2. REVIEW OF LITERATURE

The literature on AM fungi has become so voluminous in the last two decades, that it is practically not possible to review it entirely. Several reviews and books have appeared recently giving a comprehensive account on the different aspects of AM (Harley and Smith, 1983; Powell and Bagyaraj, 1984; Gianinazzi-Pearson and Gianinazzi, 1986; Hall, 1988; Smith and Gianinazzi-Pearson, 1988; Sieverding, 1991; Read et al., 1993; Smith and Read, 1997, 2002; Wright, 2005). In this context, an attempt has been made to give a brief review of literature relevant to the present work.

2.1. Mycorrhiza – Fungus - root association

The term “mycorrhizae” was first coined by Frank (1885). Mycorrhizae are non-pathogenic symbiotic soil fungi which invade on or in the root system of host plants. Three types of mycorrhizae are recognized. They are (i) Ectomycorrhizae, (ii) Endomycorrhizae, (iii) Ectendomycorrhizae.

2.2. Arbuscular Mycorrhizal Fungi (AMF)

Among the different types of mycorrhizae, arbuscular mycorrhizal fungi is most prevalent type. This is the most commonly known endomycorrhizae showing symbiotic association with many agricultural crops, shrubs, and many tropical and temperate trees. AM occur over a broad ecological range from aquatic to desert environment. The name vesicular-arbuscular fungus has been introduced by Dangeard (1900). Peyronel (1924) was the first to recognized the VAMF as *Endogone* species. Thaxter (1922) described all the identified species at that time. Mosse (1956) has demonstrated experimentally that *Endogone* species could produce VAM and fungi grew significantly faster in weight and height (Baylis, 1959). There are more than 180 species of AM fungi.
(Morton and Redecker, 2002). Taxonomically VA mycorrhizae belong to the order Glomales with three families *Glomaceae, Acaulosporaceae* and *Gigasporaceae* and six different genera *Acaulospora, Entrophospora, Gigaspora, Glomus, Scutellospora* and *Sclerocystis* (Morton and Benny, 1990).

Recently, Morton and Redecker (2002) 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 the present time, each family consists of one genus, *Archaeospora* including three species forming a typical *Acaulospora* like spores from sporiferous saccule. *Paraglomus* consists of two species forming spores of indistinguishable from those of *Glomus* species.

With the advent of molecular techniques, the classification of AMF has undergone major revision. Under the earlier classification, AMF were placed in the order Glomales within the polyphyletic division Zygomycota (Morton and Benny, 1990). An analysis of almost full-length fungal SSU rDNA sequences exposed a clear separation of the AMF from all the other included fungal groups (Schübler *et al.*, 2001). The AMF are now placed in the new division Glomeromycota. The fungi of the Glomeromycota have coenocytic to sparsely septate mycelium. They reproduce asexually though blastic development of the hyphal tip and form symbiotic relationship with photoautotrophs.

Until 2001, the AMF were grouped into the families Glomaceae, Acaulosporaceae and Gigasporaceae with six different genera according to the earlier spore characteristics base classification (Morton and Benny, 1990). Several species which produce *Glomus* types spores in fact belong to other deeply divergent lineages (Morton and Redecker, 2002) and were
placed in the families Archaeosporaceae and Paraglomaceae. Two new early branching orders, Paraglomales and Archaeosporales were required to accurately classify the fungi within Glomeromycota (Schübler *et al.*, 2001). This new classification includes Geosiphonaceae and Archaeosporales which present contains one fungus that forms endosymbiotic associations with the cyanobacterium, *Nostoc punctiforme* (Schübler, 2002) and produces spores typical to AM fungi.

The characteristic feature of VA mycorrhiza is the presence of two specialized structures namely vesicles and arbuscules. Both these structures are produced by internal mycelium but vesicles are usually formed inter and intracellularly and arbuscules formed only intracellularly. Vesicles are the organs meant for storage purpose absent in certain forms. The arbuscules are highly ramified minute arborescences within a few days of infection as hyphae penetrate mechanically and enzymatically into cortical cells. Arbuscules perform the exchange of nutrients between the fungus and root but they have short life period and are digested by their host, few days after its formation.

### 2.3. Importance of mycorrhizae

Earlier experiments proved that VA-mycorrhizal inoculation could drastically improve plant growth. Even in the unsterile soils, investigations proved that plants do respond to inoculation with efficient strains of VA mycorrhizae (Bagyaraj and Manjunath, 1980). Many workers have the opinion that the improved reproduction and plant growth is due to mycorrhizal inoculation (Xavier and Germida, 1991).

Mycorrhizae are vital for uptake and accumulation of ions from soil and translocation to hosts because of their high metabolic rate and strategically diffuse distribution in the upper soil layers. In fact, the fungus serves as a highly efficient extension of the host root system.
Minerals more than 4 cm distant from the nearest host root can be absorbed by the fungal hyphae and translocated to roots in the mycorrhizal plants. Bieleski (1973), calculated that AM fungi may increase the effective absorbing surface of a host root by as much as ten times. Ions such as P, Zn and Cu do not diffuse readily through soil. Because of this poor diffusion, roots deplete the immobile soil nutrients from a zone immediately surrounding the root. Mycorrhizal fungal hyphae extend into the soil, penetrating the zone of nutrient depletion and can increase the effectiveness of absorption of immobile elements by as much as sixty times.

The main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Ca, Mg and Mn supply to the host roots (Habarte and Manjunath, 1987; Lu and Miller, 1989; Johnson et al., 1991; Kothari et al., 1991; Lambert and Weidensaul, 1991; Li et al., 1991; Champawat and Pathak, 1993; Marschner and Dell, 1994; Smith et al., 1994; Selvaraj and Subramanian, 1995; Abdul Malik, 2000). In addition to this VA mycorrhizae exhibit other synergistic activities such as biological control of root pathogens, biological nitrogen fixation, hormone production and greater ability to withstand water stress (Giller and Cadisch, 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. They absorb and translocate water to the host. Two types of mycorrhizal fungal hyphae regulate nutrient movement; the absorbing, hyphae which are fine and highly branched hyphae that explore substrates absorbing nutrients released from adjacent soils or organic substances. In some instances, they also secrete external enzymes capable of breaking down organic materials or otherwise affecting nutrient availability.
Rhizomorphs are thought of as stress resisting organs of the fungus. AM fungi alter the kinetic properties of the root, thereby enhancing its nutrient uptake abilities. Hence it is clear that mycorrhizal fungi play a vital role in nutrient cycling and productivity of crops (Bansal and Mukerji, 1994).

2.4. AM fungi associated with medicinal plants and their influence on growth and nutrition

AM fungi present in practically all soils associated with a great variety of medicinal plants of different taxonomic groups (Jeffries, 1987). Occurrence of AM fungi in medicinal plants has been reported by several workers. Medicinal plants in India were originally reported to be non-mycorrhizal, probably due to the presence of various secondary metabolites (Mohankumar and Mahadevan, 1984). However, roots of field-grown garlic were found to be colonized by AM fungi (Shuja and Khan, 1977) and this observation has more recently been supported by many workers from Asia who found the roots of various medicinal plants to be mycorrhizal (Laksman and Raghavendra, 1990; Sullia and Prabha Sampath, 1990; Selvaraj and Subramanian, 1990; Sharma and Roy, 1991; Ueda et al., 1992; Burni et al., 1994; Srivastava and Basu, 1995; Ratti and Janardhanan, 1995).

Rao et al. (1988) examined 25 medicinal plant species growing in red sandy loam soil and harboured by VAM fungi in their root systems and observed that Mentha arvensis had maximum colonization and in Glycyrrhiza glabra had minimum colonization. Mycorrhizal spore counts in the root zone soils were highest in Digitalis lanata.

Palmarosa (Cymbopogon martini) was associated with AM fungus, Glomus aggregatum. Glass house experiments showed that inoculation of Glomus aggregatum caused an increase of two-fold growth and three-fold biomass production. These findings indicated the potential
use of AM fungi for improving the production of this medicinal plant (Gupta et al., 1990).

Physiological effects of AM infection in relation to the internal P-concentration in *Plantago major* and *Plectosperma* was examined by Baas and Kuiper (1989). AM plants had a higher rate of root respiration, biomass and higher total P and N concentration in roots compared with non-mycorrhizal *Plantago major*.

Occurrence of AM fungal association in seven aromatic *Cymbopogon* species cultivated in Jorhat, Assam, were observed in which *C. curatus* showed maximum colonization (82.2%). Arbuscules were also observed in four of the seven *Cymbopogon* sp. tested (Janardhanan et al., 1990).

Variation in the response of six cultivated species of mint (*Mentha arvensis* spp. *haplocalyx*, *M. citrata*, *M. piperita*, *M. spicata*, *M. cardiaca*, *M. gracilis* and *M. viridis*) associated with the colonization of arbuscular mycorrhizal fungi were reported. All the species of mint had abundant AM associations. Four species of *Glomus*, one species of *Entrophospora* and one species of *Sclerocystis* were isolated from the rhizosphere soil of these plants. Root colonization varied from 37.2 per cent to 56.0 per cent. The highest level of AM colonization (56.0%) was observed in the roots of *M. spicata* and *M. citrata*. Rhizosphere soil of these plants had an AM spore population ranging from 416-707 spores/100 g of soil. The highest AM spore population was observed in the rhizosphere of *M. spicata*.

The effect of AM fungus, *Glomus fasciculatum*, on isoenzyme patterns of peroxidase and polyphenol oxidase (catechol oxidase) in the roots of *Zizyphus mauritiana*, *Z. nummularia* and *Z. xylopyra* plants
were investigated after 60 days inoculation. All inoculated plants showed two additional peroxidase isoenzymes bands and one additional polyphenol oxidase band; also both peroxidase and polyphenol oxidase showed increased activities.

Krishna Naik et al. (1998) examined the response of *Citronella java* (*Cymbopogon winterianus*) to inoculation with *Glomus fasciculatum* and *Bacillus megaterium* along with super phosphate and rock phosphate at 50 and 100 per cent of the recommended level, under irrigated field conditions. The combined inoculation with *G. fasciculatum* and *B. magaterium* proved to have synergistic beneficial effects on the plant growth parameters, herbage and oil yield. Combined inoculation along with 50 per cent of the recommended level of superphosphate recorded higher herbage and oil yield compared to individual inoculation and control.

Jayanthi Srinath and Bagyaraj (1998) examined a greenhouse investigation, to study the VAM fungus, *Glomus mosseae* and plant growth promoting rhizomicroorganisms (PGPRs), *Bacillus coagulans* and *Trichoderma harzianum* on growth and nutrition of micropropagated sugarcane plantlets. It is evident that *Glomus mosseae* co-inoculated with PGPRs improve the growth and nutrition of micropropagated sugarcane. Calvet et al. (1993) reported the growth response of marigold (*Tagetes erecta* L.) significantly higher plant biomass due to inoculation with *Glomus mosseae, Trichoderma aureoviride* and *Pythium ultimum* in a peat perlite mixture. Dual inoculation with *Aspergillus fumigatus* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus was reported by Tarafdar and Maschner (1995).
The effect of inoculation with two species of VAM fungi (Glomus macrocarpum and Glomus fasciculatum) on growth and essential oil of coriander was reported by Kapoor and Mukerji (1999). The essential oil content in seeds were increased on VAM fungal inoculation. A green house investigation was conducted to screen and select an efficient VAM fungus for inoculating neem (Azadirachta indica). The neem seedlings were inoculated with nine different VAM fungi. Inoculated seedlings generally had greater plant height, stem girth, biomass, P content, Zn concentration, bio-volume index and quality index than uninoculated control plants, and this was reported by Sumana and Bagyaraj (1999). Neem seedlings responded best to inoculation with Glomus mosseae followed by G. fasciculatum.

Several studies showed natural occurrence of VAM in the rhizosphere of medicinal plants (Mukerji and Ardey, 1985; Govinda Rao et al., 1989; Lakshman and Raghavendra, 1990). Though the VAM fungi do not have host specificity, its preference to particular host plant has been observed. Srihari and Sreenivasa (1997) found Glomus macrocarpum as the efficient VAM fungus for improving growth and yield of chilli among the five different VAM fungi tested. Similarly, Glomus monosporum was found to be the best VAM fungus for the Malabar cultivar of Cardamom (Sreeramulu and Bagyaraj, 1997). Inoculation of Glomus intraradices substantially increased the biomass and nutrient uptake of Mentha citrata (Kothari et al., 1999). Earanna et al. (1999) found that Glomus fasciculatum was the best efficient VAM fungus for improving growth, biomass and P content of Coleus barbatus among the six different VAM fungi tested. Similarly, Gigaspora margarita was found to be superior in improving per cent root colonization, spore count and the population of free living N2-fixers and P-solubilizers in the rhizosphere of silver oak plants (Grevillea robusta). Similarly plant height, stem diameter, leaf area, root length, shoot and root dry weight and the concentrations of
P, Zn, Cu, Mn and Fe in shoot were significantly highest in plants inoculated with *Gigaspora margarita* as compared to other VAM fungi (Gurumurthy and Sreenivasa, 1999).

The effect of *Glomus mosseae* inoculation under different levels of phosphate on growth, bulb weight and N and P uptake by garlic (*Allium sativum* L.) and an available P$_2$O$_5$ in soil as reported by Wani and Konde (1999a). The inoculation with *G. mosseae* conjugated with different phosphate levels significantly increased bulb diameter, bulb fresh weight, N and P uptake by garlic plant and available phosphate in soil over uninoculated control.

Wani and Konde (1999b) reported the genotypical variation in root phosphatase activities, available phosphate in soil and P and S uptake of mycorrhizal garlic. All the genotypes recorded significant increase in acid and alkaline phosphatase activities due to inoculation with *Glomus mosseae* resulting in an increase in available P$_2$O$_5$ in the rhizosphere of garlic genotypes and increased P and S uptake.

Abu-Zeyad et al. (1999) examined the effects of AM fungi on the growth and yield of alkaloid, castanospermine of *Castanospermum australe*. The AM fungi, *Glomus intraradices* and *Gigaspora margarita* increased the growth and P content of plants and the yield of castanospermine in the leaves, irrespective of the P treatment. No correlation was found between the alkaloid contents of leaves from mycorrhizal seedlings and from non-mycorrhizal plants which received P.

Gupta et al. (2000) also reported AM fungal association in seven different species of *Ocimum*. Abdul-Khaliq et al. (2001) examined the influence of three species of VAM fungi, namely, *G. aggregatum*, *G. fasciculatum* and *G. mosseae* on the growth of peppermint (*Mentha*
Piperita L.) under glass house conditions; Glomus fasciculatum showed maximum plant height, shoot biomass upto 145.3 per cent followed by G. aggregatum (131.1%) and G. mosseae (87.8%) in comparison to control treatment. The per cent essential oil contents in shoot tissues from unit weight of herb yield did not show significant increase when compared to control.

Ratti et al. (2002) examined the influence of four different species of Glomus viz., G. aggregatum, G. fasciculatum, G. mosseae and G. intraradices on growth, oil content, P content and phosphatase activity of Vetiveria zizanoides. All the four species enhanced biomass, P content of root and shoot tissue, acid and alkaline phosphatase activity of the roots significantly when compared to control plants.

Efficacy of different VAM fungal species towards increasing nitrate reductase (NR) and glutamine synthetase (GS) activities in Ziziphus mauritiana was evaluated by Mathur and Vyas (1995). In general GS activity was higher in all the treatments as compared to NR activity. Addition of VAM increased the activities of both these enzymes. However, different VAM species varied in their efficacy to increase these enzymatic activities. Among the five VAM species used, G. fasciculatum was found to be the most efficient VAM species for Z. mauritiana as it increased most effectively the activities of GS and NR in Z. mauritiana.

Kunwar et al. (1999) reported the qualitative and quantitative composition of AM fungi associated with garlic. Thirty five species of AMF were isolated from the rhizosphere soils of garlic.

Anusuya and Senthilkumar (2003) reported that the combined inoculation of Glomus mosseae and Trichoderma harzianum showed the highest VAM colonization and spore number.
Khade and Rodrigues (2003) reported the incidence of AM fungal association in rhizomatous tubers and roots of *Gloriosa superba* and the diversity of AM fungi associated with it. A total of seven AM fungi belonging to various genera were recorded from the rhizosphere soil of *Gloriosa superba*. The root colonization was characterized by the presence of hyphae, arbuscular and vesicular colonization. The average root colonization of 50.38 per cent and spore density of 250.6 spores per 100 g rhizosphere soil was recorded during the study.


Recently, Rama Bhat and Kaveriappa (2004) reported the association of VAM fungi with the roots and rhizomes of *Marsilea minuta*, an aquatic fern growing in the marshes habitat. The rhizomes and roots showed the presence of hyphae and vesicles. Arbuscules were not
observed. The mycorrhizal colonization was 60-70 per cent in both rhizomes and roots.

2.5. AM – Concept of Host Specificity

Mycorrhizal associations are generally considered to benefit host plants by enhancing mineral nutrient acquisition especially with regard to phosphorus. AM associations may also improve nitrogen uptake (Barea et al., 1989). Increase in the absorption of micro nutrients, such as Mg, Cu and Zn have also been observed, but Mn uptake can be reduced (Harley and Smith, 1983; Killham, 1985; Arines and Vilarino, 1989). Other less specific changes to host physiology, which include alterations in nutrient requirements, membrane composition and metabolite levels, apparently occur even when nutrient input is negligible (Pacovsky, 1986).

There is little evidence of host specificity in most types of mycorrhizal associations (Harley and Smith, 1983; Gianinazzi-Pearson, 1984; Duddridge, 1987). Ineffective AM associations have been discovered in only a few of the many host plant and mycorrhizal fungus combinations tried in synthetic experiments (Giovannetti and Hepper, 1985). Thus relatively few endophytes can form associations with most members of the plant kingdom (Morton, 1990). Genotypic variations within a host species can influence the degree of AM formation (Azcon and Ocampo, 1981; Thomas and Ghari, 1987; Lackies et al., 1988; Sieverding and Glavez, 1988).

Root growth is usually slightly affected by AM but a detrimental reduction in root elongation occurs in some cases (Jones and Hendrix, 1987). Mycorrhizal associations have been implicated in increased host resistance to disease and other stresses. A few species of AMF are either pathogenic (Modjo and Hendrix, 1986) or non-beneficial (Muthukumar et al., 1997) to certain host plants.
It is well known that the AM are not host specific. Any AM plant species can be infected by any AM fungal species but the degree of AM infection and its effect can differ with different host endophyte combinations.

2.6. Inoculum Production

Several efforts have been made to mass multiply AM inocula. Cultures of AM fungi on plants growing in disinfested soil has been the frequently used technique to increase propagule numbers. A highly susceptible host plant should be used. It should produce abundant roots quickly and tolerate the high – light conditions required for the fungus to reproduce rapidly. Trap plants should be screened to insure that maximum levels of inoculum are achieved (Bagyaraj and Manjunath, 1980). Several host plants including sudan grass (*Sorghum bicolor* var. *sudanense*), *bahia* grass (*Paspalum notatum*), guinea grass (*Panicum maximum*), *cenchrus* grass (*Cenchrus ciliaris*), clover (*Trifolium subterraneum*), strawberry (*Fragaria* sp.), sorghum (*Sorghum vulgare*), maize (*Zea mays*), onion (*Allium cepa*) and *Coleus* (*Coleus* sp.) have been used for their suitability to multiply AM fungal inoculum. Sreenivasa and Bagyaraj (1988b) reported that rhodes grass (*Chloris gayana*) is the best host for mass multiplication of *Glomus fasciculatum*. Host plants which can be propagated from seeds are preferable to cuttings, since seeds can be more readily disinfested than cuttings. All components of the culture system should be disinfested prior to initiation. The purpose of soil disinfestation is to kill the existing AM fungi, pathogenic organisms and weed seeds. Pasteurization of soil by heating it to 85°C for two 8 h periods, 48 h apart yields good results (Sylvia and Jarstfer, 1994).

The containers used for pot cultures should be protected from contaminated soil, water and insects. Specific isolates of AM fungi should be kept well separated to reduce cross contamination. Container size
should match with the potential volume of the system within practical space constraints. Ferguson and Menge (1982) found that using large containers may give higher spore density. Light quality and irradiance, soil water content and temperature influence root colonization and spore production. Non-shaded green houses or high intensity metal-halide and sodium vapour lamps used as supplemented light give good results. Dehne and Backhaus (1986) used sodium vapour high pressure lamp with an intensity of 5000 lux for 16 h per day as an additional high source. Feldmann and Idezak (1992) found that 14 h light per day with an intensity of 1000 lux or more generated by mercury vapour lamps proved the quality of inoculum produced in the green house. But in humid tropics, intensive irradiation results in high temperature in the green houses, which are not normally air conditioned. This has a detrimental effect on plant growth. A moderate watering regime often supports optimal spore production (Nelson and Safir, 1982). Sporulation is positively correlated with temperature from 15 to 30°C for many fungi (Schenck and Smith, 1982) and hence a warm growth environment should be maintained.

Some biocides applied in the soil greatly reduce or eliminate AM fungi (Trappe, 1984) while others increase colonization and sporulation (Sreenivasa and Bagyaraj, 1988a). Though the use of selected pesticides in inoculum production has been suggested, they have rarely been used to protect against or reduce organisms that contaminate the pot cultures. AM fungal responses to P and N fertilization are strain dependent. In general, a nutrient regime low in P but high in N increases AM colonization (Sylvia and Neal, 1990).

Soil inoculum from pot cultures of many *Glomus* spp. may be stored in polythene bags for many months or even years together. Inoculum of *G. fasciculatum* produced in pot cultures and packed in polythene bags was viable after 4 years of storage (Ferguson and
Woodhead, 1982). Feldmann and Idezak (1992) found that the infectivity of *G. etunicatum* inoculum which was stored at 20 – 23°C and 30 – 50 per cent relative humidity for 3 years was reduced by only 10 – 15 per cent. However, for more reliable storage of wide range of AM fungi, the fungi can be dried *in situ* with the host plant and frozen at 70°C (Douds and Schenck, 1990). Freeze dried roots have been used as inoculum (Ganry *et al.*, 1985). In addition, spores, hyphae, vesicles and colonized roots from pot cultures have been pelletized with alginate (Ganry *et al.*, 1985; Strullu and Plenchette, 1991; Rashmi and Bagyaraj, 1997).

2.7. Selection of an efficient AM fungi by using host plant

Species of AM fungi that can either directly or indirectly increase plant growth by improving soil conditions need to be selected (Kapoor and Mukerji, 1999). Direct benefits are usually related to the enhancement of phosphate uptake by the plant, however, in some soils enhanced uptake to zinc, copper and ammonium are also important (Stribley, 1987). Indirect benefits may include increased soil aggregation or stabilization of soil associated with hyphae formed in the soil. The selected inoculants should have the ability to colonize roots rapidly following inoculation, absorb phosphate from the soil, transfer phosphorus to the plant, increase plant growth and persist in the soil as required (Raman and Mahadevan, 1996). An excellent discussion on the selection of AM fungi for possible use in agriculture has been first reported by Abbott and Robson (1982). Govinda Rao *et al.* (1983) suggested that several fungi could be screened for symbiotic response using a test-host through pot culture followed by microplot and then by field trials. Many studies have led to the selection of AM fungi for many economically important agricultural crop and medicinal plants such as *Allium cepa* (Ramana and Babu, 1999); *Ananas comosus* (Jaizme-Vega and Azcon, 1995); *Arachis hypogaeae* (Vijayakumar and Bhiravamurthy, 1999); *Acacia nilotica* (Reena and Bagyaraj, 1990); *Azadirachta indica* (Sumana and Bagyaraj, 1999);
Cajanus cajan (Ahiabar and Hirata, 1994); Capsicum (Jaizma-Vega and Azcon, 1995); Colacasia esculenta (Ganesan and Mahadevan, 1994); Cyamopsis tetragonoloba (Mathur and Vyas, 1996); Coleus aromaticus (Selvaraj et al., 1996); Citrus (Vinayak and Bagyaraj, 1990); Eleusine coracana (Tewari et al., 1993); Glycine max (Vasuvat et al., 1987); Lycopersicum esculentum (Mallesha et al., 1994); Leucaena leucocephala (Byrareddy and Bagyaraj, 1988); Cassava (Sivaprasad et al., 1990; Ganesan and Mahadevan, 1994); Musa acuminata (Jaism-Vega and Azcon, 1995); Oryza sativa (Secilia and Bagyaraj, 1994; Ammani and Rao, 1996); Phaseolus mungo (Vasuvat et al., 1987); Polianthus tuberosa (Gaur et al., 1998); Trifolium (Gazey et al., 1992); Tamarindus indica (Reena and Bagyaraj, 1990); Tectona grandis (Rajan et al., 2000); Triticum aestivum (Asif et al., 1995); Vigna mungo (Rao and Rao, 1996); Vigna radiata (Rao and Rao, 1996); Vigna unguiculata (Ahiabor and Hirata, 1994); Zea mays (Boucher et al., 1999); Zizyphus mauritiana (Mathur and Vyas, 1996); Ocimum basilicum (Hemalatha, 2002); Wedelia chinensis (Sankar, 2002); Cichorium intybus (Murugan and Selvaraj, 2003); Gloriosa superba (Elango, 2004); Andrographis paniculata (Chiramel et al., 2006); Plantago ovata (Mathur et al., 2006) and Alpinia galanga (Mani, 2004).

2.8. Benefits of arbuscular mycorrhizae

2.8.1. Nutritional

2.8.1.1. Phosphorus

Gianinazzi-Pearson and Gianinazzi (1978) assessed soluble acid and alkaline phosphatases in arbuscular mycorrhiza. There is a considerable accumulation of information about the effect of AM infection on plant growth and nutrition (Harley and Smith, 1983; Abbott and Robson, 1984). Inositol phosphatase, phospholipids and nucleic acid are the predominant P compounds, that occur both as soluble ‘P’ in soil solution, as insoluble ‘P’ adsorbed on to soil organic matter. Organic P (Po) in soils is generally mineralized to inorganic P(Pi) before it becomes
available to the plants either by simple autolysis or by enzymatic dephosphorylation.

2.8.1.2. ‘P’ uptake mechanism

Plant responses to AMF infection have received several explanations, of which ‘P’ uptake has consistently proved to be the primary factor providing a benefit. Mechanisms suggested for improved ‘P’ uptake by AMF infected root include: (1) infection and proliferation of AM hyphae in the host roots; (2) better distribution of absorbing network; (3) greater surface area and faster extension rate of active hyphae involved in absorption and (4) more favourable geometry of hyphae relative to roots and exploration of smaller pore spaces in soil.

Factors that increase surface area of the root such as root hairs and mycorrhizal hyphae, will be advantageous to the absorption of nutrients present in low concentration in soil solution. Root growth rate and radius are the two most important root parameters for ‘P’ uptake where uptake increases with root radius because of increased surface area.

P uptake depends on narrower absorbing structures, the possibility that AMF strains with narrow hyphae may be more effective in promoting growth of clover and sorghum than *G. fasciculatum* and *Gigaspora margarita*, which had hyphal diameters of 2-5 and 5-10 µm respectively (Suresh, 2006).

An increased concentration of P in mycorrhizal plant compared to non-mycorrhizal plants has been reported in *Griselinia littoralis* (Baylis, 1959); onion (Gray and Gerdemann, 1969); soybean (Bagyaraj *et al.*, 1979); sweetgum, tulip tree (Gray and Gerdemann, 1967); cotton, cowpea finger millet (Bagyaraj and Manjunath, 1980); chilli (Sreeramulu and Bagyaraj, 1986); *Citrus* sp. (Kleinshmidt and Gerdemann, 1972); barley
(Benians and Barber, 1972); *Alpinia galanga* (Mani, 2004); *Gloriosa superba* (Elango, 2004); *Sorghum bicolor* (Suresh, 2006); *Hibiscus cannabinus* (Umamaheswari, 2007).

2.8.1.3. Nitrogen

Nitrogen have been reported to stimulate as well as suppress root colonization by AM fungi. There has been several reports on the suppression of root colonization by N (Buwalda and Cole, 1982). Chambers *et al.* (1980) reported that both NO$_3^-$ and NH$_4^+$ depressed root colonization by AM fungi and suggested that the suppressive effective of NH$_4$ was due to drop in rhizosphere pH. Thompson (1986) also found that N source influenced AM colonization primarily through pH modifications. In contrast N application has been reported to increase AM fungal colonization in *Leucaena* (Aziz and Habte, 1989) and lettuce (Hepper, 1983). Sylvia and Neal (1990) found that the suppressive effect of P on root colonization was evident only when plants were N sufficient and plants N stress affects the resistance of the host colonization by AMF. AM increase nitrogen accumulation in plant roots and the various mechanisms suggested include; (i) direct uptake of combined nitrogen by AM fungi; (ii) improvement of symbiotic biological N$_2$ fixation can have indirect AM activity based on the supply of phosphate for N$_2$ fixation and functioning; (iii) interplant transfer of N, a process by which biologically fixed N by nodulated plants benefit the non-fixing plants growing near by and (iv) enzymatic activities involved in N metabolism.

Nitrogen uptake from soil by AM fungi can be affected by a number of factors, which inturn influence the predominant available forms if nitrogen i.e., NH$_4^+$ and NO$_3$. Thus factors such as organic matter content, soil texture (clay content), microbial mineralization and nitrification can greatly influence N uptake via extrametrical hyphae (Arines, 1990).
2.8.1.4. Interplant 'N' transfer through AMF

It is well documented that the increase in plant growth resulting from AM symbiosis is usually associated with increased nutrient uptake by the hyphae from the soil (Harley and Smith, 1983). It is widely accepted that a hyphal network associated with the roots of a living plant is capable of infecting the roots of other plants growing in its vicinity (Chiariello et al., 1982; Francis and Read, 1984; Newman, 1988). Francis et al. (1986) indicated that mycorrhizal connections are likely to be involved in nutrient transfer between plants. Inter-connections may also have an effect on nutrient recycling from dying to younger plants (Eason et al., 1991). Several reports have documented that a non-legume can benefit from N supplied by an inter cropped legume (Ledgard et al., 1985; Ta and Faris, 1987). Experiments using $^{15}$N as a tracer have shown that AM hyphae may be involved in nitrogen transfer from a legume to a non-legume (Van Kessel et al., 1985; Hamel et al., 1991).

2.8.1.5. Potassium and other nutrients

The role of AM fungi in the uptake of K, Ca, Mg and S is little known. Eventhough there are many reports on the effects of AM on concentration and the amounts of K in the plants these results are inconsistent and difficult to interpret (Sieverding and Toro, 1987). The ability of the extrametrical AM fungal hyphae to uptake and transport K has also been demonstrated in compartmented pots (George et al., 1992). Significant differences in growth response of soybean to different geographical isolates of G. mosseae seemed to be more related to import K rather than P nutrition of the host (Bethlenfalvay et al., 1989). The hyphae uptake of Ca (Rhodes and Gerdemann, 1978) and $SO_4^{2-}$ (Cooper and Tinker, 1978) has been shown through supplying radio isotopes ($^{44}$C, $^{35}$SO$_4$). The uptake and transport rates of Ca is very low compared to P. A substantial contribution of hyphae delivery to the host plant is not
likely under most cases because of high mobility of $\text{Ca}^{2+}$ and $\text{SO}_4^{2-}$ in the soil.

The numerous reports on the enhancement of Zn and Cu uptake by AM plants can be attributed to the uptake and transport in external hyphae to the host plant. The hyphal contribution to the uptake of Zn ranged from 16-25 per cent compared to 13 to 20 per cent for P in maize grown in calcareous soil (Kothari et al., 1991). In the same soil, Li et al. (1991) demonstrated that the delivery of Cu from the hyphae compartment ranged from 52 to 60 per cent of the total Cu uptake under restricted rooting space. In contrast, Mn uptake and concentration in plants are either unaffected but more often are lower in AM plants (Lambert and Weidensaul, 1991). The decrease in concentration of Mn in plants is most likely an indirect effect caused by AM induced changes in the rhizosphere microorganisms in population of Mn reducers (Kothari et al., 1991). The role of AM on boron (B) nutrition of the host plant is either lacking or inconclusive. AM may decrease B concentrations in host plants (Kothari et al., 1991). Plants have varying mechanisms for mobility chelating and reducing ferric (Fe) in order to facilitate uptake. Treeby (1992) indicated that AM fungi may facilitate the Fe uptake in acidic but not in alkaline soils. An increase of Fe uptake by arbuscular mycorrhizal plants has also been shown (Raju et al., 1987). Swaminathan and Verma (1979) using Zn indicate that mycorrhizal and non-mycorrhizal plants utilize same soil Zn pool similar to P. Further a direct relationship between Cu and Zn to mycorrhizal development has been demonstrated by Lambert et al. (1979) and Suresh (2006).

There have been reports that AM colonization affects sulfate ($^{35}\text{S}$) uptake by plants (Rhodes and Gerdemann, 1980). Since, sulfate is rather mobile in the soil solutions hyphal translocation do not seem critical for sulfate nutrition (Barea, 1991). But the increase in sulfate concentration
can be due to an improved phosphate nutrition mediated by AM. Harley and Smith (1983) also indicated the absence of conducive support for the role of AM in $K^+$ uptake inspite of low diffusion rates of these ions in soil solution.

An increase in $Br^-$ and $Cl^-$ and anions in plants in response to AM infection has also been reported (Buwalda et al., 1982). But these increase may unlikely be related to AM in view of their mobility in soil solutions. These ions play a role in the regulation of cellular pH, which varies for mycorrhizal and non-mycorrhizal plants.

2.9. Phytochemical compounds

Metabolism is the collection of chemical process by which an organism creates and maintains its substance and obtains energy in order to grow and function. Almost all of these chemical processes involve organic compounds. Many of the end products of metabolism are readily isolable organic compounds and have historical importance in organic chemistry. The compounds are grouped together under the heading of natural products. They are primary metabolites such as lipids, carbohydrates, proteins, nucleic acids and secondary metabolites such as alkaloids, terpenoids, steroids and flavonoids are most important (Trease and Evans, 1980).

2.9.1. Lipids and AM fungi

Studies on the lipid metabolism of AM fungi are very limited. Cooper and Losel (1978) showed that roots infected by $G. mosseae$ contained significantly more lipid than the infected roots. The mycorrhizal roots of Allium cepa, Trifolium repens and Lolium perenne contained large amount of total lipid than the non-mycorrhizal roots. Increased amount of triglyceride, diphosphatidyl glycerol, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl glycerol,
phosphatidic acid and phosphatidyl inositol are presented in the mycorrhizal onion roots than control plants.

Oil droplets have been frequently recorded in the hyphae and spores of AM fungi (Mosse and Boven, 1968; Ho and Trappe, 1975). Cox and Sander (1974) published electron micrographs of AM fungi illustrating lipid droplets in the arbuscules, vesicles and intercellular hyphae. Nordby et al. (1981) analysed the fatty acid composition in citrus roots colonized by *G. fasciculatum*. They opined that C20 fatty acids in mycorrhizal roots are likely to have been derived from the fungal symbiont, since higher plants lack the biosynthetic capacity to produce C20 fatty acids. Pacovsky and Fuller (1988) revealed that 4 soyabean cultivars infected by *G. fasciculatum* had higher level of fatty acids in both leaf and roots than non-mycorrhizal plants. Their findings further showed increased length of fatty acid chain in root and leaf of all the four cultivars.

2.9.2. AM and Proteins

Protein content was much higher in mycorrhizal than in non-mycorrhizal root extracts, in tobacco and onion (Dumas et al., 1989). A 2.6-fold higher protein content was found in mycorrhizal than in non-mycorrhizal red clover roots. Other reports have not shown such a large difference in both types of roots as have been described so far, and further deep studies of this aspect are needed. Perhaps this difference is a consequence of facts such as higher metabolic activity in AM-colonized root cells and the presence of internal and external fungal mycelium. It is difficult to speculate further because our actual knowledge of AM fungal proteins is very limited, and we do not know if the new proteins are of fungal (or) plant origin. Five polypeptides with 16, 17, 18, 22 and 30 KDA were found only in AM roots, and were considered to be AM mycorrhizins (Pacovsky, 1989). Wyss et al. (1990) have also found some
polypeptides in both the low and high molecular weight ranges, which were immuno-precipitated with an antiserum against soluble nodulins (or) membrane-bound nodulins.

Some polypeptides, at both low and high molecular weight, were more abundant in (P₂, P₆ and P₈) (or) were exclusive to (P₄, P₅ and P₇) mycorrhizal roots, others were less abundant (P₁ and P₃) in mycorrhizal than in non-mycorrhizal roots. The similarity in the degree of nodulation in both mycorrhizal and non-mycorrhizal roots allows as to discard the possibility of a bacterial origin for these polypeptides. After mycorrhizal infection, changes in polypeptide, accumulation fall into three categories: decreasing, increasing or synthesis of new proteins (Martin and Hilbert, 1991). We do not know the role of polypeptides in the symbiosis. But after IEF analysis and staining for activity of SOD, an SOD isozyme appeared only in mycorrhizal root extracts.

2.9.3. AM and Amino acids

The interactions between AM fungus and plant cell takesplace at both extracellular and intracellular level, Jeanmaire et al. (1988) showed high enzymatic activity in the interface between the cells of root and endomycorrhizal hyphae. According to Gianinazzi (1991) most metabolic process occur at the interface between the fungus and plant cell. The mycorrhizal roots possess higher pectin esterase and endo-polymethyl galacturonase activities than the non-mycorrhizal roots (Garcia-Romera et al., 1991).

Nemec and Meredith (1981) found that G. etunicatum inoculated Citrus limon leaves had higher total amino acids than control. Krishna and Bagyaraj (1983) found proteins and amino acids increased in G. fasciculatum inoculated Arachis hypogaea roots. Dumas et al. (1994) observed higher protein content in AM fungi inoculated Nicotiana
tobacum and onion (Allium cepa) roots than the control. There was no appreciable difference in free amino acid composition among the different treatments except proline and arginine which were higher in the mycorrhizal plants than in all other treatments. In T. basicola infected plants, the free amino acids level increased in the initial stages and decreased in later stages compared with the control plants. High amount of protein was shown in G. mosseae infected Trifolium pratense roots (Arines et al., 1993).

2.9.4. AM and Peroxidase

Higher peroxidase activity has been demonstrated in mycorrhizal than in non-mycorrhizal roots (Spanu and Bon-Fante Fasolo, 1988). This agrees with the results on SOD activity, in that the activities of both enzymes are associated with the defence of plants against oxidative stress produced by generating of active oxygen species (Rabinnowitch and Fridovich, 1983). More interesting is the presence of new SOD isozyme associated with the symbiosis (Palma et al., 1992). This may operate as the interface. Both nodulation (Becana et al., 1989) and environmental stress were associated with increased SOD activity.

2.9.5. AM and Phosphatases

The capacity of AM plants to utilize complex inorganic phosphate was first demonstrated by Daft and Nicolson (1969). The activity of some mycorrhizal fungi in culture to utilize some phytates (Theodorou, 1968, 1971) and presence of an active P-nitrophenyl phosphatase in Fagus sylvatica mycorrhizas (Woolhouse, 1969) has already been demonstrated. The activities of P-nitrophenyl phosphatase in mycorrhizal roots and decaying petioles of Fagus sylvatica and found that P-nitrophenyl phosphatase activity of mycorrhiza was 16 times greater than that of decaying petioles.
Mac Donald and Levis (1978) cytochemically demonstrated the presence of phosphates in *G. mosseae*. Acid phosphatase was found in lysins and growing AM fungal structures. The presence of AM specific alkaline phosphatase activity in *Allium cepa* and *Plantanus occidentalis* plants inoculated with *G. mosseae* has been reported by Gianinazzi-Pearson and Gianinazzi (1976). Qualitative changes of root soluble phosphatase activity were observed during AM infection. Gianinazzi-Pearson and Gianinazzi (1978) demonstrated the presence of soluble alkaline phosphatase specific to AM fungal infection in enzyme extracts from onion roots inoculated with *G. mosseae*. Maximum activity occurred when the infection was still in an early stage (100% arbuscular). Gianinazzi-Pearson and Gianinazzi (1983) found the increased alkaline phosphatase activity in *G. mosseae* and *Glomus* sp. inoculated soybean (*Glycine max*) roots of non-phosphate fertilized plants than in 50d old phosphate fertilized plants.

Smith and Gianinazzi-Pearson (1987) found higher alkaline phosphatase activity of *G. mosseae* than the infected roots of *Allium cepa*. Dodd *et al.* (1987) compared that the effect of individual inoculation of *G. geosporum, G. monosporum* and *G. mosseae* on acid phosphatase activity in roots of *Brassica napus, Triticum aestivum* and *Allium cepa*. They found higher acid phosphatase activity in plants infected with *G. geosporum* and *G. mosseae* than in *G. monosporum* inoculated plants. Anita *et al.* (1988) found high acid phosphatase activity in *G. fasciculatum* inoculated roots of *Trigonella* sp. Polyphosphatase activity increased in the mycorrhizal plants. Tissssserant *et al.* (1993) showed by histochemical tests the presence of alkaline phosphatase in *Glomus* spp., infected roots and *Allium porrum* and *Plantanus acerifolia*. 
2.9.6. AM and Nitrate reductase

The greater part of the nitrogen incorporated into plants is taken up as nitrate from the soil. It has not been demonstrated as yet whether the mycorrhizal fungi themselves reduce nitrate to nitrate and ammonia, and synthesize an amino acid which is transferred to the host by active transport. Proteins and/or amino acids could be made available from the plant with each degradation of an arbuscule within root. An alternative is that nitrate could be directly transferred to the plants, which then reduce it and supply the symbiotic fungus. The key enzyme for nitrate assimilation is nitrate reductase. Sequence comparison and/or DNA-DNA hybridization indicated that the PCR amplificates did indeed come from the organisms from which DNA had been isolated. A DNA hybridization experiment with the digoxigenine labeled PCR-segment and DNA isolated from about 0-5 million *Glomus* spores confirmed that this mycorrhizal fungus possesses a nitrate reductase gene (Kaldorf et al., 1994). The data indicated that the parasitic fungi tested might possess the enzyme. This somewhat surprising result is generally in accord with older enzyme activity measurement.

2.9.7. AM and Phenolic compounds

Plant phenolics, in particular flavonoids and isoflavonoids, are the most widespread classes of secondary metabolites and are known to be involved in plant-microbe interactions. The implication of phenolic compounds in mycorrhizal associations, were Dehne and Schonbeck (1979) who found that first inoculation of tomato with *Glomus mosseae* increases the total soluble phenol content of the roots. In 1984, Krishna and Bagyaraj also found that *Glomus fasciculatum* increased total soluble phenols in roots of *Arachis hypogaea*, while Codignola et al. (1989) did not report any effect of *Glomus versiforme* inoculation in the well-bound phenol content in roots of *Allium porrum* and *Ginko biloba*. Recently, El-Ghachotouli (1995) observed an increase in the total soluble phenol
content of *Pisum sativum* roots when colonized by *Glomus mosseae*, but only under a low-nitrate growth regime.

The analysis of total root phenol content may be an indication of plant reaction to a given mycorrhizal fungus, but more precise information can be obtained by analyzing specific phenolic compounds. In particular, phytoalexins, the antimicrobial phenolics induced by pathogens and intensively studied in plant pathogenic interactions, have received some attention in mycorrhizal associations on soybean. Glyceollin, principally constituted three isomers with glycollin I as a major compound, has been shown to accumulate in soybean roots when mycorrhizal infection is mature and well-developed. The absolute concentration of these phytoalexins remains low compared with tissues infected by pathogens. In particular, this is illustrated by the work of Wyss et al. (1991) who compared glyceollin accumulation in soybean roots infected by *Glomus mosseae* or *Rhizoctonia solani*. Up to 20 days after inoculation, they detected glyceollin levels in mycorrhizal roots but found rapid accumulation of glyceollin in *Rhizoctonia*-infected roots.

Recently, the phytoalexin medicarpin has been shown to increased in *Medicago truncatula* roots 7 to 13 days after inoculation with *Glomus versiforme* (Harrison and Dixon, 1993), but Volpin et al. (1994) did not detect medicarpin in roots of *Medicago sativa*. A non flavonoid phytoalexin, the furanoacetylene weyrone, was reported to accumulate in *Vicia faba* inoculated by a mixture of three *Glomus* species (Kape et al., 1992).

Many other phenolics, but not phytoalexin molecules, have been analysed in plant/mycorrhizal fungus interaction. In particular, some flavonoids and isoflavonoids with low anti-microbial properties often associated with phytoalexin induction but not considered to be true
phytoalexins, have been shown to react positively to mycorrhizal infection. Examples are coumestrol on soybean (Morandi et al., 1984), *Medicago truncatula* and *Medicago sativa* (Harrison and Dixon, 1993), daidzein on soybean (Morandi et al., 1984), *Medicago truncatula* and *Medicago sativa* (Harrison and Dixon, 1993), and *Medicago sativa* (Volpin et al., 1994), 4’, 7’ dihydroxyflavone (Harrison and Dixon, 1993).

The induction of these molecules by mycorrhizal fungi is of interest because it reflects a general induction of flavonoid/isoflavonoid metabolism, and some of them (coumestrol, daidzein, formononetin) are involved in signaling between legume and Rhizobium, in particular as Nod gene inducers (Kape and Werner, 1990). This also poses the question of their potential role in signaling between the plant and the mycorrhizal fungus.

Conjugate forms of some isoflavonoids have been identified in plant-mycorrhizal fungus inter-actions; formononetin malonyl glucoside and medicarpin malonyl glucoside were increased by Glomus versiforme inoculation of *Medicago truncatula* and *Medicago sativa* (Harrison and Dixon, 1993).

The function of isoflavonoid conjugates has not yet been clearly established. Some data suggest that medicarpin malonyl glucoside may be converted to medicarpin in alfalfa (Dixon et al., 1992). Therefore, the increased accumulation of conjugates after mycorrhizal infection could provide a source of precursors that could be rapidly metabolized in active aglycones forms when the plant is challenged by stress or pathogen attack.

Cell-wall – bound phenolics have been studied in some mycorrhizal interactions, as they are known to play a role in strengthening the cell wall and preventing invasion by pathogens (Graham and Graham,
1991). Dehne and Schonbeck (1979) observed an increase of lignin in roots of cucumber when challenged by Glomus mosseae. Although Codignola et al. (1989) did not find any effects on inoculation of Allium porrum or Ginko biloba with Glomus versiforme in root contents of the bound phenolic acids ferulic acid and p-coumaric acid, Grandmaison et al. (1993) reported a significant increase of these compounds in wall root fraction of Allium cepa inoculated with Glomus intraradices of Glomus versiforme. In addition, they observed induction of cell-wall-bound N-feruloyltyramine while soluble N-ferulolyltyramine was inhibited by the same fungi.

Thus the inoculation of plants by mycorrhizal fungi alters their root content in the metabolites of several families of phenolic compounds, like phytoalexins, isoflavonoids and some of their conjugated forms and cell-wall – bound phenolic acids. The most general effect observed is an accumulation of the majority of these phenolics. Inconstancies of results obtained by different laboratories, however, make its interpretation difficult. Information obtained by studies on the enzymes of the biosynthetic pathways of phenolic compounds is more plentiful.

2.9.8. AM and Enzymes of phenolic metabolism in roots

One important property of peroxidase is to catalyze the oxidation of cell-wall-phenolic compounds to form more hydrophobic polymers like lignin. Dehne and Schonbeck (1979) reported significantly increased level of peroxidase activity in roots of tomato inoculated with Glomus mosseae. In addition, these authors found an increase in the activities of β-glucosidases, enzymes that are involved, in particular, in the lignification process or in the release of free aglycone flavonoids.

Spanu and Bon-Fante Fasaolo (1988) found that cell-wall-bound peroxidase activity was increased in Allium porrum roots only during the
first stage of infection by *Glomus versiforme* and then declined to non-mycorrhizal levels when infection was fully developed. Gollotte (1994) found no induction of peroxidase activity in roots of pea after inoculation by *Glomus mosseae*.

The key enzyme in phenylpropanoid metabolism phenylalanine ammonia-lyase (Pal) is one of the most-studied in mycorrhizal interactions. Its activity was found to increase in roots of tomato inoculated with *Glomus mosseae* (Dehne and Schonbeck, 1979) and in roots of *Medicago sativa* inoculated by *Glomus intraradices*, but only at the early stage of infection (Volpin *et al.*, 1994), and in cell walls of *Ginkgo biloba* challenged by *Glomus versiforme* (Codignola *et al.*, 1989).

More recently levels of mRNA-encoding PAL and other major enzymes of phenylpropanoid biosynthesis were evaluated in mycorrhizal associations. PAL transcripts were found to be increased in roots of *Medicago truncatula* during *Glomus versiforme* infection (Harrison and Dixon, 1993). In bean and parsley roots, PAL and chalcone synthase (CHS) transcripts were not affected by endomycorrhizal infection (Franken and Gnadinger, 1994), whereas in *Medicago truncatula* CHS transcripts were increased in roots by *Glomus versiforme* inoculation. CHS is the first enzyme of the flavonoid/isoflavonoid pathway and is followed by chalcone isomerase (CHI) whose activity has been shown to increase in roots of *Medicago sativa* only in young infection by *Glomus intraradices* (Volpin *et al.*, 1994). Harrison and Dixon (1993) found no effect of *Glomus versiforme* inoculation on CHI transcripts of *Medicago truncatula*. Concerning a more specific enzyme of phytoalexin biosynthesis, isoflavone reductase (IFR), the penultimate enzyme of medicarpin synthesis, Harrison and Dixon (1993) found inhibition of its transcripts in roots of *Medicago truncatula* during the mature stage of *Glomus versiforme* infection. In parsley, transcripts of bergaptol-O-
methyltransferase (BMT), an enzyme involved in the bioynthesis of the furanocoumarin phytoalexins, were found in lower amounts in mycorrhizal roots (Franken and Gnadinger, 1994).

Recently, a study on roots of *Medicago truncalula* using in situ hybridization was used to localize transcript accumulation of phenylpropanoid/flavonoid metabolism enzymes after inoculation with *Glomus verisforme* (Harrison and Dixon, 1994). PAL and CHS transcripts were localized specifically in cells containing arbuscules whereas IFR transcripts that were detected at relatively high levels in cortical cells of non-colonised parts of the roots, were undetectable in arbruscular cortical cells. These results suggest, at a cellular level, the promotion by the mycorrhizal fungus of two different signaling pathways, one for the induction of PAL and CHS, and another for the suppression of IFR.

### 2.14. Synergistic effect of AMF and PGPRs on growth response of crop and medicinal plants

The agriculturally important microorganisms have their own unique place and scope in the overall context of agro-biodiversity. They play an important role in nutrient acquisition for plants viz., N, P, K and other micro elements. It is well known that a considerable number of bacterial species are also able to exert a beneficial effect on plant growth. Mostly they are associated with the plant rhizosphere, hence they are called “rhizobacteria”. This group of bacteria has been termed plant-growth promoting rhizobacteria (PGPR) (Kloeper *et al.*, 1991). PGPR include a range of organisms that live in close association with roots. PGPR is now applied to a wide spectrum of strains that have, in common, the ability to promote the growth of plants following inoculation into seeds and subterranean plant parts (Kloeper *et al.*, 1988).

Role of AM fungi and plant growth promoting rhizomicroorganisms (PGPRs) in improving plant growth is well
documented (Lakshman, 1992; Murthy et al., 1998). However the information available on the use of these beneficial microorganisms in medicinal plants is meagre (Earanna et al., 2003). Gigaspora margarita and PGPR applied to ginger increased the plant height, leaf and tiller number (Sharma et al., 1997). Response of Phyllanthus amarus to inoculation with Glomus fasciculatum and PGPRs either singly or in combination were studied under field conditions (Earanna et al., 2003). Dual inoculation of Glomus fasciculatum with either Bacillus coagulans or Trichoderma harzianum enhanced plant growth and biomass of Phyllanthus amarus (Earanna et al., 2003). Dual inoculation with Glomus mosseae plus Trichoderma harzianum was found to increase the growth, biomass and alkaloid content of Andrographis paniculata (Arpana, 2000). Coleus aromaticus inoculated with Glomus fasciculatum and PGPR increased its growth, biomass and P content (Earanna et al., 2001). Synergistic effects of AM fungi and PGPR (Lindermann and Paulitz, 1990) and yeast (Singh et al., 1991) on root colonization and subsequent sporulation have been well documented. The efficiency of PGPR and yeast viz., Saccharomyces cerevisiae was evaluated for maximization of Glomus mosseae root colonization and spore number in the root zone of rhodes grass (Chloris gayana). PGPR considerably enhanced mycorrhizal colonization compared to yeast, with Azospirillum sp. being the most efficient. They not only stimulated AM development, but also accelerated the root growth (Bhowmik and Singh, 2004).

Some soil bacteria, which have been named “mycorrhiza helper bacteria” (MHB), could enhance the development of the mycorrhizal symbiosis (Garbaye, 1994; Duponnois and Plenchette, 2003). The bacterial-growth promoting effect has been shown with different host plants, including herbaceous species (Meyer and Linderman, 1986; Paula et al., 1992; Von Alten et al., 1993; Requena et al., 1997) and tree species (Duponnois et al., 1993; Rozycki et al., 1994; Dunstan et al., 1998). The
MHB effect has been particularly well examined in symbiosis between conifers and *Laccaria bicolor* S-238 under a wide variety of experimental conditions (Duponnois and Garbaye, 1991a, b). MHBs have been found to inhibit mycorrhiza formation by fungi other than *Laccaria* spp. (Garbaye and Duponnois, 1992), to improve the persistence, of selected ectomycorrhizal fungi inoculated on seedlings in nursery soil (Duponnois et al., 1993), and to be effective in stimulating mycorrhizal development at a very low rate (Frey-Klett et al., 1997, 1999).

Lakshmipathy et al. (2001) studied the influence of AM fungus, *Glomus mosseae* singly and together with plant growth promoting rhizomicroorganisms on growth of medicinal plant *Saraca asoca*. The two PGPRs used were *Bacillus coagulans* and *Trichoderma harzianum*. The plant growth because of inoculation with *G. mosseae* and PGPRs individually enhanced plant growth and biomass significantly compared to uninoculated plants. Dual inoculation of *G. mosseae* plus *B. coagulans* further enhanced plant biomass, number of leaves, plant height and plant P content. Dual inoculation of AM fungi with PGPRs enhancing plant growth, biomass and stem girth has been observed in neem (Sumana, 1998) and *Ficus benjamina* (Jayanthi Srinath et al., 2003).

A pot culture experiment was conducted under glass house conditions to study the interactions among *Glomus mosseae*, *Pseudomonas fluorescens* and *Azospirillum brasiliense* on tomato (Muthu Raju et al., 2002). The results of the experiment showed that plant inoculated with all the three organisms had higher plant biomass and nutrient uptake and increased fruit yield. The rhizosphere soil of triple inoculated plants exhibited maximum spore count indicating increased multiplication of *G. mosseae* in the presence of PGPRs.
Certain plant growth promoting rhizomicroorganisms have been reported to enhance the activity of mycorrhizal fungi and consequently plant growth (Fitter and Garbaye, 1994; Gurumurthy, 1997). Meyer and Linderman (1986) studied the response of subterranean clover to dual inoculation with VAM fungi and plant growth promoting bacterium, *Pseudomonas putida*. They reported in population of bacteria in the rhizoplane of mycorrhiza inoculated sweet corn and clover compared to non-mycorrhizal plants.

Increased mycorrhizal root colonization and spore number due to inoculation with AM fungi in different crops have been reported by several workers (Fathima *et al.*, 1996; Gurumurthy, 1997). Addition of PGPRs along with *G. mosseae* further enhanced mycorrhizal colonization and sporulation and such kind of observations have been made by earlier workers in other crops (Sumana, 1998; Arpana, 2000; Muthu Raju *et al.*, 2002). Camprubi *et al.* (1995) observed synergistic interaction between *Trichoderma aureoviride* and *Glomus intraradices* with marked beneficial effect on *Citrus reshni*.

Lakshmipath *et al.* (2002) studied the influence of AM fungus, *G. mosseae*, singly and together with PGPRs on growth of medicinal plant *Calamus thwaitessi*. The two PGPRs used were *Bacillus coagulans* and *Trichoderma harzianum*. The plant growth because of inoculation with *G. mosseae* and PGPRs individually enhanced plant growth and biomass significantly compared to uninoculated plants. Dual inoculation of *G. mosseae* plus *B. coagulans* further enhanced plant biomass significantly compared to all other treatments, which was true for other parameters studied like plant height, number of leaves and plant P-
content. The percent mycorrhizal colonization was highest in seedlings inoculated with both *G. mosseae* and *T. harzianum*.

Thanuja (2000) determined the response of *Datura metal* and *Adathoda vasica* to diverse AM fungi and some PGPRs.

Earanna *et al.* (2002) studied the effect of inoculation with AM fungus, *Glomus fasciculatum* and PGPRs, *Azotobacter chroococcum, B. coagulans, Pseudomonas fluorescens* and *Trichoderma harzianum* on growth and phosphorus nutrition of *Catharanthus roseus*. Results revealed that inoculation with *G. fasciculatum* along with mycorrhiza helper bacterium, *B. coagulans* enhanced biomass production to the maximum extent, but not differed significantly in single inoculation with *G. fasciculatum*.

Sivakumar *et al.* (2002) determined the effect of AM fungus, *Glomus fasciculatum* and PGPR’s on the growth and biomass of *Geranium (Pelargonium graveolens)*. Plants inoculated with ‘microbial consortium’ consisting of *G. fasciculatum + Trichoderma harzianum + Pseudomonas fluorescens* performed well than other treatments and uninoculated control plants.