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ORIGINAL ARTICLE

Selenium nanoparticles induce growth and physiological tolerance of wastewater-stressed carrot plants

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Abstract

Climate changes have a direct impact on agricultural lands through their impact on the rate of water levels in the oceans and seas, which leads to a decrease in the amount of water used in agriculture, and therefore the use of alternative sources of irrigation such as wastewater and overcoming its harmful effect on plants was one of the solutions to face this problem. In the present study, the impacts of the synthesized selenium nanoparticles (Se NPs) alone or in combination with glycine betaine and proline treatments on the growth, physiological, and yield attributes of wastewater irrigated carrot plants are investigated. Furthermore, to evaluate heavy metals uptake and accumulation in edible plant parts. The usage of wastewater to carrot plants significantly increased free proline contents, total phenols, superoxide dismutase, catalase, peroxidase, polyphenol oxidase, Malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) throughout the two growth stages. While total soluble carbohydrate and soluble protein content in carrot shoots and roots were significantly reduced. Moreover, the concentrations of nickel (Ni), cadmium (Cd), lead (Pb), and cobalt (Co) in carrot plants were considerably higher than the recommended limits set by international organizations. Application of selenium nanoparticles alone or in combination with glycine betaine and proline reduced the contents of Ni, Cd, Pb, and Co; free proline; total phenols; superoxide dismutase; catalase; peroxidase; polyphenol oxidase; Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) in carrot plants. However, morphological aspects, photosynthetic pigments, soluble carbohydrates, soluble protein, total phenol, and β-Carotene were enhanced in response to Se NPs application. As an outcome, this research revealed that Se NPs combined with glycine betaine and proline can be used as a strategy to minimize heavy metal stress caused by wastewater irrigation in carrot plants, consequently enhancing crop productivity and growth.

Keywords Antioxidant enzymes · Carrot plants · Heavy metals stress · Osmolytes · Selenium nanoparticles

Introduction

One of the main issues for farmers is a lack of water, which tempts them to use wastewater for crop irrigation (Al-Zou'by et al. 2017). Different plant species have developed different mechanisms to cope with wastewater effects (Abu-Elela et al. 2021). The most common chemical contaminants in wastewater appear to be heavy metal cations, hydrocarbons, pesticides, nitrogenous compounds, pharmaceutical residues, detergents, and phosphorus (Agoro et al. 2020). Heavy metals (HMs) accumulation has received a lot of attention in recent years and becoming a more serious issue as a consequence of rapid industrialization and urbanization, the prevalent use of herbicides, pesticides, fertilizers, irrational quarrying and waste management practices (Li et al. 2021). HMs stress has an impact on plant growth as well as, indirectly, public health via the food system (Gupta et al. 2019).

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Also, Pandey et al. (2019) reported that nickel ion inhibited germination rate and early seedling growth of maize and rice; it suppressed germination and early germination percentage of maize and rice in regards to germination rate of seeds, length of roots and roots; the fresh and dry weight of seedlings. Moreover, Yaqvob et al. (2011) indicated that Fe, Pb, and Cu were found to reduce all tomato plant growth parameters.

Nanoparticles (NPs) have seen an increase in their use in industry, medicine, agriculture, and cosmetics in recent times (Adeel et al. 2019). NPs have several benefits over traditional materials, including high surface activity, more surface reaction sites, superior catalytic efficiency, and exceptional optical and magnetic characteristics (Wang et al. 2019). NPs (such as CeO_2, TiO_2, and Mn_3O_4 NPs) could indeed boost antioxidant enzyme activity, which also reduces the accumulation of reactive oxygen species (ROS) in plants, ameliorating plant stress and enhancing quality and yield (Usman et al. 2020). The progress of nanoscience opens up a new avenue for the advancement of soil remediation (Liu et al. 2021). In rice plants, foliar application of selenium and silicon NPs reduced Cd and Pb stress (Hussain et al. 2020; Soliman et al. 2022). In terms of reducing Cd toxicity in maize, foliar spraying of titanium dioxide (TiO_2) NPs outperforms soil addition (Lian et al. 2020). The use of glycine betaine (GB) and proline on plants under heavy metal stress improves growth, photosynthetic activity, oxidative stress, nutrient uptake, and reduces excessive heavy metal uptake and oxidative stress. Furthermore, GB regulates glutathione reductase (GR), ascorbic acid (AsA), and glutathione (GSH) levels in plants under HM stress. An excessive amount of GB via a genetic engineering approach can successfully improve stress tolerance, which is considered as a significant characteristic that requires further investigation (Ali et al. 2020). Proline serves as an organic osmo protectant that accumulates in plants at quite large levels when they are exposed to abiotic stressors (Dar et al. 2016). It also has a variety of activities in plants, including stress tolerance, signaling, radical scavenging, and protein stabilization, as well as acting as a stress receptor (Hayat et al. 2013).

The *Apiaceae* family's most important crop is the carrot (*Daucus carota* L.). It is a root vegetable that is found all over the world. Carrots were cultivated in Europe prior to the tenth century, according to written records. Orange carrots, which are more popular today, were developed in Central Europe during the 15th and 16th centuries. With the recognition of their high provitamin A content, orange carrots have seen a rapid rise in popularity (Iorizzo et al. 2016). Carotenoids and anthocyanins are the most abundant antioxidant pigments in carrots. Carrot cultivar variations are determined by the pigments present. Carotenoids are phytochemicals that are yellow, orange, or red in colour and are observed in most yellow and orange cultivars. The common orange carrot is high in α- and β-carotene and an excellent source of provitamin A (Dias 2012). Different researchers as well carefully investigated in Egypt the impact of wastewater irrigation on various plant species. In this regard, Elgharably and Mohamed (2016) and Merwad (2019) investigated the accumulation of heavy metals in wheat, bean, and onion plants that have been irrigated with wastewater and noticed that, in comparison to plants that were irrigated with ground water, wastewater-irrigated wheat plants had greater concentrations of Zn, Mn, Pb, Ni, and Cd in their edible parts (grains). The present study was conducted to assess whether the application of selenium NPs individually or combined with glycine betaine and proline could ameliorate the harmful effects of wastewater on carrot plants. We combined nano selenium with glycine betaine and proline in this study because they are valuable amino acids with osmoprotectant properties, and their levels vary greatly between different plants. Investigating the physiological, biochemical and yield parameters in response to our treatments. In addition to estimating heavy metals uptake and accumulation in carrot root (edible part).

Materials and methods

Chemicals and reagents

Hi-Media and Difco supplied the media ingredients and components. Chemical compounds including selenoius acid as well as other reagents (used in the following methods) were purchased from (Sigma-Aldrich, Saint Louis, Missouri, United States) at quantitative standard grade.

Seeds and water sampling

The seeds of carrot (*Daucus carota*) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Water samples were collected from each irrigation source (fresh tap water and wastewater effluent). Wastewater samples were collected from El-Rahawy drain (30°12`13.3" N latitude and 31°03′54.3" E longitude), Giza, Egypt, which receives all sewage from El-Giza governorate in addition to agricultural and domestic wastes of El-Rahway village without treatment. The wastewater samples were collected in plastic bottles and used in irrigation.

Biosynthesis of Se NPs using glutathione (GSH)

Under stirring, selenious acid (0.04 mM) was mixed with glutathione (GSH) as a product of *Saccharomyces cerevisiae* (0.2 mM) and 200 mg bovine albumin solution in 100 mL deionized water. To begin the reaction, the pH of the mixture was adjusted to 7.2 with 1.0 M sodium hydroxide. Under sonication, the reaction lasted one hour and produced red elemental Se and oxidized glutathione (GSSG). To separate GSSG from Se NPs, the red solution was dialyzed against double distilled water for 96 h, with the water changing every 24 h.

Characterization of the synthesized biogenic Se NPs

UV-Vis. spectrophotometer (JASCO V-560. UV-Vis. spectrophotometer) represented the optical behavior of the fabricated Se NPs (El-Sayyad et al. 2020), and were compared to a negative control, which consisted of reaction mixture without selenium salt. The XRD-6000 lists, Shimadzu apparatus, SSI, Japan, were used to determine the crystallinity, crystallite size, and lattice of the produced Se NPs. The diffracted X- rays' intensity was calculated as diffracted angle 2θ (El-Batal et al. 2020). The most prevailing Se NPs particle size distribution, hydrodynamic radius, and polydispersity index (PDI) were determined by Dynamic Light Scattering (Zaki et al. 2022) (DLS-PSS-NICOMP 380-USA). Further, the mean particle size, the microstructure, and the estimated shape of the biosynthesized Se NPs were assessed by High-Resolution Transmission Electron Microscope (HRTEM, JEM2100, Jeol, Japan) (Ashour et al. 2018). SEM, ZEISS, EVO-MA10, Germany, was used to study the grain size and surface morphology of Se NPs. Besides, the EDX study (BRUKER, Nano GmbH, D-12,489, 410-M, Germany) was operated to evaluate the Se elemental configuration and the investigated purity (Maksoud et al. 2020).

Methods of planting, treatments, and collection of plant samples

A field experiment was carried out during winter season of 2020–2021 under the natural environmental conditions at Botanical Garden, Botany and Microbiology Departement, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt. The carrot seeds were planted in pots (30 cm diameter) filled with 6.0 kg of clay soil. The pots were divided into 6 groups representing the following treatments: I- Fresh water (Control); II- Wastewater; III- Fresh water + Se NPs (10 ppm); IV- Fresh water + Composite of (Se NPs (10 ppm) + proline (50 ppm) and glycine betaine (50 ppm)); V- Wastewater + Se NPs (10 ppm); VI- Wastewater + composite of (Se NPs (10 ppm) + proline (50 ppm) and glycine betaine (50 ppm)). Plants in each group were treated twice (as a foliar spray) with the aforementioned treatments at 30 and 45 days after sowing. Irrigation was provided to the developed plants as needed until complete germination. The plant samples were collected for analysis at 37 (Stage I) and 52 (Stage II) days after planting. At the end of the growing season (120 days), yields from the various treatments and the control were analyzed.

Analyses of wastewater

Heavy metals contents in fresh tap water and wastewater were estimated according (Association 1995). Salinity (EC), total dissolved solids (TDs), and pH were measured (Table 1), at the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain Shams University, Cairo, Egypt.

Plant analyses

Determination of metabolic contents

Contents of total soluble proteins were assayed according to the methods of (Lowry 1951). Contents of free proline were estimated according to the method described by (Bates et al. 1973). Total phenolic compounds were carried out according to that method described by (Dai et al. 1993). Total soluble carbohydrates in yield were estimated according to the method of (Umbriet et al. 1959).

Assays of enzymes activities and lipid peroxidation

Extraction of enzymes was according to (Mukherjee and Choudhuri 1983). Superoxide dismutase (SOD) activities were estimated using the method of (Marklund and Marklund 1974). Catalase (CAT) activities were assayed according to the methods of (Aebi 1983). Peroxidase (POX) activities were determined according to the method of (Bergmeyer and Bernt 1974), The activities of polyphenol oxidase (PPO) were determined using the methods of (Kar and Mishra 1976). Lipid peroxidation was assayed as malondialdehyde (MDA) content in fresh leaves of Jute mallow according to that method described by (Heath and Packer 1968).

Determination of plant heavy metals contents

Heavy metals contents in the different samples of studied plants (edible plant part) were determined according to (Parkinson and Allen 1975). At the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain Shams University, Cairo, Egypt.

Table 1 Physicochemical analyses of the water used for irrigation

Parameters water samples	pH	EC (dS/m)	TDS (ppm)	Cations (mg L^{-1})				anions (mg L^{-1})				Macro and micro-nutrients (mg/L)						Heavy metals (mg/L)			
				Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-	CO_3^{2-}	HCO_3^-	SO_4^{2-}	P	S	Fe	Zn	Cu	Mn	Ni	Cd	Pb	Co
Fw	6.70	3.37	2156.80	6.00	4.80	0.16	22.75	24.25	0	0.60	8.85	4.81	6.68	0.12	0.01	0.04	0.09	0.05	0.03	0.02	0.05
WW	6.50	10.11	6467.20	4.32	3.66	0.14	92.88	97.12	0	0.78	3.10	5.08	32.98	0.13	0.02	0.33	0.28	0.57	0.34	0.31	0.49

Fw: Fresh water, WW: Wastewater

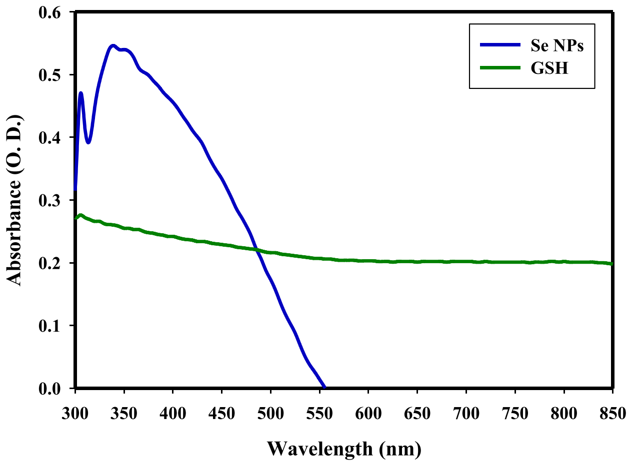

Fig. 1 UV-Vis. spectroscopy of the biogenic Se NPs, and GSH (diluted 10 times)

Statistical analysis

Statistical calculations were carried out using the computer programs SPSS version 25, Minitab version 19, and Microsoft Excel version 365 at 0.05 level of probability. The analysis of variance, one-way ANOVA, and post hoc Tukey's test were used to analyse quantitative data with a parametric distribution. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

Results

Biosynthesis and characterization of Se NPs

The production of Se NPs by GSH was observed in this study by a change in solution color to dark red which operated as a reducing agent or capping agent to reduce selenious acid into Se NPs and stabilize them in colloidal form. Filtrates of the prepared GSH were screened for their capacity for Se NPs biosynthesis. The color of the GSH seemed light, which shifted to extreme red concerning the exhibition of Se NP. The spectra exhibited the experimental peak (Fig. 1) conducting the O. D. (0.546; diluted 10 times), and the Se NPs were small in size, which was observed at 340 nm, according to the UV-Vis. analyses. Additionally, the detected peaks at 305 nm in the Se NPs spectrum corresponded to the GSH constituents. HRTEM image represented the spheroidal forms with moderately mono-dispersed Se NPs with a common size from 9.85 to 11.82 nm. The mean diameter was calculated to be 10.46 nm, as shown in Fig. 2a. The delivered mono-dispersed Se NPs were appointed to the prepared GSH; that was supposed to reduce, stabilize, and preserve agents (El-Batal et al. 2013, 2017, 2018). The DLS technique defined

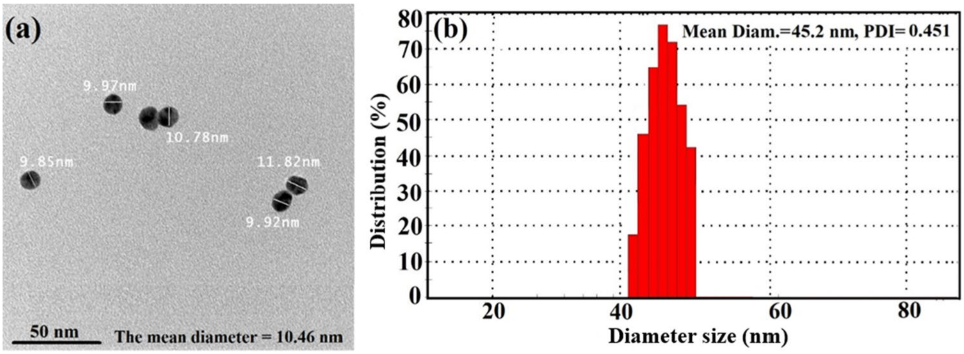

Fig. 2 Mean particle size, shape, particle size distribution and PDI determination of the synthesized Se NPs, where **a** HRTEM imaging, and **b** DLS analysis

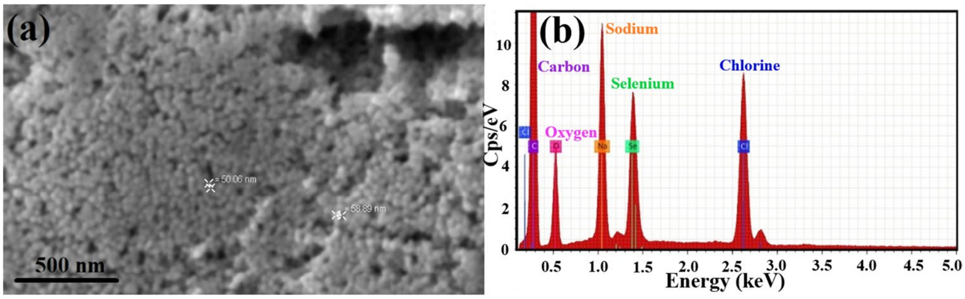

Fig. 3 Morphological shape determination, and elemental analysis of the synthesized Se NPs, where **a** SEM imaging, and **b** EDX analysis. Cps/eV: counts per second per electron-volt

the typical particle size distribution and was estimated as 45.2. The polydispersity index (PDI) can be received from DLS devices or are distinguished from electron micrographs. International standards organizations (ISOs) have demonstrated that PDI values less than 0.05 are more expected to monodisperse models. In contrast, values more than 0.7 are expected to a polydisperse diffusion of particles. Herein, for the accepted PDI values (Fig. 2b), we found that the PDI value was 0.451. The surface features and morphology of the synthesized Se NPs were investigated using SEM images. Figure 3 depicts showed the SEM image of Se NPs biosynthesized by the GSH with variable boundary size and the equivalent spherical formation located within the GSH. Additionally, the SEM image of Se NPs incorporated with GSH, exhibits uniform NPs surfaces, and the surface appearance was clear. It can be detected that Se NPs were isolated typically as a rounded particle (Fig. 3a) across the GSH, which shows as a brilliant NPs combined and stabilized with GSH. EDX study was used to establish the basic structure of the biosynthesized Se NPs and the purity, as described in Fig. 3b. Se NPs exhibited specific absorption peaks of selenium element at 1.40 keV. The lack of other elemental peaks and a massive quantity of selenium in the spectra validate the selenium element purity. The appearance of carbon, oxygen, and chlorine peaks in the synthesized samples was due to the capping and stabilizing GSH, while the presence of Na peak was due to the application of NaOH in the preparation step. EDX examination of the prepared Se NPs by GSH (Fig. 3b) represented the essential peaks that correspond to the basic atoms found in GSH (O, C, Na, K, and Cl). XRD studies were exhibited in Fig. 4 for Se NPs synthesized by using GSH. XRD for the biogenic Se NPs describes the crystal and amorphous compositions for the precursor (GSH) and the synthesized Se NPs. It must be noted 2Θ at 22.21° (donated as *) corresponds to the amorphous kind of GSH. The results of the XRD study of the biosynthesized Se NPs in (Fig. 3) displayed the diffraction characteristics regarding 2Θ at 29.89°,

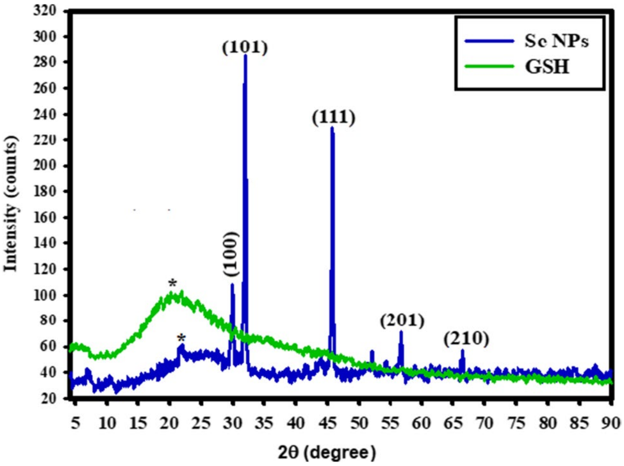

Fig. 4 Crystallinity analysis of the biosynthesized Se NPs by XRD spectrum of GSH and Se NPs

32.31°, 46.14°, 56.76°, and 66.94°, which represented the Bragg's reflections at (100), (101), (111), (201) and (210), respectively. Finally, there is only one amorphous peak at 24.09° (donated as *) for GSH (Fig. 4) involved in the synthesis and stability of Se NPs. On the other hand, the average crystallite size of the biosynthesized Se NPs was determined by using the Williamson-Hall (W-H) equation (Belavi et al. 2012; Ashour et al. 2018; Maksoud et al. 2018; Pal et al. 2018; Abdel Maksoud et al. 2019), and was observed to be 18.59 nm according to the Eq. 1.

$$\beta \cos \theta = \frac{k\lambda}{D_{W-H}} + 4\varepsilon \sin \theta \qquad (1)$$

Where DW-H is the average crystallite size, β is the full-width at half maximum, λ is the X-ray wavelength and θ is the Bragg's angle, k is a constant and ε is the strain of the samples.

Growth aspects

Lengths of shoots, roots, and number of leaves

The variations in growth parameters of carrot plants respond to wastewater irrigation as well as the use of selenium NPs alone or in combination with glycine betaine and proline are listed in (Fig. 5). Statistics revealed that when compared to control samples, wastewater irrigation dramatically improved all tested growth parameters. Furthermore, when compared to controls, treatment with selenium NPs alone or in combination with glycine betaine and proline had a positive effect on all growth parameters (Fig. 5). Concerning the impact of selenium NPs on the stressed plants, it was noticed that a single implementation with selenium NPs boosted shoot length, root length, and the number of leaves, respectively versus stressed plants. Plants treated with selenium NPs in combination with glycine betaine and proline have

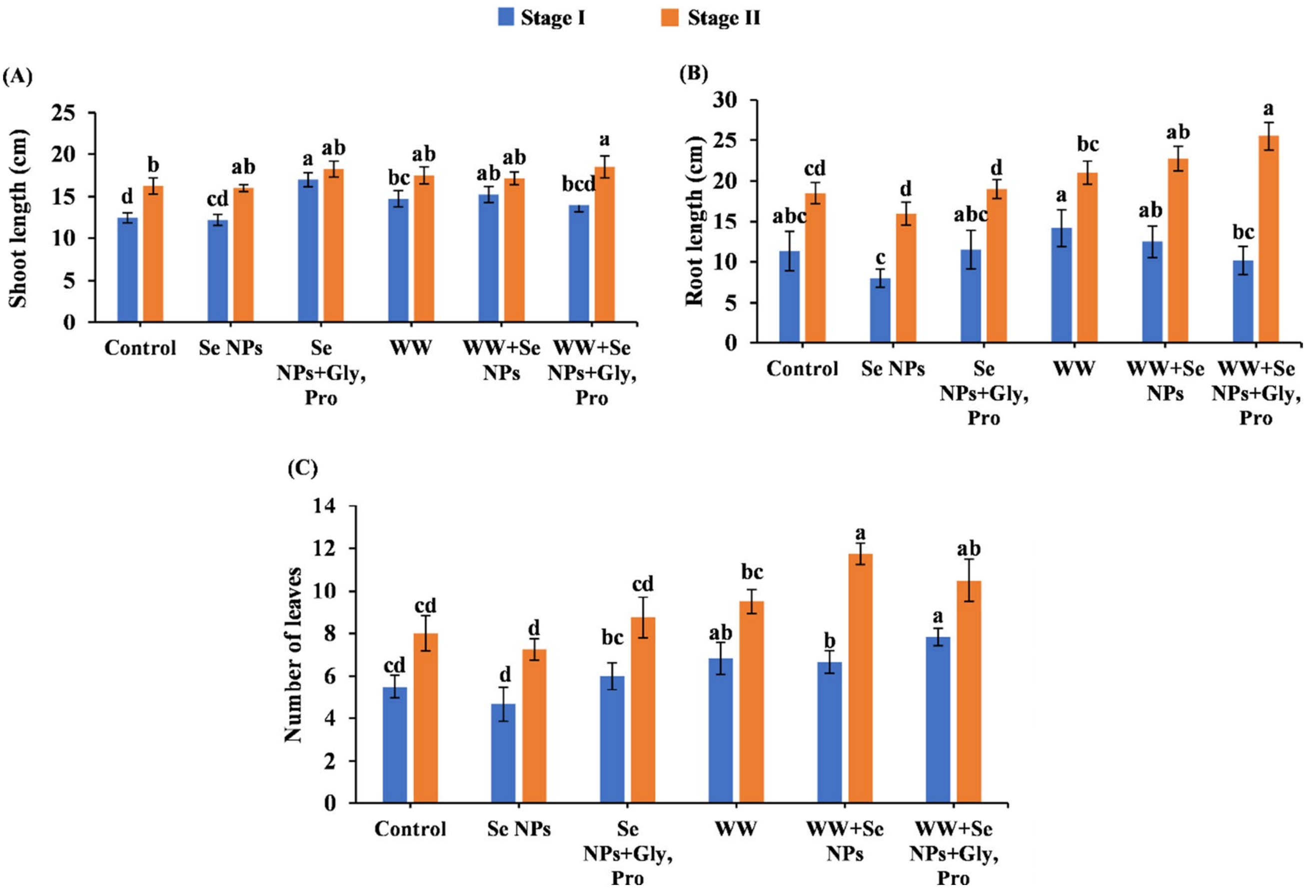

Fig. 5 Effect of Se NPs singly or in combination with glycine betaine and proline on **a** shoot length, **b** root length (cm) and **c** number of leaves / plants of carrot plant under fresh or wastewater effect. Each value is mean of 10 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

Table 2 Effect of Se NPs singly or in combination with glycine betaine and proline on fresh and dry weights (g plant^{-1}) of shoots and roots of carrot plant under fresh or wastewater effect

Treatments	Fresh weight of Shoots		Dry weight of Shoots		Fresh weight of Roots		Dry weight of Roots	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control (Fw)	3.55 ± 0.19c	4.43 ± 0.38d	0.27 ± 0.10c	0.40 ± 0.11d	9.94 ± 0.93bc	13.72 ± 1.43b	0.73 ± 0.17ab	1.47 ± 0.22a
WW	3.54 ± 0.65c	4.61 ± 0.30c	0.21 ± 0.13c	0.48 ± 0.16 cd	8.57 ± 0.84c	9.96 ± 1.72c	0.64 ± 0.11b	0.88 ± 0.19b
Se NPs	5.27 ± 0.53b	6.17 ± 0.35bc	0.36 ± 0.09bc	0.62 ± 0.06bc	9.49 ± 0.79bc	14.62 ± 0.90b	0.79 ± 0.12ab	1.51 ± 0.29a
Se NPs + Gly., Pro)	4.68 ± 0.45b	5.90 ± 0.26bc	0.71 ± 0.12a	0.92 ± 0.09a	12.18 ± 0.64a	17.19 ± 1.34a	1.01 ± 0.10a	1.87 ± 0.25a
WW + Se NPs	6.59 ± 0.55a	8.10 ± 0.22a	0.56 ± 0.07ab	0.84 ± 0.08ab	10.79 ± 0.58ab	15.47 ± 1.03ab	0.87 ± 0.14ab	1.50 ± 0.26a
WW + (Se NPs + Gly, Pro)	5.36 ± 0.32b	6.27 ± 0.37b	0.59 ± 0.09ab	0.69 ± 0.07bc	10.01 ± 0.72bc	13.84 ± 0.94b	0.95 ± 0.13a	1.72 ± 0.27a
HSD	0.44	0.30	0.11	0.11	0.70	1.16	0.14	0.26

Each value is mean of 10 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

seen the greatest increases in shoots, root length, and number of leaves. Table 2 shows the effect of wastewater and the application of selenium NPs alone or in combination with glycine betaine and proline on the fresh and dry weights of shoots and roots in carrot plants. When compared to unstressed plants, carrot plants that were irrigated with wastewater showed a significant decrease in fresh and dry weight of shoots and roots (control). Contrarily, the application of selenium NPs combined with glycine betaine and proline alone or under stress conditions led to improvements in the fresh and dry weight of shoots and roots of carrot plants.

Photosynthetic pigments

The levels of chlorophyll content (chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoids) in stressed carrot plant leaf tissue were assessed to determine the protective roles of selenium NPs application on photosynthetic pigments under wastewater irrigation stress (Fig. 6). There was a decrease in chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoid content in stressed plants when compared to the untreated control sample (Fig. 6). When compared to only freshwater irrigated plants, selenium NPs application alone or in combination with glycine betaine and proline protected photosynthetic pigments from heavy metal-induced harmful effects, as evidenced by increased contents of chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoids in response to wastewater, respectively (Fig. 6). Non-stressed carrot plants treated with selenium NPs singly or combined with glycine betaine and proline also showed increased contents of chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoids, when compared with non-stressed control plants.

Organic solutes and secondary metabolites

The impact of wastewater irrigation and single or combined application of selenium NPs on the contents of total soluble carbohydrates in carrots shoots and roots are shown in Table 3. The total soluble carbohydrates content of carrot plants is significantly reduced due to wastewater irrigation. In terms of the interaction between wastewater irrigation and selenium NPs treatment, it was discovered that the application of selenium NPs combined with glycine betaine and proline was able to increase the contents of sugars in wastewater-stressed carrot plants when compared to untreated plants. The results presented in Table 3 show the effect of wastewater and selenium NPs on the soluble protein content of carrot plants. It was discovered that carrot plants irrigated with wastewater had a significantly lower content of soluble protein than unstressed plants (control). Conversely, applying selenium NPs individually or under stress conditions improved the soluble protein content of carrot plants. Table 3 summarizes the effects of wastewater stress, selenium NPs application alone or in combination with glycine betaine + proline, and their interactions on the content of free proline in carrot plants. The content of total phenol in carrot plant leaves under heavy metal stress and the application of selenium NPs were shown in Table 4. In this study, selenium NPs significantly increased phenol levels in heavy metal-stressed carrot plants, as well as osmolytes such as soluble protein, proline, and total soluble carbohydrates in shoots and roots (Table 4).

Antioxidant enzymes

The data analysis of our results indicated that wastewater irrigation and Se NPs application, alone or in combination with glycine, had a significant impact on the antioxidant

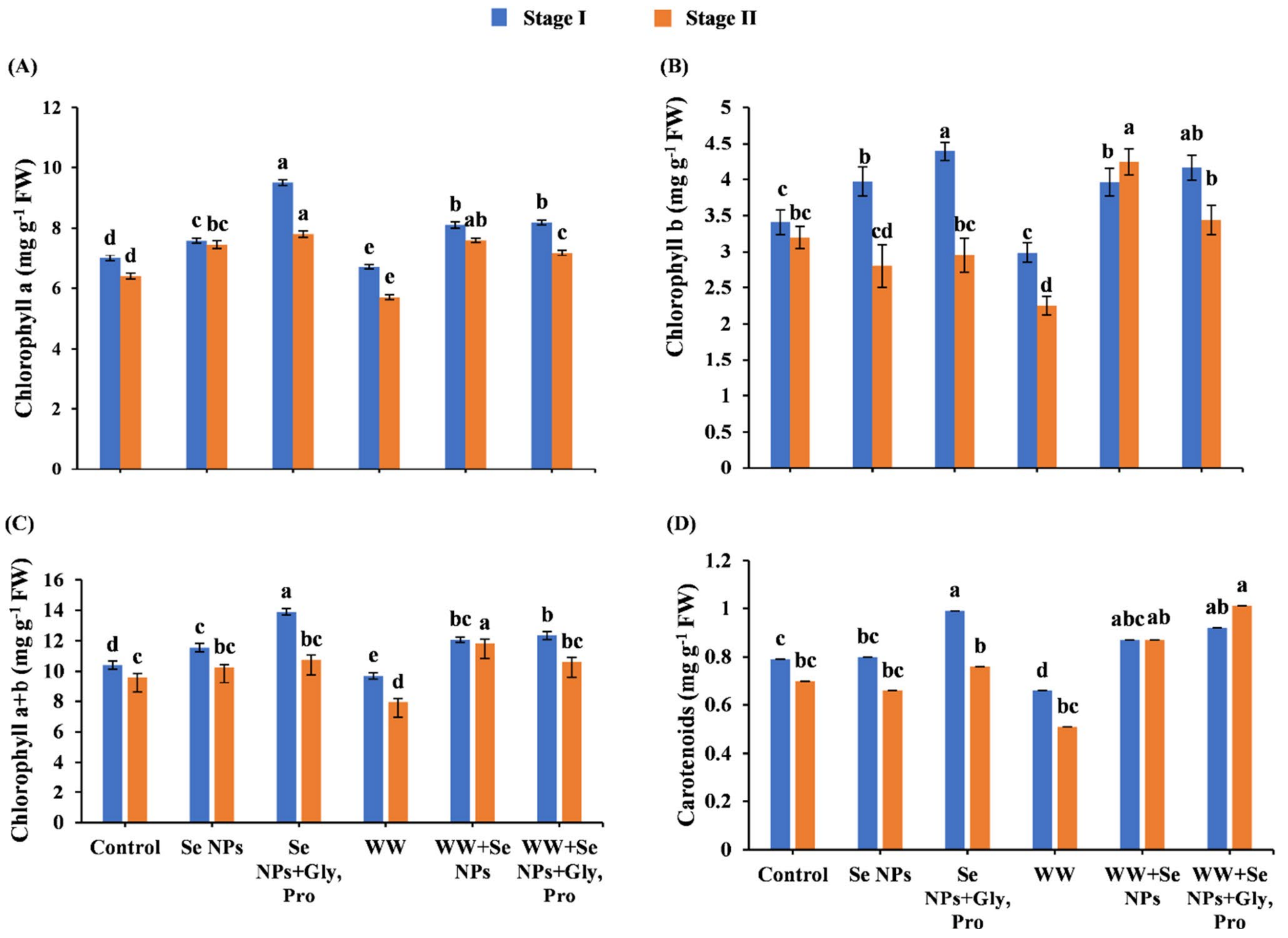

Fig. 6 Effect of Se NPs singly or in combination with glycine betaine and proline on the chlorophyll and carotenoids contents (mg g^{-1} FW) of carrot plant under fresh or wastewater effect. Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. FW: Fresh weight

Table 3 Effect of Se NPs singly or in combination with glycine betaine and proline on the total soluble carbohydrate and protein contents (mg g^{-1} dry weight) of carrot plant under fresh or wastewater effect

Treatments	Soluble carbohydrates in shoot		Soluble carbohydrates in root		Soluble proteins in shoot		Soluble proteins in root	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control (Fw)	15.38 ± 0.15d	16.49 ± 0.26e	35.58 ± 0.16d	46.50 ± 0.15e	7.94 ± 0.10c	7.02 ± 0.09c	6.82 ± 0.10d	8.23 ± 0.06e
WW	11.31 ± 0.20f	12.52 ± 0.21f	28.77 ± 0.23e	37.74 ± 0.17f	5.62 ± 0.06e	5.31 ± 0.11e	4.92 ± 0.12e	6.70 ± 0.04f
Se NPs	19.54 ± 0.18a	30.32 ± 0.16a	53.36 ± 3.08a	58.47 ± 0.19c	8.28 ± 0.08b	7.96 ± 0.07a	9.33 ± 0.07a	9.17 ± 0.08c
Se NPs + Gly., Pro)	16.75 ± 0.16c	26.51 ± 0.25c	47.69 ± 0.20b	61.04 ± 0.22a	7.61 ± 0.09d	6.41 ± 0.10d	8.17 ± 0.11c	9.82 ± 0.05b
WW + Se NPs	18.35 ± 0.12b	28.87 ± 0.19b	37.23 ± 0.15d	54.29 ± 0.20d	7.78 ± 0.12 cd	7.38 ± 0.04b	9.20 ± 0.06a	11.45 ± 0.09a
WW + (Se NPs + Gly, Pro)	14.26 ± 0.14e	23.66 ± 0.22d	41.69 ± 0.19c	59.26 ± 0.24b	8.65 ± 0.07a	8.01 ± 0.06a	8.75 ± 0.09b	8.88 ± 0.11d
HSD	0.21	0.27	1.60	0.25	0.11	0.10	0.12	0.09

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

Table 4 Effect of Se NPs singly or in combination with glycine betaine and proline on the free proline contents (mg g^{-1} dry weight) and total phenol (mg 100 g^{-1} dry weight) in shoot of carrot plant under fresh or wastewater effect

Treatments	Free proline in shoot		Total phenol in shoot	
	Stage I	Stage II	Stage I	Stage II
Control (Fw)	3.48 ± 0.05d	5.68 ± 0.02e	0.31 ± 0.03d	0.34 ± 0.05f
WW	8.69 ± 0.07a	11.76 ± 0.05a	0.50 ± 0.05a	0.52 ± 0.07a
Se NPs	3.22 ± 0.09e	6.57 ± 0.04d	0.35 ± 0.02c	0.36 ± 0.03e
Se NPs + Gly, Pro)	3.55 ± 0.06d	5.82 ± 0.07e	0.36 ± 0.07c	0.39 ± 0.04d
WW + Se NPs	4.72 ± 0.04c	8.01 ± 0.09b	0.42 ± 0.09b	0.44 ± 0.06b
WW + (Se NPs + Gly., Pro.)	5.36 ± 0.07b	7.11 ± 0.11c	0.37 ± 0.03c	0.41 ± 0.03c
HSD	0.09	0.09	0.01	0.01

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

Table 5 Effect of Se NPs singly or in combination with glycine betaine and proline on the activity of superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), and polyphenol oxidase (PPO) enzymes (unit/g. F.wt./hour) of carrot plant under fresh or wastewater effect

Treatments	superoxide dismutase (SOD) (unit/g. F.wt./hour)		catalase (CAT) (unit/g. F.wt./hour)		peroxidases (POX) (unit/g. F.wt./hour)		polyphenol oxidase (PPO) (unit/g. F.wt./hour)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control (Fw)	2.70 ± 0.24b	4.90 ± 0.42c	20.50 ± 2.12b	8.50 ± 0.71c	2.65 ± 0.35a	1.15 ± 0.49b	3.76 ± 0.68d	1.16 ± 0.17b
WW	7.60 ± 0.85a	13.20 ± 0.57a	39.25 ± 2.47a	21.25 ± 1.06a	7.15 ± 0.64a	4.85 ± 0.92a	11.72 ± 0.17a	5.52 ± 0.57a
Se NPs	2.90 ± 0.71b	5.20 ± 0.28c	23.25 ± 3.18b	9.50 ± 0.69c	3.80 ± 3.54a	0.95 ± 0.11b	4.72 ± 0.34 cd	1.80 ± 0.74b
Se NPs + Gly., Pro)	3.20 ± 0.82b	5.70 ± 0.79c	21.75 ± 1.77b	8.75 ± 0.35c	3.70 ± 2.12a	0.80 ± 0.14b	5.20 ± 0.11 cd	1.88 ± 0.99b
WW + Se NPs	4.00 ± 0.57b	6.50 ± 0.42bc	29.75 ± 3.89ab	15.50 ± 0.71b	5.35 ± 1.34a	2.30 ± 0.79b	7.96 ± 0.51b	3.60 ± 0.11ab
WW + (Se NPs + Gly., Pro)	5.10 ± 0.89ab	8.20 ± 0.25b	26.50 ± 2.12b	14.25 ± 0.33b	5.45 ± 0.92a	1.25 ± 0.35b	5.44 ± 0.34c	2.16 ± 0.23b
HSD	1.28	0.95	4.66	1.20	3.17	1.05	0.70	1.15

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

enzyme activities of carrot plants (Table 5). When carrot plants were irrigated with wastewater, the activities of peroxidase (POD), superoxide dismutase (SOD), Catalase (CAT), and polyphenol oxidase (PPO) were significantly increased compared to unirrigated plants. Table 5 shows that when carrot plants are individually treated with selenium NPs, either alone or in combination with glycine betaine and proline, there are no significant changes in antioxidant enzyme activities when compared to controls (untreated plants) in normal conditions. Under wastewater stress conditions, selenium NPs either singly or combined with glycine betaine and proline exhibit a significant boost in the activities of antioxidant enzymes (POD, SOD, CAT, and PPO) in carrot plants when compared to control plants.

Stress biomarkers

Carrot plants grown under wastewater stress had higher levels of MDA and H_2O_2 compared to non-stressed controls (Fig. 7). This was the case throughout the two stages of carrot growth. Application of selenium NPs either singly or combined with glycine betaine and proline to wastewater-stressed plants played a pivotal role in minimizing the level of MDA and H_2O_2 compared with the plants exposed to wastewater stress only (Fig. 7). These effects are more pronounced in plants grown under wastewater stress and when selenium NPs combined with glycine betaine and proline are used especially at stage II of carrot growth. Under normal conditions carrot plants sprayed with selenium NPs showed decreasing in the level of MDA and H_2O_2 respectively, as compared with that of untreated control.

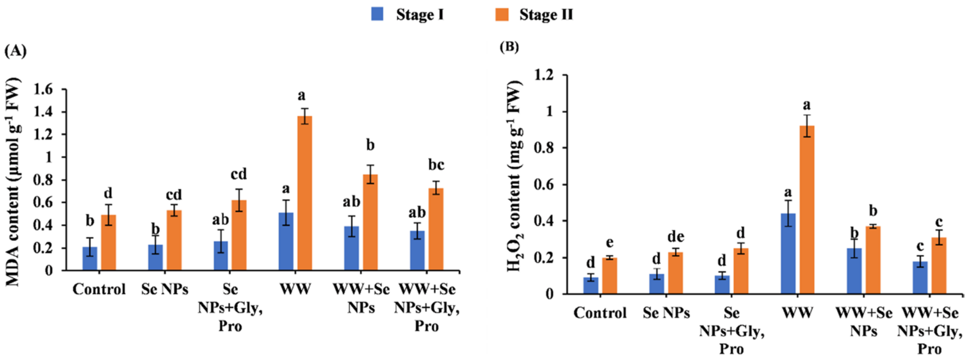

Fig. 7 Effect of Se NPs singly or in combination with glycine betaine and proline on the malondialdehyde (MDA) (μmol g^{-1} FW) and hydrogen peroxide (H_2O_2) (mg g^{-1} FW) contents of carrot leaves under fresh or wastewater effect. Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. FW: Fresh weight

Table 6 Effect of Se NPs singly or in combination with glycine betaine and proline on Yield characters of carrot plant under fresh or wastewater effect

Treatments	Shoot length (cm)	Root length (cm)	Root Diameter (cm)	Shoot FW (g $plant^{-1}$)	Shoot DW (g $plant^{-1}$)	Root FW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)
Control (Fw)	20.67 ± 2.73bc	20.00 ± 0.82 cd	6.25 ± 0.50b	5.03 ± 0.84c	0.74 ± 0.14c	23.47 ± 2.03b	1.37 ± 0.28c
WW	18.33 ± 2.34c	18.25 ± 1.26d	4.50 ± 0.58c	6.85 ± 0.71b	0.81 ± 0.17bc	17.79 ± 1.13c	0.57 ± 0.37d
Se NPs	25.17 ± 2.64a	21.25 ± 0.96c	7.88 ± 0.48a	6.97 ± 0.86b	0.97 ± 0.15abc	25.16 ± 1.20ab	2.21 ± 0.31ab
Se NPs + Gly, Pro)	23.83 ± 2.14ab	23.25 ± 0.50b	7.38 ± 0.75ab	9.36 ± 0.62a	1.28 ± 0.13a	27.49 ± 1.90a	2.92 ± 0.34a
WW + Se NPs	19.00 ± 0.89c	23.50 ± 0.58b	6.75 ± 0.96ab	8.92 ± 0.88a	1.12 ± 0.15ab	23.48 ± 2.06b	1.49 ± 0.36bc
WW + (Se NPs + Gly, Pro)	20.33 ± 0.82bc	26.75 ± 0.96a	7.50 ± 0.58ab	8.09 ± 0.74ab	1.08 ± 0.12ab	26.59 ± 0.69a	2.40 ± 0.33a
HSD	1.74	0.93	0.70	0.72	0.15	1.47	0.36

Each value is mean of 10 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine, FW = Fresh weight, DW = Dry weight

Table 7 Effect of Se NPs singly or in combination with glycine betaine and proline on β-Carotene (mg/100gm Fresh Weight), total soluble carbohydrates, proteins, and free proline (mg/g. Dry Weight) of carrot roots at yield stage under fresh or wastewater effect

Treatments	β-Carotene in roots mg/100gm FW	Soluble carbohydrates in roots mg/g. DW	Soluble proteins in roots mg/g. DW.	Free proline in roots mg/g. DW
Control (Fw)	0.51 ± 0.06bc	53.01 ± 0.26e	6.64 ± 0.10d	2.84 ± 0.04f
WW	0.45 ± 0.02c	39.00 ± 0.19f	5.88 ± 0.08e	9.24 ± 0.09a
Se NPs	0.61 ± 0.01ab	56.24 ± 0.22d	7.15 ± 0.05c	5.12 ± 0.07d
Se NPs + Gly, Pro)	0.58 ± 0.03abc	73.22 ± 0.18a	7.53 ± 0.03b	4.76 ± 0.05e
WW + Se NPs	0.66 ± 0.05a	60.57 ± 0.16c	8.12 ± 0.07a	5.42 ± 0.04c
WW + (Se NPs + Gly, Pro)	0.54 ± 0.08abc	72.52 ± 0.23b	7.08 ± 0.09c	8.71 ± 0.05b
HSD	0.06	0.26	0.10	0.06

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine, FW = Fresh weight, DW = Dry weight

Yield aspects and biochemical measurements

Heavy metal stress reduced shoot length, root length, root diameter, fresh, dry weight of shoot, and fresh, dry weight of root significantly (Table 6). The use of selenium NPs in combination with glycine betaine and proline reduced the negative effects of heavy metal stress on carrot plant yield characteristics. Concerning the implementation of selenium NPs, the results presented in Table 6 show that when carrot plants are individually treated with selenium NPs in normal conditions, there is a significant increase in yield parameters when compared to controls (untreated plants). The osmolytes and β-Carotene contents of carrot yield is significantly declined in response to wastewater irrigation. With respect to the interaction between wastewater irrigation and selenium NPs treatment, it was shown that the application of selenium NPs combined with glycine betaine and proline was able to increase the contents of osmolytes and β-Carotene in wastewater-stressed carrot plants when compared to untreated plants (Table 7).

Accumulation of heavy metals in yield

The impact of wastewater stress, the selenium NPs singly or combined with glycine betaine and proline, and their interactions on the heavy metal accumulation in carrot plants are clarified in (Table 8). Under wastewater stress conditions carrot plants showed significant increases in Ni, Cd, Pb, and Co content when compared to control plants (Table 8). However, the application of selenium NPs, selenium NPs + glycine betaine, and proline resulted in a remarkable decrease in the Ni, Cd, Pb, and Co content when compared with stressed carrot plants (Table 8).

Discussion

Selenium is essential nutrient for humans and has many important biological functions, including immune regulation, antioxidant, antiviral, and anti-cancer properties. (Fairweather-Tait et al. 2011; Rayman 2012). Selenium is primarily supplied by eating cereal crops, but the content is low in most rice-producing countries in Asia and Africa. As per the World Health Organization (WHO), approximately 15% of the planet's population is deficient in Selenium (Tan et al. 2018). So, increasing the selenium content of cereal crops with Se-containing fertilizer is essential to human health. Heavy metal stress had already been regarded as a significant issue in many terrestrial ecosystems worldwide. The industrialization has recently had a negative impact on soil and crop productivity by accumulating heavy metals. (Bashandy et al. 2020). The bioactive phytochemicals from plant extracts serve as a capping agent, preventing NPs aggregation and altering their biological activity (Fahimirad et al. 2019; Paiva-Santos et al. 2021). (Yilmaz et al. 2021), indicated that the pomegranate and watermelon peel extracts have a higher total phenolic content, and GSH which promotes in the reduction of silver ions to nanoscale-sized silver particles. The produced red color was assigned to the stimulation of biogenic Se NPs surface Plasmon alterations and provided a correct spectroscopic signal of their appearance (El-Batal et al. 2016; El-Ghazaly et al. 2017). The existence of filtrate peaks is shown by the UV-Vis spectrum of the produced GSH, which matches the literature studies (Akhtar et al. 2015; Nasiriboroumand et al. 2018). The intensity of the red color constructed was fitting to the power of the prepared GSH to biosynthesize Se NPs (El-Sayyad et al. 2020). Usually, surface plasmon resonance (SPR) is influenced by the intensity, dimension, morphological surfaces, structure and dielectric manners of the synthesized Se NPs

Table 8 Heavy metals accumulation in roots (edible part) of carrot plants at yield stage in response to Se NPs singly or in combination with glycine betaine and proline under fresh or wastewater effect

Treatments	Ni mg kg^{-1}	Cd	Pb	Co
Control (Fw)	0.014 ± 0.001c	0.017 ± 0.003c	0.033 ± 0.005d	0.007 ± 0.002d
WW	0.305 ± 0.003a	0.360 ± 0.007a	0.401 ± 0.008a	0.288 ± 0.006a
Se NPs	0.012 ± 0.005c	0.015 ± 0.002c	0.027 ± 0.003d	0.006 ± 0.001d
Se NPs + Gly, Pro)	0.009 ± 0.002c	0.011 ± 0.003c	0.018 ± 0.005d	0.001 ± 0.001d
WW + Se NPs	0.080 ± 0.008b	0.077 ± 0.005b	0.192 ± 0.009b	0.088 ± 0.007b
WW + (Se NPs + Gly, Pro)	0.061 ± 0.006b	0.075 ± 0.007b	0.149 ± 0.007c	0.052 ± 0.004c
HSD	0.008	0.009	0.011	0.007
Permissible limit*	0.10	0.10	0.20	0.10

Each value is mean of 3 replicates ± standard error of means. *Permissible limits are according to FAO/WHO (2019, 2020). Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at $P \leq 0.05$, values of the same column with the same letter are not significantly different. WW = Wastewater, Fw = Fresh water, Pro = Proline, Gly = Glycine betaine

(Kelly et al. 2003; Prasad and Selvaraj 2014; El-Baz et al. 2016; Attia et al. 2019).To investigate the average particle size and the desired shape of the biosynthesized Se NPs, HTEM images were performed, and its outcomes were compared with the DLS analysis, which operated to define particle size distribution, hydrodynamic radius, and polydispersity index (PDI) (El-Batal et al. 2016; El-Baz et al. 2016; Elkodous et al. 2019). The current values demonstrate that the biosynthesized Se NPs was a moderate mono-size spread. The particle size distribution estimated by the DLS method was found to be greater than the average particle size determined by HRTEM images. Because of the large sizes of the biosynthesized Se NPs, the DLS process assessed the hydrodynamic radius that established close to the biosynthesized Se NPs and surrounded by water layers (El-Batal et al. 2014, 2016; El-Baz et al. 2016; Hanora et al. 2016; Baraka et al. 2017), in the Se NPs biosynthesized by GSH, as shown in Fig. 2b. The EDX study is an analytic approach used for the elemental analysis or the chemical definition of the fabricated specimens (Baraka et al. 2017; Ashour et al. 2018; Maksoud et al. 2018; Elkodous et al. 2019). XRD analysis tested the crystal composition and the average crystal size of the synthesized Se NPs because it provides the state of the observed atoms (Ashour et al. 2018; Maksoud et al. 2018; Abdel Maksoud et al. 2019; Pal et al. 2019). In XRD results, all of the peaks matched the Joint Committee on Powder Diffraction Standards (JCPDS) of Se NPs with a standard card such as JCPDS File No 06-0362 (Prasad and Selvaraj 2014; Bai et al. 2017). This suggests that biogenic Se NPs crystallized in nature and formed the face-centered cubic (fcc) crystalline structure. Still, its intensity was shorter than that detected in GSH, and 2Ө was shifted due to the incorporation of Se NPs to the active GSH (El-Sayyad et al. 2020). Heavy metal stress has an impact on plant growth and metabolism, resulting in a decrease in most growth indices. Different studies have found a decrease in growth parameters as a result of heavy metal stress (Tiwari and Lata 2018). Our data are compatible with those of (Shahid et al. 2015; El-Shahir et al. 2021), who discovered that heavy metal stress significantly reduced plant height, root length, fresh and dry weights of shoots and roots. Heavy metal binds to the cell wall and the middle lamellae, enhancing pectin cross-linking and contributing to growth reduction, as well as to the plant as a whole (Khan et al. 2017). Furthermore, proline application improved the growth of chickpea plants grown under heavy metal stress (Hayat et al. 2013). Exogenous proline treatment resulted in better growth, photosynthetic efficiency, and antioxidant enzyme activity, all of which were associated with a higher yield. Selenium has been shown to boost the growth of stressed plants (Shekari et al. 2017; Shalaby et al. 2021). Moreover, (Soliman et al. 2022) proved that the applying selenium nanoparticles to wheat plants under stress or normal conditions significantly increased shoot lengths and root weights. Photosynthetic pigments are essential components of photosynthesis and play an important role in plant growth and yield attributes. In this study, heavy metal toxicity resulted in a significant decrease in chlorophyll a, chlorophyll b, and carotenoid concentrations in carrot plant leaves. Heavy metals also inhibit photosynthesis by disrupting the ultrastructure of chloroplasts and preventing the synthesis of essential pigments, inhibiting the Calvin cycle and the electron transport chain, and causing a carbon dioxide shortage by closing stomatal pores (Giannakoula et al. 2021). MDA and hydrogen peroxide accumulation could explain the decrease in photosynthetic pigments under heavy metal stress. The use of selenium nanoparticles in combination with glycine betaine and proline increased the content of photosynthetic pigments while eliminating the negative effects of heavy metal stress, as Se NPs could lower MDA and H_2O_2 levels in carrot leaves. This finding confirms the view of (Alnusairi et al. 2022), who mentioned that organic osmolytes, such as proline, are recognized to maintain cell water potential, safeguard macromolecules and enzymes from oxidative damage, increase enzyme activities, minimize H_2O_2 concentrations, and enhance tolerance of plants to oxidative stress conditions. The amino acid proline, which acts as an osmoprotectant, is more abundant in plants exposed to wastewater. The results also show that proline content significantly increased once carrot plants were irrigated with wastewater. In contrast, using the Se NPs under wastewater stress reduced the amount of proline in carrot plants compared to plants stressed by wastewater but not treated with the Se NPs (Table 5). Accordingly, proline levels in Se NPs-treated carrot plant leaves were significantly lower than in non-treated plants. Plants have developed organic solutes that regulate a variety of physiological processes in response to abiotic stress. In our experiment, the carrot plants under heavy metal stress showed higher levels of soluble carbohydrates, soluble protein, and total proline contents compared with non-stressed plants. Proline accumulation in plants may be responsible for preventing heavy metal-mediated lipid peroxidation and membrane alteration (Karakas et al. 2022). However, soluble protein, soluble carbohydrates, and free proline concentration were increased when the plants were treated with Se NPs. The increased accumulation of osmolytes has been documented to neutralize the toxic effect of metals as a result of Se NPs treatment (Zahedi et al. 2021). Exogenous application of Se NPs in combination with glycine betaine and proline to carrot plants increased total soluble carbohydrates, soluble protein, and proline, which is extremely sensitive to environmental stresses and controls the numerous genes involved in growth and metabolism by providing energy resources and carbon (Bano et al. 2021). Because of its hydrophilic nature, proline is active in chelating excess cytoplasmic metal ions, indicating a preference for coordinating nitrogen (N) or oxygen (O_2) (Sofy et al. 2020). Proline can protect enzymes and biomolecules by acting as a protein stabilizer, metal chelator, and free radical scavenger (Boguszewska and Zagdańska 2012). When applied as a foliar spray, glycine betaine has been shown to increase endogenous levels of soluble protein and proline in a variety of plant species (Shafiq et al. 2021), implying that this chemical compound plays a critical role in increasing abiotic stress tolerance by managing the mechanisms involved in growth and yield production under stress conditions. Secondary metabolites, like phenols, as well facilitate osmoregulation and ROS scavenging, bolstering the enzymatic antioxidant system's ability to withstand oxidative stress (Ali et al. 2021; Osman et al. 2021). Secondary metabolite synthesis is determined by a multitude of natural conditions, including biotic and abiotic stresses. (Ramakrishna and Ravishankar 2013; Abdel Latef et al. 2021). In this study, Se NPs significantly increased phenol levels in heavy metal-stressed carrot plants shoots. The increased accumulation of total phenols in carrot leaves reinforced the antioxidant system, preventing ROS cell damage to organelles. Regarding the application of selenium nanoparticles. In this line, (Zahedi et al. 2021) found an increase in secondary metabolite synthesis in plants in response to Se NPs application. Moreover, Soliman et al. (2022) found that Se NPs-induced phenol accumulation in wheat plants improved antioxidant efficiency and stress adaptation. Osmolytes as well scavenge ROS and mediate stress signaling for rapid elimination of stress responses to prevent oxidative damage. The prevention role of selenium nanoparticles may be related to the glycine betaine and proline combination with selenium particles. In this line, Hussain et al. (2020) illustrated that implementing glycine betaine to tobacco plants can initiate signaling pathways and induce stress resistance. The action of various enzymes such as CAT, SOD, and POD is thought to alleviate the disruption in cell homeostasis caused by ROS (Hussain et al. 2016; Ismail et al. 2022). The mechanism of ROS generation and scavenging by antioxidative capacity has been connected to plant tolerance to abiotic stresses (Sachdev et al. 2021). Under heavy metal stress conditions, the activities of enzymatic antioxidants (SOD, POD, and CAT) increased in carrot leaves, according to our findings. Similar findings in faba bean (Desoky et al. 2021) and *Vicia faba* L. Abid et al. (Abid et al. 2017) showed reduced oxidative stress caused by ROS generation. Exogenous application of Se NPs, particularly when combined with glycine betaine and proline, increased carrot antioxidant activity. Thus, improved carrot plant growth may be linked to increased antioxidant enzyme activity. Moreover, Soliman et al. (Soliman et al. 2022) found that foliar application of Se NPs increased antioxidant activity in wheat plants grown under abiotic stress conditions. Similarly, Sofy et al. (2020) mentioned that foliar application of proline modify antioxidant properties in maize plants, resulting in enhanced heavy metal stress tolerance. Excessive ROS accumulation in cell membranes compromises the structural integrity of key macromolecules such as proteins and lipids, affecting their normal development (Miller et al. 2008; Omer et al. 2022). Se NPs protected cell components from injury by absorbing excess ROS. This was accomplished by modifying antioxidant enzyme activities. Elevated lipid peroxidation and hydrogen peroxide have been noticed in heavy metal stressed plants as a consequence of unregulated ROS, which would be directly related to lipoxygenase activity (Ahanger et al. 2019; Attia et al. 2021) and could be inverted by adjustments such as Se alone or in combination with glycine betaine and proline (Zhou et al. 2020). As an outcome, in the current study, decreased ROS accumulation and oxidative damage in Se-treated carrot plants are likely associated with increased ROS-scavenging enzymes. Accordingly, plant stimulating with Se NPs increased detoxification of active oxygen species and lipid peroxidase produced by Cd stress, resulting in enhanced membrane stability and cell membrane structure prevention in Coriandrum sativum (Sardar et al. 2022; Zeeshan et al. 2023). Plant yield may be reduced due to a restriction in the growth of stressed carrot plants and a limitation in ROS scavenging compounds as a result of a reduction in photosynthetic pigments in carrot leaves. Our results are in line with those of other investigators (Badawy et al. 2021). Furthermore, the increased carrot yield caused by the use of selenium nanoparticles could be attributed to the stimulatory effect of glycine betaine and proline, which scavenges ROS and promotes the growth of stressed plants. Proline and glycine betaine have been shown in multiple research findings to reduce the effects of abiotic stress on plant growth and yield (Sofy et al. 2020; Shafiq et al. 2021). The ability of plant species to accumulate heavy metals varies greatly. These results indicate that carrot plants irrigated with wastewater accumulate higher metal concentrations, demonstrate higher plant allocation of the substrate metals in addition to interior plant mobility. These results are in line with (Fitzgerald et al. 2003; Nouri et al. 2009). Selenium NPs increased carrot plant ability to remediate toxic chemicals found in contaminated soil. The ameliorated effect of selenium nanoparticles is referred to the combination of proline and glycine betaine. Our results are following other investigators (Shafiq et al. 2021; Soliman et al. 2022) and (Sofy et al. 2020) who indicated that plants treated with proline showed a significant decrease in accumulated metals inside different plant parts.

Conclusion

Throughout the present study, selenium NPs alone or in combination with glycine betaine and proline ameliorated the negative impact of heavy metal problems generated by wastewater irrigation, resulting in significant increases in carrot plant growth traits, leaf pigments, soluble carbohydrates, protein contents, and yield criteria. Furthermore, as a mitigation method for the developed damage from heavy metal stress, using selenium NPs alone or in combination with glycine betaine and proline reduced the content of proline, malondialdehyde, and hydrogen peroxide while increasing the activities of carrot plant antioxidant enzymes. As a result, the current study suggests using selenium NPs, particularly when combined with glycine betaine and proline, to promote plant growth under normal and heavy metal stress conditions.

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Declarations

Ethics approval and consent to participate All authors approved.

Consent for publication All a authors agree for publication.

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