

REVIEW OF LITERATURE

CHAPTER II

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Of late, it is increasingly realized that mutually beneficial symbiotic systems in nature such as mycorrhizae, legume-*Rhizobium* etc., are very common and are of greater benefit than previously assumed. Further, some root colonizing bacteria (rhizobacteria) like *Azospirillum*, *Azotobacter*, *Phosphobacteria* etc., have also been found to exert beneficial effects on plant development *via* direct or indirect mechanisms in tri-partite interactions and have been defined as plant growth promoting rhizobacteria (PGPR).

The effects and potentialities of various beneficial micro-organisms as bio-inoculants in agriculture have been well documented (Subba Rao, 1988, 1995; Nasim, 2012; Hayat *et al.* 2012). The beneficial effect of single inoculation of AM fungi (Mathew and Johri, 1989), *Azotobacter* (Meshram and Shende, 1982; Lakshminarayanan *et al.*, 1987), *Azospirillum* (Tilak and Murthy, 1983; Okon and Labandera-Gonzalez, 1994) and PSB (Kathiresan *et al.*, 1995; Erturk *et al.*, 2010) in agricultural crops is well known. Over the years, the combined ability of these bio-inoculants has also been tested extensively for enhanced crop growth and yield (Tilak *et al.*, 1982; Paula *et al.*, 1991; Rakeshkumar *et al.*, 2001; Singh *et al.*, 2009). However, in forestry, the reports available on this subject are limited.

The research on mycorrhizae of forest trees in India was started at Forest Research Institute and Colleges, Dehra Dun in early seventies. Initially the work was concentrated on intensive survey and screening of tree species for endomycorrhizal association (Bakshi, 1974). Studies on the

inoculation effect of AM fungi and other beneficial microorganisms on forest trees were conducted much later in late eighties.

2.1. EFFECT OF AM FUNGI AND OTHER BENEFICIAL MICROORGANISMS ON THE GROWTH ENHANCEMENT TREES

2.1.1 Effect of AM fungi and other beneficial microorganisms on the growth enhancement of selected native fast growing forest tree species

Very few studies have been conducted involving the fast growing tree species *viz.*, *Ailanthus excelsa*, *Gmelina arborea*, *Melia dubia*, *Neolamarckia cadamba* and the bio-inoculants in the past. The same are reviewed here under.

Kandasamy *et al.* (1987) studied the growth response (shoot and root dry weights) of *Ailanthus excelsa*, *Azadirachta indica* and *Parkinsonia aculeata* to inoculation with *Glomus fasciculatum*. They recorded that AM fungus was effective in increasing biomass in all the tree species. In *A. excelsa* the percent increase in shoot dry weight and root dry weight were 62.08% and 43.18%, respectively. The percent colonization of roots was found to be 64.6%.

Balasubramanian and Srinivasan (1995) have made an attempt to identify the suitable AM fungi for *Tectona grandis*, *Dalbergia sissoo* and *Ailanthus excelsa* to produce vigorous seedlings for higher productivity by using four AM fungi *viz.*, *Glomus fasciculatum*, *G. mosseae*, *G. monosporum* and *Gigaspora margarita*. They found that AM fungal inoculation significantly increased the total biomass, leaf area and total chlorophyll of all the three tree species seedlings when compared to control. They also

reported that different AM fungi responded differently to different tree species with *G. margarita* on *Tectona*, *G. mosseae* on *Dalbergia* and *G. monosporum* on *Ailanthus* showing maximum beneficial effect.

Madan *et al.* (1995) evaluated the effect of AM fungal inoculation on the growth of *Ailanthus excelsa*, *Pongamia glabra* and *Cassia siamea* using highly alkaline-saline soils. They reported that AM fungal inoculated plants showed better survival, better shoot heights, shoot and root dry weights in all the three tree species. The colonization in roots was maximum in *A. excelsa* (55.2%) followed by *P. glabra* (49.9%) and *C. siamea* (30.0%) and there was a positive correlation between biomass production and percentage of colonization. They concluded that mycorrhizal colonization may counteract adverse soil factors such as high salinity-alkalinity and also increase growth and yield of the plants on waste lands.

Rahangdale and Gupta (1998) conducted inoculated studies using twelve AM fungi on six tree species *viz.*, *Albizia lebbbeck*, *Albizia procera*, *Acacia nilotica*, *Dalbergia sissoo*, *Gmelina arborea* and *Pongamia pinnata*. The results revealed that the inoculants were host specific and the percent root colonization, shoot height, root and shoot biomass and P content in root tissue were significantly increased in AM fungal inoculated plants. The percentage of root colonization in *G. arborea* varied from 33.33% to 80.0% depending on the isolate.

Talukdar and Thakuria (2000) inoculated AM fungi on two economically important timber yielding tree species, *Tectona grandis* and *Gmelina arborea* using degraded soils and found that AM fungi inoculation with organic amendments gave better yield in terms of shoot and root biomass.

Seema *et al.* (2000) studied the role of AM fungi (*Glomus intraradices*, *Glomus mosseae* and *Acaulospora scrobiculata*, along with mycorrhizal roots of the trap species, *Panicum maximum*) and *Azotobacter chroococcum* inoculation on growth and biomass production in *Bambusa arundinacea*, *Gmelina arborea*, *Tectona grandis* and *Dalbergia sissoo*. They observed that the inoculated seedlings exhibited improved growth and biomass. However, variations among treatments and species were observed. In general root development was more positively affected than shoot development in all species except in teak. In all 4 species root length development was observed maximum either with AM or with AM + *Azotobacter*. *Tectona grandis* and *G. arborea* doubled their biomass yield just by mycorrhization. The lowest root colonization was observed in *D. sissoo* (11.67%) and the highest in *G. arborea* (80.42%). The effect of the bio-inoculants was least in bamboo. They opined that supplementary doses of chemical fertilizers with AM and *Azotobacter* may be helpful in enriching the microbial population and thereby plant growth.

Chandra and Ujjaini (2002) inoculated AM fungi with 3 levels of soil organic matter on *Tectona grandis*, *Dendrocalamus strictus*, *Emblica officinalis*, *Albizia lebbeck* and *Gmelina arborea*. They observed that the mean responsiveness of plant biomass was increased up to 36.85% at OM (soil, sand and farm yard manure in 1: 1: 0.5). Addition of OM in soil was found to enhance the plant biomass but it was found to decrease the responsiveness to AM fungi. Root colonization percentage of plants (70% in *G. arborea*) was also enhanced with AM fungal inoculation. With increasing level of OM in soil, mycorrhizal colonization increased in uninoculated plants, while it decreased in inoculated plants.

Zambrano and Diaz (2008) studied the response of *Gmelina arborea* to *Glomus* sp. and *Azospirillum brasilense* inoculation under greenhouse conditions in two potting media viz., soil and compacted peat in Colombia. Results showed that the potting media and *A. brasilense* have significant effect on the germination of *G. arborea* seeds. A positive correlation between mycorrhization and plant height was found during the initial stage of establishment in greenhouse conditions. In addition, there was a synergic effect of both types of microorganisms on mycorrhization (55% in AM fungi and *A. brasilense* combination).

Barua *et al.* (2010) assessed the role of AM fungi on the growth of *Gmelina arborea* in arsenic amended soils at nursery stage in Bangladesh. Before sowing seeds, soils were treated with four different concentrations (10 mg kg⁻¹, 25 mg kg⁻¹, 50 mg kg⁻¹, and 100 mg kg⁻¹) of Arsenic. They recorded growth parameters (length of shoot and root, collar diameter, fresh and dry weight of shoot and root) of the plant, and mycorrhizal root colonization and spore population in the rhizosphere soil of *G. arborea*. The results indicated that mycorrhizal seedlings grew better than non-mycorrhizal seedlings. Mycorrhizal seedlings planted in soil with 10 mg kg⁻¹ arsenic showed best performance in terms of growth, biomass and mycorrhizal colonization, compared to other treatments with higher concentration of arsenic. With increasing arsenic concentration, growth of seedlings, mycorrhizal colonization rate and spore population, all decreased significantly ($p < 0.05$). The mycorrhizal seedlings had as much as 40% higher increment in total growth and 2.4 times higher increment in biomass compared to non-mycorrhizal seedlings indicating that mycorrhizal inoculation could reduce the harmful effects of arsenic on the initial growth of *G. arborea* in degraded soil at nursery stage.

There are no reports available on the effect of AM fungi and other bio-inoculants on *M. dubia* and *N. cadamba* plants.

2.1.2. Effect of AM fungi on the growth enhancement of other forest tree species

Inoculation of *Populus deltoids* seedling with AM fungi increased the growth and yield of the plant (Starkey and Brown, 1971). Janos (1975) found that the mycorrhizal inoculation doubled the growth of *Inga oerstediana* and *Vitex cooperi* seedlings. Redhead (1975) reported that growth of *Khaya grandifolia* seedlings was increased six times when inoculated with AM fungi. Inoculation of *Magnolia grandiflora* with *Glomus fasciculatum* increased seedling height two fold at six months after inoculation (Maronek *et al.*, 1980).

Kormanik *et al.* (1982) reported mycorrhizal growth responses of 43-167% in different hardwood tree species raised in sterilized sand and soil mix. Further, they reported that AM inoculation improved root growth and reduced the time required for plants to reach a saleable size. Cornet *et al.* (1985) found that *Acacia holosericea* was benefited under semi-arid conditions when inoculated with AM fungi. Inoculation of soil with AM fungi increased dry matter (80 times) yield of *Leucaena leucocephala* in exotic plantation and agro forestry systems of sub-humid tropics (Huang *et al.*, 1985).

Inoculation of *Acacia scleroxyla* with *Glomus mosseae* and *G. epigaeum* significantly increased the growth of seedlings, shoot/root ratio and total dry matter production (Borges and Chancy, 1988).

Byra Reddy and Bagyaraj (1988) studied the effect of inoculating *Leucaena leucocephala* seedlings with 7 different AM fungi. They observed that inoculated seedlings showed higher nodule number, nodule dry weight,

plant biomass and P content as compared to the control. They concluded that of the 7 mycorrhizal fungi studied, *Glomus mosseae* was found to be the best for inoculating *Leucaena* grown in a vertisol.

Srinivas *et al.* (1988) studied the growth responses of *Eucalyptus tereticornis*, *Leucaena leucocephala*, *Acacia nilotica* var. *indica* and *Casuarina equisetifolia* seedlings to inoculation with *Glomus fasciculatum*, *G. epigaeum* and *Acaulospora morrowiae* in P deficient unsterile soil. It was observed that inoculation of AM fungi increased biomass, N, P and K uptake of all the four forest tree seedlings studied.

Inoculation of *Leucaena leucocephala* with *Glomus aggregatum* increased the plant growth significantly (Manjunath *et al.*, 1989). Inoculation of AM fungi increased the growth and vigour of roots in *Tamarindus indica* (Bagyaraj and Reena, 1990). *Glomus mosseae* inoculation to *Rhododendron nilagiricum* registered 39 percent increase in seedling height (Narayanan *et al.*, 1990).

Byra Reddy *et al.* (1990) demonstrated that inoculation of *Leucaena leucocephala* with *Gigaspora margarita* resulted in reduction of 50-75% of P fertilizer required.

Mallesha and Bagyaraj (1990a) experimented single inoculation versus dual inoculation with *Glomus mosseae* and/or *Gigaspora margarita* on *Leucaena leucocephala* and reported that combined application of *Glomus mosseae* and *Gigaspora margarita* produced maximum (60%) root colonization.

The effect of AM fungi on growth and drought resistance of *Acacia nilotica* and *Leucaena leucocephala* seedlings was studied in a glasshouse experiment by Michelsen and Rosendahl (1990). It was observed that both

species responded differently to AM inoculation and/or P fertilization under drought stress conditions and the growth promoting effect of AM fungi equaled the effect of P fertilization after 12 weeks.

Reena and Bagyaraj (1990a) inoculated the seedlings of *Tamarindus indica* with 13 different AM fungi. They observed that inoculated plants had greater plant height, leaf number, stem girth, biomass, phosphate and Zn²⁺ content. They also had higher amounts of mycorrhizal spores, percent root colonization and external hyphae, as measured by percent soil aggregation. *Tamarindus indica* seedlings responded best to inoculation with *Gigaspora margarita* followed by *Glomus fasciculatum*.

Reena and Bagyaraj (1990b) screened several AM fungi including *Acaulospora laevis*, *Gigaspora margarita*, *Glomus caledonicum*, *G. fasciculatum*, *G. mosseae*, and *Glomus versiforme* for their relative symbiotic efficiency and suitability to two slow growing tree species, *Acacia nilotica* and *Calliandra calothyrsus*. They observed that *A. nilotica* seedlings responded best to inoculation with *G. mosseae*. They also observed that the inoculated seedlings had greater plant height, leaf number, stem girth, biomass and P and Zn content.

Awotoye *et al.* (1992) studied the response of four tropical N-fixing woody legumes (*Acacia auriculiformis*, *Albizia lebbbeck*, *Gliricidia sepium* and *Leucaena leucocephala*) to drought and inoculation with mycorrhizae on dry-matter production, nutrient uptake and water relations. Inoculation of *L. leucocephala* roots with AM fungi (*Glomus* and *Acaulospora* spp.) resulted in colonization of 25-68% in regularly watered plants. Drought stress reduced colonization by 8-49%. In general, AM plants survived better and had more dry matter and nutrients and a larger leaf area than uninoculated

plants. For *A. auriculiformis*, however, the AM inoculants reduced leaf area, plant dry weight and N content, although it increased P uptake. Nutrient content in drought-stressed AM plants was either greater than or comparable with the nutrient content of unstressed non-mycorrhizal plants.

Seedlings of *Acacia melanoxylon*, *A. mangium*, *A. auriculiformis* were inoculated with *Glomus* spp. AM fungi were successfully established on all seedlings. No significant increase in growth and biomass production was recorded in *A. melanoxylon*. *A. mangium* responded positively at first but effects were nullified after it was attacked by foliar disease. *A. auriculiformis* showed positive response in dry matter production (Mizoguchi, 1992).

In a study using AM fungal inoculation on bamboo, a positive correlation was obtained between the root colonization and the biomass and number of culms per rhizome (Singh *et al.*, 1992). Also, they stated that the soil with AM fungal spores can be used for inoculation in nurseries to produce healthy, vigorous bamboo seedlings, which can establish better in the main field.

The influence of AM fungi on the seedling growth of four tree species of tropical deciduous forest *i.e.*, *Caesalpinia eriostachys*, *Cordia alliodora*, *Ipomoea wolcottiana* and *Pithecellobium mangense* was investigated in a greenhouse experiment in Mexico by Huante *et al.* (1993). Dry biomass production, relative growth rate, root/shoot ratio and mycorrhizal dependency were quantified for 75-day-old seedlings. They reported that with the exception of the pioneer species *I. wolcottiana*, mycorrhizal inoculation resulted in increased biomass production, relative growth rate and leaf area. The root/shoot ratios attained for the species, however, did not show a consistent trend with colonization. Nevertheless, all species had

root/shoot ratios below 1 with inoculation and only one, *Cordia alliodora*, had a ratio greater than 1 without inoculation. The two late successional species from the mature part of the forest, *Caesalpinia eriostachys* and *P. mangense*, showed a larger mycorrhizal dependency than the two associated with disturbed environments.

Michelsen (1993) studied the effect of AM fungus (*Glomus mosseae*) or chopped roots of native plants on *Acacia abyssinica* and *A. sieberiana* using standard, non-sterile nursery soil in a tree nursery in Ethiopia. It was observed that inoculation with chopped native roots and with tropical *G. mosseae* isolate increased the shoot dry weight of *A. sieberiana* significantly, by 33 and 36%, respectively, compared with seedlings in the standard soil. Inoculation with native forest roots and the tropical *G. mosseae* increased the shoot dry weight of *A. abyssinica* by 23 and 38%, respectively. The temperate isolate of *G. mosseae* did not improve the growth of either *Acacias* significantly. Growth stimulation was accompanied by a significantly higher mycorrhizal colonization in all cases except one, whereas shoot P and Mg uptakes were generally not significantly affected. The survival of the *A. abyssinica* seedlings 6 months after their transfer from the nursery to the field was enhanced by all inoculations, although significantly so only with the tropical AM isolate. The root dry weight after 18 months in the field was also improved in this treatment. He concluded that application of a simple inoculum consisting of chopped roots of native plants with naturally occurring AM fungi can be equally effective in enhancing seedling growth in tree nurseries in the tropical region as the more elaborate use of pure cultures of AM fungi.

An investigation was carried out by Vasanthakrishna and Bagyaraj (1993) to screen and select efficient AM fungi for *Casuarina equisetifolia*. The seedlings were inoculated with 13 different AM fungi, obtained from various global sources. Inoculated plants exhibited increased plant height, biomass, and P and Zn contents. They also had increased mycorrhizal root colonization and spore numbers in soil. *Casuarina equisetifolia* seedlings responded best to inoculation with *Glomus mosseae* (local) followed by *G. versiforme* (Vasanthakrishna *et al.*, 1994).

Bhat *et al.* (1994) studied the field response of three forest tree seedlings (*Acacia nilotica*, *Casuarina equisetifolia* and *Leucaena leucocephala*) to inoculation with AM fungi. They observed that in *A. nilotica* and *C. equisetifolia*, height was not significantly enhanced by AM fungi up to one month after transplanting. In *L. leucocephala*, however, plant height was significantly increased (16.6%) by *Glomus mosseae* in the first month. *Glomus etunicatum* on *A. nilotica* and *G. fasciculatum* on *C. equisetifolia* were significantly superior to other AM fungi.

Verma and Jamaluddin (1994) studied the effect of inoculation of AM fungi on growth of four different species of bamboo *viz.*, *Dendrocalamus strictus*, *D. membranaceus*, *D. hamiltonii* and *Bambusa vulgaris* in unsterilized soil under nursery conditions by inoculating three months old seedlings with a mixture of AM fungi, *Glomus mosseae*, *G. etunicatum* and *Gigaspora* sp. After three months of treatment, the height growth in *D. membranaceus* was found maximum followed by *D. strictus*, *B. vulgaris* while *D. hamiltonii* showed minimum influence of AM inoculation. The percent root colonization by AM fungi was correlated with growth responses in all of the four bamboos.

The maximum colonization by AM fungi in roots of bamboo species was proportional to height growth of the seedlings.

Agrawal and Chauhan (1995) examined the effect of AM fungus, *Glomus fasciculatum* on *Dalbergia sissoo* seedling growth. They reported that the inoculated seedlings showed increased leaf area, root and shoot lengths and biomass.

Durga and Gupta (1995) studied the effect of AM fungi (*Glomus fasciculatum* and *Glomus mosseae*) on the initial establishment and mineral nutrition of *Tectona grandis*. Plants inoculated with AM fungi showed an increase in the concentration of phosphate, Mn and K, while N, Cu, Zn, Fe concentration decreased in the shoots. They reported that *G. mosseae* + *G. fasciculatum* inoculated plants registered greater growth mainly due to AM colonization within the root cortex, with intercellular hyphae and intracellular development of arbuscules.

Jamaluddin and Chandra (1995) studied the growth performance of *Eucalyptus* hybrid using AM fungi, *Glomus mosseae*, *G. fasciculatum* and mixed AM fungi and coal mine soil. They observed that inoculation of AM fungi increased the growth of *Eucalyptus* seedlings significantly as compared to the control.

Kanakadurga and Rama Rao (1995) studied the effect of AM fungi, *Glomus mosseae* and *G. fasciculatum* on root and shoot phenol concentration of *Terminalia arjuna*. They found that the shoot/root levels of phenols are higher in all the AM fungal treatments when compared to control.

Maksoud *et al.* (1995) observed significant increase in growth of 8 months old seedlings of *Tamarindus indica* with application of super phosphate and AM fungi (a mixture of *Glomus* and *Gigaspora*).

Mandal *et al.* (1995) found that inoculation of *A. nilotica* seedlings with *Glomus mosseae*, *Glomus constrictum* and *Gigaspora gilmorei* individually or in combination improved nodulation, dry weight, N, P and K content of the plant compared to control plants.

Manoharachary and Reddy (1995) studied the effect of AM fungi on *Dalbergia sissoo*, *Tectona grandis* and *Terminalia alata* and reported that AM fungi inoculated plants showed more growth, root and shoot dry weight and increased levels of N, P, K and Zn in plant tissues. They also reported that indigenous strains of *Glomus caledonium* and *G. fasciculatum* are more suitable for growth of forest tree species.

Mathur and Vyas (1995a) conducted an experiment to assess the efficacy of indigenous AM fungi on the growth of *Acacia nilotica* seedlings under Indian Thar desert conditions. They concluded that the indigenous AM fungi present in the Indian Thar desert soils are capable of colonizing *A. nilotica* and improved its agronomic performance through increased uptake of nutrients like N and P.

Mathur and Vyas (1995b) also studied the physiological changes in *Prosopis cineraria* seedlings by inoculating with AM fungi viz., *Acaulospora morrowiae*, *Gigaspora margarita*, *Glomus fasciculatum*, *G. mosseae* and *Scutellospora calospora*. They observed that total chlorophyll, caretenoids, soluble protein, total sugars and starch were higher in AM fungal inoculated plants. However, different AM species varied in their efficacy to increase nutrient level in *P. cineraria*.

Ragupathy and Mahadevan (1995) studied the effect of three different AM fungi viz., *Glomus mosseae*, *G. aggregatum* and *Gigaspora albida* on growth of *Peltophorum pterocarpum*. They found that the individual inoculations, especially *G. mosseae* gave better response than mixed inoculum. They believed that though many mycorrhizal strains were available in the soil, the hosts picked up its own compatible strain or isolate, which alone was responsible for increase in plant height. Competition between AM fungi may also account for preferential association.

Sarwar and Mukerji (1995) studied the influence of AM fungus *Glomus fasciculatum* on the growth of *Acacia catechu* seedlings grown in two types of nursery soils. They reported that inoculated seedlings showed better growth parameters and biomass production in both types of soils.

Sharma *et al.* (1995) conducted an experiment to identify efficient species/strains of AM fungi in enhancing survival, establishment and growth of *Acacia auriculiformis*, *Casuarina equisetifolia* and *Pterocarpus marsupium* seedlings. They reported high colonization of roots indicating that these species are mycorrhizae-dependent and for each tree species, the efficient AM species were different. The inoculated plants showed increase in growth, shoot and root biomass and P content.

Verma and Jamaluddin (1995) observed that inoculation of *Tectona grandis* seedlings with *Glomus fasciculatum* and mixed AM fungi separately showed better height growth, biomass and percent root colonization in nursery when compared to uninoculated control seedlings. The mixed AM inoculum was found to be more effective to boost the growth and biomass.

Lovelock *et al.* (1996) studied the growth response of seedlings of a tropical tree, *Beilschmiedia pendula* to AM fungi and elevated CO₂ under greenhouse conditions. They reported that inoculation with AM fungi increased relative growth rates (RGR) of the seedlings at both ambient and doubled CO₂ concentrations. RGR was correlated with the net assimilation rate (NAR) of plants. Within this general correlation, in plants with similar RGR, NAR was decreased in AM inoculated plants compared with non-mycorrhizal plants. As RGR is the product of NAR and the leaf area ratio (LAR), increases in RGR in AM fungal inoculated plants were the results of increased LAR. Thus, AM fungi increased growth rates of *B. pendula* by altering the morphology of the seedlings.

Mathur and Vyas (1996) studied the physiological changes in *Ziziphus mauritiana*, when inoculated with seven different AM fungal species, *viz.* *Glomus fasciculatum*, *G. mosseae*, *G. constrictum*, *Gigaspora margarita*, *Acaulospora morrowiae*, *Sclerocystis rubiformis* and *Scutellospora calospora*. The results showed that there were differences among the mycorrhizal fungi to enhance the plant growth, nutrient uptake and the root colonization by the endophytes. They also reported that *G. fasciculatum* was found to be the most significant in increasing plant biomass as well as the uptake of N, P, K, Ca and Mg by colonizing the roots most effectively.

Mukerji *et al.* (1996) investigated the effect of *Glomus fasciculatum* on growth of *Acacia catechu*, *Leucaena leucocephala* and *Prosopis juliflora*. Inoculation with *G. fasciculatum* increased juvenile growth and primary establishment of seedlings. It also increased shoot height, root and shoot dry weights significantly.

According to Selvaraj *et al.* (1996) inoculation of *Glomus mosseae* improved the growth performance of *Acacia leucophloea*, *A. nilotica*, *Derris indica* seedlings whereas, in *Acacia mangium* and *Delonix regia*, *Gigaspora margarita* showed better performance and also recorded an increase in P content except in shoots of *Acacia mangium* and *Derris indica*. However, in *Tamarindus indica*, the response related to plant growth, biomass and P uptake was not significant in all AM fungal treatments.

Sharma *et al.* (1996) recorded the response of *Acacia nilotica* to indigenous AM fungal inoculation and single super phosphate fertilization using degraded waste land soil and found that the overall growth of mycorrhizal plants was found to be higher over non-mycorrhizal plants at all levels of added fertilizer.

Dalberia sissoo seedlings when inoculated with twelve different AM fungi, showed greater plant height, stem girth, biomass, P and Zn contents, more number of mycorrhizal spores, percent root colonization and the seedlings responded well to *Glomus fasciculatum* followed by *Gigaspora margarita* (Sumana *et al.*, 1996).

Adjoud *et al.* (1997) studied the effect of inoculation of AM fungi on the growth of 11 *Eucalyptus* species. They reported that twenty weeks after mycorrhizal inoculation, *Eucalyptus* seedlings' stem dry weight was increased up to 49% compared to uninoculated control plants. Intensity of root colonization by a given fungus depended on the host species, but it was not related to a plant growth response. Leaf P concentration of uninoculated *Eucalyptus* seedlings varied greatly between species. Increase in leaf P concentration following mycorrhizal colonization was not necessarily associated with plant growth stimulation. The most mycorrhiza-

dependent *Eucalyptus* species tended to be those having the highest leaf P concentration in the absence of a fungal symbiont. They concluded that the mycorrhiza-dependent *Eucalyptus* species seem to have greater P requirements and consequently to rely more on the symbiotic association.

Elimination of chemical fertilizers up to 100% and higher productivity in nutritionally poor soils for *Acacia nilotica*, *Albizia lebbeck*, *Eucalyptus tereticornis* and *Prosopis juliflora* were achieved by Adholeya *et al.* (1997) using AM fungal inoculations. They conducted rehabilitation trials for wasteland reclamation with mycorrhizal inoculations to an extent of 8 years at the field station at Gualpahari (Haryana). Results of their study indicated that at the end of sixth to eighth year, there was significant improvement in biomass and nutritional status.

Dixon *et al.* (1997) reported that AM fungal inoculation of *Prosopis juliflora* seedlings in nursery significantly increased juvenile growth response. They also reported that the inoculation of tree seedlings with AM fungi improved the survival of seedlings on forestation site with 52°C rhizosphere soil temperature.

Gautam and Maitra (1998) observed that the height of *Dendrocalamus strictus* was increased by 74.7% in AM fungal inoculated plants than in uninoculated control plants. This increase in growth was a result of cumulative effect of increased phosphate and other mineral uptake, increased photosynthetic rate and improved water status brought by *Glomus macrocarpum*.

Gurumurthy *et al.* (1998) studied the effect of inoculation of AM fungus, *Sclerocystis dussii* at different P levels (0, 50, 75 and 100 kg P₂O₅/ha)

on growth and nutrition of *Tectona grandis* seedlings. They observed that the increase in percent colonization was significant up to 75 kg P₂O₅/ha while the increase in spore count was significant up to 50 kg P₂O₅/ha. Similarly plant height, stem diameter, leaf area, root length, root and shoot biomass increased significantly with increase in P levels.

Nagaveni *et al.* (1998) studied the comparative effect of inorganic fertilizer and AM fungi on growth of *Tectona grandis* plants. They observed that AM fungi inoculated seedlings showed better growth. The application of fertilizer did not show much difference in growth compared to treatments with AM fungi.

Dalbergia latifolia seedlings were inoculated with 8 different AM fungi and it responded best to inoculation with *Glomus leptotichum* as well as *G. fasciculatum* when compared to the other AM fungi. The seedlings inoculated with AM fungi had greater plant height, girth, dry weight and P content and higher spore number and mycorrhizal root colonization than uninoculated control (Sumana and Bagyaraj, 1998).

Verma and Arya (1998) studied the effect of three different inocula of AM fungi in tissue culture raised *Dendrocalamus asper* (bamboo) plantlets in two types of soil, viz., (1) sand: soil (1:1) (2) sand: soil: organic manure (1: 1: 0.5 v/v) and observed a significant positive effect of inoculum on plantlet height, dry biomass, shoot P concentration, root colonization and spore production in the sand: soil medium. In the organic manure-amended medium, these parameters further improved.

Chu (1999) studied the response of *Euterpe oleracea* seedlings (native palm tree of Amazon) to seven AM fungal species under greenhouse

conditions in Brazil. When plant growth and mineral nutrients were evaluated nine months after mycorrhizal inoculation, differential effects were observed among the AM fungal species tested, with *Scutellospora gilmorei* being the most effective one in promoting growth and nutrient content of *E. oleracea* seedlings. The increments resulted from inoculation with *S. gilmorei* were 92% in total plant height, 116% in stem diameter, 361% in dry matter production, 191% in N, 664% in P, 46% in K, 562% in Ca, 363% in Mg and 350% in Zn contents, compared to uninoculated controls. Colonized root length was positively correlated to nutrient content and plant growth. It was concluded that growth and nutrient uptake of *E. oleracea* seedlings could be significantly improved by inoculation of effective AM fungi.

The plant height, stem diameter, root length, shoot and root dry weight and concentration of P, Zn, Cu, Mn and Fe in shoot were significantly higher in *Dalbergia sissoo* plants inoculated with *Glomus fasciculatum* as compared to other AM fungi (Gurumurthy *et al.*, 1999).

Laurent *et al.* (1999) used aeroponics, a soilless plant culture method, to produce *Acacia mangium* saplings associated with AM fungi. *Acacia mangium* seedlings were first grown in multipots and inoculated with Endorize, a commercial AM fungal inoculum. They were then, either transferred to aeroponic systems or to soil. Compared to plants grown in soil, aeroponically grown saplings inoculated with AM fungal inoculum attained twice height and exhibited significantly different rates of mycorrhization, resulting in an increase in P and chlorophyll in plant tissues.

Mehrotra *et al.* (1999) studied the impact of AM fungi and fertilizers on the growth of two species of bamboo viz., *Dendrocalamus strictus* and *Bambusa nutans*. They found that inoculation with AM fungi improved the growth parameters of bamboo seedlings.

Kiers *et al.* (2000) studied the differential effects of tropical AM fungal inocula on root colonization and seedling growth of six native tree species in Panama. They concluded that (i) the seedlings of small-seeded pioneer species were more dependent on mycorrhizal inocula for initial survival and growth; (ii) although mycorrhizal fungi from all inocula were able to colonize the roots of all host species, the inoculum potential (the infectivity of an inoculum of a given concentration) and root colonization varied depending on the identity of the host seedling and the source of the inoculum; and (iii) different mycorrhizal fungal inocula also produced differences in growth depending on the host species.

Mycorrhizal Dependency (MD) of 29 agro-forestry tree species was investigated (Kumar *et al.*, 2000) by inoculating with AM fungi (mixed *Glomus mosseae*, *G. fasciculatum* and *G. epigaeum*). On the basis of the mycorrhizal colonization, seedling biomass and P contents of roots and shoots, the species were divided into three groups, those with high MD (*Azadirachta indica*, *Leucaena leucocephala*, *Gliricidia maculata*, *Sesbania grandiflora*, *Cassia siamea* and *Acacia melanoxylon*), those with moderate MD (*Tectona grandis*, *Albizia lebbek*, *Pongamia pinnata*, *Terminalia catapa*, *Sapindus emarginatus*, *Tamarindus indica*, *Syzygium cumini*, *Embllica officinalis*, *Dalbergia sissoo* etc.) and those with no MD (*Diospyros melanoxylon*, *Mangifera indica*, *Murraya koenigii*, *Polyalthia longifolia*, *Psidium guajava*, *Saraca indica* and *Ziziphus mauritiana*).

Mathur and Vyas (2000) assessed the effect of four AM fungi viz., *Scutellospora calospora*, *Gigaspora margarita*, *Acaulospora morrowiae* and *Glomus fasciculatum* on biomass production and nutrient uptake by *Tamarix aphylla*. The results showed that this tree is highly dependent on AM fungi. Among the four AM fungi tested, *Gigaspora margarita* was the most efficient one as it significantly increased the plant biomass and nutrient uptake.

Mohan (2000) reported that the local AM fungal isolate *Glomus fasciculatum* performed better in increasing plant height and biomass than other isolates on the seedlings of *Prosopis cineraria* and *P. juliflora* in arid zone soils of Rajasthan.

Rajan *et al.* (2000) conducted a greenhouse study to investigate the efficacy of nine AM fungi on *Tectona grandis*. Stumps raised in polythene bags containing inoculated AM fungi showed an increase in plant growth and plant nutritional status compared to uninoculated control. The extent of growth and nutritional status of *T. grandis* enhanced by AM fungi varied with the AM fungal species. *Glomus leptotichum* was found to be the best AM symbiont for teak in that experiment.

Sharma and Adholeya (2000) studied the response of *Eucalyptus tereticornis* grown in a greenhouse in a low P (0.67 ppm Olsen's P) soil to inoculation with mixed indigenous AM fungi. Soil was amended to achieve P levels of 10, 20, 25, 30 and 40 ppm to evaluate the growth response and dependence of *E. tereticornis* to inoculation with AM fungi. A positive response to mycorrhizal inoculation was evident at the first two levels of soil P, but not at the higher levels of soil P. Inoculated plants produced maximum dry matter (root and shoot) at 10 ppm soil P. The percentage root length colonized by AM fungi decreased from 31% to 3% as the concentration of

P increased beyond 10 ppm soil P. Higher levels of soil P depressed the AM colonization significantly. Inoculated plants had higher shoot P and N contents compared to their uninoculated counterparts at all levels of soil P. However, at the first two lower levels of soil P, inoculated plants showed significantly higher shoot P and N contents over their respective uninoculated counterparts. The increasing shoot P accumulation beyond 10 ppm did not enhance dry matter yields. Taking dry matter yield into consideration, *Eucalyptus* plants were found to be highly dependent on 10 ppm of soil P for maximum dry matter production. Any further amendment of P to soil was not beneficial for AM symbiosis or plant growth.

The effect of inoculation with AM fungi, *Glomus fasciculatum* and *G. mosseae* on dry matter production, nutrient uptake and rhizosphere microbial activity in different tree species grown in gypsum mine spoil and limestone mine spoil, respectively was studied by Rao and Tak (2001, 2002). Dry matter production was significantly enhanced upon inoculation to varying degrees depending on the tree species. The level of root colonization and spore densities in the rhizosphere increased by 14–36% and 49–81%, respectively. AM fungal inoculation resulted in enhanced activities of various soil enzymes viz., dehydrogenase, phosphatases and nitrogenase in the rhizosphere. Total uptake of many nutrients but not of K and Na, was significantly enhanced upon AM fungal inoculation.

AM fungi inoculated seedlings of *Cordia myxa*, *Artocarpus integer*, *Dalbergia sissoo*, *Pongamia pinnata*, *Mangifera indica* and *Alstonia spp.* performed better in terms of shoot height, root height and biomass (Srivastava *et al.*, 2001).

Panwar and Vyas (2002a) recorded the maximum beneficial effect with *Glomus deserticola* on *Acacia leucophloea* for nutrient uptake and enhancement of phosphatase, nitrate reductase, peroxidase and polyphenol oxidase activities, when compared to other AM fungi tested. Panwar and Vyas (2002b) also studied the relative efficiency of 8 AM fungi on growth and nutrient uptake in *Moringa concanensis*, a highly endangered multipurpose tree of Indian Thar desert. They found that inoculation of the seedlings with AM fungi increased plant biomass as well as nutrient (N and P) uptake significantly over the control. *Gigaspora margarita* was found to be the most significant AM fungus in increasing plant biomass and nutrient uptake.

Verma *et al.* (2002) studied the effect of AM fungi on growth and P uptake in *Dendrocalamus strictus* seedlings. They observed that the mixed inoculum containing six AM fungi viz., *Acaulospora scrobiculata*, *Glomus etunicatum*, *G. intraradices*, *G. mosseae*, *Gigaspora* sp. and *Scutellospora* sp., significantly enhanced height, fresh and dry biomass, tiller number and P content in shoot as compared to control. They also recorded that root colonization significantly correlated with the growth and biomass production of *D. strictus* seedlings in nursery.

Andre *et al.* (2003) reported that mycorrhization of *Acacia tortilis* sub sp. *raddiana* with *Glomus intraradices* improved root nodulation by *Sinorhizobium teranga* and *Mesorhizobium plurifarum*.

Giri *et al.* (2003) have examined the effect of AM fungus, *Glomus fasciculatum*, and salinity on the growth of *Acacia nilotica*. In saline soil, AM colonization was higher at 1.2, 4.0, and 6.5 dS m⁻¹ salinity levels in AM inoculated plants, which decreased as salinity levels further increased (9.5 dS m⁻¹). Mycorrhizal plants maintained greater root and shoot biomass

at all salinity levels compared to non-mycorrhizal plants. AM inoculated plants had higher P, Zn, and Cu concentrations than uninoculated plants. In mycorrhizal plants, nutrient concentrations decreased with the increasing levels of salinity, but were higher than those of the non-mycorrhizal plants. They concluded that mycorrhizal fungus alleviates deleterious effects of saline soils on plant growth that could be primarily related to improved P nutrition.

Kaushik *et al.*, (2003) observed that individual application of AM fungal inoculum (*Glomus mosseae*) and P fertilizer significantly increased nodule formation, N, P and K concentration in roots and shoots of *Acacia nilotica* and *Dalbergia sissoo*.

Lakshmipathy *et al.* (2003) studied the response of *Garcinia indica* to AM fungal inoculation under nursery conditions in Bangalore, India. They observed that apart from improving the plant height, number of leaves and biomass, the AM fungal inoculation resulted in improved P nutrition and in turn the root growth of the seedlings over the uninoculated ones. They also observed that the overall growth of seedlings inoculated with *Scutellospora calospora* and *Acaulospora laevis* was better.

Mamatha *et al.* (2003) studied the influence of different AM fungi on growth and nutrition of *Santalum album* plants. They observed that among all isolates, *Glomus fasciculatum* was very effective in increasing the biomass, number of leaves, plant height, P content, soil microbial count and soil enzyme activity.

Pande and Tarafdar (2003) studied the role of AM fungi on biomass production and P, Zn, Cu uptake in *Azadirachta indica*. They found that

inoculated seedlings had higher biomass as well as P, Zn and Cu accumulation than uninoculated ones. *Glomus fasciculatum* was found to be superior to *G. mosseae* or *Gigaspora margarita*.

Boby *et al.* (2004) studied the growth of *Casuarina* seedlings as influenced by AM fungi. They observed that in general, all mycorrhizal inoculations enhanced the growth of the seedlings compared to the uninoculated control. Among the different mycorrhizal fungi, inoculation with *Glomus mosseae* resulted in maximum response in terms of plant height, stem girth, root length and shoot and root biomass.

Chandra and Jamaluddin (2004) studied the effect of different mulches (stone, husks, leaves and grasses) alone and with mixed AM fungi (*Glomus mosseae*, *G. intraradices* and *Acaulospora* spp.) on growth and development of *Albizia procera* in coal mine spoil soil. Husk mulches were found more effective to growth and biomass of *Albizia procera* when used with or without AM fungi. Development of AM fungi under husks showed maximum root colonization and mixed AM fungi along with husk was found to be better in increasing growth and yield.

Grandcourt *et al.* (2004) conducted a study to understand the role of mycorrhizae in P efficiency of tree seedlings in the tropical rainforest of French Guyana. Mycorrhizal colonization, growth, P content, net photosynthesis and root respiration were determined on three occasions during a 9-month growth period for seedlings of two co-occurring species (*Dicorynia guianensis* and *Eperua falcata*) grown at three soil P concentrations, with or without arbuscular mycorrhizae. Seedlings of both species were unable to absorb P in the absence of mycorrhizal association.

Srivastava *et al.* (2004) reported that treatment with 100g of rhizosphere soil having about 500 AM fungal spores was the best dose for improving the growth of *Tecomella undulata* seedlings.

The influence of three different mixed inocula of AM fungi on the growth of *Bambusa nutans* seedlings was determined by Verma and Jamaluddin (2004). The mixed AM inocula comprised of (1) *Acaulospora scrobiculata*, *Glomus mosseae*, *Scutellospora heterogama* and *S. pellucida*; (2) *Acaulospora* sp., *A. scrobiculata*, *Gigaspora* sp., *Glomus mosseae*, and *S. pellucida*; and (3) *A. laevis*, *Acaulospora* sp., *Gigaspora* sp., and *S. pellucida*. The inoculated seedlings showed increased growth, biomass, N and P content. The improvement in growth parameters was correlated with percent root colonization of seedlings by AM fungi.

Hemavathi *et al.* (2005) investigated the effect of 5 different AM fungi on *Dendrocalamus strictus* seedlings. It was observed that inoculated plants had higher number of culms, number of leaves, plant biomass, P content and percentage of mycorrhizal roots. Bamboo seedlings responded well to inoculation with *Glomus fasciculatum* followed by *G. margarita*.

Rakesh Chugh *et al.* (2006) studied the response of *Populus deltoides* to inoculation with different AM fungi viz., *Glomus constrictum*, *G. mosseae*, *G. fasciculatum*, *Gigaspora gilmorei* and *Acaulospora laevis* in nutrient deficient sterilized soil. They observed that the plants inoculated with these mycorrhizal fungi showed better plant height, collar diameter, total number of leaves and mycorrhizal colonization. They also reported that among these fungi, *Glomus fasciculatum* was the best AM fungus for *P. deltoides*.

Richa Raghuwanshi and Upadhyay (2006) studied the growth performance of different plant species raised in saline-alkali soil in relation to various amendments. The growth got improved by the amendments, which decreased the saline-alkali soil stress conditions like pH and salt concentration. The inoculation of plants with *Glomus* sp. enhanced their performance when subjected to various conventional amendments. Maximum plant growth was observed when *Glomus* sp. inoculated plants were grown in gypsum amended saline-alkali soil.

Srivastava and Srivastava (2006) carried out work on the selection of efficient isolate of AM fungi for *Tecomella undulata*. Their investigations indicated that the indigenous consortium of AM fungi was the most suitable inoculum for *T. undulata* seedlings in biomass production and root colonization. This was followed by *G. fasciculatum* isolate.

Turjaman *et al.* (2006) determined the effect of two AM fungi, *Glomus clarum* and *Gigaspora decipiens*, on the early growth of two NTFP species, *Dyera polyphylla* and *Aquilaria filaria*, under greenhouse conditions. The seedlings of both species were inoculated with *G. clarum* or *G. decipiens* and percentage of AM colonization, plant growth, survival rate, and N and P concentrations were measured after 180 days of growth. The percentage of AM colonization of *D. polyphylla* and *A. filaria* ranged from 87 to 93% and from 22 to 39%, respectively. Colonization by *G. clarum* and *G. decipiens* increased plant height, diameter, and shoot and root dry weights. Shoot N and P concentrations of the seedlings were increased by AM colonization by as much as 70-153% and 135-360%, respectively. Survival rates were higher in the AM-colonized seedlings at 180 days after transplantation than in the

control seedlings. They suggested that AM fungi can accelerate the establishment of the planting stocks of *D. polyphylla* and *A. filaria*.

The efficiency of eight AM fungal species collected from rhizosphere soil of *Terminalia arjuna* from Indian Thar Desert was tested by Bohra *et al.* (2007). They found that mycorrhizal colonization resulted in increased accumulation of nutrients, chlorophyll, carotenoids, sugars and proteins. Among the eight AM fungi used, *Glomus fasciculatum* proved to be the most efficient.

Rama Bhat and Kaveriappa (2007) studied the inoculation effect of five species of AM fungi viz., *Acaulospora laevis*, *Gigaspora gigantea*, *Glomus fasciculatum*, *G. geosporum* and *G. macrocarpum* individually and in combinations on the growth of three tree species viz., *Gymnacranthera farquhariana*, *Knema attenuata* and *Myristica fatua* var. *magnifica*. They observed significant improvement in growth increment and increase in N, P, and K content of the inoculated plants after twelve months. Further, combined inoculations were found more effective than individual inoculations in all the three species. The effect of combined inocula of all the five AM fungi was maximum on the biomass of *G. farquhariana* and *M. fatua* var. *magnifica*, while that of combined inocula of *Gigaspora gigantea* and *Glomus macrocarpum* was maximum on the biomass of *K. attenuata*.

Turjaman *et al.* (2007) studied the effect of AM fungi on growth of milkwood tropical tree species, *Alstonia scholaris* under greenhouse conditions. They found that when *A. scholaris* seedlings were inoculated with five AM fungi viz., *Glomus clarum*, *Gigaspora decipiens*, *Glomus* sp., *Entrophospora* sp. and *Glomus* sp., they increased plant height, collar diameter, total fresh weight, total dry weight and total root length. Survival

rates were higher in AM colonized seedlings at 150 days after transplantation than those in the control. They also found that the shoot nutrient concentrations of N, P, K, Ca and Mg were significantly higher in AM fungi inoculated seedlings.

Three indigenous and one introduced (*Glomus mosseae*) AM fungi were inoculated with *Acacia mangium* in a P-deficient red lateritic soil and growth and physiological parameters were recorded. Treatment with AM fungi increased shoot height (59% to 112.5%); leaf area (131.7% to 168.3%); biomass (104.8% to 132.1%); chlorophyll content (40% to 60%) and insoluble carbohydrate (33% to 52%) over control. N, P, and K contents were also increased significantly. Root colonization by AM fungi ranged from 50% to 65%. One indigenous AM species, *Paraglomus occultum* was observed to be the most efficient followed by *Acaulospora delicata* and *Glomus mosseae*. It was concluded that efficient AM fungi has the potential to replace the need of fertilizer application in afforestation in poor soils (Ghosh *et al.*, 2008).

In view of the high mycorrhizal dependency of neem (*Azadirachta indica*), Venkateswarlu *et al.* (2008) conducted a study to know if AM fungal inoculation can enhance the azadirachtin content in seed kernels of trees grown in the field. Inoculation of neem seedlings in the nursery with *Glomus fasciculatum* and *Glomus mosseae* resulted in increased height, dry weight, root colonization and P content. In a separate experiment, field-grown neem plants inoculated in the nursery and during transplantation with *Glomus fasciculatum* were evaluated after 5 years. No significant differences were found in the tree height, girth at breast height (GBH) and fruit yield but oil percentage, total triterpenoids and azadirachtin content in kernels increased significantly as a result of AM fungal inoculation.

Guisso (2009) conducted a nursery experiment to determine the influence of an AM fungus on plant growth and nutrient uptake in tamarind (*Tamarindus indica*) and jujube (*Ziziphus mauritiana*), which are multipurpose fruit trees in Sahelian agroforestry systems. Tamarind and jujube seedlings were inoculated with *Glomus aggregatum* and grown for four months in a sterilized P deficient sandy soil (2.18 ppm P). Inoculated seedlings had greater shoot height and total biomass production. The total dry weight was increased 3 and 4 fold by *G. aggregatum*, in tamarind and jujube respectively, compared to the controls. Jujube with the highest AM root colonization had a higher P concentration (8.33 fold) in shoots than tamarind (1.62 fold), indicating greater mycorrhizal dependency of jujube seedlings. They concluded that fruit trees inoculated with AM fungi absorbed nutrients more efficiently from the soil, which resulted in improved plant growth and biomass production.

Shukla *et al.* (2009) conducted a study to investigate the effects of different light intensities and AM fungal inoculation on growth of seedlings of two tree species, *Eucalyptus tereticornis* and *Albizia procera*. The results showed that various plant growth parameters *viz.*, shoot length, dry weight and P uptake were adversely affected by low light intensity. Inoculations with AM fungi (*Acaulospora scrobiculata*, *Glomus intraradices* and *Glomus* sp.) increased the plant growth on account of all measured parameters under tested light conditions. Mycorrhizal efficiency of different AM fungi varied in narrow range. AM inoculants were more efficient at higher light intensity. While ranking the importance of two factors studied—light and AM fungi—for their effect on the growth and P uptake, inoculations with AM fungi came in the first place (explained 50–82% variation).

Zapata *et al.* (2009) evaluated the effect of AM fungi on P uptake and initial growth of *Desmoncus orthacanthos* (Arecaceae) seedlings, by conducting a 160-day bi-factorial experiment in which plants were subject to one of two levels of mycorrhizal colonization (with or without) and one of three levels of P substrate addition (4, 12, and 24 ppm). Results showed that total dry weight and leaf area responded significantly to P addition but not to mycorrhizal colonization. Phosphorus concentration in plant tissues was increased by both factors (mycorrhizae and P addition). Mycorrhizae increased relative growth rate at low P level. They concluded that AM fungi play an important role in early growth and P uptake by *D. orthacanthos* seedlings.

Arias *et al.* (2010) studied the effects of *Glomus deserticola* inoculation on *Prosopis* to Cr and Pb tolerance. The study indicated that the AM fungus was able to bind these toxic heavy metals in roots and improve the tolerance of the plants.

Manoharan *et al.* (2010) investigated the effect of AM fungus, *Glomus mosseae* on growth and physiology of *Erythrina variegata* grown in sandy loam soil with four water stress levels. Plants were harvested after 90 days and growth, physiological and microbiological parameters were determined. AM fungal inoculated plants had significantly higher plant biomass, chlorophyll (a and b), carotenoids and protein content in shoots than control plants. Mycorrhizal colonization in roots of *E. variegata* decreased significantly due to increased water stress. However, the AM fungal colonization did not decline below 11% and enabled the plants to maintain osmotic adjustments and enhanced the plants tolerance against water stress.

Fu *et al.*, (2011) investigated the growth and nutrition metabolism of *Robinia pseudoacacia* by inoculating with the *Glomus mosseae* and *G. constrictum* under different N levels in sterilized soil. The results showed that the colonization, root system vigor, biomass, leaf superoxidedismutase, catalase and nitrate reductase activity were significantly increased by inoculation with AM fungi. All the parameters were found influenced by N levels, first increasing and then decreasing with the increasing N levels, with the peak at 0.4g/kg. Effect on growth varied with different AM fungi. Inoculation with *G. mosseae* had a significant correlation with colonization rate and other indices.

Jha *et al.* (2011) conducted a study with twelve AM fungal species to identify suitable AM fungi for inoculation of *Bambusa bambos* and *Dendrocalamus strictus* at nursery stage for increasing growth and productivity. In *B. bambos*, total dry weight and P uptake were significantly increased by all studied fungi and shoot length was increased by eight AM inoculants. Maximum mycorrhizal dependency was recorded for *Acaulospora scrobiculata* (44.2%), followed by *Glomus cerebriforme* (41.6%) and *G. intraradix* (41.0%). In *D. strictus*, all tested AM inoculants significantly increased shoot length, dry shoot weight and P uptake, except *Glomus* 1. Dry root weight was significantly increased by only two inoculants namely, *G. cerebriforme* and *G. etunicatum*. Total dry weight was significantly increased by eight AM fungi. Maximum mycorrhizal dependency was recorded for *G. cerebriforme* (62.9%), followed by *G. diaphanum* (55.0%) and *G. etunicatum* (51.3%). They concluded that utilization of effective AM fungi can enhance the productivity of bamboo in Bundelkhand region.

2.1.3. Effect of *Azospirillum* on the growth enhancement of forest tree species

In forest nurseries, *Azospirillum*, *Azotobacter* and PSB are applied generally in combinations or with other symbiotic and beneficial microbes. Very few studies have been done on individual application of these bio-inoculants on forest tree seedlings.

Swaminath and Vadiraj (1988) studied the influence of *Azospirillum* on growth and dry matter production of *Leucaena leucocephala*, *Dalbergia sissoo*, *Acacia nilotica*, *Calliandra*, *Pongamia pinnata*, *Albizia lebbeck* and *Eucalyptus tereticornis* seedlings. The results after 90 days of treatment revealed that root, shoot lengths and root and shoot dry weights were greatly improved in all the species studied, but the magnitude of increase was highest in *D. sissoo*.

Rodriguez *et al.* (1991) studied the effect of inoculation of *Azospirillum brasilense* on *Casuarina cunninghamiana* seedlings. It was observed that growth of the seedlings was stimulated when inoculated with *A. brasilense* resulting in a higher biomass production than in uninoculated controls in the presence or absence of a non-nodulating strain of *Frankia*. Increase in whole plant dry weight was due to a significant increase in both shoot and root biomass, which corresponded with a higher total N content of the plants inoculated with *Azospirillum*. Such effects were not observed under inoculation with a non-nodulating *Frankia* strain.

The effects of inoculation with *Azospirillum brasilense* on germination and growth of oak (*Quercus ithaburensis*) seedlings were investigated by Zaady and Perevolotsky (1995). An inoculum concentration of 10^7 colony forming units per plant of *A. brasilense* caused significant increase in

seedling root surface area, root dry weight, foliage and shoot dry weight 30 days after inoculation. They concluded that inoculation of oak seedlings with *A. brasilense* will improve the establishment, growth and development of oak seedlings under nursery conditions.

Bhadauria *et al.* (2000) studied the effect of *Azospirillum* bio-inoculant on seedling growth and seed germination of *Embllica officinalis*. They reported that application of *Azospirillum* had positive effect on the seed germination and seedling growth of *E. officinalis*.

There are no reports available on the effect of *Azospirillum* on *Ailanthus excelsa*, *Melia dubia* and *Neolamarckia cadamba* plants.

2.1.4. Effect of *Azotobacter* on the growth enhancement of forest tree species

Azotobacter combined with chemical fertilizer, Suphala increased the culm height and better field performance of field planted *Dendrocalamus strictus* seedlings (Kinhil, 1985).

Azotobacter chroococcum treated seeds of *Cassia siamea* and *Prosopis juliflora* recorded greater germination percent and increased leaf area, fresh and dry weight of seedlings (Meshram *et al.*, 1994).

Kumar *et al.* (2008) evaluated the growth of *Jatropha curcas* on heavy metal contaminated soil amended with organic wastes (biosludge and dairy sludge) and bio-inoculant (*Azotobacter chroococcum*) under greenhouse conditions. Results showed that the plants survival rate increased with addition of amendments and bio-inoculant. Treatment T6 (heavy metal contaminated soils + dairy sludge + bio-inoculant) was found to be the best for plant growth (height and biomass).

Osman and Abd El-Rhman (2010) studied the effect of organic and bio N-fertilization on growth, productivity of fig tree (*Ficus carica*). Applying poultry manure + *Azotobacter* and poultry manure + *Azospirillum* gave the highest number of new shoots, shoot length, leaf area, total chlorophyll, number of fruits per shoot, yield, fruit volume and fruit length in the two seasons. Also, the same treatments gave the highest TSS, acidity, total and reducing sugar content and leaf minerals content (N, P, K, Ca and Mg %) in the two seasons. The results clarified that poultry manure + *Azotobacter* and poultry manure + *Azospirillum* treatments gained best vegetative growth, productivity and fruit quality.

Karthikeyan and Sakthivel (2011) studied the effect of *Azotobacter chroococcum* in rooting and subsequent growth of *Eucalyptus camaldulensis* stem cuttings. They found that *A. chroococcum* produced significant quantities of IAA for root initiation and *A. chroococcum* inoculated cuttings had higher growth than IBA treated cuttings.

There are no reports available on the effect of *Azotobacter* on *Ailanthus excelsa*, *Melia dubia* and *Neolamarckia cadamba* plants.

2.1.5. Effect of Phosphate Solubilizing Bacteria (PSB) on the growth enhancement of forest tree species

Saravanan (1991) reported that inoculation of phosphobacteria improved the height, collar diameter and shoot weight in *Acacia mellifera* and *A. farnesiana*; root length in *A. farnesiana*, number of leaves and leaf area in *A. seyal*; total dry weight in *A. seyal* and *A. farnesiana* and shoot/root ratio in *A. planifrons* and *A. farnesiana*.

Chanway and Holl (1994) inoculated four-month-old lodgepole pine (*Pinus contorta*) seedlings with *Bacillus polymyxa* and outplanted at one interior site and two coastal sites in British Columbia. They observed that the percentage of seedlings that incurred overwinter injury and that survived 13 months after outplanting were not influenced by bacterial inoculation. At Totem Field, where growth of control seedlings was greatest, inoculation had an inhibitory effect on seedling performance. At South Campus, where growth of control seedlings was intermediate compared with seedlings at Totem Field and Gavin Lake, inoculation had a slight stimulatory effect on seedling performance, but bacterial effects were not significant. However, at Gavin Lake, where seedlings attained only 14% of the biomass of those grown at Totem Field, inoculated seedlings had significantly increased stem diameter (7%), root dry weight (32%), and shoot dry weight (33%). While the effects of site history and site quality were confounded in this study, these results suggest that inoculation of lodgepole pine with *B. polymyxa* may be useful for seedlings targeted for outplanting on relatively harsh or poorer quality sites, but less so for seedlings to be planted at higher quality sites.

Shishido *et al.* (1996) studied the growth of pine and spruce seedlings and mycorrhizal colonization after inoculation with plant growth promoting fluorescent *Pseudomonas* strains Sm3-RN, Ss2-RN and Sw5-RN under greenhouse conditions. Ectomycorrhizal inoculum was introduced to seedling containers by placing 2 cc of forest floor soil around seeds at the time of sowing. Bacterial inoculation did not influence the mycorrhizal status of seedlings, but all three *Pseudomonas* strains stimulated biomass accumulation (up to 19%) of spruce and pine seedlings. For spruce, growth effects due to bacterial inoculation were similar in both mycorrhizal and non-mycorrhizal seedlings. However, statistically significant gains in pine

biomass by inoculation with strains Sm3-RN and Ss2-RN occurred only in mycorrhizal seedlings, whereas strain Sw5-RN caused significant growth promotion only in non-mycorrhizal pine. They suggested that these fluorescent pseudomonad strains enhanced spruce seedling growth through mechanisms unrelated to increased mycorrhizal colonization, but growth promotion of pine by strains Sm3-RN and Ss2-RN was facilitated by an interaction with mycorrhizae.

Enebak *et al.* (1998) studied the effects of 12 strains of PGPR on Loblolly (*Pinus taeda*) and Slash Pine (*Pinus elliottii*) seedlings by inoculating the seed at sowing under greenhouse conditions. Germination and seedling densities were determined at 21 days, and seedling biomass was measured at 12 weeks after sowing. All bacterial strains significantly increased the speed of seedling emergence over nontreated pine seed. By 12 weeks, however, no differences in stand densities were observed between bacteria-treated and non-treated seed for either pine species. Postemergence damping-off was reduced in loblolly pine when seed was treated with 3 of the 12 bacterial strains; however, postemergence damping-off on slash pine seedlings was not affected by rhizobacteria. Treatment with rhizobacteria had a significant positive and negative effect on seedling growth and biomass, which depended on tree species. Loblolly pine shoot, root lengths and shoot, root biomass were significantly reduced when seeds were treated with strains BS1 and BS2. In contrast, loblolly pine seeds treated with strains BS3, PM2, and INR7 significantly increased the root biomass of the seedlings. Slash pine seedlings had similar interactions with the bacterial strains. Strain BS1 significantly reduced shoot lengths compared with non-treated seeds, while strains 90-166, INR7, and SE49 increased shoot biomass. Slash pine root length and biomass were also reduced when

treated with strains BS1 and BS2. Unlike loblolly pine, no bacterial strain increased slash pine root length or biomass. They suggested that the effects of rhizobacteria inoculation on seedling emergence and plant growth are independent and that the effects are species specific.

Shishido and Chanway (2000) studied the colonization and growth promotion of outplanted spruce seedlings pre-inoculated with PGPR in the greenhouse. They observed that when seeds of two hybrid spruce (*Picea glauca* × *Picea engelmannii*) ecotypes were inoculated with PGPR caused significant seedling growth promotion in the greenhouse. Further, these seedlings were outplanted after 17 weeks at four reforestation sites, to evaluate seedling performance in the field. Four months after outplanting, when analyzed for relative growth rates (RGR) most strains enhanced spruce shoot or root RGRs in the field, but seedling growth responses were strain specific. For example, *Pseudomonas* strain Ss2-RN significantly increased both shoot and root RGRs by 10-234% at all sites, but increases of 28-70% were most common. In contrast, *Bacillus* strain S20-R was ineffective at all outplanting sites. In addition, seedlings inoculated with four of the six strains had significantly less shoot injury than control seedlings at all sites. The results indicated that once plant growth promotion is induced in the greenhouse, seedling RGR can increase by more than 100% during the first growing season in the field. However RGR increases of 21-47% were more common and may be more representative of the magnitude of biomass increases that can result from PGPR inoculation.

Garcia *et al.* (2004) studied effect of four bacterial strains showing *in vitro* metabolic capacities of plant growth-promoting action on the growth of holm-oak (*Quercus ilex*) and pine (*Pinus pinea*) plants at a forest nursery

before outplanting. All strains significantly increased some of the parameters studied (stem length, neck diameter and shoot dry weight), although, neck root diameter showed the smallest increase in general. An interaction plant/bacterial strain was observed. Some strains specifically increased aerial parameters in both plant species, whereas other strains did so mainly in one plant species.

Loblolly (*Pinus taeda*), slash (*Pinus elliottii*), and longleaf pine (*Pinus palustris*) seeds were inoculated in the greenhouse with rhizobacteria recovered from 4-month-old bareroot loblolly pine seedlings. Emergence and seedling densities were determined at 3- and 8-week intervals after sowing, respectively, with root and shoot biomass measured at 12 weeks. All bacterial strains significantly increased the speed of seedling emergence relative to untreated loblolly and slash pine seeds, while five strains significantly slowed longleaf germination. For loblolly and slash pine, there were no differences in percentage germination when comparing treated and untreated seed. However, all bacterial strains significantly reduced longleaf germination over nontreated seed. Treatment with rhizobacteria had significant positive and negative effects on seedling growth and biomass, depending on the tree species and bacteria isolate used. Five of the eight bacterial isolates tested increased shoot length of loblolly pine seedlings, whereas one strain increased above- and belowground biomass. Slash pine seedlings experienced growth increases with three bacterial strains. However, two bacterial strains, ALA-41G and ALA-54G, resulted in shorter slash pine shoots. Only strain ALA-3G increased shoot biomass of longleaf pine over non-treated longleaf seed. Increased seedling emergence and growth promotion by rhizobacteria collected from *Pinus* sp. is a potential

useful tool for management of these forest species. The effect may be species-specific and the use of bacteria in forest nurseries for growth promotion will need to take this into account (Enebak, 2005).

Rincon *et al.* (2005) studied the colonization of *Pinus halepensis* root by *Pseudomonas fluorescens* and interaction with the ectomycorrhizal fungus, *Suillus ganulatus*. They found that the presence of ectomycorrhiza significantly stimulated survival of bacterial in the root elongation zone where fungal colonization was higher and a synergistic effect was observed on the seedlings growth when bacteria and fungus were co-inoculated.

Solano *et al.* (2007) carried out screening of 270 PGPR isolates to improve growth of *Cistus ladanifer* seedlings for reforestation of degraded mediterranean ecosystems. Fifty-eight percent of the isolates showed at least one of the evaluated activities, with phosphate solubilisation and siderophore production being the most abundant traits. Although seven of the 11 assayed strains were phosphate solubilizers and able to produce siderophores, only one was really effective in increasing all biometric parameters in *Cistus ladanifer* seedlings. They concluded that the low diversity together with the high redundancy detected by PCR-RAPDs and the predominance of strains able to mobilise nutrients in the rhizosphere of *C. ladanifer* reveals that the plant selects for bacteria that can help to supply scarce nutrients and this type of PGPR strains would be successful in reforestation practices.

Teixeira *et al.* (2007) tested PGPR mainly *Pseudomonas* sp. and *Bacillus subtilis* obtained from the rhizosphere of *Eucalyptus* clones for rooting of cuttings and mini-cuttings planted in substrate composed of carbonized rice husk and vermiculite (1:1). Ten isolates were capable of

providing gains of up to 110% in root formation and up to 250% in root biomass over non-inoculated control cuttings. Gains in rooting varied according to clone and isolate tested. The greatest gains were obtained for the mini-cuttings exhibiting the lowest rooting efficiency. Significant increases in rooting and root dry matter of cuttings grown on rhizobacteria-inoculated substrate were found when compared to untreated or IBA treated mini-cuttings.

Mafia *et al.* (2009) evaluated the root colonization and interaction among isolates of PGPR and *Eucalyptus* species. They found that there was interaction among isolates of rhizobacteria and *Eucalyptus* species for seed germination and seedling growth. It was found that *Pseudomonas* sp. was the best rhizobacteria for growth promotion of *E. cloeziana* and *E. grandis*. *Bacillus subtilis* was the most effective inoculant for *E. globulus*. *P. fulva*, *Pseudomonas* sp. and *Stenotrophomonas maltophilia* were the most promising isolates for the *E. urophylla*.

An investigation was carried out by Yusran *et al.* (2009) on the effects of two PGPR and *Rhizobium* on the seedling growth of *Paraserianthes falcataria* and mycorrhizal development in two soils with contrasting level of pH (5 and 6) in a greenhouse. The results showed that inoculation of *P. falcataria* seeds with *Pseudomonas* sp. and *Bacillus amyloliquefaciens* in single or combined application significantly increased root and shoot dry weight, mycorrhizal root colonization and nutrient concentration (P, Zn and Cu) in the shoots of *P. falcataria* seedlings compared to untreated control. Only in the soil with pH 5, inoculation with *Pseudomonas* sp. in addition to *Rhizobium* inoculation led to a further increase of all measured variables compared to *Rhizobium* inoculation alone. In all other treatments, inoculation

with PGPR in addition to *Rhizobium* inoculation did not lead to a significant increase of any of the measured variables. The results demonstrate that plant growth-promoting rhizobacteria have the potential to promote the indigenous AM fungi establishment and the growth of *P. falcata* seedlings.

Recently, Mohan and Ayswarya Radhakrishnan (2012) have screened 23 different isolates of PSB for the growth improvement of tissue culture raised plantlets of *Tectona grandis*. They observed that out of 23 isolates screened, the isolates KED-4 (*Bacillus subtilis*) and TCO-6 (*Pseudomonas fluorescens*) were the most potential isolates for enhancing growth and biomass production of teak plantlets. They have also made an attempt to determine the synergistic effect between these 2 isolates and recorded that application of both the isolates together not only enhanced the growth but also increased the biomass and the quality of the plantlets.

Rodriguez and Fraga (1999) opined that greater attention should be paid to studies and application of new combination of PSB and other PGPR for improved results.

There are no reports available on the effect of PSB on *Ailanthus excelsa*, *Gmelina arborea*, *Melia dubia* and *Neolamarckia cadamba*.

2.1.6. Effect of combined inoculation of AM fungi and other bio-inoculants on the growth enhancement of forest tree species

Several investigations have brought to light the instances where biological activities are markedly enhanced in two or three member associations of beneficial microorganisms in the rhizosphere resulting in increased plant growth. Tinker (1980) and Barea *et al.* (1983, 2005) have reported the importance of interaction between the AM Fungi and PSB to

improve the utilization of rock phosphates by plants in general and particularly in non-acidic soils. The growth promoting effects of inoculation with combination of bio-inoculants are well documented in agriculture (Subba Rao, 1995; Constantino *et al.*, 2008, Singh *et al.*, 2009; Bahrani *et al.*, 2010). Several researchers have also studied the effect of inoculation of soil with AM fungi and other bio-inoculants on tree species. The studies are briefly reviewed below.

Dual inoculation of *Leucaena leucocephala* with *Glomus fasciculatum* and *Rhizobium* in a P-deficient soil significantly improved nodulation, mycorrhizal colonization, dry weight and N and P contents of the plants compared to control or single inoculation with either organism (Manjunath *et al.*, 1984).

The total N derived from fixation was slightly higher in *Acacia holosericea* trees inoculated with *Rhizobium* alone than with *Rhizobium* and *Glomus mosseae*. However, greater increase in plant height and dry weight was observed in dual inoculated plants (Cornet *et al.*, 1985).

Roskoski *et al.* (1986) conducted AM fungi- *Rhizobium* inoculation field trial with *Leucaena leucocephala* and *Acacia pennatula* at two sites. Result showed that inoculated plants of both species exhibited greater growth than uninoculated controls in Xalapa (acid soils), but not in LaBalsa (alkaline soils). They suggested that while the inoculation response of leguminous trees may vary with site or species, the benefits possible from the establishment of a highly effective tripartite symbiosis warrant including *Rhizobium* and AM fungi inoculation in tree legume programmes.

Basu and Kabi (1987) investigated the effect of application of bio-inoculants on the growth and nodulation of seven forest legumes viz., *Albizia lebbbeck*, *Leucaena leucocephala*, *Prosopis juliflora*, *Pongamia glabra*, *Acacia auriculiformis*, *A. arabica* and *Dalbergia sissoo*. They reported that application of bio-inoculants, either *Rhizobium* or *Rhizobium* + *Azotobacter* combined, has enhanced nodulation and growth of all the seven forest legumes significantly with *Leucaena leucocephala* registering the maximum response. Activity of the inoculants was found to increase further, at least in some cases, due to pelleting of inoculated seeds with lime.

Effectiveness among four AM fungi and *Rhizobium* in promoting growth of three legume trees viz., *Acacia auriculiformis*, *A. mangium* and *Albizia falcataria* in a P-deficient soil was studied by De la Cruz *et al.* (1988). *Glomus fasciculatum* + *Rhizobium* and *Gigaspora margarita* + *Rhizobium* were most effective for *Acacia mangium* and *Albizia falcataria*. *Scutellospora persica* + *Rhizobium*, *Gigaspora margarita* + *Rhizobium* and *Glomus fasciculatum* + *Rhizobium* were most effective for *A. auriculiformis*. Consistently poor growth was attained by seedlings inoculated with *Sclerocystis clavisporea* + *Rhizobium*, *Rhizobium* alone, or by uninoculated seedlings.

Combined application of *Azotobacter* and phosphobacteria increased 20.2% in height, 33.7% in collar diameter, 34.6% in fresh weight and 44% in dry weight over control in *Eucalyptus camaldulensis* grown in an unsterilized soil (Mohammad and Ram Prasad, 1988).

Rangarajan *et al.* (1988) investigated the effect of *Rhizobium* and AM fungi (*Glomus fasciculatum* and *Gigaspora calospora*) on *Acacia mellifera*, *A. nilotica* and *Leucaena leucocephala* seedlings. Their study revealed that

the dual inoculation resulted in increased biomass in all the three legumes over the single inoculation.

Combined application of *Glomus fasciculatum*, *Paxillus involutus* and *Frankia* to *Alnus incana* increased the root length (Chatarpaul *et al.*, 1989). Dual inoculation of *Frankia* and AM fungi resulted in increased nodule weight and acetylene reduction activity of *Alnus acuminata* (Russo, 1989) and higher N, P and micronutrient concentrations in *C. equisetifolia* seedlings (Karthikeyan and Raj, 1991).

Banwari Lal *et al.* (1990) studied the effect of inoculation of soil with *Rhizobium* and mycorrhizal fungi on N fixation and growth of *Prosopis juliflora*. They observed that the dual inoculation resulted in the enhancement of nitrogenase activity.

Gupta and Punj (1990) observed that dual inoculation of *Glomus fasciculatum* and *Rhizobium* improved the growth of *Leucaena leucocephala* over single inoculation of either organism in terms of plant dry weight, nodule dry weight and nitrogenase activity.

Mallesha and Bagyaraj (1990b) observed that co-inoculation with *Rhizobium* and *Glomus mosseae* at the time of sowing conferred maximum benefit to *Leucaena leucocephala* plants. They had more root dry weight, total shoot N and P contents as compared to the plants inoculated either first with *Rhizobium* and AM fungus 20 days later or AM fungus first and *Rhizobium* later.

Niranjan *et al.* (1990) reported an increase in nodulation, dry weight, total leghaemoglobins and nitrogenase activity to a greater extent in seedlings of *Dalbergia sissoo* inoculated with AM and *Rhizobium* than with

either organism alone or control plants. Niranjana *et al.* (2002) studied the dual inoculation effect of *Rhizobium* (cowpea miscellany) and AM fungi on growth, nodulation and N fixation in *Prosopis cineraria*. The study revealed that of several dual combinations of rhizobial isolates (PC Rhz-5, PC Rhz-7, PC Rhz-8) and AM fungi (*Gigaspora calospora*, *Glomus fasciculatum* and *G. mosseae*) tested, the seedlings of *P. cineraria* inoculated with *Rhizobium* (PC Rhz-5) + *G. mosseae* as twin symbionts recorded maximum enhancement of all parameters tested in comparison to seedlings inoculated with *Rhizobium* alone. The highest value of mycorrhizal root colonization was found in seedlings inoculated with *Glomus mosseae*. Recently, Niranjana *et al.* (2007) studied the efficiency of *Bradyrhizobium* and *Glomus fasciculatum* to promote growth of *Dalbergia sissoo* and the effect of IAA on the tripartite symbiotic association in a pot experiment. They opined that the tripartite synergistic interaction of *Bradyrhizobium* and *G. fasciculatum* with *D. sissoo* seems to be promising and can be utilized for its growth promotion in forest nurseries and reforestation of degraded areas.

In *Acacia decurrens*, Rangarajan and Narayanan (1990) observed that combined inoculation of AM fungi, *Azospirillum* and PSB increased plant dry weight by 100%. They also found that the same tetrapartite association increased height by 171.1% in *Syzygium arnotianum* and 253% in *S. cumini*.

Sharma *et al.* (1990) studied the interaction between *Rhizobium* and mycorrhizal fungi (*Glomus fasciculatum* and *Gigaspora margarita*) and their stimulatory effects on *Acacia nilotica*. They observed that dual inoculation significantly improved the plant height, plant biomass, nodulation (number and weight), N, P, sugar, chlorophyll and protein contents than single inoculation.

Thapar *et al.* (1990) studied the effect of AM fungi and *Rhizobium* on the growth of *Acacia nilotica* in saline and forest soil and observed that a suitable combination of the mycorrhizal fungi and *Rhizobium* may be useful in ensuring high biomass production and establishment of the plant species in saline soils. Thapar and Uniyal (1996) studied the response of *Acacia nilotica* to inoculation with *Glomus macrocarpum* and *Rhizobium*. They found that inoculation with the AM fungi resulted in better growth performance. Inoculation with *Rhizobium* alone and in combination with AM fungi did not show any improvement in growth over other treatments suggesting the incompatibility of *Rhizobium* strain with the host species.

In *Leucaena leucocephala*, an increase in plant height (33.25%) was observed by Young (1990) when it was inoculated with PSB. He carried out pot experiments to determine the effects of single and mixed inoculations with PSB and AM fungi on the growth of *L. leucocephala*, *Acacia confusa*, *A. mangium* and *Liquidamber formosana* in three tropical soils. The growth response of *Leucaena* to inoculation with PSB alone increased in five out of six treatments in sterilized and non-sterilized soils, with an average growth increase of 330.2%. A synergistic effect from a mixed inoculation with PSB and AM fungi on the growth of *Leucaena* occurred in unsterilized soil. Inoculation with these mixed inoculants also promoted the growth of *A. confusa* (14-63%), *A. mangium* (7-88%) and *L. formosana* (24-280%) in these three soils.

It has also been shown that combined inoculation of leguminous plants with AM fungi and either *Rhizobium* spp. or *Bradyrhizobium* spp. significantly increases the amount of nodulation, nodule biomass, overall plant growth and most importantly, level of N fixation compared to plants inoculated with nodulating bacteria alone (Azcón *et al.*, 1991).

A factorial experiment with two controlled factors was conducted in the greenhouse with *Acacia senegal* seedlings by Colonna *et al.* (1991). The substrate was a degraded sandy soil poor in available P (11 ppm — Olsen). The first controlled factor was soil sterilization, with two levels: (A) sterilized soil; (B) non-sterilized soil. The second factor was fertilization, with six levels: (1) uninoculated control; (2) inoculation with *Rhizobium* (ORS 1007); (3) inoculation with *Glomus mosseae*; (4) double inoculation with ORS 1007 and *G. mosseae*; (5) inoculation with ORS 1007 and 30 ppm P per plant; (6) inoculation with ORS 1007 and 60 ppm P. Compared to the control B1, the B5 and B6 treatments containing P increased nodule dry weight about 7 times; leaf dry weight about 4 times; total N, P and Mg 4–5 times; total K and Ca 3–4 times. The mycorrhizal inoculation had the same positive effect on plant growth and mineral composition but with lower values.

Effects of ectomycorrhizal (*Boletus suillus*) and AM fungi on drought tolerance of *Acacia auriculiformis*, *Albizia lebbbeck*, *Gliricidia sepium* and *Leucaena leucocephala* were studied by Osonubi *et al.* (1991). In *Gliricidia* and *Leucaena* both mycorrhizal inoculations stimulated greater plant growth, P and N uptake under both water stressed and unstressed condition. However, in *Acacia* and *Albizia* these parameters were stimulated by either ectomycorrhiza (*Acacia*) or AM (*Albizia*).

Bagyaraj (1992) reported that *Glomus fasciculatum*, *G. mosseae*, *G. leptotichum* and *Scutellospora* sp. were most efficient for *Dalbergia sissoo*, *D. latifolia*, *Acacia nilotica* and *A. auriculiformis*, respectively. The increase in plant biomass and height was to the extent of 34% and 24% in *D. sissoo*, 48% and 24% in *D. latifolia*, 100% and 112% in *A. nilotica* and 126% and 50% in *A. auriculiformis*. He also reported that plant biomass in *C. equisetifolia* increased by 61.44% when co-inoculated with *G. fasciculatum* and *Frankia*.

El-Gamal (1992) studied the effect of adding bio-inoculants, *Azotobacter* spp. (*A. chroococcum* and/or *A. vinelandii*) or chemical fertilizer, urea into the rhizosphere of *Sesbania sesban* seedlings which were previously inoculated with *Rhizobium sesbani* (controlled plants). Results indicated that *Sesbania* plants fortified with urea or inoculated with *A. chroococcum* and/or *A. vinelandii* into the rhizosphere gave significant increases in chlorophyll contents, total soluble carbohydrates, total soluble proteins, root, shoot lengths, dry weight of roots and shoots. Moreover, nitrogenase activity was also stimulated in *Sesbania* plants inoculated with *A. chroococcum* and/or *A. vinelandii*, while no changes were observed in nitrogenase activity in *Sesbania* plants supplemented with urea. The study suggested that the association between *Azotobacter* spp. and *R. sesbani* in the rhizosphere of *Sesbania* plants enhanced the *Sesbania* growth.

Dual inoculation of *Glomus fasciculatum* and *Rhizobium* decreased the renodulation of *Acacia nilotica* approximately by 50% as compared to single inoculation (Banwari Lal and Khanna, 1993).

Valdes *et al.* (1993) used dual inoculations of either *Glomus versiforme* and *Rhizobium loti* NGR 8 or *Glomus* sp. and *R. loti* ENCB 31 on *Leucaena esculenta*. They observed that the dual inoculations gave higher growth and P accumulation compared to treatments with a single micro-symbiont. They recommended the use of endomycorrhized plants to reduce the transplanting shock, particularly in arid zones.

Seed pelleting treatment of *Azadirachta indica* with AM fungi or *Azospirillum* or phosphobacteria alone @ 50 g kg⁻¹ of seed significantly enhanced the seed germination and seedling vigour (Vanangamudi *et al.*, 1993).

Verma *et al.* (1994) recorded a significant growth and biomass production in *Acacia nilotica* seedlings inoculated with *Glomus mosseae* and *Rhizobium*. Similar observations were made in *Casuarina* seedlings co-inoculated with AM fungus and *Frankia* spp. (Vasanthakrishna *et al.*, 1994).

Balasubramanian *et al.* (1995) observed increased plant vigour and increased performance in terms of higher nodule numbers, nodule dry weight, shoot length, root length, plant biomass and higher N build up in *Casuarina equisetifolia* seedlings due to combined inoculation of AM fungus, *Glomus fasciculatum* and *Frankia*.

Cao *et al.* (1995) reported that dual inoculation of *Leucaena leucocephala* and *Acacia mearnsii* seedlings with *Glomus mosseae*, *Glomus* spp. and *Rhizobium* significantly improved growth and number of nodules over the control.

Growth response of *Casuarina equisetifolia* under tripartite interaction between *Frankia*, *Glomus* and *Casuarina* at different P regimes was investigated by Joshaline and Balasubramanian (1995). They reported that the growth of *C. equisetifolia* can be improved considerably by the dual inoculation of *Frankia* and AM fungus, *G. moseae*.

Karthikeyan *et al.* (1995) studied the effect of dual inoculation of AM fungi and *Phosphobacterium* on the growth improvement of neem (*Azadirachta indica*) seedlings. They found that combined inoculation of AM fungi and phosphobacterium produced significant increase of total dry weight and N, P, K uptake and AM colonization over individual application and control seedlings at all tested stages. They recommended dual inoculation for afforestation of wastelands. Similar results were obtained by Kaushik and Kaushik (1995) in *Albizia lebbek* when inoculated with AM and *Rhizobium*.

Selvaraj *et al.* (1995) observed the response of two N₂ fixing tree legumes and one non-N₂ fixing tree species to various combinations of AM fungi and N fixer using N and P depleted acid soil in nursery. The results revealed that *Leucaena leucocephala* and *Acacia holosericea* responded well to the combined inoculation of *Rhizobium* and the AM fungus, *Glomus fasciculatum* while, *Cassia siamea* responded well to the dual inoculation of *Azospirillum* and *G. fasciculatum*.

Interaction of AM fungi and *Frankia* sp. on growth and nutrient uptake in *Casuarina equisetifolia* seedlings was studied by Udaiyan and Sugavanam (1995). They reported that dual inoculation of *Casuarina* with *Gigaspora margarita* and *Frankia* sp. had significantly increased growth, biomass and tissue P and K concentration when compared to single inoculation. Also the triple inoculation with AM fungi, *G. margarita*, *Glomus mosseae* and *Scutellospora nigra* along with *Frankia* sp. markedly increased growth, biomass, root colonization, sporulation and tissue nutrient concentration than single or double inoculation of AM fungi with or without *Frankia* sp.

Prosopis juliflora being a leguminous plant responded well to *Rhizobium* cultures and AM fungi application. The seedlings showed increased shoot length and biomass in nursery studies conducted at FRI, Dehradun. The seeds of *Prosopis* inoculated with *Rhizobium* (R 1115) and AM fungus (*Glomus fasciculatum*) gave healthy seedlings which had vigorous growth on nutrient deficient sodic soil (Uniyal and Thapar, 1995). These seedlings had also showed high survival percentage in poor soils and also under drought conditions.

Leguminous saplings inoculated with cultures of mycorrhizae and *Rhizobium* and non-legumes with AM fungi and *Azotobacter* when planted using top soil collected from nearby areas and farm yard manure on physically reclaimed coal mine spoil dumps at Bilaspur, Madhya Pradesh improved the physical and chemical properties of coal mine over burden as well as plant heights (Kumar and Jena, 1996).

According to Mehrotra (1996), dual inoculation of *Acacia catechu* with *Rhizobium* and AM fungi increased the shoot height and biomass to the extent of 60.5% and 40.4%, respectively, compared to inoculation with AM alone, where both the parameters were 57.2% and 35.9%, respectively.

Sekar *et al.* (1995, 1996, 1997) conducted studies on shola tree species, *Syzygium montanum*, *S. arnotianum* and *Elaeocarpus oblongus* to elucidate the influence of bio-inoculant inoculation on seedling growth. The results indicated that the combined inoculation of *Azospirillum*, PSB and AM fungi significantly increased the shoot length, collar diameter, root length, root/shoot ratio, total biomass and total P uptake in all the tree species compared to other combined or individual inoculations or uninoculated control. Among individual inoculations, AM fungi performed better than *Azospirillum* or PSB.

Combined inoculation of *Azospirillum* and phosphobacteria in *Azadirachta indica* seedlings significantly increased the seedling traits such as shoot length, root length, number of leaves at 30 and 60 days after planting (Shanmuga Moorthy, 1996).

Plant growth responses in *Acacia nilotica* on inoculation with AM fungus, *Glomus macrocarpum* and *Rhizobium* in sodic and normal soils were

studied under greenhouse conditions by Thapar and Uniyal (1996). Significant differences in growth parameters were observed in normal soil as compared to sodic soil. The overall effect of AM fungus on shoot height, biomass, collar diameter and nodule number was positive. Inoculation with *Rhizobium* and in combination with AM fungus did not show any improvements in growth over other treatments suggesting the incompatibility of *Rhizobium* strain with the host species or its ineffectiveness in sodic soil.

Meshram *et al.* (1997) studied the effect of *Azotobacter chroococcum* and AM fungus (*Glomus fasciculatum*) on biomass production of *Eucalyptus camaldulensis* on waste/barren land. The treatment included factorial combination of bio-inoculants (*Azotobacter chroococcum* and *Glomus fasciculatum*) with farm yard manure and chemical fertilizers (N + P) along with their application alone and in combination with FYM + N + P. Of all the treatment combinations, the treatment comprising of *A. chroococcum* and mycorrhizae amended with FYM and N + P resulted in maximum biomass of *E. camaldulensis* after two years of plantation on waste/barren land.

Combined application of *Azotobacter*, AM fungi with NPK to vegetatively propagated planting stock of *Dendrocalamus strictus* increased the height and number of culms (Prasad *et al.*, 1997).

Udaiyan *et al.* (1997) reported a significant increase in tissue N, P and K when *Acacia mearnsii* seedlings were inoculated with *Glomus deserticola* and *Rhizobium*, over the single inoculation and control seedlings.

The growth of *Prosopis juliflora* and its contribution to soil enrichment following inoculation with three AM isolates, *Glomus caledonius*, *Gigaspora calospora* and an indigenous strain, and two *Rhizobium* isolates, P-5 and Tal-600

were assessed by Bhatia *et al.* (1998). The trees were 6 years old and grew on a semi-arid wasteland. There was a significant increase in the biomass of closely spaced *P. juliflora* inoculated with *G. caledonius* alone. A significant reduction in the soil pH and a considerable improvement in soil P and organic carbon build-up were observed in all the treatments by the end of the experiment.

Kennedy and Chellapillai (1998) studied the effect of AM fungi, *Azospirillum* and *Phosphobacterium* on growth response and nutrient uptake of shola tree species viz., *Elaeocarpus munroii*, *Gordonia obtusa*, *Ligustrum roxburghii* and *Sideroxylon tomentosum*. All the treatments increased seedlings growth over uninoculated control plants. The plants that received the combination of AM fungi, *Azospirillum* and *Phosphobacterium* had the highest increase in height, total dry weight, AM fungal colonization and total N and P uptake.

Puthur *et al.* (1998) carried out a study to achieve cent percent transplantation success of micropropagated *Leucaena leucocephala* plantlets using *Glomus fasciculatum* and *G. macrocarpum*. Plantlets after getting their roots nodulated with *Rhizobium* (strain PRGL 001) in soilrite, were transferred to sterilized garden soil by laying inoculum of either *G. fasciculatum* or *G. macrocarpum* around their roots. Only 20% of the plantlets survived in soils lacking AM fungus, while 100% plantlets of *L. leucocephala* established very well and showed good growth in AM inoculated soil. Roots of the latter plantlets showed presence of both external and internal hyphae with well developed arbuscules and vesicles confirming the establishment of good mycorrhizal association. They concluded that the mycorrhizal association help in successful establishment of tissue culture raised plantlets of *L. leucocephala* in the field conditions by alleviating the transplantation shock.

Inoculation of *Tectona grandis* with *Azospirillum* and *Phosphobacterium* along with mulch (coir pith) increased tree height (20%) and girth (10.3%) (Subramanian *et al.*, 1998).

Sugavanam *et al.* (1998) studied the influence of six AM fungi on the growth of *Tectona grandis* individually as well as in combinations with *Azospirillum* sp. The inoculation of AM fungi or *Azospirillum* sp. increased the growth, biomass, root colonization, tissue N, P and K concentration. Significant response was observed with *Gigaspora margarita* or *Glomus versiforme* inoculation along with *Azospirillum* sp. Inoculation of AM fungi either singly or in combination with *Azospirillum* sp. had significantly lower root to shoot ratio than uninoculated control.

Gupta and Rahangdale (1999) reported that dual inoculation of *Dalbergia sissoo* and *Albizia lebbeck* with *Rhizobium* and AM fungi enhanced the growth with respect to height, biomass and nodule status.

Khan and Uniyal (1999) studied the impact of inoculation of *Acacia nilotica* seedlings with AM fungi and *Rhizobium* and *Populus deltoides* sets with AM fungi alone and in combination with fertilizers. They observed that *A. nilotica* responded positively to combined inoculation of AM fungi and *Rhizobium* whereas, *P. deltoides* responded well to AM fungal inoculation combined with normal dose of fertilizers.

Nagabhushanam and Reddy (1999) studied the influence of single and dual inoculation of AM fungi and *Rhizobium* on the growth of *Pongamia pinnata*. *Glomus aggregatum* was found to be the efficient growth promoter of *P. pinnata* seedlings. The stimulatory effect on growth of *P. pinnata* was

further enhanced in the presence of *Rhizobium*. They concluded that mixed inoculum of AM fungi along with *Rhizobium* was more beneficial for the growth and development of *Pongamia*.

Sugavanam *et al.* (1999) studied the interactions of AM fungi and *Frankia* on *Casuarina equisetifolia* under nursery conditions. Dual inoculation increased the growth and biomass of the seedlings. Among different AM fungi, *Glomus fasciculatum* followed by *G. versiforme* significantly enhanced the growth and biomass. Nodule number and nodule biomass were increased with the inoculation of AM fungi and were further increased by inoculation with *Frankia*.

Chen *et al.* (2000) studied the effects of inoculation of ECM fungus (*Laccaria lateritia*) and AM fungi (species of *Glomus*, *Acaulospora* and *Scutellospora*), alone or in combination, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla* in a glasshouse experiment. They reported that AM fungi became established much more rapidly than *Laccaria*. In plants with both types of mycorrhizas, ECM substantially increased after 2 or 3 months and the proportion of roots with AM declined. However, the proportion of roots with AM also decreased significantly in plants without ECM after 2 months. The relative susceptibility of eucalypt roots to these mycorrhizal associations varied. Treatments where *Laccaria* was applied, alone or in combination with AM fungi, resulted in the largest growth increases relative to non-mycorrhizal plants. *Acaulospora* was the most effective AM fungus for *E. urophylla* although other AM fungi also increased growth relative to the control.

Gong *et al.* (2000) tested three AM fungi (two isolates of *Glomus caledonium* and one of *G. versiforme*) and four ECM fungi (one isolate each of *Pisolithus*, *Scleroderma*, *Laccaria* and *Hebeloma*) on the seedlings of *Acacia mangium* and *A. crassicarpa*. Seedlings of both the tree species were capable of forming both ECM and AM associations and indicated strong mycorrhizal dependency on inoculant fungi. The results also indicated that inoculation with mycorrhizal fungi enhanced nodule formation by rhizobia.

The effect of inoculation with AM fungus (*Glomus fasciculatum*) with PSB on the growth and nutrients uptake of *Azadirachta indica* was examined by Kalavathi *et al.* (2000). They observed that the combined inoculation of AM fungus and phosphobacteria markedly increased the growth of *A. indica* seedlings. Both the organisms showed synergistic effect.

Acacia nilotica plants inoculated with native *Rhizobium* and AM fungus (*Glomus fasciculatum*) showed maximum growth, with significant differences between control and inoculated plants as well as between individual and combined inoculated plants. Maximum growth and uptake of N, P and K was observed in combined inoculated plants (Prasad, 2000).

Acacia nilotica seedlings inoculated with *Pisolithus tinctorius*, *G. mosseae* and *Bradyrhizobium* species showed greater total dry weight, number of nodules, weight of nodule and nitrogenase activity than the seedlings grown in soil with other organisms and uninoculated seedlings. Mycorrhizal development was not much different between the triple inoculation treatment and treatment involving *Bradyrhizobium* species and one mycorrhizal fungus (dual inoculation) (Saravanan and Natarajan, 2000).

Sastry *et al.* (2000) investigated the role of AM fungi and fluorescent *Pseudomonas* (PRS9) interactions in growth promotion and nursery establishment of *Eucalyptus* hybrid. Root length, shoot length, root and shoot fresh and dry weights were maximal at 400 AM fungal spores and 20ppm soil P. Shoot P content was maximal at 800 AM fungal spores followed by 400 AM fungal spores and vice versa in case of root P content. In general, plant growth was greater at 20ppm P. Independent of soil P levels, the quality index of mycorrhizal treatments without PRS9 was significantly higher than the treatments including PRS9 or PRS9 alone. Mycorrhizal inoculation efficiency was superior at 10ppm P irrespective of the treatment. AM alone (400 spores) significantly improved the inoculation efficiency. PRS9 in association with AM fungi inhibited growth promotion and nutrient uptake.

Singh *et al.* (2000) studied the effect of application of *Rhizobium* and AM fungi on growth of *Albizia lebbeck* and *A. procera* in severely eroded soil at Jabalpur, Madhya Pradesh. Results obtained after 4.5 years of planting exhibited best survival and growth (height and collar girth) of both *A. procera* and *A. lebbeck* which received *Rhizobium* + AM followed by *Rhizobium* and AM fungi separately. Growth parameters (survival, height and collar girth) in the treatments were significantly higher than control in both species. Further, actual values of growth parameters were higher in *A. procera* than in *A. lebbeck*.

Verma *et al.* (2000) studied the effect of *Rhizobium* and AM fungi on growth of *Dalbergia sissoo* and *Pongamia pinnata* and observed that dual inoculation resulted in better growth of seedlings in the nursery.

Chauhan and Pokhriyal (2001) studied the effect of *Rhizobium* and mycorrhizal inoculation along with pH solutions (acidic, neutral and alkaline) in *Albizia lebbeck*. They found that the neutral pH was the most pronounced and that the plants inoculated with the combined inoculum of *Rhizobium* and mycorrhiza performed the best. Also, in another study, Chauhan and Pokhriyal (2002) assessed the effect of *Rhizobium* and mycorrhiza on the nodulation and N fixation in *Albizia lebbeck* seedlings and reported that the plants performed best when inoculated with both *Rhizobium* and mycorrhiza.

Gurumurthy *et al.* (2001) studied the effect of inoculation of AM fungus, *Sclerocystis dussii* and a P-solubilizer, *Pseudomonas striata* on the growth and nutrition of *Tectona grandis* seedlings at optimum level of P tried in two forms as single superphosphate or rock phosphate. They observed that the percent mycorrhizal root colonization, mycorrhizal spore count, population of free-living N₂-fixers and P-solubilizers, activities of acid phosphatase and alkaline phosphatase were significantly higher in the dual inoculated seedlings as compared to seedlings inoculated with either *S. dussii* or *P. striata*.

Neem seedlings were inoculated with AM fungi (*Glomus intraradices* and *G. geosporum*), *Azospirillum brasilense* and PSB individually or in various combinations in unsterile soils under tropical nursery conditions by Muthukumar *et al.* (2001). They found that microbial inoculation resulted in increased mycorrhizal colonization, greater plant height, leaf area and number, collar diameter, biomass, tissue N, P, K content, and seedling quality over uninoculated control. The inoculated seedlings also had lesser root shoot ratio and low nutrient utilization efficiencies. They reported that microbial inoculation effects were greatest when seedlings were inoculated

with a combination of microbes rather than individual ones. This indicated that these microorganisms act synergistically when inoculated simultaneously with maximum response being when AM fungi were inoculated with *A. brasilense*.

Ravichandran and Balasubramanian (1999, 2001) studied the efficacy of dual inoculation of *Frankia* and AM fungus (*Glomus fasciculatum*) on the growth response of *Casuarina equisetifolia* seedlings. The results revealed that dual inoculation of *Frankia* strain (CCI3) and *G. fasciculatum* significantly improved the shoot and root length, the root volume, mycorrhizal inoculation percentage, nodule number, nodule dry weight and nodule nitrogenase activity when compared to uninoculated control. In another study, Ravichandran and Balasubramanian (2006) studied the effect of inoculation of *Frankia* and AM fungi on productivity of *Casuarina equisetifolia*. They observed that the tripartite association involving effective *Frankia* strain, efficient AM fungi and *Casuarina* seedling resulted in increased dry matter content and N and P uptake.

Van der Heijden (2001) investigated the functional significance of both AM and ECM for *Salix repens*, a dual mycorrhizal plant. The results showed that colonization of *Glomus mosseae* was low but had larger short-term effects on shoot growth and root length. *Hebeloma leucosarx* showed high ECM colonization but benefits occurred over a longer term. *G. mosseae* colonization resulted in higher shoot P uptake, shoot growth and root growth.

The effects of single and dual inoculation with *Rhizobium* and AM fungus (*Glomus mosseae*) on *Acacia saligna* plants were investigated in a greenhouse by Benbrahim and Ismaili (2002). The experiment revealed that dual inoculation significantly increased root dry weight, total dry weight

(56.9%) mycorrhizal colonization (14 fold) and N (70.9%) and P (76.3%) content of plants. Dual inoculation also increased N₂ fixation by 4.4 mg N/plant over the control.

Acacia mangium inoculated with *Rhizobium* strains BR 3609 and BR 3617 and three AM fungi (*Glomus clarum*, *Gigaspora margarita* and *Scutellospora heterogama*) showed better growth than uninoculated control plants. *S. heterogama* showed better results in both fallow and degraded soils. Dual inoculation resulted in more nodules and higher AM fungal colonization (Kayode and Franco, 2002).

Nagaveni and Vijayalakshmi (2002) inoculated *Azotobacter chroococcum* and AM fungi singly as well as in combination on the seedlings of *Eucalyptus camaldulensis*, *Wrightia tinctoria* and *Bombax ceiba* under nursery conditions. Better response was observed in both AM fungi alone and AM + *Azotobacter* treatments and least response was observed in *Azotobacter* alone treatment.

Rojas *et al.* (2002) reported that adding mycorrhizal fungi and fluorescent *Pseudomonas* sp. to clear-cut soils increased nodulation and N-fixation of red alder (*Alnus rubra*) and snowbrush (*Ceanothus velutinus*) plants.

Interaction between the AM fungus *Glomus mosseae*, *Azotobacter chroococcum* and *Azospirillum brasilense* in soil and their consequent effect on growth and nutrition of neem seedlings were studied under glasshouse conditions by Sumana and Bagyaraj (2002). The results revealed that dual inoculation of *G. mosseae* and *A. chroococcum* resulted in maximum plant biomass, N and P uptake; biovolume index and quality index of neem

seedlings. It also increased the mycorrhizal root colonization and spore number in soil of the root-zone. They also reported that the dual inoculation improved plant growth more than the single inoculation and uninoculated control.

Suvarna *et al.* (2002) studied the effect of dual inoculation of *Rhizobium* and *Glomus macrocarpum* on *Albizia lebbbeck* under greenhouse conditions. The results indicated that the shoot length, shoot, root and nodule dry weight were significantly higher in dual inoculated seedlings. Maximum shoot N content was found in dual inoculated seedlings whereas, maximum shoot P content was found in AM treatment followed by dual inoculation.

The effects of dual inoculation with *Frankia* and mycorrhizal fungi on Tibetan seabuckethorn (*Hippophae tibetana*) in pot cultures were investigated by Tian *et al.* (2002). The results showed that AM fungi and *Frankia* can stimulate the growth and the N fixation ability of host plants, respectively, yet the stimulation by dual inoculation on the growth and N fixation ability of the host plants was more significant.

Jayanthi *et al.* (2003) studied the influence of the AM fungus, *Glomus mosseae* and the PGPR, *Bacillus coagulans* and *Trichoderma harzianum* on the growth and nutrition of micropropagated *Ficus benjamina* plantlets under greenhouse conditions. The AM fungus was inoculated either singly or in combination with the PGPRs. Plants showed maximum plant height, biomass, P content, mycorrhizal root colonization, spore numbers and populations of *T. harzianum* and *B. coagulans* in root zone soil when all the three organisms were inoculated together. They concluded that when *G. mosseae* co-inoculated with PGPRs enhanced growth and nutrition of *F. benjamina*.

Experiments were conducted to determine the effect of inoculation with *Rhizobium*, the ectomycorrhizal fungus, *Hebeloma mesophaeum* and the AM fungus, *Glomus caledonium* on growth and N fixation of black locust (*Robinia pseudoacacia*) seedlings grown *in vitro* and in pot culture (Tian *et al.*, 2003). The results showed that both mycorrhizal fungi and *Rhizobium* stimulated the growth and N fixing ability of inoculated seedlings. Inoculation with all three microbes together produced the most beneficial effects on N fixation, mycorrhizal development and seedling growth.

Vasishth *et al.* (2003) studied the growth and nutrient uptake in *Acacia catechu* seedlings after inoculation with *Rhizobium* and AM fungi. The results indicated that the inoculants, individually and in combinations, significantly increased growth parameters like shoot length, phytomass, nodule number and dry weight, N, P, and K concentration over control.

Inoculation with *Azospirillum*, PSB and AM fungi on silk cotton tree (*Bombax ceiba*), improved seed germination, seedling growth, chlorophyll, total soluble carbohydrates, reducing sugars, total free aminoacids, buffer soluble proteins and phenolics (Vijayakumari and Janardhanan, 2003).

Lalitha and Santhaguru (2004) studied the response of tree-legumes to dual inoculation with *Rhizobium* and *Glomus fasciculatum*. On the basis of percentage of root colonization and plant biomass accumulation in tree-legumes (*Albizia lebbek*, *A. amara*, *Acacia auriculiformis*, *A. mellifera*, *Enterolobium saman*, *Erythrina indica*, *Pithecellobium dulce*, *Leucaena leucocephala* and *Pongamia glabra*) *Glomus fasciculatum* was found to be the most efficient AM fungus among five species tested, *viz.*, *Glomus mosseae*, *G. fasciculatum*, *G. microcarpum*, *Sclerocystis pachycaulis* and *Gigaspora nigra*. Dual inoculation with *Rhizobium* and *Glomus fasciculatum* enhanced

the plant biomass accumulation (93 - 443%), total N content (134 - 337%) and total chlorophyll content in leaves (232 - 630%) as compared to the single inoculation with either *Rhizobium* or *G. fasciculatum* alone.

Rajendran and Devaraj (2004) conducted an experiment to study the productivity of *Casuarina equisetifolia* in farm forestry. Seedlings inoculated with *Azospirillum*, *Phosphobacterium*, AM fungi and *Frankia* individually and in combinations were planted in farmland. Growth and biomass were estimated 24 months after planting by harvesting the plant. The height of the tree ranged from 9.87 to 11.90 m and the girth at breast height (GBH) ranged from 16.8 to 23.2 cm. The maximum height, GBH and total biomass were obtained in the combined application of *Azospirillum*, *Phosphobacterium*, AM fungi and *Frankia*. The combination of AM + *Frankia* among double inoculations, and combination of *Azospirillum*, AM and *Frankia* among triple inoculations have also promoted total height, GBH and total biomass production significantly.

Ratha Krishnan *et al.* (2004) studied the influence of inoculation of bio-inoculants (*Azospirillum*, *Azotobacter*, PSB and AM fungi) on growth and biomass productivity of *Simarouba glauca* seedlings. The results indicated that the combined application of *Azotobacter* and PSB significantly increased the shoot length and collar diameter. The combined inoculation of *Azotobacter* and AM fungi registered the maximum root length, number of leaves, root/shoot ratio, quality index and highest AM colonization percentage. Whereas, the total dry matter production and relative growth rate were higher for the combined application of *Azospirillum*, PSB and AM fungi. PSB and AM fungi combination recorded the highest total leaf area.

Aseri and Rao (2005) studied the interactive effect of bio-inoculants (*Azospirillum brasilense*, *Azotobacter chroococcum* and *Glomus mosseae*) with chemical fertilizers on growth, rhizosphere activity and nutrient uptake of Ber (*Ziziphus mauritiana* var. *rotundifolia*). Plant height and shoot dry weight were significantly improved upon inoculation with N fixing bacteria or AM fungus with maximum growth when coupled with 50% of recommended N and P fertilizers. Application of P fertilizer significantly reduced percent root colonization and density of AM fungal spores. Similarly, nitrogenase activity of the rhizosphere soil was also decreased in the presence of N fertilizer. Maximum N uptake was observed with N fixing bacteria while, P with AM fungus.

Lakshman (2005) studied the growth response and biomass production of *Albizia lebbeck* seedlings by inoculation of AM fungi, *Rhizobium* and molybdenum spray. The results revealed that inoculation with bio-inoculants and Mo spray significantly improved the shoot and root length, percentage of AM colonization, number of leaves and shoot dry weight.

Tamilselvi (2005) reported that inoculation of *Acacia auriculiformis* and *A. mangium* with suitable AM fungus (*Glomus fasciculatum*), ECM fungus (*Pisolithus albus*), symbiotic (*Rhizobium*) and asymbiotic (*Azospirillum*) N fixers resulted in increased growth and seedling quality. Inoculation of *G. fasciculatum*, *P. albus*, *Rhizobium* and/or *Azospirillum* increased in shoot and root length, diameter, tissue nutrients, dry matter production, volume and quality index over the other combined inoculations and uninoculated seedlings in both the acacias. This was attributed to the increased P provided by the mycorrhizal fungi which in turn could have helped N fixers to fix increased amount of N. Synergistic action of all the microbes in the rhizosphere of both the acacias was observed.

Gopal Krishnan *et al.* (2006) studied the effect of dual inoculation (*Rhizobium* + AM fungus) on the seedlings of *Albizia lebbbeck* under glasshouse conditions. They observed that the dual inoculation improved the shoot and root length, leaf area, chlorophyll content, plant biomass, nodulation status, N and P uptake, amount of N₂ fixed and root colonization over the uninoculated control.

Muthukumar and Udaiyan (2006) assessed the effect of bio-inoculants (*Glomus aggregatum*, *Bacillus polymixa*, *Azospirillum brasilense*) on seedling growth promotion of bamboo (*Dendrocalamus strictus*) in two soil types (alfisol, vertisol) with or without fertilizer application. Bamboo seedlings were grown in the presence or absence of bio-inoculants either individually or in all combinations for 180 days in field soil under tropical nursery conditions. Shoot, rhizome and root length, dry masses, nutrient concentrations and AM fungal colonized root lengths were determined at harvest. Combined inoculation of AM fungi, PSB and *A. brasilense* resulted in maximum growth response both under fertilized and unfertilized conditions in both soil types. Fertilizer application enhanced the efficiencies of N, P and K uptake, but reduced their usage efficiencies. Though soil type did not affect microbial inoculation response, fertilizer application significantly affected plant response to inoculation.

Shah *et al.* (2006) conducted an experiment by inoculating *Frankia*, *Rhizobium* and *Azospirillum* either singly or in combination with AM fungi on *Casuarina equisetifolia*, *Acacia nilotica* and *Eucalyptus tereticornis*. The inoculation resulted in high biomass built up over control. *C. equisetifolia* and *A. nilotica* developed more nodules with higher nodule dry mass and high nodule

nitrogenase activity compared to control. They also observed that the dual inoculations viz., *Frankia* + AMF, *Rhizobium* + AMF and *Azospirillum* + AMF showed higher productivity than single inoculation in all the tree species tested.

Swaminathan and Srinivasan (2006) studied the influence of microbial inoculants (*Azospirillum*, *Azotobacter*, PSB, AM fungi) on growth and survival of *Tectona grandis* seedlings. The inoculants were mixed with nursery potting mixture used for filling the poly pots. Biometric observations were recorded six months after treatment and also their survival in the main field-tested for one year. They observed that *Azotobacter* gave best performance with respect to shoot length, shoot weight and leaf area and combined inoculation of PSB + AM fungi gave higher root length and biomass, dry matter production and fine root weight. The survival was better in seedlings inoculated with PSB + AM fungi.

Karthikeyan *et al.* (2007) tested the effect of application of bio-inoculants viz., AM fungi, *Azospirillum* and *Phosphobacterium*, individually and in various combinations on *Azadirachta indica* seedlings under nursery conditions. Their studies revealed that the seedlings inoculated with bio-inoculants resulted in increased plant height, collar diameter and biomass. They also reported that the combined application of the bio-inoculants resulted in healthy and vigorously growing seedlings in nursery.

Ayswarya (2008) investigated the efficiency of different bio-inoculants, compost and vermicompost on the growth of *Tectona grandis* clones. Among the different treatments, the plantlets grown with mixed inoculum of bio-inoculants (AM fungi, *Azospirillum* and PSB) and 10g or 15g of vermicompost showed more shoot height, root length, collar diameter, shoot and root biomass, volume index and quality index. This was followed by the

treatment with AM fungi + phosphate solubilizing bacteria + 10g or 15g of vermicompost. Treatment with triple combination of bio-inoculants and 15g compost also showed better growth performances. In general, the application of combination of bio-inoculants and biomanures showed significantly higher and better growth performances of the tissue culture raised plantlets of teak when compared to the uninoculated (control) plantlets during different periods of the observation.

A factorial pot experiment was carried out by Diouf *et al.* (2008) in a greenhouse to determine effect of inoculation of AM fungus (*Glomus aggregatum*), *Rhizobium* (strains ISRA 604 and ISRA 605) and P fertilization as triple super phosphate on the growth of *Gliricidia sepium*. Mycorrhizal colonization, nodulation, plant growth, P and N contents, and N fixed were determined. The results showed that frequency and intensity of mycorrhization were highest at 0, 20 and 40 mg P kg⁻¹ soil applied however, rhizobial inoculation has influenced frequency of mycorrhization. In the *Rhizobium*-AM fungus interaction, plant shoot N content increased when trees were inoculated with *G. aggregatum* whatever the rhizobial strain inoculated. The inoculated plants exhibited the highest total N in whole plant as well as in shoots and roots than in non-inoculated plants.

Sitepu *et al.* (2008) studied the effect of PGPR and AM fungus on tree species *viz.*, *Shorea* spp., *Alstonia scholaris*, *Acacia crassicarpa*, and *Agathis lorantifolia*. They found that double inoculation of PGPR and AM fungus increased N and P content of *Agathis lorantifolia* seedlings 6.5 months after inoculation. The most improved N and P contents (12.8 and 2.08 mg/plant) were achieved by the seedlings inoculated with a combination of PGPR and *Gigaspora* sp.

Siviero *et al.* (2008) evaluated the effect of inoculating three AM fungi (*Glomus clarum*, *G. intraradices* and *G. etunicatum*) with three N-fixing bacteria (two *Rhizobium* sp. and one *Burkholderia* sp.) on growth and survival of *Schizolobium amazonicum*, a fast growing legume tree. Two methods of planting were used: direct sowing or transplantation of seedlings after initial growth in nursery. They found that *G. intraradices* was more effective in promoting plant growth when inoculated in seed, and the bacterial strains had no effect when inoculated alone or with AM fungi. However, in seedlings the dual inoculation was found to be more effective. At 210 days both the *Rhizobium* species (Rhi1 and Rhi2) associated with *G. clarum* or *G. etunicatum* increased plant growth. At 390 days *G. clarum* associated with *Burkholderia* (LEM6) or Rhi1 increased most of the parameters evaluated, including biomass and wood production. The presence of microorganisms showed significant differences when compared with non-inoculated plants. They concluded that some microbial combinations were effective in stimulating plant growth, but further experiments were necessary to evaluate which N-fixing bacteria and AM fungi was more effective for each planting systems for *S. amazonicum*.

Verma *et al.* (2008a) studied the effect of AM fungi, *Azospirillum*, PSB and a companion fungus (*Aspergillus fumigatus*) on growth of aonla (*Emblica officinalis*) in nursery. Dual application of AM fungi and PSB produced maximum plant height followed by combination of all the four bio-inoculants. Maximum diameter of seedlings was obtained in *Azospirillum* treatment followed by *Azospirillum* + companion fungus. Maximum root colonization was in AM fungi + companion fungus and AM fungi +

companion fungus + *Azospirillum*. They recommended the application of AM fungus and companion fungus or *Azospirillum* and companion fungus to boost the growth of aonla in nursery.

Verma *et al.* (2008b) studied the effect of application of bio-inoculants, *Azospirillum*, PSB and AM fungi on the production of planting propagules (stumps) of *Tectona grandis* in nursery. They observed that seed germination was maximum in *Azospirillum* treatment followed by its combination with AM fungi and PSB. Maximum height of seedlings was recorded in AM fungi + *Azospirillum*, AM fungi + PSB and AM fungi + PSB + *Azospirillum* combinations after 5 months. Based on the profit index application of AM fungi along with *Azospirillum* was found the best treatment to produce quality planting propagules of teak in nursery.

The effects of N fixing *Azotobacter* and phosphate solubilizing *Bacillus megaterium* on the growth of two trees, Teak (*Tectona grandis*) and Indian redwood (*Chukrasia tubularis*) were tested under nursery conditions by Aditya *et al.* (2009). The pot mixture was inoculated with both N fixing bacteria and PSB. The co-inoculation effects were also monitored along with the application of either of single super phosphate or rock phosphate alone or in combination of both. Statistically significant positive effect upon co-inoculation was observed on the seedling height and collar diameter for *C. tubularis*. However, in *T. grandis* the co-inoculation had significantly increased the seedling height but not the collar diameter.

Aggarwal and Goyal (2009) evaluated the growth of *Populus deltoides* and *Toona ciliata* over a period of 6 months, in nursery soil amended with 10% fly ash (v/v), 5% distillery waste (v/v), 20% farmyard manure (v/v) and microbial consortium of *Pseudomonas striata* and

Azotobacter sp. @ 30 ml/pot in different combinations. Analysis of biometric parameters such as plant height, collar diameter and total dry biomass indicated that the treatment (T8) comprising of fly ash @ 10%, farmyard manure @ 20% and microbial consortium @ 30 ml/pot promoted growth of *P. deltooides*. They suggested addition of fly ash, farmyard manure and microbial inoculants to amend potting mixture for improving survival rates and plant growth in nurseries.

Arriagada *et al.* (2009) studied the improvement of growth of *Eucalyptus globulus* and soil biological parameters by amendment with sewage sludge and inoculation with AM and saprobe fungi. They investigated the effects of sewage sludge application with or without saprobe fungi (*Coriopsis rigida* and *Trichoderma harzianum*) or AM fungi (*Glomus deserticola* and *Gigaspora rosea*) on *Eucalyptus globulus*. Inoculation with both AM and saprobe fungi in the presence of sewage sludge significantly increased plant growth and dry shoot weight. The AM fungi induced a significant increase in fluorescein diacetate activity but did not increase beta-glucosidase activity. Dual inoculation with *G. deserticola* and either of the saprobe fungi had positive effects on K, Ca, Mg and Fe contents.

Aseri *et al.* (2009) studied the influence of selected N fixing bacteria and AM fungi alone or in combination, on the growth and biomass production of aonla (*Emblica officinalis*). The study revealed positive response of bio-inoculants in nursery seedlings followed by transplantation in the harsh field conditions of the Thar desert. In both nursery and field experiments, the combined treatment of *Azotobacter chroococcum* and *Glomus mosseae* was found to be the most effective. Besides enhancing the rhizosphere microbial activity and concentration of various metabolites and

nutrients, these bio-inoculants also helped in better establishment of aonla plant under field conditions. Significant improvement in the plant height, plant canopy, collar diameter and fruit yield was evident in five-year-old aonla plants in field conditions.

Bisht *et al.* (2009) conducted a study to evaluate the effects of AM fungi, *Rhizobium leguminosarum* strain DSP2 and *Pseudomonas fluorescens* strain GRPr on the growth and nutrient acquisition of *Dalbergia sissoo*. Mollisol and Entisol were used to compare the effects of different soils. A tetrapartite interaction of AM fungi, *P. fluorescens*, *R. leguminosarum* and *D. sissoo* showed improved plant growth response in the Entisol compared to uninoculated plants. The interaction of AM fungi with DSP2 was found; however, AM fungi did not show the same growth responses in combination with either GRPr or DSP2 and GRPr regardless of soil type. AM fungi and GRPr showed decreased plant growth, suggesting that enhanced plant growth was dependent on the type of bacteria - AM fungi combination. They concluded that in the case of *D. sissoo*, choice and testing of the combination of beneficial organisms is necessary to get desired plant growth promotion.

Matias *et al.* (2009) inoculated two native species, *Centrosema coriaceum* (Leguminosae) and *Tibouchina multiflora* (Melastomataceae), with selected strains of AM fungi and/or a rhizobium strain and used to revegetate an iron ore area in Brazil. After 48 months of plantation, the plant growth, survival index, physical and chemical soil properties, leaf N and P, and soil P were evaluated. Inoculated plants benefited in all the analyzed aspects. While *T. multiflora* presented increased P content in leaves and soil rhizosphere only in inoculated plants, *C. coriaceum* showed the highest

P content in both leaves and soil independent of inoculation. Although the AM inoculated plants presented more intense root colonization, the same mycorrhizal species were found in both inoculated and non-inoculated plants of *C. coriaceum* and *T. multiflora*. However, species of the Gigasporaceae family were favored in the *C. coriaceum* rhizosphere, as compared with *T. multiflora*. In addition, *C. coriaceum* was able to select in its rhizosphere a rhizobial strain efficient in P solubilization and a large and efficient population of phosphorus solubilizing microorganisms, preferentially composed of fungi favouring a tripartite action of rhizobia, fungi and AM fungal populations as strategies to solubilize soil phosphate for survival and growth in the rupestrian field.

Co-inoculation effect of *Glomus mosseae*, *G. fasciculatum*, mixed AM fungi (*Glomus* spp. [except *G. mosseae*, *G. fasciculatum*] with *Acaulospora* spp., *Sclerocystis* spp. and *Gigaspora* spp.), *Rhizobium* sp. and *Trichoderma viride* on the growth of *Acacia catechu* seedlings was studied by Parkash and Aggarwal (2009). Inoculated seedlings had a pronounced positive effect on all growth parameters such as height, fresh and dry weights of roots and shoots, AM spore count, percent mycorrhizal colonization and root nodule number compared to uninoculated seedlings. Phosphorus uptake was also higher in inoculated seedlings than in the control. They concluded that combined inoculation provides a good scope for commercially utilizing the efficient strains of AM fungi with other beneficial rhizosphere microflora in the establishment of slow growing seedlings ensuring better survival and growth.

Raja *et al.* (2009) investigated the influence of AM fungi and its interaction with other microbial inoculants *viz.*, *Azospirillum* spp., *Azotobacter* spp. and PSB on plant biomass, nutrients and biochemical

constituents in *Jatropha curcas* under field conditions. They reported that combined application of all microbial inoculants (*Azospirillum* + PSB + *Azotobacter* + AM fungi) significantly enhanced fresh biomass, total soluble protein and phenols over other inoculants and uninoculated control. The study indicated effectiveness of bio-inoculants in increasing plant growth, nutrients and biochemical constituents of *Jatropha* plants.

Rajeshkumar *et al.* (2009) conducted a greenhouse nursery study to assess the interaction between an AM fungus, *Glomus geosporum*, a N fixing bacterium, *Azotobacter chroococcum* and a mycorrhiza helper bacterium, *Bacillus coagulans* in soil and their consequent effect on growth and nutrition of *Melia azedarach* seedlings. Triple inoculation of *G. geosporum*, *A. chroococcum* and *B. coagulans* resulted in maximum plant biomass, N, P, Zn and Cu uptake and biovolume and quality index of *M. azedarach* seedlings. It also increased the mycorrhizal root colonization and spore numbers in the rhizosphere soil of the inoculated plants over uninoculated control plants. The enzyme activity, namely acid phosphatase and dehydrogenase, in the root zone soil was found to be high in the 3-combination treatments and low in the uninoculated control. Triple inoculation with *G. geosporum* + *A. chroococcum* + *B. coagulans* proved to be the best microbial consortium for inoculating *M. azedarach* at nursery level in order to get healthy and vigorously growing seedlings.

Seema *et al.* (2009) studied the effect of inoculation of bio-inoculants (AMF, *Azotobacter* & PSB) and chemical fertilizer (NPK) individually and in combinations on growth and nutrient acquisition of *Tectona grandis* under nursery conditions. They observed that in all the combinations, growth and nutrient uptake were significantly higher in comparison to uninoculated

seedlings but showed variation with treatments. AMF + *Azotobacter* combination was found to be the most effective (3.91 times higher biomass) than others (effective between 28.18-302.28%). *Azotobacter* alone was found to be the least effective (28.18%) treatment.

Zubek *et al.* (2009) studied the response of three endangered plant species, *Plantago atrata* and *Pulsatilla slavica*, which are on the IUCN red list of plants, and *Senecio umbrosus*, which is extinct in the wild in Poland, to inoculation with AM fungi and soil bacteria to select the most effective microbial consortium for application in conservation projects. Individuals of these taxa were cultivated with (1) native AM fungi isolated from natural habitats of the investigated species, (2) a mixture of AM fungi strains available in the laboratory, and (3) a combination of AM fungi lab strains with rhizobacteria. The plants were found to be dependent on AM fungi for their growth; the mycorrhizal dependency for *P. atrata* was 91%, *S. umbrosus*-95%, and *P. slavica*-65%. They found that the applied inocula did not significantly differ in the stimulation of the growth of *P. atrata* and *S. umbrosus*, while in *P. slavica*, native AM fungi proved to be less efficient. They concluded that AM fungal application could improve the *ex situ* propagation of these three threatened taxa and may contribute to the success of *S. umbrosus* reintroduction.

Aggangan *et al.* (2010) studied the growth response of *Acacia mangium* seedlings to inoculation of AM fungi and four isolates of the ectomycorrhizal fungus, *Pisolithus tinctorius*. AM inoculants were: *Glomus etunicatum*, *G. fasciculatum*, *G. macrocarpum* and *Gigaspora margarita* and mixed species. They reported that generally, ECM fungi promoted height and diameter growth of *A. mangium* more than the AM inoculants. The Korean

Pisolithus increased plant dry weight by 122–145%, mixed AM inoculants by 61–97%, and *Glomus* and *Gigaspora* by 45–72% over the control. Mycorrhizal root colonization was positively correlated with plant dry weight and N, P, K, Ca, Na, Fe and Cu contents. In conclusion, the results provided strong evidence for benefits of mycorrhizal inoculation on *A. mangium* seedlings under glasshouse conditions.

Joseph *et al.* (2010) studied the response of *Tectona grandis* seedlings to inoculation with different microbial bio-inoculants and their combinations. They found that inoculation with bio-inoculants increased the seedling quality and seedling growth in *Tectona grandis*. The growth response produced by AM fungus (*Glomus fasciculatum*), *Azospirillum brasilense* and *Phosphobacterium* (*Bacillus megaterium* var. *phosphaticum*) was found to be the best.

Muthukumar and Udaiyan (2010) investigated the role of tetrapartite associations between an AM fungus (*Glomus geosporum*), PSB (*Paenibacillus polymyxa*), *Frankia* and *Casuarina equisetifolia* on growth, nutrient acquisition, nutrient utilization and seedling quality of *C. equisetifolia*. They found that inoculation of seedlings with bio-inoculants stimulated seedling growth, the efficiency of nutrient uptake and improved seedling quality. However, microbial inoculation generally reduced the efficiency of nutrient utilization in dry matter production (nutrient use efficiency). Inoculation of *P. polymyxa* or *Frankia* increased the extent of AM colonization, which resulted in the accumulation of the nutrients. Seedlings inoculated with *Frankia* and *G. geosporum* had more, and heavier nodules compared to seedlings inoculated with *Frankia* alone. Dual inoculation of microbes was

more effective than individual inoculations. The growth response of seedlings to inoculation involving all the microbes was greater than the response to either individual or dual inoculations.

Pavan Kumar (2011) investigated the influence of inoculation with AM fungi, *Rhizobium*, *Azotobacter* and PSB singly and in combination on the growth of *Albizia lebbbeck* seedlings. He found that mycorrhizal association and resting spore population showed an increasing trend till the age of 180 days. Dual inoculations were found to be more effective for both AM colonization and biomass production. Dual inoculation involving mycorrhizae and *Azotobacter*, *Rhizobium* and PSB performed better than single inoculations of mycorrhizae. AM fungi + *Rhizobium* combination showed better P uptake and N fixation than AM fungi alone. Among different AM fungi, *Glomus fasciculatum* formed better colonization and induced better growth. AM fungal species varied in their efficiency in supporting the seedling growth. They suggested that mixed inoculums might be more efficient than single inoculum for field inoculations.

Singh *et al.*, (2011) studied the PGPR isolated from root nodules of native *Acacia senegal* (*Bacillus licheniformis*, *Sinorhizobium saheli*) and *Prosopis cineraria* (*S. kostiense* and *S. saheli*) and their effect on seed germination and seedling traits in two genotypes of *A. senegal*. The treatments with *Bacillus licheniformis* or *S. kostiense*, inoculated either individually or as coinoculants, had positive effect on phenotypic traits of germination. Two *A. senegal* genotypes exhibited significant differences with regard to all the phenotypic traits. On the other hand, treatments with *S. saheli* isolated from either *A. senegal* or *P. cineraria* had negative effects on germination and related phenotypic traits. They reported that there was a

synergistic effect between *B. licheniformis* and *S. kostiense* which had positive effects on phenotypic traits of germination. Whereas, mixed inoculation treatments with *S. saheli* and *B. licheniformis* had negative effects on phenotypic traits of germination.

Vipin Parkash and Ashok Aggarwal (2011) studied the effect of co-inoculation of endomycorrhizal fungi with other beneficial microbes on establishment and growth of *Eucalyptus saligna* seedlings. They found that dual inoculations of mixed VAM + *Rhizobium* sp. showed maximum increase in seedling height; *Glomus mosseae* + *Rhizobium* sp. showed more root P content; and mixed VAM + *Rhizobium* sp. and *G. mosseae* + *Rhizobium* sp. showed more shoot P content in inoculated seedlings. Triple inoculation also exhibited significant positive response on growth of *E. saligna* seedlings. Mixed VAM + *Rhizobium* sp. + *Trichoderma viride* had more increase in height, VAM spore number and percentage mycorrhizal root colonization; *G. mosseae* + *T. viride* + *Rhizobium* sp. treatment showed more root P content; *G. mosseae* + *Rhizobium* sp. + *T. viride* followed by mixed VAM + *Rhizobium* sp. + *T. viride* treatment showed more shoot P content. Although, dual inoculations had good response but triple inoculations had more pronounced and significant response on growth and development of *E. saligna*.

Gong *et al.* (2012) assessed the effects of *Glomus mosseae* and *Rhizobium* strain NWYC129 on the growth of black locust (*Robinia pseudoacacia*) seedlings and the quality of weathered soft rock soils under pot conditions. They observed that black locust seedlings could form strong symbiotic relationships with AM fungi and *Rhizobium*. Dual inoculation with *G. mosseae* and *Rhizobium* had the best effects on shoot dry weight, root dry weight, shoot height, root length and stem diameters of the

seedlings, compared with single and non-inoculation. Inoculation with *G. mosseae* and/or *Rhizobium* clearly enhanced the content of organic matter in weathered soft rock soils, and significantly improved the transport rate of soil phosphorus from the rhizosphere soils to plant tissue compared with non-inoculation. Dual inoculation showed the greatest activities of urease, alkaline phosphatase, invertase and catalase among the five treatments. They concluded that dual inoculation with *G. mosseae* and *Rhizobium* NWYC129 was highly beneficial for improving the growth of black locust seedlings.

Soliman *et al* (2012) investigated the effect of AM fungi and *Sinorhizobium teranga* individually or in combination, on alleviation of salt stress, growth and development of *Acacia saligna*. They observed that co-inoculated (AMF + R) stressed plants were able to maintain a higher osmotic potential of cells leading to significantly rapid growth, enhanced nodulation, N, P, K, Ca, total carbohydrates percentages and chlorophyll contents as well as proline in leaves, and significantly reduced the Na percentage.

Umashankar *et al.* (2012) evaluated the performance of microbial inoculants *viz.*, *Trichoderma*, P-solubilizer (*Bacillus coagulans*) and N fixer (*Azotobacter*) as well as chemical fertilizer (urea, rock phosphate and muriate of potash in 1:1:1 ratio and applied at 5g/plant) for growth promoting activity of silver oak. Height and girth of the plants treated with microbial inoculants recorded at 30, 60, 90 and 120 days after transplanting showed significantly higher values than control. The seedlings treated with microbial inoculants have also showed better height and girth than the seedlings treated with chemical fertilizers.

There are no reports available on the combined effect of bio-inoculants viz., AM fungi, *Azospirillum*, *Azotobacter* and PSB on *Ailanthus excelsa*, *Gmelina arborea*, *Melia dubia* and *Neolamarkia cadamba* plants.

2.2. EFFECT OF AM FUNGI AND OTHER BENEFICIAL MICROORGANISMS ON PLANT DISEASE CONTROL

AM fungi are capable of reducing the effects of various root fungal pathogens and plant parasitic nematodes. Reduced susceptibility or increased tolerance of roots to certain soil-borne pathogens is frequently associated with an established mycorrhizal colonization (Dehne, 1982). Roots colonized by AM fungi exhibit high chitinolytic activities. Increased lignification of the cell wall in the roots by AM is said to prevent penetration of the roots by *Fusarium* species and results in less infection (Dehne and Schonbeck, 1979). Increase in synthesis of secondary metabolites like lignin, ethylene and phenols (Dehne, 1982), as well as phytoalexins (Allen *et al.*, 1980; Morandi *et al.*, 1984) may all contribute to these “protective” effects.

2.2.1. AM fungi in disease control on forest tree species

Dehne (1982) reviewed and summarized most of the research work and results on interactions between AM fungi and plant pathogens done in two decades earlier. He suggested that the influence of AM fungi on disease incidence and development depends on the variable factors like plant pathogen, symbiotic fungus and environmental conditions. Mostly, the interactions between pathogen and symbiont are mediated by the host. AM fungi were also reported to be antagonistic to the phytoparasitic nematodes besides being beneficial to a wide range of host plants (Bagyaraj *et al.*, 1979; Hussey and Roncadori, 1982; Sitaramaiah and Sikora, 1982; Smith, 1987).

Kaushik *et al.* (2000) studied the impact of *Glomus mosseae* inoculation on root pathogens in *Acacia nilotica* and *Dalbergia sissoo* seedlings. They reported that AM inoculation significantly increased the plant growth and vigour, *i.e.*, plant height, root and shoot dry weight in both the species. Previously AM fungi inoculated plants significantly increased the survival percentage against both *Rhizoctonia solani* and *Fusarium oxysporum* on *A. nilotica* and *D. sissoo*.

The effect of *Trichoderma harzianum*, *Pseudomonas fluorescens*, organic matter (OM), AM fungi and *Azospirillum* sp. on *Fusarium* wilt and growth of *Gmelina arborea* seedlings was investigated by Singh *et al.* (2003). Germination was found to be maximum in organic matter alone followed by *T. harzianum* + AM fungi and organic matter + *Azospirillum* combinations. Mortality was maximum (41.7%) in *F. oxysporum* alone. No mortality was recorded in *T. harzianum* + OM + AM fungi + *F. oxysporum* and *T. harzianum* + OM + *F. oxysporum* + *Azospirillum* combinations. Root colonization by AM fungi was maximum in *T. harzianum* + AM fungi combination (92.5%) followed by *T. harzianum* + AM fungi + *F. oxysporum* combination (90.0%). The results indicated that many treatment combinations can effectively control the disease and improve growth of seedlings.

Tabin *et al.* (2009) examined the ability of *Glomus fasciculatum*, an AM fungus to reduce the damping-off incidence of *Aquilaria agallocha* seedlings. Dual inoculations (AMF + pathogen) restricted the progression of the pathogen (*Pythium aphanidermatum*) in the root tissues of the seedlings. Mycorrhizal inoculation not only reduced the percentage of disease incidence, but also significantly increased host plant height, total biomass and dry matter. They concluded that inoculation of AM fungi in the

rhizosphere soil of *Aquilaria* improves plant-soil interaction by enhancing nutrient status, plant growth, promote co-existence of other microbes and protect the host against pathogens.

2.2.2. PGPR in disease control on forest tree species

PGPR are also known to induce resistance to many root diseases as well as foliar diseases. Enebak and Carey (2000) studied the effect of inoculation of PGPR viz., *Bacillus pumilis* and *Serratia marcescens* on the fusiform rust in loblolly pine, *Pinus taeda* seedlings. They found that when the pine seeds were treated with PGPR at the time of sowing, and seedlings were infected with *Cronartium quercuum* f. sp. *fusiformis* later, and assessed after six months, the seedlings from the seed treated with PGPR had significantly less number of galls. They proposed that the PGPR appeared to have induced systemic resistance to fusiform rust in loblolly pine, resulting in less infection over untreated control seedlings.

DeLong *et al.* (2002) studied the inhibitory effect of fluorescent pseudomonad bacteria isolated from paper birch, *Betula papyrifera* and Douglas-fir, *Pseudotsuga menziesii* on the root pathogen, *Armillaria ostoyae* *in vitro*. They found that the fluorescent pseudomonad bacteria could reduce the radial growth of *A. ostoyae* as well as reduce the biomass of the fungus in dual culture tests. Also, the cell-free bacterial culture filtrates added to the growth medium reduced growth of *A. ostoyae*.

Sanjay Arya and Kaushik (2003) investigated the biological control potential of six biocontrol agents, *Trichoderma viride*, *T. harzianum*, *Gliocladium virens*, *Bacillus* sp., *B. subtilis* and *Pseudomonas fluorescens* against *Rhizoctonia solani*, *R. bataticola*, *Fusarium oxysporum*, *F. moniliformae*,

F. solani and *Phythium aphanidermatum* causing damping-off in forest nurseries. The results revealed that *P. fluorescens*, *Bacillus* sp. and *T. viride* significantly inhibited mycelial growth of the fungi *in vitro*. Seed treatment of *T. viride* and *P. fluorescens* proved superior to other fungal and bacterial biocontrol agents in reducing disease incidence compared to untreated controls.

Mohanani (2007) screened *Trichoderma harzianum*, *T. viride*, and *Pseudomonas fluorescens* for two consecutive years to study their efficacy against the seedling damping-off pathogens, *Rhizoctonia solani* and *Cylindrocladium quinqueseptatum* along with solarization of nursery beds by tarping the moistened soil by thick polythene sheets. The antagonists were introduced in the nursery beds by soil amendment and seed coating either singly or in different combinations using *Eucalyptus tereticornis* and *E. grandis* as the host plants. Solarization increased the temperature in the top layer of soil (2.5–5 cm depth) up to 51°C and reduced the pathogen inoculum considerably. Among various treatments, *T. harzianum* as soil application was reported to be the most effective one against damping-off followed by the combination of soil solarization and *T. harzianum* soil application.

Shukla *et al.* (2007) studied the wilting in *Dalbergia sissoo* and use of antagonistic *Pseudomonas fluorescens* in its control. They reported that in the seedlings which were first dipped in *Fusarium solani* spore suspension and later in *P. fluorescence* broth culture, the bacterium gave a positive protective cover against *Fusarium* (58.33% survival). In seedlings which were first treated with *P. fluorescens* the protective action was washed off in the spore suspension of *Fusarium* (8.37% survival). In simultaneous *Pseudomonas* +

Fusarium solutions, again the *Pseudomonas* was dominant in protecting the seedlings (58.33% survival).

Singh *et al.* (2008) studied the efficiency of rhizosphere competent *Bacillus subtilis* in controlling root rot fungus, *Macrophomina* and growth enhancement of *Pinus roxburghii*. They found that *B. subtilis* exhibited strong antagonistic activity against *M. phaseolina* and other phytopathogens including *Fusarium oxysporum* and *Rhizoctonia solani*. The cell-free culture filtrate of the bacterium inhibited the growth of *M. phaseolina*. Pot trial study resulted in statistically significant increase in seedling biomass besides reduction in root rot symptoms in inoculated chir-pine seedlings, as compared to control.

Shukla and Gupta (2009) screened 11 strains of *Pseudomonas fluorescens* against *Fusarium solani*, the causal organism of wilt disease in *Dalbergia sissoo*. They found that Pf-8 strain collected from Khirabad, Yamuna Nagar (Haryana) was highly antagonistic, inhibiting the growth of *F. solani* under laboratory conditions.

There are no reports available on the effect of bio-inoculants *viz.*, AM fungi, *Azospirillum*, *Azotobacter* and PSB individually and in combinations on *Fusarium* wilt control in *Ailanthus excelsa*, *Melia dubia* and *Neolamarckia cadamba* plants.

The foregoing survey of literature on the use of bio-inoculants on tree species in India reveal that most of the studies are done on exotic tree species, like *Eucalyptus* and *Casuarina* involving Ectomycorrhizae, AM fungi and *Frankia* or on legumes like Acacias and Albizias involving *Rhizobium* and AM fungi. The studies on the economically important fast growing native tree

species are very scanty and only a few studies are reported on *Ailanthus excelsa* and *Gmelina arborea*, using mostly single bio-inoculant (AM fungi) that too under nursery conditions.