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Toxicity of Cadmium on Seed germination, plant growth and Antioxidant Enzymes in Pea (*Pisum sativum sp*.)

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Abstract: In the present study, a novel approach has been made to evaluate the effect of cadmium in pea in terms of germination, plant growth, and relevant enzymes activity. Pea (***Pisum sativum sp***.), an important pulse crop consumed by humans, was selected as a test plant. During the present investigation pea seeds were grown in pot culture in triplicate containing different concentrations of cadmium viz., 1.0, 2.0, 4.0, 8.0 and 16.0 ppm respectively. Changes in the physiological and biochemical activities were observed. At the high cadmium concentration, germination percentage was decreased as compared to control. There was also observed considerable reduction in shoot length, root length along with the number of leaves. There was a marked increase in peroxidase, catalase and lipid peroxidase activity by the application of the test chemical was observed in different concentration of cadmium. The results suggest that the activities of peroxidase, catalase and lipid peroxidase of pea (***Pisum sativum sp***.), plant are inhibited under cd stress affecting their growth.

Key words: Cadmium, plant growth and antioxidative enzymes, ***Pisum sativum sp***.

INTRODUCTION

Cadmium (Cd), a non-essential element, is among the most hazardous environmental pollutants for humans, animals and plants even at low concentrations[1-3]. Cd is not an essential nutrient for plants, and it

can accumulate at higher levels in aerial organs[4,5], inducing phytotoxicity manifested in leaf roll, chlorosis, growth reduction, and eventually death[1,5,6]. It has been demonstrated that Cd affects a wide range of physiological and metabolic activities in plants: for example, Chl *a* and *b* content[2], the activities of photosynthetic carbon reduction cycle enzymes[7], mineral distribution[4,5], photosynthetic processes[8,9] and oxidative stress[10]. The intensity of the effects depends on the species, metal concentration and duration of exposure[1,8].

MATERIALS AND METHODS

Seeds of pea were surface sterilized with 0.1% $HgCl_2$ followed by three rinses in sterile distilled water and germinated in pot. The earthen pots (size ~30 cm; diameter ~30 cm depth) filled with fertile/ normal soil used to allow the germination of pea seeds to raise normal seedlings. Cd treatment in the form of $CdCl_2$ was given with distilled water at 1.0-16.0 ppm concentrations. The observations taken at specific time intervals (15, 30, 45 and 60 days after treatment. The plants were harvested for various physiological and biochemical estimations.

Plant height: The shoot height was recorded from the ground level to the top of the plants at specific time intervals. The values were averaged and expressed as plant height per plant in centimeters shown as Fig.1.

Number of leaves: All physiologically active leaves present in plants were counted at different time intervals till pods stage shown as Fig.1.

Lipid peroxidation analysis: The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation by the method described by Heath and Packer[11]. Plant tissue was homogenized in TCA. The homogenate was centrifuged at 15,000 g for 5 min. To take Supernatant, 0.5% of TBA was added. The mixture was heated at 95°C for 30 min. and then quickly cooled in ice bath. After centrifugation at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm. Lipid peroxidase activity was expressed in terms of µmol MDA g^{-1} Fresh tissue.

Catalase analysis: Catalase activity was determined by the method of Euller and Josephson[12] . 2 ml of potassium phosphate[12] buffer (pH = 7.0), 1 ml distilled water, 1 ml enzyme extract (2.5%) and 1ml H_2O_2 (0.5%) in test tubes were added and incubated for 10 minutes. After 10 min 2 ml of 4 N H_2SO_4 was added to stop the reaction. For blank 2 ml of 4 N $H_2SO_4$was added prior to the addition of 1 ml H_2O_2. After ten minutes the final volume of both blank and sample was titrated against 0.01 N $KMnO_4$ with the help of burette and 100 ml conical flask. Catalase activity was expressed in terms µmol decomposed H_2O_2 g^{-1} fresh weight of tissue.

Peroxidase Analysis: Peroxidase activity was determined by the method of Luck [13]. 2 ml of Potassium phosphate buffer (pH = 6.0), 1 ml distilled water, 1 ml p-phenyl diamine, 1ml H_2O_2 (0.5%) and 1 ml enzyme extract(2.5%) in test tubes were added and incubated for 10 minutes. After 10 min 2 ml of 4 N H_2SO_4 was added to stop the reaction. For blank 2 ml of 4 N $H_2SO_4$was added before adding enzyme extract. The final volume of both blank and sample was centrifuged at 4^0C at 5000 rpm for 10 minutes and optical density was read at 485 nm wavelength. The peroxidase activity was expressed in terms of ΔOD/gm fresh weight of tissue.

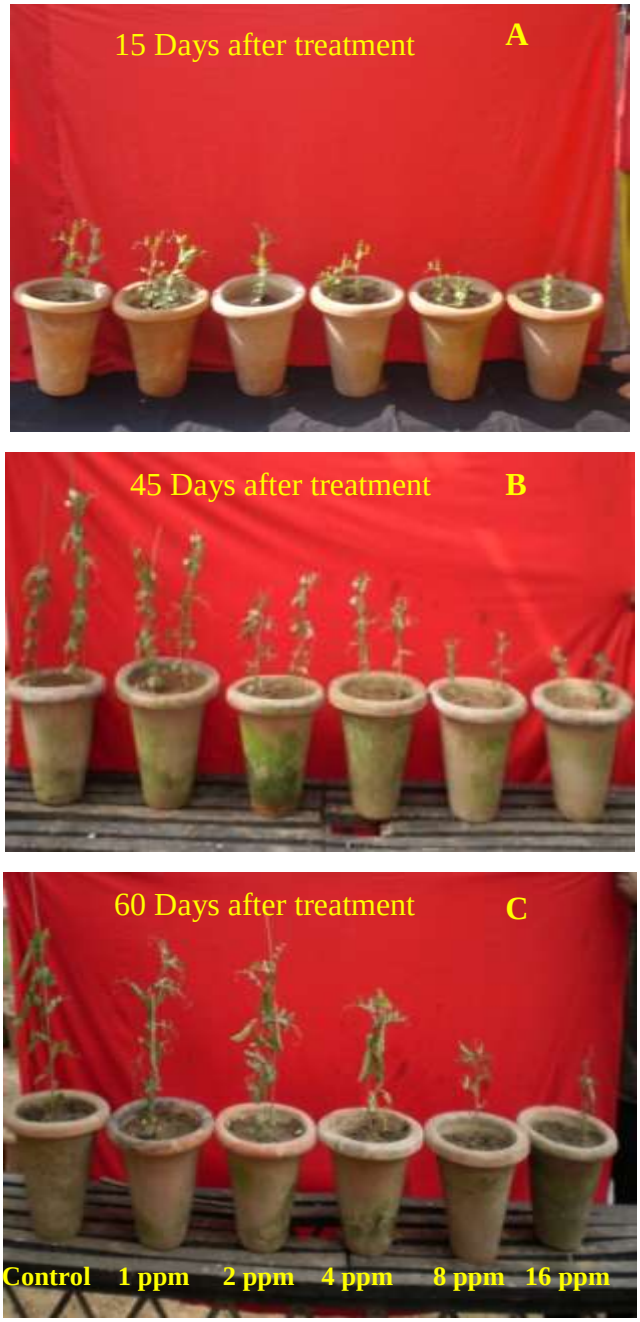

Fig. 1: Influence of differential cadmium levels in plant morphology in *Pisum sativum* var. Arkel. A-C indicate acquisition of shoot appearance. The pots were irrigated with cadmium treatment levels (1, 2, 4, 8 and 16 ppm) once in a week, followed by normal irrigation to the level of field capacity.

RESULT

The root and shoot lengths were recorded in pea as influenced by cadmium contaminated irrigation water (1, 2, 4, 8 and 16 ppm). The shoot length growth behavior was recorded in due course of time i.e., 15-60 days at the interval of 15 days. The lower level of cadmium treatment (1 ppm) could cause loss in shoot length ca. 15-5% within 30 days, which could get further enhanced to the level of 63-50% in cases treated with four fold higher cadmium solution (16 ppm). Similarly the root length growth behavior was recorded in due course of time i.e., 15-60 days at the interval of 15 days. The lower level of cadmium treatment (1 ppm) could cause loss in root length ca. 21-24% within 30 days which could get further enhanced to the level of 80-56% in case treated with four fold higher cadmium solution (16 ppm) as shown in Fig.2A and 2B.

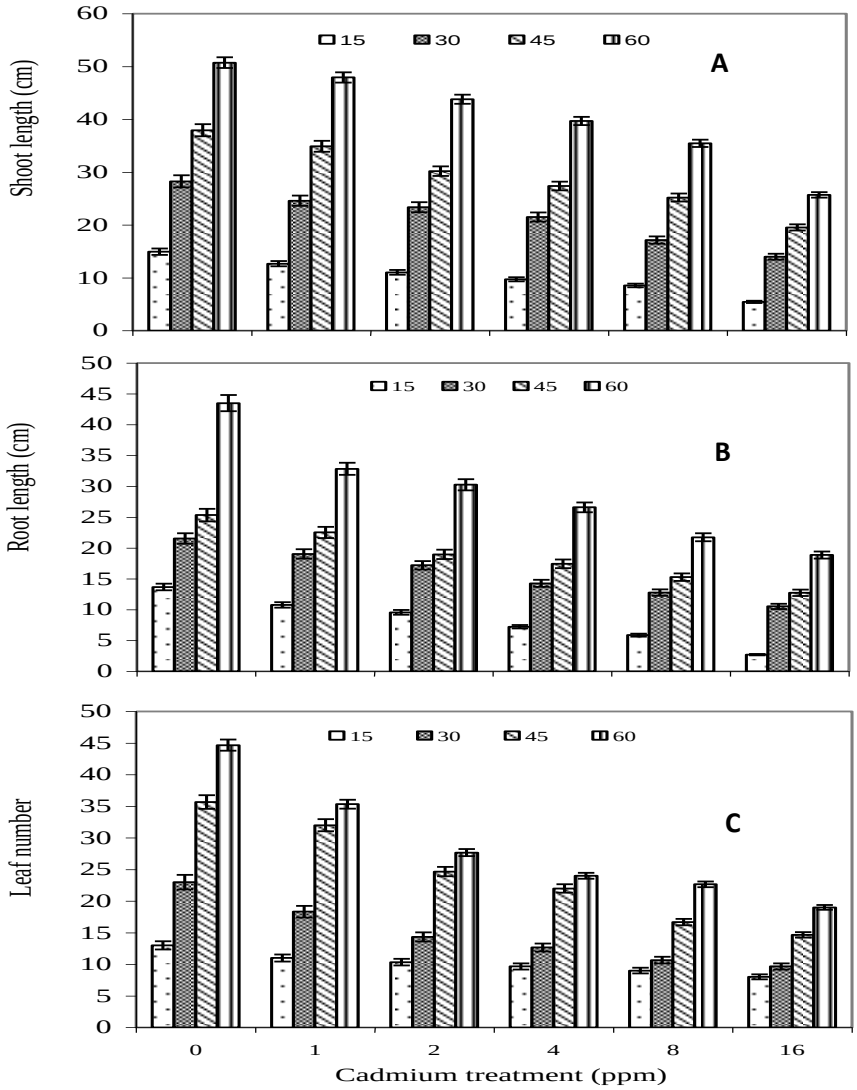

Fig. 2: Effect of cadmium on shoot length (A), root length (B), and number of leaves(C) in *Pisum sativum* var. Arkel. The plants grown under the application of different concentrations (1, 2, 4, 8 and 16 ppm) for a period of 60 days. Values are mean (n=3) with S.E. (±).

The total number of leaves have shown down regulation in retaining their number almost 15-38% depending upon the treatment levels within 15 days as shown by pea. The enhancement in days after treatment have been found correlated in an increasing order in response to loss in total number of leaves. The pea cultivar has shown ca. 20% loss (1 ppm) in total leaves after 60 days in comparison to 38, 46, 49 and 58% in case treated with 2, 4, 8 and 16 ppm levels of the cadmium after 60 days after treatment shown as Fig.2C.

The effect of cadmium irrigation in pea plant was also correlated with certain stress inducible enzymes such as peroxidase and catalase. These enzymes genes generally gets switched on during adverse experiences by plants. The data shown in Fig. 3 clearly opted increasing trends in pea plant. Almost their intrinsic abilities in relation to increase in peroxidase and catalase activity (%) are correlated with cadmium levels, trends as observed with the increase in peroxidase activities (Fig. 3A, B). Both these enzymes are stress mitigating biomolecules therefore; biologically both of them have behaved as per biological rule in supporting the biological system. Similarly lipid peroxidase was also increased with increasing concentration of cadmium (Fig. 3C).

DISCUSSION

Cadmium (Cd) is one such environmental toxicant, which persists and prevails as toxic heavy metal among animals and plants[14].Increasing the concentration of $CdCl_2$ during the germination stage had suppressed the seed germination of *B. rapa* as found also by Asgharipour *et al*., Shaimma *et al*., Heidari and Sarani[15-17].Shan *et al.* reported that high Cd concentrations resulted in a reduction in seedlings growth, expressed as shoot and root length, induced root browning in peanut[18].

The peroxides produced in response to oxidative stress are converted to water by the antioxidant enzyme catalase, thus preventing membrane damage[19,20]. The increase in peroxidase can develop a physical barrier in the cell by increasing lignin biosynthesis resulting in thickening of tissues, hence protecting the cell from ROS damage[21,22]. Our result clearly correlated with the results reported in *Brassica juncea*[20,22], sunflower cotyledons[23] and radish[24] as the shown in Fig. 3 B.

Cadmium chloride treatments effectively increased the activity of the catalase enzyme (CAT) in *B. rapa* leaves[16].The increase in CAT activity after Cd treatments may be due to the scavenging role of CAT to H_2O_2, which could be quenched by the induction of specific enzymes like CAT[25] as shown in Fig.3A.

Shafi *et al*. reported that MDA content was increased[26] under salt and Cd stress in wheat plants *Soybean*[27] as the shown in Fig. 3C. Although Cd does not generate ROS directly, it generates oxidative stress via interference with the antioxidant defense system[28] and increases MDA content in plants due to increased lipid peroxidation.

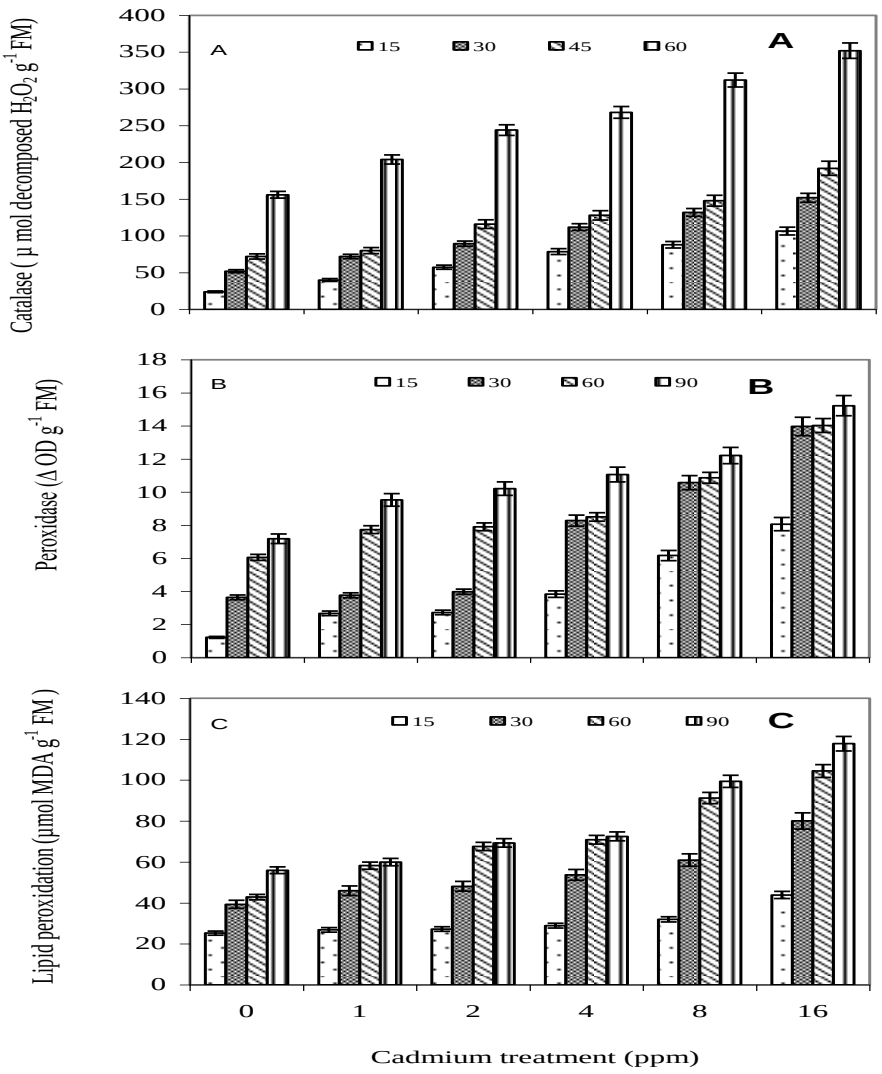

Fig. 3: Effect of cadmium contaminated water on enzymes activities of peroxidase (A) catalase (B) and lipid peroxidase (C) in *Pisum sativum* var. Arkel . Plants were exposed to 1, 2, 4, 8 and 16 ppm cadmium for a period of 60 days. Data shown are mean values S.E .(±) of independent experiments done in three replicates (n=3).

CONCLUSION

Consequently our findings as reported have extended an overview about pea cultivation under the influence of differential levels of the cadmium. It is found that pea may be preferred to be cultivated in agro-climate areas either free from cadmium or may be less affected to ensure crop productivity in relation to national economy (socio-economy) and food safety security for the masses.

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