DISCUSSION
Forests are the cornerstone of life on this planet. They regulate climate, conserve soil and are the natural reservoirs of fresh water. Not only do they provide food and shelter to more than 80% of the world’s terrestrial biodiversity, but also they continue to be home to 300 million people even in this age of globalization. Further, they provide multiple benefits to mankind apart from meeting the aesthetic and recreational needs of man. Today, forests cover only 31% of the earth’s land area, making it difficult to support all life forms inhabiting them while also maintaining global ecological balance.

The rapidly growing Indian economy has implications for all the sectors of economy, including forestry. The societal demands on forests are becoming more diversified and rising faster than the capacity of forests to supply them. Forest degradation and loss of biodiversity taking place on an unprecedented scale, is fast eroding the very basis of the livelihood of forest dependent communities. Forestry has the prime objective of developing and protecting forests for their maximum productivity on a sustainable basis. With increasing demand for industrial wood, fuel and timber, plantation forestry especially outside the forest area has gained importance in recent years.

*Ailanthus excelsa, Gmelina arborea, Melia dubia* and *Neolamarckia cadamba* are important trees of tropical regions, especially Indian subcontinent and grown either as monoculture plantations or in agroforestry systems because of the demand for its’ wood. Low soil fertility in tropical regions results in poor plant growth. This is significant especially in the case of forest trees, since they are generally transplanted without considering the fertility status of soil.
In the present study, efficacy of AM fungi, nitrogen fixers and phosphate solubilizing bacteria on seed germination and disease control in nursery and the growth of above selected tree species under nursery as well as field conditions were studied. The study can throw light on the identification of suitable combinations of different bio-inoculants with positive synergistic interactions, for a given set of environmental conditions. By inoculation of such compatible combinations in the forest tree nurseries, the performance attributes of the seedlings can be improved, which in turn increase the outplanting survival rate and field performance potential.

Experiment 1

Diseases cause major losses both in nurseries and plantations in forestry. In nurseries, they cause heavy damage to seedlings reducing both quantity and quality of planting stocks. Forest nurseries in India produce many million plants annually. These comprise mainly seedlings and to some extent stem cuttings and micro-propagated plantlets in case of clones. Wide range of fungal and bacterial pathogens attack seedlings of both exotic and indigenous tree species like Acacias, Albizias, Casuarinas, Dalbergias, Deodar, Eucalypts, Pines, Sandal, Sal, Teak etc., in different parts of the country (Sharma et al., 1985; Sankaran et al., 1986; Mohanan and Sharma, 1993; Mohan and Manokaran, 2001; Mohan et al., 2002).

Among the nursery diseases, root rot is one of the most prevalent and highly destructive diseases causing heavy loss of tree seedlings. It is caused by different soil fungi such as *Fusarium* and *Rhizoctonia* which are quite prevalent in forest nurseries in India (Mehrotra, 1990; Mohanan, 1999; Shivanna, 2003). These feeder root pathogens infect immature and meristematic cortical tissues of roots and cause necrosis. Chemical control
measures are generally used to manage these fungal diseases. However, ecological damage and pathogen resistance resulting from the use of chemical compounds are putting pressure on governments/growers to find environmentally safe alternative disease management strategies. There is therefore, increasing interest in biological control methods to suppress the growth of plant pathogens and to stimulate natural plant disease resistance.

Many beneficial microbes suppress the growth of plant pathogens through competition for nutrients, the production of inhibitory metabolites, and/or parasitism thereby naturally limiting the spread of plant disease in the environment.

The recent development in organic farming, has bought with it, the usage of biofertilizers, biopesticides and organic manures. Application of bio-inoculants such as Arbuscular Mycorrhizal (AM) fungi, Ectomycorrhizal (ECM) fungi, *Rhizobium* and Plant Growth Promoting Rhizobacteria (PGPR) such as *Azospirillum*, *Azotobacter*, Phosphobacteria and bio-control agents such as *Trichoderma* to seed/soil during raising of seedlings decrease or stop the growth of various soil-borne and root pathogens through different mechanisms of action (Bagyaraj, 1996; Dowling and O’ Gara, 1994; O’ Sullivan and O’ Gara, 1992).

Valuable data generated on induced suppression of soil-borne pathogens by AM fungi and PGPR have no doubt proved their potentiality in controlling plant pathogens (Dehne, 1982; Chakravarty and Mishra, 1986; Sanjay Arya and Kaushik, 2003). But, experiments have only usually tested single AM fungus. Also, there are certain pathogenic antagonists, *Pseudomonas*, *Bacillus* and other PGPR which cooperate with mycorrhizal fungi for biocontrol. The phytosanitary role of mycorrhizal fungi can be made
more effective when integrated with other PGPR. Thus, for exploiting the prophylactic activity of bio-inoculants in a best way, the right combinations of factors should be found out, the most important being the selection of appropriate/efficient combination of bio-inoculants.

In the present study, an experiment was conducted to test the efficacy of different bio-inoculants (AM fungi and PGPR) individually and in combinations on seed germination and survival of seedlings of selected four different fast growing native tree species against the plant pathogenic fungus, *Fusarium oxysporum* causing root rot in nurseries. It was observed that application of bio-inoculants individually and in different combinations showed increased seed germination, better survival and growth of seedlings of all the four tree species even in the presence of the pathogen, *F. oxysporum*.

Maximum percentage of seed germination was recorded in combined application of all four bio-inoculants along with *Fusarium* in all the four species. This was followed by AMF + Azo + *Fusarium* in *A. excelsa*, *G. arborea* and *N. cadamba* and AMF + Azoto + *Fusarium* in *M. dubia*. These findings are in accordance with those of Vanagamudi *et al*. (1993) who reported that seed inoculation of *Azadirachta indica* with AM fungi or *Azospirillum* or PSB significantly enhanced the seed germination of *A. indica*; Bhadauria *et al*. (2000) who reported that application of *Azospirillum* had positive effect on the seed germination of *Emblica officinalis*. Vijayakumari and Janardhanan (2003) have also found that inoculation with *Azospirillum*, PSB and AM fungi on *Bombax ceiba* improved seed germination and Singh *et al*. (2003) have reported that germination was found to be improved with seed inoculation of *Trichoderma harzianum* + AM fungi and organic matter + *Azospirillum* combinations in *Gmelina arborea*. 
Recently, Verma et al. (2008b) have also observed that teak seed germination was maximum with application of *Azospirillum* and combination of AM fungi + PSB; Mafia et al. (2009) have reported that PGPR promoted seed germination in *Eucalyptus* species; and very recently, Singh et al. (2011) have reported that inoculation with *Bacillus licheniformis* and *Sinorhizobium saheli* had positive and synergistic effect on seed germination of *Acacia senegal*. The improved germination of seed with application of bio-inoculants may be due to modification of the soil environment surrounding the seed by AM fungi and PGPR. Similar finding was made by Zambrano and Diaz (2008) who reported that *Glomus* sp. and *Azospirillum brasilense* had significant effect on the germination of *G. arborea* seeds. The germination percentage of seed in DAP + *Fusarium* treatment of *A. excelsa* (47.71%) and *N. cadamba* (23.63%) was found to be less than that of uninoculated controls (51.57% and 41.55%, respectively). This may be due to the chemical nature of the fertilizer affecting the small sized and soft coated seed of these tree species.

Perusal of the data in Tables 3, 20, 37 and 54 indicate that in general, the survival percentage of seedlings was minimum (5.37%, 8.03%, 10.00% and 15.96% in *A. excelsa*, *G. arborea*, *M. dubia* and *N. cadamba*, respectively) in *Fusarium* alone treatment (T2). Whereas, all the treatments involving bio-inoculants + *Fusarium* (T3 to T13) showed significantly higher survival percentage of seedlings. The present findings are in accordance with those of Singh et al. (2003) who reported that maximum mortality was observed in *F. oxysporum* treatment and many treatment combinations involving AM fungi, *Azospirillum*, *Trichoderma harzianum* and organic matter could effectively control the *Fusarium* wilt disease in *Gmelina arborea*. The findings of the study are also quite in agreement with the observations made

The reduction/suppression of disease incidence and improved survival of seedlings of all bio-inoculants applied tree species may be due to the inhibition of pathogen’s spore germination by inoculated AMF and PGPR and improved nutrient uptake leading to increased host vigour (Kaushik and Mandal, 1991).

**Experiment 2**

**Growth response:** In all the four tree species, the shoot height, root length, collar diameter, leaf area, shoot dry weight, root dry weight and total dry weight were higher in the seedlings inoculated with bio-inoculants than uninoculated control (Tables 4-6, 21-23, 38-40 and 55-57). The present findings were quite in agreement with the results reported by earlier researchers on the native tree species. Kandasamy *et al.* (1987) while working with *Ailanthus excelsa* and two other species reported that inoculation with AM fungus, *Glomus fasciculatum* increased the shoot and root dry weights (62.08% and 43.18%, respectively). Balasubramanian and Srinivasan (1995) have also reported that inoculation with four AM fungi significantly increased the total biomass, leaf area and total chlorophyll of *Ailanthus excelsa, Tectona grandis* and *Dalbergia sissoo* seedlings. Madan *et al.* (1995) reported that AM fungal inoculated *Ailanthus excelsa, Pongamia glabra* and *Cassia siamea* seedlings showed better shoot height, shoot and
root dry weight in highly alkaline-saline soils. In the subsequent years Rahangdale and Gupta (1998) reported that all the twelve AM fungi have contributed to significant increase in shoot height, root and shoot biomass in all the six tree species tested including *Gmelina arborea*.

Talukdar and Thakuria (2000) also reported that AM fungi inoculated *Gmelina arborea* and *Tectona grandis* with organic amendments gave better yield in terms of shoot and root biomass in degraded soils. Chandra and Ujjaini (2002) reported that when *Gmelina arborea* and four other species were inoculated with AM fungi + organic matter, the plant biomass increased up to 36.85%. Recently, Barua et al. (2010) have found that length of shoot and root, collar diameter, fresh and dry weight of shoot and root were improved in AM fungi inoculated *Gmelina arborea* plants. They also observed that 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.

The significant effect of AMF and PGPR on growth parameters of tree seedlings in the current study may be due to the increased production of auxins and gibberellins by the bio-inoculants. The findings are also in conformity with the studies conducted by Dela Cruz et al. (1988) and Dubey et al. (1997). The increase in seedling biomass production may be strongly correlated with improved accumulation of N due to *Azospirillum* and *Azotobacter* and P due to AM fungi and PSB inoculation (Ratha Krishnan et al., 2004).

In general, seedlings inoculated with combination of all four bio-inoculants showed maximum values for the growth parameters at both 90 DAI and 180 DAI in all the four tree species. Further, among the
treatments involving bio-inoculants, dual combinations, especially the ones involving a N fixer and a P solubilizer/mobilizer were found to be better than single inoculations. These findings are in accordance with the findings of Seema et al. (2000) who reported that AM fungi and *Azotobacter chroococcum* inoculation on *Gmelina arborea, Bambusa arundinacea, Tectona grandis* and *Dalbergia sissoo* resulted in improved growth and biomass. Recently, Zambrano and Diaz (2008) have also reported a positive correlation between mycorrhization and plant height and a synergic effect between AM fungi, *Glomus* spp. and *Azospirillum brasilense* on *Gmelina arborea* with two potting media.

No comparable reports are available on *Melia dubia* and *Neolamarckia cadamba*. However, similar phenomenon has been reported earlier in related tree species belonging to same family like *Azadirachta indica* (Kalavathi et al. 2000; Muthukumar et al. 2001; Sumana and Bagyaraj. 2002; Karthikeyan et al., 2007), *Chukrasia tubularis* (Aditya et al., 2009), *Melia azadirach* (Rajeshkumar et al., 2009) and *Coffea canephora* (Thammaiah et al., 2003; Prem Kumari and Balasubramanian, 1993).

The effect of bio-inoculants on the growth performance (shoot height, root length, collar diameter, leaf area, shoot dry weight, root dry weight and total dry weight) varied with the treatment and the tree (host) species. Balasubramanian and Srinivasan (1995) also reported that different AM fungi responded differently to different tree species with *Gigaspora margarita* on *Tectona*, *Glomus mosseae* on *Dalbergia* and *G. monosporum* on *Ailanthus* showing maximum beneficial effect. Rahangdale and Gupta (1998) reported that twelve AM fungi produced different growth response on six tree species tested. Seema et al. (2000) also reported variations among treatments and species while working with AM fungi and *Azotobacter chroococcum* and three tree species. The greater increase in shoot height, collar diameter and total
dry weight in treatment with combination of all the four bio-inoculants and dual inoculations involving AMF/PSB and Azo/Azoto, in all the tree species may be due improved accumulation of both N due to \textit{Azospirillum} and \textit{Azotobacter} (Wong and Stenberg, 1979) and P due to AM fungi and PSB inoculation (Habte and Manjunath, 1987; Saravanan, 1991).

**Growth indices:** In all the four tree species, Aggregate Growth Rate (AGR) and Net Assimilation Rate (NAR) were higher in the seedlings inoculated with bio-inoculants than uninoculated control. Relative Growth Rate (RGR) in general, was found to be higher in inoculated \textit{A. excelsa} and \textit{N. cadamba} seedlings while no significant difference was found in the other two tree species. However, Leaf Area Ratio (LAR) was found to be lower in inoculated seedlings of all the tree species, which indicates that lesser leaf area in inoculated seedlings is able to produce more dry matter (Tables 7, 24, 41 and 58). These findings are in accordance with the findings of earlier researchers on other tree species. Huante \textit{et al.} (1993) reported that AM fungal inoculation resulted in increased biomass production and RGR in \textit{Caesalpinia eriostachys}, \textit{Cordia alliodora} and \textit{Pithecellobium mangense}. Lovelock \textit{et al.} (1996) reported that inoculation with AM fungi increased RGR of the tropical tree, \textit{Beilschmiedia pendula} seedlings. Shishido and Chanway (2000) reported that hybrid spruce (\textit{Picea glauca} × \textit{Picea engelmannii}) seedlings pre-inoculated with PGPR (\textit{Pseudomonas} sp. and \textit{Bacillus} sp.) exhibited significantly higher shoot and root RGRs (increased by 10-234%) four months after outplanting, at all the four sites tested. Ratha Krishnan \textit{et al.} (2004) reported that inoculation of \textit{Azospirillum}, \textit{Azotobacter}, PSB and AM fungi on \textit{Simarouba glauca} seedlings resulted in increased RGR. Recently, Zapata \textit{et al.} (2009) reported that AM fungi increased RGR of \textit{Desmoncus orthacanthos} (Arecaceae) seedlings at low P level.
Increase in growth rates of AM fungi and PGPR inoculated plants may be due to alteration of morphology (more leaf area) and physiology (improved nutrient accumulation and photosynthesis) of the seedlings. NAR and LAR are correlated with RGR of plants. The higher the NAR the more efficient the species, which usually translates into higher growth rates (Larcher, 2003). In all the tree species, inoculation with AM fungi and PGPR increased both total plant growth, as measured by increased AGR, and the efficiency of growth, as measured by increased NAR. This increased growth efficiency allowed the plant to have a smaller shoot system (decreased LAR), which is the source, while still enhancing the size of the root system (see increased root/shoot ratio), which is a sink (Leopold and Kriedemann, 1975).

Quality indices: The root: shoot ratio is an important measure for seedling survival. It relates the water absorbing area (roots) to the transpiring area (shoot). A good ratio- one which indicates a healthy plant is 1:1 to 2:1 root: shoot mass (Jaenicke, 1999). Significant difference was found between the bio-inoculants/DAP treated seedlings and the uninoculated control in M. dubia and N. cadamba whereas, no significant difference was found in A. excelsa and G. arborea between treated and untreated seedlings. However, the root shoot ratio was higher in all the treated seedlings of the four species than the controls and many treatments showed desired values of >1.0 (Tables 8, 25, 42 and 59). This indicates that the bio-inoculants tend to improve the root shoot ratio of inoculated seedlings by increasing the root biomass. The seedlings of uninoculated control in all the four species had root shoot ratios of less than one, which means that shoot biomass was too high compared to root biomass indicating that these seedlings were of sub-optimal quality and unlikely to withstand the adverse conditions in most planting sites.
A less rigorous, but non-destructive, index is the ‘sturdiness quotient’ which compares height (in cm) over root collar diameter (in mm). A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. A sturdiness quotient higher than 6 is undesirable (Jaenicke, 1999; Gregorio et al., 2007). Among the four tree species studied, seedlings of A. excelsa, M. dubia and N. cadamba had desirable sturdiness quotient values (<6.0) at both the ages of observation whereas, the seedlings of G. arborea showed sturdiness quotient values of >6.0 which indicates that these seedlings were basically lanky. However, in both the cases, the inoculation of seedlings with AM fungi and PGPR has resulted in improvement of sturdiness quotient values towards <6.0 (Tables 8, 25, 42 and 59).

In all the four tree species, the volume index and the quality index were higher in the seedlings inoculated with bio-inoculants than uninoculated control (Tables 9, 26, 43 and 60). These findings are in accordance with the findings of earlier researchers on native and related tree species like Azadirachta indica (Sumana and Bagyaraj, 2002), Tectona grandis (Ayswarya, 2008; Rajeshkumar et al., 2009) Melia azedarach (Rajeshkumar et al., 2009) as well as exotic ones like Eucalyptus hybrid (Sastry et al., 2000), Simarouba glauca (Ratha Krishnan et al., 2004) and Acacia auriculiformis and A. mangium (Tamilselvi, 2005).

Sumana and Bagyaraj (2002) also reported that dual inoculation of G. mosseae and A. chroococcum resulted in maximum volume index and quality index of neem seedlings while Rajeshkumar et al. (2009) reported that triple inoculation of G. geosporum, A. chroococcum, and B. coagulans resulted in maximum volume index and quality index of Melia azedarach seedlings.
**Nutrient studies:** With regard to total N, P and K contents and uptake in all the four species, the seedlings inoculated with bio-inoculants showed higher values than uninoculated control (Tables 10-16, 27-32, 44-49 and 61-66). Similarly, the SAR values for N, P and K were higher in seedlings inoculated with bio-inoculants in all the four species (Figures 1, 3, 5 and 7). In general, again the tetra-partite combination (AMF + Azo + Azoto + PSB) followed by dual application of bio-inoculants involving a N fixer and a P mobilizer/solubilizer proved superior to treatments involving individual inoculations. This is in accordance with the observation of Rahangdale and Gupta (1998) who reported that P content in root tissue significantly increased in all the AM fungi inoculated six tree species including *Gmelina arborea*.

No comparable studies on nutrient content/uptake are available on the other three tree species. However, several researchers have studied this aspect in other native and related tree species. The results of the present study are in agreement with the previous studies in species like *Azadirachta indica* (Muthukumar *et al*., 2001; Sumana and Bagyaraj, 2002), *Ziziphus mauritiana* var. *rotundifolia* (Aseri and Rao, 2005), *Dendrocalamus strictus* (Muthukumar and Udayian, 2006); *Emblica officinalis* (Aseri *et al*., 2009), *Melia azedarach* (Rajeshkumar *et al*., 2009) and *Albizia lebbeck* (Pavan Kumar, 2011). Further, several researchers have also reported that dual combinations were found better than individual inoculations (Sumana and Bagyaraj, 2002; Aseri and Rao, 2005; Pavan Kumar, 2011).

The increased N uptake may have resulted either from *Azospirillum* (Scott *et al*., 1978) or from the increased N absorption induced secondarily by increased P uptake made possible by AMF (Young *et al*., 1988) and/or PSB (Kuccy, 1987). Enhanced P uptake due to combined inoculum is
attributable to the concurrence presence of the AMF and PSB which solubilize immobile organic phosphates to inorganic phosphate by organic acid produced by PSB (Raj et al., 1981) and phosphatase enzyme secreted by AMF (Bartlett and Lewis, 1973). According to Hattingh (1975), increased P uptake was due to existence and continued growth of mycorrhizal roots extending beyond the vicinity of the root surface and exploits the immobile phosphorus. Results from the study have shown that the combined inoculation of all the four bio-inoculants might have played a significant role in improving the growth response and nutrient uptake of four native tree species under nursery condition.

**Persistence studies:** Plant colonization is largely determined by availability of mycorrhizal fungal propagules. The results indicated that all the four tree species have innate property of naturally colonizing with AM fungi and pronounced response was seen in all the four test species, when sufficient propagules were given for mycorrhizal association.

In the present study, the highest persistence of inoculated AM fungi in terms of percent root colonization and spore number in rhizosphere was recorded in treatments involving combined application of all four bio-inoculants, followed by dual inoculations involving AMF in all the tree species. Similarly, persistence of PGPR in terms of bacterial population was observed maximum in treatments involving combined inoculation of all four bio-inoculants and the dual inoculations involving respective PGPR.

The AM fungal colonization in roots was maximum due to combined inoculation of AMF + Azo + Azoto + PSB in all the four species, with percent colonization of 60.26%, 67.41%, 51.95% and 76.31%, in *A. excelsa*, *G. arborea*, *M. dubia* and *N. cadamba*, respectively (Tables 16, 33, 50 and 67). The average
percent root colonization in these species was found to be 57.05%, 61.17%, 47.93% and 74.20%, respectively. The improvement in growth parameters was correlated with percent root colonization of seedlings by AM fungi. This may be due to increased absorption of P by mycorrhization (Grandcourt et al., 2004). A direct correlation was observed between percent root colonization and soil spore population of AM fungi in different samples. Similar observations were made by Kandasamy et al. (1987), Balasubramanian and Srinivasan (1995) and Madan et al. (1995) in A. excelsa inoculated with AM fungi; and Rahangdale and Gupta (1998), Seema et al. (2000), Chandra and Ujjaini (2002), Singh et al. (2003) and Barua et al. (2010) in G. arborea inoculated with AM fungi; and Zambrano and Diaz (2008) in G. arborea with dual inoculation of AM fungus and Azospirillum.

Mycorrhizal parameters (root colonization and spore numbers) were considerably high (about 3 to 12 times) in AM fungi inoculated plants. The increased AM colonization in the roots is due to addition of AM fungal inoculum and may be due to the presence of phosphate solubilizing bacteria (Azcon et al., 1976) or due to higher uptake of N and P (Manjunath et al., 1984; Dela Cruz, 1988). Good colonization means greater absorption of nutrients and hence might have resulted in better growth.

Among the four tree species, least colonization was observed in M. dubia (41.35 to 51.95%) compared to A. excelsa (45.57 to 60.26%), G. arborea (53.15 to 67.41%) and N. cadamba (70.17 to 76.37%). This indicates the symbiotic difference of mycorrhizal fungi with different host plants. It also explains the importance of host specificity for determining the role of AM fungi. Similar findings were reported by Selvaraj et al. (1996)
while working with six tropical tree species. The difference in root colonization between the treatments with AM fungi within a given species may be due to the effect of the co-inoculant.

It was observed from the study that the Microbial Inoculation Effect (MIE) varied with the treatment and the seedlings inoculated with combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB) showed the highest value of MIE, followed by dual inoculations when compared to all other treatments in all the four tree species tested (Figures 4 (A), 6 (A), 8 (A) and 10 (A)). This indicates that these organisms act synergistically when inoculated in combinations. Similar findings were reported by Muthukumar et al. (2001) in neem.

The root colonization AM fungal spores and PGPR found in uninoculated control of all the tree species indicate the presence of native AM fungi and PGPR in the nursery soils. In spite of their presence in uninoculated control, the seedling growth response (seedling height, collar diameter, leaf area and total dry weight) was much less than the AM fungi and/or PGPR inoculated seedlings. It is concluded that the naturally occurring AM fungi and PGPR in soil may be insufficient as expressed by Powell and Daniel (1978) and there is need to apply suitable AM fungi and PGPR to increase the growth and biomass production.

**Bio-inoculants vs DAP:** In the present study, though the growth performance was found to be high with DAP, it was interesting to note that the combined inoculation of all the bio-inoculants as well as dual inoculations involving a N fixer and P solubilizer/mobilizer were found to be equally effective in all the tree species. There are no comparable reports of using DAP/chemical fertilizer on the selected tree species. However,
several workers have reported similar phenomenon in other tree species. Byra Reddy et al. (1990) demonstrated that inoculation of *Leucaena leucocephala* with *Gigaspora margarita* resulted in reduction of 50-75% of P fertilizer required. El-Gamal (1992) reported that adding bio-inoculants, *Azotobacter* spp., (*A. chroococcum* and/or *A. vinelandii*) or chemical fertilizer (urea) into the rhizosphere of *Sesbania sesban* seedlings resulted in significant increase in both chlorophyll contents, total soluble carbohydrates, total soluble proteins, root, shoot lengths, dry weight of roots and shoots. Verma et al. (1994) concluded that inoculation of *Acacia nilotica* with AMF (mixed spore) singly or in combination with *Rhizobium* is equally beneficial as that of chemical fertilizers. Nagaveni et al. (1998) observed that application of inorganic fertilizer did not show much difference in growth of *Tectona grandis* compared to AM fungi treatments. Mehrotra et al. (1999) reported that inoculation of *Dendrocalamus strictus* and *Bambusa nutans* with AM fungi improved the growth parameters of bamboo seedlings as compared to chemical fertilizers.

Further, Aseri and Rao (2005) while working with *Ziziphus mauritiana* reported that growth enhancement with inoculation of nitrogen fixing bacteria was at par with that of 100% N fertilizer dosage. Muthukumar and Udaian (2006) reported that combined inoculation of AM fungi, PSB and *Azospirillum brasilense* on *Dendrocalamus strictus* resulted in maximum growth response both under fertilized and unfertilized conditions in two soil types. Seema et al. (2009) reported that AMF + *Azotobacter* combination was found to be the most effective (3.91 times higher biomass) on *Tectona grandis* than other treatments including chemical fertilizers. Recently,
Umashankar et al. (2012) reported that *Grevillea robusta* seedlings treated with microbial inoculants (*Trichoderma, Bacillus, Azotobacter*) have showed better height and girth than the seedlings treated with chemical fertilizers.

Production of seedlings is one of the most important stages in the establishment of forest stands. A successful, high yielding stand is closely dependent on the quality of planted seedlings, which should be capable of resisting adverse field conditions and grow into trees with an economically desirable volume. Several variables are used to evaluate seedling quality, including shoot height, root configuration, collar diameter, number of leaves, ratio of shoot to root, ratio of stem base diameter to shoot height, dry and fresh matter weight of shoot and root, shoot stiffness and nutritional aspects (Paiva and Gomes, 1993). Wendling et al. (2005) demonstrated that rating seedlings according to height class has been widely used in commercial nurseries. However, Fonseca et al. (2002) argued that variables used for evaluating seedling quality should not be studied separately, that way avoiding the risk of selecting higher and yet weaker seedlings while discarding smaller, sturdier ones. The Dickson quality index (DQI) is a tool to evaluate seedling quality as a function of total dry matter, shoot height, root length, collar diameter, shoot dry matter and root dry matter (Dickson et al., 1960). DQI is considered a promising integrated measure of morphological traits (Johnson and Cline, 1991) and thought to be a good indicator of seedling quality as its calculation computes robustness and biomass distribution while considering several important parameters (Fonseca et al. 2002). Binotto et al. (2010) have reported that among different variables studied, the variables relating to dry matter showed the highest correlations with the DQI, followed by collar diameter. Shoot height was effective to indicate seedling quality if analyzed together with collar
diameter. Hence, in the present study, among different variables, the effect of bio-inoculants on total dry weight, collar diameter and seedling height along with DQI were primarily considered for assessing seedling quality and synergistic effect of bio-inoculants.

In general, all treated seedlings exhibited improved growth and biomass over control but there were variations among treatments and species (Tables 4-6, 21-23, 38-40 and 55-57). The response to individual inoculations was comparatively low in all the tree species, although the AMF and PGPR multiplied in root zone. Among individual inoculations, AMF has showed better performance. However, in combined inoculations, both AMF and PGPR have performed discernibly high, as compared to that of any individual treatment. Further, dual combinations involving a N fixer and a P solubilizer/mobilizer were found to be highly effective indicating the synergistic effect. Pavan Kumar (2011) reported that dual inoculations involving mycorrhizae + Azotobacter and Rhizobium + PSB performed better than single inoculations of mycorrhizae on Albizia lebbeck seedlings. Singh et al. (2011) reported a synergistic effect between Bacillus licheniformis and Sinorhizobium saheli with their dual inoculation on native Acacia senegal which had positive effects on seed germination and seedling traits. The present study is also in conformity with the above studies and previously identified positive synergistic response of bio-inoculants in different tree species (Felker et al., 2005; Tamilselvi, 2005; Niranjan et al., 2007; Seema et al. 2009; Muthukumar and Udaiyan, 2010).

Muthukumar et al. (2001) reported that microbial inoculation effects were greatest and that these microorganisms act synergistically when neem seedlings were inoculated simultaneously with a combination of microbes rather than individual ones with maximum response being when AM fungi
were inoculated with \textit{A. brasiliense}. Sumana and Bagyaraj (2002) reported that dual inoculation of \textit{Glomus mosseae} and \textit{Azotobacter chroococcum/Azospirillum brasiliense} resulted in maximum plant biomass, N and P uptake; biovolume index and quality index of neem seedlings more than the single inoculations and uninoculated control. Ratha Krishnan \textit{et al.} (2004) reported that the dual application of \textit{Azotobacter} and PSB significantly increased the shoot length and collar diameter, while the dual inoculation of \textit{Azotobacter} and AM fungi registered the maximum root length, number of leaves, root/shoot ratio, quality index and highest AM colonization percentage in \textit{Simarouba glauca} seedlings. Aditya \textit{et al.}, (2009) demonstrated the synergistic effect of N fixing \textit{Azotobacter} sp. and PSB, \textit{Bacillus megaterium} on the growth of \textit{Chukrasia tubularis} and \textit{Tectona grandis} seedlings under nursery conditions.

In the present study, though the dual inoculations were found to be superior to single inoculations, it was observed that the combinations of AMF + PSB and Azo + Azoto did not give the same synergistic effect as that of a N fixer + P mobilizer/solubilizer. Some earlier researchers also reported that combinations of P solubilizer and P mobilizer did not enhance the plant growth. Sastry \textit{et al.} (2000) reported that \textit{Pseudomonas} inoculations in association with AM fungi inhibited growth promotion and nutrient uptake in \textit{Eucalyptus} hybrid. Bisht \textit{et al.} (2009) evaluated the effect of AM fungi, \textit{Rhizobium leguminosarum} and \textit{Pseudomonas fluorescens} on \textit{Dalbergia sissoo} in two soil types and reported that combined inoculation of all the three bio-inoculants showed improved plant growth whereas, combination of AM fungi and \textit{Pseudomonas} showed decreased plant growth. They also reported positive interaction of AM fungi with \textit{Rhizobium}; however, AM fungi did not
show the same growth responses in combination with PSB, *Pseudomonas*
regardless of soil type. These findings clearly indicate that enhanced plant
growth is dependent on the type of bacteria-AM fungi combination involved.

**BC ratio:** In the present study, it was observed that the bio-inoculants
treated plants especially, combined application of all bio-inoculants and dual
inoculations involving N fixer and P solubilizer/mobiliser having good quality
index and attaining good height and collar diameter gave better benefit cost
ratio than other treatments (Tables 17, 34, 51 and 68; Figures 4 (B), 6 (B), 8
(B) and 10 (B)). In terms of benefit cost analyses of the bio-inoculants, it is
well known that the beneficial effect of the bio-inoculants is multifaceted,
and does not result solely from improved nutrient uptake. Thus it may not
be appropriate to compare the cost of inoculation directly with the cost of
chemical fertilizer additives that achieve equivalent growth response in
target plants. Indeed, the benefits of chemical soil additives are usually short
lived, unless slow release formulations are used, and hence are not
sustainable in low input or natural ecosystems. If the bare economics of the
relative costs of chemical fertilizers versus bio-inoculants are compared, the
latter will be little more costly in the short term. However, in terms of overall
plant health and sustainability, the benefits of establishing an effective AM
symbiosis and PGPR are much wider and more long lasting.

**Experiment 3**

The ability of AM fungi and PGPR to produce a dramatic response in
plant growth through increased uptake of essential nutrients, production of
hormones, resistance to various biotic and abiotic stresses, has been well
documented and several workers have exploited these abilities of
bio-inoculants in different crops all over the world. However, the studies on
this subject in forestry are limited and most of them are confined to laboratory or greenhouse pot trials. Moreover, research on these bio-inoculants in comparison with chemical fertilizers is scanty. In view of this, field trials were undertaken to determine the outplanting performance of four selected tree species inoculated with these bio-inoculants.

Perusal of the data in Figures 4 (C), 6 (C), 8 (C) and 10 (C) and Tables 18-19, 35-36, 52-53 and 69-70 indicate that in general, the survival and growth performance of plants in terms of plant height and basal diameter/DBH was found to be significantly higher in all the treatments involving bio-inoculants/DAP than uninoculated control in all the tree species. These growth parameters were found to be maximum in combined application of all four bio-inoculants followed by DAP (alone) in all the four tested tree species. These were followed by dual applications of Azo + PSB in A. excelsa, AMF + Azo in G. arborea, AMF + Azoto in M. dubia and Azoto + PSB in N. cadamba.

The results of the present field study are in conformity with the performance of bio-inoculants applied plants of other tree species reported by many researches in India and elsewhere (Kinhal, 1985; Roskoski et al., 1986).

*Azotobacter* combined with chemical fertilizer, Suphala increased the culm height and better field performance of *Dendrocalamus strictus* plants in the field (Kinhal, 1985). Roskoski et al. (1986) reported that AM fungi + *Rhizobium* inoculated plants of *Leucaena leucocephala* and *Acacia pennatula* exhibited greater growth than uninoculated controls in acid soils, but not in alkaline soils.
Michelsen (1993) reported that the survival of *Acacia abyssinica* plants in the field was enhanced by AM fungus (*Glomus mosseae*) and inoculation also improved the root dry weight after 18 months. Chanway and Holl (1994) have reported that *Bacillus polymyxa* inoculated *Pinus contorta* seedlings when planted in poor quality site showed significant increase in stem diameter (7%), root dry weight (32%), and shoot dry weight (33%). Bhat *et al.* (1994) reported that plant height was significantly enhanced (16.6%) by AM fungus, *Glomus mosseae* in *Leucaena leucocephala* one month after transplanting in the field.

Elimination of chemical fertilizers up to 100% and higher productivity (biomass and nutritional status) in nutritionally poor soils for *Acacia nilotica*, *Albizia lebbeck*, *Eucalyptus tereticornis* and *Prosopis juliflora* were achieved at the end of sixth to eighth year by Adholeya *et al.* (1997) using AM fungal inoculations. Meshram *et al.* (1997) reported that inoculation of *Azotobacter chroococcum* and AM fungus (*Glomus fasciculatum*) amended with FYM and N+P resulted in maximum biomass of *Eucalyptus camaldulensis* after two years of plantation on waste/barren land. Puthur *et al.* (1998) reported that cent percent of the micro-propagated *Leucaena leucocephala* plantlets established very well and showed good growth in AM inoculated (*Glomus fasciculatum* and *G. macrocarpum*) sterilized garden soil by alleviating the transplantation shock. Inoculation of *Tectona grandis* with *Azospirillum* and *Phosphobacterium* increased tree height (20%) and girth (10.3%) in mulched plots (Subramanian *et al.*, 1998).

Shishido and Chanway (2000) reported that hybrid spruce (*Picea glauca × Picea engelmannii*) seedlings pre-inoculated with PGPR (*Pseudomonas* sp. and *Bacillus* sp.) exhibited significantly higher shoot and root RGRs (increased by 10-234%) four months after outplanting, at all the four sites tested.
Singh et al. (2000) reported that inoculation of *Rhizobium* + AM fungi on *Albizia lebbeck* and *A. procera* resulted in best survival and growth (height and collar girth) of both the species in severely eroded soil after 4.5 years of planting. Individual inoculations also significantly improved the growth parameters. Rajendran and Devaraj (2004) reported that combined application of *Azospirillum*, Phosphobacterium, AM fungi and *Frankia* resulted in maximum height, GBH and total biomass of *Casuarina equisetifolia* 24 months after planting in the field apart from AM + *Frankia* and *Azospirillum*, AM + Azo + *Frankia* combinations which had significant effect.

Swaminathan and Srinivasan (2006) reported that the survival of *Tectona grandis* plants in the main field after one year of transplantation was better in seedlings that were previously inoculated with PSB and AM fungi in the nursery. Venkateswarlu et al. (2008) reported that field-grown neem (*Azadirachta indica*) plants inoculated in the nursery and during transplantation with *Glomus fasciculatum* showed no significant difference in the tree height, girth at breast height (GBH) and fruit yield after 5 years but oil percentage, total triterpenoids and azadirachtin content in kernels increased significantly as a result of AM fungal inoculation. Aseri et al. (2009) reported that combined treatment of *Azotobacter chroococcum* and *Glomus mosseae* was found to be the most effective in better establishment of *Emblica officinalis* plants under harsh field conditions and significantly improving the plant height, plant canopy, collar diameter and fruit yield in five-year-old plants. Raja et al. (2009) reported that application of combined microbial inoculants, *Azospirillum* + PSB + *Azotobacter* + AM fungi significantly enhanced the fresh biomass, total soluble protein and phenols as well as relative water content in *Jatropha curcas* over other inoculants and uninoculated control under field conditions.
Chemical fertilizers are known to enhance plant growth by direct supply of plant nutrients. However, their effect is often temporary. In the present field study also it was observed that the plant height in DAP (alone) treatment which was equal to or higher than combined application of all four bio-inoculants at 6 MAP was found to be less than combined application of all four bio-inoculants at 24 MAP, especially in *G. arborea*, *M. dubia* and *N. cadamba*. Further, chemical fertilizers have been reported to produce hazardous effects on the soil through loss of valuable micro-flora, apart from increasing the soil alkalinity or acidity. Sharma and Adholeya (2000) reported that higher levels of soil P depressed the AM colonization significantly (decreased from 31% to 3%) as the concentration of P increased beyond 10 ppm soil P in *Eucalyptus tereticornis* due to application of P fertilizer. Karanja *et al.* (1999) reported that the roots of *Grevillea robusta* treated with rock phosphate and AM fungi were not colonized with AM fungi even after 12 months. Stamford *et al.* (1997) reported that AM fungi were unable to develop efficiently and colonize the roots of *Mimosa caesalpinifolia* in the soil treated with P-fertilizer, phosphogypsum/triple superphosphate and AM fungi. Aseri and Rao (2005) reported that application of P fertilizer significantly reduced percent root colonization and density of AM fungal spores and application of N fertilizer decreased the nitrogenase activity of the rhizosphere soil in *Ziziphus mauritiana* var. *rotundifolia*.

Application of bio-inoculants to the seedlings increased the growth parameters to the tune of 14.36% to 61.49% in *A. excelsa*, 8.85% to 51.98% in *G. arborea*, 17.42% to 57.67% in *M. dubia* and 13.11% to 52.02% in *N. cadamba* over respective uninoculated controls in nursery (Tables 71, 74, 77 and 80). Similarly, the percent increase in nutrient (N, P and K) uptake ranged between 48.93% and 83.48% in *A. excelsa*, 43.7% and 85.73% in *G. arborea*.
34.09% and 70.84% in *M. dubia* and 41.69% and 78.38% in *N. cadamba* (Tables 72, 75, 78 and 81). The percent increase in growth parameters of inoculated plants in the field over control was 8.68% to 27.77% in *A. excelsa*, 17.27% to 34.81% in *G. arborea*, 7.94% to 28.28% in *M. dubia* and 10.10% to 30.92% in *N. cadamba* (Tables 73, 76, 79 and 82).

The results of the present field study indicate that once plant growth promotion is induced in the nursery, seedlings can withstand transplanting shock and perform better in terms of their survival and collar diameter/DBH and plant height during the initial growing phase in the field. Similar results were reported by Zapata *et al.* (2006) on *Desmoncus orthacanthos* using AM fungi. Further, the effect of bio-inoculants on the growth performance (plant height and basal diameter/DBH) varied with the tree (host) species. (Enebak *et al.*, 1998; Singh *et al.*, 2000; Enebak, 2005) Singh *et al.* (2000) also observed that the actual values of all growth parameters were higher in *Albizia procera* than in *A. lebbeck*. Previously, Enebak *et al.* (1998) and subsequently, Enebak (2005) while working with *Pinus taeda*, *Pinus elliottii* and *Pinus palustris* also suggested that the effects of rhizobacteria inoculation on seedling emergence and plant growth are independent and species specific.

Inoculation of tree seedlings with AM fungi and PGPR may be more useful especially for seedlings targeted for outplanting on relatively harsh or poor quality sites (Chanway and Holl, 1994; Ghosh *et al.*, 2008). In the present study, microbial inoculation with AM fungi and PGPR to the seedlings of the selected four native fast growing tree species improved the growth, biomass and nutritional status of seedlings under nursery conditions which further resulted in better performance of these seedlings in the field conditions.
The health and the growth of the seedlings in nursery are very critical for their outplanting performance in the field. Seedlings usually undergo transplantation shock after their planting in the field and may suffer low survival rate especially in poor and adverse soils. In the adoption process of the seedlings to new and adverse environments in a complex and natural situation, tetra-partite interaction of AM fungi-PGPR-soil-plant plays very important role. From a practical viewpoint, raising successful plantation, apart from using superior plant genotype, is dependent upon soil managements by providing sufficient nutrient for plants to grow well and by giving protection from the soil borne diseases. It is then necessary to focus on rhizosphere soil because AM fungi and PGPR have abilities to alter and regulate such micro-scale of soil environments. Preparation of seedling inoculated with bio-inoculants before their outplanting is environmentally benign, less expensive and relatively easy for nursery application.

The growth substrates used in seedling production are usually devoid of sufficient beneficial microorganisms. By introducing such microorganisms to the substrates in sufficient quantity, it would be possible to lower fertilizer and pesticide inputs and grow the plants in a more sustainable way (Cordier et al., 2000). Rodriguez and Fraga (1999) opined that greater attention should be paid to studies and application of combination of PSB and other PGPR for improved results. Combined inoculation provides a good scope for commercially utilizing the efficient strains of AM fungi and PGPR for beneficial effects in the primary establishment of tree seedlings ensuring better survival and improved growth.

The overall outcome of the nursery and field trial was a positive response of all the four tree seedlings to inoculation with bio-inoculants
compared to the control. Statistically significant increase in plant growth parameters for all the four native tree species under nursery and field conditions, confirmed the positive effect of bio-inoculants, especially the dual inoculations of N fixing and P solubilizing/mobilizing bio-inoculants. These results were really interesting because in some of the previously reported trials with tropical trees, AM fungal inoculation failed to improve tree seedling growth in the nursery (Cuenca et al., 1990) as well as the field (Bhat et al., 1994; Venkateswarlu et al., 2008). Probably, in cases of co-inoculation of different tree species, selection of the N fixing and P solubilizing / mobilizing organisms are very important parameters.

In conclusion, this study revealed that the co-inoculation of N fixing and P solubilizing/mobilizing organisms improved the plant growth (primarily seedling height, collar diameter, leaf area and biomass) and nutrition (N, P and K uptake) of Ailanthus excelsa, Gmelina arborea, Melia dubia and Neolamarckia cadamba. This combination can be utilized for promotion of plant growth in tropical region, concomitantly saving considerable amount of chemical N and P fertilizers under nursery and field conditions.