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APPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI ON CADMIUM (cd) TOLERANCE IN GREEN GRAM (Vigna radiata.(L) R. Wilczek

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Abstract

The present investigation was carried out to determine the effect of cadmium(Cd) on enzyme activity of green gram (Vigna radiata.(L) R. Wilczek.The green 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 green gram. All result when compared with control show that cadmium metal adversely affects the growth of green gram by reducing enzyme activity.The present research work was to carried out the effect of different concentrations of cadmium with AMF on, enzymatic activity, of green gram grown under pot culture experiment.

Keywords: Green Gram, Cadmium, Catalase, Peraxidase.

Introduction

Environmental pollution has been converted into an explanation focus of distress for all the nations worldwide, as not only the developing countries but developed nations as well are affected by and suffer from it. Pollution has many forms, the air we breathe, the water we drink, the ground where we cultivate our food crops and even the increasing noise, we hear every day-all contribute to health problems and lesser quality of life.Heavy metals are defined as metallic elements that have a relatively high density compared to water. With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure.Environmental pollution is increasing with each passing year and inflicting serious and permanent injury to the world. Environmental contamination is different types,namely water, soil, noise,air, and light-weight. These cause damage to the living system.

The most important effluent discharging industries are daneryes,textiles,paper mills,iron and steel industreies,fertilizers units etc.industries effluents containing organic and inorganic compounds various forms of heavy metals suspended solids and other materials which naturally affect the water quality as well as ecosystem. 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 technology that use plants to reduce, remove, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for 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.

Materials and Methods

The seeds green gram (co-7) was obtained from Tamil nadu Agricultural University (TNAU), Coimbatore and Tamil nadu. 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 green 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

500 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 min at 12,500 rpm. The supernatant was used for enzyme assay.

Assay

The activity of catalase enzyme 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 peroxidantoxido 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 Discution

Catalase

The effect of different concentrations of cadmium with AMF on Catalase contents (min-1 mg-1 protein).) in leaf of green gram at 15,30,45, 60 and 75 DAS is given in Figure 1. The highest Catalase content of leaf. (3.450,7.185,9.350,15. 587, and 13.346min-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 (0.639,1.137,1.986,2.832, and 2.171 mg g-1 fr. wt.) was observed in 12.5mg(cd)T9 concentration of cadmium treated plants at 15,30,45, 60 and 75 DAS respectively.

The reduction of proteins content and enzyme activity was inhibited due to heavy metal treatment Schützendübel and Polle, 2002. Kanwal et al., 2016 indicate that AMF inoculated wheat plants with different Zn concentrations showed increased plant growth than nonmetal treated plants. Some studies (Li et al., 2011) reported that mycorrhizal colonization does not decrease in plants growing with high metal contents. Ling-Zhi et al., 2011reveled that the AMF enhances the resilience of crop plants through its active participation in nutrient uptake and maintaining cell water content. Tripathi and Tripathi, 1999 concluded the protein content in Albizia lebbak has been interpreted either due to reduced de novo synthesis of proteins or increased decomposition of proteins into amino acids.

Moreover, cadmium stress, decrease in protein control was related with increased protease activity in soybean (Balestrasse et al. 2003). Verma et al. (2012) showed that the soluble protein content decreased in seedlings with increasing concentration of cadmium chloride over the control seedlings of Sesbania sesban. The amino acids can directly or indirectly influence the physiological activities of the plant (Sharma, 1985; Shafiq and Iqbal, 2005; Street et al., 2007). Total free amino acid was increased with the increasing concentration of heavy metals as reported by Bhardwaj et al. (2009). The heavy metals have decreased the content soluble sugar with increasing concentration, which are more important constituent is manufactured during photosynthesis and broken-down during respiration by plants (Hemalatha et al., 1997). Dhir et al. 2004 demonstrated proline content were accumulated in shoots of B. juncea, Triticum aestivum and Vigna radiate with the influence of cadmium. Zengin and Munzuroglu (2006) found that proline accumulation increased with the exposure to cadmium in hydrophytes of Ceratophyllum, Wolffia, and Hydrilla. Recent study (Ferrol et al. 2016) reported that the plants inoculated with AMF under heavy metal stress may result in the expression of specific genes, which are responsible for the production of proteins (including metallothioneins) that increase the resistance of plants to stress.

Shaaban et al., 2015 reported that the total soluble protein content was significantly decreased with increasing the heavy metal (Cd or Pb) concentrations as compare to VAM inoculation increased significantly the total soluble protein content with influence of heavy metal levels. Additionally, VAM fungi may be can detoxify the heavy metals via exudation of metal-binding proteins (Howe et al., 1997).

Hayat et al., 2011 reveled that the Increase in proline accumulation in Cd-stressed plants has earlier been demonstrated in tomato. Hashem et al., 2016 suggested that the proline content were enhanced under stress conditions. Further enhancement of proline in AMF-inoculated plants supports the potential role of AMF and proline in plants. Shekoofeh et al. (2012) reported that the AMF-induced enhancement of proline content and subsequent mitigation of salt stress in Ocimumbasilicum. Proline accumulation in response to heavy metal stress, osmotic stress, drought, and high levels of salinity has been one of the indicator physiological factors (Ashraf and Foolad 2007). Although metal-induced proline accumulation in plant tissues has been observed (Andrade et al., 2009; Fariduddin et al., 2009), reports on the effects of mycorrhizal symbiosis in proline or soluble amino acid contents are scarce or null under metal stress conditions.
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) 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). 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), 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). 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) demonstrated that the antioxidant enzymes were increases with the Cd treatments 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.

Potassium is a key nutrient in the plants tolerant to stress and acts catalytic for many of the enzymatic processes in plants (Arun et al., 2005; Azmat et al., 2010). The nutrient such as Fe, Mn and Zn were reduced in maize due to chromium treatments as reported by Sharma and Pant (1994)
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Haider et al., 2006 concluded that the decrease in concentration of Mn may have caused reduction in the concentration of chlorophyll that may be related to the reduction in quantum field of oxygen evolved in photosynthesis. Magnesium acts as cofactor in sugar synthesis when it is available in greater amount in the metabolic environment (Rajkumar and Narayanaswamy, 2004; Haider et al., 2006). Meharg and Cairney 2000 concluded that the enhanced nutrient supply, mainly phosphorus to the host plant by the AMF may attenuate the effect of physiological stress caused by Cd.

The results (Callahan et al., 2007) revealed that the plants under metal stress may also alter its amino acid composition as a consequence of metal effects on nitrogen metabolism and the involvement of several nitrogen compounds in metal detoxification. Potassium is an important macroelement involved in several processes including enzyme activation, stomatal movements and stress tolerance (Abd_Allah et al., 2015). Alqarawi et al. (2014) demonstrated that the increased uptake of essential microelements in AMF inoculated stressed plants as compared to uninoculated counterparts.

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Recent study (Zhang et al., 2018) reported the Inoculation with G. mosseae increased the Cd content in L. perenne roots but had no significant effect on the Cd content in aboveground plant parts. This might be related to the Cd immobilization by the AMF or the increased biomass in the aboveground parts of L. perenne, which diluted the Cd concentration. Marschner and Rimmington 1996 mentions that the Utilization of arbuscular mycorrhizal fungi in heavy metal accumulator plant species has been proved beneficial in terms of increasing nutrient absorption rates of heavy metal.
Hashem et al. 2016 suggest that the increase of cadmium accumulation in cadmium treated plants was observed which was reduced to some extent by AMF inoculation particularly in shoots. Reduced accumulation of cadmium in shoots of AMF inoculated plants confer a role of AMF in the selective uptake of toxic ions. The mycorrhizal fungi act as Protect under heavy metal toxicity might vary considerably depending upon host fungal association (Andrade et al. 2008). Kaldorf et al. 1999 suggested that the mycorrhizal symbiosis include dilution of the metal ions by increased root or shoot growth, increased metal and phosphorus ratio, exclusion by precipitation of polyphosphate granules, and active compartmentalization into plastids. Davies Jr. et al., 2001 reported that the influence of inoculation with mycorrhizal fungi as improvers of plant tolerance and phytoaccumulation of Cr.

Miransari, 2011 revealed that the AMF inoculated plant can make ability to take up heavy metals from an increased soil volume and concentrate metals in the roots. Joner et al., 2000 concluded that the effect of AMF in decreasing heavy metal stress has been assigned to the selective immobilization of the toxic metal within the root tissues that are colonized by the fungus or to the high metal sorption capacity of the extraradical mycelium of the AMF.

Bhaduri and Fulekar, 2012 results showed that AMF exhibited tolerance for Cd up to 100 mg/l and accumulated 88.07% in its tissues with no visual symptoms of toxicity, whereas those in non-AMF showed marked growth reduction at the same concentration with a metal accumulation of 73.2%. The AMF might help to chelate Cd in plant vacuoles and cell walls, and its arbuscular structure or extraradical mycelium result to Cd accumulation in plant belowground organs (Yao et al., 2014; Li et al., 2016). Study (Li et al., 2016; Luo et al., 2017) reveled that the inoculation with AMF relieve Cd toxicity by decreasing contaminants accumulation in oryza sativa, especially of shoot and root.

Besides the increased biomass with inoculation diluting Cd concentration in plants, AMF could decrease Cd bio-available by secreting glomalin binding Cd, increasing soil pH, and mobilized toxic metals in extraradical mycelium to diminish Cd absorption (González-Chávez et al., 2004; Wang et al., 2012; Wu et al., 2015). Recent study reported (Sun et al. 2018) concluded that AMF inoculation increased the biomass and P concentrations of all the cultivars. The Cd concentrations in the roots were higher than those in the shoots of all cultivars irrespective of inoculation, but the AMF had different effects on Cd accumulation in highland and lowland cultivars. The relatively high accumulation of cadmium in root tissue and the minimal transfer of cadmium from roots to shoots have been documented in other studies (Kukier and Chaney, 2002; Rodda et al., 2011). The tendency of mycorrhizal fungi to preferentially increase cadmium accumulation in plant roots has also been documented in subterranean clover (Joner and Leyval,1997)

Peroxidase

The effect of different concentrations of cadmium with AMF on Peroxidase contents (min-1 mg-1 protein).) in leaf of green gram at 15,30,45, 60 and 75 DAS is given in Figure 2. The highest peroxidase content of leaf. (5.847,8.198,10.347,13.154, and 9.932 mg g-1 fr. wt.), was recorded in 12.5mg (cd)cadmium treated plants at 15,30,45, 60 and 75 DAS respectively. The lowest Peroxidase content of leaf (0.938,1.136,2.231,2.938,2and 2.034 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).

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Table no:1. Effect of cadmium and AMF on the peroxidase activity of green gram(vigna mungo.L)

Treatments with cadmium + AMF	**Peroxidase (mg/g^{-1} fresh weight)**				
	Day After Sowing (DAS)(ppm)				
	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

Table no:2. Effect of cadmium and AMF on the catalyse activity of green gram (vignamungo.L)

Treatments with cadmium + AMF	**Catalyse (mg/g^{-1} fresh weight)**				
	Day After Sowing (DAS)(ppm)				
	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

Conclution

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 green 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 werehigh 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 a symbiotic fungal association with the higher plants tried to reduce the toxic nature of the industrial waste specifically on the cadmium alife scavenging mechanism to protect the agricultural field in to from the antagonistic action of cadmium.

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