Literature Review

Soil is a complex and dynamic biological system which experiences a remarkable set of transformations over time, as energy, chemical elements and water are processed. Over time, primary minerals are weathered and lost. Although new secondary minerals may be formed during soil development, the soil's primary minerals are decomposed and its acid-neutralizing capacity gradually consumed. Moreover, soil is a structured, heterogeneous and discontinuous system, generally poor in nutrients and energy sources (in comparison with the concentrations optimal for nutrient microbial growth *in vitro*), with microorganisms living in discrete microhabitats. The chemical, physical and biological characteristics of these microhabitats differ in both time and space. The microbial population in soil is very diverse. Bacteria, actinomycetes and fungi are three major groups of soil inhabiting microorganisms. Microorganisms are ubiquitous in nature and form vital components of all known ecosystems on earth. One of the fascinating aspects of microorganisms is that some of them have evolved to thrive under conditions that are too harsh for animals as well as plants. The high microbial diversity is due to their ability to survive and multiply in diverse habitats, including anaerobic and other extreme conditions owing to their metabolic versatility and flexibility to utilize whole substrate as nutrient source. It is important to study microbial diversity not only for basic scientific research, but also to understand the link between diversity in relation to community structure and function. There is now a great interest to search for microorganisms which have potential to be used for sustainable crop production by promoting plant growth and suppressing soil borne plant diseases.

The rhizosphere is the critical interface between biota and geologic environment, fundamentally important to soil formation. The plant roots along with the rhizosphere are networks within the bulk soil, biological hotspots where respiration, gas exchange, nutrient and moisture use, and localized supplies of organic matter are most concentrated. In contrast, the bulk soil is a more oligotrophic environment, especially with respect to supply of root-derived organic matter. More than anything, reactive organic reductants and microbial activity are concentrated near roots compared with the soil system as a whole.

Root systems are symbiotic systems in which cells of plants, fungi, and bacteria are intimately associated, both structurally and functionally. This state of existence sometimes renders difficulty in isolation of plant part from that of the microbe. The fact that fungi and bacteria colonize root tissues in "endorhizospheres" suggests that concepts of continuity rather than those of class may be in order for how we think of the rhizosphere and soil. In place of class concepts of rhizoplane, rhizosphere and bulk soil, a continuum seems much more pertinent between (a) root–microbe system, which includes all cells of plant roots,

mycorrhizal fungi and closely associated non-mycorrhizal fungi and bacteria; (b) rhizosphere surrounding these cells, a volume which is immediately affected by the functioning of the root–microbe system and depends on chemical reaction, chemical element, microorganism, and soil type; and (c) bulk soil, the soil not immediately affected by the active functioning of roots, but which may be transformed by rhizospheres over pedogenic time.

Rhizosphere microflora

The study of rhizosphere in the recent times has gained substantial ground and recognition, owing to escalation of recent advances in study of microbial community. Hiltner (1904) defined "the rhizosphere as the zone of soil immediately adjacent to legume roots that supports high levels of bacterial activity". Wallace (2001), defined it as "the rooting zone of the plants including the root, soil attached to the root and adjacent soil. Ryan and Delhaize (2001) further added " the rhizosphere is a densely populated area in which the roots must compete with the invading root systems of neighboring plant species for space, water, and mineral nutrients, and soil- borne microorganisms, including bacteria, fungi, and insects feeding on an abundant source of organic material". However, the term has further been broadened to include both the volume of soil influenced by the root and the root tissues colonized by micro-organisms (Pinton *et al.*, 2001).The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity (McCully 2005). Rhizosphere can be divided into ecto and endo rhizosphere. The term endorhizosphere is used to describe the multi-layered microenvironment, which includes a mucoid layer on the root surface, the epidermal layer of the root tissue including the root hairs and the cortical cells.

Rhizosphere has been regarded as 'hot spot' for microbial colonization and activity (Bolton *et al.*, 1993). Actively growing roots release organic compounds, such as sloughed off cells, secretions, lysates and exudates, into the rhizosphere. The activity of microbes in the rhizosphere is expected to be higher and qualitatively different in the rhizosphere as compared to microbes in bulk soil. Such root-released products can be highly specific for a given plant species or even a particular cultivar and plants are thought to selectively enrich their rhizospheres for microorganisms that are well adapted to the utilization of specific released organic compounds (Lynch and Whipps, 1990).

The rhizosphere has been investigated with an increasing awareness that the microbiology of this microhabitat is very closely geared to the health of the plant. There has been accumulation of considerable volumes of literature on soil microorganisms. However, a summarized review has been presented in the following paragraph:

Ansari *et al.* (1986) have studied rhizosphere and rhizoplane mycoflora of barley infected with *Ustilago hordei* and discussed about the biochemical changes that occur due to infection. Higher fungal population and higher number of fungal species were encountered in the infected plants in comparison to their healthy counterparts. Gopinath *et al.* (1987) have reported the colonization of *Fusarium sp.* in sorghum seeds and their significance was observed. Thirty high yielding cultivars of sorghum analyzed, showed severe infection of *Fusarium moniliforme, F. oxysporum, F. semitectum* and *F. solani. F. semitectum* infected the embryonic tissue in 93% seeds, while *F. semitectum* and *F. solani* colonized the embryo in 8 and 5% seeds respectively. But *F. oxysporum* did not colonize the embryo.

Shaik and Nusrath (1987) observed *Trichoderma viridi* and *Aspergillus niger* as a part of microflora of wilt resistant cultivar while susceptible cultivar showed a predominance of *Fusarium udum* and *Fusarium spp.* during all the stages of plant growth. Rangaswami (1988) discussed soil plant microbe interrelationships and concluded that the balance between the rhizosphere micro flora and plant pathogen, vis-à-vis, the soil microflora and the plant pathogens are important in host pathogen relationship. Unless the plant pathogen is capable of competing with other soil organisms and penetrating the barrier of mantle consisting of a wide variety and large number of the rhizosphere microorganisms, it could not reach the root surface, and cause infection.

Watanabe (1988) assayed a total of 22 soil samples from various habitats all over Japan and isolated 10-15 *Pythium* spp. from 21-46 samples in the respective districts by trapping methods, mainly with cucumber seeds as a trapping substrate. Pathogenic species were more in cultivated soil than in the uncultivated one. Among 10 identified species of *Pythium, P. aphanidermatum* and *P. deliense* have been predominantly isolated from the plants and soils of the Thailand by Chamswarng and Gesnara (1988). Neweigy *et al.* (1988) investigated antagonism of microbial isolates from rhizosphere of board bean plants against *F. solani* and *Rhizoctonia solani*. Of the 110 fungal isolates tested 13 isolates antagonized *R. solani* and 9 isolates antagonized *F. solani* . Of the 5 efficient fungal antagonists, 3 were of the genus *Trichoderma* and 2 were of the genus *Aspergillus. Bacillus* species and *Streptomyces* species were also found to be antagonistic against *F. solani* and *R. solani*. A total of 116 fungal species were isolated from rhizosphere, rhizoplane and non-rhizosphere zone of four Indian mangrove plants viz. *Rhizophora mucronata, Avicennia officinalis, Heritiera minor* and *Caropa moluccensis* from the Sunderbans, West Bengal. The highest number of fungi were isolated from *Rhiozophora mucronta* while the least from *Carapa moluccensis*. Salt tolerant fungi were also isolated by supplementing the medium with 3% and 6% NaCl (Garg, 1988).

Out of the ninety six individual strains obtained from the cotton rhizosphere which were tested for bio-control of pre-emergence damping off of cotton by *Phythium ultimum,. Pseudomonas fluorescens* strains 3551 and 3580 controlled the disease (Loper, 1988). Certain other strains (2-79 and 13-79) of *P. flurorescens* applied as a seed treatment, protected wheat against take all disease , caused by *Gaeumannomyces garminis* var. *triticii* and increased the yield at an average of 11% (Weller, 1988). Hyphomycetes fungi including species of *Aspergillus, Penicillium* and *Trichoderma* which showed *in-vitro* antagonism against *Phytopthora*, were effective as agents for biological control of *Phytophthora* root rot of *Azalea* and *Citrus*. Bio-contol of *Phytophthora* which causes bark infection of sweet orange seedlings in the green house and of matured lemon tress in the field was also obtained with *Aspergillus flavipes, A ocharcens, A wentii* and *Penicillum fumicolossum* . Kapoor and Kar (1989) have reported on antagonism of *Azotobactor* and *Bacillus* to *Fusarium oxysporum* f. sp. *lycopersici* and concluded that bacterial antagonists (*Bacillus* sp.) showed greater inhibition of tomato wilt pathogen (*F. oxysporium* f. sp. *lycopersici*) and produced antifungal antibiotic in culture.

Khalis *et al.* (1990) have studied the rhizosphere fungi of umbelliferous hosts and discussed it in the relation to its root exudates. Root exudates affected the growth and activity of fungi in rhizosphere. The rhizosphere mycoflora of coriander and arum have been studied in the relation to qualitative composition of root exudates. Khanna *et al.* (1990) have isolated, characterized and screened antagonists of *Pseudomonas tolasii* for biological control and observed that many isolates caused complete suppression in the appearance of blotch symptoms on whole mushroom cap and blocks, when tried in combination with pathogenic *P. tolasii*. One of these biological antagonist, an isolate of *P. fluorescence* biovar 1 proved quite effective in controlling the blotch incidence on *Agaricus bisporus* beds.

Kapoor and Kumar (1991) have studied the temperature effects on the antagonistic activity of fungal and bacterial antagonists against isolates of *Fusarium oxysporyum* and *Fusarium solani.* They reported that fungal and bacterial antagonists expressed clear antagonistic activity in the temperature regions at 20-27^{0}C and 20-25 ^{0}C respectively, but were most effective at lowest temperature (20^{0}C) tested. In general, antagonistic activity decreases with decrease in temperature. *F solani* isolated KHFs -41 and *F. oxisporyum* isolate DF -13 were most sensitive to fungal and bacterial antagonists, respectively. Amewowor *et al.* (1991)

isolated a number of myxomycetes and associated microorganisms from the root zones of the cabbage and broad bean in the field plots. The soil in the field plots had been studied qualitatively throughout a growing seasons and population compared with those in soil, 30 cm distant from the stem bases. All organisms were more abundant in the root zones than in non-root zone of the soil.

Ten bacteria isolated from the soybean (*Glycine max*) rhizosphere and ten isolates from soil were compared with respect to several characteristics that have been suggested as important to successful root colonization. Rhizosphere and soil isolates differed significantly in their ability to move along soybean root (Hozore *et al.*, 1991). Rhizosphere bacteria were able to colonize root segments further from the point of inoculation in greater number than soil bacteria. Rhizosphere and soil bacteria did not differ significantly in rates and extent of growth, in either exudates collected from germinating seeds or inorganic salt solution.

Hee (1991) conducted studies on the selection and identification of antagonist rhizobacteria in relation to controlling soil borne disease of vegetables. 926 isolates of rhizobacteria from 3 different kinds of selective media and 30 isolates of plant pathogenic bacteria were dual cultured with 10 species of important soil borne plant pathogenic fungi, respectively and their antagonism measured by their inhibition zone. The population density of rhizobacteria in the same field was different according to the crop species planted and the isolation frequency of the antagonistic bacteria from the species of plant was also markedly different according to the fields or regions from where the soils were collected for the effective isolation of rhizobacteria, M523 and King's B media were more suitable than D+ medium. Of 926 rhizobacteria isolated from the soils of 22 plant species. 63 isolates were selected to be antagonistic to *Phytophthora capsici* ,54 to *Rhizoctonia solani* and 17 to *Fusarium oxysporum* f.sp. *lycopersici*, respectively. Of these one isolate RB 173 was finally selected as the most effective antagonist to the 9 species of soil borne plant pathogenic fungi and was identified as *Pseudomonus fluorescens*.

Antagonistic fluorescent *Pseudomonus* have frequently been suggested to be important natural antagonist of plant pathogens. These bacteria have been considered very useful, particularly in relation to the microbial suppression of the "take-all" fungus *Gaeumannomyces graminis* var. *tritici* following wheat monoculture and for the management of rice disease. Arya and Mathew (1993) have studied the rhizosphere microflora of pigeon pea and discussed the qualitative and quantitative incidence of microorganisms after solarization. They isolated microorganisms from non-rhizosphere and rhizosphere seeds of 4

different varieties of pigeon pea after mulching with 300 gauge polyethylene sheet for 15 days. *Fusarium udum* a severe pathogen of *Cajanus cajan* causing wilt disease could not be recovered from the non-rhizosphere soil after mulching for 45 days. They suggested that the suitability of soil for a crop depends not only on its chemical and physical properties but also on its microbial population.

Sen and Gupta (1994) have studied the incidence of the Fusarial wilt of *Robinia psedoacacia* L. in relation to effect of some important edaphic factors. The effect of various soil factors, viz., temperature, moisture , pH and type on the incidence for three species of the *Fusarium* viz., *Fusarium oxysporum, F.equiseti* , and *F. semitectum* was studied. The soil temperature between 20-30^{0}C, moisture of 60% and pH 6.0 were most favourable to all *Fusarium* species. Yephit *et al.* (1995) have worked on *Fusarium* wilt in Carnation and discussed the effect of culture resistance on the propagule persisting in soil. They have taken six carnation cultivars with different degrees of resistance to *Fusarium oxysporum* f. sp *dianthi* race 2 which persisted in naturally infected soil in the field and in containers with artificially infertile soil at three inoculum concentrations.

Soil suppressiveness to *Fusarium* wilt and dry root rot legumes induced by incorporation of the cruciferous crop residues were studied by Sharma *et al.* (1995). They reported that continuous cropping of leguminous crops on the same piece of land has made sandy soils conductive to *Fusarium* wilt and dry root rot (*Macrophomina phaseolina*). Soil suppressiveness may be induced by changes in the microflora environment through addition of antagonistic microorganisms on the nutritional amendments. Bhattacharya and Bora (1995) have studied the rhizosphere microflora of the tea in relation to age of the plants. They have collected the rhizosphere soil of 5, 35 and 75 years old tea plants during different seasons. Fungi were found to be dominant in autumn, while bacteria in rainy-summer, and actinomycetes in spring.

A new solid medium has been developed for the enumeration and isolation of soil and rhizosphere microorganisms. This medium, named rhizosphere isolation medium contains glucose and 15 of the 20 common amino acids. The absence of five other amino acids, namely, aspartic acid, asparagine, cysteine, proline, and threonine, inhibits the growth of *Bacillus mycoides*, a commonly encountered bacterium that rapidly spreads on agar media and complicates the isolation and enumeration of other microorganisms. Compared with a

similar medium containing Casamino Acids, rhizosphere isolation medium had half as many colonies of *B. mycoides*, with each colony approximately half the diameter. The two media had similar total numbers of bacterial colonies. Isolates were divided into taxononomic groups, roughly corresponding to species and genus, by fatty acid methyl ester analysis and numerical methods. There were 24 genera and 41 species were found among the isolates from rhizosphere isolation medium, while 19 genera and 35 species were found in the isolates from the medium prepared with Casamino Acids. No major group of bacteria was found to occur only on one medium or on the other, indicating that the five missing amino acids had no great effect on organisms other than *B. mycoides*. This medium may prove useful in soil and rhizosphere studies in which the growth of *B. mycoides* is undesirable (Buyer, 1995).

Pandey *et al.* (2000, a) isolated four antagonistic bacterial isolates, *Bacillus subtilis*, *Bacillus sp.*, *Pseudomonas corrugata* 1 and *P. corrugata* 2, from the rhizosphere of tea plants growing in different geographical locations in India. These were tested as microbial inoculants for hardening of tissue-cultured tea plants raised in the laboratory prior to the transfer to open land. Bacterial inoculations resulted in enhanced survival (up to 100, 96, and 88%), as against 50, 52, and 36% survival observed in the corresponding control plants, in rainy, winter and summer seasons, respectively. Rhizoplane and rhizosphere soil analyses showed that the major biotic factor responsible for mortality following the transfer of tissue culture raised plants to soil was fungal attack (*Fusarium oxysporum*). Bacterial inoculations also resulted in plant growth promotion of tissue culture as well as seed raised plants of tea.

Pandey *et al.* (2000, b) outlined through a detailed study conducted from various tea growing locations in India, that species of *Penicillium* and *Trichoderma* were dominant in the rhizosphere of established tea bushes. *Penicillium erythromellis, P. janthinellum, P. raistrickii, Trichoderma pseudokoningii* and *T. koningii* were found to be closely associated with tea roots. While seasonal fluctuation in occurrence was observed in the case of *Penicillium spp.*, the population of *Trichoderma* spp. showed less variation during the year. Both species were sensitive to low temperature. In general, fungi associated with the tea rhizosphere were found to prefer a mesophilic temperature range (15 °C to 35 °C). The dominant species of *Penicillium* and *Trichoderma* also exhibited tolerance to lower temperatures, i.e., 5 to 10 °C on agar plates. Most fungi were able to grow in a wide range of pH (4 to 12). Lowering of soil pH in the rhizosphere of tea bushes was positively correlated with the age of the bush and may have affected the development of a specific microbial community in the rhizosphere. Pandey and Upadhyay (2000) reported that rhizosphere of

healthy pigeonpea plant was heavily colonised by *Aspergillus niger* and *Penicillum* sp than those diseased by *Fusarium udum*. *A. niger* also showed moderate antagonism and suppression of the pathogen colony. Resident *Trichoderma* and *Gliocladium* was highly antagonistic but none of the bacterial isolates was antagonistic to the pathogen. *T. viride* formed loops, coiled and ruptured the cell wall of the pathogen. Mechanism of parasitism between *F. udum* and *G. virens* resulted in twisting, air bubbling and disintegration of pathogen hyphae while *T. harzianum* caused severe vacuolation, shrinkage and coagulation of cytoplasm of pathogen hyphae. Kallurmath and Rajasab (2000) isolated that two species of *Aspergilli* along with ten other fungi from rhizophere of onion (*Allium cepa* L.) were isolated. Aspergilli in general were dominant, contributing 38.59% to the total rhizosphere mycoflora. Among the isolates *Aspergilli*, *A.niger* and *A.flavus* were comparatively dominant. *A.niger* was particularly most dominant on onion bulbs with the progress of their maturity.

Pandey *et al.* (2001) reported dominant fungi in the rhizosphere of established tea bushes and their interaction with the dominant bacteria under *in situ* conditions. The population of *Penicillium* and *Trichoderma* species were inversely correlated with the population of two most dominant rhizosphere bacteria, *Bacillus subtilis* and *B. mycoides*. Both *Bacillus* species have been shown to have antagonistic activity against these two fungi under *in vitro* condition. The study demonstrated the existence of a similar antagonism under in situ conditions in the rhizosphere of established tea bushes.

Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilizers, biopesticides, phytostimulators and bioremediers. *Pseudomonas*, one of the best root colonizers is therefore used as a model root colonizer. Lugtenberg et al. (2001) focused on (a) the temporal spatial description of root colonizing bacteria as visualized by confocal laser scanning microscopical analysis of autofluorescent microorganisms, and (b) bacterial genes and the traits used for the colonization of root and of animal tissues, indicating the general importance of a study.

Plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* were isolated from the rhizosphere of rice and sugarcane by Kumar *et al.* (2002). Among 40 strains that were confirmed as *Pseudomonas fluorescens*, 18 exhibited strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum*, mainly through the production of

antifungal metabolites. Genotyping of these *P. fluorescens* strains was made by PCR-RAPD analysis, since differentiation by biochemical methods was limited.

Mathur *et al.* (2004) reported that the plant rhizosphere is an important zone where many micro-organisms both friend and foe exits. The microflora associated with a plant rhizosphere is generally influenced by the soil type, pH, temparature and stages of plant growth. Parveen *et. al.* (2004) studied the mode of antagonism of *Trichodrma viride* against *Alternaria triticina* causing leaf blight of wheat. When studied *in vitro* by employing dual culture technique, *Trichoderma viride* as a biocontrol agent inhibited the growth of the pathogen, its mycelial strands coiled around the hyphae of the test pathogen forming a rope like structure and finally disintegrating the test pathogen, *Alternaria triticina.*

Singh and Sindhan (2004) evaluated four antagonists viz. *Trichoderma viride* , *Trichoderma harzianum, Gliocladium virens* and *Aspergillus nidulans* as seed, soil and combined (seed and soil) treatment for the control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycoperssici* in green house. *Trichoderma viride, Trichoderma harzianum* and *Gliocladium virens* as seed treatment @10g/kg seed were effective in controlling seedling mortality up to 85% and were at par with carbendazin. After emergence they provided 100% protection; *A. nidulaus* was least effective. All the antagonist treated plants had longer roots and shoots, and more leaves per plant as compared to plants in control.

Aspergillus niger, Trichoderma harzianum, Trichoderma viride, and *Penicillium aurantiogrisem* a bacterium (B1) and *Bacillus subtilis* were isolated from the rhizosphere (Sharma and Champawat, 2004). Amongst the various rhizospheric microorganisms, *Trichoderma viride* and from rhizoplane microorganisms *Gliocladium virens* and bacterium (B2) proved effective against *Fusarium oxysporium* under experimental condition. They also reported four fungi viz. *Aspergillus niger. Trichoderma harzianum, Trichoderma viride* and *Penicillium aurantiogriseum* and two bacteria viz. bacterium (B1) and *Bacillus subtilis* isolated from rhizosphere. The spore of *F. oxysporum* germinated minimum in association with rhizospheric *Trichoderma viride.* The rhizoplane microorganisms *G. virens* and bacterium (B2) exhibited minimum spore germination of *Fusarium oxysporum.*

Oyeyiola (2009) isolated and identified fungi present in the rhizosphere and rhizoplane of Okra (*Hibiscus esculentus*). The fungi were *Penicillium frequetans, Penicillium oxalicum, Penicillium palitans, Rhizopus stolonifer, Rhizopus oligosporus, Rhizopus oryzae, Aspergillus niger, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus clavatus, Mucor hiemalis, Mucor racemosus, Alternaria herbarum and Alternaria triticina.* Among them *R. stolonifer, A. niger* and *A. clavatus* were predominant in both the rhizosphere soil and the

rhizoplane, while *P. oxalicum* and *A. herbarum* were predominant in the rhizosphere soil only. *Mucor hiemalis, Penicillium frequetans, P. oxalicum, A. clavatus, P. palitans* and *A. triticina* were present in the rhizosphere soil and the rhizoplane, but they were absent from the non-rhizosphere soil. The rhizosphere soil contained a greater spectrum of fungal species than either the rhizoplane or the non-rhizosphere soil. The experimental soil was sandy loam in texture. The rhizosphere effect increased progressively with increase in plant age until the 6th week after seed sowing and then declined.

Mulaw *et al.* (2010) reported that the production of *Coffea arabica* in the southwestern is affected by tracheomycosis caused by a soil-born fungus *Gibberella xylarioides*. The use of endemic antagonistic strains of mycoparasitic *Trichoderma* species would be a nature conserving means to combat this disease. They have used molecular methods to reveal that the community of *Trichoderma* in the rhizosphere of *C. arabica* in its native forests, is highly diverse and includes many putatively endemic species. (rRNA) gene fragments.

AM Fungi

Arbuscular Mycorrhizal fungi (AMF) are most ubiquitous in terrestrial ecosystems and form mutualistic relationship with more than 80 percent of major group of vascular plants. An apparently low taxonomic diversity coupled with a broad geographic range had led to the view that AMF are a rather homogenous group, both functionally and morphologically. It is routinely possible, however, to find 10-30 spore types in the soil at a single site. This high local diversity against a background of low global diversity is paradoxical if all fungi are equally capable of colonizing all plants. The apparent high genetic diversity of AMF is similarly paradoxical in relation to the low morphological diversity. Improved methods of isolation from soil, and of microscopy and molecular analysis have shown that there may be substantial diversity present in field soils, but the relationship between morphological and molecular diversity is still unclear.

Root colonization with AMF is a dynamic process, which is influenced by several edaphic factors such as nutrient status of soil, seasons, VAM strains, soil temperature, soil pH, host cultivar susceptibility to VAM colonization and feeder root condition. There has been growing appreciation of the importance of plant and fungal interaction especially AMF on terrestrial ecosystem (Giovannetti *et al.* 2006; Rodrigues, 2008). Mycorrhiza form critical link between the plant and soil structure and make a large direct contribution to soil fertility and quality through contribution of soil organic matter. The assumed primary benefit to plants of the Mycorrhizal symbiosis is an increased uptake of immobile nutrients, especially

phosphorus that are mobilised by the fungus. However, there is increasing evidence that AMF have a range of other effects, for example, protection against plant parasites (Aggarwal *et al.* 2006 a; Bhargava *et al.* 2008) , water stress tolerance (Newsham *et al.*1995) alleviation of salt stress (Evelin *et al.* 2009) and in sustainable maintenance of plant health and soil fertility (Wright and Upadyaya, 1998; Jeffries *et al.* 2003 a). This evidence of multiple functions, host selectively (or non- random distribution) and higher diversity than is apparent from cultures and morphology leads us to conclude that communities of AMF are much more diverse than previously thought. At the community level, recent experiments have shown that AMF are major players and may influence plant species diversity (van der Heijden *et al.* 1998; Klironomos *et al.*2000) and recruitment of plant species into the population (Kiers *et al.* 2000). Colonisation of host plants by AMF exhibits significant spatial and temporal heterogeneity (Merryweather and Fitter, 1998 ; Helgason *et al.*1999). High spore diversity has been found in the field (Bever *et al.* 1996; Eom *et al.* 2000) and the number of spores produced by each AM fungus may be host- dependant (Sanders and Fitter, 1992 ; Bever *et al.*1996).Traditionally , AMF community diversity has been measured using spore counts from field soils, but such counts are a measure of the sporulation activity of the fungi rather than a direct measurement of diversity, and maybe highly variable and almost impossible to correlate among habitat types (Morton *et al.*1995). Molecular genetic analysis provides a way around this obstacle as it has the potential to identify objectively that taxa present in the roots of the plants.

The plant provides the fungal partner with carbon and the fungus improves the plant nutrient uptake from the soil. These fungi have long been considered obligate symbionts with plants, since growing the AM fungus without a host plant has not been possible. Isolated spores can germinate and produce hyphae, but they die if no host root is found. This view has recently been challenged by an experimental study showing that AMF can grow and form spores *in vitro*, if provided with a carbon source and stimulated by particular bacterial strains (Hildebrandt *et al.*, 2006). Whether this can also occur in nature is not yet known. Spores are asexual, multinucleate and are produced directly by the mycelium, either inside or outside the root. In some species, small sporocarps can be produced, where several spores are surrounded by a peridium-like structure. Typical glomeromycotan spores are globose, relatively big (40-800 μm) and with a multilayered wall that can be smooth or ornamented. Evidence of sexual reproduction has not been reported so far in the Glomeromycota. The hyphae lack septa (cross walls between hyphal cells) and can grow both outside (extraradical) and inside the roots (intraradical). This coenocytic structure allows the nuclei to move along the hyphae

(Bago *et al.*, 1999). The intraradical mycelium typically produces highly branched structures called arbuscules, inside the cortical cells of roots (Arum-mycorrhizal type). In some other cases, hyphal coils are formed instead (Paris-mycorrhizal type). The variability of structures along this Arum–Paris continuum has ecological, functional and taxonomic significance that are not fully understood yet (Dickson *et al*, 2007). Many species of Glomeromycota also form large intraradical, globose, storage cells called vesicles. Because of this, glomeromycotan fungi are sometimes also referred to as vesicular-arbuscular mycorrhizal fungi (VAM). Glomeromycotan fungi do not disseminate solely by spores. New plant hosts can be colonised from hyphal fragments present in the soil or growing from colonised root fragments. Alternatively, roots can be colonised by extraradical mycelium extending from a previously established mycorrhiza. The latter may give rise to an extensive mycelial network connecting the root systems of several plants from the same or different species. Nonetheless, the relative importance of the two dispersal methods and the establishment of hyphal networks in nature have not been evaluated. There are some widespread and frequent AM species detected in molecular studies, without any known stage of spore formation. This suggests that there might be species that rarely or never sporulate.

The phylum Glomeromycota was established as a monophyletic group, distinct from the Zygomycota in which they were previously placed. Phylogenetic studies based on molecular data placed the Glomeromycota as a sister group to the Basidio- and Ascomycota (Lutzoni *et al.*, 2004, James *et al.*, 2006). Traditional taxonomy in the Glomeromycota has mainly been based in spore morphology and ontogeny. The structures and characters of the mycelia, e.g. arbuscules, vesicles, coils are of exiguous taxonomical value. Fewer than 200 species, grouped in eleven genera, are described. Most of them have been described after being isolated and grown in pot cultures using a handful of host plant species. Glomeromycotan fungi produce relatively large (40–800 mm) spores with layered walls, containing several hundreds to thousands of nuclei (Becard and Pfeffer 1993). Spores may be formed singly, in clusters or aggregated in so-called sporocarps (Gerdemann and Trappe 1974).

Different functional groups of bacteria such as N_2-fixing bacteria (Secilia and Bagyaraj, 1987), plant growth-promoting rhizobacteria, phosphate-solubilising bacteria (Toro *et al.*, 1996) and antagonists of plant pathogens (Citernesi *et al.*, 1996; Budi *et al.*, 1999) have been reported to be associated with the rhizosphere of different plants colonised by AMF. Some bacteria have also been found to be associated with AM fungal structures such as external hyphae (Toljander *et al.*, 2006) and spore or spore walls (Xavier and Germida, 2003; Roesti

et al., 2005). Bacteria have also been reported to live inside the spores of certain AM fungal isolates (Bianciotto *et al.*, 2003).

Mycorrhizal contribution to agri-ecosystem

Pot experiments were conducted by Louis (1990) to determine the phosphorus response curves of inoculated and uninoculated mungbeans and rice. Two soil types, Luisiana clay and macolod clay loam were used for mungbeans and only Macolod clay loam was used for rice. Phosphorus levels were set at ½, ½, 1, 2x and 4x the recommended rate. In Louisiana clay, mungbean inoculated with mycorrhiza had a better response to P application compared with uninoculated ones. When soil was un-amended with P no significant difference between uninoculated and inoculated plants in all the fermentors used was observed. However, uninoculated plants died two months after sowing, while inoculated plants survived until the maturity stage. Differences on growth improvement attributed to mycorrhiza depended on the amount of P added and the species of VA endophytes used. Uninoculated plants had no response to increasing addition of P . In Macolod clay loam, it was noticeable that inoculated mungbeans were more vigorous, green, and healthier in appearance compared with uninoculated ones. However, the quantitative differences in all the parameters used were statistically insignificant. Both inoculated and uninoculated plants increased similarly in height biomass and pod yield with increasing additions of P. Rice inoculated with mycorrhizae, regardless of VA endophytes, had a better response to P application compared with uninoculated ones when grown in Macolod clay loam. Significant differences among treatments were observed with regard to mean height and biomass of rice. The same trend was observed with regard to group weight. The differences within treatments. At lowest level of P applied, *Gigaspora* sp. and *G. mosseae* were already highly effective as shown by the increase in al parameters used. Optimum growth reasons of uninoculated plants was obtained when P added was at recommended rates and further additions had only slight effect on mean height and no effect on biomass.

Communities of vesicular arbuscular (VAM) mycorrhizal fungi was studied by Johnson *et. al.* (1991) in a along term crop rotation experiment at two location. Spores of mycorohizal fungi were counted and identified in experimental plots with a cropping history of either corn (zea-mays) or Soybean (*Glycine max).* Mycorrhizal fungi communities were affected by both location and cropping history, at Waseca, *Glomus aggregatum.* Schonbeckek & Smith, *G. leptotichum* Schenek & and Smith & *G. occultum* walker spores were more abundant in the soil with a corn history than a soybean history. Densities of *G. aggretatum*

spores were negatively correlated with soil pH at Waseea, but were unrelated to pH at Lamberton where the mean soil pH was lower. Our results indicate that mycorrhiza fungal species are individualistic in their response to cropping history and edaphic factor.

Influence of inoculation with Vesicular-Arbuscualr-mycorhizal fungi on the growth of Asparagus *(Asparagus officienalis)* seedling was examined by Mizonobell *et. al.* (1991) using *Glomus* spp. '301' and '401' which were bred by Kyowa Kakko Kogyo Co. Ltd. Difference in shoot length began to appear clearly between mycorrhizal fungus inoculated and uninoculated seedling 60 days after the inoculation, and higher density (100 spore) of *Glomus* spp. '301' spores gave a higher mycorryzal infection level and made shoots longer. It was revealed that both *Glomus* spp. '30' & '401' had an effect on the growth of asparagus seedlings & *Glomus* spp. '401' promoted the seedling growth more intensively than '301' VAM infection was observed only in the feeder root. In a VAM infected seedling, the number and thickness of storage roots increased, where as feeder roots were shortened.

Screening for the best species of AMF associated with mahogany was done. Growth of host plants at four months significantly increased as a result of AMF inoculation Mahogany seedling inoculated with a mixture *Glomus* inoculated with a mixture of *Glomus etunicatum* and *Glomus mosseae* produced the highest biomas. Availability of soil moisture proved to be the greatest factor in influencing plant growth while AMF inoculation could increase nutrient and water uptake of mahogany seedling resulting to higher growth rates.

AM fungi are an intimate link between the roots of most plants and soils, thereby affecting the development of host plants and host soils. The role of VAM fungi in improving plant nutrition and their interactions with other soil biota have been investigated with reference to host plant growth, but little is known about how these interactions affect soil structure. The impact of cultural practices and the particular role that AMF play in improving soil structure are discussed by Schreiner and Bethlenfalvay (1995) in the context of sustainable farming. Natural mycorrhyzal potential has been carried out in a representative area of a decertified semi arid ecosystem in the southeast of Spain. Many indigenous plants from the field site were mycorrhizal, including the dominat *Anthyllis cytisoides,* which had high levels of colonization by arbuscular mycorrhizal fungi (AMF). Low numbers of AMF spore were present in the soil, although a range of species, including *Scutellospora calospora, Glomus coronatum, Glomus constricim,* and several *Acaulospora* species, was represented.

Crop nutrition and Plant growth

The relationship between the development of arbuscular mycorrhizas and increased growth and nutrition of the host was recognized by Asai as early as 1944. Pioneering work on the potential of mycorrhizas in plant nutrition was carried out by Mosse (1957) on apples, Baylis (1967) on *Griselinia* and other New Zealand plants and Gerdemann (1965) on *Liquidambar* and maize. McGonigle (1988) evaluated seventy-eight field trials with AM fungi and found that inoculation increased yield by about 37%. Though at that time, it was not clear whether the observed increase in yield could be correlated to increased nutrient uptake facilitated by AM fungi, later developments have shown that indeed, AM fungi increase yield through improved nutrition Since roots of most plants are colonized by mycorrhizas, unequivocal proof that AM colonization increases nutrient uptake has been difficult to obtain. Nevertheless, the role of AM fungi in increasing nutrient uptake and plant growth has now been documented. It is now established that even when there is no increase in nutrient uptake efficiency in tissue concentrations or in total plant nutrient content, the fungal partner can make a significant contribution to nutrient uptake.

The development of research on roles of AM fungi in nutrient uptake has been closely linked to the knowledge of soil chemistry, particularly in relation to pools and availability of phosphorus (Van Aarle, 2009). The amount and form of phosphorus in soil and the factors affecting its availability are important in determining the ways in which AM fungi influence uptake by plants (Comerford, 1998). Phosphorus, one of the most essential elements of plants, is not easily available from soil because, in spite of being present in relatively large amounts, much of it is insoluble. Indirect evidence that AM roots can be more efficient in nutrient uptake than non-mycorrhizal roots came from the observation that responsive AM plants are both larger and contain higher concentrations of P in their tissues than uncolonized controls. The explanation first suggested was that AM colonization increased efficiency of absorption by roots. However, this is not the only possible explanation for the increases. Increased total root length or efficiency in AM plants would certainly contribute to increased total uptake, but would not necessarily lead to elevated tissue concentration. If growth keeps pace with P upake, tissue concentrations remain constant, for they are dependent on the relative rates of uptake and growth. It is not known exactly what mechanisms are involved by AM fungi in the P uptake of inorganic and organic P. External phosphatase can make organically bound P available for uptake. It is however not believed that a large amount of phosphatases is excreted by AM fungal extraradical mycelium (Olsson *et.al*, 2002). The

uptake of P by AM fungi must be an active process since the difference in electrochemical potential between soil and extraradical mycelium is large and since the inorganic P concentration inside the mycelium is high compared with the soil solution. It is generally accepted that the uptake of P takes place partly via proton cotransport, which is driven by a membrane-bound proton-ATPase (Becard *et.al*, 2004). Proton-ATPases were found to be expressed in the extraradical mycelium of two *Glomus* species, indicating that P uptake is mediated by a high-affinity transporter of which the functioning depends on the electrochemical gradient of proton generated by a plasma membrane proton-ATPase (Ferrol *et al*, 2002).

Elevated concentrations may also result from increased carbon AM plants, leading to C-limitation and 'luxury' accumulation of P (Smith and Read, 2008). More direct evidence of increased efficiency of P absorption was obtained by expression uptake on the basis of amount of absorbing tissue. The first demonstration of increased inflow of P in AM roots was in highly responsive *Allium cepa* colonized by *Glomus* sp. (Sanders and Tinker, 1971, 1973). They calculated that AM fungi contributed about 70% of the P absorbed by the AM plants. Direct confirmation of the role of AM fungi in improved plant nutrition was obtained by Cavagnaro *et al.* (2006), who, using mutant tomato with reduced AM colonization, showed that AM fungal populations not only contribute to nutrition, but also enhance food quality in terms of nutrient densities. Effects of AM fungi on nutritional value of crops deserve particular attention particularly in the light of concerns that use of highly purified fertilizers and other modern agricultural practices are reducing micronutrient densities below those required for human health (Welch, 2002; Welch and Graham, 2002). Another area of current investigation is to explore the probability of AM fungi increasing beneficial compounds in the plants like antioxidants.

Other than P, evidence is also now accumulating that the AM pathway makes considerable contribution to plant N uptake from soil, regardless of total N uptake and N responses. In most cases, improved nodulation and N_2 fixation in AM plants appears to be the result of relief from P stress and possibly uptake of some essential micronutrients, which result in both a general improvement in growth and indirect effects upon the N_2 fixing system. Experiments using ^{15}N have shown that hyphal N transfer occurs between AM fungi and host roots (Hawkins *et al.* 2000; Mader *et al.* 2000). However, the translocation of N has not been correlated with increased plant N content or growth. There is also evidence now that the efficiency of Zn and Cu is increased in AM plants. Further, the interactions between AM colonization and accumulation of heavy metals and other toxic elements is an area of

considerable interest in relation to both production of safe food and bioremediation programmes.

In order to enhance the sustainability of agro-ecosystems, there is a shift towards low-input instead of conventional high-input agricultural systems. In these low-input systems the Mycorrhizal symbiosis is regaining its importance. This can lead to better fungal development in soil, higher root colonization, and an enhanced nutrient uptake.

Plant protection by AMF

Plants and pathogens interact with a wide variety of organisms throughout their lifecycle. These interactions can significantly affect plant health in various ways. The types of interactions were referred to as mutualism, protocooperation, commensalism, neutralism, competition, amensalism, parasitism, and predation. While the terminology was developed for macroecology, examples of all of these types of interactions can be found in the natural world at both the macroscopic and microscopic level. And, because the development of plant diseases involves both plants and microbes, the interactions that lead to biological control may take place at multiple levels of scale. From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. Mutualism is an association between two or more species where both species derive benefit. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact, such as those between plants and mycorrhizal fungi.

Different hypothesis have been proposed to explain bioprotection by AM fungi. These include (a) improvement of plant nutrition and root biomass in Mycorrhizal plants, which could contribute to an increased plant tolerance and compensate for root damage caused by a pathogen, (b) changes in root system morphology, (c) modification of antagonistic microbial populations in the mycorrhizosphere, and (d) competition between AM fungi and pathogenic fungi to colonize root tissues, with the possible induction of resistance mechanisms.

Many of the microbes isolated and classified as BCAs can be considered facultative mutualists involved in protocooperation, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions. Further down the spectrum, commensalism is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited. Most plant-associated microbes are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in overtly positive or negative consequences to the plant. And, while their presence may present a

variety of challenges to an infecting pathogen, an absence of measurable decrease in pathogen infection or disease severity is indicative of commensal interactions. Neutralism describes the biological interactions when the population density of one species has absolutely no effect whatsoever on the other. Related to biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism. In contrast, antagonism between organisms results in a negative outcome for one or both. Competition within and between species results in decreased growth, activity and/or fecundity of the interacting organisms. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant. Direct interactions that benefit one population at the expense of another also affect our understanding of biological control. Parasitism is a symbiosis in which two phylogenetically unrelated organisms coexist over a prolonged period of time. In this type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defense systems. Lastly, predation refers to the hunting and killing of one organism by another for consumption and sustenance. While the term predator typically refer to animals that feed at higher trophic levels in the macroscopic world, it has also been applied to the actions of microbes, e.g. protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance. Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualisms between microbes and their plant hosts or from manipulating antagonisms between microbes and pathogens.

The role of Mycorrhizal fungi in control of various soil borne plant diseases has been reviewed by many workers (Jalali and Jalali, 1991; Jeffries *et al*, 2003, Aggarwal *et al*, 2006; Sharma *et al*, 2009). Besides, the association of VAM fungi with plant nematodes and the beneficial effect of mycorrhizal symbiosis on plant growth had led to investigations into the potential of VAM fungi to limit yield losses due to nematodes (Bhargava *et al*, 2008).

Soil-borne pathogens causing particularly wilts and root-rots, are responsive to differential soil conditions including dynamics of microbial activity in the zone of root influence. V-A mycorrhizal colonization have been shown to promote or inhibit the development of plant diseases, either by marked shifts in the rhizosphere environment to the benefit or detriment of potential pathogen, and /or alternation of the host plant to benefit or hinder, pathogen's progression and development.

It has been demonstrated that changes in the soil environment, particularly in terms of its water and nutrient status, are able to appreciably affect the germination, growth, development and pathogenic behaviour of many soil microorganisms. In pathogenic fungi, quiescence of spores, mycelial lysis, and formation of resistant structures can be induced by microbial competition for nutrient and even for space. And the exogenous addition of nutrients can reverse the impact of such competition.

Numerous studies have clearly shown that mycorrhizal root system are less susceptible to the attack of soil pathogens than non-mycorrhizal systems. Becker and Gerdemann (1977) reported that roots of onion were less susceptible to *Pyrenophore terrestris*, casual agent of pink root disease. In fact, those segments of root system which have mycorrhizal colonization were observed to be directly proportional to disease resistance. Jalali (1988) demonstrated that mycorrhizal inoculations resulted in significant reduction in the host infection by *Fusarium* and *Rhizoctonia.* Drastic growth suppression of *Fusarium oxysporum* f.sp. *ciceri* in chickpea was observed when subjected to soil-inoculation with *Glomus* sp. Similar response was observed on *Rhizotonia solani* when speed-pelleting with sporocarps of the mycorrhizal endophyte was done. Baltruschat and Schonbeck (1975) reported significant reduction in the number of chalmydospores of the root-rotting fungus *Thielaviopsis basicola* on mycorrhizal tobacco roots than non-mycorrhizal ones. On the other hand, multiplication of tomato aucuba mosaic virus increased when tomato plants were subjected to mycorrhizal inoculation. The mycorrhizal colonization alerts the host metabolism, which may result in an increase or decrease in host resistance. Certain chemical, physiological and morphooogical alterations in the host plant are known to be induced by the mycorrhizal infection some of which may be correlated to the alerted host resistance / susceptibility. It is, however, an important feature that mycorrhiza strengthen the cell walls by increasing lignification and the production of other polysaccharides. In such tissue the growth of the pathogen is likely to be suppressed, as was shown with *Fusarium oxysporum*. A stronger vascular system increases the flow of nutrients, confer greater mechanical strength and inhibit effects from potential

pathogen.Vesicular-arbuscular fungus reduces *Rhizoctonia* disease incidence. VAM fungus, *Glomus* sp. was isolated from field with rice-mungbean-corn cropping pattern. It was used to challenge the pathogen *Rhizoctonia solani* on rice, corn and mungbean in pot experiment. *Glomus* sp. was unable to control *R. solani* in mungbean as the pathogen caused damping-off in seedlings a few days after sowing and before the mycorrhizal fungus could establish itself in the soil and roots. On rice, however, sheath blight incidence was reduced by about 30% when the VAM fungus was added to steamed soil at seedling stage or when the sclerotia of the pathogen were added a month after seedling germination. Disease incidence was reduced even (45%) when both *Glomus* and *R.solani* were added to the soil seeding time. With corn grown in steamed soil, the presence of the VAM fungus reduced disease incidence from 66.6% to 8.3% and in natural field soil, from 16.6% to no disease occurrence.

Aspergillus niger (mycoparasite) and VA endophytes jointly as well as individually significantly suppressed occurrence of *Rhizoctonia solani.* The positive influence of endomycorrhizal fungi (possibly *Glomus mosseae*) on the excessive root proliferation in the maize seedlings in *in situ* experiments was established. Evidence was documented for the secretion of growth promoting metabolites like IAA and kinetin on associative growth and development of maize roots and fungal symbionts. Such a study under controlled conditions with micro propagated plants, allowed identification of promoters involved and accurate determination of which step in the rooting process is blocked in recalcitrant crops (Verma,1995). The arbuscular mycorrhiza of *Gigaspora margarita* and transformed Ri T-DNA carrot root was formed by dual culture technology. Some morphological attributes i.e. infection and distribution of hyphae in root, bidirectional movement of hyphal cytoplasm flow, the formation of extrametrical auxiliary cells, hyphal wound healing and spore formation, development and maturity were investigated and the physiological significance in relation to these morphological characteristics was discussed. The structure in symbiosis affected nutrient absorption and translation in plants.

Jalali and Jalali (1991) showed that suppressive effect had direct correlation with the ability of the mycorrhizal symbiont to develop in the absence of available soil phosphate. Soil conditions favourable for V-A mycorrhizal colonization were observed to be not conducive for the growth of test pathogens. High chitinase activity of the mycorrhizal tissue may restrict the growth of the root pathogen in the host. The inhibition of chlamydospore production of *Thielaviopsis basicola* is also found to be due to an increased level of arginine in mycorrhizal roots. In studies of colonization patterns of tomato roots by the Mycorrhizal fungus *Glomus*

mosseae and the pathogen *Phytophthora parasitica*, Cordier *et. al* (1998) showed that proliferation of the pathogen is greatly reduced in Mycorrhizal root system of tomato, compared with non mycorhhizal ones. Moreover, they observed that the host cells containing typical haustoria-like arbuscules structures of the Mycorrhizal fungus were not infected by *P. parasitica* and that pathogen proliferation was reduced not only in mycorrhizal parts but also in nonmycorrhizal parts of mycorrhizal root systems. Cytomolecular phenomena underlying bioprotection against *P. parasitica* in *G. mosseae*-colonized root systems of tomato has been demonstrated.

Various mechanisms also allow VAM fungi to increase a plant's stress tolerance. This includes the intricate network of fungal hyphae around the roots which block pathogen infections. Inoculation of apple-tree seedlings with the VAM fungi *Glomus fasciculatum* and *G. macrocarpum* suppressed apple replant disease caused by phytotoxic myxomycetes . VAM fungi protect the host plant against root-infecting pathogenic bacteria. The damage due to *Pseudomonas syringae* on tomato may be significantly reduced when the plants are well colonized by mycorrhizae (Garcia-Garrido and Ocampo 1989). The mechanisms involved in these interactions include physical protection, chemical interactions and indirect effects. The other mechanisms employed by VAM fungi to indirectly suppress plant pathogens include enhanced nutrition to plants; morphological changes in the root by increased lignification; changes in the chemical composition of the plant tissues like antifungal chitinase, isoflavonoids, etc. alleviation of abiotic stress and changes in the microbial composition in the mycorrhizosphere (Linderman, 1994).

Effectiveness of arbuscular mycorrhizal fungi in the protection of common bean plant (*Phaseolus vulgaris* L.) against Fusarium root rot disease was investigated by Al-Askar and Rashad (2010) under natural conditions in pot experiment. A mixture of arbuscular mycorrhizal fungi consisting of propagated units of *Glomus mosseae*, *Glomus intraradices*, *Glomus clarum*, *Gigaspora gigantea* and *Gigaspora margarita* in suspension form (10^6 unit L^{-1} in concentration) was used at dilution of 5 ml L^{-1} water. The obtained results demonstrated that, arbuscular mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected bean plants. On the other hand, mycorrhizal colonization significantly increased the tested growth parameters and mineral nutrient concentrations. While, infection with *Fusarium* root rot disease negatively affected the mycorrhizal colonization level in bean roots. Finally, mycorrhizal colonization led to a significant increase in the phenolic content and the activities of the investigated defense

related enzymes (phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase enzyme). From the obtained results, it can be concluded that the application of arbuscular mycorrhizal fungi as a biocontrol agent played an important role in plant resistance and exhibit greater potential to protect bean plants against the infection with *F. solani*. In studies of colonization patterns of tomato roots by the Mycorrhizal fungus *Glomus mosseae* and the pathogen *Phytophthora parasitica*.

Mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected bean plants. These results are in agreement with that of Dar *et al.* (1997), who found that inoculation of common bean plants with *Glomus mosseae* decreased root rot by 34 to 77%. Many authors have reported that the AM colonization can reduce root disease caused by several soil borne pathogens (Abdalla and Abdel-Fattah, 2000;Yao *et al.*, 2002; Chandanie *et al.*, 2009). Among the potential mechanisms involved in the resistance of mycorrhizal systems, the induction of plant defenses is the most controversial (Wehner *et al.*, 2009). Where a number of biochemical and physiological changes has been associated with mycorrhizal colonization.

Alteration in isoenzymatic patterns and biochemical properties of some defense-related enzymes such as chitinases, chitosanases and β-1,3-glucanases have previously been shown during mycorrhizal colonization (Pozo *et al.*, 2002). These hydrolytic enzymes are believed to have a role in defense against invading fungal pathogens because of their potential to hydrolyze fungal cell wall. Stimulating the host roots to produce and accumulate sufficient concentrations of metabolites which impart resistance to the host tissue against pathogen invasion have been reported also (El-Khallal, 2007).

Direct (via interference competition, including chemical interactions) and indirect (via exploitation competition) interactions have been suggested as mechanisms by which AM fungi can reduce the abundance of pathogenic fungi in roots. These have generally been proposed in response to observations of negative correlations in the abundance of AM fungal structures and pathogenic microorganisms in roots (Filion *et al.*, 2003).

Presumably, pathogenic and AM fungi exploit common resources within the root, including infection sites, space and photosynthates within the root (Whipps, 2004). Interference competition may also arise if carbon availability within intercellular spaces and the rhizosphere (Graham, 2001) or the number of infection loci within the root system (Vigo *et al.*, 2000) is reduced as a result of AM fungal colonization. Moreover, increasing the richness

of AM fungal taxa colonizing the root system may result in more intense competition with a pathogenic fungus.

VAM Fungi in biocontrol of fungal pathogens

VAM fungi	Host Plant	Disease	Pathogen	Reference
G. fasciculatum	Tomato	Wilt	*Fusarium oxysporum* f.sp. *lycopersici*	Caron *et al.* 1986
G. fasciculatum	Green gram Black gram Chick pea Sugar cane	Root rot Wilt	*Macrophomina phaseolina* *Fusarium moniliforme and Cephalosporium sacchari*	Kheri and Chandra 1990 ; Jalali *et al.* 1990 : Chandra *et al.* 2000
G. mosseae	Tomato	Blight	*Phytophthora parasitica*	Cordier *et al,* 1998
G.mosseae	Tomato and Egg plant seedlings	Wilt	*Verticillium dahliae*	Karagiannidis *et al.* 2002
G.mosseae *G.intraradices* *G.clarum* *G.aiganlea* *Gigaspora margarita*	Common bean plant	Rot	*Fusarium solani*	Al.Askar and Rashid, 2010
G. fasciculatum	Pea	Root rot	*Aphanomyces enteiches*	Vidyasekharan, 1990
Gigaspora	Pigeon pea	Pegion pea blight	*Phytophthora drechsleri* f.sp.*cajani*	Bisht *et al.* 1985
G. geosporum *G. mosseae*	*Dalbergia sissoo*	Wilt	*Fusarium solani*	Singh *et al.* 2000
G. mosseae *G. fasciculatum*	Soybean	Blight	*Phytophthora megasperma* var.*sojae*	Graham and Menge, 1982
G. mosseae	Poinsettia	Rot Damping off	*Rhizoctonia solani* *Pythium ultimum*	Graham and Menge, 1982

Another group of rhizosphere influencing root-microbe interaction are the plant growth promoting Rhizobacteria (PGPR) which describe soil bacteria that colonise the roots of plants flowing inoculation onto seed and that enhance plant health. PGPR are known to participate ecosystem processes including biocontrol of pathogens. *Pseudomonas* and *Bacillus* are the

genera most commonly known as PGPR, but many other genera also exhibit PGPR activities (Chakraborty *et al.* 2006). Microbial populations in the rhizosphere where a typical response exterted by a kind of bacterial population called " mycorrhiza-helper bacteria (MHB) known to stimulate mycelia growth/ formation of AMF and secretes compound responsible for enhanced root exudation. This in turn stimulates AMF mycelia in the rhizosphere. The bacteria have been found adhering to the AMF hyphae as well as embedded within the spore walls. Bacteria adhering to AMF mycelium may utilize hyphal exudates or use mycelium as vehicle for colonization of rhizosphere.

The behaviour of target and non-target microorganisms at the soil-root interface is largely mediated by factors which manipulate host physiology, and the quantitative and qualitative nature of root exudates. Among the many factors, pesticide applications are able to induce substantial changes in the zone of root influence. Not only are pathogens affected, but changes in the function of mycorrhizal fungi are expected to exert measurable influence on the spectrum of root exudation, as well as the mineral nutrition of host plant favouring a result potential for predisposition to disease.

Synergistic effects of bacteria and mycorrhizal fungi been studied with respect to their combined beneficial impacts on plants. Both ectomycorrhizal (Garbaye, 1994) and endomycorrhizal (Meyer and Linderman, 1986) fungi can interact with different bacterial species. These interactions occur in the zone of soil surrounding the roots and fungal hyphae; commonly referred to as the 'mycorrhizosphere'. The interactions between bacteria and AM fungi have potentially beneficial functions, including the majority of those where PGPR (Meyer and Linderman, 1986; von Alten *et al.*, 1993; Kloepper, 1978) including N_2-fixing bacteria (Secilia and Bagyaraj, 1987) are involved. Plant growth-promoting rhizobacteria are usually in contact with the root surface, or rhizoplane, and increase plant yield by one or more mechanisms such as improved mineral nutrition, disease suppression, or phytohormone production (Weller, 1988; Lugtenberg *et al.*, 2001; Broek and Vanderleyden, 1995). An additional possibility is that the beneficial effects of some PGPR bacteria are due to their interactions with AM fungi. Some reports have shown that PGPR have a strong stimulatory impact on the growth of AM fungi (Linderman, 1997). For example, increased mycelia growth from Glomus mosseae spores caused by an unidentified PGPR has been reported by Azcon (1992). These results suggest that selected PGPR and AM fungi could be co inoculated to optimize the formation and functioning of the AM symbiosis.

Apart from having effects on AM fungal growth, PGPR have been suggested to possess a variety of other direct mechanisms to support the mycorrhizal symbiosis. Garbaye (1994) proposed the term 'mycorrhization helper bacteria' for rhizobacteria that increased the ability of the root to establish symbiotic interactions with ectomycorrhizal fungi. He suggested a number of possible mechanisms for the helper effect, including stimulation of root development, enhanced susceptibility of the root to ectomycorrhizal fungal colonization, or enhancement of the recognition process between root and fungus. Several reports have also demonstrated enhanced AM fungal colonization levels in roots in the presence of PGPR. For example, association of *Pseudomonas putida* with indigenous AM fungi resulted in a clear growth enhancement of clover plants (Meyer and Linderman, 1986), Some PGPR may have properties that support both mycorrhizal establishment and function, supporting the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms.

Specific interactions between AM fungi and PGPR most likely occur, and certain groups of bacteria have been shown to be established to a much higher extent in the mycorrhizosphere compared with other groups. This was shown, for example, by Andrade and colleagues (1997) who found that bacteria of the genera Arthrobacter and Bacillus were most frequent in the hyphosphere, the zone of soil surrounding individual AM fungal hyphae, whereas *Pseudomonas* spp. were most abundant in the rhizosphere of *Sorghum bicolor*. This study and others (Artursson *et al* ., 2005) suggest that Gram-positive bacteria may be more commonly associated with AM fungi than Gram-negative bacteria, but this possibility needs to be more rigorously confirmed. It is noteworthy, however, that the bacterial groups most commonly reported to interact synergistically with AM fungi are mainly Gram-positive bacteria and γ-proteobacteria supporting their hypothesis that some members of these phylogenetic groups are more integrally associated with AM fungi than others.

Several PGPR have been shown to be excellent root colonizers (Barea et al., 2002) and a number of surface components have been demonstrated to play a role in the physical interactions between such bacteria and plant roots (Bianciotto and Bonfante, 2002). Several bacteria reported to be good root colonizers, for example, some *Pseudomonas* spp., are also capable of adhering to AM fungal hyphal surfaces, suggesting that the mechanisms involved could be fairly similar. Close cell-to-cell contact between, for example, rhizobia and their host plant roots is an important prerequisite for the formation of the nodules during endosymbiosis, and one may speculate whether similar correlations exist between attachment of bacteria to AM fungal hyphal surfaces and changes in fungal growth or performance.

However, little information is available concerning the extent to which PGPR colonize AM fungal hyphae. Bianciotto and colleagues (1996) reported that some *Rhizobium* and *Pseudomonas* species attached to germinated AM fungal spores and hyphae under sterile conditions, and that the degree of attachment varied with the bacterial strain. However, no specificity for either fungal or inorganic surfaces could be detected among the bacteria tested. Based on their results, these authors suggested that interactions between rhizobacteria and AM fungi were mediated by soluble factors or physical contact.

Endocellular bacteria are reported in only a few fungi including some Glomeromycota species (AM fungi and Geosiphon pyriforme) (Scannerini and Bonfante, 1991; Bianciotto *et al.*, 2000; Perotto and Bonfante, 1997) and in the ectomycorrhizal basidiomycete *Laccaria bicolour* (Bertaux *et al.*, 2003). Regarding the AM fungi, their cytoplasm harbours bacteria-like organisms, which have been observed by microscopy in several AM fungal species (*Glomus versiforme, Acaulospora laevis, Gigaspora margarita*) (Mosse, 1970; MacDonald and Chandler, 1981; Scannerini and Bonfante, 1991; Bonfante *et al.*, 1994). Further investigation of these structures, including the demonstration of their prokaryotic nature,was long regarded as a task too complicated because they could not be cultured. However, by using morphological observations in combination with molecular analyses, Bianciotto and colleagues (1996) succeeded in showing that they actually were of true bacterial origin. They also demonstrated the AM fungal endosymbiotic properties of these bacteria, that they were able to complete their life cycles within fungal cells, and that the bacterial cells were Gram-negative and rod-shaped. Several additional characteristics of the endosymbiotic bacterial genome have since been reported (Ruiz-Lozano and Bonfante, 2000; Minerdi *et al.*, 2001; Minerdi *et al.*, 2002a; Minerdi *et al.*, 2002b).

Endosymbiotic bacteria have been detected in several members of the Gigasporaceae; actually the only fungal species in this family among the evaluated ones, reported not to contain such bacteria was *Gigaspora rosea* (Bianciotto *et al.*, 2000). In the five other species belonging to the Gigasporaceae, intracellular bacteria were detected through all the steps of the fungal life cycle: spores, germtubes, and extra- and intraradical hyphae, except arbuscules (Bianciotto et al., 1996b). The AM fungus most extensively studied for its endosymbiotic bacteria is *G. margarita* isolate BEG 34, which was also the first fungus in which these prokaryotic cells were further investigated

(Bianciotto *et al.*, 1996b). Recent studies have indicated an average of about 20 000 bacteria per *G. margarita* spore (Bianciotto *et al.*, 2004; Jargeat *et al.*, 2004). These bacteria were initially assigned to the genus *Burkholderia* on the basis of their 16S ribosomal RNA gene

sequence, but were recently reassigned to a new taxon termed *Candidatus Glomeribacter gigasporarum* (Bianciotto et al., 2003). In spite of several attempts, these bacteria have never been grown on cell-free media (MacDonald and Chandler, 1981; Scannerini and Bonfante, 1991; Bianciotto *et al.*, 2004; Jargeat *et al.*, 2004), which is the reason why they are assigned to the provisional Candidatus designation for uncultured bacteria (Murray and Schleifer, 1994; Murray and Stackebrandt, 1995).

Soil microorganisms, particularly PGPR, can influence AM formation and function and, conversely, mycorrhizas can affect the microbial populations, particularly PGPR in the rhizosphere (Linderman 1992, 1994; Fitter and Garbaye 1994; Barea 1975,). The analysis of microbe – microbe interactions is crucial to an understanding of the events which occur at the root – soil interface and, particularly, to those related to the microbial colonization of the root surface, or the processes of root infection/colonization by pathogens or mutualistic symbionts (Lynch 1990). These interactions must be taken into consideration when trying to manage AM fungi and PGPR for the biological control of plant pathogens or for the biogeochemical cycling of plant nutrients (Barea *et al.* 2002).

Once the AM status has been established in plant roots, reduced damage caused by soil-borne plant pathogens has been shown. To account for this, several mechanisms have been suggested to explain the enhancement of plant resistance/tolerance in mycorrhizal plants (Linderman 1994, 2000; Azcón-Aguilar and Barea 1992, 1996). One of the proposed mechanisms is based on the microbial changes produced in the mycorrhizosphere. In this context, there is strong evidence that these microbial shifts occur, and that the resulting microbial equilibria could influence the growth and health of the plants. Although this effect has not been assessed specifically as a mechanism for AM-associated biological control, there are indications that such a mechanism could be involved (Azcón-Aguilar and Barea 1992, 1996; Linderman 1994, 2000). In any case, it has been demonstrated that such an effect is dependent on the AM fungus involved, as well as the substrate and host plant (Azcón-Aguilar and Barea 1996; Linderman 2000). Since specific PGPR antagonistic to root pathogens are being used as biological control agents (Alabouvette *et al.* 1997), it has been proposed to try to exploit the prophylactic ability of AM fungi in association with these antagonists (Linderman 1994, 2000;Azcón-Aguilar and Barea 1996; Barea *et al.* 1998; Budi *et al.* 1999).

Several studies have demonstrated that microbial antagonists of fungal pathogens, either fungi or PGPR, do not exert any anti-microbial effect against AM fungi (Calvet *et al.* 1992; Barea et al. 2002; Edwards *et al.* 1998; Vazquez *et al.* 2000). This is a key point to

exploit the possibilities of dual (AM fungi and PGPR) inoculation in plant defense against root pathogens. In particular, Barea *et al.* (1998) carried out a series of experiments to test the effect of *Pseudomonas* spp. producing 2,4-diacetylphloroglucinol (DAPG) on AM formation and functioning. Three *Pseudomonas* strains were tested for their effects on AM fungi: a wild type (F113) producing the antifungal compound DAPG; the genetically modified strain (F113G22), a DAPG-negative mutant of F113; and another genetically modified strain [F113 (pCU203)], a DAPG-overproducer. The results from *in vitro* and in soil experiments demonstrate no negative effects of these Pseudomonas strains on spore germination, and a stimulation of hyphal growth of the AM fungus *Glomus mosseae*. Concentrations of the antifungal compound DAPG which were far in excess of those reached in the rhizosphere of *Pseudomonas*-inoculated plants exhibited negative effects on germination of AM fungal spores, but more realistic concentrations of DAPG did not affect AM fungal development. A soil microcosm system was also used to evaluate the effect of these bacteria on the process of AM formation. No significant difference in AM formation on tomato plants between F113, F113G22 and F113 (pCU203) was observed, with the F113 and F113G22 strains resulting in a significant increase in the percentage of the root system becoming mycorrhizal. Therefore, these strains behaved as MHB. In a field experiment, none of these *Pseudomonas* strains affected: (a) number and diversity of AM fungal native population; (b) the percentage of root length that became mycorrhizal; (c) AM performance. Furthermore, the antifungal Pseudomonas improved plant growth and nutrient (N and P) acquisition by the mycorrhizal plants (Barea *et al.* 1998).

It should be mandatory to detect the cohesiveness of both AMF and PGPR participating in a particular rhizosphere while maintaining the healthy rhizosphere. The key step is to ascertain whether an antifungal biocontrol agent will negatively affect the AMF populations. There is need to exploit the possibilities of dual (AMF and PGPR) inoculation to provide plant defense against root pathogens. The success of these microbes will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculants formulation and delivery. It has also been established that combined uses of both AMF and PGPR increases the efficacy towards pathogen control (Sharma *et al.* 2009). But the magnitude of pathogen suppression varies from rhizosphere to rhizosphere, which needs to be deciphered and customized. Hence, there is a need to modulate the mycorrhizosphere to maintain higher hyphosphere activity to mange resident AMF and PGPR for improving the plant and soil health and should be the key aim of

the applied research in the future. With the growing interest in reducing chemical inputs, companies involved in the manufacturing and marketing of bioformulations (AMF-PGPR biocontrol consortium) should experience continued growth. However, stringent quality control measures must be adopted so that farmers get quality products. New, more effective and stable formulations also will need to be developed.