Chapter-1

Introduction
INTRODUCTION

Legumes are well known for their ability to fix atmospheric nitrogen (N) and enhance N pool of soil, leading to increase in crop productivity both in conventional or derelict soil. They provide pulses which are source of different amino acids, proteins and minerals in human diet. The vegetarian population, especially, relies on pulses for dietary protein requirements. They are used as food in a variety of forms, besides, this that they fix inert environmental N symbiotically in association with root nodule bacteria and shows no or little dependence on N fertilizers (Ma et al., 2006). They are able to grow under a wide range of climatic conditions and edaphic factors such as soil moisture, toxicants, pH and texture etc. The estimated amounts of N which can be fixed by leguminous crops range from 70-100 Kg ha\(^{-1}\) year\(^{-1}\) in peas and beans to over 300 Kg ha\(^{-1}\) year\(^{-1}\) for clover or lucerne (Postgate, 1982). Excessive metal concentration on the other hand causes undeniable damage to *Rhizobium*, legumes and their symbiosis.

Plants are constantly exposed to adverse environmental conditions that negatively affect their growth and productivity. Heavy metal (HM) toxicity is one of the major abiotic stresses leading to hazardous effects in plants and alters physiological and metabolic processes (Villiers et al., 2011). They become toxic when their concentration exceeds a beyond certain threshold level which further depends upon plant species, edaphic conditions and the type of the metal. Among HMs, Cadmium (Cd) is one of the most toxic, nonessential and mobile element present in soil that adversely affect plant growth and yield. (Sanita di toppi and Gabbielli, 1999; Qadir et al., 2004; Rahmanian et al., 2011). The toxicity of Cd in arable field is mainly due to the application of pesticides, industrial processes, fossil fuel combustion, cement manufacture, non-ferrous metal production, Cd-containing sewage sludge and phosphate fertilizers (Angelone and Bini, 1992; Sanita di toppi and Gabbielli, 1999; Solis-Domínguez et al., 2007; Nazar et al., 2012, Sandalio et al., 2001). The high mobility of this metal in the soil-plant system makes its entrance easier into the food chain (Dalcorso et al., 2018). Although, Cd is not essential for plant growth, but it is readily taken up by roots and translocated into the leaves of many plant species (Prasad, 1995). The degree to which plants are able to take up Cd by their roots depends on its concentration in the soil and its bioavailability,
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Inhibited by the presence of organic matter, pH, redox potential, temperature and concentration of other elements (Eriksson et al., 1996; Ciecko et al., 2001; Yu et al., 5; Singh et al., 2008). Despite the different mobility of Cd in plants, its immobilization in roots is more than other parts (Benavides et al., 2005). Cadmium is only immobilized in the root portion but also translocated to the aerial part (Yonis, 7; Anjum et al., 2008). Physiological effects of Cd stress have been well documented in higher plants, especially those of agricultural importance such as legumes and cereals (Anjum et al., 2008; Farooqui et al., 2009). The tolerance mechanisms in plant require the coordination of several complex physiological and biochemical processes to counter the detrimental effects of this contaminant (Hossain et al., 2012). Legumes and cereals show visual symptoms like reddish brown spots on hairy and secondary leaves such as blackening, browning, burning and weakening stems and leaves. (Anjum et al., 2008; Farooqui et al., 2009). Cadmium stress causes severe signs of chlorosis and necrosis on the leaf lamina between the veins and pinal chlorosis in chickpea cultivars (Faizan et al., 2011). The reduction in with biomass and yield with increased levels of Cd has been primarily attributed to reduced photosynthesis (Verma and Dubey, 2002; Wahid et al., 2007) which may be to the decline in photosynthetic pigments and rubisco activity (Bibi and Hussain, 5; Wani et al., 2008b). Cadmium toxicity results in inactivation and denaturation of the enzymes, proteins, blocking of functional groups of metabolically important molecules, substitution or displacement of essential metal ions from biomolecules, formation of modification and disruption of membrane integrity (Ramesh, 2008; Siers et al., 2011) which is finally attributed to altered activities of several key enzymes (Sharma and Dietz 2006; Dubey, 2011). It also disturbs redox homeostasis directly by stimulating the formation of reactive oxygen species (ROS) and oxidation of membrane lipids.

The tolerance mechanisms in plant require the coordination of several complexes physiological and biochemical effects to counter the detrimental effects of (Hossain et al. 2012) and plants have evolved a sophisticated antioxidant defense system to scavenge them. Among antioxidant enzymes, superoxide dismutase constitutes the primary step of cellular defense and dismutates superoxide radicals to hydrogen peroxide and oxygen. Accumulation of hydrogen peroxide which is a strong
oxidant is prevented in cell by catalase and peroxidase (Maksymiec, 2007; Dube et al., 2009).

Nitrogen is a key macromolecule and earth can be conceived as immersed in an ocean of N. However, atmospheric N is not available to plants because of its relatively inert nature under normal temperature and pressure. The process of symbiotic N₂ fixation is of immense importance both from economic and environmental point of view. Industrial fixation of atmospheric N needs great energy inputs and is not environment friendly because of the attendant risk of leakage of ammonia into the atmosphere.

A dramatic change in microbial composition and their activity occur in metal-enriched soil which adversely affects (Khan et al., 2009a; Krujatz et al., 2011) nutrient pool, soil fertility, symbiosis and yield of legumes like *Trifolium pratense* (McGrath et al., 1988), *Cicer arietinum* (Hayat, 2011; Wani et al., 2008b; Wani and Khan, 2010), *Vigna radiata* (Wani et al., 2007a) and *Pisum sativum* (Wani et al., 2008a). There are numerous reports where alleviated Cd level limits the rhizobial growth, their host legume, the total soluble protein content and N fixation to concomitantly reduce the crop yield (Moftah, 2000; Broos et al, 2005; Figueira et al., 2005). Mechanisms of HM tolerance in *Rhizobium* are diverse and may involve energy-dependent efflux of the metal (Grass et al., 2000; Muson et al., 2000; Franke et al., 2001; Saltikov and Olson, 2002), precipitation of the metals as insoluble salts (Blake et al., 1993), alteration of membrane permeability for the metal (Levine and Marzluf, 1989), immobilization of the metal within the cell wall (Cervantes and Gutierrez-corona, 1994), production of chelating agents (Silver and Phung, 1996) biochemical transformation of the metal ions (Williams and Silver, 1984) and immobilization or transformation of metals to make them inactive (Nies, 1992). The synthesis of antioxidant enzymes in the inoculated legumes plays a pivotal role in protecting them from oxidative stress (Figueira et al., 2005; Corticeiro et al., 2006).

Maintenance of a healthy and pollution free environment is a current issue of global importance. The degradation of the soil by chemical fertilizers, fungicides, pesticides and weedicides has a chemophobia among the scientific community, all over the world. Mycorrhiza is a term which designates a symbiotic non-pathogenic relationship between a group of fungi and plant roots. These fungi have emerged as
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Bio-fertilizer, a cheap and environmental friendly alternative to expensive fertilizers (Srivastava et al., 1996) and the greatest advantage given by them is the greater resistance to the nutrient uptake of nutrients such as P, Zn, Cu, Ca, K, Fe, Mg, Mn, Cl and N (Harrier, 2001; Singh and Kapoor, 9) resulting in enhanced plant growth. (Barea, 1991; Tarafdar and Kumar, 1996). Application of these fungi in soil can enhance the plants resistance to biotic and abiotic stresses (Asrar and Elhendi, 2011; Abdel-Fattah et al 2002; Ruiz-Lozano, 3) and reduce other soil stresses such as high salt levels, toxicities of mine spoils and fills and HMs (Garg and Bhandari, 2014). Arbuscular mycorrhizal fungi can enhance plant resistance to Cd stress by improving the plant's nutritional status particularly P uptake and subsequently enhancing its growth (Janousková and likova, 2010; Miransari, 2011a). Symbiosis of this fungi can effectively immobilize HMs through the chitin, free amino, hydroxyl and carboxyl groups present in the wall (Joner et al., 2000; Garg and Chandel, 2010), binding of HM to the hyphae's metal sorption capacity of extraradical mycelium (Gohre and Paszkowski, 2006; g and Bhandari, 2014), precipitation and detoxification in soil matrix (Saraswat Rai, 2011), adsorption of metal on the root surface or its accumulation within the roots (Joner et al., 2000; Garg and Chandel, 2010), dilution of metal by increased root shoot growth (Mohammadi et al., 2011) and chelation by siderophores, allathionines as well as polyphosphate granules (Upadhyaya et al., 2010). Arbuscular mycorrhizal fungi carry genes which improve the capability of ROS scavenging and reduce Cd concentration in plants. In general, several ecological adverse effects of HMs on plants and soil microorganisms have been reported with varying degree of effects on legumes.

The positive synergistic interactions among components of the tripartite ncient association result in improved rates of N2 fixation, phosphorus uptake and p production under conditions of reduced N and P uptake (Azcon et al., 1991; vier and Germida, 2003; Faizan et al., 2004). However, the synergistic or additive ractions among the components of the tripartite association (Rhizobium-AMF-ume) under HM stress have not yet explored.

Considering the importance of legumes in maintaining soil fertility and inflicting reports on the effects of Cd on rhizospheric symbionts, some attentions
have been focused to study the role of AM fungi and Rhizobium in plant metabolism and in the alleviation of Cd stress. Therefore, the present research work was undertaken with the following objectives.

✓ To screen and select legumes based on Cd sensitive grown in soil supplemented with different levels of Cd.
✓ To study the influence of Rhizobium application in the alleviation of Cd-induced effects in Cd least sensitive and Cd most-sensitive legumes.
✓ To study the application of AM fungi in the alleviation of Cd-induced effects in Cd least sensitive and Cd most-sensitive legumes.
✓ To study the influence of dual inoculation of Rhizobium and AM fungi in the alleviation of Cd-induced effects in Cd least sensitive and Cd most-sensitive legumes.