IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **11** | Issue 2 | July, 2016 | 282-287

DOI: 10.15740/HAS/IJPS/11.2/282-287
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RESEARCH ARTICLE

Effects of $CdCl_2$ and Arbuscular mycorrhizal fungi (AMF) on the growth and nutrient content of black gram (*Vigna mungo* L.)

■ **A. KRISHNAN AND P.S. SHARAVANAN**

SUMMARY

Remediation of sites polluted with toxic metals is mainly challenging, unlike organic compounds, metals cannot be degraded and the cleanup regularly requires their removal. However, this energy-intensive approach can be prohibitively luxurious. In addition, removing process often employs strict physicochemical agents which can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem. Arbuscular mycorrhizal fungi (AMF) affords a gorgeous system to advance plant-based environmental clean-up. The experimental plants black gram were raised in pots, the pot containing 2 kg of soil with AMF various levels of cadmium chloride ($CdCl_2$) (control, 5, 10, 15, 20 and 25 mg/kg soil). Five replicates were maintained for each level. Morphological parameters like root and shoot length, nodule number, total leaf area and dry weight of root and shoot. The nutrient content of TN, P, K, Ca, Mg, Zn, Co, Fe and Mn of black gram (*Vigna mungo* L.) were recorded in 30th DAS of plants. The Cadmium chloride ($CdCl_2$) treatment at all levels decreased the various growth, and nutrient contents of black gram leaves. The AMF with ($CdCl_2$) treatments plants increased all the parameters. AM-fungi have an important role in promotion of biological and chemical properties of plants under ($CdCl_2$) stressed environment.

Key Words : $CdCl_2$, AMF, Morphological parameters, Nutrient content, Black gram

How to cite this article : Krishnan, A. and Sharavanan, P.S. (2016). Effects of $CdCl_2$ and Arbuscular mycorrhizal fungi (AMF) on the growth and nutrient content of black Gram (*Vigna mungo* L.). *Internat. J. Plant Sci.*, **11** (2): 282-287, **DOI: 10.15740/HAS/IJPS/11.2/282-287**.

Article chronicle : Received : 23.04.2016; **Revised :** 07.05.2016; **Accepted :** 14.06.2016

The definition "heavy metals (HMS)" is based on the density of the elemental form of the metal, on the reactivity of metal, on atomic number and on other chemical properties and toxicity (Duffus, 2002). Most heavy metals are categorized as toxic and accessible, based on the classification of (Wood 1974), and their concentrations in soil vary between 1 to 100,000 mg/kg (Blaylock and Huang, 2000). The plant toxicity of heavy metals differs according to plant species; for flowering plants the toxicity may appear as AS(III)~ Hg>Cd>Tl>Se(IV)>Pb>Bi~Sb (Fergusson, 1990).

A few heavy metal elements such as Cu, Fe, Mn, Ni and Zn are necessary for normal growth and

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development of plants. These metals are required in several enzyme-catalyzed or Redox reactions, in electron transfer, and have structural function in nucleic acid metabolism (Gohre and Paszkowski, 2006). In disparity, metals like Cd, Pb, Hg, and As are not essential and may be toxic to plants at very low concentrations in soils (Mertz, 1981). Heavy metals occur in terrestrial and aquatic ecosystems from both natural and anthropogenic sources, and are also emitted into the atmosphere.

The biological half-lives of these HMS are long and have potential to accumulate in different body organs and thus, produce unwanted side. Cd (Cadmium) is one of the most highly dispersed metals by anthropogenic activities (Alloway, 1995). The agricultural soils are contaminated by fertilizer impurities (Cd^{2+}), use of refuge derived compost and sewage sludge (Cd^{2+}). Cd is easily taken up by plants because, geochemically, it is quite mobile element in water and soil ecosystems. Cd has a bad reputation for being highly toxic and threatening to plant growth (Iqbal and Mehmood, 1991).

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. The arbuscular mycorrhizal fungi (AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species (Selvaraj and Chellappan, 2006). The AMF are significant in the remediation of contaminated soil as accumulation (Jamal *et al.*, 2002). The external mycelium of AMF allows for wider exploration of soil volumes by spreading beyond the root exploration zone, thus, providing access to greater quantities of heavy metals present in the rhizosphere (Khan *et al.*, 2000). Higher concentrations of metals are also stored in mycorrhizal structures in the root and in fungal spores. AM fungi can also increase plant establishment and growth despite high levels of soil heavy metals due to improved nutrition (Taylor and Harrier, 2001), water availability (Auge, 2001), and soil aggregation properties associated with this symbiosis (Kabir and Koide, 2000). So the present investigation has been carried out to find out the effect of $CdCl_2$ with AMF on morphological parameters and nutrient contents of black gram at 30th DAS of seedlings.

MATERIAL AND METHODS

Seed materials :

The seeds of black gram (*Vigna mungo* L.), varieties UID -19 were obtained from Sekar Agro Centre, Dharmapuri, Tamil Nadu, India.

Inoculum preparation :

The arbuscular mycorrhizal fungus species employed was *Glomus mosseae*. The inoculum used consisted of soil containing spores (800 to 1000/100g dry soil), hyphal fragments and fine roots of onion infected with *Glomus mosseae.* Uniform sized and healthy seeds of black gram were selected for the pot culture experiments.

Irrigation schedule :

Pre-sowing irrigation was given to ensure uniform germination. Irrigation was given at 2 DAS with due care to avoid excess flooding of water. Uniform irrigation was given for two times per day. Five plant samples were randomly collected at 30th days of plants and they were used for observations of morphological parameters like root and shoot length, root nodules, total leaf area, fresh and dry weight of the black gram seedlings.

Weed management :

Hand weeding was done two times at 10th and 20th days after sowing in order to remove the weeds from the pots.

Shoot length and root length (cm/plants) :

Five plants were randomly selected for recording the root length and shoot length of crop plants. They were measured by using centimetre scale.

Fresh weight and dry weight :

Five plant samples were randomly selected at 30th days of black gram seedlings. They were separated into root and shoot. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air over at 80°C for 24 hr and then their dry weight were also determined.

Estimation of macro and micronutrient contents :

The plant samples were collected and estimation of nutrient content *viz.*, total nitrogen (TN) (Jackson, 1958 quoted by Yoshida *et al.*, 1972), phosphorus (P) (Black, 1965 quoted by Yoshida *et al.*, 1972) potassium (K) (Williams and Twine, 1960) calcium (Ca) and magnesium (Mg) (Yoshida *et al.*, 1972) zinc (Zn), copper (Co), iron (Fe) and manganese (Mn) (DeVries and Tiller, 1980).

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi (AMF) is soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots (Barea and Jeffries, 1995). About 95 per cent of the world's plant species belong to characteristically mycorrhizal families (Smith and Read, 1997) and potentially benefit from AM fungus-mediated mineral nutrition due to the fundamental role played by these glomalean fungi in biogeochemical element cycling (Jeffries *et al.*, 2003).

The different level of cadmium chloride ($CdCl_2$) treatments at 30th DAS of black gram seedlings at all levels decreased, the germination percentage (33.7%), shoot length (5.9 cm), root length (3.0 cm), fresh weight (8.48 mg/g fr. wt.) and dry weight (4.1 mg/g fr.wt) when compared to control (Table 1). At the same time cadmium chloride ($CdCl_2$) with AMF inoculation treatment increased with all levels germination percentage (58.65%), shoot length (7.2 cm), root length (4.2 cm), fresh weight (10.21 mg/g fr. wt.) and dry weight (4.98 mg/g fr.wt).

AMF develop intensively inside roots and within the soil by forming an extensive extra-radical network and this helps plants considerably in exploiting mineral nutrients and water from the soil. Phosphorus is the key element obtained by plants through the symbiosis and the evidence to support this is extensive (Smith and Read, 1997). In exchange, mycorrhizal plants provide the fungus with photosynthetic C, which in turn is delivered to the soil *via*., fungal hyphae.

Research revealed that AM fungi promote increased growth in crops due to increased nutrient uptake especially in marginal soils (Liasu *et al.*, 2002). This root fungus facilitates resistance to soil borne pathogens and also promotes resistance to soil pollutants including heavy metals and hydrocarbons in some cases (Killham and Firestone, 1983). Researchers also reported that AM fungi promotes the uptake of metal ions (Leyval, *et al.*, 1991 and 1997; Citterio *et al.*, 2005), or decreased uptake (Citterio *et al.*, 2005) and or having no effects on metal uptake (Trotta *et al.*, 2006). While conflicting reports have been given on the effects of AM fungi on phytoextraction of metal including heavy metals from polluted soil (Lasat, 2002 and Liu *et al.*, 2007).

Another explanation the protection and enhanced capability of greater uptake of minerals result in greater biomass production, a pre-requisite for successful remediation. The potentials of phytoremediation of contaminated soil can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi most appropriate for contaminated site (Joner and Leyval, 1997). Mycorrhizae have also been reported in plants growing on heavy metal contaminated sites and indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the heavy metal contaminated soil. (Hildebrandt *et al.*, 2007).

The nutrient content of black gram seedlings the

Table 1 : Effect of $CdCl_2$ and arbuscular mycorrhizal fungi (AMF) on the growth parameters on black gram (*Vigna mungo* L.) at 30th days of plants

$CdCl_2$ concentration (mg/kg) with AMF	Germination (%)	Shoot length (cm/plant)	Root length (cm/plant)	Fresh weight (mg/gfr. wt.)	Dry weight (mg/g dry wt.)
Control	93 ± 4.65	17.45 ± 0.8725	8.12 ± 0.406	24.8 ± 1.24	11.32 ± 0.566
5	76.55 ± 3.8275	13.82 ± 0.691	6.34 ± 0.317	21.65 ± 1.0825	9.11 ± 0.4555
5 +AMF	81.4 ± 4.07	15.22 ± 0.761	7.6 ± 0.38	24.018 ± 1.2009	10.24 ± 0.512
10	70 ± 3.5	10.6 ± 0.53	5.02 ± 0.251	18.78 ± 0.939	8 ± 0.4
10 +AMF	78.6 ± 3.93	13.45 ± 0.6725	6.4 ± 0.32	21.54 ± 1.077	9.805 ± 0.49025
15	62 ± 3.1	8.9 ± 0.445	4.08 ± 0.204	15.23 ± 0.7615	6.4 ± 0.32
15 + AMF	71.8 ± 3.59	10.85 ± 0.5425	4.92 ± 0.246	18.3 ± 0.915	7.82 ± 0.391
20	51.4 ± 2.57	6.1 ± 0.305	3.55 ± 0.1775	12.202 ± 0.6101	4.54 ± 0.227
20 +AMF	69.3 ± 3.465	7.82 ± 0.391	4.2 ± 0.21	15.1 ± 0.755	5.24 ± 0.262
25	33.7 ± 1.685	5.9 ± 0.295	3 ± 0.15	8.48 ± 0.424	4.1 ± 0.205
25 + AMF	58.65 ± 2.9325	7.2 ± 0.36	4.12 ± 0.206	10.21 ± 0.5105	4.98 ± 0.249

different level of cadmium chloride ($CdCl_2$) treatments at 30th DAS at all levels decreased the T N (2.33 mg/g dr. wt.), (P) (0.66 mg/g dr. wt.), K (2.0 mg/g dr. wt.), Ca (0.2 mg/g dr. wt.), Mg (0.14 mg/g dr. wt.), Zn (0.23 mg/g dr. wt.), Co (0.019 mg/g dr. wt.), Fe (0.17 mg/g dr. wt.) and Mn (0.023 mg/g dr. wt.) were recorded at 30th DAS of seedlings (Table 2).

The cadmium chloride ($CdCl_2$) with AMF inoculation treatment increased the nutrient content *viz.*, TN (3.8 mg/g dr. wt.), (P) (1.18 mg/g dr. wt.), K (3.54 mg/g dr. wt.), Ca (0.37 mg/g dr. wt.), Mg(0.29 mg/g dr. wt.), Zn (0.30 mg/g dr. wt.), Co (0.028 mg/g dr. wt.), Fe (0.432 mg/g dr. wt.) and Mn (0.3 mg/g dr. wt.) were recorded at 30th DAS of seedlings.

The role of AMF on nutrient uptake (TN, P and microelements), on the growth of AM crops, as well as on possible mechanisms of nutrientuptake, have been widely studied, as recently reviewed by Jeffries *et al.*, (2003); Al-Karaki (2006); Cardoso and Kuyper (2006); Gohre and Paszkowki (2006); Gregory (2006); Martin *et al.* (2007) and Cavagnaro (2008). It is now generally recognized that AMF enhance the uptake of nitrogen (N) and of relatively immobile soil nutrients such as phosphorus (P), sulfur (S),copper (Cu), zinc (Zn), and boron (B).

AMF sorption of heavy metals is a passive mechanism of ion immobilization on the surface of microbial cells including processes like adsorption, ion-exchange, complexation, precipitation, and crystallization on and within what may often be a multilaminate, microfibrillar cell wall rich in negatively charged ligands such as phosphoryl, carboxyl, sulfhydryl, hydroxyl, and phenolic groups (Leyval and Joner, 2001).

This finding supports results from numerous studies reporting that AM fungi often protect plants against high accumulation of toxic elements in the shoots, as it was reported for Cu (Gonzalez-Chavez *et al.*, 2004), Al (Rufyikiri *et al.*, 2000), Cd (Gonzalez-Chavez *et al.*, 2004;Yu *et al.*, 2004), Pb (Malcova *et al.*, 2003), U (Rufyikiri *et al.*, 2004) and As (Yu *et al.*, 2009). In addition, glomalin, aninsoluble glycoprotein which is contained in hyphae and spores of AM fungi, had a high binding capacity for Cu, Cd and Pb (Gonzalez-Chavez *et al.*, 2004), hence, it could act as a biostabilizer for there mediation of polluted soils. Glomalin could be considered for heavy metals sequestration in hyphae and spores of AM fungi as well. The effect of AMF in decreasing heavy metal stress has been assigned to the selective immo- bilisation of the toxic metal within the root tissues that are colonised by the fungus (Kaldorf *et al.*, 1999) or to the high metal sorption capacity of the extraradical mycelium of the AMF (Joner *et al.*, 2000).

Phytoremediation is a newly emerging as a biobased and low cost, alternative technology in the cleanup of contaminated soils. AM fungi occur in the soil of most ecosystems, including polluted soils. By acquiring phosphate, micronutrients and water and delivering aproportion to their hosts they enhance the host nutritional status. Similarly,Cd Cl_2 is taken up via the fungal hyphae and can be transported to the plant. Thus, in some cases mycorrhizal plants experience enhanced Cd Cl_2 uptake and root-to-shoot transport while in other cases AM fungi contribute to Cd Cl_2 immobilization within the soil. The

Table 2 : Effect of $CdCl_2$ and arbuscular mycorrhizal fungi (AMF) on the nutrient content (mg/g dry wt.) of glack gram (*Vigna mungo* L.) at 30th days of plants

$CdCl_2$ concentration (mg/kg) with AMF	TN	P	K	Ca	Mg	Zn	Co	Fe	Mn
Control	8.3± 0.415	3.5±0.175	7.2±0.36	1.5±0.075	0.84±0.042	0.8± 0.04	0.088±0.0044	0.98±0.049	0.783±0.03915
5	6.1±0.305	2.75±0.1375	6.4±0.32	0.83±0.0415	0.71±0.0355	0.73±0.0365	0.068±0.0034	0.742±0.0371	0.704±0.0352
5 +AMF	7.6±0.38	4.1±0.205	7.02±0.351	1.14±0.057	0.79±0.0395	0.76±0.038	0.074±0.0037	0.95±0.0475	0.79±0.0395
10	5.56±0.278	2.02±0.101	5.75±0.2875	0.72±0.036	0.59±0.0295	0.62±0.031	0.059±0.00295	0.602±0.0301	0.532±0.0266
10 +AMF	7±0.35	3.95±0.1975	6.6±0.33	1±0.05	0.63±0.0315	0.5±0.025	0.067±0.00335	0.86±0.043	0.721±0.03605
15	4.6±0.23	1.12±0.056	4±0.2	0.62±0.031	0.43±0.0215	0.46±0.023	0.039±0.00195	0.47±0.0235	0.387±0.01935
15 + AMF	5.85±0.292	3.3±0.165	5.4±0.27	0.83±0.0415	0.57±0.0285	0.53±0.0265	0.05±0.0025	0.69±0.0345	0.59±0.0295
20	3.1±0.155	0.87±0.0435	2.9±0.145	0.41±0.0205	0.23±0.0115	0.3±0.015	0.031±0.00155	0.289±0.01445	0.243±0.01215
20 +AMF	4.22±0.211	2.3±0.115	4.38±0.219	0.54±0.027	0.32±0.016	0.44±0.022	0.045±0.00225	0.562±0.0281	0.38±0.019
25	2.33±0.116	0.66±0.033	2±0.1	0.2±0.01	0.14±0.007	0.23±0.0115	0.019±0.00095	0.17±0.0085	0.023±0.00115
25 + AMF	3.8± 0.19	1.18±0.059	3.54±0.177	0.37±0.0185	0.29±0.0145	0.3±0.015	0.028±0.0014	0.432±0.0216	0.3±0.015

result of mycorrhizal colonization on remediation of contaminated soils depends on the plant–fungus–heavy metal combination and is influenced by soil chemical and physical conditions.

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