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Physiological and Growth Responses of Castor (*Ricinus Communis* L) Under Cadmium Stressed Environment

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Abstract

Cadmium (Cd) is considered as phytotoxic in nature, its toxicity on the plant development decrease the antioxidative enzymes activities under stress environment. Castor (*Ricinus Communis* L.) is a metal tolerant plant and its ability to survive in highly polluted soils. Castor plant exhibited the high level of the Cd stress in the soil and buildup the antioxidants i.e., super oxide dismutase (SOD), peroxidase (POD) and malondialdehyde (MAD) on the top of the ground parts under Cd stress. Castor plant grown in the treated soil for 30 days in various levels of Cd 0, 10, 25 and 50 mg kg^{-1} soil treatments. Stress caused by heavy metal toxicity effects on reduced the plant growth, biomass, of castor plant respectively under 25- 50 mg kg^{-1} stress as against to control treatment. Our results indicated that castor significantly enhanced the Cd contents in root, stem and leaves. The POD and SOD enzyme activities were significantly increased 215.30 µmol/g^{-1} and 53.20 U/g respectively under 50 mg kg^{-1} stress as against control. While, MAD and chlorophyll content 3.11% and 0.48%, proline content 2.23 to 1.75 µg^{-1} were decreased under 25 and 50 mg kg^{-1} Cd stress as against control. According to Pearson's correlation the our research work exposed strongly positive relationship with root, shoot, proline and malionaldihyde. Although the destructive relationship was demonstrated by PoD and SOD enzyme activities. Hence, this study recommended that castor can grow in highly polluted soils for phytoremediation.

Keywords: Antioxidant enzymes; castor; cadmium stress; chlorophyll content

Introduction

The cadmium (Cd) is one of the most hazardous metals in the soil and known to be big concern for global agriculture (Xu *et al*. 2018; Yang *et al*. 2013; Huang *et al*. 2011) . In uncontaminated soils worldwide, the average abundance of Cd is 0.36 mg/kg, although the values can vary between continents or countries and soil types, the Cd is a non-essential toxic heavy metal and taken up by plants easily and can translocate to different plant parts (Liu *et al*., 2014). Almost soil Cd concentration range 5-20 ppm is required for remedial action for toxic plants (Adriano, 2001). Castor is an ideal and suitable plant especially used in contaminated soils for remediation and stress tolerate plant (Xi *et al*., 2012). Castor can also eliminate the large amount of Cd from contaminated soils and it's better than other crops i.e., mustard (*Brassica juncea*) (Bauddh and Singh 2012a,b). Budh *et al*. 2016, reported that the R. Communis L. has been found good antioxidant defense system against Cd stress condition. Another study shows that the castor plants can tolerate elevated levels of heavy metals through several developed mechanisms, such as activation of antioxidant enzymes, exclusion, accumulation of proline, and phytochelatins (Yeboah *et al*. 2020). The toxicity of Cd produced oxidative stress in plants and alters the activity of the enzymes such as superoxide (SOD), peroxidase (POD) and non-enzymatic antioxidants glutathione (GSH) consequently reduce the plant growth (Chaoui *et al*., 1997; Khan *et al*., 2007; Szollosi *et al*., 2009). The major changes occurred in the morphology and physiology of the plants(Chhajro *et al*., 2016; 2018;Chaoui *et al*., 1997; Szollosi *et al*., 2009). Countless researchers have been found that Cd could reduce directly or indirectly chlorophyll content and a number of essential nutrients uptake in the plants *(Ci et al*., 2009) . The aim of this study was to evaluate the extent of accumulation of Cd in castor grown under Cd contaminated soil and its effect on metal uptake by plant biomass and antioxidant enzymes mechanisms (MDA, POD and SOD) of castor under contaminated soil. Our research work is hypothesized that the *Ricinus communis* can be used for a successful rehabilitation of contaminated soils having potential ability to tolerate the metals. The castor may be useful indicator for enhancing metal tolerance under Cd stress condition.

Materials and Methods

Collection of soil samples and experiment design: Surface soil samples (0-20 cm) were collected from an arable field of the Huazhong Agricultural University (HZAU) Wuhan, P.R. China. Five sub-samples were collected randomly and composited into one sample. The Cd salt, Cd $(NO_3)_2$. $4H_2O$ was used as the pollutant source and it was added to the soil at different

concentrations of Cd salt i.e. 0, 10, 25 and 50 mg kg^{-1} soil.

Conducting pot experiment: This pot experiment was conducted in a greenhouse condition having 25°C temperature and 65% average relative humidity. Seeds of castor plant were obtained from the Tonglvshan mine, Daye city, Hubei Province, China. Castor plant grown in the treated soil for 30 days in various levels of 0, 10, 25 and 50 mg kg^{-1} soil treatments.

Determination of soil and plant samples: The soil samples were consisted of five sub-samples at randomly. The plants related materials present in soil were removed. Soil was air dried, ground into fine powder to pass through a 0.15 mm nylon mesh were measured physico-chemical properties of soil and the plant samples (leaf, shoot and roots) were washed thoroughly to remove soil and dust particles, oven dried at 75^0C for overnight and ground into fine powder for processes to determine the Cd concentrations using an atomic absorption spectrophotometer (Spectr-AA 220FS, Varian, USA).

The determination of enzymatic activities of castor plant: To examine the enzymes superoxide dismutase (EC 1.15.1.1), SOD, Enzyme classification number (ECN) peroxidase (EC 1.11.1.11), POD and malondialdehyde MAD ($CH_2(CHO)_2$) content was determined by measuring the inhibition of the reduction of nitro-bluetetrazolium (NBT) described by (Lacan and Baccou, 1998). The plant leaf samples (0.5 g) were homogenized in 5 ml of 0.2 mol L^{-1} of sodium phosphate buffer (pH 7.8). The homogenized mixture was centrifuged at 12,000 rpm for 15 minutes at 4^0C and aliquot was collected in falcon tubes so as to proceed with the enzyme determination absorbance tests was measured at 470 nm as described by (Wu *et al*., 2015). SOD in the plant samples was determined by analyzing the ability to reduce the photochemical activity of nitro blue tetrazolium as described by (Wu *et al*., 2015). The MDA content was determined as described by (Heath and Packer, 1968) .

By using following formula:

MDA (Ecmol / l) = 6.45 (OD_{532} - OD_{600}) - 0.56 OD_{450}.

Chlorophyll and Proline contents: Chlorophyll was extracted in 85% (v/v) aqueous acetone and absorption measured in an atomic absorption spectrophotometer model (Spectr-AA 220FS, Varian, USA) at 664 nm.

Shimadzu UV-1201 model spectrophotometer at 645 and 663 nm.

Proline Determination

Proline determination was extracted from a sample of 0.5 g fresh shoot material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of (Turkan and Bor, M and Ozdermir, 2003: Bates *et al*. 1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as µ mol proline g-1 FW.

Statistical analysis: All data were analyzed with (SPSS IBM Statistics version 21) and Microsoft Office Excel 2013, as the mean value with the standard deviation was found significant using analysis of variance (ANOVA) $p < 0.05$ value was considered as a significant difference test.

Results

Increased industrialization and urbanization have decidedly contributed to soil contamination by Cd which impairs plant growth development and antioxidant enzymes in castor under stress condition. Castor is a suitable plant used in contaminated soils for remediation and tolerate metal toxicity environments.

The development of growth parameters & accumulation of cadmium: Plant height and biomass of the castor plant was significantly reduced under Cd stress environment as compared to control. Although all the Cd toxicity stresses exposed that castor was extremely affected by the plant growth and biomass under 25- 50 Cd mg kg^{-1}, which caused 71% - 116%, 166% - >200% and 48% - 84% reduction in stem, root and plant height respectively, as against to control (Table 1).

Table 1. To evaluate the effect of Cd stress on plant height and the biomass of castor plant under contaminated soil

Cd Stress (mg kg^{-1})	Stem weight (g pot^{-1})	Root weight (g pot^{-1})	Plant height (cm)
0	15.77 ± 0.42	10.77 ± 0.40	56.10 ±0.06
10	11.60 ± 0.31	8.71 ± 0.35	42.12±0.58
25	9.22 ± 0.38	6.23 ± 0.35	38.40±0.10
50	7.30 ± 0.25	4.30 ± 0.25	30.50±0.21
Data are presented as the mean ± SD (n = 3). Significant differences from the control are indicated as $p<0.05$.			

Cd uptake in the castor plant such as (root, stem and leaves) was examined to assess the ability of castor to remove Cd, when exposed different levels of Cd in the soil at 25 and 50 mg kg^{-1} Cd stress treatments as against control as shown in (Table 2). The increased level of Cd toxicity can significantly increase its concentration in all parts of the plant. However, the increment was more marked, when Cd was applied at 25 and 50 mg kg^{-1}. The increment of castor plant tissue in the order of roots <stem < leaves was significantly increased Cd concentration with increasing Cd concentrations in the soil at 25 and 50 mg kg^{-1} Cd stress as against control. The Cd stressed castor plants treated with 50 mg kg^{-1} Cd were exhibited 2 fold, 1.8 fold and 1.5 fold in leaves,

stem and roots respectively as compared to control treatments (Table 2)

Table 2. The distribution of castor plant tissue grown under Cd stress condition in Cd contaminated soil

Cd Stress (mg kg^{-1})	Leaves (µg pot^{-1})	Stem(µg pot^{-1})	Root (µg pot^{-1})
0	0.04±0.001	0.05±0.01	0.14±0.01
10	99.03 ±0.01	124.82±0.01	130.45±0.14
25	145.05±0.05	167.03 ±0.08	170.54±0.28
50	160.09±0.06	188.04±0.10	200.34±0.56
Data are presented as the mean ± SD (n = 3). Significant differences from the control are indicated as $p<0.05$.			

Table 3. The response of chlorophyll and carotenoid content and proline content of castor plant grown under contaminated soil.

Cd Stress (mg kg-1)	Chlorophyll (µg-1)	Carotenoid (µg-1)	Proline (µg-1)
0	2.23 ± 0.001^{a}	0.15 ± 0.002^{c}	64 ± 0.001^{a}
10	2.01 ± 0.002^{b}	0.19 ± 0.002^{b}	59 ± 0.002^{b}
25	1.82 ± 0.003^{c}	0.23 ± 0.002^{ab}	57 ± 0.002^{bc}
50	1.75 ± 0.002^{d}	0.27 ± 0.002^{a}	49 ± 0.002^{c}
Data was analyzed by one way analysis of variance Duncan multiple range at $p < 0.05$. Different Values are means ± SD of thrice replicates. Indicate significant difference between the treatments.			

Table 4. Relationship of plant height with physiological parameters

	PH	RT	ST	Clf	Crt	Pr	MAD	POD	Sod
RT	0.91**								
ST	0.92**	0.99**							
Clf	0.26ns	0.56ns	0.57ns						
Crt	0.14ns	0.45ns	0.45ns	0.99**					
Pr	0.99**	0.89**	0.89**	0.26ns	0.15ns				
MAD	0.78*	0.92**	0.93**	0.80**	0.72*	0.78*			
POD	-0.92**	0.85**	-0.84**	-0.04ns	0.08ns	-0.88ns	-0.60*		
Sod	-0.92**	-0.81***	0.02ns	0.15ns	-0.80**	-0.89**	-0.89**	0.55ns	
Rpt	0.08ns	0.24ns	0.24ns	0.49ns	0.49ns	0.09ns	0.37ns	0.02ns	0.06ns

Chlorophyll content of castor plant expressed as chlorophyll a, b, proline and carotenoid contents value was shown in Table 3. The results indicate the chlorophyll and proline content of castor plants were dramatically decreased under Cd stress. The castor plant experienced significant reduction exposed in chlorophyll content under 50 mg kg^{-1}, treatments as compared to control (Table 3). Chlorophyll contents were

Relationship of plant height with physiological parameters

According to Pearson's correlation, the strongly positive

Antioxidant enzymes inhibition in Cd contaminated soil: Antioxidant enzymes play a key role in the management of oxidative stress. Accordingly, the present parameters were evaluated to the effect of Cd levels on the antioxidant enzyme activities under Cd-stressed. Cd stress lead to a significant variation into the antioxidant defense in the castor plants. The antioxidant enzymes activity in the castor plant was significantly increased under Cd stress as compared to control (Figure 1). The maximum increase occurred at 50 mg Cd kg^{-1} soil treatment as compared to the control. In this study, the significantly decreased under 25- 50 mg kg^{-1} Cd stress were noted values 2.23 to 1.75 μg^{-1} as compared to control. Another hand, proline contents of castor plants were also decreased under Cd stress was noted having values 64 to 49 μg^{-1} in 0 and 50 mg kg^{-1} treatments, respectively. In Table 3, while, it can be seen that the carotenoid significantly increases under 50 mg kg^{-1} treatments as compared to control relationship exposed between root, shoot, proline, malionaldihyde (Table 1). Although a destructive relationship was demonstrated by PoD and SOD. results related to two key antioxidant enzymes activities such as peroxidase (POD) super oxide dismutase (SOD) was significantly greater (215.30 Ug^{-1} and 53.20 U g^{-1}) under Cd in 50 mg kg^{-1} treatment as compared to control. Contrary malondialdehyde (MAD) activity in the castor plant was significantly decreased under 50 mg kg^{-1} Cd stress as against control (Figure 2). While, the investigation of alteration results indicated that the MAD was significantly decreased 3.11% under Cd stress as against to control ($p < 0.05$)

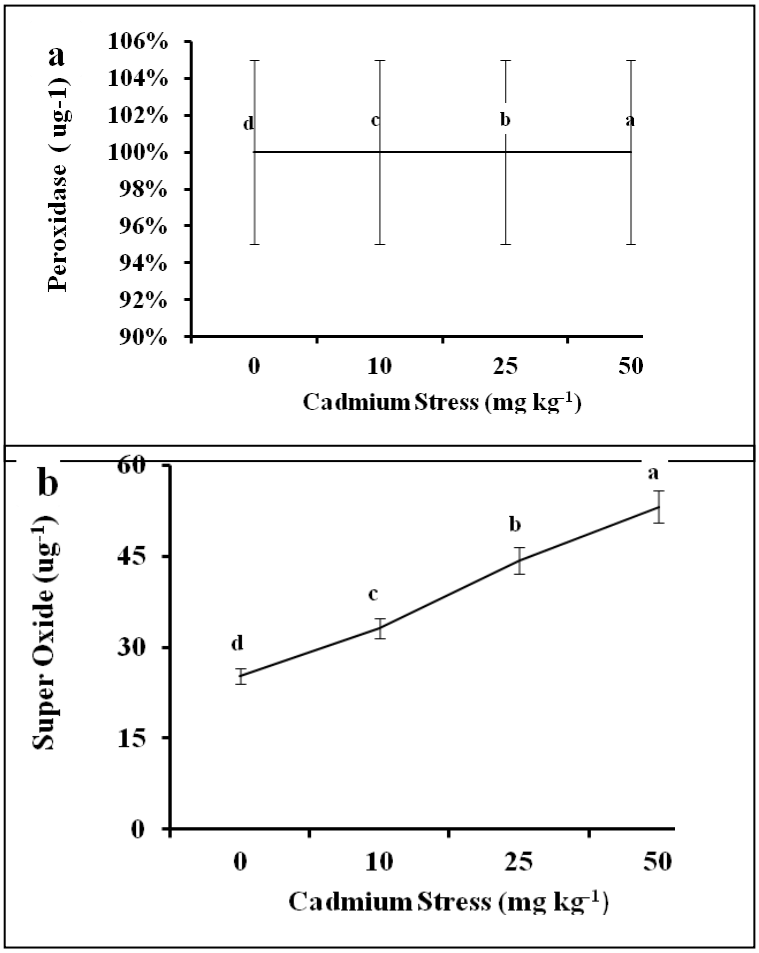

Figure 1 a&b: Peroxide (POD) and superdismute (SOD) antioxidant enzymes content under Cd stress. Data was analyzed by one way analysis of variance Duncan multiple range at $p < 0.05$. Different letters indicate the significant difference between the treatments.

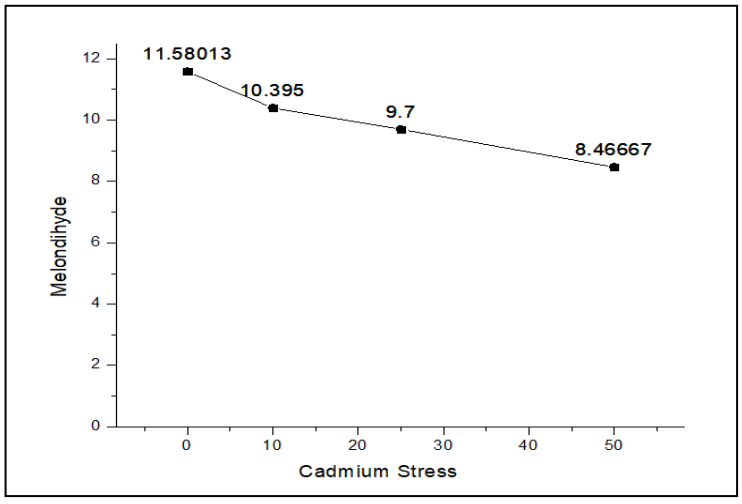

Figure 2. The malonaldehyde (MDA) antioxidant enzymes of castor plants grown under Cd stress. Data was analyzed by one way analysis of variance Duncan multiple range at $p < 0.05$. Different Values are means ± SD of thrice replicates. Indicate significant difference between the treatments.

Discussion

The effects of Cd on biomass, protein, antioxidative enzymes and proline were examined High Cd level in soil cause significant reduction in malondialdehyde (MAD), Chlorophyll and proline content. Metal toxicity by addition of Cd has been widely found in different plant species and it has shown that Cd can cause a selection of phototoxic symptoms (Farooq *et al*., 2013). Recently (Arshad *et al*., 2016) found that the Cd concentrations in shoot and root of wheat were observed greater under 100 µM Cd treatment than in the 0 µM Cd treatment. (Shi and Cai, 2009), also reported that the Cd inhibited the

growth and biomass declined under Cd stress condition. The numerous studies indicated that the oxidative stress and morphological characteristics of castor plants exposed Cd stress. According to Pearson's correlation, the strongly positive relationship exposed between root, shoot, proline, malionaldihyde. Although a destructive relationship was demonstrated by PoD and SOD. Our results showed that the maximum plant growth reduction was noted under the highest Cd (25 and 50 mg kg^{-1}) treatments, which caused 71% - 116%, 166% - >200% and 48% - 84% reduction in stem, root and plant height respectively as against to control. Our results are agreed with (Zhang *et al. 2*015; Zhang *et al*. 2010) stated that the highest Cd concentrations in plant shoots from154.30 and 122.77 $mgkg^{-1}$ and soil Cd concentration of 200 mg kg^{-1}. The highest amounts of accumulation in plant shoots from Kangding and Yajiang were recorded 700.5 and 1403.2 µg pot^{-1}, respectively. Several scientists found that the higher accumulation of Cd in roots was varied in different plant species (Daud et al. (2009) and (Ekmekci *et al*. 2008). Furthermore, many authors have reported that increased antioxidant enzymes activities when plants are exposed to diverse kind of environmental stress (Khan *et al*. 2007; Wu *et al*. 2007). The POD and SOD enzyme activities were significantly increased in the response of Cd 50 mg kg^{-1} Cd stress as against control. Similar results also observed by (Gallego *et al*., 1996; Guo *et al*., 2016) Several studies showed that Cd toxicity disturbed the biochemical and metabolic processes in cotton plants (Vaculík *et al*., 2009). While, in accordance with the results found by Weinstein the metals-induced oxidative damage, reducing the enzyme activity even more when added copper treatments, the our results indicated that the malondialdehyde (MAD) was significantly decreased 3.11% under Cd stress as against to control ($p < 0.005$) in the castor plant (Figure 2). MAD antioxidant enzyme activities of castor plant were decreased under Cd stress as against control. Similar results in chamomile plants related to Cd and Cu uptake(Zhou and Qiu, 2005) that promotes the MDA contents due to lipid peroxidation (Roychoudhury *et al*., 2012) stated that the proline is likewise involved in antioxidant protection the role of this amino acid in protecting plants against damage by reactive oxygen species. Another study reveled that significant increase in leaf MDA production when treated with Cd (Srivastava *et al*. 2005). Chlorophyll content was significantly decreased under different Cd stress were noted values 2.23 to 1.75 $µg^{-1}$ as against control (Table 3). Another hand, proline content of castor plants were also dramatically decreased under Cd stress were noted values 64 to 49 $µg^{-1}$ in 0 and 50 mg kg^{-1} treatments (Table 3), respectively. Our results are agreed with(Farooq *et al*., 2013), they found that the chlorophyll and proline content was declined under Cd stress as compared with control, may be due to more oxidative damage that inhibited the protein contents.

Conclusions

The present study indicated that castor (*Ricinus communis* L.) plant might be a vigorous potential to build up and tolerate the Cd stress in polluted soil and Cd has negative effects on the morphological and physiological changes in R. communis. Cd induced the oxidative stress via increasing antioxidative enzymes activities such as (SOD and POD), which build up the higher Cd under 50 mg kg^{-1} Cd stresses. The plant development, MDA enzyme activity, chlorophyll content and proline content of castor plants were significantly reduced under 50 mg kg^{-1} Cd stress. Thus, this study showed that 50 mg kg^{-1} Cd could be a safer option for increasing the Cd uptake by *Ricinus Communis* L. and minimizing the Cd toxicity under polluted soil. Our results highlighted that the castor is a good choice to remediate and tolerate the Cd stress environment.

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