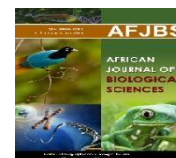


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### Evaluation of Effect of Cadmium Stress on Growth and Antioxidant Enzymes Activities of *Jatropha curcas* L.

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#### Abstract

The essential processes of plants, including photosynthesis, seed germination, seedling growth, and other physiological processes, may be harmed by traces of heavy metals. Therefore, we aimed to evaluate the effect of cadmium stress on growth, and its effect on activities of antioxidant enzymes of *Jatropha curcas* L. The *J. curcas* plants were cultivated at open fields at Defence Institute of Bio-Energy Research, Haldwani. *J. curcas* strain DARL-2 were exposed to cadmium (Cd) stress concentrations at 0 (control), 10, 20, 30, 40, 50, 80, 100, 150, 200 and 300 mg/kg. Both control and treated pots were watered with deionized water at regular interval. All experimental pots were conducted under aseptic condition equipped with cold fluorescent light ( $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with adjusted photoperiod of 12/12 h (day/night) at  $500 \text{ mg L}^{-1} \text{ CO}_2$  and  $70.00 \pm 2.00 \%$  RH. Results revealed that leaf growth parameters *viz.* opening of cotyledonary leaves, emergence of true leaf, and specific leaf area ( $\text{cm}^2/\text{g}^{-1}$ ) were decreased non-significantly ( $p > 0.05$ ) as with the increasing concentrations of cadmium. Whereas, the leaf area ( $\text{cm}^2$ ) was increased non-significantly at 20 mg/kg ( $82.09 \text{ cm}^2$ ), 40 mg/kg ( $98.67 \text{ cm}^2$ ), and 50 mg/kg ( $101.62 \text{ cm}^2$ ), concentrations as to compared control ( $79.04 \text{ cm}^2$ ). The antioxidant enzyme activities *viz.* super oxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), malondialdehyde (MDA), and catalase in *J. curcas* leaves subjected to cadmium stress increased with increasing concentrations of cadmium. Furthermore, increasing activities of antioxidant enzymes were directly proportional to increasing concentrations of cadmium. In conclusion, leaf growth parameters of *J. curcas* were affected up to cadmium concentration of 300 mg/kg. The increasing cadmium concentrations initiate gradual and proportional changes in the determined stress indicator parameters. Our study outcome would certainly be useful to find strategies to alleviate *J. curcas* from heavy metal stress and also to explore and enhance its phytoremediation potential.

**Keywords:** Cadmium stress, *Jatropha curcas* L, Leaf growth, Antioxidant enzymes

## Introduction

Over the past few decades, heavy metal-related environmental contamination has gained significant attention due to the harmful effects it has on the local plants and animals. These pollutants are introduced to the soil and water by mining, pesticides, fertilizers, vehicle exhaust, and industrial discharge. High levels of heavy metal contamination in soil and water also present a risk to the ecosystem because, although their toxic effects reduce agricultural yield, their uptake by plants puts them in the food chain and has detrimental effects on human health. Lead, cadmium, arsenic, chromium, and mercury are examples of heavy metals that are exceedingly toxic and can cause oxidative bursts and tissue damage, which can result in acute or chronic poisoning in both humans and animals (Shourie, 2022).

Studies reported by various research investigators in the literature have documented the toxicity of metals in plants (Hediji *et al.*, 2021; Uveges *et al.*, 2002). The essential processes of plants, including photosynthesis, seed germination, seedling growth, and other physiological processes, may be harmed by traces of heavy metals. Plants react to heavy metal stress in a number of ways, including oxidative burst, increased antioxidant synthesis, and modifications in the permeability of cell membranes. A common occurrence connected to the majority of heavy metal toxicity is growth inhibition (Peralta *et al.*, 2000; Reichman, 2002). It has been reported that seed germination parameters such as germination percentage, germination index, emergence and growth of plumule and radicle, root and shoot lengths, and root and shoot dry matter are affected by heavy metal concentration levels ranging from 10 to 200 ppm, with varying effects (Liu *et al.*, 1994; Jiang and Liu, 2000).

*Jatropha curcas* L. belonging to family *Euphorbiaceae*, is a plant with multiple benefits. Its seeds contain approximately 40-45% oil and therefore it is a potential oilseed crop to be used as feedstock for biodiesel production (Jonas *et al.*, 2020). It is also known for its ethnopharmacological uses as an antimicrobial agent, a potential antioxidant and for its anti-inflammatory activity, due to the presence of large number of bioactive phytochemicals (Bastos *et al.*, 2021). It is also being explored for its phytoremediation potential as it has shown high tolerance towards heavy metals when grown in contaminated soils (Garcia Martin *et al.*, 2020). *J. curcas* is a stress-resistant, perennial plant, growing well in marginal or poor soil, therefore there is special interest in its cultivation in barren and arid regions (Kashe *et al.*, 2018). With these viewpoints, the present study was conducted with the main objective to evaluate the effect of cadmium stress on seed germination and seedling growth, and its effect on activities of antioxidant enzymes of *Jatropha curcas* L.

## Materials and Methods

### *Plant material and cadmium (Cd) concentrations*

The *J. curcas* plants were cultivated at open fields at Defence Institute of Bio-Energy Research, Haldwani (29.22°N and 79.52°E; 424 m asl). Seeds of *J. curcas* strain DARL-2 were cleaned by washing with tap water for multiple times, and soaked overnight in a solution (0.1%, w/v) of carbendazim based broad spectrum systemic fungicide. Seeds were then allowed to dry in shed

for 24 h. The dried seeds were then established on moistened filter paper placed in glass plates for possible germination at room temperature. After germination, seedlings of uniform size were selected and transplanted into pots containing autoclaved mixture of sand and soil in 1:1 ratio. Cadmium as  $\text{CdCl}_2 \cdot 6\text{H}_2\text{O}$  (Himedia) solution was added in the pots to obtain the Cd concentrations of 0 (control), 10, 20, 30, 40, 50, 80, 100, 150, 200 and 300 mg/kg (Figure 1). Experiment was conducted with ten replicates of each treatment and all replication pot had three seedlings. Both control and treated pots were watered with deionized water at regular interval. All experimental pots were conducted under aseptic condition equipped with cold fluorescent light ( $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with adjusted photoperiod of 12/12 h (day/night) at  $500 \text{ mg L}^{-1} \text{ CO}_2$  and  $70.00 \pm 2.00 \% \text{ RH}$ .



**Figure 1:** Cadmium treatment at different concentrations for *J. curcas*

Assessments parameters were measured as per ISTA (International Seed Testing Association) standard methods. At end of the one month, the opening of cotyledonary leaves, emergence of true leaf, leaf area ( $\text{cm}^2$ ), and specific leaf area ( $\text{cm}^2/\text{g}^{-1}$ ) were also measured.

#### **Tissue extraction**

0.5 g of *J. curcas* leaf tissue was homogenized in 1.5 mL of 0.1 M isolating buffer consisting of potassium-phosphate (1M stock solution of 90.8 mL  $\text{K}_2\text{HPO}_4$  and 9.2 mL 1M stock solution of  $\text{KH}_2\text{PO}_4$  [0.1M potassium-phosphate buffer (pH=7.8), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM diethylenetriaminepentaacetic acid (DTPA), 1 mM 1,4-dithiothreitol (DTT), 5 mM ascorbate] in ice-cold mortar (Hegedus *et al.*, 2001). The suspension was centrifuged for 20 minutes, at 10000 g,  $4^\circ\text{C}$ . The supernatant was aliquoted into 1.5 mL Eppendorf tubes and used for enzyme activity measurements.

### Determination of antioxidant enzyme activities

The antioxidant enzyme activities viz. super oxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), malondialdehyde (MDA), and catalase were assayed using standardized ELISA kit-based assay methods.

### Statistical Analysis

Data based on replicates (at least six times) were subjected to an analysis of variance (ANOVA) test to determine the significance between the different treatments using CropStat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines. The treatment means were compared by Least Significant Difference (LSD) test at a significance level of  $p \leq 0.05$ .

### Results

The effect of cadmium stress on leaf growth parameters in terms of opening of cotyledonary leaves, emergence of true leaf, leaf area ( $\text{cm}^2$ ), specific leaf area ( $\text{cm}^2/\text{g}^{-1}$ ) were represented in Table 1. Results depicted that leaf growth parameters viz. opening of cotyledonary leaves, emergence of true leaf, and specific leaf area ( $\text{cm}^2/\text{g}^{-1}$ ) were decreased non-significantly ( $p > 0.05$ ) as with the increasing concentrations of cadmium. Whereas, the leaf area ( $\text{cm}^2$ ) was increased non-significantly at 20 mg/kg ( $82.09 \text{ cm}^2$ ), 40 mg/kg ( $98.67 \text{ cm}^2$ ), and 50 mg/kg ( $101.62 \text{ cm}^2$ ), concentrations as compared control ( $79.04 \text{ cm}^2$ ).

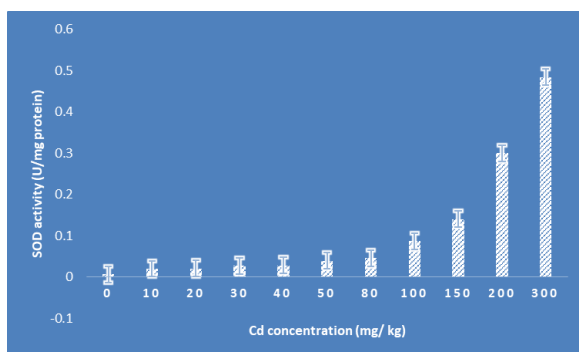
**Table 1:** Effect of cadmium stress on leaf growth parameters in terms of opening of cotyledonary leaves, emergence of true leaf, leaf area, specific leaf area in *J. curcas* leaves

Treatment Cd (mg/kg)	Opening of Cotyledonary Leaves	Emergence of True Leaf	Leaf Area ( $\text{cm}^2$ )	Specific Leaf Area ( $\text{cm}^2/\text{g}^{-1}$ )
Control	94.84	93.77	79.04	52.55
10	86.02	84.44	74.72	47.39
20	86.95	85.77	82.09	45.83
30	92.11	92.66	68.16	41.42
40	87.56	86.66	98.67	38.14
50	86.95	87.33	101.62	34.57
80	82.10	82.00	77.07	34.28
100	82.71	78.66	71.00	30.28
150	73.92	76.44	57.08	30.30
200	59.37	54.00	20.09	15.53

<b>300</b>	46.33	49.55	9.00	8.24
<b>SEM</b>	2.55	2.62	3.07	1.21
<b>LSD</b>	7.17	7.35	8.86	3.48

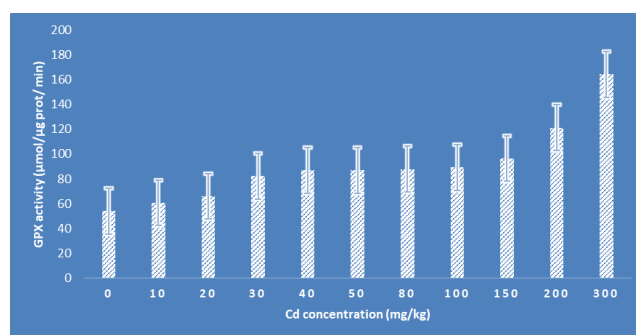
Values are expressed as mean; n=10

The effect of cadmium stress on the antioxidant enzyme activities *viz.* super oxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), malondialdehyde (MDA), and catalase were represented in Table 2. Results delineated that leaf antioxidant activities *viz.* SOD (U/mg protein), GPX ( $\mu\text{mol}/\mu\text{g}$  protein/min), APX ( $\mu\text{mol}/\mu\text{g}$  protein/min), GR ( $\mu\text{mol}/\text{g}$  flwt), MDA ( $\mu\text{mol}/\text{g}$  flwt), and catalase ( $\mu\text{mol}/\mu\text{g}$  protein/min) were increased with increasing concentrations of cadmium. Furthermore, increasing activities of antioxidant enzymes were directly proportional to increasing concentrations of cadmium. Highest and lowest antioxidant enzyme activities *viz.* SOD (U/mg protein), GPX ( $\mu\text{mol}/\mu\text{g}$  protein/min), APX ( $\mu\text{mol}/\mu\text{g}$  protein/min), GR ( $\mu\text{mol}/\text{g}$  flwt), MDA ( $\mu\text{mol}/\text{g}$  flwt), and catalase ( $\mu\text{mol}/\mu\text{g}$  protein/min) were observed at the highest and lowest concentrations of cadmium respectively.



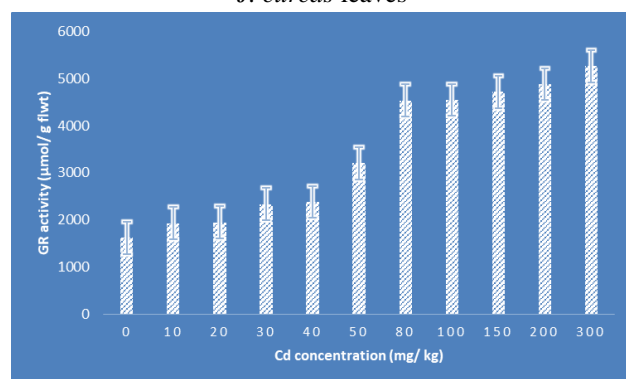
Values are expressed as mean; n=10

**Figure 1:** Effect of cadmium stress on SOD activity of *J. curcas* leaves



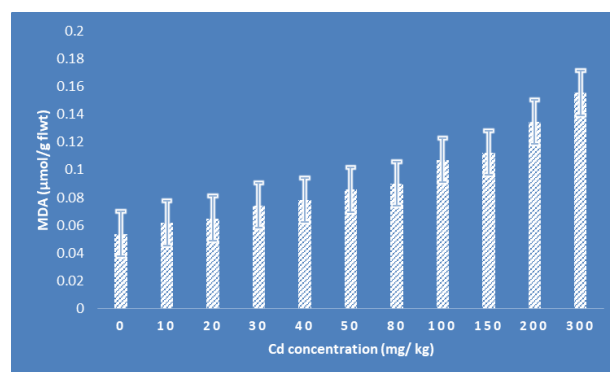
Values are expressed as mean; n=10

**Figure 2:** Effect of cadmium stress on GPx activity of *J. curcas* leaves



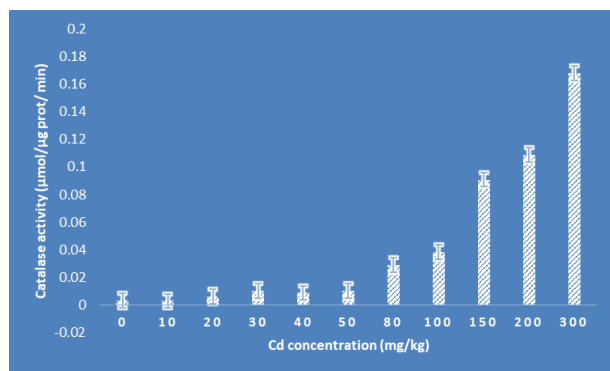
Values are expressed as mean; n=10

**Figure 3:** Effect of cadmium stress on GR activity of *J. curcas* leaves



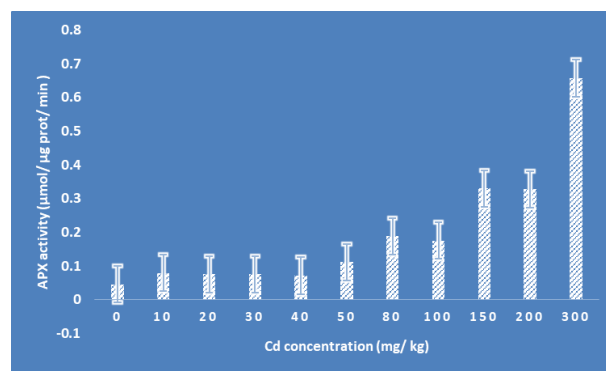
Values are expressed as mean; n=10

**Figure 4:** Effect of cadmium stress on MDA activity of *J. curcas* leaves



Values are expressed as mean; n=10

**Figure 5:** Effect of cadmium stress on catalase activity of *J. curcas* leaves



Values are expressed as mean; n=10

**Figure 6:** Effect of cadmium stress on APX activity of *J. curcas* leaves

## Discussion

In less developed parts of the world, the excessive amount of industrial waste discharge is increasing the toxicity of heavy metals. The agriculture's ability to sustain itself would be threatened by this issue (Sabir *et al.*, 2011). When heavy metal concentrations rise above a certain point, plant growth is hampered, which lowers yield (Jalloh *et al.*, 2009). Heavy metals are deposited in the environment by both natural and human activity, and they are a major source of pollution (Gaur and Adholeya, 2004). Appenroth (2010) claims that the main human-caused sources of metal contamination are the use of pesticides, smelting, mining, industries, rock erosion, and numerous other activities. Because soil is the primary medium used by plants to grow, its quality needs to be given careful consideration. Because of its toxicity and solubility, cadmium is regarded as a major soil pollutant (Jain *et al.*, 2007). Plants can readily absorb cadmium, and it can reach at elevated levels.

Significant sources of Cd include mining, pesticides, phosphate fertilizers, and industrial waste (Milone *et al.*, 2003). Other factors, such as the burning of fossil fuels and the use of tainted water, alter the productivity and quality of the soil, lowering crop yields (Dourado *et al.*, 2013). The toxicity of Cd affects membrane functions and the biosynthesis of chlorophyll (Vitoria *et al.*, 2001). Even at lower concentrations, Cd is extremely toxic because it is a non-essential element. Due to the water-soluble nature of Cd salts, plant roots can readily absorb and transfer Cd to aerial parts (Daud *et al.*, 2009). The health of humans and animals is greatly at risk from consuming these parts because, even in small amounts, they are extremely toxic (Li *et al.*, 2015). With this context we aimed to evaluate the effect of cadmium stress on growth, and its effect on activities of antioxidant enzymes in *Jatropha curcas* L.

Our study findings revealed that leaf growth parameters *viz.* opening of cotyledonary leaves, emergence of true leaf, and specific leaf area ( $\text{cm}^2/\text{g}^{-1}$ ) were decreased non-significantly ( $p > 0.05$ ) with the increasing concentrations of Cd. Whereas, the leaf area ( $\text{cm}^2$ ) was increased non-significantly at 20 mg/kg, 40 mg/kg, and 50 mg/kg, concentrations. These findings were in accordance with literature findings wherein cadmium considerably inhibits the germination of *Cassia siamea* and *Leucaena leucocephala* (Shafiq and Iqbal, 2005; Shafiq and Iqbal, 2008). Furthermore, fresh weight, dry weight and relative water content were also found to be

greatly decreased in Mung bean on exposure to 200 ppm of Cd (Shourie and Vijayalakshmi, 2018). Cadmium was found to be more toxic than iron and zinc. The metal tolerance of germinated seeds was also found to differ significantly between species (El Rasafi *et al.*, 2016). Leaf area is one of the six most important traits that drives plant form and function (Diaz *et al.*, 2016). This descriptor has been widely used to describe a range of variables including growth, productivity, photosynthetic efficiency, soil characteristics including salinity and acidity, transfer and exchange of heat, carbon, nutrients and water, which in turn affect plant yield (Diaz *et al.*, 2016; Cristofori *et al.*, 2007). Thus, a correct determination of the leaf area becomes even more important in crop species, since the leaf is the organ of greater influence with the environment and it is through this that the agronomic studies are based on the important decision making.

Formation of reactive oxygen species is a general phenomenon in plants (Gill and Tuteja, 2010; Kovacik *et al.*, 2017), also increased antioxidative enzyme activity ensuing upon stress posed by Cd is well documented in the literature (Pospisil, 2012). In concurrence with literature findings results of our study on antioxidant enzyme activities *viz.* super oxide dismutase (SOD), glutathione peroxidase (GPx), ascorbate peroxidase (APX), glutathione reductase (GR), malondialdehyde (MDA), and catalase in *J. curcas* leaves subjected to Cd stress increased with increasing concentrations of Cd. Furthermore, increasing activities of antioxidant enzymes were directly proportional to increasing concentrations of Cd. The activity of antioxidative enzyme, the level of MDA supplies characterization of the physiological state of the plant, since peroxidase is localized in the cell wall, the cytosol, the vacuole and the apoplast, while APX is located in chloroplasts, mitochondria, glyoxysomes and peroxisomes, moreover ascorbate is the substrate of APX and a non-enzymatic antioxidant in the same time (Pereira *et al.*, 2005).

### **Conclusions**

Heavy metal pollutant like cadmium undoubtedly causes deleterious effects in plants. In our study leaf growth parameters of *J. curcas* were affected up to concentration of 300 mg/kg. Moreover, results our study delineated that increasing cadmium concentrations initiate gradual and proportional changes in the determined stress indicator parameters especially outcome our research investigation provided evidence pertaining to lipid oxidation caused by cadmium stress. The ability of the *J. curcas* plant to produce biodiesel is well known. Additionally, it is thought to be a significant plant in pharmacology. The results of our study will undoubtedly be helpful in determining how to lessen the heavy metal stress that *J. curcas* is under as well as in enhancing and exploring its potential for phytoremediation.

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### Authors Contribution

Kamal Kant Patra was involved in the execution of all the laboratory work and data analysis; Keshamma E was provided the research guidance and manuscript editing. All authors have read and approved the final manuscript before its submission.

### Conflicts of Interests

None to declare.

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