Chapter 2

Review of Literature
2. Review of Literature

2.1. Plant Growth Promoting Rhizobacteria (PGPR)

It has been suggested that PGPR strains can promote plant growth and development either directly and indirectly (Castro et al., 2009). Direct stimulation includes biological nitrogen fixation, producing phytohormones like auxins, cytokinins and gibberellins, solubilizing minerals like phosphorus and iron, production of siderophores and enzymes and induction of systemic resistance, while indirect stimulation is basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for niches within the rhizosphere (Van Loon, 2007).

2.1.1. Indole-3-acetic acid (IAA)

IAA (indole-3-acetic acid) is the member of the group of phytohormones and is generally considered the most important native auxin (Ashrafuzzaman et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, and differentiation, and gene regulation (Ryu and Patten, 2008). Diverse bacterial species possess the ability to produce the auxin phytohormone IAA. Five isolates of PGPR namely Pseudomonas aeruginosa, P.putida, B. subtilis, Paenibacillus polymyxa and B. boronophilus were characterized for the production of IAA. IAA significantly increased the shoot length, root length, and dry matter production of chick pea seedlings (Yadav et al., 2010). A potential isolate of P. fluorescens which effectively inhibited the mycelial growth of Macrophomina phaseolina under in vitro condition was also characterized as good producer of IAA (Kharwar et al., 2007). Usha Rani et al., (2012) reported that among the sixteen isolates from pigeon pea rhizosphere, seven were found to be highest IAA producers and exhibited
antagonistic activity against soil fungi. Shobha and Kumudini, (2012) screened seven stains of *B. megaterium* for IAA production which also inhibited the mycelial growth of *F. oxysporum*.

2.1.2. Ammonia production

It has been reported that ammonia production indirectly influences the plant growth. *B. subtilis* strain MA-2 and *Pseudomonas fluorescens* strain MA-4 was efficient in ammonia production and significantly increased biomass of medicinal and aromatic plant such as *Geranium* (Mishra *et al*., 2010). However, ammonia production was observed less frequently in *Azotobacter* isolates. Ammonia production was detected in 95% of the isolates from the rhizosphere of rice (Joseph *et al*., 2007).

2.1.3. HCN production

A secondary metabolite produced commonly by rhizosphere Pseudomonads is Hydrogen Cyanide (HCN), a gas known to negatively affect root metabolism and root growth (Schippers *et al*., 1990) and is a potential and environmentally compatible mechanism for biological control of weeds (Heydari *et al*., 2008). The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules (Charest *et al*., 2005). The strains of fluorescent *Pseudomonads* in black pepper were found to produce volatile and non volatile metabolites including HCN against fungal pathogens (Diby *et al*., 2001). Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi (Ramette *et al*., 2003). Fluorescent *Pseudomonads* isolated from the rhizosphere soils of bajra (*Pennisetum glaucum*), jowar (*Sorghum vulgare*), rice (*Oryza sativa*) and maize (*Zea mays*) produced HCN (Suresh *et al*., 2010), which suggested that these isolates can be used as potential biofertilizers and also as biocontrol agents.
2.1.4. Siderophores

Under iron-limiting conditions PGPB produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion (Whipps, 2001). Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe3+ complexes that can be taken up by active transport mechanisms. Siderophores are also important for some pathogenic bacteria for their acquisition of iron (Miethke and Marahiel, 2007). Siderophores have been demonstrated to play a major role in plant disease suppression by some bacterial biocontrol agents which inhibit the growth or the metabolic activity of plant pathogens by sequestering iron (Haggag Wafaa and Abo Sedera, 2000). Tailor and Joshi, (2012) carried out extensive screening for the siderophore producing bacteria from the sugarcane rhizosphere soil. Seven isolates were found to produce more than 85% siderophore units. Amongst them *Pseudomonas fluorescens* was found to be the most efficient siderophore producer (96% SU). Rakh *et al.*, (2011) reported that *Pseudomonas cf. monteilii* 9 was an efficient producer of siderophore, which also exhibited antagonistic activity against *Sclerotium rolfsii*. Soil bacterial isolates including *Azotobacter vinelandii* MAC 259, *Pseudomonas* and *Bacillus cereus* UW 85 produce siderophores which can be used as efficient rhizobacteria to increase the crop yield (Husen, 2003). Siderophores directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria, would suppress the growth of pathogenic organisms viz., *F. oxysporum* and *R. solani*, function as stress factors in inducing host resistance (Haas and Defago, 2005). *B. megaterium* from tea rhizosphere produces siderophores which helps in plant growth promotion and disease reduction (Chakraborty *et al.*, 2006).

2.1.5. Phosphate solubilization

Phosphorus (P) is a major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of
solubilising the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Chen et al., 2006; Rodriguez et al., 2006). The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture. The use of phosphate solubilising bacteria as inoculants increases the P uptake by plants (Igual et al., 2001). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solubilising Microorganisms (PSM) including bacteria have provided as an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. A total of 27 oxalotrophic phosphate solubilizing bacteria were isolated from rhizosphere soil of oxalogenic plant. These isolates were studied for their plant growth promoting factors like indole acetic acid production, cell wall degrading enzyme activities, cellulase, chitinase and proteolytic enzyme. The results showed that rhizospheric oxalate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture (Bartakke et al., 2012). PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to chickpea that represent a possible mechanism of plant growth promotion under field condition (Verma et al., 2001; Verma et al., 2010). Several species of fluorescent Pseudomonas such as P. fluorescens NJ101 (Bano and Musarrat, 2004), P. aeruginosa (Jha et al., 2009) and Bacillus sp. (Ahmad et al., 2008) have been reported as good phosphate solubilizers. Dhindani et al., (2013) characterized the Pseudomonas aeruginosa strain DSM 50071 for its highest phosphate solubilizing aility.

2.2. **Trichoderma spp**

*Trichoderma* species are among the most frequently isolated fungi and are present in the root ecosystems. The fungi are opportunistic avirulent plant symbionts and functions as parasites and antagonists of many phytopathogenic
fungi, thus protecting plants from diseases (Harman et al., 2004). *Trichoderma* strains act as biocontrol agent against fungal phytopathogens either directly or indirectly. In indirect mechanism the inhibition may be by competition for nutrients and space, modification of the environmental conditions, antibiosis, and induction of plant defensive mechanism, however direct mechanism encompasses mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the *Trichoderma* strain, the antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration (Gajera et al., 2013).

Strains of *Trichoderma* added to the rhizosphere protect plants against numerous classes of pathogens, e.g. those that produce aerial infections, including viral, bacterial and fungal pathogens, which points to the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants (Harman, 2004). At a molecular level, resistance results in an increase in the concentration of metabolites and enzymes related to defensive mechanisms, such as the enzymes, phenyl-alanine ammonio-lyase (PAL) and chalcone synthase (CHS), involved in the biosynthesis of phytoalexins (HR response), chitinases and glucanases. These comprise pathogenesis-related proteins (PR) (SAR response) and enzymes involved in the response to oxidative stress (Woo et al., 2006).

### 2.2.1. Mycoparasitism

Mycoparasitism, the direct attack of one fungus on another, is a very complex process that involves sequential events, including recognition, attack, subsequent penetration and killing of the host. It is believed that fungi secrete exochitinases constitutively at low levels. When chitinase degrade fungal cell walls, they release oligomers that induce exochitinases and the attack begins (Tahia et al., 2004). During this process *Trichoderma* secrets cell wall degrading enzymes (CWDEs) that hydrolyze the cell wall of the host fungus, subsequently
releasing oligomers from the pathogen cell wall (Kubicek et al., 2001). It is believed that *Trichoderma* secretes hydrolytic enzymes at a constitutive level and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation (Harman et al., 2004). Once the fungi come into contact, *Trichoderma spp.* attach to the host and can coil around it and form appressoria on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to lectins on the target fungi (Inbar et al., 1996). Sharma, (2011) observed the interaction between *Trichoderma spp* and *Fusarium oxysporum*, the sequence of events noticed was categorized as pre-contact antagonistic interaction, chemo-attractive intermediate phase and, finally, parasitic interaction. They concluded that most of the *Trichoderma* isolates showed considerably good antibiosis and parasitism. Anwar et al., (2008) reported the parasitic activity between *T. viride* and *Rhizoctonia solani* which revealed that *T. viride* hyphae formed peg like structures and ran along the host hyphae but remained adhered to the host hyphae. The cytoplasm of host hyphae also looked disintegrated which might be due to the production of lytic enzymes such as chitinase, glucanase and cellulase released by the bio agents, which are capable of degrading the cell wall of *Rhizoctonia solani*. Ravi Chandran and Reddi Kumar, (2012) studied the interaction between *Trichoderma* spp and *F. solani*, and reported that during hyphal interaction, the antagonist produced various structure like haustoria, coiling and penetration followed by leakage of nutrients from the hyphae of the test pathogen.

2.2.2. **Competition for nutrients**

Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens (Chet et al., 1997). For instance, in most filamentous fungi, iron uptake is essential for viability (Esiendle, 2004), and under iron starvation, most fungi excrete low-molecular-weight ferrie-iron specific chelators, termed siderophores, to mobilize environmental iron. Subsequently, iron from the ferri
siderophore complexes is recovered via specific uptake mechanisms. Root extudates are major source of nutrients in soil which are excreted from the tips, thus colonization in the rhizosphere of the root tip by an antagonist might reduce infection by *Fusarium* like pathogens (Cook and Baker, 1983)

### 2.2.3. Cell wall degrading enzyme production

The extracellular enzymes produced by *Trichoderma* strains may be correlated with the antagonism. *Trichoderma* directly attacks the plant pathogen by excreting lytic enzymes such as chitinases, β-1, 3-glucanases and proteases (Elad *et al.*, 1982, Haran *et al.*, 1996). The enzymes, cellulase and β-1,3-glucanase produced by *Trichoderma* might be involved in hydrolysis of *P. aphanidermatum* cell wall during antagonism (Thrane *et al.*, 1997). De Marco and Felix, (2002) observed that the biocontrol potential of an Indian *Trichoderma* isolate against *C. perniciosa* was due to protease activity. *Trichoderma* spp showed antagonism against *S. rolfsii, R. solani, S. sclerotiorum*. They also observed that the chitin and cellulose provided in the medium was utilized and thus concluded that they are strong producer of cellulase and chitinase enzyme. Krishna kumar *et al.*, (2012) isolated 12 species of *Trichoderma* and tested against *S.rolfsii, Colletotrichum gloeoporioides* and *C. capsici*. All the isolates produced β-1,3-glucanase and chitinase enzymes. Lunge and Patil, (2012) isolated three chitinolytic *Trichoderma* species namely *T.harzianum, T. viride, T. flavofuscum* and suggested that the chitinolytic activity was the better tool for antagonism of *Trichoderma* species. The *in vitro* study of Gajera *et al.*, (2008) revealed that *T. viride* exhibited more antagonism against *F. oxysporum f.sp.ciceri* and it was positively correlated with extracellular enzymes such as protease, chitinase, and β-1,3-glucanase. There are many reports demonstrating that chitinases and β-1,3 glucanases are effective features associated with the ability of *Trichoderma* to control plant pathogens (Brimner and Boland 2003; Kubicek *et al.*, 2001).
2.3. Mycorrhiza- Root fungus association

More than hundred and fifty years ago, it was reported that tree rootlets are nourished by certain fungal mycelia which mantle them. Gasparrini, (1856) stated that a fungal mantle was found around roots of chestnut, hazel and pine. The term ‘mycorrhiza’ was introduced by Frank, (1885). Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi with the roots of higher plants (Sieverding, 1991). Till recently the actual beneficial aspects of mycorrhizae were less known. But last few decades of research works found out the relevance of mycorrhizae and today scientists utilize these fungi for exploring its benefits. Three types of mycorrhizae are recognized. They are (i) Ectomycorrhizae, (ii) Endomycorrhizae, (iii) Ectendomycorrhizae.

2.3.1. Arbuscular mycorrhizal Fungi

The endomycorrhizae are characterized by inter and intracellular fungal growth in root cortex, forming specific fungal structures, referred to as vesicles and arbuscles. Vesicles are the organs meant for storage purpose which are absent in certain forms. The arbuscles perform the exchange of nutrients between the fungus and root but they have short period and are digested by their host, few days after formation. This characteristic growth gives the endomycorrhiza the alternate name vesicular arbuscular mycorrhizae. Vasicular Arbuscular mycorrhiza fungi [(V)AMF] are distributed worldwide, which are currently classified in the order Glomales. The taxonomy is further divided into suborders based on the presence of vesicles in the root for the suborder Glominae or absence of vesicles in the root for the suborder Gigasporineae. The term vesicular arbuscular mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in Glomales, but because a major suborder lacks the ability to form vesicles in roots, (V)AM is now the preferred form of writing. The order Glomales is further divided into families and genera according to the method of spore formation. Taxonomically Arbuscular
mycorrhiza fungi belong to the order Glomales with three families Glomaceae, Acaulosporaceae and Gigasporaceae and six genera Acaulospora, Entrophospora, Gigaspora, Scutellospora, Sclerocystis and Glomus (Morton and Benny, 1990). There are more than 180 species of AM fungi (Morton and Redecker, 2002).

It has been recently reported that two ancestral clades of AM fungal species were discovered from deeply divergent ribosomal DNA sequences. They are classified as two new families Archaeosporaceae and Paraglomaceae. At present time, each family consists of one genus, Archaeospora including three species forming a typical Acaulospora like spores from sporiferous saccule. Paraglomus consists two species forming spore which are indistinguishable from those of Glomus species.

2.3.2. Role of Arbuscular Mycorrhizal Fungi

The ubiquitous nature of arbuscular mycorrhizal fungi and their beneficial role in plant growth are well documented. By forming an extended intricate hyphal network, AMF can efficiently absorb mineral nutrients from soil and deliver them to their host plants in exchange for carbohydrates. AMF can also enhance tolerance to abiotic stresses such as drought and metal toxicity (Meharg and Cairney, 2000). Therefore, it is evident that AMF are an important associate for crop plants in sustainable agriculture.

Enhanced uptake of P (phosphate) is generally regarded as the most important benefit that AMF provide to their host plant, and plant P status is often the main controlling factor in the plant–fungal relationship (Graham, 2001). AMF may also enhance plant uptake of N from organic sources (Hodge et al., 2001), this may be associated with increased growth and yield of host plants (Earanna et al., 2002; Rajan et al., 2004). It is also observed that, if colonization by AMF is disrupted, uptake of P, growth and in some cases yield can be significantly reduced (Thompson, 1994). Also there is considerable evidence to suggest that
AMF are able to increase the host plant’s tolerance to water stress (Davies et al., 2002; Auge et al., 2004) including that caused by high salinity (Al-Karakí et al., 2001).

It has been shown that the AM fungi significantly increased the phosphorus content in many plants (Gaur et al., 2000). The hyphae of AMF can explore an area around the root far exceeding that available to root hairs. It is due to the ability of hyphae to make immobile the fixed elements (P, Zn, and Cu) in acid and alkaline soils more available to plants, especially in the plants with course root systems (Allen et al., 1995).

The mycorrhizal fungi produce enzymes, auxins, vitamins, cytokinins and other substances that increase rootlet size and longevity (Gopinathan and Raman, 1991). They protect the rootlets from pathogens (Rabie, 1998), alleviate environmental stresses on plants (Ruiz-Lozano et al., 2001), improve plant tolerance to drought and polluted environments (Auge, 2001; Vivas et al., 2003), and accelerate plant establishment (Caravaca et al., 2003). At present, AMF are considered as an important component in the restoration and reestablishment of the vegetation in fragile or degraded ecosystem functioning (Dhillion and Gardsjord, 2004).

2.3.3. Distribution of AMF in the rhizosphere of various crops and plant communities

Arbuscular mycorrhizal fungi constitute a group of important mutualistic symbiotic soil microorganisms which are associated with the roots of angiosperms, gymnosperms, pteridophytes and gametophytes of some mosses even also in some aquatic plants (Beck-Nielsen and Madsen, 2001). Colonization by arbuscular mycorrhizal fungi assures good survival and growth of plants (Vijaykumar and Abraham, 2001, Lakshman and Patil, 2004). The role of endomycorrhizal fungi in improving the growth and development of the host plant in a variety of plants under different habitats studied. Remarkable work has
been done in India on the distribution of AM fungi in agricultural fields, forest soil and many other horticultural fields (Kulkarni, 2007).

There are reports on the association of AMF in several cultivated crops such as sorghum, maize, ragi (Sunilkumar and Garampalli, 2010), wheat (Vyas et al., 2006), rice (Bhattacharjee, and Sharma, 2011), mungbean (Hindumathi and Reddy, 2011), soybean (Heggo and Angle, 1990), tubers, (Khade and Rodrigues, 2003), medicinal plants (Sharma and Jha, 2012), bamboos (Mohanan and Manoj Sebastian, 1999), ornamental plants (Muthukumar and Udaiyan, 1994), oil crops sunflower (Chandrasekaran et al., 1995), sesam (Selvaraj and Subramanian, 1995), groundnut (Gupta and Krishnamurthy, 1996), vegetable crops (Iqbal and Gautam, 1996) and chilli (Boonlue et al., 2012).

Similar reports on plants including plantation crops such as cardamom (Sivaprasad, 1998), lime (Michel-Rosales and Valdes, 1996), onion (Mallesha et al., 1994), banana (Khade and Rodrigues, 2004), papaya (Khade and Rodrigues, 2008), mulberry (Katiyar et al., 1995), coffee (Theodoro et al., 2003, Muleta et al., 2007), tobacco (Abdul Malik, 2000) and sweet potato (Tholkappian et al., 2000) have been reported. The occurrence of AM fungi has been reported in many plant communities such as forests (Sengupta et al., 1998), grasslands, tepper and praires (Sanders and Fitter, 1992), deserts (Khaliel, 1988) and mangroves (Sengupta and Chaudhuri, 1989).

### 2.3.4. Ecology of AM fungi

Arbuscular mycorrhizal fungi (AMF) are geographically ubiquitous and occur over a broad ecological range. AMF although widely distributed, there is limited knowledge of occurrence of individual or dominant species in relation to soil and climate. Several biotic and abiotic factors influence the AM in various ways. The major factors affecting the diversity, abundance and distribution of arbuscular mycorrhizal fungi in agricultural fields are (i) seasonal variations (ii) climatic factors and (iii) edaphic factors.
2.3.4.1. Seasonality

AM fungi are dominant in tropical condition than in subtropical ones. Seasonal variations can have a remarkable influence on the occurrence of AM spores in soils (Lugo et al., 2003, Mndyam and Jumpponen, 2008). The spore population is found maximum during the cropping season and decreased simultaneously in the post harvest season (Vyas and Srinivastava, 1988). Environmental conditions have a significant effect on the distribution, density and floristic composition of AM fungi (Muthukumar, 1996; Sugavanam and Udaiyan, 1997). Karthikeyan and Selvaraj, (2009) reported that species richness was highest during summer and late summer seasons and they were significantly correlated with soil pH, soil calcium, phosphorous and percent root colonization. Similarly the highest number of AMF spores was found in rainy season while moderate in winter and least in summer (Nisha et al., 2010). Similar observations were reported by Khade and Rodrigues, (2008) where colonization of AM fungi in Carica papaya exhibited variations depending on edaphic factors and seasonal patterns in the weather.

Singh et al., (1992) reported variation in spore density and AM root colonization of lemon seedlings with the change of season and soil depth. Seasonal fluctuation in number of AM spores in soils of sand dunes (Beena et al., 1999), tropical forest (Singh and Tiwari, 1999) and deciduous forest (Brundrett and Kendrick, 1988) has been reported. Mago and Mukerji (1994) observed that percent root colonization with AM fungi in relation to seasonal changes in some members of Lamiaceae. AM colonization was found to be the lowest during winter and highest during late summer.

Selvaraj et al., (1994) reported the seasonal variation in the incidence of AM fungi in roots and rhizosphere of clay loam alkaline soils of Pitchavaram, India. Beena et al., (1997) reported that colonization of AM fungi, species richness and spore density in the rhizosphere were highest during monsoon. Beena et al., (2000) also reported seasonal variations of AM fungal association.
with *Ipomoea pes-caprae* of coastal sand dunes. It has been reported that AM fungal sporulation and colonization are seasonal and dependent on host species and maximum sporulation and colonization was registered in rainy season in case of *Theobroma grandiflorum* and *Paullinia cupana* (de Oliveira and de Oliveira, 2005). They suggested that the maximum sporulation in this season could be correlated with the fact that during this period most photosynthate is allocated to roots and rhizomes, which helped fungal symbiont to produce more spores (Gemma and Koske, 1988). Lugo and Cabello, (2002) studied seasonal variation of AM fungi in a mountain grass land, they reported that higher number of vesicles, arbucules and maximum root colonization during summer season.

### 2.3.4.2. Climatic factors

Climatic factors influences root colonization and spore population of AMF (Udaiyan et al., 1996).

#### 2.3.4.2.1. Rainfall and relative humidity

The ecological studies to pin point the effect of climatic factors on population dynamics, infectivity and effectivity of AM fungi are very few. A significant relationship between AM fungal infection and rainfall in citrus (Michelini et al., 1993) and a reduction in the intensity and percentage of AM fungal infection in three species of savanna grasses during dry season were reported. A similar drop in the infection levels of sugarcane was found in Barbados during the dry season (Chinnery et al., 1987). A positive relation between AM fungal spore numbers and relative humidity in *Acacia fernesiana* and a negative relationship in *A. planiferons* were found (Udaiyan et al., 1996).

#### 2.3.4.2.2. Temperature

Temperature fluctuations with different seasons influence the AM spore population and root colonization directly or indirectly. Staddon *et al.*, (2002) reported that the intensity of mycorrhizal fungi changes with increase or decrease in temperature. Schenck and Schroder, (1974) studied the effect of temperature
on AM establishment and registered the greatest root colonization between 28-34 °C. A significant correlation of AM root colonization with temperature has been observed in *Plantago lanceolata*, while there was no significant effect of temperature on *Holcus lanatus* (Heinemeyer and Fitter, 2004). Schenck and Smith (1982) observed that the growth response in soybean varied with different combinations of soil temperature and AM species. Maximum shoot growth and mycorrhizal colonization at 30 °C with *Gigaspora calospora* and *Glomus intraradices* and at 36 °C with *G.ambisporum* inoculation have been reported (Smith and Roncodori, 1986).

2.3.4.2.3. Wind

AM fungi dispersal is mainly activated by wind. The large spores of AM fungi can be suspended in moving air currents (Tommerup, 1982) and wind dispersal has been observed in arid ecosystems (Warner *et al*., 1987). Wind dispersal mechanism is responsible for introduction of AM fungi to new geographic locations (Brundrett, 1991).

2.3.4.2.4. Light

AMF obtain their carbon source from the plants and thus rely on the photosynthesis and the translocation of photosynthates to the root. Since, light control the rate of photosynthesis it can strongly affect mycorrhizal formation. But shading reduces AM colonization and spore production. Day length plays an important role in AM development, but the effect of light depends on the photosensitivity of host species (Redhead, 1975). Photoperiod may also be more important so that the light intensity at optimal day length increase the root colonization (Daft and El-Giahmi, 1978).

2.3.4.3. Edaphic factors

Arbuscular mycorrhizal fungal taxa have a specific multidimensional niche determined by the plant species that are present at the site and by edaphic factors such as pH, moisture content, phosphorus (P) and nitrogen (N)
availability (Ahulu et al., 2006). These factors could also affect the crop production in different agro ecosystems (Porras-Soriano et al., 2009).

2.3.4.3.1. Soil pH

It is well acknowledged that soil pH could have major impact on diversity pattern of AM in natural and agricultural ecosystems (Porter et al., 1987). Intensity of AM root colonization and spore density has shown to depend on soil pH (Sylviya and Williams, 1992). Maximum number of AM spores and root colonization was reported at pH 7.2-7.4 by Selvaraj et al., (2001). However Sankaranarayanan and Sundrababu, (2001) reported that pH 6-7 was best for mycorrhizal development. The soil pH was found to have no correlation with both infections of root tissues by VA-mycorrhizal fungi and spore densities (Akond et al., 2008). The distribution of AM fungi was also affected by soil pH. *Glomus* spp occurred in soil with a wide pH range. The greater the soil alkalinity, the more *Glomus* spp were found, while in soils with greater acidity, more *Acaulospora* were isolated. *Scutellospora* occurred mostly in soil with pH of 6.0-7.0, and *Gigaspora* was distributed mainly in acid soil (Gai and Liu, 2003).

2.3.4.3.2. Soil moisture

The soil moisture is found to be correlated with spore density. The increase in spore population may be due to increase in soil moisture. Similar reports have been reported by Mohankumar and Mahadevan, (1999). Khan, (1974) reported low spore density in the month of May. It indicates that spore density correlated with moisture content. Hindumathi and Reddy, (2011) reported that the moisture content exhibited significant negative correlation with percent root colonization and positive correlation with spore density indicating that the moisture content could accelerate root colonization and spore density. They also suggested that the average moisture levels of about 17% - 20% have been apparently favorable for high spore density and maximum root colonization.
2.3.4.3.3. Phosphorus

Phosphorus is one of the key macronutrients required for plant growth and metabolism. AM spore colonization and spore density was greater in the soil having low P- values (Janaki Rani and Manoharchary, 1994). Wu et al., (2006) reported significant negative correlation between percent root colonization and available soil P content indicating that lower available P could accelerate the colonization on Citrus roots. Bruce et al., (1994) concluded that at very early stages of colonization P exerts its effect via reduced growth of infection units, and in P-deficient plants, root colonization increases almost linearly with time and is substantially reduced by P amendment. However, small additions of P to low available P soil can increase root colonization (Bolan et al., 1984). When P limits the growth of plants, infection of roots by AMF may lead to an increase in plant growth through an improved uptake of P (Harley and Smith, 1983). Mycorrhiza formation by Scutellospora calospora was unaffected by the addition of P to the soil, whilst Glomus sp. was observed to decrease in both percent colonization and mycorhizal root length with the addition of P to the soil (Pearson and Schweiger, 1994).

2.3.4.3.4. Soil nitrogen

Hayman, (1975) showed that ‘N’ fertilizers had a large negative effect on the mycorrhizal colonization. Following application of 300kg N and (NH₄)₂ SO₄. Menge, (1984) noted that daily fertilization of citrus with more than 100ppm ‘N’ as a mixture of NO₃⁻ and NH₄⁺ retarded mycorrhizal development. Davis and Young, (1985) reported NO₃⁻ salts to be more inhibitory to development than NH₄⁺ salts. Johnson et al., (2003) demonstrated that N enrichment impacts mycorrhizal allocation across a wide range of grassland ecosystems. Such changes are important because they suggest an alteration in mycorrhizal functioning that, in turn, may be imparted in plant community composition and ecosystem function. The soil available N showed high correlation with root colonization and specially spore populations (Ghorbani et al., 2012). There are
several reports that soil N could suppress root colonization by VAM fungi (Chambers et al., 1980; Buwalda and Goh, 1982; Johnson et al., 1984). The suppressive effect of soil N on AM fungi has been attributed primarily to pH changes associated with variations in soil N (Thompson, 1986).

2.3.5. Mass inoculum production

The production of inoculums is one of the hindrances in the large scale production of arbuscular mycorrhizal fungi (Silva et al., 2002). AM fungi require living plants with profuse root system for their association and proliferation to increase the number of infection sites. Fast-growing plants with short life cycle and rapid infectibility by large range of AM fungi are generally preferred (Ijdo et al., 2010), especially monocots such as maize (Zea mays L.), sorghum (Sorghum bicolor (L.) Moench), onion (Allium cepa L.), bahia grass (Paspalum notatum Flugge) and other grasses, are ideal trap plants (Gaur and Adholeya 2002; Douds et al., 2005).

Several studies have been carried out to determine the best combinations of AMF and host species, in order to increase spore production (Bagyaraj and Manjunath, 1980; Hetrick and Bloom, 1986; Struble and Skipper, 1988). Different methods have been used for mass production of AM fungi, such as aeroponic (Hung and Sylvia, 1988), hydroponics (Hawkins and George, 1997) and the monoxenic cultivation (Sylvia and Jarstfer, 1994), but, the most widely used is pot culture, where the fungi are usually maintained and multiplied in conjunction with suitable host plant roots (Ferguson and Woodhead, 1982).

Culture of AM fungi with a suitable host plant and the desired symbiont in soil substrate under pot conditions is the classical, cheapest and most-preferred method of multiplication for large-scale production of AM fungal propagules (Selvaraj and Chellappan 2006; Kapoor et al., 2008).

Different practices like use of waste substrates along with the traditional substrate (soil-sand mixture) are being tried for mass culture of AM fungi these
days. Addition of any substrate into soil provides minerals in addition to beneficial elements that ultimately enhance the growth of AM fungi as well as plant. Tanwar et al., (2010) found an increase in AM spore population, % root colonization, fresh and dry root and shoot weight as an effect of sugarcane bagasse and ash. Chauhan et al., (2011) used two host plant species viz., *Triticum aestivum* (Wheat) and *Hordeum vulgare* (Barley) with tea residue for mass production of *Glomus mosseae*.

Three plant species *viz.*, wheat, lemon grass and lily grass were examined for mass production of consortium of *A. laevis*. AM fungus present in the rhizosphere soil after adding different concentration of fresh and decomposed apple pomace as substrate was recorded. It was also observed that plants having higher AM colonization showed higher AM spore production showing a positive correlation. They not only stimulated AM development, but also accelerated the root and shoot growth (Chauhan et al., 2013).

### 2.3.6. Influence of AMF on Biological characteristics

Mycorrhiza is the mutualistic association between soil –borne fungi with the roots of higher plants. AM fungi are known to colonize a number of tropical plants including vegetables (Reddy et al., 2006). These fungi use some of the root exudates and modify root physiology thereby altering the microbial equilibrium on the root surface (Mc Allister et al., 1995). They are well recognized as bio-fertilizer due to their manifold advantages provided to the host plant besides increasing nutrient and water uptake (Bohra et al., 2007).

Patil et al., (2013) studied the co- inoculation effect of AM fungi (*Glomus fasciculatum*), *Azospirillum brasilensce* and Phosphate solubilizing bacteria (*Bacillus polymyxa*) on height, dry weight of root and shoot, percent root colonization, spore number, P and N uptake by Finger millet (*Eleusine coracana* Gaertn). They have reported that the single inoculation of AM fungi and combined inoculation with *A. brasilensce* and *B. polymyxa* were found to be
moderately increase all the growth parameters. However the plant inoculated with *G. fasciculatum* + *A. brasilense* + *B. polymyxia* showed significantly highest growth parameters.

Nedorost and Pokluda, (2012) studied the effect of arbuscular mycorrhizae on tomato plants (*Lycopersicon esculentum*) in the pot experiment. In their studies three different fertilization regimes (optimum – H1, stress a – H2, stress b - H3) and three different mycorrhizal treatments (control – Ctrl, *Glomus mosseae* – Gm, *Glomus intraradices* – Gi) were used. Economical parameter (yield), nutritional characteristic (vitamin C content, phosphates and minerals content, total antioxidant capacity), and level of root colonization also were studied. They concluded that yield of the tomatoes was influenced by the basic dose of the fertilization, especially in the H2 and H3 treatment. The highest yield was in the H2 treatment in Gm (938 g per plant). The positive effect of the inoculation resulted in the increased content of the vitamin C. The highest significant influence was observed in the H2 treatment (plants inoculated with Gi) with the average content of the vitamin C 289 mg.kg$^{-1}$. The average rate of the colonization was in the range from 39% to 65%.

Spores of ten species of AM fungi were isolated from the rhizosphere soils of *Sorghum bicolor* (L.) from different localities of Madurai and Sivagangai districts of Tamil Nadu. *Gluconacetobacter diazotrophicus* was isolated from stem tissues of sugarcane (*Saccharum officinarum* L.) from Madurai district. The AM fungi in association with *G. diazotrophicus* were evaluated on the basis of root colonization, fresh weight and dry matter yield, N, P, soluble sugars and photosynthetic pigments in leaves of *S. bicolor*. All these parameter were significantly higher in dually inoculated plants especially in *G. diazotrophicus* + *Glomus fasciculatum* combination (Meenakshisundaram and Santhaguru, 2011).

Prasad et al., (2012) conducted a pot experiment to investigate the potential effect of arbuscular mycorrhizal fungi (*Glomus mosseae* and
Acaulospora laevis) and phosphate solubilizing bacteria (Pseudomonas fluorescens) with different levels of superphosphate on Chrysanthemum indicum L. They measured different plant growth parameters such as mycorrhization’s characteristics, phosphatase activity and phosphorus uptake at 100 days after inoculation. Their results revealed that inoculation with A.laevis + P. fluorescens at medium concentration of superphosphate showed maximum height, fresh and dry root weight, AM root colonization, AM spore count, alkaline phosphatase activity, acidic phosphatase activity and the percent phosphorus uptake in shoot and root and root length was maximum in G. mosseae + A. laevis + P. fluorescens treated plants and also found that leaf area and fresh and dry shoot weight were maximum in the treatment (G. mosseae + A. laevis + P. fluorescens) at low concentration of superphosphate. From the obtained results they concluded that the use of AMF increases nutrient acquisition from an organic fertilizer source by enhancing acidic phosphatase (ACP) and alkaline phosphatase (ALP) activity, thus facilitating P acquisition and improving plant growth.

Seedlings of Wedilia chinensis infested with seven different indigenous AM fungi viz., Acaulospora deligata, Glomus aggregatum, G. feugianum, G. fasciculatum, G. rubiforme, Gigaspora margarita and Scutillospora heterogama showed a general increase in growth parameters such as plant height, total dry weight than those of the uninoculated plants. However, the difference in shoot length and root length was significant between the treatments. Seedlings raised in the presence of G. fasciculatum showed an increase in shoot and root length as well as in dry weights, followed by those grown in the presence of G. aggregatum than other treatments. The nutritional status of W. chinensis seedlings, viz., Phosphorus, potassium, zinc, copper, magnesium and iron content, were also significantly higher in plant inoculated with G. fasciculatum followed by G. aggregatum (Nisha and Rajeshkumar, 2010a). Variation in plant nutrient status in relation to the fungal species for the medicinal plant species is well documented (Rajan et al., 2004).
The effect of two rhizobacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) and commercial product containing multiple strains of arbuscular mycorrhizal fungi (AMF) and an NPK fertiliser on the growth and yield of habanero chilli (*Capsicum chinense* Jacquin) have been evaluated. All treatments were applied as single or combined inoculants, under nursery and field conditions, in a completely randomised design. The biofertilizers were applied to the roots by coating or dipping, with the inoculants in a solid or liquid support, respectively. At 30 days after inoculation, populations of $2.5 \times 10^6$ to $1.3 \times 10^6$ cfu g soil$^{-1}$ of *A. brasilense* and $10.3 \times 10^5$ to $2.6 \times 10^5$ of *A. chroococcum* were detected in the rhizosphere of the crop. The prevalence of colonisation of plants inoculated with AMF ranged from 35 to 57%, with the greatest values recorded for the treatment involving single biofertilization by root coating. In the nursery phase, single biofertilization promoted a higher growth and nutrient content in the crop than combined biofertilization. However, in the field phase the combined biofertilization increased the nutrient content of the plant leaves, which was significantly greater than observed in the NPK treatment. The highest yields were recorded for the treatments involving a single inoculation of *A. chroococcum* and for those with the multi-strain of AMF, with average values of 2.5 and 2.3 kg plant$^{-1}$ respectively, compared with 1.0 kg plant$^{-1}$ obtained with the treatment in which NPK fertilizer was applied (Constantino *et al.*, 2008).

Oseni *et al.*, (2010) have carried out a greenhouse experiment to evaluate the responsiveness of tomato (*Lycopersicon esculentum* L.) seedlings to arbuscular mycorrhizal inoculation in a soilless medium on the transplant production performance. In their experiment the tomato variety RODADE was inoculated with BIOCULT mycorrhiza granules (consisting of both *Glomus etunicatum* and *G. intraradices*) and hydroponically grown on vermiculite for six weeks. Shoot and root fresh weight and dry weight, leaf area, stem length, leaf area ratio (LAR), relative growth rate (RGR), unit leaf rate (ULR), mycorrhiza dependency (MD) and growth response to arbuscular mycorrhiza (AM) fungi...
were measured at 14, 28, 42 days after inoculation. They have observed that the AM fungi inoculated seedlings exhibited better transplant performance due to their higher shoot fresh weight (avg. 11.28 g plant-1), high shoot/root ratio (avg. 0.236), higher root biomass (avg. 2.17 g plant-1) higher RGR (7.34 mg g-1 day-1) and ULR 1.28 mg cm-1day-1). AM inoculation resulted in 23.3% root colonization by the fungi in the vermiculite medium. The use of AM fungi appeared to provide benefits to the development of tomato seedling transplants in a soilless nursery condition. Wang et al. (2008) reported that inoculation at initial stage of plant development can promote AM symbiosis, which leads to increase in plant growth in the nursery and improving performance after planting in the field.

A field study was conducted to evaluate the effectiveness of arbuscular mycorrhizal fungi (AMF) and phosphorus levels (100, 150 and 200 kg) for increasing biomass yield and ajmalicine content in a medicinal plant (Catharanthus roseus). The plants treated with 150 and 200 kg P2O5/ha along with AMF had the maximum plant height, number of leaves, root biomass, phosphorus content, root colonization, spore count and ajmalicine content 120 days after planting when compared with the control plants. They suggested that these treatments could be recommended for enhancing biomass and alkaloid content in C. roseus (Karthikeyan et al., 2008).

Arumugam et al., (2010) investigated the effect of Rhizobium and Arbuscular mycorrhizal fungi inoculation, both individually and in combination on growth and chlorophyll content of economically important plant Vigna unguiculata L. A significant increase over control in root length (45.6 cm), shoot height (12.2 cm), dry weight of root (0.4 g) and shoot (1.8 g), total number of nodules (39.6 nos.), dry weight of nodules (0.5 g), percentage of mycorrhizal infection (96.6%), chlorophylls a (0.83 mg/g f.wt.), b (1.19 mg/g f.wt) and total chlorophyll (2.24 mg/g f.wt) was recorded in dual inoculated (AM fungi and Rhizobium) plants than plants with individual inoculation. Hence they suggested
that the dual inoculation with AM fungi and *Rhizobium* biofertilizer is more effective than the individual treatment.

Combined inoculation of VAM fungi and *Bradyrhizobium japonicum* increased the root colonization of VAM (28.6%), root nodulation (30.3%), fresh weight (14.5%), dry weight (15.5%) and seed weight (15.3%) in soybean (Jalaluddin, 2005).

Sensory *et al.*, (2013) examined the effect of AMF on seedling growth of melon. They used three different AMF species (*Gigaspora margarita, Glomus intraradices, G. etunicatum*) and observed that the root colonization ranged from 38.9% to 54.9%, and improved seedling traits. Among three species *G. intraradices* had higher positive mycorrhizal dependency.

Root colonization of *Glomus fasciculatum* improved the essential oil production, growth parameters as well as nutrient uptake in *Ocimum basilicum* (Rasouli Sadaghiani *et al.*, 2010). Synergistic effects of dual inoculation of *A. chroococcum* strains with AM fungi were observed for boll weight and seed cotton yields (Paul *et al.*, 2011). Mirzakhani *et al.*, (2009) recorded that the seed yield of safflower have been significantly affected by co-inoculation of AM fungi with Azotobacter, because these biofertilizer can fix the nitrogen, Phosphorus in soil and enhance the absorption of elements by plants.

A pot experiment was carried out to study the effect of mycorrhizal associations on the growth of sunflower under moisture stress conditions at three levels of soil phosphorus. Mycorrhizal plants showed high leaf area and total dry matter at 100% field capacity (FC) and 50 mg P/kg oil. The cumulative water use and water use efficiency (WUE) was also high in mycorrhizal plants. WUE increased with the P level and reduced with moisture stress. Stomatal conductance and transpiration rate was high in mycorrhizal plants. Phosphorus levels also influenced stomatal conductance and transpiration rates. Both mycorrhizal plants and high soil P levels increased partitioning of biomass more
towards root, resulting in higher root to leaf weight ratio. The plants with mycorrhizal associations had low stomatal and mesophyll limitations for photosynthesis (Nagarathna, 2007)

Borde et al., (2009) revealed that the *G. fasciculatum* significantly increased the plant height, total biomass, bulb diameter, and more allin and allinase enzyme activity. Dhanasekar and Dhandapani, (2012) reported that the sunflower (*H. annuus* L) responded well to the application of biofertilizer (*Azospirillum*, *Azotobacter*, *phosphobacter*, *Rhizobacter*). These fertilizer increased the plant height, number of leaves, stem diameter, percentage of seed filling and seed dry weight on two sunflower varieties (TCSH-1, SSH-48).

The shoot dry matter, shoot height and root length of onion inoculated with mycorrhizal fungi at the third sampling date (35 DAT) increased by 32% ; 22% and 20% respectively compared to uninoculated onion plants. Also, the growth parameters of leek plants inoculated with AMF at the third sampling date (35 DAT) increased by 25%; 24.8% and 28% respectively compared to the uninoculated leek plants. On the other hand, the shoot P concentration and P uptake of onion and leek plants with and without Mycorrhizal fungi (AMF) increased with increasing plant growth stages. The increase in P concentration and P uptake of onion and leek plants inoculated with Mycorrhizal fungi was rapid in the second growth stage (from 25 to 35 DAT) compared to the first growth stage (from 15 to 25 DAT). A highly significant correlation between root length and P uptake of plant (onion and leek with and without AMF) was observed. This study however indicated that beneficial responses due to mycorrhizal inoculation may need some time (35 DAT or more under the same conditions of this experiment) to develop its structure into the plant roots.

Morovvat et al., (2012) studied the effect of *G. intraradices* on different form of organic phosphorus in the sunflower rhizosphere soil. It has been concluded that there was significant increase in Fe- P fractions in the AM treated
sunflower and this could be attributed to high secretion of iron specific chelates such as hydroxymate siderophore. Ramakrishnan and Ezhil bama, (2010) conducted an experiment to find out the effect of *Azospirillum* and arbuscular mycorrhizae on finger millet var.CO.12. Both the inoculations were applied by seed, seedling and soil application methods. Various growth biometrics *viz.* plant biomass, root and shoot growth at 30 and 60 days after transplanting in main field significantly increased over untreated control.

Solaiman *et al.*, (2012) investigated the effect of AM fungi and *Rhizobium* sp on growth characteristics of three different varieties of chickpea (*Cicer arietinum* L.) namely BARI chola -3, 4, 5. In their experiment it was observed that the nodulation, dry matter production, root colonization and spore population of AM fungi, growth and mycorrhizal dependency was increased in chola -3.

Guru *et al.*, (2011) revealed the single and dual inoculation of *G.fasciculatum* on different growth parameters. They observed that the dual inoculation of *G. fasciculatum* improved the plant height, dry weight and available N, P and fruit yield of tomato plants. Sandeep kumar *et al.*, (2011) studied the synergistic effect of AM fungi and *Azotobacter* and phosphate solubilizing bacteria on the growth characteristics of three fiber yielding plants *Corchorus capsularis* L., *Crotalaria juncea* L. and *Hibiscus cannabinus* L. They revealed that the inoculation of *G. fasciculatum* with *Azotobacter* significantly improved the plant height, shoot, root dry weight and phosphate uptake.

**2.3.7. Influence of AMF on Biochemical characteristics**

The effect of association of two arbuscular mycorrhizal fungi, *Glomus macrocarpum* and *G. fasciculatum*, on the concentration and composition of essential oil in coriander (*Coriandrum sativum*) was studied. VAM inoculation increased the essential oil concentration in fruits by as much as 43%. Although significant variation in effectiveness of the two fungal species was observed, the quality of essential oil was significantly enhanced on mycorrhization. GC
characterisation of essential oil showed increased concentration of geraniol and linalool in plants inoculated with *G. macrocarpum* and *G. fasciculatum* respectively (Kapoor, 2002).

Six VA-mycorrhizal fungi were tested for their ability to increase the biomass by colonization of roots and phosphatase activity in three varieties of papaya (*Carica papaya* L). Phosphatase activity due to mycorrhizal colonization showed that both the acid and alkaline phosphatase activity increased significantly in all the three varieties of papaya. Acid phosphatase activity was higher than alkaline phosphatase activity (John Kennedy and Rangarajan, 2001).

Papaya (*Carica papaya* cv. Coorg Honey Dew) plants inoculated with the VA mycorrhizal fungi *Glomus mosseae* and *G. fasciculatum* in sterilized nursery soil showed improved plant height, dry matter as well as P, N and Zn concentrations with no or low levels of phosphorus application. There was an enhanced alkaline and acid phosphatase activity on the root surface and also in the enzyme extract of the root of papaya (Mohandas, 1992).

Prabhu *et al.*, (2013) have shown that the effect of AM inoculation in the protein content showed a greater increase in mycorrhizal plants than in control seedlings and water stressed plants. Their SDS PAGE study revealed prominent protein expression pattern in AM infected soil samples compared to non-infected soil samples.

Total soluble sugar content was increased in the roots of medicinal plants inoculated with *Glomus fasciculatum* (Kavatagi and Lakshman, 2011). VAM fungi significantly increased the net photosynthesis by increasing total chlorophyll and carotenoid contents ultimately increasing carbohydrate accumulation. The VAM fungi also increased stomatal resistance, thereby reducing the rate of transpiration (Mathur and Vyas, 1995). Shrestha *et al.*, (1995) have shown that photosynthesis and transpiration rates of mycorrhizal *Satsuma mandarian* trees were higher than non-mycorrhizal trees. The
chlorophyll content, fresh weight and leaf area were higher in mycorrhizal plants than in non-mycorrhizal plants but differences were significant only under draught stress conditions (Morte et al., 2000).

Ariya and Buchn, (2013) reported that cotton plants inoculated with AM fungi brought about significant changes in chlorophyll a, b and total chlorophyll content. This increase may be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Hayman, 1983; Sampath and Ganesh, 2003; Rajasekaran et al., 2006) or due to the presence of large and more numerous bundle sheath chloroplasts in the inoculated leaves (Krishna and Bagyaraj, 1984).

2.3.8. Efficacy of AM fungi in plant defence

It is a well-known fact that the establishment of AM symbiosis in a plant results in changing physiological and biochemical properties of the host significantly (Linderman, 1992). Among these changes, altered root exudates play a key role to modify the composition and activity of microbial population in the rhizosphere (Bansal and Mukerji, 1994). In addition, substances released by the extraradical mycelium of AM fungi may also have a strong impact on microbial population around the roots (Filion et al., 1999).

The role of AM fungi in disease control has been studied in a number of plant pathogen-host species combinations. Different AM fungal species have been studied and found to be effective in reducing plant diseases caused by pathogens such as species of Cylindrocladium, Fusarium, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Sclerotinium, and Verticillium on different host species (Harrier and Watson, 2004). Biocontrol of R. solani diseases using AM fungi has been studied by many researchers. Guillen et al., (2002) Chandanie et al. (2006) found that pre-inoculation of cucumber (Cucumis sativus L.) seedlings with AM fungus G. mosseae 10 days before transplanting into soil infested with R. solani provided 48% protection against damping-off disease. In

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In contrast, Guenoune *et al.* (2001) found that the defense responses of alfalfa roots to the pathogenic fungus *R. solani* were significantly reduced in roots simultaneously colonized with the AM fungus *G. intraradices*.

Systemic acquired resistance (SAR) plays an important role in the ability of plants to defend themselves against pathogens. SAR occurs in all or most plants in response to colonization of AM fungi. A number of biochemical and physiological changes has been associated with AM colonization including the production of antifungal or oxidative enzymes (Pozo and Azcon-Aguilar, 2007) cell death and deposition of lignin (Saldajeno *et al.*, 2008).

Several AMF species have been found to control soil borne pathogens such as species of *Aphanomyces*, *cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotium* and *Verticillium* (Harrier and Watson, 2004). Matsubara *et al.*., (2001) showed that *Glomus fasiculatum* and *Gigaspora margarita* decreased root rot disease due to *Fusarium oxysporum* f. sp.*asparagi* in asparagus under green house conditions. It has been shown that *Glomus clarum* effectively decreased root necroses caused by *Rhizoctonia solani* in Cowpea (Abdel –Fattah and Shabana, 2002). Khaosaad *et al.*, (2007) reported that *Glomus mosseae* systemically reduced all the diseases caused by *Gaeumannomyces graminis* var. *tritici* in barley. Thygesen *et al.*, (2004) recorded a possible mycorrhiza induced tolerance against Pea root rot caused by the pathogen *Aphnomyces euteiches*, further more the degree of tolerance induction differed between the two AM fungi used *viz.*, *Glomus claroideum* and *G. intraradices*. El- Haddad *et al.*, (2004) found that treatment with the mixture of AM fungi *viz.*, *Glomus intraradices*, *G. mosseae*, *G. clarum*, *Gigaspora margarita*, *G. gigantea* significantly reduced the white rot disease in Onion caused by *Sclerotium cepivorum* in green house and field experiment. Root-rot index was reduced to 1 when *M. phaseolina* inoculated chick pea plants was treated singly with *G. intraradices* (Akhtar and Siddiqui, 2009).
2.3.9. Mechanisms of Bioprotection

There are several biochemical mechanism which have been postulated in plant protection against pathogens. These include;

2.3.9.1. Improved Nutrient status of the host plant

It is well known that AM fungi can improve the nutrient status of their plants (Smith and Read, 2008). Several mineral nutrients especially P, are allocated through the symbiosis to the plant in exchange for carbon (Pearson and Jakobsen, 1993). There is an evidence that plants took up larger amounts of nutrients through their AM fungi (Glomus intraradices and G. mosseae) have increased tolerance for pathogenic infections (Karagiannidis et al., 2002). Aysan and Demir, (2009) suggested that the colonization of Glomus mosseae and G. fasciculatum significantly reduced the disease severity caused by Sclerotinia sclerotiorum in common bean by increased nutrient uptake especially nitrogen and phosphorus. It seems that enhanced plant growth and improved nutrient assimilation and possibly a physical barrier have probably imparted altered resistance to the plants (Dar et al., 1997).

Akkopru and Demir, (2005) observed about 17% reduction in Fusarium wilt of tomato after inoculation of plants with G. intraradices. In addition, the changes in nutrient uptake in the root system, a mycorrhizosphere effect and activation of plant defense mechanisms are thought to be responsible for disease inhibition by AM fungi. Jia et al., (2004) reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in Vicia faba because of the enhanced P supply due to AM fungi inoculation. In addition to phosphate, AM fungi enhance uptake of nitrogen, potassium, calcium, copper, magnesium, iron and zinc (Clark and Zeto, 2000). The obvious contribution to reduction of root rot disease is due to increased nutrient uptake, particularly P and other mineral, because AM symbiosis results in more resistant or tolerant to pathogen attacks (Linderman, 1994). The root rot severity of Chickpea inoculated with Macrophomina phaseolina was reduced by Glomus intraradices. Kumar et al.,
(2004) reported that increased uptake of P and other minerals reduced the root rot incidence in chick pea. AM enhanced both the growth and yield measurements of Rhizoctonia-inoculated potato plants and significantly reduced the harmful effects of the disease (Mohammed et al., 2008). Tabin et al., (2009) reported that dual inoculations of Glomus fasciculatum with Pythium aphanidermatum restricted the progression of damping-off disease of Aquilaria agallocha seedlings. In addition they suggested that, inoculation of AM fungi in the rhizosphere soil improves plant soil interaction by enhancing nutrient status, plant growth, promote co-existence of other microbes and protect the host against pathogens.

2.3.9.2. Competitive interaction with pathogenic fungus

Direct and indirect interactions have been suggested as mechanisms by which AM fungi can reduce the abundance of pathogenic fungi in roots. Pathogenic and AM fungi exploit common resources within the root, including infection sites, space, and photosynthate within the root (Whipps, 2004). Increasing the richness of AM fungal taxa colonizing the root system may result in more intense competition with a pathogenic fungus. Cordier et al., (1996) showed that Phytophthora development was reduced in AM fungal colonized and adjacent uncolonized regions of AM root systems, and that in the former the pathogen does not penetrate arbuscle containing cells. This means that localized competition occurs and even in the absence of systemic resistance, resistance was still induced at some distance from the AM colonized tissue. Linderman, (1994) proposed that the growth of AM fungi and pathogen depends on host photosynthates and that they compete for carbon compounds reaching the root. When AM fungi have primary access to photosynthates, the higher demand may inhibit pathogen growth. Vigo et al., (2000) investigated the impact of colonization by the arbuscular mycorrhizal fungus Glomus mosseae on tomato root necrosis caused by the soil pathogen Phytophthora parasitica. From their results they concluded that the reduction in the number of infection loci is one mechanisms via which AMF achieve bio control of pathogen.
2.3.9.3. Architectural changes in the root system

Gutjahr et al., (2009) reported that AM fungal colonization influences the architecture of rice root system. Interactions between changes in the root system and protection of plant roots from pathogen attack have been demonstrated for several AM species. Matsubara et al., (1995) have observed that egg plants colonized by *Glomus etunicatum* or *Gigaspora margarita* contained higher lignin concentrations in the first order and second order roots compared to non mycorrhizal plants when *Verticillium dahlia* was present. Saldajeno et al., (2008) revealed that mycorrhizal plants deposited host cell wall thickening containing lignins, none sterified pectins and callose around the sites of pathogen infection, whereas, non mycorrhizal plants did not contain, the lignin which acts as a physical barrier to stop the pathogen invasion.

2.3.9.4. Microbial community changes in the rhizosphere

AM symbiosis is known to alter the microbial population composition quantitatively and qualitatively in the mycorrhizosphere. Different factors such as altered exudation patterns, putative direct AM fungal effects, different root size and architecture, altered physiology may contribute to quantitative and qualitative microbial community changes in mycorrhizosphere caused by AM fungi (Toljander et al., 2007). Larsen et al., (2003) reported that changes due to the influence of single AM fungi on mycorrhizosphere microbial communities may in turn lead to reduction of fungal pathogen populations.

2.3.9.5. Activation of plant defense compounds

Mechanisms that can account for the disease control ability of AM fungi may include competition for infection site and host photosynthates, root damage compensation, morphological changes in the host root and changes in microbial communities in the mycorrhizosphere. Modifications in plant physiology following mycorrhizal colonization may explain the decrease in susceptibility to pathogens. These effects may arise through changes in lignin formation (Saldajeno et al., 2008), production of phenolic compounds (Zeng, 2006),
induction of new isoforms of the hydrolytic enzymes like chitinase and β-1, 3–glucanase (EL- Khallal, 2007). The appearance of *Verticillium* wilt was reduced by AM fungus infection and the effects were more apparent in *Glomus etunicatum* than *Gigaspora margarita* in eggplant (Matsubara et al., 1995).

Plant pre-inoculation with native AMF decreased nematode colonization and reproduction (Pena et al., 2006). They suggested that AMF are crucial for the control of root feeding nematodes in natural systems and illustrate that locally operating mechanisms are involved in this process. The tomato plants pre-colonized by the arbuscular mycorrhizal fungus *G.mosseae* showed decreased root damage by the pathogen *Phytophthora nicotianae* var. *parasitica* (Pozo et al., 1996).

Hindumathi and Reddy, (2012) investigated the synergistic effects of inoculation with predominantly occurring indigenous arbuscular mycorrizal fungi *G. constrictum* Trappe individually or in combination with *Rhizobium* and charcoal rot pathogen *Macrophomina phaseolina* on the extent of nodulation, nodules dry weight, nodule nitrogen, biomass, nutrient uptake (NPK) grain yield of soybean and suppression of *M. phaseolina* at harvest and suggested that the dual inoculation of *G. constrictum* and *Rhizobium* not only improved the biomass yield but also exhibited more tolerance to *M. phaseolina*.

The inoculation of *G. fasciculatum* and *G. mosseae*, *Acalospora laevis* in the root system of maize reduced the black bundle disease incidence caused by *Cephalosporium acremonium* (Veerabhadrarwamy and Garampalli, 2011). Their study suggested that AM fungi can be used as bio-control agent as well as growth promoter as it shows negative antagonistic interaction with soil borne plant pathogens and exhibited the ability to supply macro and micro nutrients to the host plants.

Induction of secondary metabolites responses has been reported in other beneficial microbe- plant interactions involving arbuscular mycorrhizal fungi.
Zolfaghari et al., (2013) revealed that the inoculation of arbuscular mycorrhizal fungi cause systemic induction of monoterpenic pathways in *Ocimum basilium*, suggesting that the inoculation with *G. mosseae* and/or *G. fasciculatum* can significantly increase the productivity and reduce the amount of fertilizer required for economically valuable herbaceous crops.

Careful perusal of the literature indicated that there is very limited or no work regarding the diversity of AM in this economically important oil seed crop. Also there is no work on the biocontrol charcoal rot pathogen *M. phaseolina* which hampers sunflower production. Hence the present investigation was carried out in sunflower to fulfill the lacuna that is available.