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Foliar application of Selenium reduced cadmium uptake in *Abelmoschus esculentus* L.

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ABSTRACT

This study investigated the effects of foliar application of selenium (Se) against Cadmium (Cd) toxicity on the plant growth, biochemical analysis and Cd and Se content in Okra (*Abelmoschus esculentus*) plant. The experiment was carried out under greenhouse conditions. Cadmium as added to the soil at six concentrations (0, 5, 25, 50, 100 and 250 as $CdCl2$) mg/kg). For each Cd treatment, three concentration of Se (0, 0.5 and 1.0 µM) were applied as foliar spray. The results showed that increasing Cd concentration in the soil decreased the plant growth as well as increased the antioxidant activity. Application of Se to Cd challenged plants caused an increase in pigment content and enzyme activity. More importantly, low doses of Se reduced the shoot Cd content in the okra plant; however, high level of Se negatively influenced plant growth under Cd toxicity. Overall, Se reduced the cadmium toxicity in plants and the effects were dose-dependent.

INTRODUCTION

Plants are capable of taking up and accumulating heavy metals from the soil. Such accumulation in crop plants of toxic heavy metals threatens human health when consumed. Cadmium (Cd) is a non-essential element and one of the most toxic heavy metals due to its high toxicity for all living organisms. Cadmium (Cd) negatively impacts photosynthesis, chlorophyll production, and various metabolic processes, including carbohydrate metabolism and nutrient uptake and transport (Akonac and Canal, 2023). Cadmium toxicity leads to notable reductions in the growth and development of both aerial and root portions of plants (Qin et al., 2020). Additionally, Cd induces oxidative stress by generating reactive oxygen species (ROS), which disrupt the plant's antioxidant defence mechanisms (Farooqi et al., 2009). Selenium (Se) is acknowledged as a vital micronutrient for both humans and animals, serving a crucial function in numerous biological processes. It is an integral part of selenoproteins, which are essential for antioxidant defence and the proper functioning of the thyroid. The global prevalence of selenium deficiency, particularly in regions with low soil selenium levels, can lead to significant health issues for many people. Sufficient selenium intake is linked to a variety of health benefits, including a lower risk of certain diseases such as cancer, cardiovascular conditions, and immune system disorders (Pilon-Smits, 2005). At low concentrations, Se plays a crucial role in antioxidant processes, such as enhancing glutathione peroxidase activity and maintaining hormonal balance in plant cells (Filek et al., 2008). For example, applying low levels of Se (5 and 10 µM) has boosted the growth and photosynthetic capacity of cucumber seedlings exposed to NaCl salinity (Hawrylak-Nowak, 2009). Selenium has a protective and antioxidant function that reduces oxidative stress. It also mitigates stress by enhancing the plant's antioxidant capacity, both through increasing the activity of enzymatic antioxidants and boosting non-enzymatic antioxidants (Azizi et al., 2020). In agriculture, Se is applied to plants as soil application or foliar spraying, of which, foliar application is generally more effective than soil application as the element tends to be adsorbed onto soil particles, as is often the case with Oxisols.

Okra (*Abelmoschus esculentus* L.) is a significant vegetable in the subcontinent, belonging to the Malvaceae family. Originally native to Ethiopia and Sudan, it is now predominantly cultivated in India. As an ancient crop, okra holds substantial medicinal value and has been cultivated across Africa, the Americas, Asia, and Europe (Ariyo, 1993; Oyenuga, 1969; Oyelade et al., 2003; Hamon, 1991). It is susceptible to various abiotic stresses, including drought, chilling temperatures, frost, and water logging. Therefore, this research was conducted to investigate the effects of foliar spray selenium on physiological and biochemical characteristics of okra plant under cadmium stress.

Materials and Methods

2.1. *Plant material, experimental conditions:*

Okra (*Abelmoschus esculentus* L. Moench), Arka Anamika (Bhindi) variety was used in this experiment. Seeds of okra were procured from agro seed stores, Tirunelveli. Seeds were surface sterilized with 0.1% sodium hypochlorite for 5 min. Seeds were thoroughly washed with distilled water and sown in 26 x 13.5 cm plastic pots. The bottom was covered with plastic tray for water retention. Each pot was then filled with 2000 g of red soil. Cadmium, as $CdCl_2$, was mixed thoroughly with the soil and at six different levels (5, 25, 50, 100 and 250 mg Cd kg^{-1}) while sodium selenate as the source of Se was applied at three concentrations (0, 0.5 and 1.0 μM) as foliar spray twice a week after three-leaf stage. The soil was additionally supplied with N(NH_3NO_3), P(KH_2PO_4), K(K_2SO_4) fertilizer to avoid severe N deficiency as reported by Chi et al., (2017). The fertilizers were applied on days 0, 15, 30 and 45). Each treatment was replicated five times. Four seeds were germinated in each pot and the pots were placed inside the greenhouse. The pots were watered every alternate day to ensure sufficient water content. Plant samples were collected on 15th day, 30th day and 60th day. After 60 days the plants were harvested from the pots and separated into shoot, root, fruit and used for further analyses.

2.2. *Plant biomass*

The roots were separated from the aerial part and then rinsed three times with tape water followed by deionized water. The shoots and roots were oven-dried at 60˚C for overnight and weighed for dry matter content.

2.3. *Determination of photosynthetic pigment*

According to the method of Hiscox and Israelstam (1979), the content of chlorophyll a and b as well as total carotenoids were determined spectrophotometrically. The samples were collected from the third leaf and pigments were extracted using DMSO (Dimethyl sulfoxide) in a water bath for 30 minutes at 60˚C. The absorbance of the resulting extracts was measured at 645, 663 and 470 nm against the DMSO blank.

2.4. *Determination of soluble protein, proline and peroxidase*

The soluble protein concentration was determined according to Bradford (1976) using with bovine serum albumin (BSA) as the standard protein. Free proline content in leaves was determined according to Bates et al. (1973). Plant materials were homogenized in 10 mL of 3% aqueous sulphosalicylic acid and filtered. To 2 mL of filtrate in a test tube, 2 mL of glacial acetic acid and 2 mL ninhydrin were added and heated in the boiling water bath for 1 h. After that 4 mL toluene was added to the reaction mixture. Toluene layer was separated and the absorbance was recorded at 520 nm and proline concentration was calculated using a proline standard calibration curve. For the determination of peroxidise, fresh leaf tissue (1 g) was homogenized in 3 ml ice- cold phosphate buffer (0.1M, pH 7.0), centrifuged at 18,000 g at 5˚C for 15 min and supernatant was used for the assay of the activity of POD. The activity of POD was determined in terms of oxidation of guaiacol by measuring the increase in absorbance at 470 nm (Putter, 1974). The reaction solution was composed of 3 ml buffer (pH 7.0), 0.05 mL guaiacol, 0.1 mL enzyme extract and 0.03 mL hydrogen peroxide solution. The reaction was started by addition of H_2O_2 and the increase in absorbance was recorded at 470 nm.

2.5 Total Cadmium determination

Samples were dried in an oven at 60˚C. A weight of 0.1 g of dried ground sample was placed in a 75 mL Kjeldahl Straight digestion tube and 5 mL of concentrated HNO_3 was added (Modified from Mahfoud et al. 2018). The sample was digested in a KJEL-E-TEK (Kjeldahl) Block digester for 45 min at 90˚C, and then the temperature was increased to 170˚C and maintained until a clear solution was obtained. After cooling, the solution was filtered with whatman No.42 filter paper; made up to 25 mL in a volumetric flask by adding distilled water. It was then transferred to a 50 mL Polyethylene bottles. The resulting solutions were appropriately diluted with distilled water and analyzed by ICP-OES (Perkin Elmer Optima 5300 DV).

2.6. *TF (Translocation Factor) and BFs (Bioaccumulation Factor)*

The translocation factor (TF) of Cd from shoot to root was calculated as follows:

TF= Cd in shoots (μg g^{-1}) / Cd in roots (μg g^{-1})

The Bio-accumulation factor (BFs) was calculated as follows:

BCF= Cd in shoots (μg g^{-1}) / Cd concentration in soil (mg kg^{-1})

2.7. *Data analysis*

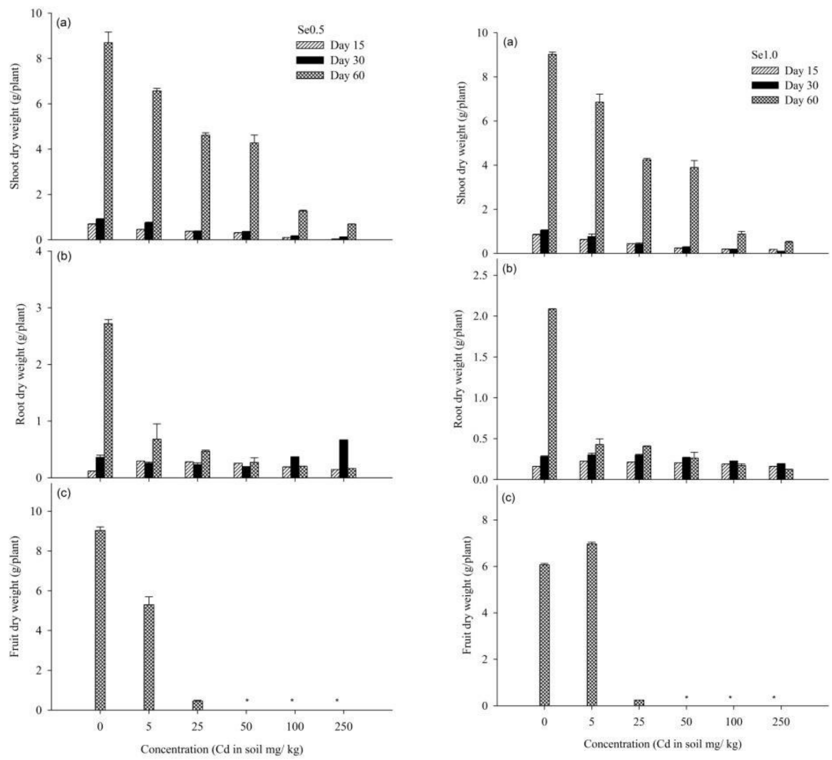
The experiments were established in a completely randomised block design, with six treatments, five replications per treatment and four plants per pot. The data were processed using IBM SPSS Statistics 22.0 version. The values presented are mean (±SD) from three independent replicates. Data were analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests at $P \leq 0.05$ confidence levels.

Results and Discussion

1.) Growth parameter of Okra plants

Shoot dry weight, root dry weight and fruit dry weight of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray with Se at 0.5 and 1.0 μ are presented in Figure 1. In plants exposed to 100 and 250 mg Cd, a strong growth reduction and a drastic decrease in the dry weight of shoot, root and fruit was observed. In addition, high Cd exposure caused stunted plant growth, chlorosis and brown root. Okra plants treated with 0.5 μM selenium under cadmium stress exhibited the maximum increase in both shoot and root dry weight at high level Cd treatments on 60 day old plants followed by 30 and 15 day old plants. At 100 and 250 mg kg^{-1} Cd addition, the low dose of Se foliar application significantly improved the growth of okra plants under different concentrations of Cd stress. Significant increases in the shoot dry weight treated with Se at 0.5 μM kg^{-1} compared with Se application at 1.0 μM. Comparatively, the highest growth response was observed in Se 0.5 μM kg^{-1} and its toxic resist to growth of the *Abelmoschus* plant particularly in the variety of Arka Anamika. Similar results were found in lupine plants treated with Se (Akonac and Canal, 2023). Most importantly, under Cd toxicity conditions,

Toxicity may cause change in plant growth, morphology, physiology and yield. Cd is easily taken up by plants, reducing growth, changes in nitrogen and carbohydrates metabolisms in the plant (Gulser and Sonmez, 2012). Cd damages photosynthetic activity, seed germination, plant biomass mechanisms, crop yield and also interferes with its normal development by disrupting nutrient uptake (Haider et al., 2021). In this study, it was found that above 100 and 250 mg Cd kg^{-1} with Se foliar spray treatments caused a decrease in shoot, root dry weight. But 50 mg Cd kg^{-1} with Se foliar application treatments could resist the Cd toxicity and improved the plant growth significantly. These findings were consistent with the previous research work. In a study carried out with okra plant, 100 and 250 mg Cd kg^{-1} applied in soil caused a decrease in both shoot and root dry weight (Mahalakshmi & Selvam, 2025). However, Se 1.0 μM kg^{-1} treatment did not cause improvement in plant growth in Cd toxic conditions. Mozafariyan et al., (2014) reported that low level of selenium application increased the yield of pepper under cadmium stress. Selenate has a crucial role in curing heavy metal toxicity at low doses. Same results were reported by Azizi et al., (2020) that increasing Cd stress continuous reductions of vegetative traits of fresh and dry weight of root, dry weight of savory plant., Similarly, toxic heavy metal effect on plant growth of radish in Cd contaminated soil was alleviated with low doses Se treatment (Qi et al., 2021). However, high doses of Se inhibited the growth of radish (Chi et al., 2017). In contrast, High doses of Se spray could improve the growth of tomato under Cd stress and increase the dry weights of roots (Lima et al., 2019).



Fi
gure 1: *Shoot dry weight (a), root dry weight (b) and fruit dry weight (c) of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray with Se at 0.5 and Se1.0 µM. Values are means of three independent replicates. (Note: *-indicate no fruit formation).*

2.) Pigments

Selenium foliar spray influenced the photosynthetic pigment content. Treatment with 0.5 and 1.0 µM selenium increased the chlorophyll a and chlorophyll b content during the initial stages of growth when compared with 30 and 60 days growth. After 60 days of growth, with 0.5 µM Se treatment, the concentrations of chlorophyll a, b and carotenoids decreased from 7.01 to 2.72 mg/g FW, from 4.91 to 2 mg/g FW and from 0.65 to 0.27 mg/g FW with increasing Cd concentration from 0 mg/kg to 250 mg/kg. Interaction effects between Cd and Se, the pigment content (Chlorophyll a, b and carotenoids) decreased with increasing Cd concentration. Azizi et al., (2020) reported the similar results, demonstrating Se doses increased the chlorophyll pigments in the savory seedlings exposed to Cd. Heavy metals disrupt compound formations by inhibiting the biosynthesis of LHC II compound proteins (Katznelson et al., 1962). Carotenoids play an important role in the protection against oxidative stress and these pigments could detoxify and can reduce the toxic effects of free radicals (Di Toppi & Gabbrielli, 1999). Plants could uptake selenate by the sulphate transport in the plasma membrane. Application of selenate in wheat plant improved the net photosynthesis, stomatal conductance and chlorophyll content (Sepehri et al., 2019; Khan et al., 2015). Cadmium damages the photosynthetic activity and inhibits the uptake of essential nutrients from the soil (Haider et al., 2021). The low amount of chlorophyll in the leaf can occur when synthesis of photosynthetic pigments is inhibited by Cd, nonetheless increasing the amount of selenium can reduce the chloroplast degradation and increase chlorophyll content (Cai et al., 2011). Similarly, selenium can reduce the negative effects of Cd on chlorophyll content in rice leaves because of free oxygen radicals by break down of photosynthetic pigments (Liu et al., 2004).

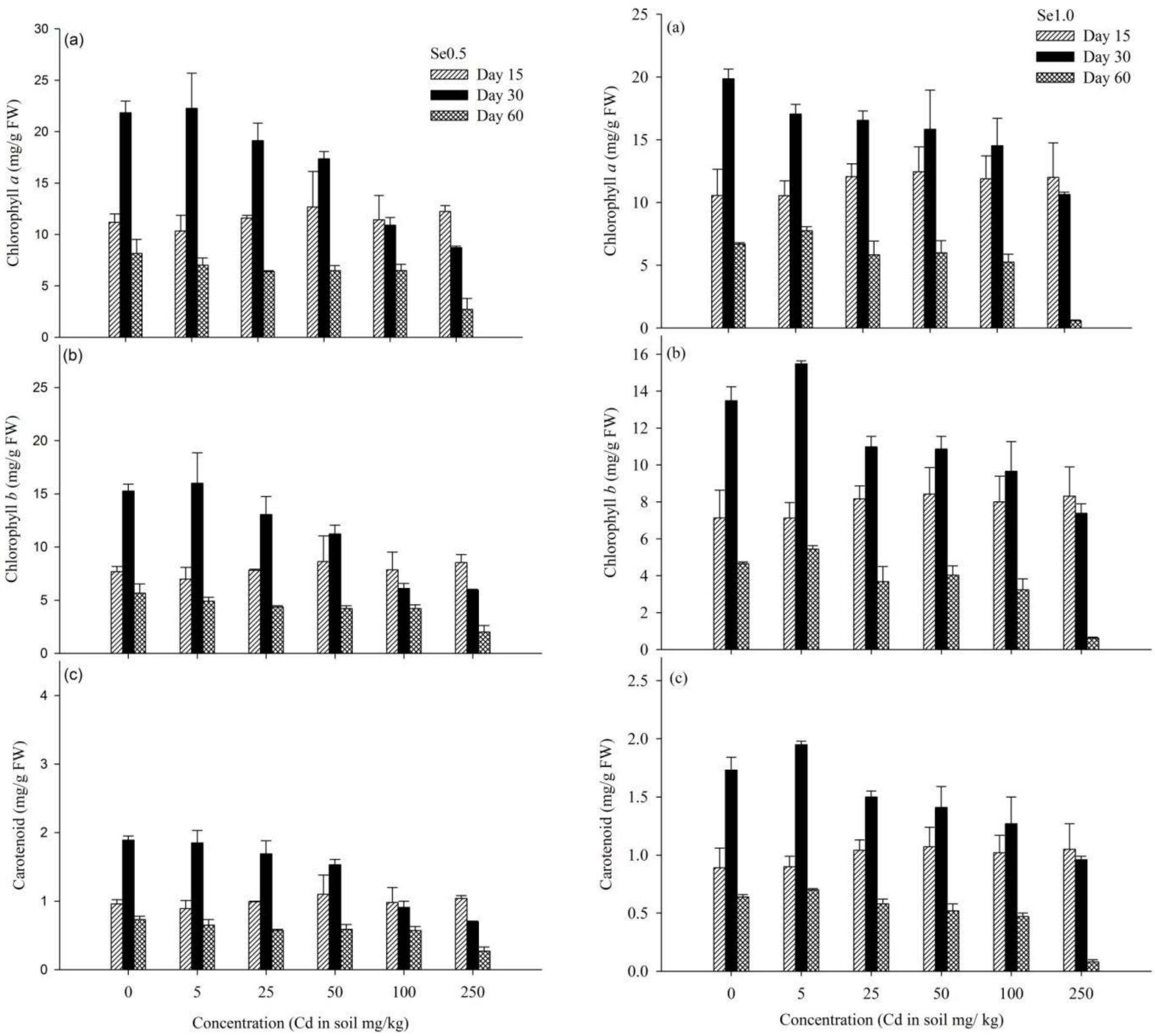


Figure 2: Pigment content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray with Se at 0.5 and Se1.0 µM. Values are means of three independent replicates.

3.) Protein

Foliar spraying Se significantly increased the protein content under increasing Cd concentration. On the 15th day, Se 0.5 µM treatment significantly increased the protein content from 0.13 to 0.36 mg/g FW compared than Se 1.0 µM treatment. The highest increase in protein content found after 60 days of growth in both Se treatments compared with control. Under Cd stress, Se increased the protein content with increasing Se concentration. Similar results were reported by Yorgancilar et al., (2009), who observed that the protein content of the lupine plant was higher with increasing the higher Se treatments.

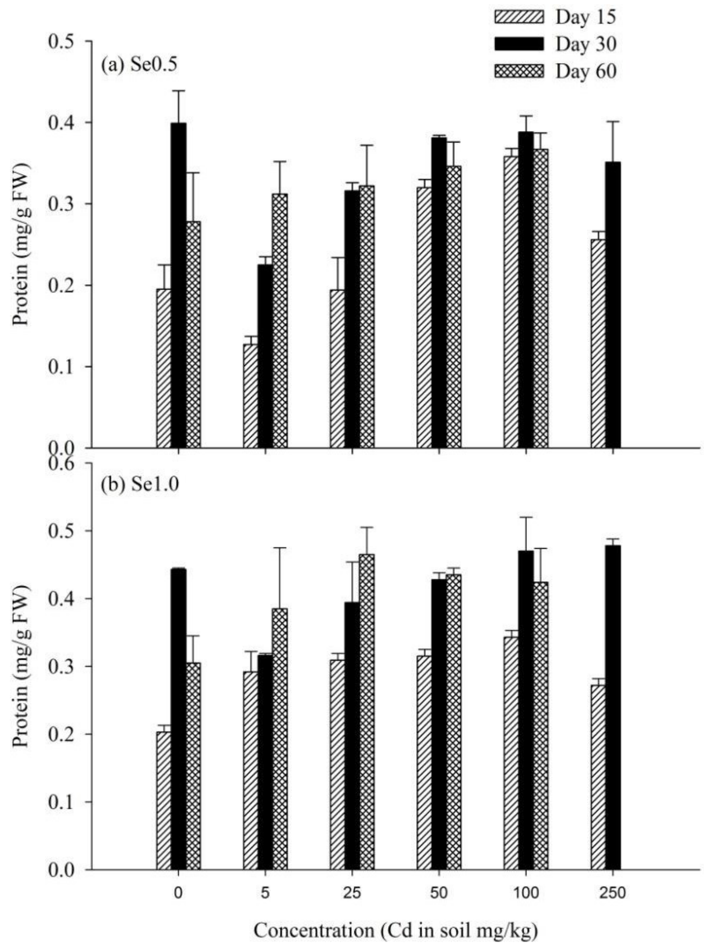


Figure 3: Soluble protein content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray of Se at 0.5 μM (a) and 1.0 μM (b). Values are means of three independent replicates.

4.) Proline

The results show that proline accumulation significantly decreased with increasing Cd treatment in presence of Se. Similar results were reported by Schiavon et al., (2016) who observed that increasing selenium treatment significantly reduced proline levels in radish leaves and also affected root proline levels. This reduction in proline content suggests selenium may impact osmotic regulation and stress responses in plants, potentially altering their metabolic processes. While comparing plants of different age, 30 day old plants produced more proline with increasing Se treatment compared with control. In contrast proline accumulation in savory plants subjected to cadmium (Cd) showed a considerable increase in proline levels with higher Cd concentrations (Azizi et al., 2020). This indicates that both Cd stress and Se application affect proline accumulation, potentially influencing the plant's response to stressful conditions.

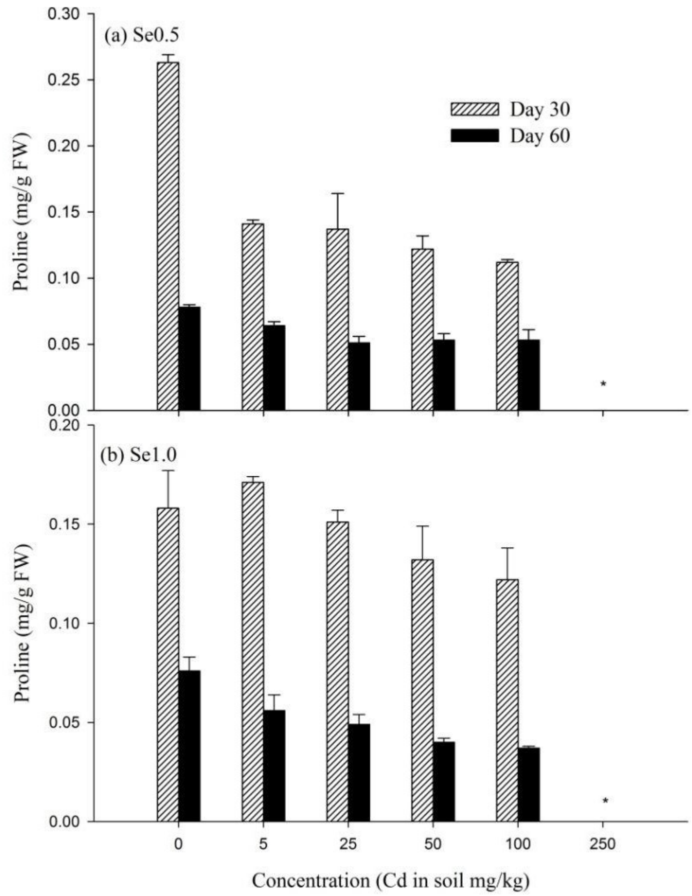


*Figure 4: Proline content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray of Se at 0.5 µM (a) and 1.0 µM (b). Values are means of three independent replicates. (Note: Day 15 -indicate there was no sample and *-indicate that no plant).*

5.) Peroxidase enzyme

The results regarding peroxidase (POD) activity in Okra plants under Cd stress indicated that the activity of this antioxidant increased with increasing concentrations of Cd. The increase in POD activity is important to help in the detoxification of reactive oxygen species (ROS). Comparing with 30 days old and 60 day olds plants, peroxidase activity was more in 15 days plants with increasing concentration of Cd. Similar result was reported that foliar application of Se enhanced the activity of peroxidase enzyme with increasing concentrations of Cd in savory plants (Azizi et al., 2020). The activity of POD increased with higher concentrations of Cd while foliar application of Se enhanced the activity of POD in the okra plants. Chi et al., (2017) reported that increasing Se treatment increased the POD activity level in tomato plant when applied in the form of foliar spray. Conversely, certain tomato varieties showed reduced POD activity with Se spray. Selenium plays a significant role in boosting the antioxidant defence system of okra plants under Cd stress. Similar results were reported in pak choi plant that the activity of POD increased with increasing treatment of selenate (Yu et al., 2018). This study found that the foliar application of Se can effectively mitigate the toxic effects of Cd by enhancing the activity of antioxidant enzymes like POD, which is crucial for protecting plants from oxidative stress caused by heavy metals.

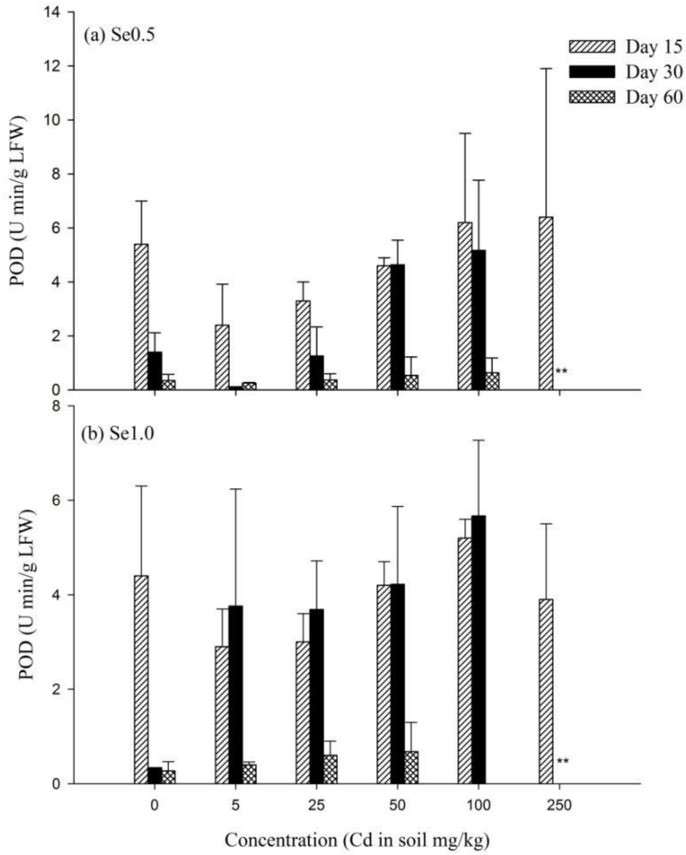


*Figure 5: Peroxidase (POD) enzyme content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray of Se at 0.5 µM (a) and 1.0 µM (b). Values are means of three independent replicates. (Note: *-indicate there was no plant).*

9.) Cadmium and Selenium accumulation and translocation

When exposed to the Cd, the content of Cd in roots was higher than other parts (shoots and fruits). Increasing Cd treatment decreased the okra shoot Cd content when treated with foliar spray of Se. When Cd alone treatment, the root Cd content was higher than at shoots and fruits. When Se was administered, the content of Cd was reduced in the following order: Root > Shoot > Fruit of okra plant. This reduction of Cd content in the plant could be linked with Se application probably Se suppressed the translocation Cd on the upper portion of the plant through foliar application of Se. The TFs and BFs were more marked in plants treated with Se at 0.5 µM. Similarly, Hawrylak-Nowak et al., (2014) reported that the TF value for Cd is low and Se ions showed antagonistic effects on the uptake and accumulation of Cd in plants and decreased its content in roots. Similarly, Se application decreased the Cd content in *Brassica napus* and wheat plant parts from pods to root (Zhao et al., 2019; Wu et al., 2020). With foliar application of Se in okra plant, the shoot Se content increased with increasing Cd treatment at both Se0.5 and Se1.0 µM level (from 10 µg/g to 19 µg/g and 12 µg/g to 15 µg/g). Meanwhile, Se0.5µM treatment had more Cd accumulation in the root compared with high Se treatment. Selenium foliar application increased root Cd content when supplied at Se0.5µM, Which means transport of Cd from root and shoot decreased while the Cd was around the root while a small part is translocated to the shoot. Ions that attach to this compartment cannot enter the cell in the aerial parts of plants. Azizi et al., (2020) and Zhang et al., (2020) reported that addition of Se decreased the Cd content on their shoots and fruits which depends on the Se doses level in savory and strawberry plant. The fruit Cd content was decreased from 36 µg/g to 7 µg/g and from 29 µg/g to 6 µg/g in presence of Se at 0.5 and 1.0 µM, respectively. Therefore, foliar application of Se reduced the Cd accumulation on their parts. A low translocation of cadmium within the plant, which could be a mechanism of this vegetal species to defend itself toward this toxic, metals (Pedrero et al., 2008).

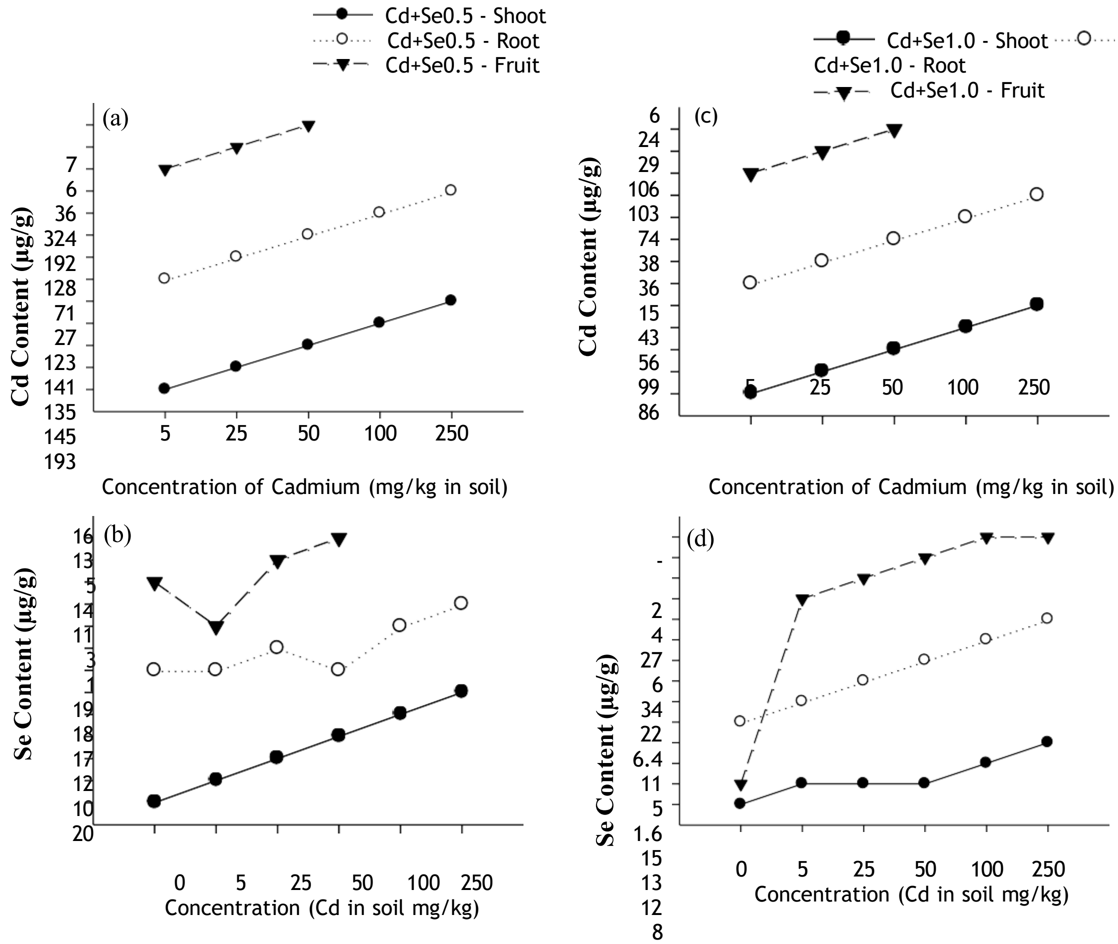


Figure 6: Cd content (a) and Se content (b) in Cd amended plants with 0.5 µM Se; Cd content (c) and Se content (d) Cd amended plants with 1.0 µM Se.

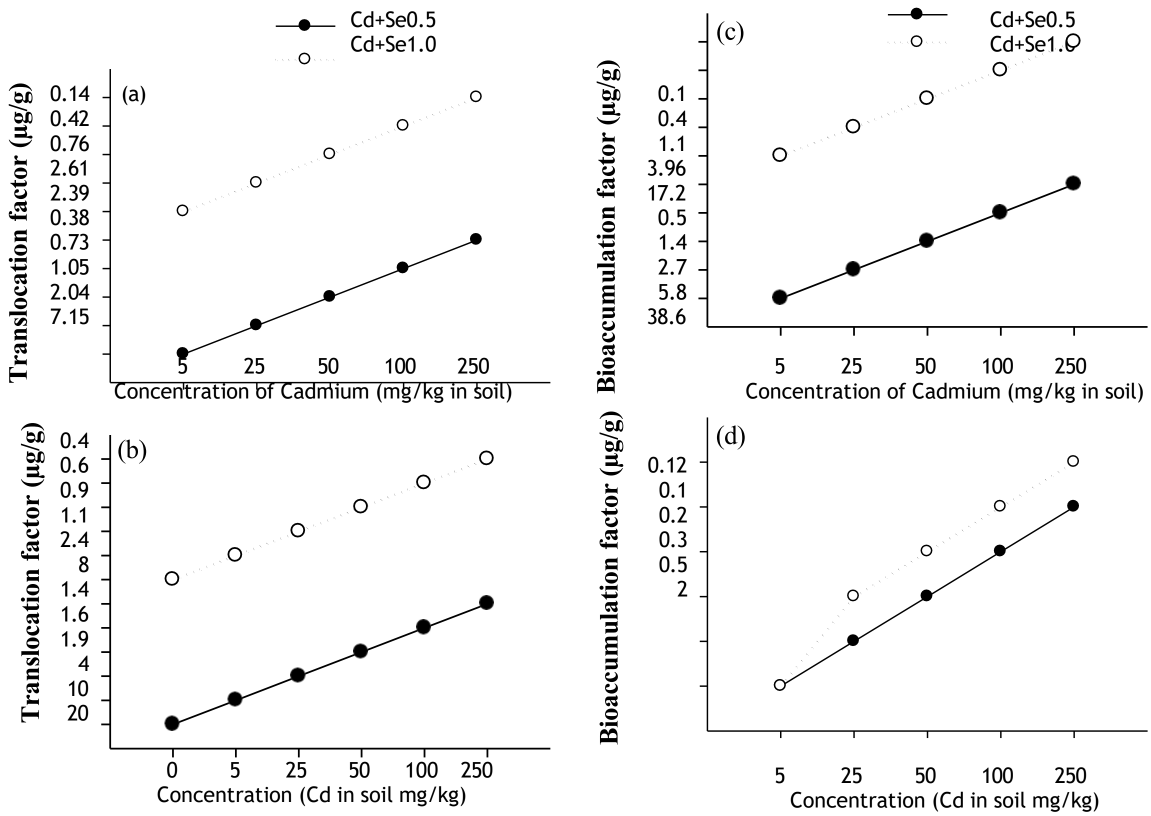


Figure 7: Cd-Translocation factor (a) Bioaccumulation factor (c) and Se-Translocation factor (b) Bioaccumulation factor (d) of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg and foliar spray with Se.

CONCLUSION

Foliar application of Se reduced Cd content in *Abelmoschus esculentus* plant. The application of 0.5 µM and 1.0 µM of Se is effective in improving the toxicity of Cd increasing plant growth and diminishing root Cd in okra plant. Therefore, it was concluded that addition of Selenate at low and medium levels can promote to alleviate the toxic effects of Cd in okra. Moreover the effectiveness of selenium in reducing Cd toxicity depends on the dose as well as the form of application.

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