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Catalase and peroxidase activity in response to cadmium contamination in maize (*Zea mays*) inoculated with AM fungi and *Pseudomonas*

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Abstract
Heavy metal Cadmium (Cd^{2+}) tolerating AMF Arbuscular Mycorrhizal fungi *Glomus* sp. (AM_1) and a bacteria *Pseudomonas* sp. (PS_1) both were isolated from cadmium contaminated area of Coimbatore district of Tamil Nadu. Isolates along with two standard cultures *G. mosseae* (AMs) and *Pseudomonas putida* (PSs) in single and combinations were used for green house study in Cd spiked soil at varying levels i.e., (0, 75 and 125 ppm). Phytochemical changes in the maize plants were studied on 30 and 60 DAS. Anti-oxidant enzymes *viz.*, catalase and peroxidase were estimated in all the treatments. The treatment T_6 is the combination of *Glomus* sp. (AM_1) and *Pseudomonas* sp. (PS_1) inoculated plants had recorded the highest catalase activity in shoot (6.49 H_2O_2µg/g/min) and root (6.82 H_2O_2µg/g/min), peroxidise activity in shoot (6.25 Changes in absorbance min/g) on 30 DAS. The results indicates that the enzyme activity was enhanced when, it was inoculated with microbial consortia.

Keywords: Cadmium, glomus, *pseudomonas*, catalase, peroxidase

Introduction
Heavy metal toxicity in plants reduces the nutrient and water uptake as well as translocation. It also increases the oxidative damage and suppress the plant growth. Though, mitigating of various heavy metal toxicity through application of nutrients, organic amendments and other plant growth regulators is a emerging technique. Added to that, heavy metal cadmium (Cd^{2+}) is to interact with higher plants at physio and biochemical levels, hence reducing the plant growth (Ali and Gill, 2022) [1]. Among these metals Hg, Ag, Cd, As, Sb and Pb have no function in plants as well as in microorganisms as nutrients and seem to be less or more toxic to them (Niess, 1999) [16]. It also reduces the population of soil microbes (Moreno *et al*., 1999) [15]. Since, cadmium is not an essential element for plant growth, it negatively disturbs plant growth and its development. It is also documented as a very important soil pollutant due to its large solubility in water leads to more toxicity (Pinto *et al*., 2004) [17].
Cadmium (Cd^{2+}) is prone to generate the toxic oxygen species and free radicles thus inducing the oxidative stress to the plants. The Cellular enzymatic and non-enzymatic antioxidant enzymes helping the plants in alleviating the cellular oxidative damage induced by the cadmium toxicity. These reactions happen in various cellular and subcellular levels (Hegedus *et al*., 2001) [11]. Gratao *et al.* (2005) [9] also reported that, various enzymes are being activated during the exposure of plants to cadmium stress. Production of enzymes *viz*., peroxidise, catalase, superoxide dismutase and glutathione reductase are the major ROS scavenging mechanisms in plants. Superoxide dismutase (SOD) is an important enzyme in protecting cells against oxidative stresses. The enzyme SOD dismutates superoxides and converts the radical O_2- to hydrogen peroxide (H_2O_2) and oxygen (O_2). But, H_2O_2 is also toxic to plant cells and further it has to be detoxified by antioxidant enzymes like catalase and peroxidase to water and oxygen.
Bioremediation is an environment-friendly process which utilizes microorganisms *viz*., bacteria, fungi and algae and plants to help in the restoration of contaminated soil to its original state Saha, *et al*., 2021 [19]. It harnesses the metabolic capabilities of these microorganisms and plants to convert harmful cadmium Cd^{2+} into less harmful forms, creating an eco-friendly and sustainable solution to Cd contaminated soil.

Biosorption and bioaccumulation are the biological processes which facilitate in removing heavy metal from polluted resources Kumar et.al., 2021 [14].

Applications of plant growth-promoting rhizobacteria (PGPR) is a very important factor in bacterial-assisted Cd bioremediation (Zulfiqar, et.al., 2022) [24]. Belimov *et al*., 2005[2] reported that, many PGPR microorganisms, namely *Rhodococcus* sp. 4N-4, *Flavobacterium* sp. 5P-4, *Variovorax paradoxus* 2C-1, have widely been used as a bio-inoculant for Cd mitigation. Similarly, Sinha and Mukherjee, 2009 [20] also found that *Pseudomonas aeruginosa* can be used for bio-remediation of Cd $^{2+}$ contaminated soil. Dell Amico et.al., 2005 [6] reported that *Pseudomonas tolaasii* RP23, *P. Fluorescens* RS9, can be used for mitigating Cd toxicity in various agricultural and horticultural crops grown in Cd-contaminated soils.

AM fungi are soil microorganisms which forms a symbiosis association with the most of the higher plants, creating a direct physical linkage between plant roots and soil. *Glomus mosseae* and *Glomus intraradices* were enhanced the photosynthetic activity and plant growth in comparison to the other plants in metal contaminated area. Plants infected with AM fungi may produce some of the chemical molecules such as phytochelatins, metallothioneins and glutathione which may chelate Cd complexes (Garg and Bhandari 2014) [8]. Glomalin produced by AM fungi mycelia can also bind more metals and it can significantly immobilize the heavy metals and enhances the tolerance to the host plants which grows in extreme environmental conditions (Zhang *et al*. 2019) [22],. Therefore AM fungi and *Pseudomonas* used in consortia used for growing the crop plants in Cd contaminated soils.

Materials and Methods

Experiment was conducted in the green house of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore using the standard cultures *Glomus mosseae* and *Pseudomonas putida*. 5 kg of sterilized soil (double) was filled in the pots (30x28 cm size) and mixed with 300:20:200 of N: P: K mg/kg of soil (recommended dose). ¼th N and 100 % P and K was mixed in the soil (basal dose). ¾th of N was applied at 45th DAS. Cadmium (Cd^{2+}) was applied @ 75 and 125 mg/kg of soil as $CdCl_2$ before 12 h of sowing. All the microorganisms *G. mosseae* (AM s) and *Glomus* sp. (AM $_1$) were applied as a thin layer below 5 cm to the seeds @ 50g per pot having 8-10 spores /g and 48 h old liquid cultures of *Pseudomonas viz*., PS $_1$ and *Pseudomonas putida* (PS s) were applied @ 50 ml per pot with a cell load of 10^9 cells/ml. Maize seeds - var. CO 1 were surface sterilized with 0.1 % $HgCl_2$ for 3 minutes before sowing and three plants per pot was maintained. Factorial experiment was used with 3 replications. Cd $^{2+}$ concentration was factor 1 (Cd0 ppm, Cd75 ppm and Cd125 ppm) and microorganisms as single and combinations were factor 2. (T_1 to T_9). Randomly collected plant samples (30 and 60 DAS) were used for estimating peroxidase and catalase activity. Activity of both enzymes were measured in shoot and root (Sadasivam and Manickam, 1992) [18]. Peroxidase activity was calculated and expressed as change in OD/min/g of sample and catalase activity was calculated and expressed as g of H_2O_2 /g/ min.

Treatment details

T_1 (UI) – Un-inoculated control
T_2 - PS $_1$ – *Pseudomonas* sp.,
T_3 - PS $_S$ – *Pseudomonas putida*
T_4 - AM $_1$ – *Glomus* sp.,
T_5 - AM $_S$ – *Glomus mosseae*
T_6 – T_2+T_4 (PS $_1$ – *Pseudomonas* sp., + AM $_1$ – *Glomus* sp.)
T_7 – T_3 + T_4 (PS $_S$ – *Pseudomonas putida* + AM $_1$ – *Glomus* sp.,)
T_8 – T_2+T_5 (PS $_1$ – *Pseudomonas* sp., + AM $_S$ – *Glomus mosseae*)
T_9 – T_3+T_5 (PS $_S$ – *Pseudomonas putida* + AM $_S$ – *Glomus mosseae*)

Results

Peroxidase activity

The enzyme peroxidase activity was measured in root and shoot of maize plants on 30 and 60 DAS. Peroxidase activity was increased with increasing levels of Cd on both 30 and 60 DAS was observed. Peroxidase activity was increased in the combined inoculation of AM fungi with bacteria irrespective to root or shoot. The treatment T_7 (*Glomus* sp. (AM $_1$) and *P. putida* (PS s)) has recorded the highest peroxidase activity (10.37 Changes in absorbance/min/g of fresh root tissue) followed by T_9 (*G. mosseae* (AM $_S$) with *P. putida* (PS s)) (9.92 Changes in absorbance min/g of fresh root tissue) at 125 ppm on 30 DAS. (Table1).

Table 1: Effect of microbial consortium of AMF and *Pseudomonas* sp. On peroxidase activity in root of maize crop on 30 and 60 DAS at different Cd levels

Treatments/Concentration of Cd (ppm)	Peroxidase activity in Root (Changes in absorbance min/g)					
	30 DAS			60 DAS		
	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$
T_1(UI)	0.14	1.53	2.89	0.20	1.98	3.29
T_2 (PS $_1$)	4.83	3.98	5.90	5.13	4.62	3.28
T_3 (PS $_S$)	4.19	3.13	1.90	4.92	3.92	2.16
T_4 (AM $_1$)	5.78	7.92	9.92	6.48	7.39	6.29
T_5 (AM $_S$)	5.53	7.08	9.58	6.39	7.28	6.28
T_6 (AM $_1$ x PS $_1$)	8.20	6.25	7.53	8.19	8.75	7.16
T_7 (AM $_1$ x PS $_S$)	6.38	8.34	10.37	5.19	7.09	8.18
T_8 (AM $_S$ x PS $_1$)	6.53	8.96	9.09	7.92	8.84	8.36
T_9 (AM $_S$ x PS $_S$)	5.30	7.87	9.92	5.72	7.63	9.26
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.161	0.323		0.151	0.303	
C	0.093	0.186		0.087	0.175	
T x C	0.279	0.559		0.261	0.525	

The treatment T_6 (*Glomus* sp. (AM $_1$) and *Pseudomonas* sp. (PS $_1$)) was shown the higher activity (6.25 Changes in absorbance min/g of fresh shoot tissue) followed by T_8 (*G. mosseae* (AM $_S$) with *Pseudomonas* sp. (PS $_1$)) (6.10 Changes in absorbance min/g of fresh shoot tissue) and the control (0.61 Changes in absorbance min/g of fresh shoot tissue) (Table. 2) at 125 ppm on 60 DAS. Increasing trend was observed in roots over shoot in all the treatments. There was 66.45 per cent increase of peroxidase activity in root over shoot on 30 DAS in T_7 (*Glomus* sp. (AM $_1$) with *P. putida* (PS s)) at 125 ppm.

Table 2: Effect of microbial consortium of AMF and *Pseudomonas* sp. On peroxidase activity in shoot of maize crop on 30 and 60 DAS at different Cd levels

Treatments/Concentration of Cd (ppm)	Peroxidase activity in shoot (Changes in absorbance min/g)					
	30 DAS			60 DAS		
	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$
T_1(UI)	0.09	1.17	1.93	0.47	0.53	0.61
T_2 (PS $_1$)	3.15	2.38	4.20	1.35	2.08	3.94
T_3 (PS $_S$)	3.01	1.96	1.32	0.81	1.29	1.73
T_4 (AM $_1$)	4.12	6.33	7.39	1.92	2.61	4.78
T_5 (AM $_S$)	4.63	5.92	7.18	0.51	1.28	0.95
T_6 (AM $_1$ x PS $_1$)	7.24	5.23	5.97	3.20	5.46	6.25
T_7 (AM $_1$ x PS $_S$)	5.21	7.92	6.23	2.81	4.20	5.82
T_8 (AM $_S$ x PS $_1$)	5.72	7.20	7.92	3.59	5.91	6.10
T_9 (AM $_S$ x PS $_S$)	4.10	6.38	8.01	2.64	3.69	5.97
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.127	0.255		0.084	0.168	
C	0.073	0.147		0.048	0.097	
T x C	0.221	0.443		0.145	0.291	

Catalase activity

The enzyme catalase activity was measured in root and shoot of maize plants on 30 and 60 DAS. Catalase activity was increased with increasing levels of Cd on both 30 and 60 DAS was observed as in case of peroxidase activity in the present study. The treatment T_6 (*Glomus* sp. (AM $_1$) and *Pseudomonas* sp. (PS $_1$)) had recorded the highest catalase activity in shoot (6.49 H_2O_2µg/g/min) Table. 3 and root (6.82 H_2O_2µg/g/min) Table. 4 at 125 ppm on 30 DAS and the percentage increased in root over shoot was 5 per cent on 30 DAS. All other treatments show increased enzyme activity in root over shoot on both 30 and 60 DAS. Root catalase activity was increased in T_8 (*G. mosseae* (AM $_S$) and *Pseudomonas* sp. (PS $_1$)) on both 30 and 60 DAS (7.01 H_2O_2µg/g/min and 7.28 H_2O_2µg/g/min) at 125 ppm respectively. Irrespective to the isolate, standard AM species inoculated treatments showed highest enzyme activity. The combined inoculation increased the catalase activity than the sole treatments.

Table 3: Effect of microbial consortium of AMF and *Pseudomonas* sp. on catalase activity in shoot of maize crop on 30 and 60 DAS at different Cd levels

Treatments/Concentration of Cd (ppm)	Catalase activity in shoot (H_2O_2µg/g/min)					
	30 DAS			60 DAS		
	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$
T_1(UI)	0.32	0.99	1.73	0.67	1.52	2.20
T_2 (PS $_1$)	0.94	1.76	3.57	1.62	3.20	4.10
T_3 (PS $_S$)	0.77	2.10	2.71	1.20	2.19	3.92
T_4 (AM $_1$)	1.28	2.76	3.90	1.78	3.80	4.20
T_5 (AM $_S$)	0.97	2.21	3.18	1.45	3.23	4.81
T_6 (AM $_1$ x PS $_1$)	2.16	4.98	6.49	2.14	4.60	6.92
T_7 (AM $_1$ x PS $_S$)	1.98	3.76	5.28	2.28	4.12	6.10
T_8 (AM $_S$ x PS $_1$)	2.31	5.42	6.31	2.01	5.30	7.01
T_9 (AM $_S$ x PS $_S$)	1.85	3.41	5.96	1.43	3.97	5.73
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.080	0.162		0.090	0.181	
C	0.046	0.093		0.052	0.104	
T x C	0.140	0.280		0.156	0.314	

Table 4: Effect of microbial consortium of AMF and *Pseudomonas* sp. on catalase activity in root of maize crop on 30 and 60 DAS at different Cd levels

Treatments/Concentration of Cd (ppm)	Catalase activity in root (H_2O_2µg/g/min)					
	30 DAS			60 DAS		
	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$	$Cd_{0\ ppm}$	$Cd_{75\ ppm}$	$Cd_{125\ ppm}$
T_1(UI)	0.51	1.22	1.94	0.86	1.93	2.57
T_2 (PS $_1$)	0.98	1.92	4.01	1.35	4.10	4.92
T_3 (PS $_S$)	0.82	2.84	3.18	1.38	2.81	4.26
T_4 (AM $_1$)	1.39	3.17	4.27	1.92	4.18	4.17
T_5 (AM $_S$)	1.26	3.01	3.94	1.27	3.92	5.36
T_6 (AM $_1$ x PS $_1$)	2.96	5.72	6.82	2.53	5.26	6.84

T_7 (AM $_1$ x PS $_S$)	2.64	4.28	6.15	2.96	5.10	6.18
T_8 (AM $_S$ x PS $_1$)	2.96	6.02	6.92	2.91	5.82	7.28
T_9 (AM $_S$ x PS $_S$)	2.47	4.28	6.27	1.96	4.23	6.29
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.091	0.184		0.099	0.198	
C	0.052	0.106		0.057	0.114	
T x C	0.158	0.318		0.171	0.343	

Discussion

Accumulation of oxygen free radicals was enhanced in the plant system, when it was exposed to heavy metal Cd^{2+}. Plants responds to these deleterious oxidative state and was alleviated by the activities of cellular enzymes and other antioxidants (Cuypers *et al*., 2000) [4]. The enzyme peroxidase can convert peroxides into non-reactive oxygen species. Peroxidase and catalase activity was increased with increasing levels of Cd in all the treatment. The combined inoculation increased the enzyme activity to 10.37 changes in absorbance/min per g of fresh root tissue over shoot 6.23 changes in absorbance/min per g of fresh shoot at 125 ppm of Cd. This finding was agreed with Chaoui *et al*. (2004) [3], who reported that excessive uptake of Cd induced a strong increase of peroxidase activity. Dong *et al*. (2006) [7] found that Cd treatment resulted in a marked increase of peroxidase activity in both leaves and roots. Peroxidase activity in leaves was high at early days and it was reduced in later growth period. The same was found here, the peroxidase activity was reduced on 60 DAS compared to 30 DAS. Since, Peroxidase is involving in biosynthesis of lignin molecule that could built up a physical barrier against toxic heavy metals (Hegedus *et al*., 2001) [11].

Gwozaz *et al.* (1997) [10] reported that catalase activity was decreased at higher concentration of heavy metals. Catalase activity increased with stress level (Zhang *et al*., 2003) [23]. In this study the antioxidant enzyme catalase was increased with increasing metal concentrations. Combined inoculation of AM fungi with *Pseudomonas* increased the antioxidative enzyme to 6.31 H_2O_2/µg/g/min over control 1.73 H_2O_2µg/g/min. Enzyme activity was increased on 60 DAS, because of these increased levels the mechanism of antioxidative defence was very active. But Yang *et al*. (2008) [21] reported that catalase activity was increased with the treatment 0 to 250 mg/l of Cd and then decreased with higher concentrations. The increase in antioxidant enzyme activity observed might be due to the direct response of generation of super oxide radical because of Cd stress. The increase in catalase enzyme was due to the accumulation and toxicity of H_2O_2 in the plant system by Cd stress.

Conclusion

Cadmium toxicity in plants and in soil was reduced by the inoculation of Arbuscular mycorrhizal fungi and *Pseudomonas* sp. was observed in the experimental results. Increased enzyme activity was noted with increasing levels of Cd in all the treatments and in both 30 and 60 DAS. The treatment T_6 (*Glomus* sp. (AM $_1$) and *Pseudomonas* sp. (PS $_1$)) had recorded the highest catalase activity in shoot (6.49 H_2O_2µg/g/min) and root (6.82 H_2O_2µg/g/min), peroxidise activity in shoot (6.25 Changes in absorbance min/g) on 30 DAS. The anti-oxidant enzymes in the plants helped the Cd $^{2+}$ bio-accumulation in root and also translocation of the heavy metal Cd^{2+} was reduced in shoot and grain (data not given). In conclusion that Arbuscular mycorrhizal fungi and *Pseudomonas* sp. can be used for managing the soil contaminated with heavy metal Cd^{2+} as a low-cost technology for improving the crop productivity as well as reducing the transport in food chain.

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