Selenium Nanoparticles Improves Morpho-Physio-Biochemical Traits and Cadmium Stress Tolerance in Garden Mint using Seedling Root Dip Feeding

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Abstract. Due to extensive global contamination of agricultural soils with cadmium (Cd), there is a dire need for cost-effective and practical methods to ensure the production of safe food. The recent introduction of micronutrients in nano form has been found to reduce the accumulation of Cd in crops. A greenhouse pot-culture experiment was done to investigate the effects of selenium nanoparticles (0, 10, 20 and 30 mgL-1) applied by seedling root dipping technique on the growth, biomass, physio-biochemical and antioxidant potential of mint grown under induced cadmium stress (25 mg kg-1of soil using CdCl2). Findings revealed that application of selenium nanoparticles considerably improved tissues biomass, photosynthesis, and antioxidant enzyme activities in the mint plants. In addition, the application of Se nanoparticles in optimal concentration improved the relative water contents (7.26%), and decreased the electrolyte leakage (32.96%) in plant tissues. Selenium nanoparticles exhibited a dose-additive effect in reducing the levels of malondialdehyde cadmium in tissues of mint plants. Selenium nanoparticles at 20 mg L-1 demonstrated more efficacy than other levels under control and Cd stressed conditions. These findings indicate that seedling root dip feeding of selenium nanoparticles is an efficient approach and could be recommended for remediation of Cd contaminated soils.

1 Introduction

The sustainability of agro-ecosystems is being seriously threatened by heavy metal (HM) contamination of agricultural fields [1]. Many toxic metals are recognized as soil contaminants due to their widespread and potentially harmful effects on plants grown in such soils [2, 3;4]. Cadmium (Cd), one of the HMs, is a non-essential element, very toxic to plants. Nearly 20 million hectares of cultivable land worldwide are known to be contaminated by Cd [5;6]. Food safety and human health are at stake if Cd accumulates in edible roots, shoots and seeds [7]. Plants exposed to Cd experience changes in cellular redox homeostasis

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revealed by increased malondialdehyde (MDA) content, chlorosis, deformed chloroplast machinery, decreased water and nutrients uptake and accumulation, impaired transpiration and photosynthesis [8, 9]. Furthermore, due to the accumulation of Cd in the biological system, increased reactive oxygen species are generated, which ultimately cause destruction of proteins, causing a devastating effect on the total protein content [10, 11]. Plants tolerate oxidative stress by activating their own defensive mechanisms, made up of a variety of enzymes [12, 13,14].

Selenium (Se) is a beneficial and essential micronutrient for humans and animals, which acts as the cofactor and coenzyme in improving the immune system of human body [15]. According to the world health organization (WHO) reports, around 15 percent of the global population suffer from Se deficiency [12, 16]. In addition, the positive effects of Se on plants have been recently reported in some plants during biotic and abiotic stress such as [17;18]. In plants under the interaction of Cd and Se application, Se usually mitigates Cd toxicity through subsiding oxidative stress, forms an insert complex or reduces Cd uptake and rehabilitees damaged cells [17, 19]. Vital biological processes are exerted by Se through activating selenoenzymes, which can boost antioxidant capacity, fortify ROS scavenging, and protect cell membrane [17, 20]

There are numerous approaches for mitigating the negative effects of Cd on crop growth, biomass production and yield [21]. Seedling root dipping with some stress ameliorants is a low-cost and bio-safe strategy of mitigating Cd toxicity in plants during seedling stage of plant growth [22]. The elite characteristics of nanoparticles (NPs) may be utilized to improve seed germination and crop production [23]. Recent studies have demonstrated that selenium nanoparticles (SeNPs) may improve nutrients uptake, growth and yield of applied plants [24,25]. Additionally, post emergence application of SeNPs alleviates abiotic stress in plants [26].

Garden mint (Mentha arvensis L.) an important industrial, medicinal and aromatic plant, have numerous applications in the flavorings, food, cosmetic and pharmaceutical industries [27]. Menthol, as principal secondary metabolite is extracted commercially from this plant [28]. It is a medicinal and culinary herb that plays an important role in human health [29]. Mint's native name is "Podina" and it is widely used in India, Pakistan and the rest of the world. It is one of the most extensively utilized crop in the food business as well as in the pharmaceutical and cosmetics industries [30]. There is very little information about the physiological limits of seedling root dipping and various levels of SeNPs in vegetable crops. Moreover, there is almost no information about the role of SeNPs on early growth, and yield of mint under Cd stress. Therefore, the current study was designed to investigate the influence of SeNPs root dipping technique on the growth biochemical, water related and osmolyte attributes of mint under Cd stress.

2 Materials and Methods

2.1 Experimental Design and Treatments

A pot experiment was carried out in greenhouse at the Department of Environmental Science, The University of Lahore, Pakistan. Experimental treatments consisted of mainly two factors: cadmium stress (C_1 = control; C_2 = 25 mg kg^{-1} of soil *w/w* using $CdCl_2$), and root dipping in SeNPs levels, L_1 = 0 mg L^{-1} (distilled water); L_2 = 10 mg L^{-1}, L_3 = 20 mg L^{-1} and L_4 = 30 mg L^{-1} employing a completely randomized design (CRD) under the factorial arrangement and replicated thrice. Total (2 × 4 × 3 = 24) pots were used for the study. Selenium nanoparticles, were obtained from the NanoSany Corporation, Iran.

2.2 Crop Management

The experimental soil was collected from a farmland area at a depth of 0-30 cm (sandy clay loam) near the site of experiment. The physicochemical properties of the experimental soils were appraised following [31]. The initial physico-chemical attributes are pH = 7.5; electrical conductivity = 1.94 dSm^{-1}; organic matter = 0.65%; available phosphorous = 5.06 mg kg^{-1}; available potassium = 154 mg kg^{-1}; and available nitrogen (NH^{+4}) = 2.03 mg kg^{-1}. The Cd source was cadmium chloride ($CdCl_2$). Soil was spiked by 25 mg kg^{-1} of soil. Cd-free soil was used as a control. For 15 days, 5 kg of Cd-spiked or Cd-free soil was placed in plastic pots under shaded region. To make treatment concentration of various treatments, the Se nano-powder was weighed at 10, 20 and 30 mg and was dissolved into a liter of water. The mixtures were ultrasonicated to achieve uniform dispersions [32]. Garden mint plants with roots were initiated from rhizome cuttings (10 cm long) from local nursery. They were transferred into laboratory of biology and them were sterilized of laminar air flow system in the following order with 10% ethanol, 15% sodium hypochlorite for 2-3 minutes each and washed 3 times with sterile water. Before transplanting garden mint, seedlings were dipped into the SeNPs for 5 minutes. Initially seven seedlings were transplanted into the pots. Then, the five seedlings were maintained had better growth than the rest of the into pots. A Hoagland solution (50%) consisting of moderate strength was applied to cater to the nutritional needs of plants at a rate of 1 L per week per pot. Based on the physical observations of the plants in pots, the necessary agronomic practices such as irrigation and weeding were carried out regularly as required during the experiment. Upon completion of a 75-days period, garden mint plants were carefully taken out of pots and evaluated by morpho-physio-biochemical, attributes.

2.3 Growth and biomass attributes

Three plants were collected and their roots and shoots were separated for the measurements of growth variables. To eliminate sticky soil particles distilled water was used for plant wash and then those were air-dried. The fresh weight of the root and leaves was then determined using an analytical balance. To estimate the dry weight of the roots and leaves discreetly, they were oven-dried at 70°C for 48 hours.

2.4 Photosynthetic attributes

Leaf samples (5) were crushed in a test tube having 85% acetone (v/v) for 24 h and was placed in dark condition for pigment extraction. After centrifugation for 10 min at 4000 × g at 4°C the absorbance was calculated at 470, 647, and 664.5 nm using the spectrophotometer (Halo DB-20/ DB-20S, UK), the value in the supernatant was measured and then according to Lichtenthaler method [33], the contents of chlorophyll a, b and carotenoids were determined. The total chlorophyll concentrations were measured by the addition of chlorophyll a and b.

2.5 Membrane damage attributes

To test the electrolyte leakage (EL) level, a small piece of leaf was dipped in purified deionized water. The sample's first EL measurement was obtained following a 2-hour incubation at 32°C, and its second EL reading was obtained following a 20-minute incubation at 121°C [34]. To calculate the EL level of samples, the following formula was used: $EL = (EC1/EC2) \times 100$.

2.6 Enzymatic antioxidants activity and lipid peroxidation attributes

The supernatant extracted from 1 g of lettuce leaves with 50 mM phosphate buffer was centrifuged (~15,000×g for 10 minutes) for determination of enzyme activity. By following the procedure reported by Velikova et al. [35] peroxidase (POD) activity was determined. Catalase activity (CAT) and superoxide dismutase activity (SOD) was determined according to Aebi [36] and Beauchamp and Fridovich [37] protocols. The MDA contents was assayed by the thiobarbituric acid reaction method [38].

2.7 Water related attributes

The method of Turner and Kramer [39] was employed for RWC measurement, and the following formula was used for the calculation: $RWC = [(FW - DW) / (TW - DW)] \times 100$; Where FW = fresh weight; TW = Turgid Weight; DW = Dry Weight

2.8 Osmolytes attributes

A fresh leaf sample (0.5 g) was collected and ground with a buffer (pH value~ 7.2) with protease inhibitors of 1 μM along with saline phosphate buffer. In 1L of deionized water, the dissolution of 1.37 mM NaCl, 2 mM KH_2PO_4, 2.7 mM KCl, and 10 mM Na_2HPO_4 was carried out for the preparation of the saline buffer. By adding HCl to this solution, the pH of the buffer was adjusted. After that, the solution was autoclaved and centrifuged for 5 mins for the separation of the supernatant. Proline contents were determined by following the protocols of Maehly and Chance [40], while soluble sugars and soluble proteins were determined by a method defined by Giannakoula et al. [41] and Bradford assay [42], respectively.

2.9 Cadmium Accumulation

About (0.5 g) of mung bean seeds were digested in an di-acid mixture. A flame atomic absorption spectrophotometer (HITACHI Z-2000) for measuring atomic absorption recorded the amounts of Cd^{+2} in the mungbean seeds by following the protocols of Abbas et al. [43].

2.10 Statistical analysis

Collected data was tested using Fisher's Analysis of Variance (ANOVA) technique. The highest Significant Difference (HSD) test (5% probability level) was applied for comparison of means. Regression and correlation analyses were computed by using the Minitab-19 statistical software. All statistical computations were performed with Statistix software version 10 (Analytical Software, Tallahassee, USA), and for the graphical work, Microsoft Excel (2013 version) was employed in this study.

3 Results

3.1 Growth and biomass attributes

Significant reduction in the plant height (22.41%), root length (16.39%), shoot length (21.88%), root fresh weight (14.28%), root dry weight (33.33%), shoot fresh weight (25.53%) and shoot dry weight (26.92%) of garden mint plants. Whereas, application of Se nanoparticles by seedling root dipping technique enhanced the growth and biomass attributes both in control and Cd amended treatments (Figure 1). Increased doses of Se led to a gradual improvement in the fresh and dry biomass of both shoot and root but gradual decrease was noticed where 30 mg L^{-1} was applied across both Cd-contaminated and control conditions. The most significant growth and biomass indices were noticed when seeding roots of garden mint were dipped in Se nanoparticles solution (20 mg L^{-1}) in both control and Cd-contaminated treatments.

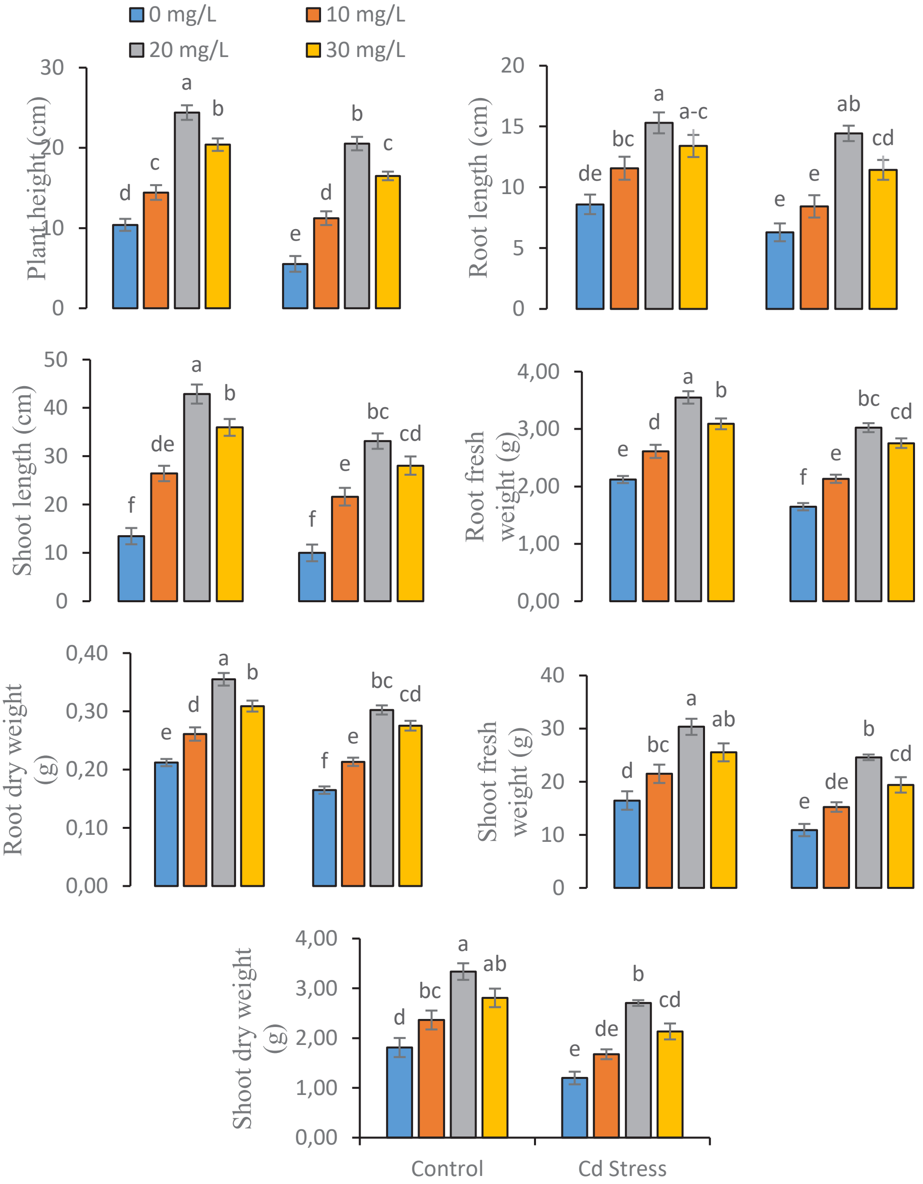

Fig. 1. Effect of various doses of Se nanoparticles on the growth attributes of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.2 Photosynthetic attributes

Figure 2 exhibited the detrimental effects of Cd toxicity on the photosynthetic attributes of mature garden mint leaves. The addition of cadmium resulted in a decrease across all measured photosynthetic parameters in garden mint plants. The application of Se nanoparticles (20 mg L^{-1}) showcased the highest levels among all photosynthetic attributes in plants subjected to both control and Cd-stressed conditions. The presence of Cd-induced toxicity led to reductions in chlorophyll *a* content (15.78%), chlorophyll *b* contents (24.32%), Total chlorophyll contents (17.11%) and carotenoid content (15.59%) in comparison to non-stressed plants. However, the maximum dose of Se nanoparticles demonstrated a decrease in the photosynthetic attributes garden mint plants under both control and Cd-stressed conditions.

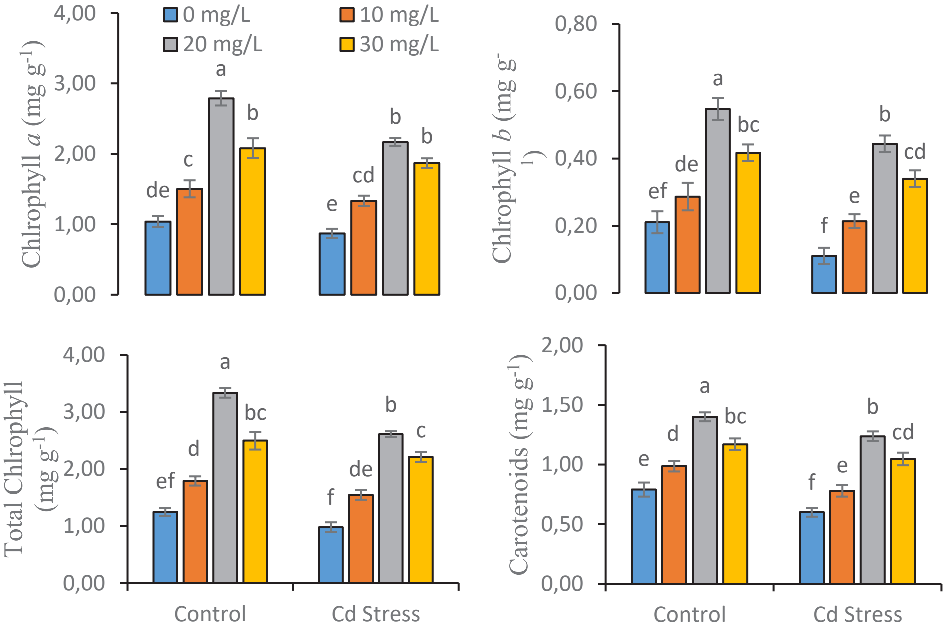

Fig. 2. Effect of various doses of Se nanoparticles on the photosynthetic attributes of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.3 Water related and membrane damage attributes

The study revealed significant impacts on relative water content and electrolyte leakage in Cd-affected garden mint plants compared to the control group. Under Cd stress, relative water content decreased by 7.26%, while electrolyte leakage increased by 32.96%. Furthermore, the Se nanoparticles resulted in increased relative water content and reduced membrane damage in mint plants. The highest relative water content and least membrane damage were observed in Cd-affected and control plants that received supplementation of Se nanoparticles (20 mg L^{-1}) using seedling root dipping technique. Among the treatments, plants affected by Cd and treated solely with maximum level of Se nanoparticles displayed inferior response compared to the other applications of Se nanoparticles (Figure 3).

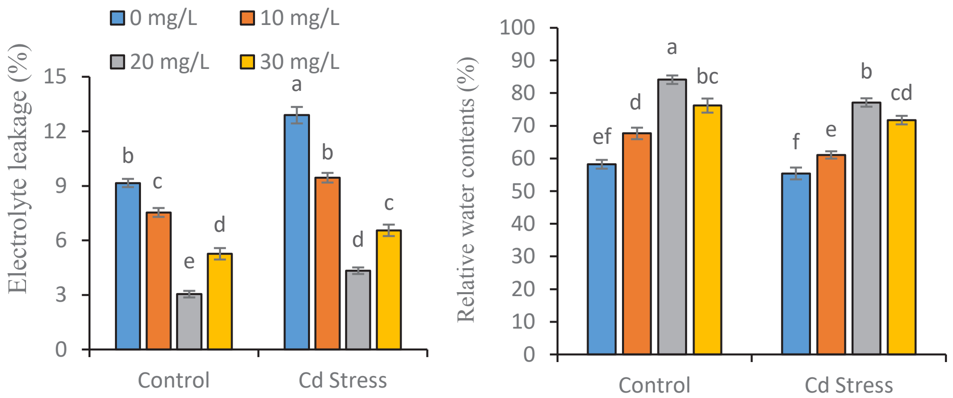

Fig. 3. Effect of various doses of Se nanoparticles on the water related and membrane damage attributes of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.4 Osmolyte attributes

The application of Se nanoparticles, whether under Cd stress or control conditions, demonstrated elevated levels of soluble sugars and soluble proteins in the mint plants. The induced stress from Cd resulted in decreased levels of soluble sugars (11.85%) and soluble proteins (18.83%) in mint plants. Notably, the use of Se nanoparticles at the rate of 20 mg L^{-1} displayed increased levels of soluble sugars and soluble proteins in both conditions, as depicted in Figure 4.

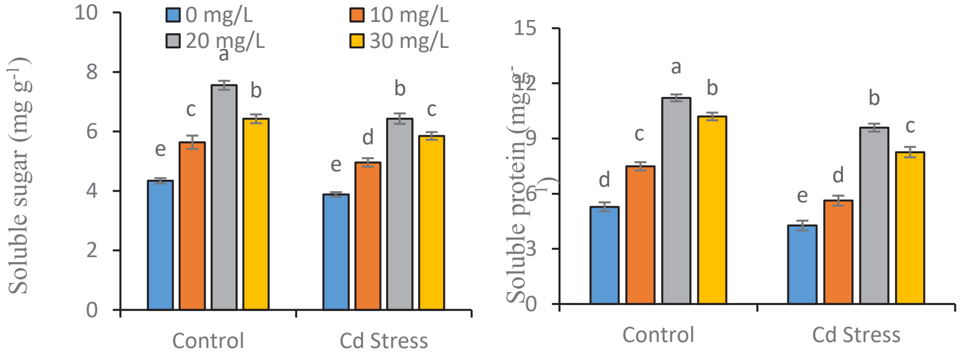

Fig. 4. Effect of various doses of Se nanoparticles on the osmolyte attributes of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.5 Enzymatic and lipid peroxidation attributes

Under cadmium stress, the activity of enzymatic antioxidants and lipid peroxidation attributes showed an increase compared to the control conditions. Induced Cd stress increased the superoxide dismutase activity (SOD) (21.17%), peroxidase activity (POD) (7.34%), catalase activity (CAT) (8.38%) and malonaldehyde (MDA) contents (10.73%). The application of Se nanoparticles using seedling root dipping technique led to additional improvements in SOD, CAT, and POD activities, effectively mitigating Cd stress (Figure 5).

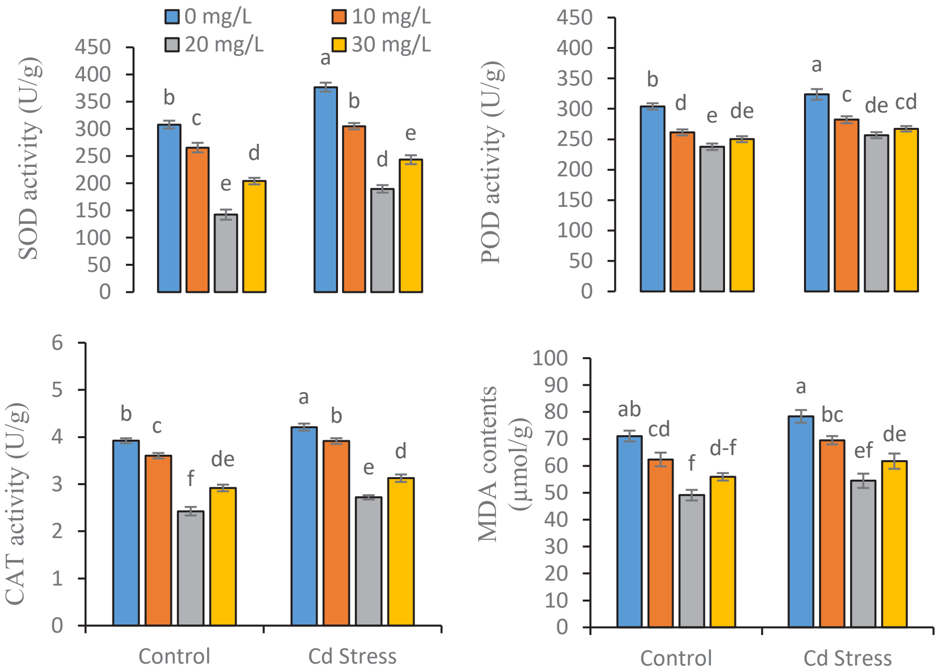

Fig. 5. Effect of various doses of Se nanoparticles on the enzymatic antioxidants and lipid per oxidation attributes of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.6 Cadmium accumulation in plant parts

Cadmium stress and the different treatments involving seedling root dipping applied Se nanoparticles had a significant impact on the accumulation of Cd in the roots and leaves of garden mint plants grown under both stressed and normal conditions. The stress induced by Cd led to an increase in root and leaf Cd content compared to the control group under normal conditions. The maximum accumulation of Cd in roots (1.54 μg^{-1}) and leaves (1.25 μg^{-1}) was observed under induced Cd stress conditions, specifically in treatments without Se nanoparticles application. Conversely, the lowest accumulation of Cd in both roots and leaves was recorded in treatments involving seedling root dipping application of selenium nanoparticles at a rate of 20 mg L^{-1} (Figure 5).

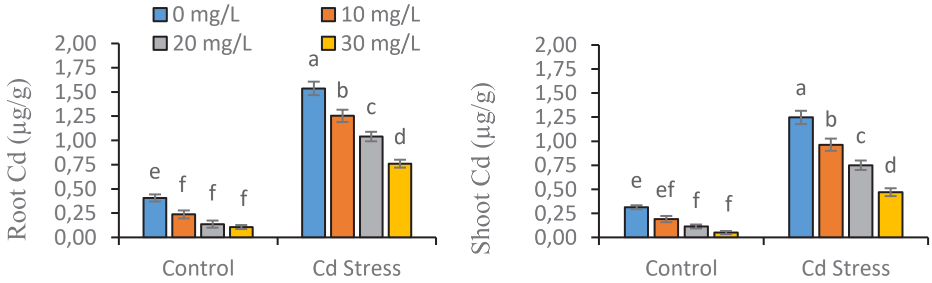

Fig. 6. Effect of various doses of Se nanoparticles on the Cd accumulation in various plants parts of garden mint under Cd stress using seedling root dipping technique; The lowercase letters showed the

significant variations across treatments means; values denote the means with standard deviation replicated three times.

3.7 Principal component analysis

Garden mint plants cultivated on Cd contaminated soil with root dipping technique of nano selenium treatments shown to have a connection between Cd, growth and physio-biochemical characteristics using loading plots of principal component analysis (Figure 6). The first primary components, PC1, make up the largest percentage of all components and account for 90.0% of the entire database. PC1 makes up 90.0% of this dataset, whereas PC2 makes up 6.0% of it. The first set of variables that PC1 has a positive correlation with are: growth, biomass and photosynthetic attributes are positively correlate. The factors related to SOD, CAT, POD, MDA, EL and Cd accumulation are slightly negative with rest of the attributes.

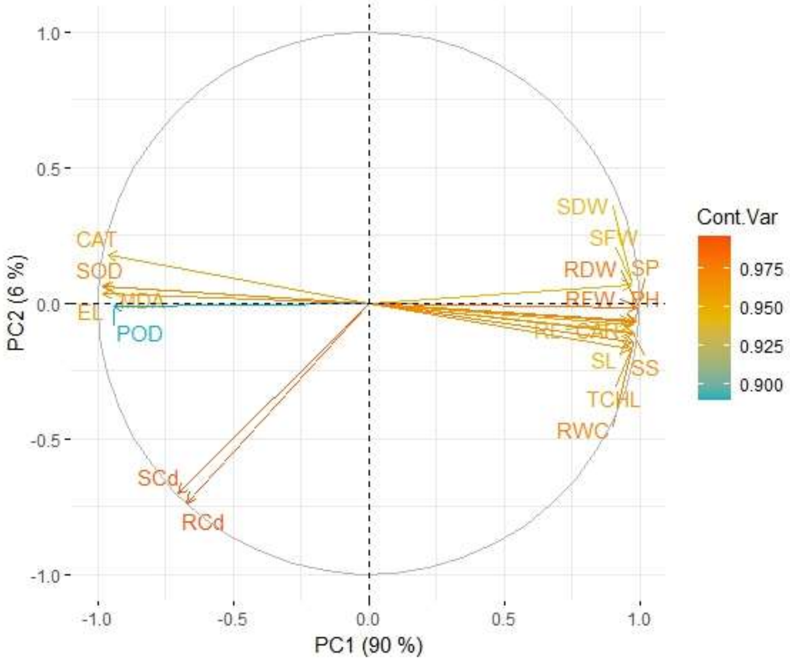

Fig. 7. Loading plot of principal component analysis (PCA) on various attributes of garden mint under various levels of nano selenium grown in Cd contaminated soil; RL = Root length; SL = Shoot length; RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; TCHL = Total chlorophyll contents; EL = Electrolyte leakage; RWC = Relative water contents; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; MDA = Malonaldehyde contents; Cd-R; Cadmium contents in roots; Cd-S = Cadmium contents in shoot

4 Discussion

The research suggests that Se NPs significantly improved the growth and biomass of mint plants grown under Cd stress. Cadmium stress inhibited the mint growth, however, Se NPs enhanced plants vigor root elongation and better shoot growth [44, 45]. Our results are aligned with Farooq et al. [46], reported that Se NPs improved plants growth under arsenic stress. In addition, Se NPs improves biomass by improving photosynthetic efficiency [47]. The negative effects of Cd on plants growth and biomass were mitigated by Se NPs by controlling nutrients uptake, altering plants hormone levels and by improving antioxidant defense system [14]. These findings revealed use of Se NPs is a promising approach to enhance plants productivity and to mitigate heavy metals stress under stressful conditions.

Cadmium resulted in decrease of photosynthetic parameters and a significant improvement in these parameters of has been noticed in those mint plants treated with Se NPs. Shah et al. [10] reported the likewise findings where Cd reduced the photosynthesis in *Brassica oleracea* plants. Increased chlorophyll contents because of Se NPs, suggest the enhanced synthesis as well as the improved efficiency of photosynthetic pigments [48, 49]. It has been observed that treatment of Se NPs increase plants photosynthetic activities, so enhance chlorophyll contents may be because of improvement in light absorption and its better utilization. This indicated that Se played a vital role in the improvement of photosynthesis in those plants exposed to stress [50].

The ROS overproduction shows the oxidative stress due to different types of stresses including Cd stress [51]. In current study, the overproduction of ROS was cause by Cd stress, which resulted in membrane lipid peroxidation. Recent studies demonstrated the heavy metals-induced oxidative injury to various plants species such as Cr in maize [52], Cd in lettuce [53] and Pb & Cu in citrus [41]. Nevertheless, exogenous application of Se NPs recovered the oxidative injury in mint plants by Cd stress. This decrease in oxidative injury by Se NPs in plants is may be due to enhancement of plants defense system under heavy metals stress [54]. Ahmad et al. [45] reported that Se NPs overcome the ROS by reducing oxidative stress through modulation of antioxidant enzymatic activities in sesame plants. The decrease in oxidative stress by Se NPs might be due to scavenge of ROS by Se NPs application to plants grown in a stressful environment [47, 55]. Thus, plants treated with Se NPs, reveal lower level of lipid peroxidation, suggest decrease in membrane damage while improvement in membrane integrity[56]. El-Badri et al. [57] reported that Se NPs maintained the membrane integrity in rapeseed, so promoted healthy physiological processes in stressed plants.

The current study examined the antioxidant enzymes activities in mint plants under Cd stress. Plants activate their natural defense under stressful environment such as under heavy metals stress [58, 59]. The supplementation with Se NPs further enhances the plants defense system by stimulation the antioxidant enzymes activities such as SOD, POD, CAT and APX. Such enzymes play their role in scavenging the ROS and detoxify the oxidative byproducts, so preserve the cellular homeostasis [60]. The SOD effectively detoxifies the oxidative free radicals and inhibition of hydrogen peroxide and lipid peroxidation is reported by POD and CAT, respectively [61]. Thus, it is concluded that use of Se NPs is a promising approach to improve plants defense and to reduce the oxidative stress towards plants grown in a stressed environment.

The effect of Se NPs was assessed on Cd uptake by mint plants under Cd stress. Results revealed that it has observed the high concentrations of Cd in both root and shoot tissue as compared with respective control plants. The concentrations of Cd uptake were increased by increasing level of Cd in a dose-additive manner. Plants exposed to cadmium experience a variety of physiological and biochemical disturbances that result in growth inhibition and toxic symptoms [62 - 64]. However, the exogenous application of Se NPs significantly reduced the uptake of Cd to the roots of mint plants as well as restricted the translocation of Cd from root to shoot and our results are aligned with previous studies [14. 54]. This reduction in the uptake and translocation of Cd by mint plants might be due to the potential of NPs to modify the dynamics of Cd mobilization, possibly by affecting the expression of metal transporters and chelation mechanism [65,66]. Cadmium might have been sequestered by Se NPs in root tissues, or might be complexed with Se, so became less bioavailable [67].

Due to these above-mentioned mechanisms, the uptake and translocation of Cd may be minimized with the application of Se NPs. Thus, exogenous application of SeNPs, offers a viable strategy of reducing cadmium toxicity in plants. This strategy may find use in phytoremediation and agricultural techniques meant to improve crop resilience under stressful environments.

5 Conclusion

The plants treated with Se nanoparticles using the seedling root dipping technique exhibited better response compared to control plants under Cd stress. Based upon findings it was noticed that more improvements in relative water content, levels of soluble sugars, enzymatic activity, photosynthetic pigment content, as well as both dry and fresh biomass was noticed by the optimal level of selenium nanoparticles. Our findings strongly support the use of Se nanoparticles to mitigate Cd stress and enhance the growth of mint plants facing Cd stress. Moreover, integrating Se nanoparticles with other soil and irrigation management practices can further fortify the resilience of mint crop against metal stress. However, before proposing commercial recommendations, economic considerations must be thoroughly assessed.

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