



## Growth, accumulation and biochemical responses induced by cadmium in *Jatropha curcas* L.

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**Abstract:** *Jatropha curcas* L. attracting considerable global interest due to its potential use as a biofuel crop as well as its potential to grow in any type of soil type. The phytotoxicity imposed by cadmium (Cd) and its detoxifying responses of *Jatropha curcas* L. have been investigated. *J. curcas* L. exposed to various concentrations of cadmium (0, 10, 25, 50 and 100  $\mu$ M) and accumulated good amount of cadmium in various plant parts with increasing concentration. Effect on biomass, photosynthetic pigments and protein level were evaluated as gross effect, while lipid peroxidation reflected oxidative stress cadmium induced lipid peroxidation with increase in metal concentration. Toxic effects of cadmium on plant were reflected by the reductions in photosynthetic pigments, protein at higher concentrations. Enzymatic and non-enzymatic antioxidants were monitored as plants primary and metal detoxifying responses. Enzymes viz., superoxide dismutase, guaiacol peroxidase and ascorbate peroxidase showed stimulation at lower concentrations except catalase which showed declining trend. Initially, cysteine, and non-protein thiols was found increased at lower concentrations, which depleted with increase in concentration. However plant showed progressive increase in proline content at all concentrations. The study concludes that plant could grow under cadmium stress and protect themselves from phytotoxicity of Cd by altering various metabolic processes.

**Key words:** *J. curcas*, Biofuel, Cadmium, Antioxidant, Cysteine, Nonprotein thiols and proline contents, Photosynthetic pigments

### Introduction

"600,000 km<sup>2</sup> of wasteland that is available in India over 300,000 km<sup>2</sup> are suitable for *Jatropha* cultivation." These are the words of Dr. Abdul Kalam, the former President of India, indicating the importance of *Jatropha* for the production of biodiesel (Convention 2006). Being second most populous country, India ranked sixth in terms of energy demand in the world, India has a major challenge to meet its energy requirements. The problem of fulfilling the fuel demand is becoming serious day by day, in this situation the use of biofuel is the most convincing alternative of conventional fuel. There are number of seed bearing plant species which are rich in oil and have excellent fuel properties, but the members of family Euphorbiaceae are most promising plant species to meet the requirement of biofuel. *Jatropha curcas* L. a plant of the same family is attracting considerable global interest due to its potential use as a biofuel crop. *Jatropha curcas* L. is a non-edible oil-yielding perennial shrub and cultivated as a medicinal plant in many tropical and subtropical countries. It grows almost anywhere, even on gravelly, sandy and saline soil, its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. It is suitable for preventing soil erosion and shifting of sand dunes. Various parts of the plant hold potential for use as a source of oil, animal feed or medicinal preparations. The hardy *Jatropha* is resistant to drought and pests, and produces seeds containing 27-40% oil (Achten *et al.*, 2007).

Now a days main focus is given on reclamation and remediation of the contaminated land by planting the accumulator plants. Previous studies showed that besides having the medicinal

properties and rich oil content *Jatropha curcas* L. is known to grows well on heavy metal contaminated land (Kumar *et al.*, 2007). Out of many heavy metals cadmium is one of highly toxic environment pollutant. Cadmium is the non-essential heavy metal, toxic to almost all living beings. Power stations, heating systems, metal working industries, nickel-cadmium batteries and phosphate fertilizers and geo-chemical weathering of rocks are the main emission sources of cadmium (Toppi and Gabbrielli, 1999). Due to long biological persistence and taking its entry via food chain cadmium is known to be neurotoxic, mutagenic and carcinogenic in human beings (Stohs *et al.*, 1995).

Chlorosis, necrosis, growth retardation, water imbalance, phosphorus and nitrogen deficiency, reduced manganese transport and accelerated senescence are known phytotoxic symptoms caused by cadmium (Benavides *et al.*, 2005). Cadmium toxicity causes oxidative stress in plants either by producing reactive oxygen species (ROS) like superoxide (O<sub>2</sub><sup>-</sup>), hydroxyl radicals (OH<sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by autooxidation and fenton reaction. or by decreasing enzymatic and non-enzymatic antioxidants due to its high affinity towards sulfur containing peptides and protein (Benavides *et al.*, 2005; Romero-Puertas *et al.*, 2004). However, plants possess their own defense mechanisms like cellular antioxidants and antioxidant enzymes which protect various vital physiological processes from damage by ROS produced under various heavy metal stresses (Rai *et al.*, 2004).

Defense system of the plant to reactive oxygen species constitutes enzymes like SOD, CAT, APX, GPX and other antioxidant compounds such as cysteine, proline, non protein thiol

and carotenoids *etc.* (Larson, 1988). SOD constitutes the first line of defense converting superoxide radical to hydrogen peroxide ( $H_2O_2$ ), which is then reduced to water and oxygen either by APX in ascorbate–glutathione cycle or by GPX and CAT in cytoplasm and other cellular compartments (Mishra *et al.*, 2006).

Present study aimed to examine the accumulation potential and response of *Jatropha* plants against oxidative stress posed by Cd at low, moderate and high concentrations with reference to gross effect on biomass, photosynthetic pigments and protein, antioxidant enzymes *viz.*, SOD, APX, GPX, CAT and other antioxidants *viz.*, cysteine, non protein thiol and proline to observe the stress exerted by the metal and level of tolerance and detoxification strategy adopted by the plants.

### Materials and Methods

Seeds of *J. curcas* L. were sown in plastic pots (12 in. diameter) filled with acid washed sand (0.01M HCl), placed in glasshouse. After the seed germination sand was irrigated daily with nutrient solution (Hewitt, 1966). Plants after 30 days of germination treated with different concentrations of cadmium (0, 10, 25, 50 and 100  $\mu$ M) for the period of 10 days. After harvesting, plants were washed with double distilled water, blotted and separated in leaves and roots and used for the study of various parameters.

Biomass of the cadmium treated and untreated plants was observed by recording the fresh weight and dry weight of the whole plant. Metal accumulation was estimated by the method of Piper (1942). Chlorophylls content was calculated using the formula given by Arnon (1949) as amended by Lichtenthaler (1987) and carotenoid was calculated by Duxbury and Yentsch (1956). Total sugar was estimated by the method of Dubais *et al.* (1956). The level of lipid peroxidation in fresh leaves was measured in terms of malondialdehyde (MDA) content by the thiobarbutaric acid (TBA) reaction method Heath and Packer (1968). The protein content was estimated according to method of Lowry *et al.* (1951). The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) by method of Beauchamp and Fridovich (1971). The activity of ascorbate peroxidase (APX) was measured according to the method of Nakano and Asada (1981) by estimating the rate of ascorbate oxidation. Guaiacol peroxidase (GPX) activity was assayed according to the method of Hemeda and Klein (1990). The activity of catalase (CAT) was measured by monitoring  $H_2O_2$  decomposition according to method of Aebi (1974). Cysteine content in leaves was measured following the method of Gaitonde (1967). Free proline content in leaves was estimated according to the method given by Bates *et al.* (1973). For estimation of non-protein thiol (NP-SH) content supernatant was reacted with Ellmans reagent, according to method of Ellmans (1959). The data observed in the experiment were statistically analyzed for the calculation of standard error (SE) and student *t*-test was administered for testing the hypothesis.

### Results

Plant showed increase in height and fresh weight upto 25  $\mu$ M over control after that it showed significant decrease in height and fresh weight. Dry weight increased only at the concentration of 10  $\mu$ M after that it showed decreasing trend. Moisture content of plant increased at all concentrations over control. Accumulation of Cd was increased with increase in concentration in all the root, stem and leaves. Maximum accumulation was found at 100  $\mu$ M in roots. While accumulation was found to be minimum in leaves.

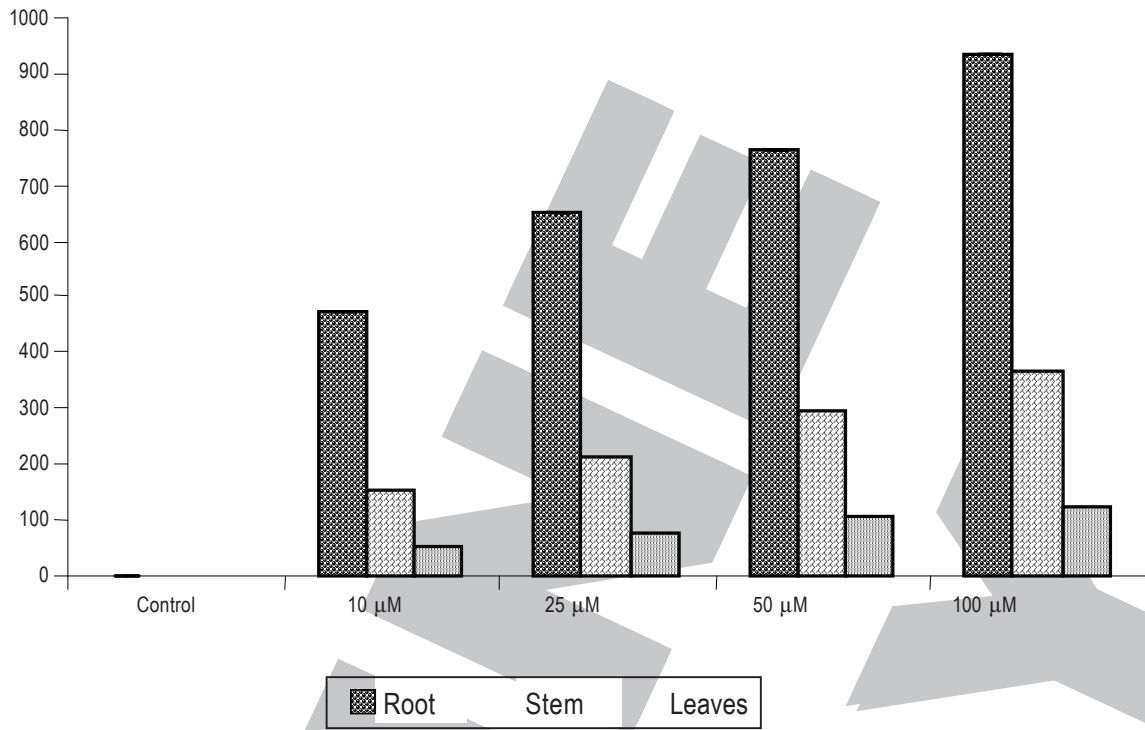
Photosynthetic pigments increased upto 10  $\mu$ M over control after that they decreased progressively with increasing concentration. Maximum decrease was observed at 100  $\mu$ M. Total sugar increased only at 10  $\mu$ M over control beyond this concentration it decreases progressively at all the concentrations over control. Total protein increased upto 25  $\mu$ M over control after that it decreases sharply. The maximum decrease was observed at 100  $\mu$ M both in total sugar and protein. MDA was found to be increased at all the concentrations with maximum at 100  $\mu$ M.

Antioxidant enzymes generally showed stimulation at lower concentration over control followed by decrease at higher concentrations. SOD activity increased significantly upto 10  $\mu$ M beyond that it showed continuous declination with maximum declination at 100  $\mu$ M. Significant increase in APX activity was shown upto 25  $\mu$ M over control. At higher concentrations of 50  $\mu$ M and 100  $\mu$ M APX activity showed declination over control. GPx activity showed stimulation progressively with increasing concentrations over control. The maximum increase was observed at 50  $\mu$ M. CAT activity showed progressive declination with increase in concentrations over control. Maximum declination was noticed at 100  $\mu$ M.

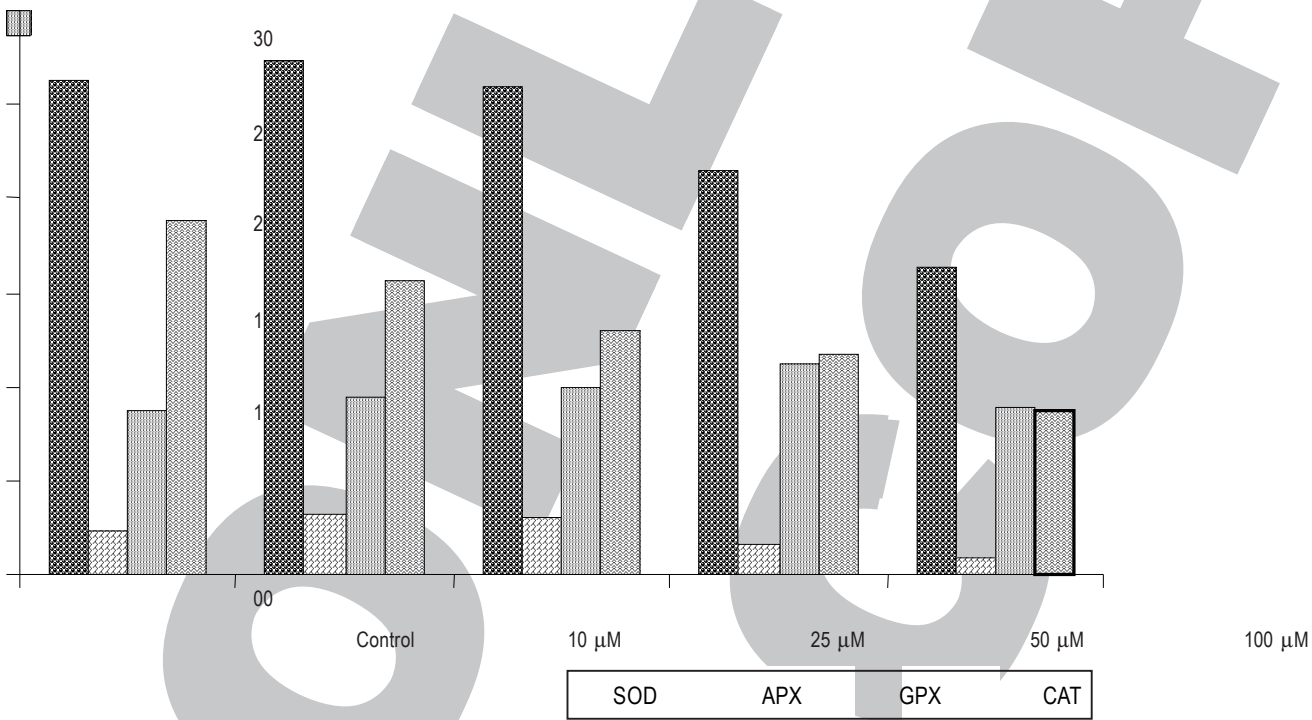
Cysteine level increased with increase in cadmium concentration upto 50  $\mu$ M over control, maximum increase was observed at 25  $\mu$ M. NP-SH content increased at all the concentrations with maximum increase was noticed at 50  $\mu$ M. Proline was found to be increased progressively with increasing the concentrations.

### Discussion

*J. curcas* was able to tolerate 100  $\mu$ M chromium with some physiological and biochemical changes. This suggests this plant has a high adaptability to cope-up with cadmium stress. Toxic effect of Cd was evident from curtailed growth and reduced biomass. The roots accumulated more cadmium than the stem and leaves in all treatments. High metal confinement in the root tissues may be due to immobilization of Cd by cell wall (Vecchia *et al.*, 2005) and extracellular carbohydrates (Wagner, 1993), which may be an important defense strategy, adopted by plants. Plants showed slightly better growth upon exposure to lower exposure, this effect has been attributed particularly to enhanced uptake of nutrients like P and K.



**Fig. 1:** Shows the accumulation of cadmium in root, stem and leaves at different concentrations cadmium content ( $\mu\text{g g}^{-1}$  dry weight)



**Fig. 2:** Represent the activity of various antioxidant enzyme (unit  $\text{mg}^{-1}$  protein) namely SOD, APX, GPX and CAT

**Table - 1:** Plant growth parameters in different concentrations of cadmium

Treatments ( $\mu\text{M}$ )	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Moisture (%)
Control	24.9 $\pm$ 0.41	16.7 $\pm$ 0.45	2.68 $\pm$ 0.36	83.9 $\pm$ 0.53
10 $\mu\text{M}$	27.3 $\pm$ 0.20	18.9 $\pm$ 0.48	2.78 $\pm$ 0.28	85.2 $\pm$ 0.39
25 $\mu\text{M}$	25.1 $\pm$ 0.46	17.3 $\pm$ 0.33	2.01 $\pm$ 0.16	88.3 $\pm$ 0.36
50 $\mu\text{M}$	21.3 $\pm$ 0.45	13.7 $\pm$ 0.43	1.33 $\pm$ 0.22	90.2 $\pm$ 0.41
100 $\mu\text{M}$	17.4 $\pm$ 0.38	8.21 $\pm$ 0.26	0.97 $\pm$ 0.40	88.1 $\pm$ 0.32

Plant showed increase in height and fresh weight upto 25  $\mu\text{M}$  over control after that it showed significant decrease in height and fresh weight. Dry weight increased only at the concentration of 10  $\mu\text{M}$  after that it showed decreasing trend. Moisture content of plant increases at all the concentrations over control

**Table - 2:** Shows the effects of different concentration of cadmium on photosynthetic pigments

Treatments ( $\mu\text{M}$ )	Chlorophyll a ( $\text{mg g}^{-1}$ FW)	Chlorophyll b ( $\text{mg g}^{-1}$ FW)	Total chlorophyll ( $\text{mg g}^{-1}$ FW)	Carotenoid ( $\text{mg g}^{-1}$ FW)
Control	0.95 $\pm$ 0.55	0.47 $\pm$ 0.09	1.42 $\pm$ 0.74	0.42 $\pm$ 0.67
10 $\mu\text{M}$	1.10 $\pm$ 0.78	0.51 $\pm$ 0.19	1.61 $\pm$ 0.89	0.47 $\pm$ 0.04
25 $\mu\text{M}$	0.91 $\pm$ 0.94	0.41 $\pm$ 0.08	1.32 $\pm$ 0.92	0.41 $\pm$ 0.01
50 $\mu\text{M}$	0.87 $\pm$ 0.16	0.33 $\pm$ 0.76	1.22 $\pm$ 0.03	0.37 $\pm$ 0.83
100 $\mu\text{M}$	0.73 $\pm$ 0.67	0.21 $\pm$ 0.32	0.94 $\pm$ 0.076	0.29 $\pm$ 0.54

Values are the mean of three replicate  $\pm$  SD

**Table - 3:** Represent the total sugar, protein and lipid peroxidation (MDA) at different concentrations of cadmium

Treatments ( $\mu\text{M}$ )	Total sugar ( $\mu\text{g g}^{-1}$ )	Total protein ( $\text{mg g}^{-1}$ FW)	MDA ( $\mu\text{mole g}^{-1}$ FW)
Control	6.33 $\pm$ 0.27	12.5 $\pm$ 0.89	4.6 $\pm$ 0.09
10 $\mu\text{M}$	6.98 $\pm$ 0.42	13.01 $\pm$ 0.97	5.1 $\pm$ 0.13
25 $\mu\text{M}$	5.67 $\pm$ 0.16	12.82 $\pm$ 0.29	5.89 $\pm$ 0.17
50 $\mu\text{M}$	4.08 $\pm$ 0.34	9.38 $\pm$ 0.82	7.11 $\pm$ 0.36
100 $\mu\text{M}$	3.38 $\pm$ 0.09	7.28 $\pm$ 0.37	7.76 $\pm$ 0.21

Values are the mean of three replicate  $\pm$  SD

**Table - 4:** Represent the level of cysteine (nmole  $\text{g}^{-1}$  FW), NP-SH (nmol  $\text{g}^{-1}$  FW) and proline ( $\mu\text{mol g}^{-1}$  FW) at different concentrations of cadmium

Treatments	Cysteine	NP-SH	Proline
Control	32.1 $\pm$ 1.87	43.7 $\pm$ 2.33	0.38 $\pm$ 0.031
10 $\mu\text{M}$	35.0 $\pm$ 0.97	45.33 $\pm$ 1.72	0.42 $\pm$ 0.042
25 $\mu\text{M}$	37.9 $\pm$ 0.86	46.1 $\pm$ 0.97	0.49 $\pm$ 0.26
50 $\mu\text{M}$	33.2 $\pm$ 0.32	46.7 $\pm$ 0.43	0.53 $\pm$ 0.006
100 $\mu\text{M}$	28.5 $\pm$ 0.27	44.3 $\pm$ 1.28	0.59 $\pm$ 0.015

Values are the mean of three replicate  $\pm$  SD

Cadmium reduced the foliar contents of total chlorophyll, Chl-a, and Chl-b contents in *J. curcas* at higher concentrations. This might be attributed to the toxicity of cadmium to chlorophyll biosynthesis of the plant. Significant reduction in photosynthetic pigments may be due to oxidation of the photochemical apparatus (Somashekaraiah 1992), reduction in chloroplast density and size (Baryla *et al.*, 2001), Fe deficiency, phosphorus deficiency or reduced Mn transport (Benavides *et al.*, 2005). Cadmium also reduced carotenoid contents in plant which served as accessory pigments for photosynthesis and also protected the plants from

photo-oxidation. Similar, observations have also been made in other plants exposed to cadmium (Mishra *et al.*, 2006).

Increased sugar synthesis at lower concentration may be due to the metal-induced alteration of carbohydrate metabolism. Reduced sugar synthesis in plants by cadmium might be due to lower synthesis or diversion of metabolite to other synthesis processes.

Increase in protein level at lower metal concentrations may be attributed to induction of stress proteins (Toppi and Gabbrielli, 1999). At higher concentrations of metal, however, there was significant decrease in protein content, which may be due to Cd induced oxidation of proteins, mediated by  $\text{H}_2\text{O}_2$  and due to increased proteolytic activity. Protein degradation and proteolytic activity have been proposed as an index of oxidative stress (Romero-Puertas *et al.*, 2004).

In the present study, there was a gradual increase in MDA content with increase in concentration indicating damage to the membrane. In chloroplasts and peroxisomes Cd causes ROS generation, these ROS cause severe lipid peroxidation due to removal of hydrogen from unsaturated fatty acids leading to formation of lipid radicals and reactive aldehydes (Romero-Puertas *et al.*, 2004).

Cadmium induced reactive oxygen species in plants to mitigate and repair the damage initiated by reactive oxygen species, plants have evolved a complex system involving of antioxidant enzymes. Increase in the SOD activity at lower concentrations may be attributed to the increased production of active oxygen species (Somashekaraiah *et al.*, 1992). A reduction in SOD activity at higher Cd concentration may be attributed to an inactivation of enzyme by  $\text{H}_2\text{O}_2$ , which is produced in different cellular compartments and also from a number of non-enzymatic

and enzymatic processes in cells (Dixit *et al.*, 2001; Sandalio *et al.*, 2001; Schützendübel *et al.*, 2002).

Ascorbate peroxidase (APX) is the member of the ascorbic acid–glutathione cycle, and plays a crucial role in eliminating poisonous H<sub>2</sub>O<sub>2</sub> from plant cells. In this study, APX activity inhibited at all the concentrations except at 10 µM in plant. This is similar to the results earlier described in bacopa plants growing under cadmium stress (Mishra *et al.*, 2006). This might be due to the harmful effects of the over production of H<sub>2</sub>O<sub>2</sub> or its poisonous active oxygen derivatives. GPX activity showed better response as compared to APX. GPX thus seemed to play a lead role as compared to APX. GPX is thought to be a stress marker enzyme (Castillo, 1986).

GPX participates in the lignin biosynthesis and might build up a physical barrier against poisoning of the heavy metals (Rai *et al.*, 2004). Therefore, hyperactivities of GPX strategy adopted by the plant to overcome the toxicity of the cadmium, seemed to play a lead role as compared to APX. CAT activity in the present study decreased at all the concentration under Cd stress. CAT is sensitive to O<sub>2</sub><sup>-</sup> radicals and thus their increasing levels under Cd stress may result in inactivation of enzyme (Cakmak, 2000). The decrease may also be associated with degradation caused by induced peroxisomal proteases or may be due to photoinactivation of enzyme (Sandalio *et al.*, 2001). This is similar to the results earlier described in bacopa plants growing under cadmium stress (Mishra *et al.*, 2006).

Cellular antioxidants (thiols, carotenoids, ascorbate, *etc.*) may also play an important role in inducing resistance in plants to metals by protecting labile macromolecules against attack by free radicals which are formed during various metabolic reactions leading to oxidative stress (Kumar *et al.*, 2002). In present study the concentration of NP-SH and increased with the increase in Cd concentration and decreased at higher concentration (50 and 100 µM Cd). The increase in cysteine content following metal exposure may be due to stimulated sulfate reduction pathway (Mishra *et al.*, 2006).

During the present study, higher accumulation of proline in cadmium treated plants has been observed which might be attributed to the strategies adapted by plants to cope up with toxicity (Rai *et al.*, 2004). Proline has multiple functions, such as, osmoticum, scavenger of free radicals, protector role of cytoplasmic enzymes, source of nitrogen and carbon for post stress growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential (Rout and Shaw, 1998).

It may be concluded from the present study that *J. curcas* besides having the properties of petrocrop it could grow as well in cadmium rich environment and accumulate fairly good amount of cadmium in roots followed by stem and then leaves. Cadmium accumulation affects various physiological processes of plant cadmium-induced oxidative stress was tolerated by this plant

through the hyperactivity of antioxidant defense system. Thus this plant has a high adaptability to cope-up with cadmium stress at lower concentration.

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### References

- Convention: India eNews Pvt. Ltd.. <http://indiaenews.com/2006-06/10698-kalam-attend-jatropha-planters-convention.htm>. (2006).
- Achten, W.M.J., Mathijs E., Verchot, L., Singh, V.P., Aerts, R. and Muys, B.: *Jatropha* biodiesel fueling sustainability. *Biofuels, Bioproducts and Biorefining. The Jatropha Archives*, **1**: 283-291 (2007).
- Aebi, H.: Catalase. In: H.U. Bergmeyer (Ed.) *Methods of enzymatic analysis*. Verlag Chemie, Weinheim, Academic Press Inc., New York. p. 680 (1974).
- Arnon, D.I.: Copper enzymes in isolated chloroplasts: Polyphenoloxidases in *Beta vulgaris*. *Plant Physiol.*, **24**: 1–15 (1949).
- Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C. and Havaux, M.: Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: Causes and consequences for photosynthesis and growth. *Planta*, **212**: 696–709 (2001).
- Bates, L.S., Waldren, R.P. and Teare, J.D.: Rapid determination of proline for water stress studies. *Plant Soil*, **39**: 205-207 (1973).
- Beauchamp, C. and Fridovich, I.: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, **44**: 276–287 (1971).
- Benavides, M.P., Gallego, S.M. and Tomaro, M.L.: Cadmium toxicity in plants. *Braz. J. Plant Physiol.*, **17**: 21-34 (2005).
- Cakmak, I.: Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.*, **146**: 185–205 (2000).
- Castillo, F.J.: Extracellular peroxidases as markers of stress?, In: H. Greppin (1986).
- Dixit, V. Pandey, V. and Shyam, R.: Differential oxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *J. Exp. Bot.*, **52**: 1101-1109 (2001).
- Dubais, M.K.A., Hamilton, J.K., Rebox and Smith, F.: Calorimetric dubais: Method of determination of sugar and related substances. *Anal. Chem.*, **28**: 350-356 (1956).
- Duxbury, A.C. and Yentsch, C.S.: Plantkton pigment monograph. *J. Mar. Res.*, **15**: 93-101 (1956).
- Ellman, G.L.: Tissue sulphhydryl groups. *Arch. Biochem. Biophys.*, **82**: 70-77 (1959).
- Gaitonde, M.K.: A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochem. J.*, **104**: 627–633 (1967).
- Heath, R.L. and Packer, L.: Photoperoxidation in isolated chloroplasts 1. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, **125**: 189–198 (1968).
- Hemeda, H.M. and Klein, B.P.: Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *J. Food Sci.*, **55**: 184-185 (1990).
- Hewitt, E.J.: Sand and water culture methods used in the study of plant nutrition. Tech. Commun., 22, 2<sup>nd</sup> Edn, Commonwealth Agricultural Bureaux. Farnham Royal Bucks, England (1966).
- Kumar, A., Vajpayee, P., Ali, M.B., Tripathi, R.D., Singh, N., Rai, U.N. and Singh, S.N.: Biochemical responses of *Cassia siamea* Lamk grown on coal combustion residue (fly-ash). *Bull. Environ. Contam. Toxicol.*, **68**: 675–683 (2002).
- Larson, R.A.: The antioxidants of higher plants. *Phytochemistry*, **27**: 969-978 (1988).

- Lichtenthaler, H.K.: Chlorophyll and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, **148**: 350-385 (1987).
- Lowry, O.H., Rosenberg, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275 (1951).
- Mishra, S., Srivastava, S., Tripathi, R.D., Govindarajan, R., Kuriakose, S.V. and Prasad, M.N.V.: Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant Physiol. Biochem.*, **44**: 25-37 (2006).
- Nakano, Y. and Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, **22**: 867-880 (1981).
- Piper, C.S.: Soil and Plant Analysis. Waite Agricultural Research Institute. The University, Adelaide, Australia (1942).
- Rai, V., Vajpayee, P., Singh, S.N. and Mehrotra, S.: Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, praline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Science*, **167**: 1159-1169 (2004).
- Romero-Puertas, M.C., Rodriguez-Serrano, M., Corpas, F.J., Gomez, M., del Rio, L.A. and Sandalio, L.M.: Cadmium induced subcellular accumulation of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in pea leaves. *Plant Cell Environ.*, **27**: 1122-1134 (2004).
- Rout, N.P. and Shaw, B.P.: Salinity tolerance in aquatic macrophytes: Probable role of proline, the enzymes involved in its synthesis and C4 type of metabolism. *Plant Sci.*, **136**: 121-130 (1998).
- Sandalio, L.M., Dalurzo, H.C., Gómez, M., Romero-Puertas, M.C. and del Rio, L.A.: Cadmium induces changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, **52**: 2115-2126 (2001).
- Schutzendubel, A., Nikolova, P., Rudolf, C. and Polle, A.: Cadmium and H<sub>2</sub>O<sub>2</sub> induced oxidative stress in *Populus canescens* roots. *Plant Physiol. Biochem.*, **40**: 577-584 (2002).
- Somashekaraiah, B.V., Padmaja, K. and Prasad, A.R.K.: Phytotoxicity of cadmium ions on germinating seedlings of mungbean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. *Physiol. Plant*, **85**: 85-89 (1992).
- Stohs, S.J. and Bagchi, D.: Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med.*, **18**: 321-336 (1995).
- Toppi, S.D.L. and Gabbriellini, R.: Response to cadmium in higher plants. *Environ. Exp. Bot.*, **41**: 105-130 (1999).
- Vecchia, F.D., Rocca, N.L., Moro, I., De Faveri, S., Andreoli, C. and Rascio, N.: Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium. *Plant Sci.*, **168**: 329-338 (2005).
- Wagner, R.C.: Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.*, **51**: 173-212 (1993).

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