अरबस्कुलर माइकोराइजा फफूँदी एवं *स्युडोमोनास स्ट्रीयाटा* द्वारा कैडमियम विषाक्तता की जैव उन्नति

Bioamelioration of cadmium toxicity by arbuscular mycorrhizal fungus and *Pseudomonas striata*

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Bioamelioration of cadmium toxicity by arbuscular mycorrhizal fungus and *Pseudomonas striata*

By

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CERTIFICATE

This is to certify that the thesis entitled, **"Bioamelioration of cadmium toxicity by arbuscular mycorrhizal fungus and *Pseudomonas striata*"**, submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, by **Mr. Chethan Kumar, G.**, in partial fulfillment of the requirements for the award of the degree of **Master of Science in Microbiology**, is a record of ***bona fide*** research work carried out by him under my guidance and supervision and that no part of the thesis has been submitted any where for the publication or for any other degree or diploma.

The assistance and help received by him during the course of this investigation has been duly acknowledged.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Cadmium is the most mobile and toxic among the commonly occurring heavy metals. It can be easily absorbed and translocated to feed and food crops in quantities that are not only phytotoxic but may be harmful to animal and human health (Weissenhorn and Leyval, 1995). During the last decade cadmium has been enriched in agro-ecosystems by atmospheric deposition, application of sewage sludge (Moreno, 1999) mineral P fertilizers, soil amendments (Pathak *et al.,* 2002) and fly ash (Karana and Haktaniu, 1997) and thus concern has grown with regard to contamination of the food chain and decrease of soil fertility (Babich and Stotzky, 1978; Jackson and Alloway, 1992).

In soils, metals may (i) occur as microbiologically inactive forms such as insoluble precipitate, (ii) be adsorbed by clay-sized minerals and (iii) occur as humic solid organic complexes (Blum, 1989). Among the various elements of inorganic origin that are responsible for soil pollution, heavy metals such as Cd and Pb are by far the most important. Once these metals enter the soil, they remain there for long periods of time without being destroyed by the soil microorganisms, whereas molecules of organic origin can be microbially degraded (Blum, 1989).

Phosphatic fertilizers contain varying amounts of heavy metals such as Cd, Hg and Pb as impurity. The cadmium content in various phosphatic fertilizer ranges between 89-188 mg/kg (Pathak and Ahmed, 1995; Singh and Biswas, 1976). According to Cook and Morrow (1995), Phosphatic fertilizer contain from 10-200 ppm cadmium. An effective process to remove cadmium from phosphatic rock fertilizers is not known to exist until now. Phosphatic fertilizers contribute 0.3-1.2 g/ha/yr cadmium to the soil (Mortvedt *et al.,* 1987). In India, rock phosphate deposits contain low ‘P’ content and are not utilized for the manufacture of ‘P’ fertilizers. Due to this reason rock phosphate with high P_2O_5 contents is imported for the purpose of manufacturing P fertilizers. The cadmium impurity present in the rock phosphates utilized for P manufacture fertilizer ranges from 10-90 ppm.

Sewage sludges and pesticides are other minor sources of cadmium in soils (Filipek, 1994; Krishna Murti *et al.*, 1996). The sewage sludge of some major cities of India varied in cadmium content; Ahmedabad (3.5 mg/kg), Delhi (5.5 mg/kg), Nagpur (1.5 mg/hg), Chennai (8.3 mg/kg), Jaipur (7.3 mg/kg) and Kolkata (3.25 mg/kg) (Juwarkar RAPA, FAO, 1994). Soil types which fix phosphorus also fix cadmium (Filipek, 1994; Krishna murti *et al.,* 1996; Smolders and Mclaughlin, 1996; Weggler Beaton *et al.,* 2000). Cadmium uptake by crops is a serious concern because of its potential toxicity to humans (Smolders and Mclaughlin, 1996; Weggler Beaton *et al.,* 2008). Cadmium strongly adsorbs in soil and acidified soil shows enhanced uptake of cadmium. Current hazard from Cd through phosphate fertilizer application is alarming and requires constant monitoring (Tiller, 1989).

Traditionally 'P' solubilising microorganisms are utilized for efficient use of inert sources of phosphorus including rock phosphate (Gaur, 1990). These microorganisms also increase the efficiency of soluble form of fertilizer applied to the soil. The majority of the commercially used phosphate solubilising microorganisms mediate the solubilisation by acid production (Gaur, 1990). It is also found that each unit decrease in pH results in 2 fold increase in concentration of metal e.g. Zn, Ni, Cd in the soil solution (Christensen, 1984; Sanders *et al.,* 1986). Soil properties affects Cd uptake in plants. Its availability increases in acidic and sandy soils, therefore, potatoes which are grown in acidic sandy soils have high content of cadmium.

AMF colonize 80% of vascular plant species (Trappe, 1987) and have the capacity to reduce excess plant uptake of heavy metal (Leyval *et al.,* 1997). AMF are known to secrete a glycoprotein glomelins which is known to bind/sequester heavy metals (Nichols, 2003; Gonzalez-Chavez *et al.,* 2004). AM mycelium has a high metal sorption capacity (Joner *et al.*, 2004). AMF indirectly influences rhizospheric characteristics e.g.: changes in pH (Li *et al*., 1991), microbial communities (Olsson *et al*., 1998) and root exudation patterns (Laheurte *et al*., 1990). These factors collectively influence metal mobility/ availability (Joner *et al*., 2000).

A variety of secreted microbial products play a role in sequestration of heavy metals at the cell wall level (Macaskie and Dean, 1990). The interaction with proteins is well known (Spiro, 1981). Alleviation of Cd toxicity by AM has been reported (Heggo *et al.*, 1990). AM is also known to protect the plant against the harmful effects of cadmium by its greater accumulation in fungal structure than the host tissue (Turner *et al.*, 1993). Phosphate rich granules localized within fungal vacuoles have been reported to contain calcium, iron, aluminum and cadmium (Turnar *et al*., 1993).

Therefore experiment was aimed to see the cadmium amelioration potential of dual inoculation with PSB and AMF on okra as a test crop cultivated in soils amended with varying cadmium concentrations. The present investigation was initiated with the following objectives:

1. Screening AMF isolates for cadmium tolerance under *in vitro* conditions.
2. To assess the potential of PSB (*Pseudomonas striata*) and AMF in reducing effect of cadmium on a test crop.

2. REVIEW OF LITERATURE

2.1 The toxicity of cadmium in general

Cadmium (Cd) is ubiquitous in the human environment and has been recognized as one of the most deleterious heavy metal pollutants (Robards and Worsfold, 1991; Christine, 1997). It may easily move from soil to food plants through root absorption and accumulate in their tissues (Oliver, 1997). In this way, Cd may enter the food chain and affect human health (Adriano, 1986). Among many heavy metals polluting soil, Cd is of concern because of its potential harmful effects on not only humans and animals, but also the most adverse effects on microbial biomass and its activity. Microbial biomass plays an important role in the biological cycles of almost all the major plant nutrients and in maintaining soil fertility (Smith, 1996; Jose *et al.,* 2002; Yao *et al.,* 2003). Cd can also cause change in the size, composition and activity of soil microbial community (Giller *et al.,* 1998). There are many studies on this topic (Brookes, 1995; Nannipieri *et al.,* 1997; Giller *et al.,* 1998).

Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries of urban traffic. It is widely used in electroplating, pigments, plastic stabilizers and nickel-cadmium batteries (Sanita di Toppi and Gabrielli, 1999). It is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto *et al.,* 2004). Concentrations in soils vary from 0.1-1.0 mg/kg in agricultural soils not receiving sewage sludge. Genotoxicity and ecotoxicity of cadmium in animals have been also reported (Degreave, 1981; Bhattacharya and Chaudhuri, 1995). Baker *et al.,* (1990) reported that Cd never occurs in isolation in natural environments, but mostly as a 'guest' metal in Pb-Zn mineralization. Wagner (1993) estimated that non-polluted soil solutions contain Cd concentrations ranging from 0.04 to 0.32 mM.

2.2. Cadmium toxicity in higher plants

Soil solutions which have Cd concentration varying from 0.32 to about 1mM can be regarded as polluted to a moderate level (Sanita di Toppi and Gabrielli, 1999). Regarding its potential toxicity for soil organisms and soil microbial processes, Duxbury (1985) classified Cd as an element of "Intermediate" toxicity. Although the toxic effects of cadmium on biological systems have been reported by several authors (Bingham *et al.,* 1976; Mukherjee *et al*., 1984; Obata and Umebayashi, 1997; Das *et al.,* 1997; Sanita di Toppi and Gabrielli, 1999), the mechanism of Cd toxicity are not completely understood yet. Cadmium can alter the uptake of minerals by plants through its effects on the availability of minerals from the soil, or through a reduction in the population of soil microbes (Moreno *et al.,* 1999). Stomatal opening, transpiration, and photosynthesis have been reported to be affected by cadmium in nutrient solutions, but the metal is taken up into plants more readily from nutrient solutions than from soil (Sanita di Toppi and Gabrielli, 1999). Cholorosis, leaf rolls and stunting are the main and easily visible symptoms of cadmium toxicity in plants. Chlorosis may appear to be Fe deficiency (Haghiri, 1973), phosphorous deficiency or reduce Mn transport (Godbold and Hutterman, 1985). The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis (Alcantara *et al*., 1994). In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants (Das *et al.,* 1997). Cd also reduced the absorption of nitrate and its transport from root to shoots, by inhibiting the nitrate reductase activity in the shoots (Hernandez *et al*. 1996). Appreciable inhibition the nitrate reductase activity was also found in plants of *Silene cucubalus* (Mathys, 1975). Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatment (Balestrass *et al.,* 2003). Metal toxicity can affect the plasma membrane permeability, causing a reduction in water content; in particular Cd has been reported to interact with the water balance (Barcelo *et al.,* 1986; Poschenrieder *et al.,* 1989; Costa and Morle, 1994). Cadmium treatments have been shown to reduce ATPase activity of the plasma membrane fraction of wheat and sunflower roots (Fodor *et al.,* 1995). Cadmium produces alterations in the functionality of membrane fraction of wheat and

sunflower roots (Fodor *et al.,* 1995). Cadmium produces alterations in the functionality of membranes by inducing lipid peroxidation (Fodor *et al.,* 1995) and disturbances in chloroplast metabolism by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO2 fixation (Srobart *et al.,* 1985; De Filippis and Ziegler, 1993).

Several studies have suggested that an oxidative stress could be involved in Cd toxicity, by either inducing oxygen free radical production or by decreasing enzymatic and non-enzymatic antioxidant (Somashekaraiah *et al*., 1992; Stohs and Bagchi, 1995; Shaw, 1995; Gallego *et al.,* 1996; Sandalio *et al.,* 2001; Balestrasse *et al.,* 2001; Fornazier *et al.,* 2002; Cho and Seo, 2004). The accelerated senescence observed in nodules of soybean plants treated with Cd has been attributed to the oxidative stress generated by the metal (Balestrasse *et al.,* 2004).

Experiment conducted on rice crop in Vietnam (MARDI, 2004) indicated that 1.0-3.0 mg Cd/l delayed the seed germination. Also 20-40 mg/kg of Cd caused severe damage to rice crop leading to a yield decline by 80%.

The levels of cadmium in four prevalent mushroom species in South Africa are reported. Poisonous type mushrooms had relatively high levels of cadmium. *Agaricus xanthodermus* had Cd 29.5 mg per kg by dry weight, while *Rhizina undulata*, which grows on dead wood recorded Cd 20 mg kg $^{(-1)}$ by dry weight (Jonnalagadda *et al.,* 2006).

Non oilseed sunflower (*Helianthus annus* L.) is naturally higher in cadmium (Cd) than many other grain crops. Because raising soil $_{P}$H usually depresses Cd uptake by most species, a study was designed to determine if application of agricultural limestone to neutralize soil acidity would decrease Cd uptake by sunflower plants grown on different soil in the production area of North Dakota. The result indicated that limestone application did not reduce Cd uptake and transfer to kernels of sunflower, in contrast with most species studied (Yin-Ming Li *et al.,* 1996).

2.3 Heavy metal hyperaccumulator plants

The metal hyperaccumulation characteristic is not common in terrestrial higher plants and less than 0.2% of all angiosperms have been identified as metal hyperaccumulators (Baker *et al.,* 2000). Hyperaccumulators of Ni Zn, Cd, Pb, Cu, As, CO, and Mn have been reported (Brooks *et al.,* 1974; Brown *et al.,* 1995; Baker *et al.,* 2000; Ma *et al.,* 2001). The species belong to the *Brassicaceae* or *Cruciferae* family, which is well representing among the reported hyperaccumulators. *T. caerulescens* is best known as a Zn hyperaccumulator, although it also hyperaccumulates Cd and Ni (Assuncao *et al.,* 2003). *Brassica juncea* is a heavy metal-accumulator plant with a high biomass, making it a good candidate for application in phytoremedition strategies (Salt *et al.,* 1995, 1998; Pilon-Smits and Pilon, 2002; Clemens *et al.,* 2002). Recently, transgenic approaches have shown that, in this species, Cd accumulation may be further increased by ectopic expression of the rate- limiting enzyme for glutathione biosynthesis, namely glutamylcysteine synthetase (Zhu *et al.,* 1999).

Vegetable crops such as spinach, peas, okra, radish, cauliflower, brinjal, and tomato are known to accumulate cadmium in considerable amounts and make the element to enter into food web. Cadmium and lead levels were found to be higher than standards irrespective of whether clean water or wastewater was used for irrigation but with significantly higher values with wastewater irrigation (Sharma and Agrawal, 2006). The metal ion was found in leaf (0.17–0.24 mg per kg fresh weight) and fruit (0.07–0.18 mg per kg fresh weight) portions of all the sampled vegetables: bitter gourd (*Momordica charantia* L.), cauliflower (*Brassica oleracea* L.), eggplant (*Solanum melongena* L.), fenugreek (*Trigonella foenumgraecum* L.), okra (*Abelmoschus esculentus* L.), onion (*Allium cepa* L.), pumpkin (*Cucurbita pepo* L.), and spinach (*Spinacia oleracea* L.). Leafy tissue accumulated Cd about twice that of the fruit portion (Qadir and Ghafoor, 2000).

2.4 Cadmium homeostasis

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms that includes uptake and accumulation of metals through binding to extracellular exudates and cell wall complexation of ions inside the cell by various substances, for example, organic acids, amino acids, ferritins, phytochelatins and metallothioneins; general biochemical stress defense responses such as the induction of antioxidative enzymes and activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Verkleil and Schat, 1990; Prasad, 1999; Sanita di Toppi and Gabrielli, 1999; Hall, 2002; Cho *et al.*, 2003).

2.5 Cadmium mobilization, uptake and transport

The bioavailability of some metals is limited because of low solubility in oxygenated water and strong binding to soil particles. Both the acidification of the rhizosphere and the exudation of carboxylates are considered potential targets for enhancing metal accumulation (Clemens *et al.*, 2002) The degree to which higher plants are able to take up Cd depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentration of other elements. With the exception of Fe, which is solubilized by either reduction to Fe (II) or extrusion of Fe (III)- chelating phytosiderophores (Hirsch, 1998), little is known about active mobilization of trace elements by plant roots. In particular, the uptake of Cd ions seems to be in competition for the same transmembrane carrier with nutrients, such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni, (Clarkson and Luttge, 1989; Rivetta *et al.* 1997). The cell membrane plays a role in metal homeostasis, preventing or reducing into the cell.

Despite the different mobility of metal ions in plants, the metal content is generally greater in roots than in the above-ground tissues (Ramos *et al.*, 2002). In most environmental conditions, Cd enters first the roots, and consequently they are likely to experience Cd damage first (Sanita di Toppi and Gabrielli, 1999). Cd easily penetrates the root through the cortical tissue and it translocated to the above-ground tissues (Yang

et al., 1998). As soon as Cd enters the roots, it can reach the xylem through an apoplastic and/or a symplastic pathway (Cataldo *et al.,* 1995), complexed by several ligands, such as organic acids and/or phytochelatins (Senden *et al.,* 1992, Salt *et al.,* 1995). Normally, Cd ions are mainly retained in the roots, and only small amounts are transported to the shoots (Cataldo *et al.,* 1983). In general, the content of Cd in plants decreases in the order: root>stems>leaves>fruits>seeds (Blum, 1997). Hinesly *et al.,* (1984) reported that soil pH greatly influences Cd uptake and transportation in corn. Cadmium concentrations in maize and ryegrass were negatively related to product of cadmium in soil and pH (Tudoreanu and Phillips, 2004). A positive coefficant between soil pH and cadmium in ryegrass may derive from ionic competition, for example, sodium has been demonstrated to increase plant cadmium (Chiy and Phillips, 1999).

With the exception of the recently described Cd-carbonic anhydrase of marine diatoms (Lane and Morel, 2002), no biological function has been reported to date for the metals Pb and Cd. Thus, it is unlikely that metal transporters with specificities for the respective metal cations exist (Clemens, 2001). However, these cations without specific metal transporters are likely to enter cells through cation transporters with broad substrate specificity (Clemens, 2001). Cd and Zn have been found to be co-accumulated in aerial parts of *Arabidopsis hallleri* (Bert *et al.* 2003) plants. This shows that Cd and Zn uptake are genetically correlated, suggesting that the metals are taken up (partly, at least) by the same transporter(s) or that their transporters, when different, are controlled by common regulators. *Arabidopsi halleri* L. previously known as *Cardaminopsis halleri* L. Hayek, is one of the two species known to hyperaccumutor and usually occurs on Zn, Cd and Pb contaminated sites (Bert *et al.,* 2003). Differences in grain cadmium accumulation between two wheat species (*Triticum aestivum* and *Triticum turgidum* var. *durum*) may not only result from differences in root Cd influx, but seem to be associated with differences in plant-internal Cd allocation (Hart *et al.,* 1998).

Several cation transporters have been identified in recent years with the use of molecular technique, largely owing to the complementation of *Sacharomyces cerevisiae* mutants (Clemens, 2001). Recently, several plant transports have been identified that show affinity for both Zn and Cd.

2.6 Cadmium accumulation and detoxification

In general, plant accumulation of a given metal is a function of uptake capacity and intracellular binding sites. At every level, concentration and affinities of chelating molecules, as well as the presence and selectivity of transport affect metal accumulation rates (Clemens *et al.*, 2002). The strategies for avoiding heavy metal toxicity are diverse. A first barrier against Cd stress, operating mainly at the root level, can be the immobilization of Cd by means of the cell wall (Nishizono *et al.*, 1989) and extracellular carbohydrates (mucilage, callose) (Verkleij and schat, 1990; Wanger, 1993). In roots and leaves of bush bean, Cd ions seem to be mostly bound by pectic sites and histidyl group of the cell wall (Leita *et al.*, 1996). However, the importance of these mechanisms may vary in accordance with the concentration of Cd supplied, the species involved, the exposure time, etc. (Sanita di Toppi and Gabrielli, 1999).

2.7 Mycorrhizas

Arbuscular mycorrhizal (AM) fungi are vital components of nearly all terrestrial ecosystems, forming mutually beneficial (mutualistic) symbioses with the roots of around 80% of vascular plants and often increasing phosphate (P) uptake and growth (Smith and Jakobsen, 2003). Mycorrhizas are among the extracellular strategies to avoid metal toxicity (Marschner, 1995; Jentscke and Godbold, 2002). However, only few studies have presented direct evidence of the alleviation of metal toxicity by mycorrhizal fungus (Leyval *et al.* 1997; Jentshcke and Godbold, 2000; Schutzendubel and Polle, 2002), especially those regarding the toxic effects of Al (Schier and McQuattie, 1996), Ni (Jones and Hutchinson, 1986), Zn (Brown and Wilkins, 1985) and Cd (Jentshke *et al.* 1999).

AM fungal isolates differ in their effect on heavy metal uptake by plants (Leyval, and Haselwandter, 1997). Some reports indicate higher concentrations of heavy metals in plants due to AM (Joner and Leyval, 1997), whereas others have found a reduced plant concentration; for example, Zn and Cu in mycorrhizal plants (Heggo, and Chaney, 1990). Thus, selection of appropriate isolates could be of importance for a given phytoremediation strategy.

AM fungi are of importance as they play a vital role in metal tolerance (del Val *et al*., 1999) and accumulation (Jamal *et al.*, 2002; Zhu *et al.*, 2001). External mycelium of AM fungi provides a wider exploration of soil volumes by spreading beyond the root exploration zone (Khan *et al.*, 2000; Malcova and Gryndler, 2003), thus providing access to greater volume of heavy metals present in rhizosphere. A greater volume of metals present is also stored in the mycorrhizal structures in the root and in spores. For example, concentrations of over 1200 mg/kg of Zn have been reported in fungal tissues of *Glomus mosseae* and over 600 mg/kg in *G. vesiforme* (Chen and Li, 2001). Another important feature of this symbiosis is that AM fungi can increase plant establishment and growth despite high levels of soil heavy metals (Enkhtuya and Vosátka, 2002), due to better nutrition (Feng and Christie, 2001; Taylor and Harrier, 2001), water availability (Auge, 2001) and soil aggregation properties (Kabir and Koide, 2000) associated with this symbiosis.

AM fungus is significant in the ecological improvement of rhizosphere (Medina, and Azcón, 2003; Azcón-Aguilar, and Barea, 2003). Several heavy metal-tolerant AM fungi have been isolated from polluted soils, which can be useful for reclamation of such degraded soils as they are found to be associated with a large number of plant species in heavy metal-polluted soil. Gildon and Tinker isolated a mycorrhizal strain which tolerated 100 mg kg–1 of Zn in the soil. Considerable amount of AM fungal colonization was also reported in an extremely polluted metal mining area with HCl-extractable Cd soil concentration of more than 300 mg kg–1 (Gildon and Tinker, 1983). Similarly, Weissenhorn *et al.,* (1993) isolated mycorrhizal fungi from two heavy metal-polluted soils, which were found to be more resistant to Cd than a reference strain. Sambandan *et al.,* (1992) reported 15 AM fungal species from heavy metal-contaminated soils from India. Of the 15 AM species isolated, *Glomus geosporum* was encountered in all the sites studied. The percentage colonization ranged from 22 to 71% and spore count was as high as 622 per 100 g soil.

A *Glomus* sp. isolated from the roots of the violet plant improved maize growth in a polluted soil (Hildebrandt and Bothe, 1999) and reduced root and shoot heavy metal concentrations in comparison to a common *Glomus* isolate or non-colonized controls

(Kaldorf and Hildebrandt, 1999). Weissenhorn *et al.,* (1995) suggested a high tolerance of indigenous AM fungal population to elevated metal concentrations in soil and inside the roots. AM fungal colonization up to 40% was reported in spite of high Cd (1220 mg kg–1) and Pb (895 mg kg–1) concentrations. They further reported abundance of AM fungi (100 spores per 50 g soil) in two agricultural soils close to a Pb–Zn smelter.

Persistence of heavy metal tolerance of the arbuscular mycorrhizal fungus *Glomus intraradices* has been shown by Radka Sudová *et al.,* in 2007. In *G. intraradices*, the extent of presymbiotic hyphal extension was generally increased with Cd and Pb concentrations, even at concentrations that partially inhibited spore germination (Teresa and Iris, 2004).

2.8 Several possible mechanisms to metal tolerance of AM fungi are

1. Immobilization of metals in the fungal biomass is one such mechanism involved (Zhu and Laidlaw, 2001; Li and Christie, 2000).
2. Reduced transfer, as indicated by enhanced root/shoot Cd ratios in AM plants, has been suggested as a barrier in metal transport (Joner and Briones, 2000; Tullio and Rea, 2003). This may occur due to intracellular precipitation of metallic cations with PO_4.
3. Accumulation of Cd, Ti and Ba in fungal structures than in the host plant cells (Turnau and Oberwinkler, 1993).
4. Uptake into hyphae may be influenced by absorption on hyphal walls as chitin has an important metal-binding capacity (Zhou, 1999).
5. Chelation by such compounds as siderophores and metallothionens released by fungi or other rhizosphere microbes, and sequestration by plant-derived compounds like phytochelatins or phytates (Joner and Leyval, 1997).
6. Other possible metal tolerance mechanisms include dilution by increased root or shoot growth, exclusion by precipitation into polyphosphate granules, and compartmentalization into plastids or other membrane-rich organelles (Turnau and Kottke, 1993).

7. Indirect mechanisms include the effect of AM fungi on rhizosphere characteristics such as changes in pH (Li, 1991) microbial communities and root-exudation patterns (Leyval and Berthelin, 1990).

The mechanisms involved in conferring tolerance to heavy metal toxicity has been proved difficult to resolves since large difference in plant and fungal species in the response to metals has been observed (Hall, 2002). In Norway spruce seedling treated with Cd, fungus can only increase the tolerance of its host, fungi at the cellular level are probably similar to those of higher plants. Detoxication of Cd in *Paxillus involotus* involved binding of Cd to the cell walls and accumulation Cd in the vacuole (Blaudez *et al.,* 2000).

Cell wall and plasma membranes

The interaction of the metals with the cell wall has been by Ernst *et al.,* (1992). Most of the cell-wall-associated heavy metals are bound to polygalacturonic acids, to which the affinity of metal ions vary according to the metal (Ernst *et al.,* 1992).The plasma membrane is the first "living" structure that is target for heavy metal toxicity and consequently, could also be involved in tolerance. Such toxicity could result from various mechanisms including the oxidation and cross-linking of protein thiols, inhibition of key membrane proteins such as H^+-ATPase, or changes in the composition and fluidity of membrane lipids (Meharg, 1993). A direct effect of Cd and Cu has been reported on the lipid composition of membranes (Fodor *et al.,* 1995; Hernandez and Cook, 1997; Quartacci *et al.,* 2001). Moreover, Cd treatment has been shown to reduce ATPase activity of the plasma membrane fraction of wheat and sunflower roots (Fodor *et al.,* 1995).

Cadmium chelation

One recurrent general mechanism for heavy metal detoxification in plants and other organisms is the chelation of the metal by a ligand such as acids, amino acids, peptides and polypeptides (Rauser, 1999) and, in some cases, the subsequent compartmentalization of the ligand-metal complex. Vacuolar compartmentalization

prevents the free circulation of Cd ions in the cytosol and forces them into a limited area (Sanita di Toppi and Gabrielli, 1999). Extracelluar chelation by organic acids, amino acids, peptides, and polypeptides (Rauser, 1999) such as malate efflux from root apices is stimulated by exposure to aluminum tolerance in wheat (Delhaize and Ryan, 1995). Some aluminum-resistant mutants of *Arabidopsis* also have increased organic acid efflux from roots (Larsen *et al.,* 1998).

Metallothioneins and Phytochelatins

The two best-characterized heavy metal-binding polypeptides involved in chelation and sequestrartion of heavy metals include the metallothioneins (MTs), gene-encoded, cysteine-rich polypeptides, and the phytochelations (PCs), which, in contrast, are enzymatically synthesized, cysteine-rich peptides (Cobbet, 2000). MTs were first identified as Cd-binding proteins in mammalian tissues and are classified based on the arrangment of Cys residues (Robinson *et al.,* 1993; Cobbett and Goldsbrough, 2002). These peptides are distributed widely in the plant kingdom; it was proposed that PCs were the functional equivalent of MTs (Grill *et al.,* 1985). Subsequently, numerous examples of MT-like genes, and in some cases MT proteins, have been isolated from a variety of plant species and it is now apparent that plants express both of these Cys-containing metal-binding ligands.

PCs were first identified in 1983 in the yeast *Schizosaccharomyces pombe* (where they were called cadystins) (Cobbett, 2000), and have subsequently been identified in a wide variety of plant species and in some other microorganisms (Grill *et al.*, 1989; Cobbett and Goldsbrough, 2002). Numerous physiological, biochemical, and genetic studies have confirmed that the tripeptide glutathione (GSH; Glu-CysGly) is the substrate for PC biosynthesis. Although a number of structural variants of PCs, for example, $(GluCys)_n$-Ala, $(GluCys)_n$-Ser, and $(GluCys)_n$-Glu have been identified in some plant species, they are assumed to be functionally analogous and synthesized via essentially similar biochemical pathway (Rauser, 1999) . PC synthesis from GSH is catalysed by a transpeptidase named phytochelation synthase (EC 2.3.2.15), which is a constitutive enzyme requiring post-translational activition by heavy metals (Grill *et al*., 1989; De

Knecht *et al.*, 1995; Klapheck *et al.*, 1995). Phytochelatin synthase (PCS) has been shown to be activated only in the presence of heavy metal ions, in particular Cd, Ag, Pb, Cu, Hg, Zn, Sn, Au, and As, both in vivo and vitro (Cobbett, 2000). The reaction involves the transpetidation of the –GluCys moiety of GSH onto a second GSH molecule to from PC (n=2) or onto a PC molecule to produce a PC (n+I) oligomer (Cobbett, 2001). The capacity to synthesize PCs is supposed to be present in al higher plants (Gekeler *et al.*, 1989), the majority of algae (Ahner *et al.*, 1995) and several fungi (Grill *et al.*, 1986; Miersch *et al.*, 2001) Kinetic studies using plant cell cultures demonstrated that PC biosynthesis occur within minutes of exposure to Cd and is independent of *de novo* protein synthesis. The enzyme appears to be expressed independently of heavy metal exposure (Cobbett and Goldsbrough, 2002).

2.9 Bacteria

Bacteria are also known to possess heavy metal tolerance. In one study *Rhizobium leguminosarum* bv. *viciae* strain expressing different degree of tolerance to metal stress is a novel aspect in bacterial protection against heavy metal deleterious effects (Etelvina Maria and Ana Isabel, 2005).

Eleven cadmium-tolerant bacterial strains were isolated from the root zone of Indian mustard (*Brassica juncea* L. Czern.) seedlings grown in Cd-supplemented soils as well as sewage sludge and mining waste highly contaminated with Cd. The bacteria also showed increased tolerance to other metals including Zn, Cu, Ni and Co (Belimova *et al.*, 2005).

2.10 Soil enzymes and effect of cadmium on soil enzymes

Cadmium has toxic effect on dehydrogenase activity (DHA) and is used as bioindicators of the toxic effect of Cd in soil. In soils rich in heavy metal, dehydrogenase and phosphatase activity were sometimes higher than soils with a low heavy metal content (Marzadori *et al.*, 1996). They also stated that soil physicochemical characters e.g. pH, organic carbon, moisture content and the content of heavy metals of pedologic origin, all influence soil enzyme activity.

Enzymes are one of the main targets of heavy metal ions and prolonged exposure of soils to heavy metals results in marked decrease in soil enzyme activity (Tyler *et a*l., 1989). Metal interaction with ligand groups of enzymes largely defines their toxicity, and the inhibition of enzymes may be due to masking of catalytically active groups or protein denaturation (Das *et al.,* 1997). In addition, heavy metal excess may stimulate the formation of free radicals and reactive oxygen species (Dietz *et al.,* 2002). In order to cope with highly toxic metals, or to maintain the level of essential metals within physiological ranges, plants have evolved complex mechanisms that serve to control the uptake, accumulation and detoxification of metals.

The literature cited above indicates the potential of arbuscular mycorrhiza and some bacteria in bioremediation of soils polluted with heavy metals like cadmium. Therefore, an attempt was made to screen the AM fungi as a possible biological tool to alleviate effect of soil cadmium.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Microorganisms

The strain of bacteria *Pseudomonas striata* and five strains of arbuscular mycorrhizal fungi used in this study were collected from the culture collection, Division of Microbiology, Indian Agricultural Research Institute, New Delhi.

3.1.2 Media and supplements

3.1.2.1 Mass multiplication of standard AMF strains (by Trap culture technique, Mark Brundrett, 1999) (Plate 1).

This was done by pot culture method using sterilized soil with Maize as a trap crop or host for growth and multiplication of AMF.

Five strains mass multiplied were:

1. *Glomus fasciculatum*
2. *Glomus etunicatum*
3. *Glomus intraradices*
4. *Scutellospora gilmori*
5. *Scutellospora calospora*

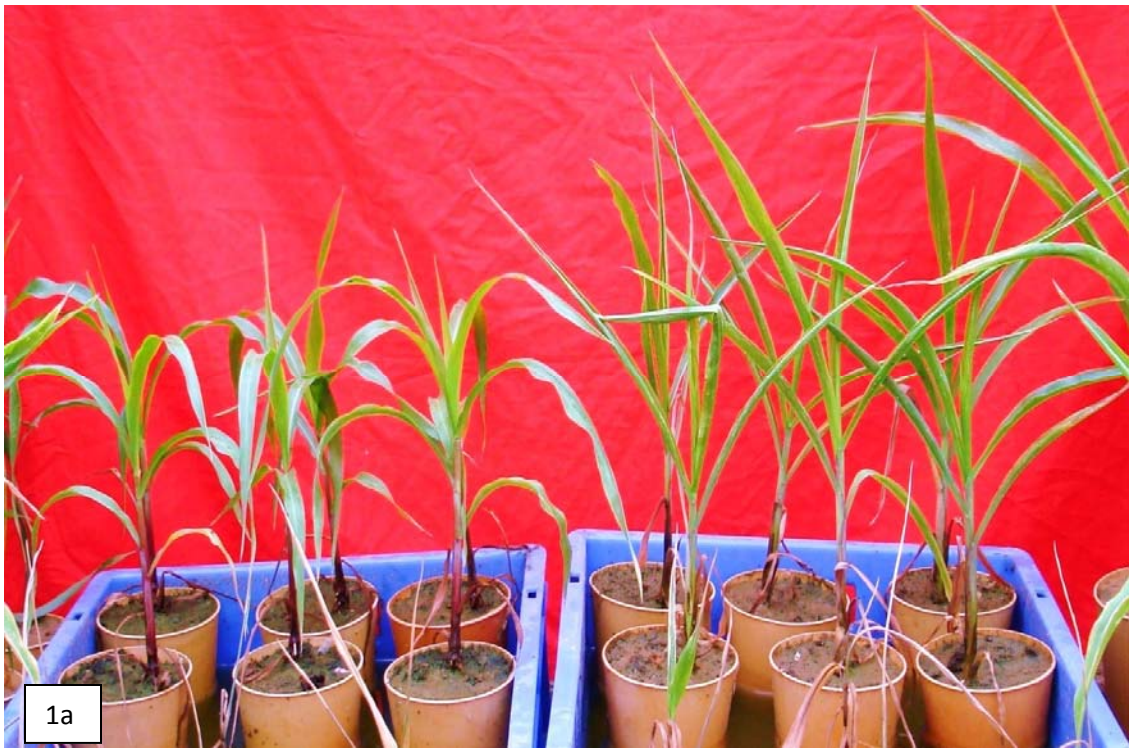
1a. Trap Culture Technique Using maize

AMF strains used are

1. *Glomus fasciculatum*
2. *Glomus etunicatum*
3. *Glomus intraradices*
4. *Scutellospora gilmori*
5. *Scutellospora calospora*

1b. Control pots

Plate I



1a

1b

3.1.2.1 Media composition for *Pseudomonas striata* (Pikovskaya, 1948)

S. No.	Pikovskya medium	g/l
1.	Glucose	10.0
2.	Yeast extract	0.5
3.	Tri Calcium phosphate (TCP)	5.0
4.	NH_4SO_4	0.5
5.	NaCl	0.2
6.	KCl	0.2
7.	$FeSO_4$	Traces
8.	$MnSO_4$	Traces
9.	$MgSO_4.7H_2O$	0.1
10.	Agar	15.0
11.	Distilled water	1000 ml
12.	PH	7.0±0.2

3.2 METHODS

3.2.1 Evaluating five strains of AMF for their symbiotic characteristics

The symbiotic characteristics of AMF such as percentage root infection, number of spores per gram of soil were evaluated as follows:

To estimate the percentage root infection the staining protocol for Arbuscular Mycorrhizae colonized roots described by Phillips and Hayman (1970) was used. Following is the list reagents required and procedure followed.

Reagents

- 10% KOH: mix 10 gm of KOH in 90 ml distilled water.
- 2% HCl: 2 ml of concentrated HCl in 98 ml of distilled water.
- 0.05% Trypan blue stain solution.

- Staining solution: trypan blue solution + lactic acid + glycerol (1:1:1 by volume).
- De-staining solution: lactic acid + glycerol + water (1:1:1 by volume).

Procedure

The fine terminal feeder roots were collected from five different portions of the root system. Preparation of root samples by washing the collected roots with tap water to remove adhering soil particles and the plant roots cut into approximately a centimeter length. Root segments of a length of 0.5-1.5 cm were placed in holders so that the sample fits in the lower 50% of the container. Added 10% KOH (w/v) to each container, making sure that the sample is completely covered and that the fluid did not fill more than half of the container. The heat treatment was done by boiling in a waterbath (by heating at 90^{o}C in 10% KOH for 1-2 h). For greenhouse-grown maize roots 2-3 weeks old, only 15 minutes of autoclaving (with no prior KOH soaking) was necessary. Field grown adventitious roots of maize that are 1cm in diameter and 2-3 months old required overnight soaking and 1 hour of autoclaving. Some tissues may require multiple soaking and more than an hour in the autoclave. Removed samples from autoclave/waterbath and rinsed in tap water three times. Added 5% HCl and allowed it sit for about 3-4 minutes. Poured out the HCl and added trypan blue stain. Allowed it to sit overnight. Removed excess stain and immersed the roots in destaining solution for few minutes and examined the roots under a dissecting scope. Methods of examination was by (i) light microscope: Roots were examined in small segments between two microscope slides (a version of the “squash”method), and quantification was done using an ocular or stage grid graticule or micrometer. The tissue was easily smashed and spread out here, allowing for visualization of more fungal structures. (ii) Stereo (Dissecting) scope: Whole roots were examined using a petri dish that has been fitted with a gridded piece of plexiglass. Roots were placed in the dish with water, and the grid is placed over them. Methods of quantification was by calculating the percent colonization of the sample by use of only two categories (“mycorrhizal” or “non-mycorrhizal”) and subdividing it into various categories of fungal structures (i.e. vesicles, arbuscules, hyphae).

$$\text{Percentage root infection} = \frac{\text{Sum of mycorrhizal infections observed in root segments}}{\text{Number of root segments examined}} \times 100$$

3.2.2 To estimate the spore count of soil (spore isolation/quantitative estimation of propagules from the soil and microscopic counting):

For isolation of spores from root-soil mixtures, the mixtures of roots and rhizosphere soils were collected from a depth of 5-30 cm. The root-soil mixtures collected were subsequently placed in plastic bags and transferred to the laboratory. In the laboratory, they were air dried and stored in a refrigerator at 4^{o}C until processing. Spores of arbuscular fungi were isolated by using the wet sieving and decanting method described by Gerdemann and Nicolson (1963).

Procedure: 100g of air-dried root-rhizosphere soil mixture was placed into a glass container with 1000 ml of tap water. The root-soil mixture was vigorously mixed with a glass rod for 5-10 minutes. After 10-second pause enabled to settle heavier particles and organic material, the remaining soil-root-hyphae-spore suspension was slowly poured through a set of six sieves (43, 63, 75, 150, 250, and 500 microns). The sieves used were those with pores of diameters of 0.5 (the top one), and 0.045 mm the last one. Most spores retained on the 0.045 mm sieve. The top sieves isolated large sporocarps and spores associated with roots. The extracts were washed away from the sieves to Petri dishes of a diameter of 10 cm and to beakers and volume made to 100ml. Using a dissecting microscope, spores, aggregates, and sporocarps were picked by means of pipette and needle. The number of spores (spore count) was examined by transferring 1-5 ml from the beaker into counting plate under stereomicroscope. The results expressed as number of spores per unit of soil (per gm of soil).

3.2.3 Screening the cadmium tolerance of AMF strains (*In vitro* screening):

AMF strains collected from trap culture technique were screened to evaluate their tolerance to different cadmium levels under *in vitro* conditions. Experiment was conducted to evaluate the ecotoxic effects of cadmium solution on spore germination of different AMF. Screening conducted with different cadmium levels using cadmium acetate solution. Spore germination of different AMF and their hyphal growth (microscopic studies) were the selection criteria. On the basis of this preliminary screening the most tolerant AMF was identified for conducting pot experiment.

The concentrations of cadmium acetate for laboratory screening of AMF were 2.5 ppm, 5 ppm, 10 ppm, 25 ppm, and 50 ppm.

Procedure

Germinating spores on agar, on membranes above soil or soil solutions or in soils is to examine tolerance of spores to varying culture conditions. Spores were extracted, washed repeatedly in tap water. In one set of experiments a filter paper (0.45 µm pores) was pre-moistened with cadmium acetate solution; 25-30 spores were collected in a pasteur pipette and transferred to the filter under a stereomicroscope. Spores were gently redistributed so that none are touching; filters were kept in petridishes and kept for incubation. In another set of experiments a deepot was filled with a pre-sterilized sand and soil mix (4:1 v/v). Filters were folded in half and then in half again (with the spores inside). They were buried in the sand-soil mix of the deepot, moistened with cadmium acetate solutions of varying above mentioned concentrations. Deepots were covered with foil, and then placed in a rack in growth room. Filters were gently removed after 2-3 weeks, opened, and placed in a glass petri dish containing hot 0.05% direct blue stain. After immersion for 30 seconds, the filter was transferred to a clean petri dish and examined. Some spores with intact germ tubes were picked up with fine-tipped forceps and transferred to glass slides for microscopic studies.

3.3 POT CULTURE EXPERIMENT

A pot experiment with Okra – *Abelmoschus esculentus* as test crop was conducted, to evaluate the effect of different levels of cadmium on soil micro-biological parameters under the influence of cadmium tolerant microorganism. The details of levels of cadmium, microbial cultures used and experimental plan are given below (Plate 2).

Crop: Okra – *Abelmoschus esculentus*

Microorganisms

i. *Pseudomonas striata*
ii. *Glomus intraradices*

Cadmium acetate solution levels (mg/kg): 0, 2.5, 10, 25

Cadmium concentration (mg/kg)	No organisms (uninoculated)	*P. striata*	*G. intraradices* (AMF)	*P. striata* + *G. intraradices*
0	4 replications	4 replications	4 replications	4 replications
2.5	4 replications	4 replications	4 replications	4 replications
10	4 replications	4 replications	4 replications	4 replications
25	4 replications	4 replications	4 replications	4 replications
Total	16	16	16	16

Total treatments: (4+4+4+4) x 4 = 64 pots

2a. *Abelmoschus esculentus* in soil containing 2.5 ppm cadmium concentration inoculated with *Glomus intraradices + Pseudomonas striata*

2b. Effect of inoculation with *Pseudomonas striata* and *Glomus intraradices* on growth of okra plants at 10 ppm soil cadmium level

Plate II

2a

2b

Pot size: 1.5 kg

The soil used for raising *Abelmoschus* sp. consisted of a mixture of soil: sand: vermiculite. It was autoclaved at 15 lb pressure for 1 hour for three consecutive days.

Four-five seeds as per plan of the experiment were sown per pot in all the treatments. After germination thinning was done to maintain uniformly single plant per pot. Soil sampling was done from the rhizosphere region at harvest stage of the plants. At each sampling following parameters were estimated using standard protocols.

i. Dehydrogenase enzyme Assay (Casida *et al.,* 1964)
ii. Alkaline Phosphatase (Tabatabai and Bremner, 1971)
iii. Acid Phosphatase (Tabatabai and Bremner, 1971)
iv. Percentage root infection of Okra plants by AMF (Phillips and Hayman, 1970)
v. Plant height
vi. Plant root length
vii. Plant fresh weight

3.4 Enzyme Estimations

3.4.1 Estimation soil enzyme dehydrogenase (Casida *et al.*, 1964)

Reagents

- Calcium carbonate ($CaCO_3$), reagent grade
- 2,3,5-Triphenyltetrazolium chloride (TTC), 3%; Dissolved 3 g of TTC in about 80 ml of water, and adjusted the volume to 100 ml with water.
- Methanol, analytical reagent grade.
- Triphenyl formazan (TPF) standard solution: Dissolved 100 ml of TPF in about 80 ml of methanol, and adjusted the volume to 100 ml with methanol. Mixed thoroughly.

Twenty g of air-dried soil (< 2 mm) and 0.2 g of $CaCO_3$ were mixed thoroughly, and placed 6 g of this mixture in each of three test tubes. To each tube, 1 ml of 3% aqueous solution of TTC and 2.5 ml of distilled water were added. Those samples were incubated at 37^{o}C for 24 hours. Then, 10 ml of methanol was added. The solution was filtered through a glass funnel plugged with absorbent cotton. Volume was made to 100 ml with methanol. OD was recorded at 485 nm. The result was calculated with the standard curve.

3.4.2 Estimation of soil enzyme phosphomonoesterases (Acid and Alkaline phosphatase (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977)

Reagents

- Toluene, Fisher certified reagent
- Modified universal buffer (MUB) stock solution: Dissolved 12.1 g of tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid H_3BO_3 in 488 ml of 1 N sodium hydroxide (NaOH) and diluted the solution to 1 liter with water. Stored it in a refrigerator.
- Modified universal buffer (MUB), pH 6.5 and 11: Placed 200 ml of MUB stock solution in a 500 ml beaker containing a magnetic stirring bar, and the beaker placed on a magnetic stirrer. Titrated the solution to pH 6.5 with 0.1 N hydrochloric acid (HCl), and adjusted the volume to 1 liter with water. Titrated another 200 ml of the MUB stock solution to pH 11 by using 0.1 N NaOH, and adjusted the volume to 1 liter with water.
- p-Nitrophenyl phosphate tetrahydrate in about 40 ml of modified universal buffer (MUB) pH 6.5 (for assay of acid phosphatase) and pH 11 (for assay alkaline phosphatase), and diluted the solution to 50 ml with MUB of the same pH. Stored the solution in a refrigerator.
- Calcium chloride ($CaCl_2$) 0.5 M: Dissolved 73.5 g of $CaCl_2.H_2O$ in about 700 ml of water, and diluted the volume to 1 liter with water.

- Standard p-nitrophenol solution: Dissolved 1.0 g of p-nitrophenol in about 70 ml of water, and diluted the solution to 1 liter with water. Stored the solution in a refrigerator.

One g of soil was placed in a 50 ml-Erlenmeyer flask, 0.2 ml of toluene was added, a ml of MUB (pH 6.5 for assay of acid phosphatase and pH 11 for assay of alkaline phosphatase), 1 ml of p-nitrophenyl phosphate solution made in the same buffer, and swirled the flask for a few seconds to mix the content. The samples were incubated at 37^{o}C for 1 hour. Then, 1 ml M $CaCl_2$ and 4 ml of 0.5 M NaOH, swirled the flask for a few second and filtered the soil suspension through Whatman No. 2. OD was recorded at 420 nm.

3.5 Estimation of Plant Parameters

3.5.1 Estimation of fresh weight of plant

Plants were harvested at 1.5 months age (45 DAS). Then, fresh plant weight noted.

3.5.2 Plant height

Plant height was noted at the harvest stage of plants.

3.5.3 Plant root length

Root length was noted at the harvest stage of plants.

4. RESULTS

4.1 Evaluating the strains of AMF for their symbiotic characteristics

4.1.1 Root infection (%)

The five strains evaluated showed varying degree of root infection in maize crop. Among the five strains evaluated, two namely *Scutellospora calospora* (64.33%) and *Glomus intraradices* (58.88%) showed the highest root infection (Table 1). This observation was recorded at the harvest stage of maize (60 DAS) (Plate 3-6). The other three strains tested showed 56.33%, 19.8% and 46% root infection in case of *Glomus etunicatum, Glomus fasciculatum* and *Scutellospora gilmori* respectively.

4.1.2 Estimation of propagules (spores) in rhizosphere of maize

Spore count in rhizosphere of maize *i.e.* number of spores (propagules) per gram of rhizospheric soil was greatest in *Glomus intraradices* (151) followed by *Glomus fasciculatum* (146) at harvest stage of maize (Table 2). The remaining three strains scored less in spore count with 76, 57 and 111 per gram of soil in case of *Scutellospora calospora, Glomus etunicatum* and *Scutellospora gilmori* respectively.

4.1.3 Screening the cadmium tolerance of AMF strains (*In vitro* screening)

Under *in vitro* screening at 0, 2.5, 10, 25 and 50 ppm concentration of cadmium only *G. intraradices* showed spore germination and mycelial growth out of the five AM strains tested.

Table 1: Percentage root infection by strains of AMF in maize grown by Trap culture method

Test strain	Root infection (%)
Control	15
Glomus intraradices	58.88
Scutellospora calospora	64.33
Glomus etunicatum	56.33
Glomus fasciculatum	19.8
Scutellospora gilmori	46

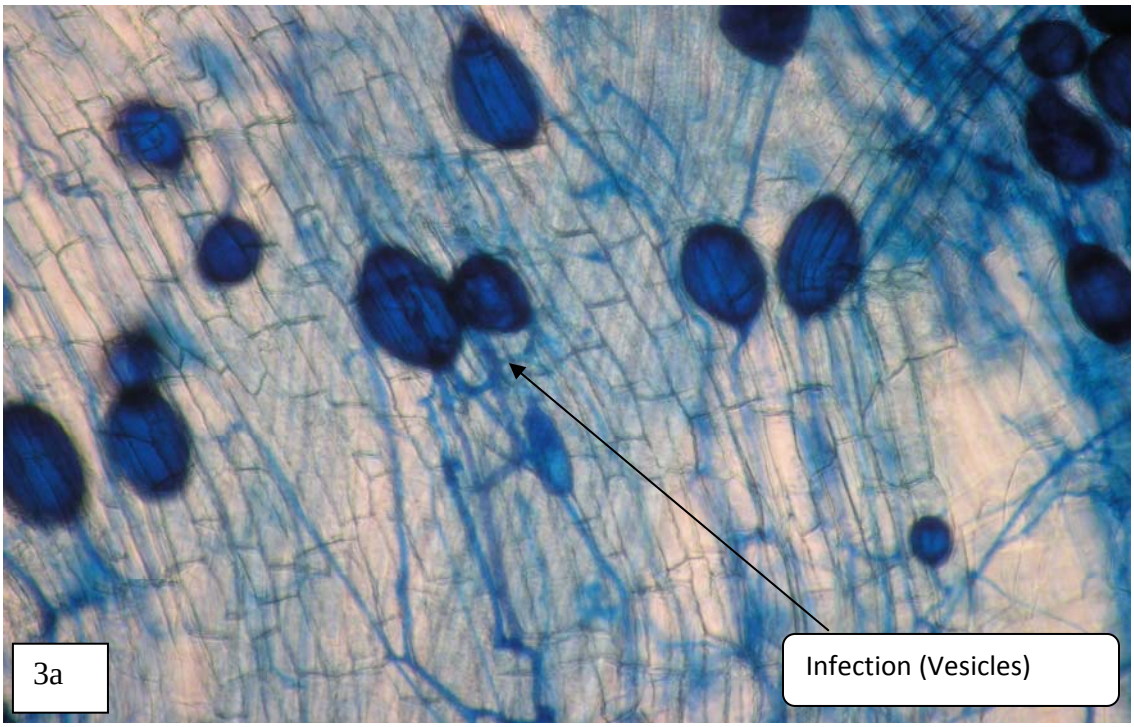
Table 2: Estimation of propagules (spores) in rhizosphere of maize

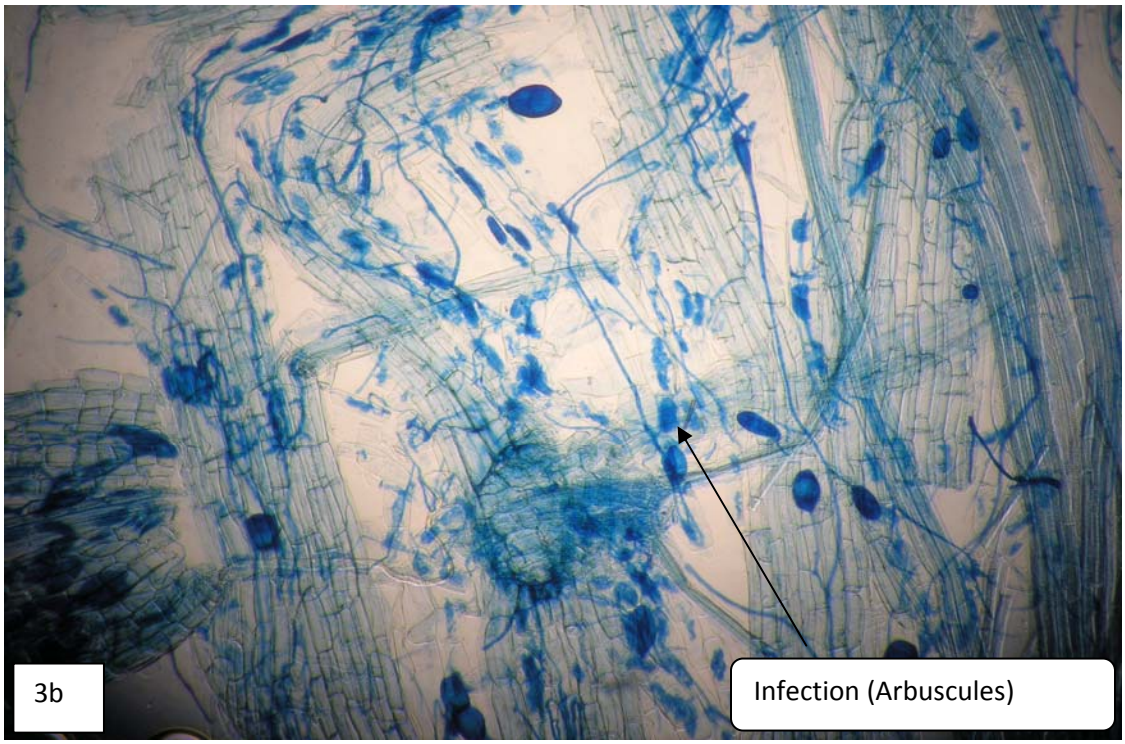
Test strain	Spore count (number of spores/gram of soil)
Control	33
Glomus intraradices	151
Scutellospora calospora	76
Glomus etunicatum	57
Glomus fasciculatum	146
Scutellospora gilmori	111

3a. Root segment of Maize infected with AMF (*Glomus etunicatum*)

3b. Root segment of Maize infected with AMF (*Glomus fasciculatum*)

Plate III

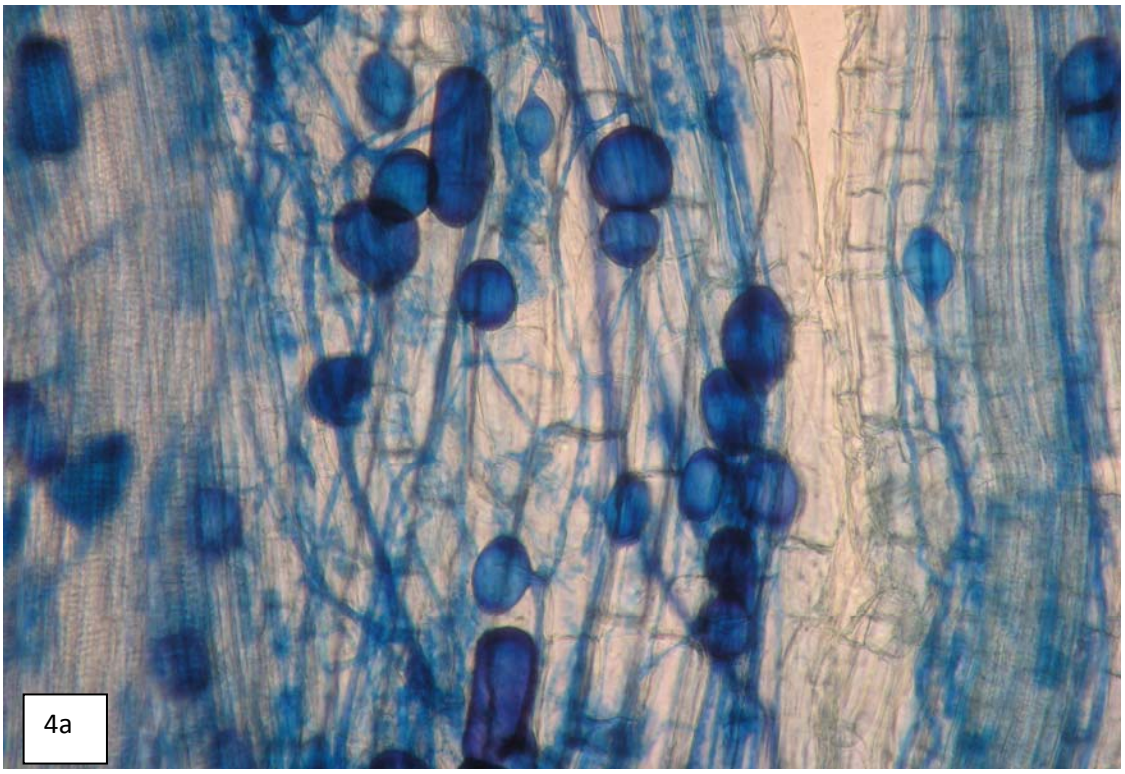


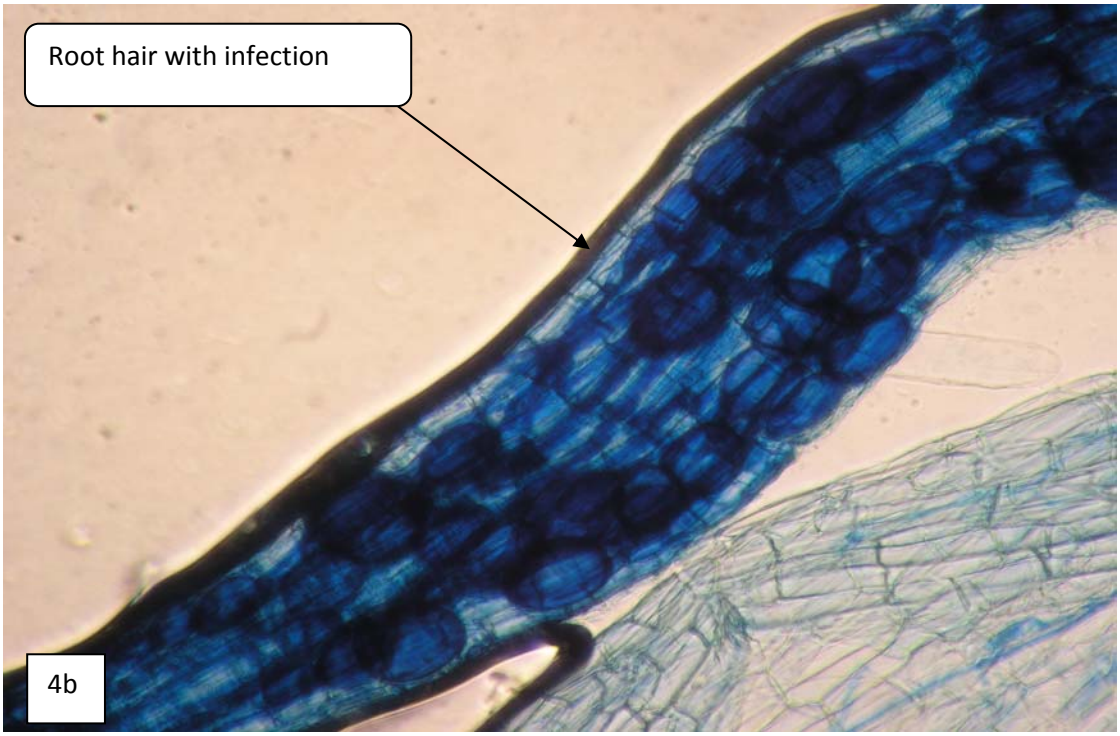


4a. Root segment of Maize infected with AMF (*Glomus intraradices*)

4b. Root segment of Maize infected with AMF (*Glomus intraradices*)

Plate IV

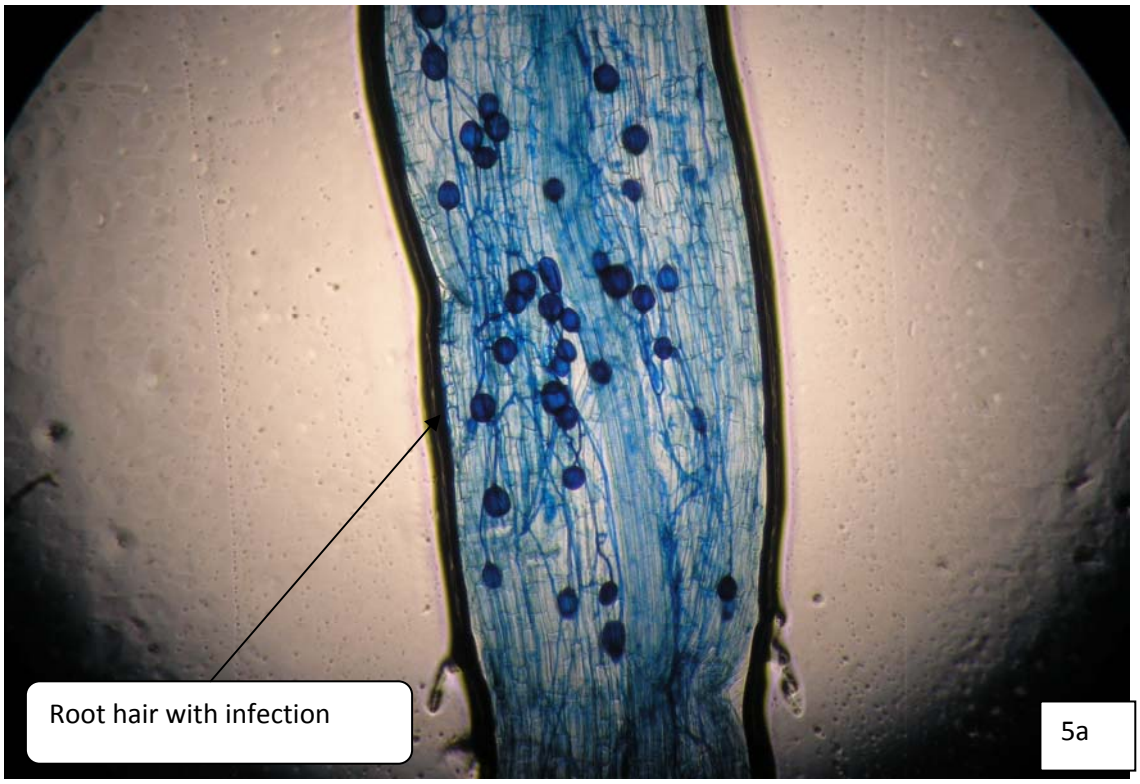


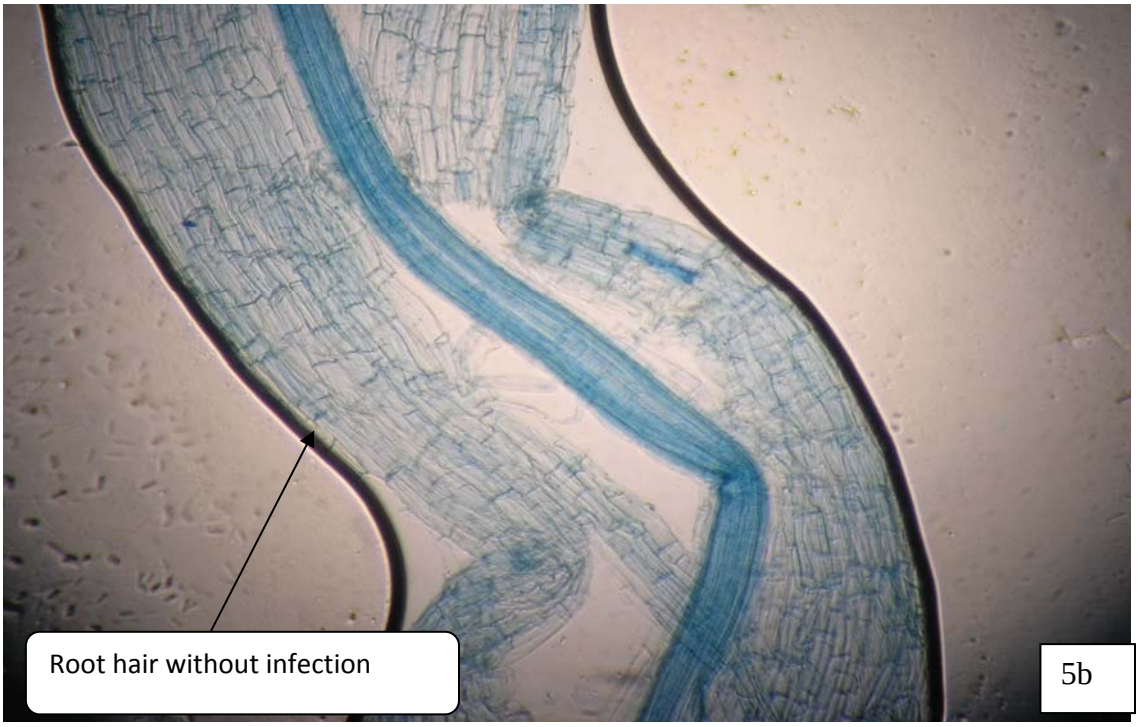


5a. Root segment of Maize infected with AMF (*Scutellospora gilmori*)

5b. Root segment of Maize showing no infection with AMF

Plate V

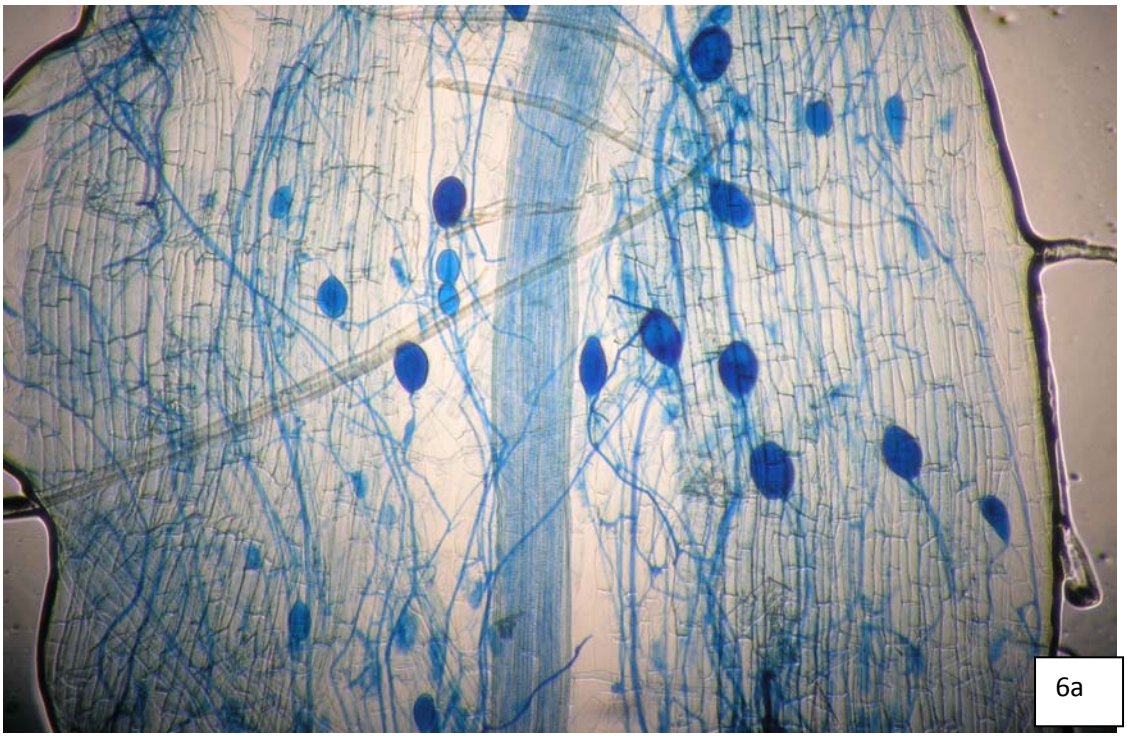




6a. Root segment of Maize infected with AMF (*Scutellospora calospora*)

6b. Root segment of Maize showing no infection with AMF

Plate VI



6a

6b

4.2 Pot culture experiment with okra

4.2.1 Effect of different soil treatments on plant parameters

4.2.1.1 Plant height

Effect of different soil cadmium concentration and microbial inoculation on plant height is given in table 3.

A highly significant (P≤0.05) difference in plant height was observed at different cadmium levels and also under different inoculation treatments.

A reduction in plant height was recorded with increasing soil cadmium levels. At 25 ppm cadmium shoot length of 12.82 cm and at 10 ppm 13.53 cm. Higher plant height noted in case of zero ppm cadmium (15.41 cm) and 2.5 ppm cadmium (14.13 cm). Similarly low plant height in uninoculated (11 cm) and high in dual inoculation (15.29 cm) was noted. Gradual increase in plant height with respect to dual inoculation (15.29 cm) compared to individual inoculation with *P. striata* (14.53 cm) or *G. intraradices* (15.06 cm) was noticed.

4.2.1.2 Root length

Significant difference in root length were observed between different soil cadmium levels at P≤0.05 noticed (Table 4). High cadmium levels i.e. 25 ppm and 10 ppm reduced the root length to 10.6 cm and 11.56 cm respectively, whereas higher root length was noted at low cadmium level 2.5 ppm (12.69 cm) and zero cadmium level (13.19 cm). Similarly, uninocuated treatments showed lower root length (9.97 cm) compared to dual inoculation (13.13 cm). At 25 ppm highly significant gain in root length following dual inoculation (11.75 cm) was registered over uninoculated plants.

4.2.1.3 Fresh weight

Table 5 indicates the fresh weight of okra with the CD value of 0.80 (P≤0.05). Results showed significant difference between the treatments. Uninoculated conditions recorded lower plant fresh weight (2.89 g) than the dual inoculated treatment (6.14 g).

Table 3: Effect of soil cadmium concentration on plant height (shoot length) of okra (cm)

Treatment (I)	Cd Concentration (ppm) (C)				Mean
	0	2.5	10	25	
Uninoculated	12.38	10.75	10.50	10.38	11.00
P. striata	16.13	15.00	14.25	12.75	14.53
G. intraradices	16.50	15.25	14.50	14.00	15.06
P. striata + G. intraradices	16.63	15.50	14.88	14.13	15.29
Mean	15.41	14.13	13.53	12.82	
S. E. (±)	1.07				
CD at 5%	3.18				

Table 4: Effect of soil cadmium concentration on root length of okra plant (cm)

Treatment (I)	Cd Concentration (ppm) (C)				Mean
	0	2.5	10	25	
Uninoculated	11.50	10.25	10.00	08.13	09.97
P. striata	13.50	13.25	11.25	11.00	12.25
G. intraradices	13.75	13.50	12.00	11.50	12.69
P. striata + G. intraradices	14.00	13.75	13.00	11.75	13.13
Mean	13.19	12.69	11.56	10.60	
S. E. (±)	1.29				
CD at 5%	3.83				

Table 5: Effect of soil cadmium concentration on fresh weight of okra (gm)

Treatment (I)	Cd Concentration (ppm) (C)				Mean
	0	2.5	10	25	
Uninoculated	3.63	3.03	2.75	2.13	2.89
P. striata	5.30	4.94	4.50	4.23	4.74
G. intraradices	6.40	5.10	4.60	4.28	5.10
P. striata + *G. intraradices*	10.34	5.15	4.65	4.40	6.14
Mean	6.42	4.56	4.13	3.76	
S. E. (±)	0.27				
CD at 5%	0.80				

Similarly, high cadmium dose (25 ppm) resulted in decreased plant fresh weight (3.76 g) compared to cadmium less condition (6.42 g). The combined inoculation with *P. striata* and *G. intraradices* at zero ppm cadmium gave the highest fresh weight of plant (10.34 g) whesreas, least fresh weight was recorded in uninoculated okra plant at 25 ppm soil cadmium.

4.2.2 Effect of different soil cadmium levels and different inoculants on soil parameters

4.2.2.1 Soil dehydrogenase activity

Statistically significant differences were noticed in the soil DH enzyme activity at different soil cadmium levels and under microbial inoculations (Table 6). The interaction effect of different soil cadmium levels and inoculations with respect to soil DH activity was at par under inoculated conditions. Soil DH activity was found to be unaffected by 25 ppm soil cadmium concentration due to the influence of microbial inoculants. Effect of microbial inoculation in reducing cadmium effect on soil DHA was statistically significant at $P \leq 0.05$. Maximum DH activity was recorded in uncontaminated soil where dual inoculation with *P. striata* and *G. intraradices* were used and least value was registered at 25 ppm soil cadmium not receiving any microbial inoculation. At the highest soil cadmium concentration individual inoculation with *P. striata* or *G. intraradices* and their combined inoculation were statistically supported identical soil DH enzyme activity. Inoculation effect was most pronounced at 2.5 ppm soil cadmium where microbial inoculants significantly improved soil DH activity over the uninoculated soil.

4.2.2.2 Alkaline phosphatase activity

As the soil cadmium concentrations increased from 2.5 ppm a significant decline in enzyme activity was noticed. Soil enzyme alkaline phosphatase activity in uninoculated soil and dual inoculated soil was significantly different. Similarly, its activity differed significantly with respect to different cadmium levels (Table 7). Microbial inoculation significantly reduced the effect of cadmium on soil alkaline phosphatase activity as noticed in dual inoculation (0.76 nmol of p-nitrophenol/g of

Table 6: Interaction effect of dual inoculation and cadmium concentration on soil dehydrogenase activity

Treatment (I)	Dehydrogenase activity (µg of TPF/g of soil/day) Cadmium concentration (ppm) (C)				Mean
	0	**2.5**	**10**	**25**	
Uninoculated	1.68	1.48	1.27	1.19	1.41
P. striata	2.40	2.50	1.60	1.45	1.99
G. intraradices	2.47	2.27	1.56	1.47	1.94
P. striata + *G. intraradices*	3.12	2.63	1.86	1.5	2.28
Mean	2.42	2.22	1.57	1.40	
SE(±)	0.22				
CD at 5%	0.62				

Table 7: Interaction effect of dual inoculation and cadmium concentration on soil alkaline phosphatase activity

Treatment (I)	Alkaline phosphatase activity (nmol of p-nitrophenol/g of soil/h) Cadmium concentration (ppm) (C)				Mean
	0	2.5	10	25	
Uninoculated	0.83	0.59	0.61	0.58	0.65
P. striata	0.81	0.57	0.59	0.59	0.64
G. intraradices	0.98	0.69	0.66	0.58	0.73
P. striata + *G. intraradices*	1.13	0.83	0.97	0.76	0.92
Mean	0.94	0.67	0.71	0.63	
SE(±)	0.03				
CD at 5%	0.10				

soil/h) compared to no inoculation (0.58 nmol of p-nitrophenol/g of soil/h) at 25 ppm cadmium level. Microbial inoculation significantly improved the soil alkaline phosphatase activity as is evident at zero and 2.5 ppm soil cadmium levels. Among the inoculants dual inoculation was highly effective at all the soil cadmium concentrations tested. At 25 ppm soil cadmium the uninoculated and the individual inoculation with *P. striata* and *G. intraradices* were identical in terms of alkaline phosphatase activity.

4.2.2.3 Acid phosphatase activity

The acid phosphatase activity in uninoculated and inoculated treatment was statistically different at P≤0.05 (Table 8). Cadmium had no effect even in higher dose (25 ppm) on activity of acid phosphatase due to presence of both microorganisms in case of dual inoculation (2.59 nmol of p-nitrophenol/g of soil/h) compared to uninoculated condition (0.76 nmol of p-nitrophenol/g of soil/h). Similarly higher cadmium dose significantly reduced the activity of acid phosphatase (1.91 nmol of p-nitrophenol/g of soil/h) compared to zero cadmium level (3.04 nmol of p-nitrophenol/g of soil/h). Inoculation with microorganisms checked the steady decline in soil acid pkosphatase activity with increasing soil cadmium level as was observed under uninoculated soils. *P. striata* and *G. intraradices* were comparable but dual inoculation was significantly better than single inoculation.

4.2.3 Effect of different soil cadmium levels on plant and microbial parameters

4.2.3.1 Percentage root infection of okra under different soil cadmium levels

Statistically insignificant difference was noticed under different soil cadmium conditions with respect to root infection percentage of okra crop by *G. intraradices.* All the *Glomus* inoculated treatments were more or less equally infected by the organism showing no statistical difference. Table 9 presents the extent of root infection following inoculation with AMF under four different cadmium concentrations. A concentration upto 25 ppm had no adverse effect on colonization potential of AMF as it was comparable to lower levels and unspiked soils. At all the levels of cadmium tested *P. striata* co inoculation did not favour root colonization by AMF.

Table 8: Interaction effect of dual inoculation and cadmium concentration on soil acid phosphatase activity

Treatment (I)	Acid phosphatase activity (nmol of p-nitrophenol/g of soil/h) Cadmium concentration (ppm) (C)				Mean
	0	2.5	10	25	
Uninoculated	2.42	0.98	0.91	0.76	1.27
P. striata	2.58	2.72	2.65	2.21	2.54
G. intraradices	3.48	2.62	2.77	2.07	2.74
P. striata + *G. intraradices*	3.68	2.77	2.66	2.59	2.93
Mean	3.04	2.27	2.25	1.91	
SE(±)	0.07				
CD at 5%	0.20				

Table 9: Percentage root infection of Okra under different cadmium concentration (in pot culture experiment)

Treatment	Root infection (%) Cadmium concentration (ppm)			
	0	**2.5**	**10**	**25**
Uninoculated	18.00	09.00	11.50	12.00
P. striata	16.50	17.00	14.50	14.00
G. intraradices	78.00	73.50	72.33	75.50
P. striata + G. intraradices	72.00	68.00	79.88	63.50

5. DISCUSSION

Plants with exceptionally high metal accumulating capacity often have a slow growth rate and produce limited amounts of biomass when the concentration of metal in the contaminated soil is very high and toxic. To maximize the chance of success of phytoremediation PGPR and AMF, soil microbes that inhabit the rhizosphere, are utilized in the nutrient poor agricultural soils. They increase HM sequestration capacity of plants by recycling nutrients, maintaining soil structure, detoxifying chemicals, and controlling pests while decreasing toxicity of metals by changing their bioavailability. Meanwhile, plants provide the microorganisms with root exudates such as free amino acids, proteins, carbohydrates, alcohols, vitamins or hormones, are important sources of nutrients. PGPR and AMF improve plant growth and development in heavy metal contaminated soils by assisting root growth and branching. Gamalero *et al*. found that the total root length, surface area, and volume in tomato and cucumber roots increased with two strains of a PGPR *P. fluorescens* (92rk and P190r). The plants also experienced similar results with AMF *Glomus mosseae* BEG12. The plants developed increased root surface area, volume, number of tips and degree of root branching. The authors ascribed these findings both to modification of root architecture, with PGPR and AMF, and to a greater absorption surface area due to mycelium of AMF. The PGPR and AMF often change the HM speciation from bioavailable to non- bioavailable by changing the oxidation state of the metal. PGPR and AMF can reduce the toxicity of heavy metals by decreasing the bioavailability of toxic HM or increasing the bioavailability of nontoxic HM (Denton, 2007).

This experiment was carried out to see the effect of individual inoculation of *P. striata* and arbuscular mycorrhizal fungus or their combined inoculation on the soil enzyme activities and plant growth parameters in spiked soils with cadmium.

Since ‘P’ fertilizers are major contributors of soil cadmium, commercially used phosphorous solubilising bacterium *P. striata* and phosphorous mobilizing fungus were used in the present investigation to assess their impact in sequestering the soil cadmium. The objective was to know if the use of bioinoculants can reduce the passage

of cadmium into plant system and their possible impact on soil enzymes active in various ‘P’ transformations. The results of the experiments are discussed here under.

5.1 After screening five AMF for their efficiency to infect host using trap culture technique, *Glomus intraradices* was selected. *Glomus intraradices* showed the highest root infection (58.88%) and rhizospheric spore counts (151 g^{-1} soil). *Glomus intraradices* exhibited tolerance to high level of cadmium under *in vitro* screening. Based on these studies *G. intraradices* selected for pot culture experiment (Figure 1,2).

5.2 In general, the inoculation with *P. striata* and *G. intraradices* were effective in significant improvement of shoot length of okra under cadmium spiked soils. Their dual inoculation was statistically at par. These results are supported by similar observations made by Heggo *et al.* (1990) and Turner *et al.* (1993). A maximum plant height of 16.63 cm was recorded following dual inoculation with *P. striata* and *G. intraradices.* This was significantly higher than uninoculated plants under uncontaminated soil as well as at soil cadmium concentration of 25 ppm. This shows that dual inoculation is effective both under uncontaminated soils as well as contaminated soils in promoting plant height (Table 3) (Figure 3).

5.3 In the present study, it was noticed that the dual inoculation with *P. striata* and *G. intraradices* supported good growth of root under uncontaminated soils (Figure 4). However, it was statistically identical to the individual inoculation with *P. striata* and *G. intraradices* with increasing levels of soil cadmium. The beneficial effect of microbial inoculation was evident as significantly better root development was recorded in all the treatments receiving microbial inoculation in comparison to uninoculated plants. these observations are in accordance with those of Kundu and Gaur (1980), Hetrick and Bloom (1984).

5.4 The biomass of the okra plant also registered beneficial effect of combined inoculation over the single inoculation both under cadmium stress as well as unstressed soils. At zero cadmium level *G. intraradices* was better than *P. striata* but their combined

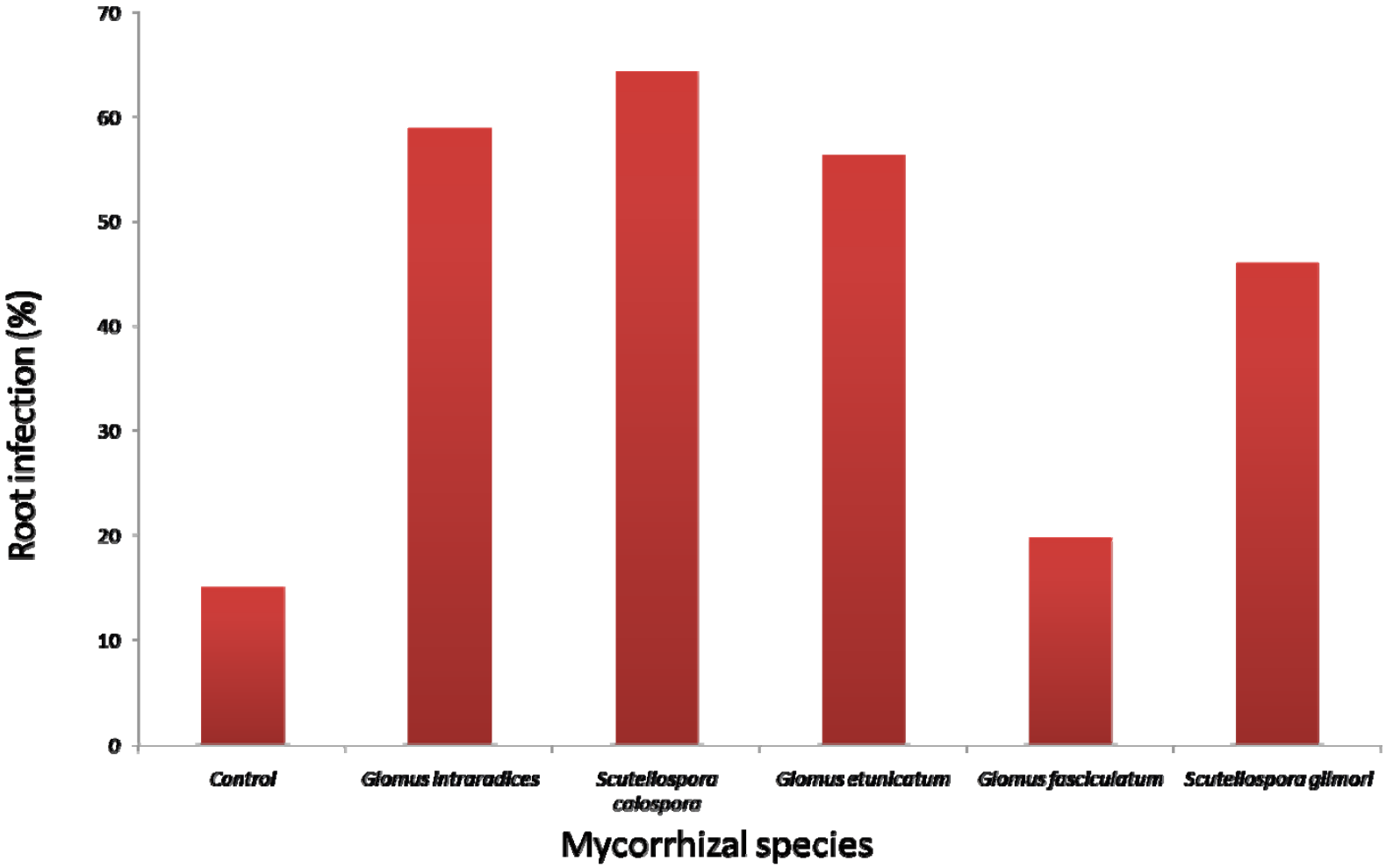


Fig. 1. Percentage root infection by strains of AMF in maize grown by Trap culture method

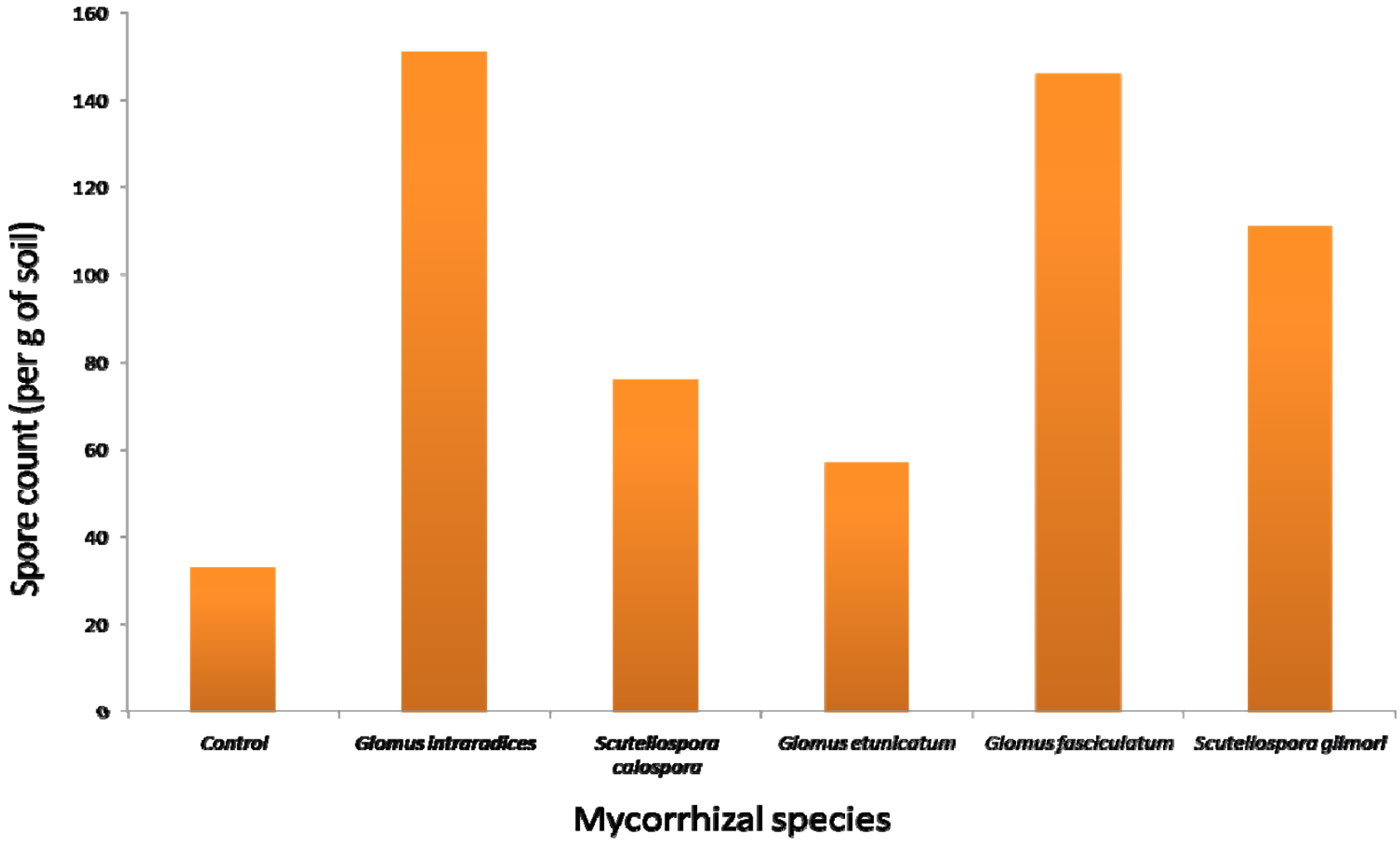


Fig. 2. Estimation of propagules in rhizosphere of maize

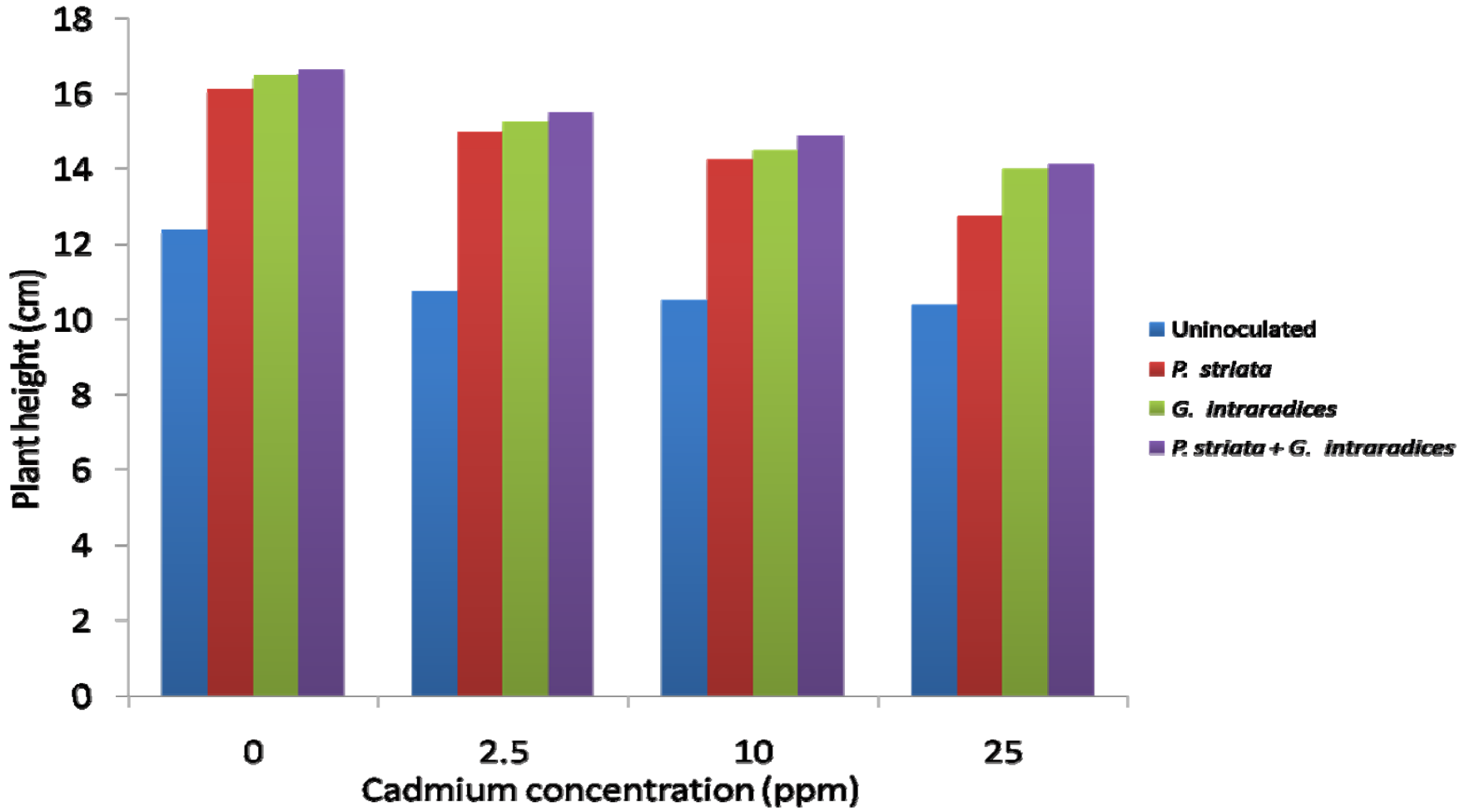


Fig. 3. Effect of soil cadmium concentration on plant height (shoot length) of okra (cm)

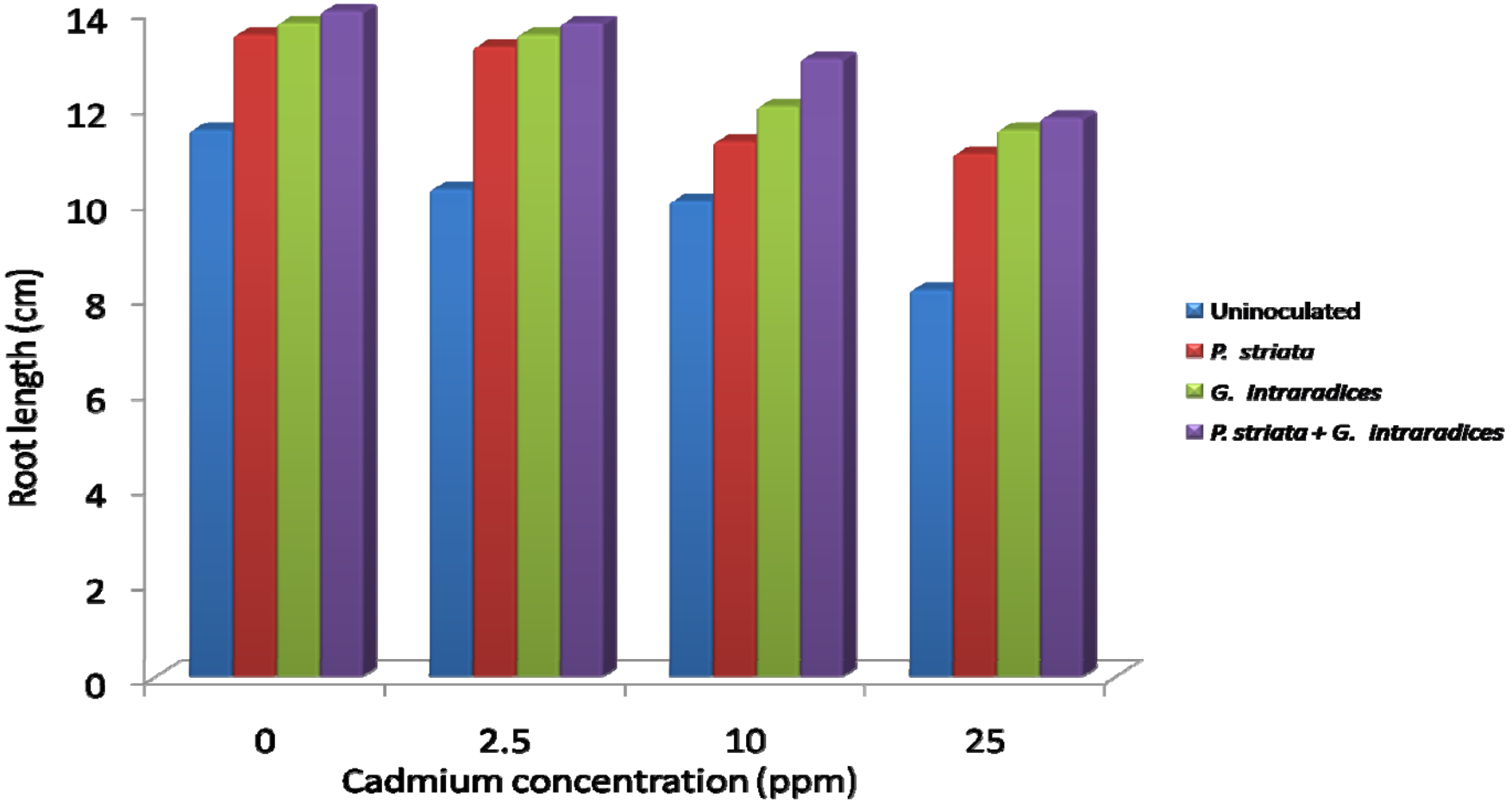


Fig. 4. Effect of soil cadmium concentration on root length of okra plant (cm)

inoculation was better than the individual inoculation. However, this trend was not so pronounced at 25 ppm of cadmium which showed statistically identical values for individual as well as combined inoculation (Figure 5). At 2.5 ppm and 10 ppm the response of okra plants in terms of biomass accumulation to the microbial inoculants was not so pronounced as at zero ppm cadmium. This indicates that inoculatiojn with the *P. striata* or *G. intraradices* helps in overcoming the cadmium stress. The above observations may be expressed to the fact that microorganisms helps in metabolically resist or acclimatize to heavy metal stress.

5.5 The data presented in table 6, 7 and 8 revealed the impact of microbial inoculation on soil enzyme activities under cadmium stress conditions. Soil dehydrogenase activity, acid and alkaline phosphatase activity demonstrated a declining trend with increasing levels of soil cadmium (Figure 6, 7, and 8). The present observation is supported by number of researchers, Das *et al.* (1997) Salt (1995), Karaca and Haktanir (1997). All the three soil enzymes estimated showed a positive response to the combined inoculation with *P. striata* and *G. intraradices* which was statistically better than the uninoculated soils at zero ppm cadmium level. A sharp decline in enzyme activity acid phosphatase was recorded at 25 ppm cadmium. Heavy metalhas been recognized as a major factor impeding the soilmicrobial processes.

An inhibition of the enzyme activity dehydrogenase, acid and alkaline phosphatase with increased levels of soil cadmium was noticed. However, this inhibition could be minimized following microbial inoculation. The beneficial effects were more pronounced under dual inoculation conditions. The maximum concentration of cadmium used in the present study (25 ppm) did not completely eliminate the enzyme activity. This may be attributed to the relative tolerance of the microflora to high concentrations in the range of 0 to 8000 ppm where the inhibition is clearly visible. Gould *et al.* (1979) using hydroponic technique demonstrated that microbial inoculation significantly improved the enzyme activity and it also stimulates the plant root to secrete more of acid phosphatase enzyme.

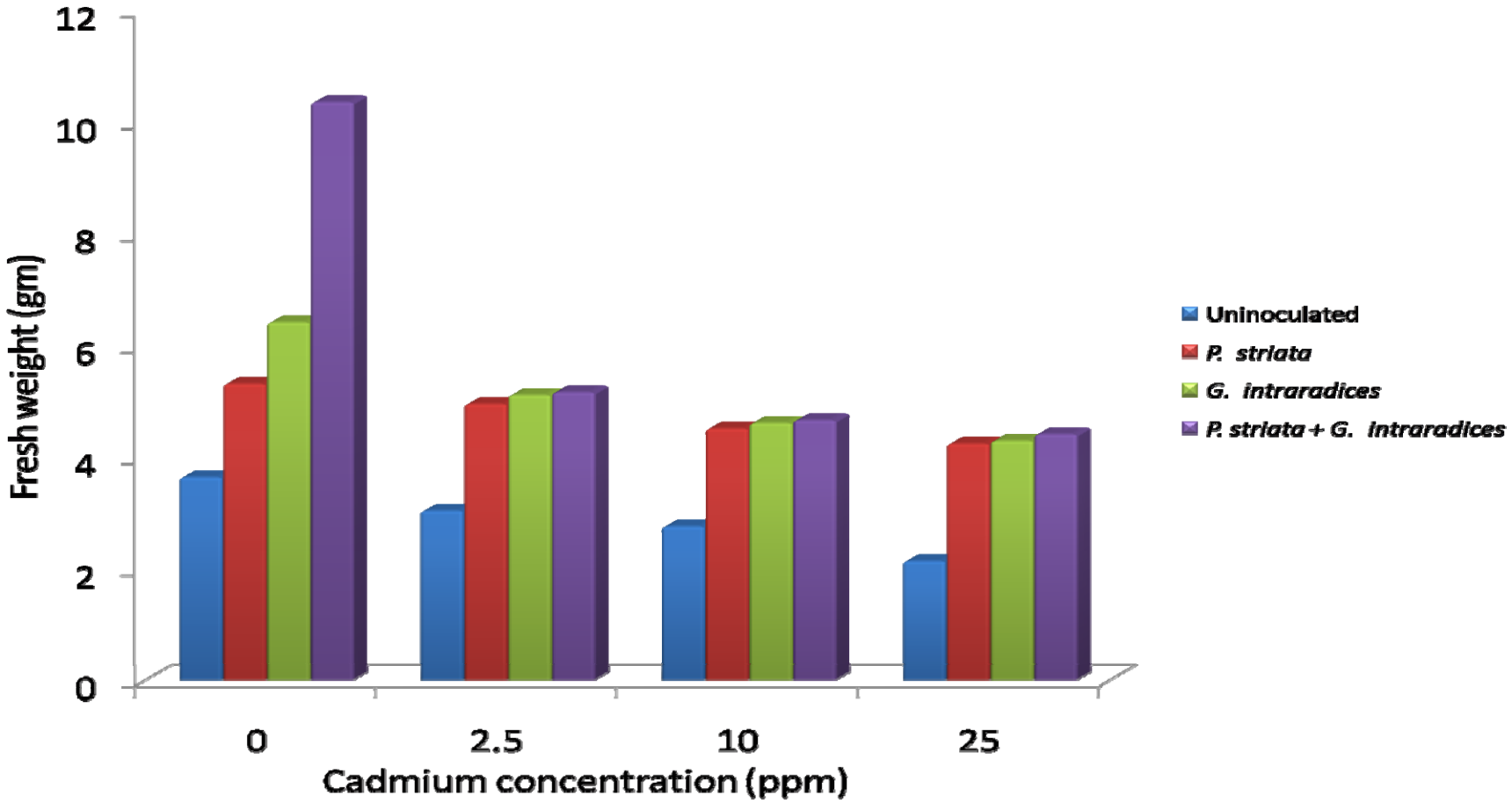


Fig. 5. Effect of cadmium concentration on fresh weight of okra (gm)

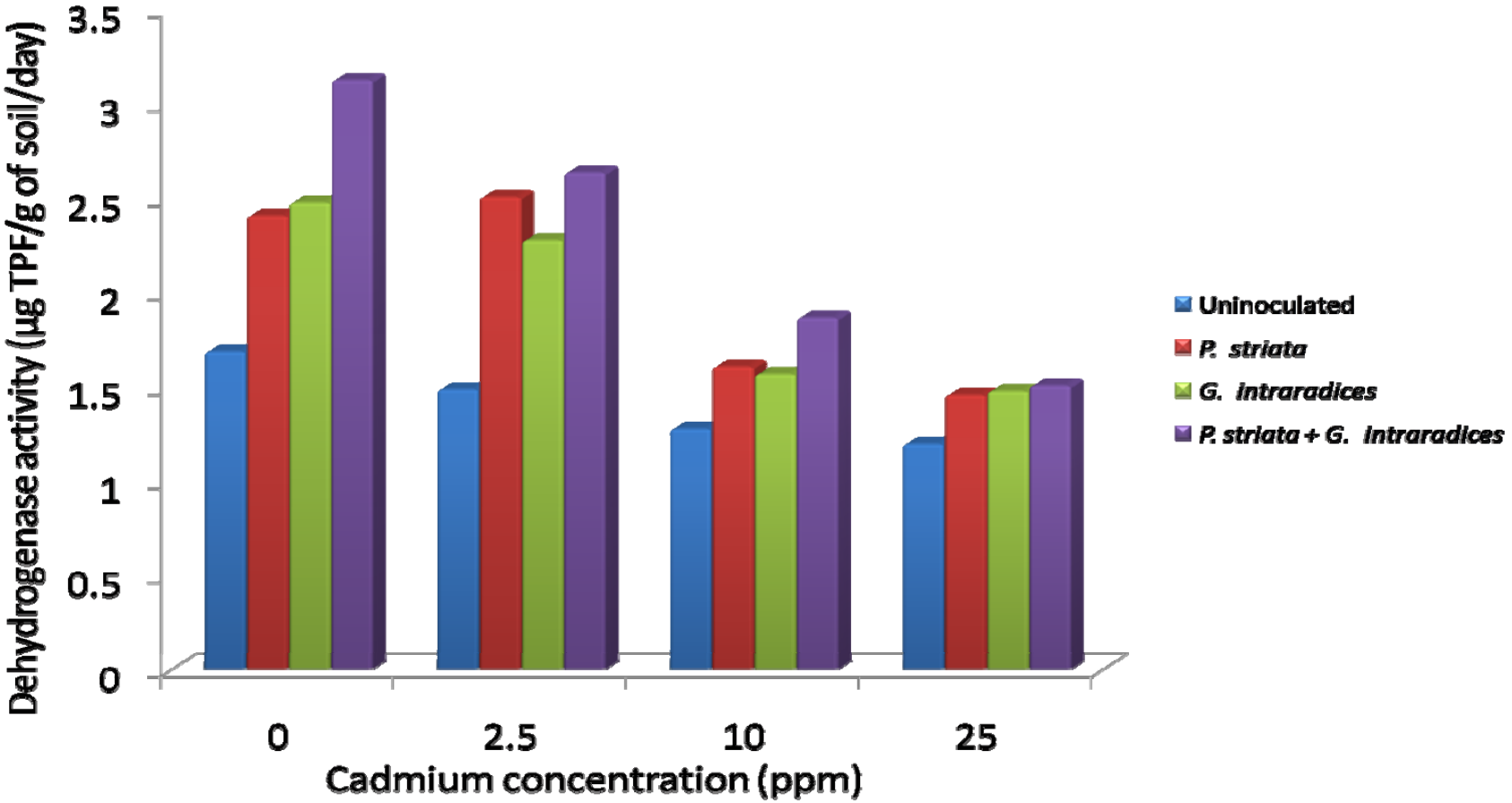


Fig. 6. Interaction effect of dual inoculation and soil cadmium concentration on dehydrogenase activity

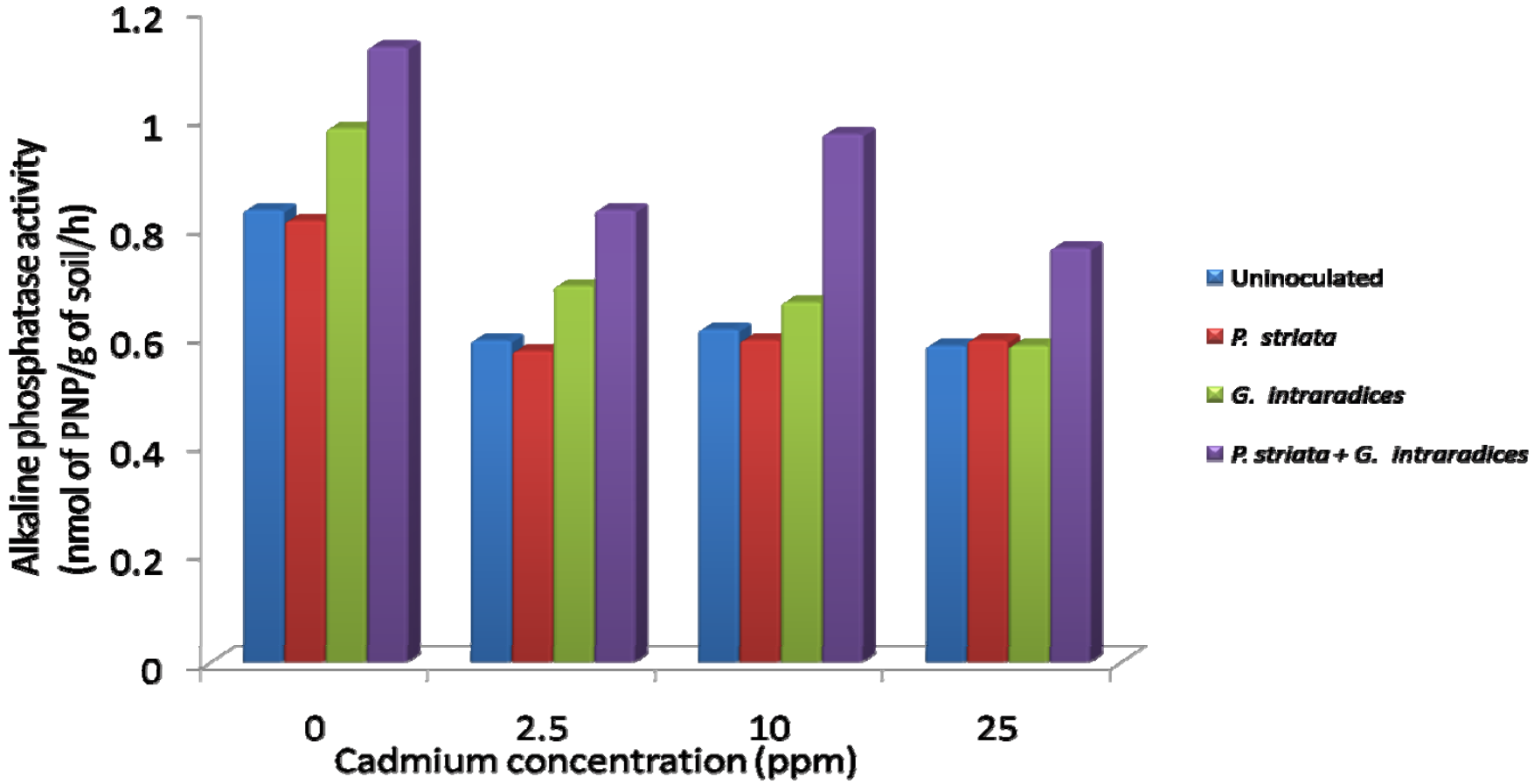


Fig. 7. Interaction effect of dual inoculation and soil cadmium concentration on alkaline phosphatase activity

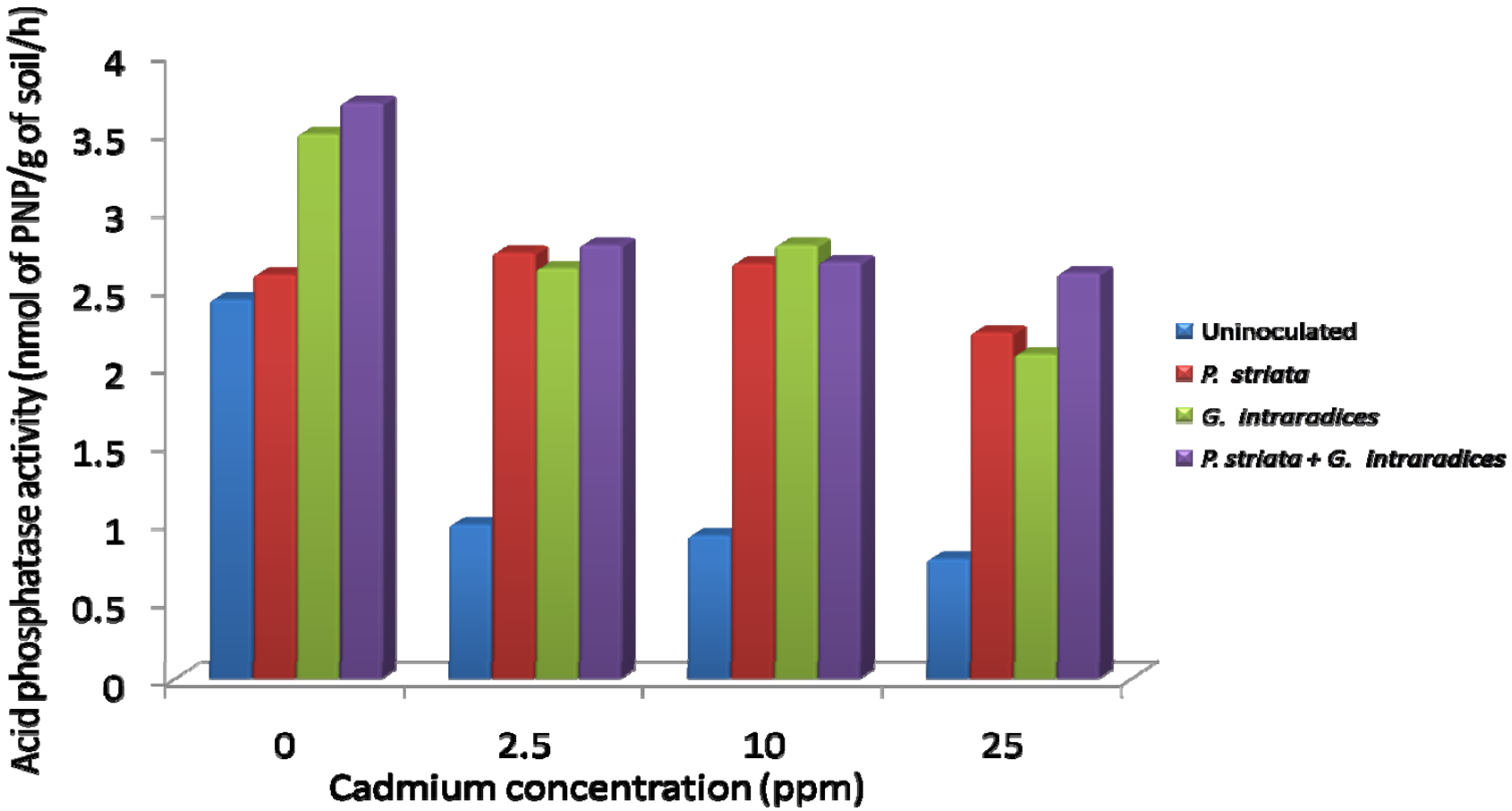


Fig. 8. Interaction effect of dual inoculation and soil cadmium concentration on acid phosphatase activity

5.6 In general the percentage root infection of okra grown under cadmium stress ranging from zero to 25 ppm did not show appreciable decline. This could be probably attributed to the relative tolerance of the AM fungus used in the present study to cadmium concentrations. The relative intolerance of the AM fungi could be due to secretion of glycoprotein glomalins which is known to bind/sequester heavy metals. AM mycelium has a high metal sorption capacity (Joner *et al.*, 2004) (Figure 9).

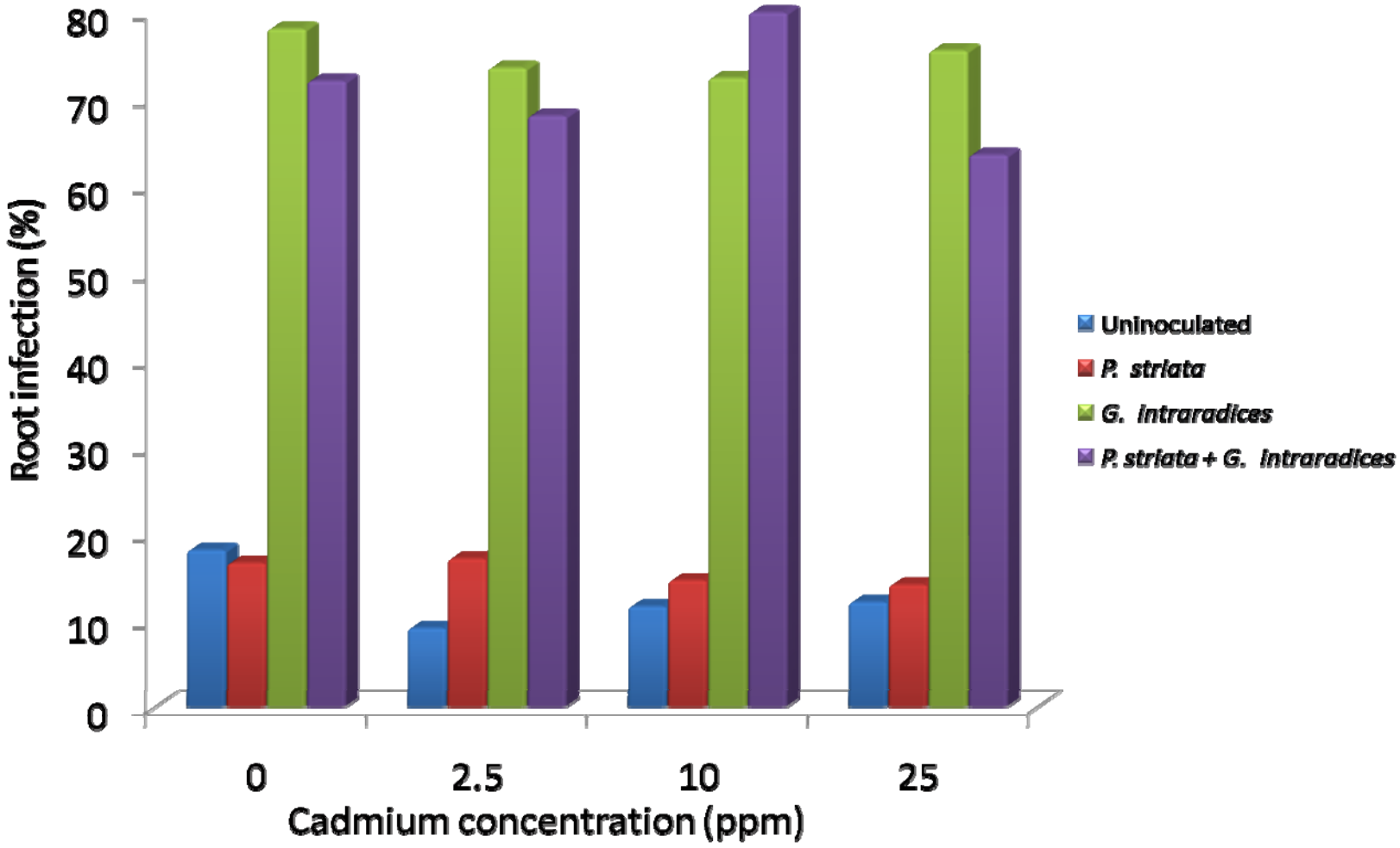


Fig. 9. Percentage root infection in okra

6. SUMMARY AND CONCLUSION

Phosphorous being a macronutrient is one of the limiting factor for crop production. Worldwide P-deficiency is overcome by addition of phosphatic fertilizers and rock phosphates. Phosphatic fertilizers and rock phosphate contain varying amounts of heavy metals such as Cd, Hg and Pb as impurity. Of these cadmium is the most mobile and toxic. An effective process to remove cadmium from phosphatic fertilizers is not known to exist. Cadmium entering into agricultural soils persists for long period of time thereby influencing parameters.

Soil application of rock phosphate along with phosphate solubilising microorganisms is commonly used agricultural practice to improve phosphorus availability to the crops. The present investigation was undertaken to assess the impact of cadmium contamination on soil microbiological parameters and role if any of the bioinoculants in overcoming the abiotic stress.

In vitro and *in vivo* screening was undertaken to evaluate cadmium resistant AMF using maize as a trap crop. Based on high percentage infection and spore count *G. intraradices* was selected for further experimentation. A pot culture experiment was undertaken using okra as a test crop for assessing the impact of cadmium toxicity with the objectives (i) screening AMF isolates for cadmium tolerance under *in vitro* conditions, (ii) to assess the potential of PSB (*Pseudomonas striata*) and AMF in reducing effect of cadmium on a test crop.

(i) The results of a highly significant difference in plant height were observed at different cadmium levels and also under different inoculation treatments. Higher plant height noted in case of zero ppm cadmium and 2.5 ppm cadmium. Similarly, low plant height in uninoculated and high in dual inoculation noted. Gradual increase in plant height with respect to dual inoculation compared to individual inoculation with *P. striata* or *G. intraradices* noticed.

(ii) High cadmium levels i.e. 25 ppm and 10 ppm reduced the root length, whereas higher root length was noted at low cadmium level 2.5 ppm and zero cadmium level. Similarly, uninocuated treatments shown lower root length compared to dual inoculation.

(iii) Fresh weight showed significant difference between the treatments. Uninoculated conditions recorded lower plant fresh weight than the dual inoculated treatment. Similarly, high cadmium dose (25 ppm) resulted in decreased plant fresh weight compared to cadmium less condition.

(iv) Statistically significant differences were noticed in the soil DH enzyme activity at different soil cadmium levels and under microbial inoculations. Soil DH activity was found to be unaffected by 25 ppm soil cadmium concentration due to the influence of microbial inoculants.

(v) Soil enzyme alkaline phosphatase activity in uninoculated soil and dual inoculated soil was significantly different. Microbial inoculation significantly reduced the effect of cadmium on soil alkaline phosphatase activity as noticed in dual inoculation compared to no inoculation.

(vi) Acid phosphatase activity in uninoculated and inoculated treatment was statistically different. Cadmium had no effect even in higher dose (25 ppm) on activity of acid phosphatase due to presence of both microorganisms in case of dual inoculation. Similarly higher cadmium dose significantly reduced the activity of acid phosphatase compared to zero cadmium level.

(vii) Statistically insignificant difference was noticed under different soil cadmium conditions with respect to root infection percentage of okra crop by *G. intraradices.* All the *Glomus* inoculated treatments were more or less equally infected by the organism showing no statistical difference.

Abstract

Experiments were conducted under *in vitro* to evaluate cadmium tolerance ability and cultural characteristics of five different strains of Arbuscular mycorrhizal fungus. Based on results of these initial screening, the cadmium tolerant strain with good cultural characterstics *Glomus intraradices* was selected for main pot culture experiment. This AMF was used for pot culture along with *Pseudomonas striata* using Okra (*Abelmoschus esculentus*), as test crop to evaluate the effect of varying cadmium concentration and effect of individual or dual inoculation on growth and development of okra plants. Also , to evaluate their possible role in retaining the activity of soil enzymes involved in 'P' transformation. The treatments comprised no inoculation, individual inoculation of *P. striata* and AMF (*G. intraradices*) and dual inculation of okra using the both organisms in soil having 0, 2.5, 10 and 25 ppm level of cadmium. Under pot culture condition, significant decrease in plant parameters such as plant height, root length, fresh weight was noticed at higher cadmium levels (10 and 25 ppm). Significant reduction of cadmium toxicity effect on plant parameters under dual inoculation was observed even under elevated cadmium levels (10 and 25 ppm). Dual inoculation also caused reduction of toxic effect of cadmium on soil parameters like dehydrogenase activity, acid and alkaline phosphatase activity. Under *in vivo* condition significant ($P \leq 0.05$) decline in plant parameters and soil enzyme activity due to higher cadmium dose 10 and 25 ppm was noticed. However, under presence of both organisms, dual inoculation condition insensitivity to high cadmium dose up to 25 ppm by plant and soil parameters was noticed. Microbial inoculation significantly reduced the effect of cadmium on plant and soil parameters.

अरबस्वुफलर माइकोराइजा फफूँदी एवं *स्युडोमोनास स्ट्रीयाटा* द्वारा कैडमियम विषाक्तता की जैव उन्नति

सारांश

अरबस्वुफलर माइकोराइजा पफपूँफदी के पाँच प्रजातियों का कैडमियम सहिष्णुता तथा संर्वध्न विशिष्टताओं का प्रयोगशाला में मुल्यांकन हेतु प्रयोग निस्पादित किया गया। प्रारंभिक छँटाई के आधर पर उन्नत कैडमियम सहिष्णुता गुण वाले *ग्लोमस इन्ट्रारेडिसेस* प्रजाति को मुख्य गमला संर्वध्न प्रयोग के लिए चुना गया। कैडमियम के विभिन्न सांद्रताओं का, परीक्षण पादप के रूप में चुने गये भिन्डी ;*एबलमसकस एसवुफलेंटसद्ध* की वृ(तथा विकास पर प्रभाव, अरबसवुफलर माईकोराइजा पफपूँफदी एवं *स्युडोमोनास स्ट्रीयाटा* के एकल तथा दोहरी टीकाकरण के साथ आकलित किया गया। पफॉस्पफोरस परिवर्तन में शामिल एन्जाइम की गतिविध्यिों को बनाये रखने मे इनकी संभावित भुमिकाओं का भी आंकलन किया गया। 0, 2.5, 10 तथा 25 पी.पी.एम. स्तर कैडमियम मिश्रित मृदा ने असंरोपित, *पी. स्ट्रीयाटा* तथा ए. एम.एपफ. ;*जी. इन्ट्रारेडिसेसद्ध* एकल टीकाकृत एवं द्विटीकाकृत भिन्डी को उपचार के तौर पर लिया गया। गमला सर्वध्न स्थिति में, पौधे के मापदंड जैसे उँचाई, जड़ की लंबाई, ताजा वजन इत्यादि में कैडमियम की अध्कि सांद्रता ;10 तथा 25 पी.पी.एम.द्ध पर सार्थक कमी पाई गयी। कैडमियम के उच्च स्तर ;10 तथा 25 पी.पी.एम.द्ध पर द्विटीकाकृत उपचार ने कैडमियम विषाक्ता के प्रभाव को कम करती प्रतीत हुई। वास्तविक अवस्था में कैडमियम की उच्च खुराक 10 तथा 25 पी.पी.एम. पर पौध मापदंड एवं मृदा एन्जाइम गतिविधि में सार्थक ;पी ढ 0.05द्ध गिरावट पाई गयी। पिफर भी, दोनो जीवों की उपस्थिति में दोहरी टीकाकृत अवस्था में अध्कि कैडमियम खुराक, 25 पी.पी.एम तक पौधे की असंवेदन शीलता सुचित की गयी। सुक्ष्मजीव टीकाकरण पौधे तथा मृदा मापदंडो पर कैडमियम के प्रभाव को कम प्रतीत हुई।

REFERENCES

Alcantara, E., Romera, F. J. Cañete, M., De La Guardia, M. D. (1994). Effects of heavy metals on both induction and function of root Fe(III) reductase in Fe-deficient cucumber (*Cucumis sativus* L.) plants. *J. Exp. Bot.* **45:** 1893-1898.

Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R. and Barea, J. M. (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Applied Soil Ecology.* **22:** 29–37.

Babich, H. and Stotzky, G. (1997). Effect of camium on fungi and on interactions between fungi and bacteia in soil: influence of clay minerals and pH. *Appl. Environ. Microbial.* **33(5):** 1059-1066.

Babich, H. and Stotzky, G. (1997). Sensitivity of various bacteria including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. *Appl. Environ. Microbial.* **5:** 99-105.

Baker, A. J. M. (1981). Accumulators and excluders–strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, **3:** 643–654.

Balestrasse, K. B., Benavides, M. P., Gallego, S. M. and Tomaro, M. L. (2003). Effect on cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. *Plant Biol.* **30:** 57-64.

Balestrasse, K. B., Gallego, S. M. and Tomaro, M. L. (2004). Cadmium-induced senescence in nodules of soybean (*Glycine max* L.) plants. *Plant Soil* **262:** 373-381.

Balestrasse, K. B., Gardey, L., Gallego, S. M. and Tomaro, M. L. (2001). Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. *J. Plant Physiol.* **28:** 497-504.

Barceló, J., Poschenrieder, C., Andreu, I. and Gunsé, B. (1986). Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). Effects of Cd on water potential, relative water content and cell wall elasticity. *J. Plant Physiol.* **125:** 17-25.

Bhattacharya, M., Chaudhuri, M. A. (1995). Heavy metal (Pb^{2+} and Cd^{2+}) stress-induced damages in *Vigna* seedlings and possible involvement of phytochelatin-like substances in mitigation of heavy metal stress. *Indian Journ. of Environ. Bull.* **33:** 236-238.

Bingham, F. T., Page, A. L., Mahler, R. J. and Ganje, T. J. (1976). Yield and cadmium accumulation of forage species in relation to cadmium content of sludge-amended soil. *J. Environ. Qual.* **5:** 57-59.

Blum, W. H. (1997). Cadmium uptake by higher plants. In: Proceedings of extended abstracts from the Fourth International Conference on the Biogeochemistry of Trace Elements, Berkeley, USA. University of California. 109-110.

Blum, W.E.H. (1989). Soil pollution by heavy metals: causes, processes, impacts and need for future actions: In: Proceedings of third meeting. Steering Committee for the conservation and management of the environment and naturals habitats (CDPE), Strasbourg.

Brundrett, M. C. (1991). Mycorrhizas in natural ecosystems. In: Macfayden A, Begon M & Fitter AH (eds) *Advances in Ecological Research.* Academic Press, London. **21**: 171-313.

Brundrett, M. C., Abbott, L. K. and Jasper, D. A (1999). Glomalean mycorrhizal fungi from tropical Australia. I. Comparison of the effectiveness and specificity of different isolation procedures. *Mycorrhiza,* **8:** 305-314.

Brundrett, M. C., Piché, Y and Peterson, R. L. (1984). A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany*, **62:** 2128-2134.

Brundrett. M., Melville, L and Peterson, L. (1994). Practical Methods in Mycorrhizal Research. Mycologue Publications Ltd. Waterloo.

Casida, L. E., Klein, D. A. and Santoro, T. (1964). Soil dehydrogenase activity. *Soil Science,* **98:**371-376.

Cho, U. H. and Seo, N. H. (2005). Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. *Plant Science,* **168:** 113-120.

Christensen, T. H. (1984). Cadmium soil sorption at low concentrations. Its effect on time, cadmium load, pH and calcium. *Water, air and soil pollution,* **21:** 105-114.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta.* **212:** 475-486.

Cobbett, C. S. (2000). Phytochelatins and their role in heavy metal detoxification. *Plant Physiology* **123:** 825-832.

Colbet, C. and Goldsbrough, P. (2002). Phytochelatins and Metallothioneins: Roles in Heavy Metal Detoxification and Homeostasis. *Annual Review of Plant Biology* **53**: 159-182.

Costa, G. and Morel, J. L. (1994). Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiol. Biochem.* **32:** 561-570.

Das, P., Samantaray, S. and Rout, G. R. (1997). Studies on cadmium toxicity in plants: a review. *Environ. Pollution* **98:** 29-36.

Degreave, N. (1981). Carcinogenic, teratogenic and mutagenic effects of cadmium. *Mutation Res.* **86:**.115-122.

del Val, C., Barea, J. M. and Azcòn-Aguilar, C (1999). Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Appl. Soil Ecol*. **11:** 261–269.

del Val, C., Barea, J. M. and Azcòn-Aguilar, C. (1999). Diversity of arbuscular mycorrhizal fungus populations in heavy metal contaminated soils. *Appl. Environ. Microbiol*. **99**: 718–723.

Duxbury, T. (1985). Ecological aspects of heavy metal responses in microorganisms. *Adv. Microb. Ecol.* **8:** 185-235.

Feng, G., Song, Y. C., Li, X. L. and Christie, P (2003). Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Appl. Soil Ecol*. **22:** 139–148.

Fodor, A., Szabó-Nagy, A. and Erdei, L. (1995). The effects of cadmium on the fluidity and H^+-ATPase activity of plasma membrane from sunflower and wheat roots. *J. Plant Physiol.* **14:** 787-792.

Fornazier RF, Ferreira RR, Vitória AP, Molina SMG, Lea PJ, Azevedo RA (2002) Effects of cadmium on antioxidant enzyme activities in sugar cane. *Biol. Plant.* **45:**91-97.

Gallego SM, Benavides MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Sci.* **121:**151-159.

Gaur, A., and Adholeya, A (2004). Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science,* **86:** 528-534.

Gerdemann J. W. (1968). Vescicular Arbuscular Mycorrhiza and Plant Growth. *Annual Review of Phytopathology,* **6(1):** 418.

Gerdemann, J. W., Nicolson T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* **46**: 235-244.

Gildon, A. and Tinker, P. B (1981). A heavy metal-tolerant strain of a mycorrhizal fungus. *New Phytologist*, **95:** 263–268.

Gildon, A. and Tinker, P. B. (1981). A heavy metal-tolerant strain of a mycorrhizal fungus. *New Phytol*. **95**: 263–268.

Gildon, A. and Tinker, P. B. (1983). Interactions of vesicular-arbuscular mycorrhiza infections and heavy metals in plants. The effects of infection on uptake of copper. *Trans. Br. Mycol. Soc*. **77**: 648–649.

Goldbol DL, Hutterman A (1985) Effect of zinc, cadmium and mercury on root elongation on *Picea abies* (Karst.) seedlings and the significance of these metals to forest die-back. *Environ. Pollution* **38:**375-381.

Haghiri F (1973) Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc and soil temperature. *J. Environ. Qual.* **2:**93-96.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*. **53:** 1-11.

Heggo, A., Angle, J. S. and Chaney, R. L (1990). Effects of vesicular arbuscular mycorrhizal fungi on heavy-metal uptake by soybeans. *Soil Biology and Biochemistry*, **22:** 865–869.

Hernández LE, Cárpena-Ruiz R, Garate A (1996) Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J. Plant Nutr.* **19:**1581-1598.

Hernández LE, Cooke DT (1997) Modification of the roots plasma membrane lipid composition of cadmium-treated *Pisum sativum*. *J. Exp. Bot.* **48:**1375-1381.

Howden, R., Andersen, C. S., Goldsbrough, P. B. and Cobbett, C. S. (1995). A cadmium-sensitive, glutathione-deficient mutant of *Arabidopsis thaliana*. *Plant Physiology* **107:** 1067-1073.

Howden, R., Goldsbrough, P. B., Andersen, C. S. and Cobbett, C. S. (1995) Cadmium-sensitive, *cad*1 mutants of *Arabidopsis thaliana* are phytochelatin deficient. *Plant Physiology* **107:** 1059-1066.

Jackson, A. P., Alloway, B. T. (1992). The bioavailability of cadmium to lettuce and cabbage in soils previously treated with sewage sledges. *Plant Soils* **132:** 179-186.

Joner, E. J. and Leyval, C (1997). Uptake of 109Cd by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytologist*, **135:** 353–360.

Joner, E. J. and Leyval, C. (2001). Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. *Biol. Fertil. Soils*. **33**: 351–357.

Joner, E. J., Leyval, C. and Briones, R (2000). Metal binding capacity of arbuscular mycorrhizal mycelium. *Biology and Fertility of Soils*, **226**: 227–234.

Joner, E. J., Ravnskov, S. and Jakobsen, I. (2000). Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radiolabelled inorganic and organic phosphate. *Biotechnol. Lett.* **22**: 1705–1708.

Kabata-Pendias, A. and Pendias, H. (1992). Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL.

Kabir, Z. and Koide, R. T (2000). The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. *Agriculture, Ecosystems & Environment*, **78:** 167–174.

Karaca, A., Haktanır, K. (1997). Determination of Cd contents of phosphate fertilizers and raw phosphates. *Turkish J. of Agriculture and Forestry*. **21 (4):** 327-334.

Karaca, A., Haktanır, K. (1997). Effects of Afsin-Elbistan power plant emission on the physical, chemical, and biological properties of nearby soils. *Ph. D. Thesis*.

Karaca, A., Naseby, N.C., Lynch, J. (1999). Effect of Cd contamination with sewage sludge and phosphatase fertiliser amendments on soil enzyme activities, available Cd and other ion contents of soil. *Soil Biology and Biochemistry* (in press).

Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S. and Hayes, W. J (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, **41**: 197–207.

Laheurte, F., Leyval, C. and Berthelin, J. (1990). Root exudates of maize, pine and beech seedlings influenced by mycorrhizal and bacterial inoculation. *Symbiosis*. **9**: 111–116.

Leyval, C., Singh, B. R. and Joner, E. J. (1995) Occurrence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. *Water Air Soil Pollut*. **84**: 203–216.

Leyval, C., Turnau, K. and Haselwandter, K (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza*, **7:** 139–153.

Li Y. M, Chaney R. L., Schneiter A. A., Miller J. F., Elias E. M., Hammond J. J. (1997). Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax. *Euphytica*. **94:** 23-30.

Li, X. L. and Christie, P. (2000). Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. *Chemosphere*. **42**: 201–207.

Li, X. L., George, E. and Marschner, H (1991). Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New Phytologist*, **119**: 397–404.

Loeffler, S., Hochberger, A., Grill, E., Gekeler, W., Winnacker E-L. and Zenk, M. H. (1989). Termination of the phytochelatin synthase reaction through sequestration of heavy metals by the reaction product. *FEMS Letters* **258:** 42-46.

Mathys W (1975) Enzymes of heavy metal-resistant and non-resistant populations of *Silene cucubalus* and their interactions with some heavy metals in vitro and in vivo. *Physiol. Plant.* **33:**161-165.

McLaughlin, M. J. and Singh, B. R. (1999). Cadmium in soils and plants. *Developments in Plant and Soil Sciences* **85:** 1-7.

McLaughlin, M. J., Bell, M. J., Wright, G. C. and Cozens, G. D. (2000). Uptake and partitioning of cadmium by cultivars of peanut (*Arachis hypogaea* L.). *Plant and Soil* **222:** 51-58.

Mclaughlin, M. J., Tiller, K. G., Naidu, R. and Stevens, D.P. (2005). Review: the behaviour and environmental impact of contaminants in fertilizers. *Australian Journal of Soil Research* **34(1):** 1-54.

McLaughlin, M.J., Maier, N.A. , Rayment, G.E., Sparrow, L.A., Berg, G., McKay, A., Milham, P., Merry, R.H., and Smart, M.K. (1997). Cadmium in Australian potato tubers and soils. *J. Environ. Qual.* **26:** 1644–1649.

Moreno, J. L., Hernandez, T., Garcia, C (1999). Effect of cadmium- containing sewage sludge compost on dynamics of organic matter and microbial activity in an arid soils. *Biol. Fert. Soils* **28:** 230-237.

Mortvedt, J.J. (1982). Studies on the chemical form of cadmium contaminants in phosphate fertilizers. *Soil Sci.* **134:** 185–192.

Mortvedt, J.J. (1987). Cadmium levels in soils and plants from some long-term soil fertility experiments in the United States of America. *J. Environ. Qual.* **16:** 137–142.

Mortvedt, J.J. (1996). Heavy metal contaminants in inorganic and organic fertilizers. *Fert. Res.* **43:** 55–61.

Mukherjee, A., Sharma, A., Talukdeer, G. (1984). Effects of cadmium on cellular systems in Higher organisms. *The Nucleus* **27:** 121-139.

Obata, H., Umebayashi, M. (1997). Effects of cadmium on mineral nutrient concentrations in Plants differing in tolerance for cadmium. *J. Plant Nutr.* **20:** 97-105.

Oliver, D. P., Gartrell, J. W., Tiller, K. G., Correll, R., Cozens, G. D., Youngberg, B. L. (1997) Differential responses of Australian wheat cultivars to cadmium concentration in wheat grain. *Australian Journal of Agricultural Research.* **46:** 873-886.

Olsson, P. A., Francis, R., Read, D. J. and Söderström, B. (1998). Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids. *Plant Soil*. **201**: 9–16.

Pathak, H (2002). Fertilizer use and environmental quality. *Fert. News.* **47 (11):** 13-20.

Pathak, H., Bhatia, A., Shiv prasad, Shalini Singh, Sushil Kumar, Jain, M.C. and Singh, P. (2003). Effect of DCD, FYM and moisture regimes on nitrous oxide emission from alluvial soil in rice-wheat cropping system. *J. Indian Soc. Soil Sci.* **51:** 139-144.

Pathak, H., Biswas, D.R. and Singh, R. (2003) Fertilizer use and environmental quality. *Fert. News.* **48(11):** 5-11.

Pawlowska, T. E. and Charvat, I (2004). Heavy-Metal Stress and Developmental Patterns of Arbuscular Mycorrhizal Fungi. *Applied and Environmental Microbiology,* **70 (11):** 6643–6649.

Pearson, J. N. and Jakobsen, I. (1993). The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with 32P and 33P. *New Phytol*. **124**: 489–494.

Pfleger, F. L., Stewart, E. L. and Noyd, R. K. (1994). Role of VAM fungi in mine land revegetation. *Mycorrhizae and Plant Health* (eds Pfleger, F. L. and Linderman, R. G.), The American Phytopathological Society, MN, USA, 47–82.

Phillips, J. M. and Hayman D. S. (1970). Improved procedure for clearing roots and staining parasite and mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society.* **55:** 158-161.

Pinto, A. P., Mota, A. M., de Varennes, A., Pinto, F. C. (2004). Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Sci. Tot. Environ* **326:** 239-247.

Pinto, E., Sigaud-Kutner, T. C. S., Leitão, M. A. S., Okamoto, O. K., Morse, D., Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *J. Phycol.* **39:** 1008-1018.

Poschenrieder C, Gunsé B, Barceló J (1989) Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. *Plant Physiol.* **90:**1365-1371.

Rillig, M. C. and Steinberg, P. D (2002). Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification. *Soil Biology and Biochemistry,* **34**, 1371–1374.

Robards, K. and Worsfold, P. (1991). Cadmium: Toxicology and Analysis. *Analyst.* **116:** 549-568.

Sally E. S., F. Andrew S. and Iver J. (2003). Mycorrhizal Fungi Can Dominate Phosphate Supply to Plants Irrespective of Growth Responses. *Plant Physiol.* **133.**

Salt, D. E., Blaylock, M., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I., Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.* **13:** 468-474.

Salt, D. E. and Rauser, W. E. (1995). MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107:**1293-1301.

Salt, D. E., Prince, R. C., Pickering, I. J., Raskin, I. (1995). Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109:** 427-433.

Salt, D. E., Smith, R. D., Raskin, I. (1998). Phytoremediation. Ann. Rev. Plant Physiol. *Plant Mol. Biol.* **49:** 643-668.

Sambandan, K., Kannan, K. and Raman, N (1992). Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu. *Journal of Environmental Biology*, **13**: 159–167.

Sandalio, L. M., Dalurzo, H. C., Gomez, M., Romero-Puertas, M. C. and del Río, L. A. (2001). Cadmium induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **52:**2115-2126.

Sanita` di Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher plants. *Environmental and Experimental Botany* **41:** 105-130.

Singh, B. R. and Myhr, K. (1998). Cadmium uptake by barley as affected by Cd sources and pH levels. *Geoderma* **84:** 185-194.

Smith, S. E. and. Read, D. J. (1997). *Mycorrhizal Symbiosis*, Academic Press, San Diego, USA.

Smolders, E., and M.J. McLaughlin. (1996). Effect of Cl on Cd uptake by Swiss chard in unbuffered and chelator buffered nutrient solutions. *Soil Sci. Soc. Am. J.* **60:** 1443–1447.

Somashekaraiah, V., Padmaja, K., Prasad, A. R. K. (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*)-involvement of lipid peroxides in chlorophyll degradation. *Physiologia plantarum.* **85:** 85-89.

Stobart, A. K., Griffits, W., Bukhari, I. A. and Sherwood, R. P. (1985). The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiol. Plant.* **63:**293-298.

Stohs, S. J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med.* **18:** 321-336.

Taylor, J. and Harrier, L. A. (2001). A comparison of development and mineral nutrition of micropropagated *Fragaria* × *ananassa* cv. Elvira (strawberry) when colonized by nine species of arbuscular mycorrhizal fungi. *Appl. Soil Ecol*. **18:** 205–215.

Tonin, C., Vandenkoornhuyse, P., Joner, E. J., Straczek, J. and Leyval, C (2001). Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza* **10**: 161–168.

Tullio, M., Pierandrei, F., Salerno, A. and Rea, E (2003). Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium-polluted and unpolluted soil. *Biology and Fertility of Soils*, **37**: 211–214.

Turnau, K. and Mesjasz-Przybylowicz, J. (2003). Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza*. **13**: 185–190.

Turnau, K., Kottke, I. and Oberwinkler, F. (1993). Element localization in mycorrhizal roots of *Pteridium aquilinum* L. Kuhn collected from experimental plots treated with cadmium dust. *New Phytol*. **123**: 313–324.

Turner, Ryszka, P., Gianinazzi-Pearson, V. and van Tuinen, D (2001). Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza* **10**: 169–174.

Verkleij, J. A. C. and Schat, H. (1990). Mechanisms of metal tolerance in higher plants. In: Shaw J. (eds), Heavy metal tolerance in plants: evolutionary aspects, 179-193. CRC Press, Boca Raton.

Wagner, G. J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.* **51:** 173-212.

Weggler-Beaton, K., McLaughlin, M. J., and Graham, R. D. (2000). Salinity increases cadmium uptake by wheat and Swiss chard from soil amended with biosolids. *Aust. J. Soil Res.* **38:** 37–45.

Weissenhorn, I. Leyval, C. and Berthelin, J. (1995). Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a soil polluted by atmospheric deposition from a smelter. *Biology and Fertility of Soil*, **19:** 22–28.

Weissenhorn, I., Glashoff, A., Leyval, C. and Berthelin, J. (1994). Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant Soil*. **167**: 189–196.

Weissenhorn, I., Leyval, C. and Berthelin, J. (1993). Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant and Soil*. **157**: 247–256.

Weissenhorn, I., Leyval, C., Belgy, G. and Berthelin, J. (1995). Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza*. **5**: 245–251.

Weissenhorn, I., Mench, M. and Leyval, C. (1995). Bioavailability of heavy metals and arbuscular mycorrhiza in a sewage sludge amended sandy soil. *Soil Biol. Biochem*. **27**: 287–296.

Zhang, G., Fukami, M. and Sekimoto, H. (2002). Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Research* **77:** 93-98.

Zhou, J. L. (1999). Zn biosorption by *Rhizopus arrhizus* and other fungi. *Appl. Microbiol. Biotechnol*., **51**: 686–693.

Zhu, Y. G., Christie, P. and Laidlaw, A. S. (2001). Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere*. **42**: 193–199.