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Enzyme activity of arbuscular mycorrhizal fungi on cadmium tolerance in black gram (*Vigna mungo.* (L*)* hepper)

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Abstract
The present study was conducted to determine the effect of cadmium(Cd) on enzyme activity of black gram (*vigna mungo.*(L*)*hepper**)**.The black gram seeds were treated under control, 2.5, 5, 7.5, 10, and 12.5 mg of Cd. Every treatment was replicated thrice in a randomized block design. Observations were complete on enzyme activity of catalase,peraxidase of black gram .all result when compared with control show that cadmium metal adversely affects the growth of black gram by reducing enzyme activity.The present research work was to find out the effect of different concentrations of cadmium with AMF on, enzymatic activity, of black gram grown under pot culture experiment.

Keywords: black gram ,Cadmium , catalase, peraxidase

1. Introduction
Environmental pollution is increasing with each passing year and inflicting serious and permanent injury to the world. Environmental pollution is of different types namely air, water, soil, noise and light-weight. These cause damage to the living system. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Mycophytoremedial is a term functional to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for private or public applications. Arbuscular mycorrhizal fungi (AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species.

2. Materials and methods
The seeds Black gram (co-7) was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore and Tamilnadu. The uniform seeds are selected for the experimental purpose. Source of Cd (Cadmium chloride (cdcl2) stock solution prepared by dissolving the molecular weight of (Cd) and different concentrations *viz.*, (garden soil -Control,T1-2.5mg,T2-2.5mg+AMF,T3-5mg,T4-5mg+AMF,T5-7.5mg,T6-7.5mg+AMF,T7-10mg,T8-10mg+AMF,T9-12.5mg,T10-12.5mg+AMF) of (Cd) the solution were prepared freshly at the time of experiments. The pods were filed with 5 Kg of garden soil, selected black gram seeds were sown in the pods irrigated with normal tap water was maintained as the control.

AM Fungi
The AM Fungi (*Glomus fasciculatum*) were collected from Department of Microbiology Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

Enzymatic Activities
The activities of catalase, peroxidise were estimated and recorded at different days intervals.

Catalase
Catalase activity was assayed as described by Chandlee and Scandalios (1984).

Extraction
Five hundred milligrams of frozen material was homogenized in 5 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4 for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay.

Assay
The activity of enzyme catalase was measured using the method of Chandlee and Scandalios (1984) with modification that the assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH – 7.0), 0.4 ml of 15 mM H2O2 and 0.04 ml of enzyme extract. The decomposition of H2O2 was followed by the decline in absorbance at 240 nm. The enzyme activity is expressed in units 1 mM of H2O2 reduction per minute per mg protein.

Peroxidase (Kumar and Khan, 1982)
Donor: Hydrogen peroxidant oxido reductase
Assay mixture of peroxidase contained 2 ml of 0.1M phosphate buffer (pH 6.8), 1ml of 0.01M pyrogallol, 1ml of 0.005M H2O2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25C after which the reaction was terminated by adding 1ml of 2.5N H2SO4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5N H2SO4 at zero time. The activity is expressed in unit mg-1 protein. (One unit is defined as the change in the absorbance by 0.1 min-1 mg-1 protein).

Result and Disscution

Catalase

The effect of different concentrations of cadmium with AMF on Catalase contents (min-1 mg-1 protein).) in leaf of black gram at 15,30,45, 60 and 75 DAS is given in Figure 1. The highest Catalase content of leaf.(3.45,7.18,9.35,15.58, and 13.34 min^{-1} mg^{-1} protein.) was recorded in 2.5mg(cd)+AMF(T2) treated plants at 15,30,45, 60 and 75 DAS respectively. The lowest protein content of leaf (0.63,1.13,1.98,2.83,and 2.17 min-1 mg-1 protein.) was observed in 12.5mg(cd)T9 concentration of cadmium treated plants at 15,30,45, 60 and 75 DAS respectively.

Peroxidase

The effect of different concentrations of cadmium with AMF on Peroxidase contents (min-1 mg-1 protein).) in leaf of black gram at 15,30,45, 60 and 75 DAS is given in Figure 2. The highest peroxidase content of leaf.(5.84,8.19,10.34,13.15, and 9.93 min^{-1} mg^{-1} protein.) was recorded in 12.5mg (cd)cadmium treated plants at 15,30,45, 60 and 75 DAS respectively. The lowest Peroxidase content of leaf (0.93,1.13,2.23,2.93,2 and 2.03 min^{-1} mg^{-1} protein.) was observed in 2.5mg(cd)+AMF treated plants at 15,30,45, 60 and 75 DAS respectively.

The enzymes like catalase and peroxidase were low in AMF then the control. Then it was found increase with increasing heavy metal concentrations. Antioxidant enzymes such as CAT, APX and SOD play a vital role in increasing defensive mechanisms towards more ROS production (Foyer et al., 2005. Yang *et al.*, 1995 reported that the enzymatic activities such as POX and CAT were increased in wheat, maize, cucumber and *Sorghum* under cadmium toxicity. In *P. aureus* cadmium increased the activities of guaiacol POX and ascorbate POX, along with the detection of new isoenzyme of POXs in both roots and leaves (Chaoui *et al.*, 1997).

Rabie et al., 2013 suggested that the VAM fungi increase of protein synthesis as well as induction of antioxidant enzymes in maize plants. It might be to avoid heavy metal mediated oxidative stress. Tong et al. 2004 suggested that the plants in AMF showed consistent increase in antioxidant enzymes with increase in concentration and better growth rate as compared to non-AMF as possible mechanisms for plant protection against high accumulated toxic heavy metals in shoots. The study reported that the (Qu et al., 2009) [7] inoculation with G. mosseae could significantly improve the activities of secondary metabolism-related enzymes including polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase in Cabernet sauvignon roots, alleviating injuries to plant cell membranes caused by Cd stress.

The study reported that the effects of autochthonous microorganisms (AMF and/or plant growth promoting bacteria) on the antioxidant activities of plants growing in a multi heavy metal contaminated soil (Azcón et al. 2010) [8]. Azcon et al. 2010 concluded that the AMF inoculation enhanced the activity of CAT, ascorbate peroxidase, or GR and decreased the levels of oxidative damage to plant bio molecules due to metal stress. Garg and Aggarwal (2012) [9], studied that the AMF inoculated Cajanus cajan have significantly higher levels of SOD, CAT, POX as well as GR and were more tolerant to high soil Cd and Pb contents than non inoculated plants.

The higher enzyme activity were observed in AMF than non-AMF with increasing concentrations. It is possible that roots in mycorrhizal associations make available powerful physiological defense against Cd to cope with toxicity (Bhaduri and Fulekar, 2012) [10]. Bhaduri and Fulekar, 2012 reported that the antioxidant enzyme activities increased on exposure to cadmium stress and AMF inoculation further increased the activity resulting in quick ROS scavenging and hence averting oxidative stress for better stress adaptation in Ipomoea aquatic. The study (Hashem et al. 2016) [11] demonstrated that the antioxidant enzymes were increases with the Cd treatmens in C. italic. However, AMF inoculation suggests the importance of AMF in mitigating the deleterious impacts of cadmium stress. Hence AMF can be potential approach for enhancing the tolerance level of C. italic.

Kanwal et al., 2016 suggested that the AMF is able to maintain mycorrhizal symbiosis in Zn toxic soils and significantly increase the plant growth, productivity and nutrient contents. Hashem et al., 2016 demonstrated that the enzymatic activities such as SOD, CAT, POD, GR and APX increased in Cd-stressed tomato plants and that their activity was further enhanced by inoculation with AMF. This further increase in the activities of antioxidants suggests the role of AMF in mediating quick scavenging of ROS.

Table 1: Effect of cadmium and AMF on the catalyse activity of black gram(*vigna mungo*.L)

Treatments with Chromium + AMF	Catalyse (mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75
Control	2.86±0.08	4.35±0.13	6.18±0.18	9.61±0.28	9.15±0.27
2.5 mg Cr	2.14±0.06	3.25±0.09	4.85±0.14	7.18±0.21	6.35±0.19
2.5 mg Cr + AMF	3.45±0.10	7.18±0.21	9.35±0.28	11.58±0.34	10.35±0.31
5 mg Cr	1.93±0.05	2.35±0.07	3.73±0.11	5.75±0.17	4.37±0.13
5 mg Cr + AMF	2.03±0.06	4.15±0.12	4.95±0.15	6.77±0.20	5.19±0.15
7.5 mg Cr	1.23±0.03	2.13±0.06	2.94±0.08	3.84±0.11	2.15±0.06
7.5 mg Cr + AMF	1.85±0.05	3.75±0.11	3.67±0.11	5.13±0.15	3.94±0.11
10 mg Cr	0.76±0.02	1.98±0.05	2.13±0.06	2.88±0.08	2.75±0.08
10 mg Cr + AMF	0.98±0.03	1.56±0.04	2.87±0.09	3.34±0.10	3.16±0.09
12.5 mg Cr	0.55±0.02	0.35±0.01	1.23±0.03	1.93±0.05	1.17±0.03
12.5 mg Cr + AMF	0.63±0.02	1.13±0.03	1.98±0.06	2.83±0.08	2.17±0.06

Table 2: Effect of cadmium and AMF on the peroxidase activity of black gram(*vigna mungo*.L)

Treatments with Chromium + AMF	Peroxidase (mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75
Control	0.93±0.02±	1.13±0.03	2.23±0.06	2.93±0.08	2.03±0.06
2.5 mg Cr	3.98±0.11	5.78±0.17	8.95±0.26	12.16±0.36	7.35±0.22
2.5 mg Cr + AMF	5.84±0.17	8.19±0.24	10.34±0.31	13.15±0.39	9.93±0.29
5 mg Cr	2.03±0.06	2.96±0.08	4.34±0.13	5.99±0.17	4.07±0.12
5 mg Cr + AMF	2.78±0.08	4.39±0.13	6.83±0.20	10.75±0.32	6.35±0.19
7.5 mg Cr	3.53±0.10	4.15±0.12	5.78±0.17	6.39±0.19	5.18±0.15
7.5 mg Cr + AMF	3.87±0.11	5.59±0.16	7.88±0.23	11.10±0.33	6.98±0.20
10 mg Cr	2.13±0.06	3.16±0.09	4.15±0.12	7.93±0.23	4.19±0.12
10 mg Cr + AMF	1.93±0.05	2.23±0.06	3.93±0.119	4.38±0.13	3.83±0.11
12.5 mg Cr	0.54±0.01	0.93±0.02	1.78±0.05	2.19±0.06	1.93±0.05
12.5 mg Cr + AMF	1.97±0.05	2,13±0.06	3.98±0.11	4.18±0.12	3.14±0.09

Conclusion

cadmium (Cd) is one of several heavy metals that cause severe environmental contamination in soil, sediments and groundwater. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Mycophytoremedial is a term functional to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for private or public applications. Arbuscular mycorrhizal fungi (AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species.

The present investigation has been carried out to find out the effect of cadmium an am fungi on seed enzyme activities of black gram plants the co-7 varieties of blackgram seeds were obtained from the Tamil Nadu agricultural university Coimbatore. The cadmium chlorite salts were used for the treatment purpose

The enzyme activity aspects such as catalyse,peracidase were high at AMF as compare control. But, the higher level of cadmium inhibited all enzymes like catalase, and peroxidase were low in AMF then the control. Then it was found increase with increasing heavy metal concentrations. Then it was found increase with increasing heavy metal concentrations. The present investication have enhanced on tremendous increase in the productivity by using AM fungi which is an symbiotic fungal association with the higher plants tried to reduce the toxic nature of the industrial waste specifically on the chromium an life scavenging mechanisms to protect the agricultural field in to from the antagonistic action of chromium.

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