by assessing total amino acids, proline, total soluble proteins, and total soluble carbohydrates. Data indicate that salt stress (150 mM NaCl) increased total amino acid content by 62.3% compared to the control. SeNPs treatment alone led to a 48.9% increase compared to the control, while SeNPs priming under salt stress reduced amino acid accumulation by 25.9% compared to salt stress alone. This suggests that SeNPs help regulate amino acid metabolism under stress conditions. However, data show that proline content increased by 91.3% under salt stress compared to the control, indicating an osmoprotective response. SeNPs treatment alone had a minimal effect (3.9% increase compared to control). However, under salt stress, SeNPs priming reduced proline accumulation by 26.8% compared to salt stress alone, suggesting a role in reducing

oxidative stress damage. Furthermore, Salt stress elevated the total soluble proteins content by 89.1% compared to the control. SeNPs treatment alone resulted in a 9.6% increase, while SeNPs priming under salt stress reduced the protein content by 29.7% compared to salt stress alone. This suggests that SeNPs help maintain protein stability under stress. Additionally, salt stress significantly increased carbohydrate content by 45.2% compared to the control, likely as an adaptive response. SeNPs treatment alone increased carbohydrates slightly (5.1% compared to control). Under salt stress, SeNPs priming reduced carbohydrate accumulation by 22.5% compared to salt stress alone, indicating improved stress adaptation and metabolic balance. The high R^2 values (≥ 0.8776) and highly significant P -values ($P \leq 0.001$) confirm strong statistical reliability. These results suggest that

Fig. 3 Effect of selenium nanoparticles (SeNPs; 40 mg L⁻¹) on malondialdehyde content (MDA), an indicator of lipid peroxidation (a), and electrolyte leakage, an indicator of membrane stability (b), in 21-day-old salt-stressed *Vicia faba* (L.) seedlings. Values represent the mean of three replicates $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments according to Duncan's multiple range test ($P \leq 0.001$)

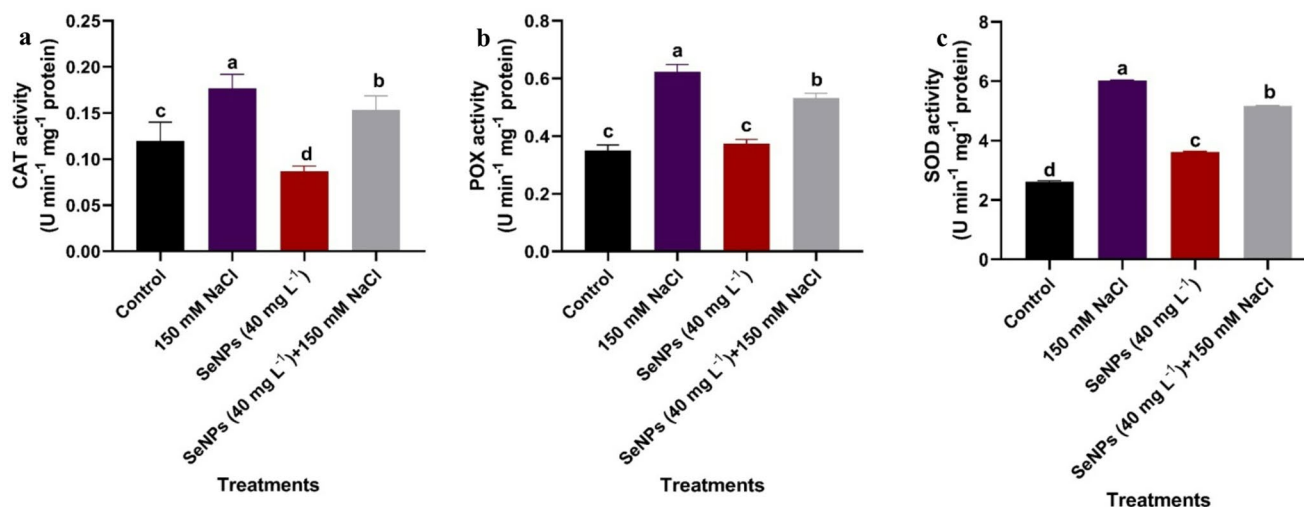
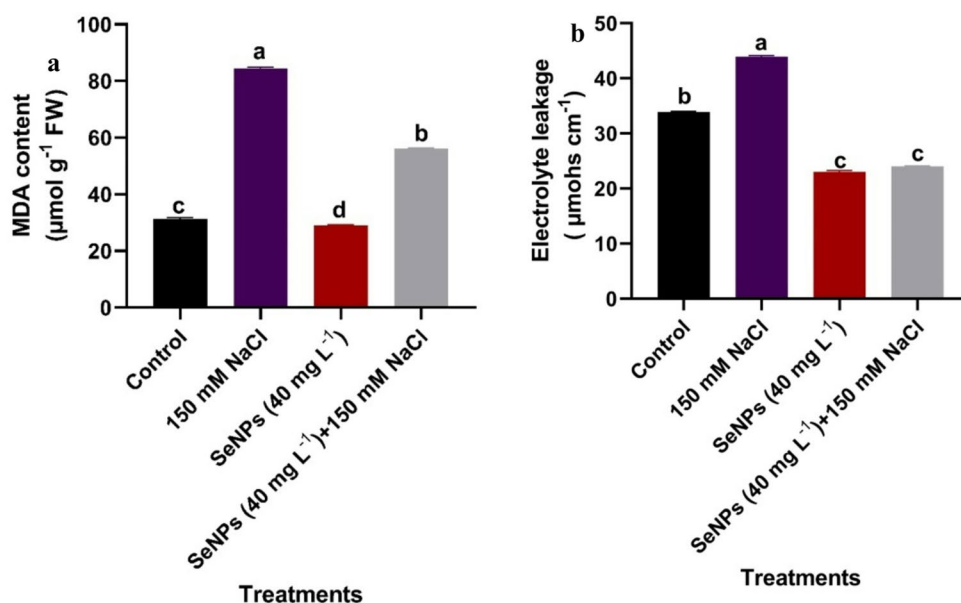


Fig. 4 Effect of selenium nanoparticles (SeNPs; 40 mg L⁻¹) on antioxidant enzyme activities, including catalase (CAT) (a), peroxidase (POX) (b), and superoxide dismutase (SOD) (c), in 21-day-old salt-stressed *Vicia faba* (L.) seedlings. Values represent the mean of three

replicates $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments according to Duncan's multiple range test ($P \leq 0.001$)

Table 2 Effect of selenium nanoparticles (SeNPs; 40 mg L⁻¹) on total amino acid content, proline content, total soluble protein content, and total soluble carbohydrate content in 21-day-old salt-stressed *Vicia Faba* (L.) seedlings. Salinity stress was induced using sodium chloride (NaCl, 150 mM). All biochemical contents are expressed on a dry weight basis (DW). Values represent the mean of three replicates $\pm$ standard deviation (SD). Different lowercase letters within the same column indicate significant differences among treatments according to duncan's multiple range test ($P \leq 0.001$). R² represents the coefficient of determination

Treatments	Total amino acids(mg g ⁻¹ DW)	Proline(mg g ⁻¹ DW)	Total solubleproteins(mg g ⁻¹ DW)	Total solublecarbohydrates(mg g ⁻¹ DW)
Control	31.20 $\pm$ 3.4 ^d	3.33 $\pm$ 0.47 ^c	3.86 $\pm$ 0.4 ^d	6.63 $\pm$ 0.30 ^c
150 mM NaCl	50.63 $\pm$ 3.3 ^a	6.37 $\pm$ 0.28 ^a	7.30 $\pm$ 1.3 ^a	9.63 $\pm$ 0.23 ^a
SeNPs (40 mg L ⁻¹)	46.46 $\pm$ 2.5 ^b	3.46 $\pm$ 0.44 ^c	4.23 $\pm$ 0.73 ^c	6.97 $\pm$ 0.36 ^c
SeNPs (40 mg L ⁻¹) + 150 mM NaCl	37.46 $\pm$ 0.4 ^c	4.66 $\pm$ 0.40 ^b	5.13 $\pm$ 1.3 ^b	7.46 $\pm$ 0.44 ^b
R ²	0.8776	0.9367	0.9385	0.9500

SeNPs priming mitigates salt stress effects by regulating osmolytes accumulation, reducing oxidative damage, and stabilizing protein metabolism, thereby improving stress tolerance in *Vicia faba* (L.) seedlings.

4 Discussion

Salinity is a major abiotic stress that restricts plant growth and productivity by disrupting osmotic balance, nutrient uptake, and metabolic processes (Zorb et al. 2019). In the present study, exposure of *Vicia faba* seedlings to 150 mM NaCl significantly reduced plant height, leaf area, and both fresh and dry biomass. These reductions align with earlier findings in faba bean (Khorasaninejad and Hemmati 2020) and other legumes, confirming that high salinity levels impair photosynthetic capacity and cell turgor. Seed priming with green-synthesized selenium nanoparticles (SeNPs) markedly alleviated these inhibitory effects. Plants treated with SeNPs exhibited improved shoot and root growth and higher biomass compared to the salt-stressed control. This enhancement may be attributed to SeNP-mediated regulation of ion homeostasis, which promotes potassium uptake and reduces sodium accumulation (Handa et al. 2019). Similar growth improvements under SeNP treatments were reported in wheat (Karimi et al. 2020) and tomato (Ahmad et al. 2018), suggesting that SeNPs enhance cellular stability and metabolic recovery under saline environments.

The improvement in photosynthetic efficiency and pigment content observed in SeNP-treated *Vicia faba* seedlings under salinity stress can be attributed to selenium's protective role in maintaining the structural and functional integrity of the photosynthetic apparatus, particularly photosystem II (PSII) (Azimi et al. 2021). Selenium enhances chlorophyll biosynthesis by stimulating the activity of δ -aminolevulinic acid dehydratase, an enzyme critical for tetrapyrrole synthesis, while reducing chlorophyll degradation through the suppression of chlorophyllase activity (Djanaguiraman et al. 2010). Moreover, SeNPs preserve thylakoid membrane stability by preventing lipid peroxidation and protein oxidation induced by reactive oxygen species (ROS), thereby sustaining electron transport and photochemical efficiency (Shahraki et al. 2022; Faisal et al. 2024). The enhanced quantum efficiency of PSII in SeNP-treated plants may also result from the upregulation of antioxidant enzymes that protect the D1 protein from photoinhibition (Feng et al. 2013). This protective mechanism ensures sustained CO₂ fixation and improved photosynthetic performance under saline conditions. Collectively, these findings confirm that SeNPs play a crucial physiological role in maintaining pigment stability, protecting photosynthetic machinery, and ensuring energy balance during stress adaptation.

Salinity-induced oxidative stress leads to excessive production of reactive oxygen species (ROS), which damage lipids, proteins, and membranes (Hasanuzzaman et al. 2012; Sachdev et al. 2023). In this study, salt-stressed plants showed elevated levels of malondialdehyde (MDA) and electrolyte leakage, indicating oxidative injury. Application of SeNPs substantially reduced both parameters, demonstrating their protective role in maintaining membrane integrity. This antioxidative effect was further supported by significant decrease in catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) activities in SeNP-treated plants. These enzymes play complementary roles: SOD catalyzes the conversion of superoxide radicals into H₂O₂, while CAT and POX subsequently detoxify H₂O₂ into water and oxygen (Zhao et al. 2021; Xu et al. 2022). Similar results of antioxidant enzyme activity have been observed in SeNP-treated *Phaseolus vulgaris* (El-Sayed et al. 2023) and wheat seedlings (Alnusairi et al. 2021), confirming that SeNPs strengthen redox homeostasis and cellular protection mechanisms under stress.

Osmolyte accumulation is an adaptive strategy that helps the plant maintain osmotic balance under salinity stress (Jiang et al. 2017). In this study, SeNP treatment enhanced proline, total soluble carbohydrate, and total protein contents in salt-stressed *Vicia faba* plants. Proline acts as a compatible solute that protects macromolecules, stabilizes membranes, and scavenges free radicals (Majumder et al. 2009). The observed increase is consistent with reports by Karimi et al. (2020), who found that SeNPs promoted osmoprotectant accumulation in wheat under salinity stress. Similar effects have been reported in olive plants under drought (Regni et al. 2021) and in strawberries under salinity (Liu et al. 2024), where SeNPs enhanced chloroplast enzyme activity and light-harvesting efficiency. Selenium plays a dual physiological role in regulating osmolyte metabolism depending on environmental conditions. Under optimal growth conditions, selenium stimulates the accumulation of proline, soluble sugars, and proteins by enhancing nitrogen assimilation, carbon metabolism, and antioxidant capacity (Hasanuzzaman et al. 2011). This mild induction acts as a metabolic primer, strengthening plant defense readiness against future stress events. However, under saline conditions, selenium often reduces the overaccumulation of osmolytes such as proline and soluble sugars. This reduction indicates effective mitigation of oxidative and osmotic stress, reflecting a lower requirement for osmolyte-based protection (Shahraki et al. 2022). Selenium enhances the efficiency of antioxidant enzymes and maintains membrane stability, reducing reactive oxygen species (ROS) formation and preventing excessive osmolyte synthesis as a compensatory response (Feng et al. 2013). Furthermore, selenium-mediated regulation of ion homeostasis by decreasing Na⁺ uptake and increasing

K⁺ retention helps maintain osmotic balance and protein stability, leading to improved stress adaptation (El-Badri et al. 2022). Thus, SeNP treatment fine-tunes osmolyte accumulation through a balanced modulation of metabolic and redox pathways, optimizing energy allocation under both normal and saline conditions.

The results of this study are largely consistent with previous reports on SeNP-mediated stress tolerance; however, the novelty lies in using *Trigonella foenum-graecum* (fenugreek) seed extract for SeNP synthesis and applying it to *Vicia faba*. Fenugreek-derived biomolecules such as saponins, flavonoids, and galactomannans likely serve as effective reducing and capping agents, enhancing nanoparticle stability and biological activity. To our knowledge, this is the first report demonstrating that fenugreek-synthesized SeNPs can improve salinity tolerance in faba bean through modulation of antioxidant defense and osmolyte metabolism.

While the present findings confirm the potential of green-synthesized SeNPs as biostimulants under salt stress, several aspects warrant further investigation. The optimal SeNP dosage requires careful assessment, as excessive concentrations may lead to phytotoxicity or altered physiological responses. Moreover, plant responses to SeNPs can vary among species and environmental conditions. Future research should evaluate long-term ecological impacts, nanoparticle persistence in soil, and molecular mechanisms underlying SeNP-induced stress tolerance under field conditions. Overall, fenugreek-mediated SeNPs significantly mitigated salinity stress in *Vicia faba* by improving growth, stabilizing photosynthetic pigments, enhancing antioxidant enzyme activities, and regulating osmolyte accumulation. These findings highlight the promise of SeNPs as eco-friendly, plant-based nanobiostimulants for sustainable crop production in saline environments.

5 Conclusion

This study demonstrates that green-synthesized selenium nanoparticles (SeNPs), derived from fenugreek (*Trigonella foenum-graecum*) seed extract, serve as effective biostimulants for enhancing salt stress tolerance in *Vicia faba* seedlings. SeNP priming significantly improved germination, growth performance, and photosynthetic pigment content under salinity stress. The treatment also mitigated oxidative damage by reducing malondialdehyde (MDA) levels and electrolyte leakage, while upregulating antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX). Additionally, SeNP treatment promoted the accumulation of osmolytes such as proline, soluble proteins, and carbohydrates. These coordinated effects contributed to osmotic adjustment, improved

ion homeostasis, and stabilized redox balance, collectively enhancing the physiological resilience of plants under saline conditions. Therefore, eco-friendly, plant-based SeNPs represent a sustainable and promising approach to improving crop productivity and stress tolerance in salt-affected environments.

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Afaf A. Nessem: Responsible for conceptualization, visualization, methodology, data validation, software application, data collection, and took part in reviewing and editing the manuscript.

Esraa H. Ismael: Focused on methodology, data validation, and data collection.

Walaa A. Abo-Shanab: Responsible for conceptualization, visualization, methodology, data validation, software application, data collection, drafting the initial manuscript, as well as reviewing and editing.

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Data Availability The authors confirm that all data presented in this manuscript are original to their research. Supplementary data related to the experiment have been included in the manuscript. Additional data can be provided upon request by contacting the corresponding author.

Declarations

Conflict of interest The authors affirm that there are no conflicts of interest related to this work.

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