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Effect of cadium stress on the protein content, lipid peroxidaton and antioxidant enzymes in okra (*Abelmoschus esculentus* L.)

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Abstract

This study was conducted to assess the role of antioxidants enzymes protection from heavy metal stress in Okra (Abelmoschus esculentus.L). different concentrations of cadmium nitrate [$Cd(NO_3)_2$ viz. (20ppm, 40ppm, 60ppm, 80ppm and 100ppm)] were applied to seedlings which resulted in gradual decline in root length, shoot length, number of leaves, lipid peroxidation etc thus indicating the primary physiological response of Cd^{2+} stress. Enzymatic activities analysis of different antioxidant enzymes such as peroxidase, catalase and superoxide dismutase revealed that response varied differentially with the increase in the Cd stress. Increased activities of SOD, APX and CAT enzymes under heavy metals treatments may be considered as circumstantial evidence for tolerance mechanism evolved by the Okra and can be used as biomarker for Cd stress in plants.

Keywods: Antioxidant enzymes; Cadmium stress; Lipid peroxidation, okra;

Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Cd is a one such non-essential and deleterious heavy metal pollutant that is released into the soil from various industrial, mining and farming practices and is reported as one of the top 20 toxins which affect the human health by entering in the food chain [1]. Cd is a non-redox-active metal, metal that it is easily taken up by plant roots growing on Cd-contaminated soils and transported to above ground plant parts [2,3]. The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil [4] but this threshold is continuously exceeding because of several human and agricultural activities. Plants exposed to high levels of Cd causes reduction in photosynthesis, water uptake, and nutrient uptake. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips, and finally death [5,6].

Increased conc. of Cd has been observed to induces the generation of reactive oxygen species (ROS) including superoxide radical ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). which causes cell death due to oxidative stress such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acids [7,8]. To repair the Cd-induced inhibitory effects of ROS, plants employ ROS-detoxifying antioxidant defense mechanisms. Among antioxidative enzymes, superoxide dismutase constitutes the primary step of cellular defense and dismutates $O2^{\bullet -}$ to H_2O_2 and O_2. To scavenge ROS and avoid oxidative damage, plants possess the antioxidative enzymes superoxide dismutase

(SOD), catalase (CAT) and peroxidase (POX), glutathione peroxidase, ascorbate peroxidase and glutathione reductase, as well as non enzyme antioxidants such as ascorbic acid and glutathione [9,10]. Cd toxicity in crop has been found to be associated with the ability of antioxidant machinery to scavenge Cd-induced production of excessive ROS in plants [1,11].

Heavy metal contamination of soil resulting from wastewater irrigation is a cause of serious concern particularly in poor tropical regions mainly due to the potential health impacts by consuming contaminated vegetables. The present study was conducted to determine the effect of different conc. of cadmium (Cd^{2+}) on physiological and antioxidative enzymes on okra plants (*A. esculentus* L.) and explore their contrasting inhibitory and stimulatory effects on productivity of this plant species.

Material and Methods

Seed of okra (*A.esculentus* L) were germinated in Petri dishes in a growth chamber at 25°C, 12 h light /12 h dark period, (illumination of 2500 Lux, Philips T2 40W/33 lamp). Seven-day old seedlings were subjected to viz. 20, 40, 60, 80 and 100 ppm concentrations of $Cd(NO_3)_2$ for 24 hours. The total seedling was harvested after 48 hours of stress and stored at -20°C for analysis of different physiological and biochemical parameters.

Lipid peroxidation was measured in terms of content of malondialdehyde (MDA, ε = 155 $mmol^{-1}$ cm^{-1}), a product of lipid peroxidation, following the described method [12]. 0.5 g seedling were homogenized in 10 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 5 min. To 1 ml aliquot of supernatant, 4 ml 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. MDA content was expressed as nmol MDA per g fresh weight.

The activity of catalase were analyzed as described in the method [13]. The assay mixture consist of 50 µL of the enzyme extract, 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, and 20 mM H_2O_2 in a total volume of 1.5ml.The decrease of H_2O_2 was monitored by reading the absorbance at 240 nm at the moment of H_2O_2 addition and 1 min later. The difference in absorbance (DA_{240}) was divided by the H_2O_2 molar extinction coefficient (36 $M^{-1}.cm^{-1}$) and the enzyme activity expressed as mmol of H_2O_2 $min^{-1}.mg^{-1}$ protein.

Specific APX activity was assayed using the assay mixture consisted of 50 µL of the enzyme extract, 50 mM phosphate buffer (pH 6.0), 0.1 µM EDTA, 0.5 mM ascorbate, and 1.0 mM H_2O_2 in a total volume of 1.5 ml as per the method described by Nakano and Asada [14]. Ascorbate oxidation was monitored by reading the absorbance at 290 nm at the moment of H_2O_2 addition and 1 min later. The difference in absorbance (DA_{290}) was divided by the ascorbate molar extinction coefficient (2.8 $mM^{-1}.cm^{-1}$) and the enzyme activity expressed as mmol of H_2O_2 min^{-1} $.mg^{-1}$ protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H_2O_2.

The SOD activity was determined using the protocol described by Roth and Gilbert, [15]. One millilitre of reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 100 mM EDTA, 20 ml enzyme extract and 10 mM pyrogallol. The enzyme activity [U (mg protein) 21] was calculated by monitoring the reaction

mixture for 120 s (at 60 s intervals) at 420 nm on a Nanovue® device.

Results and discussion

Morphological features and overall growth pattern of seedlings indicated that cadmium stress in okra caused inhibition of shoot and root growth and reduction in the fresh mass of the seedlings. The shoot and root length decreased with the increase in concentration of $Cd(NO_3)_2$in okra. The decrease varied from 35.66- 44.44% and 31.2-42.6% in shoot and root tissues respectively (Fig 1). Similar reports of retardation of shoot and root growth length in other plants has been described previously in response to Cd and other heavy metal treatments [16,17]. The growth inhibition may be consequence of Cd interference with the vital metabolic processes such as photosynthesis and translocation of photosynthetic products and essential nutrients [1,18].

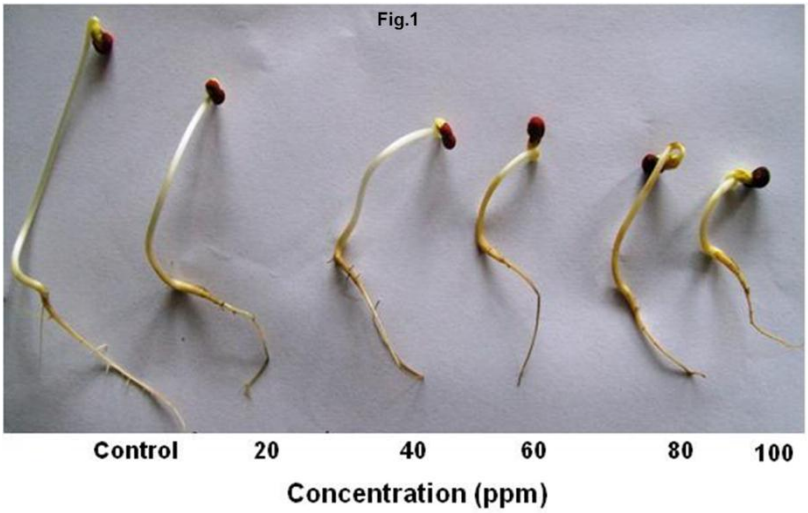

Figure.1 Effect of different conc. of cadmium on the shoot and roots of Okra variety

Measurement of MDA level is routinely used as an index of lipid peroxidation under stress conditions. Exposure to Cd resulted in transient response of accumulation of lipid peroxidation molecules in okra (Fig.2). Maximum increase in MDA level was observed at 40 ppm (125.4%) conc. whereas at other conc. of cadmium there was a decrease (10.24 to 38.26%) in activity as compared to control samples. However these results does not confer with the earlier studies which reported an increased activity of MDA response to Cd stress as in *Pisum sativum* [19] and *Vigna mungo* [20].

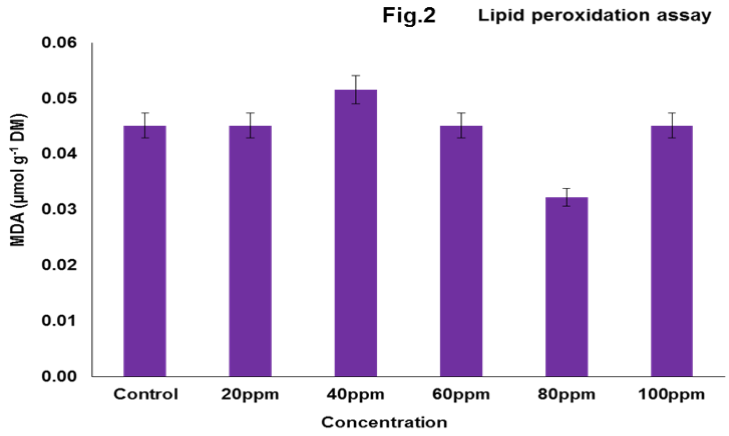

Figure 2. Effect of different Cadmium conc. on the Lipid peroxidation assay

The protective mechanisms developed by plants to scavenge free radicals and peroxides over-produced in response to heavy metals exposure include several antioxidative enzymes such as APX, CAT, and SOD.[10,21]. In response to the increase in the conc. of Cd stress, peroxidase activity get induced at 20 and 60 ppm by 12.56% and 80.41% respectively whereas a slight decrease was observed at 40, 80 and 100 ppm conc. as compared with the activity of control samples (Fig.3a). The enhancement of POX activity could have resulted from either ionic microenvironment or tissue specific gene expression in plants. Moreover, POX participating in lignin biosynthesis could build up a physical barrier against toxic heavy metals [22,23].

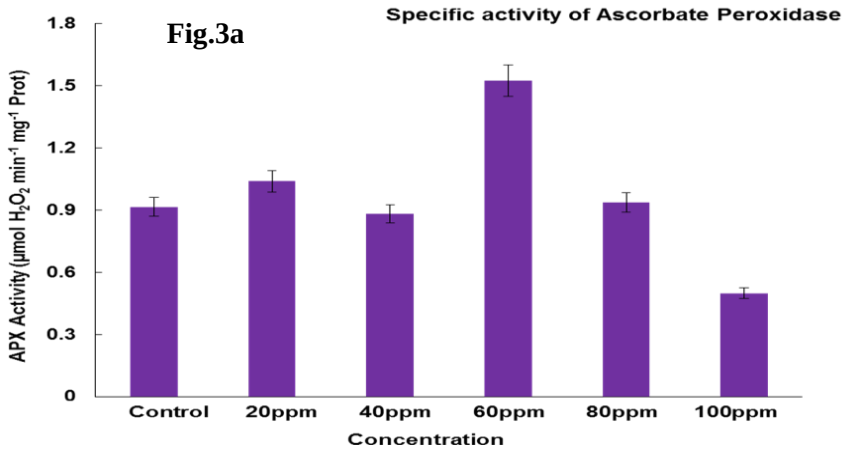

Figure 3a. Effect of different Cd conc. on the specific activity of Ascorbate peroxidase.

Similarly increased Cd conc. induced the activity of catalase enzyme in the seedlings by 5.08% to 95.93% except at 80 ppm where a decrease was observed (Fig. 3b). CAT eliminates H_2O_2 by breaking it down directly to form water and oxygen. CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert ~6 million molecules of H_2O_2 to H_2O and O_2 per minute, whereas APX is involved in scavenging of H_2O_2 in water-water and ascorbate-glutathione cycles and utilizes ascorbate as the electron donor. Therefore, these enzymes are of utmost importance for the removal of ROS under stressed conditions [23].

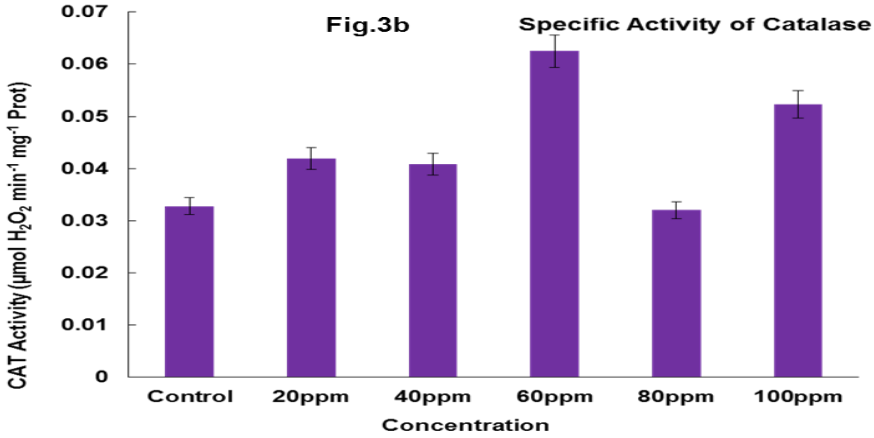

Figure 3b. Effect of different Cd conc. on the specific activity of catalases.

As compared to non-treated control samples, there was a consistent increase of 20 to 64% in the SOD enzyme except of a lower conc. of 20 ppm where a dip was seen (Fig. 3c). Superoxide Dismutase (SOD) represents a key element of the enzymatic system that protects the plant cell against deleterious peroxidation reactions [24]. It was reported that the activation of SOD enzymes exerts a pivotal role during metal stress resistance and for the maintenance of overall defense system of plants subjected to this kind of oxidative damage [1,25,26].

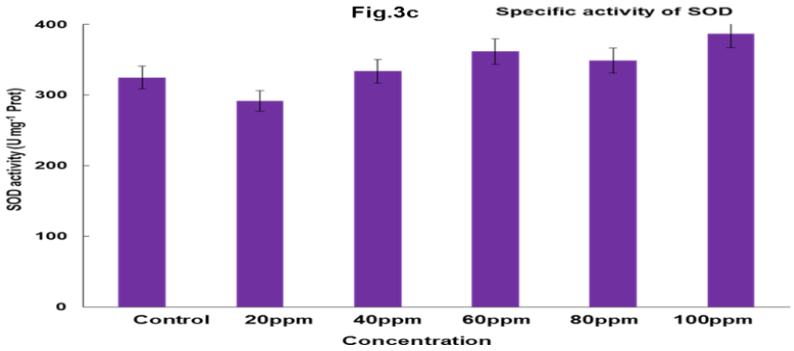

Figure 3c. Effect of different Cd conc. on the specific activity of superoxide dismutase enzyme.

The observations made in this study suggest that there is accumulation of reactive oxygen species, as indicated by the differential activity of lipid peroxidation resulting from the cadmium stress and subsequent detoxification of these AOS by POX, CAT and SOD enzymes can be used as a biomarker in the areas contaminated with heavy conc of Cd, due to its higher antioxidant potential and lesser susceptibility to Cd stress.

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