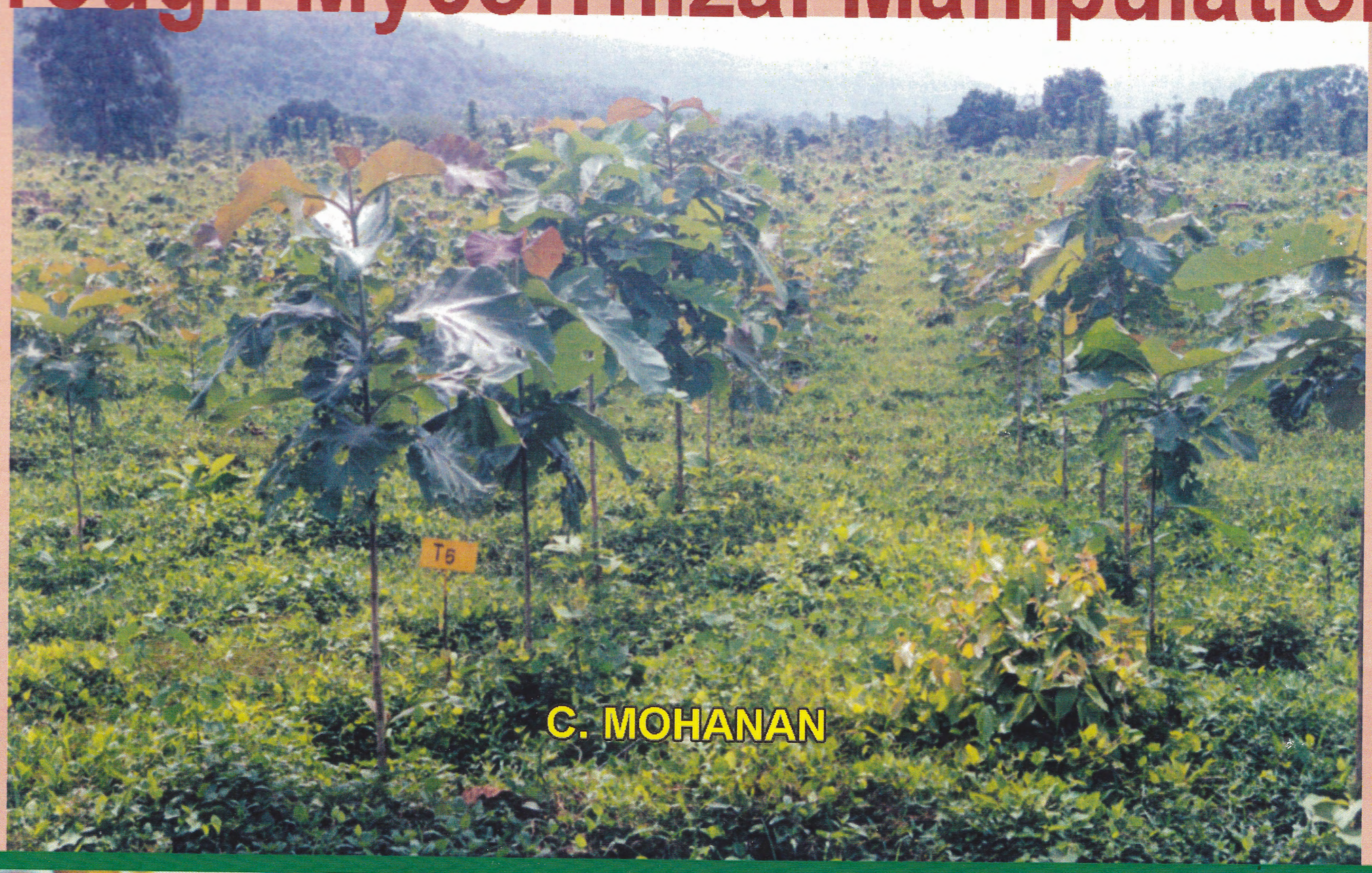
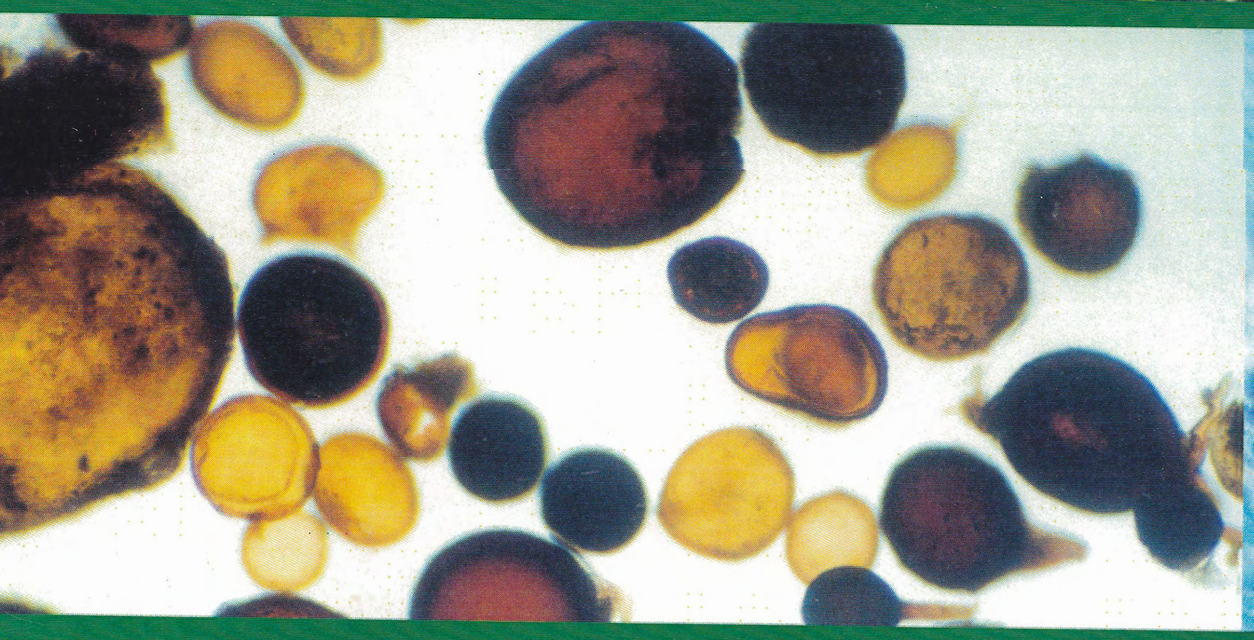
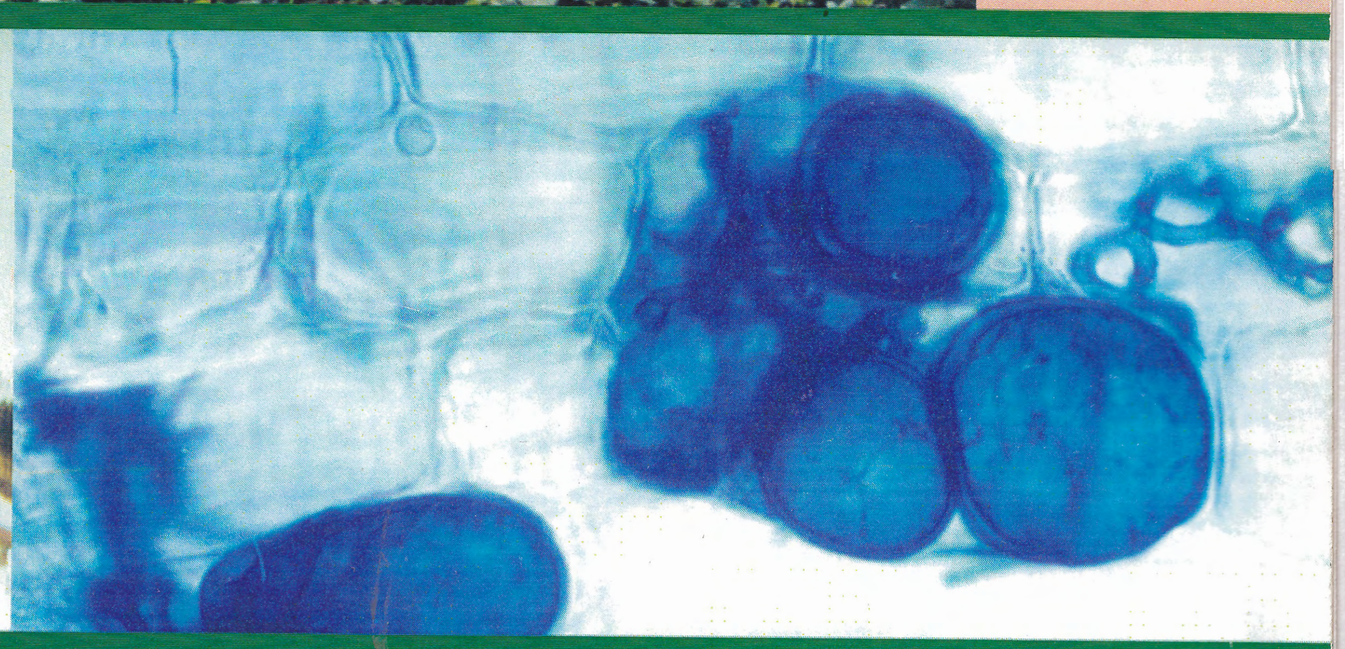
Productivity Improvement of Teak Through Mycorrhizal Manipulations



C. MOHANAN





KFRI

KERALA FOREST RESEARCH INSTITUTE

An Institution of Kerala State Science, Technology and Environment
Peechi, Thrissur 680 653 Kerala

JUNE 2005

KFRI Research Report No. 276 ISSN0970-8103

PRODUCTIVITY IMPROVEMENT OF TEAK THROUGH MYCORRHIZAL MANIPULATIONS

(Final Report of the Research Project No. KFRI 376/01)

C. Mohanan

Forest Pathology Discipline
Division of Forest Protection

KFRI

Kerala Forest Research Institute

An Institution of Kerala State Council for Science, Technology and Environment
Peechi 680 653 Kerala

June 2005

ABSTRACT OF THE PROJECT PROPOSAL

1. Project No. : KFRI 376/01
2. Project Title : Productivity improvement of teak through mycorrhizal manipulations
3. Objectives :

   i. To assess the mycorrhizal associations with teak and AM fungal inoculum density in teak plantation soils in various eco-climatic zones in southern India.

   ii. To evaluate the AM fungal dependency of teak and establish the efficiency of AM fungal species/strains in increasing the growth of teak plants in glasshouse and nursery trials.

   iii. To standardize technology for production and application of efficient AM fungi in root trainers.

   iv. To study the performance of potential AM fungi inoculated teak seedlings in degraded, stress-prone areas in pilot-scale field trial.

4. Date of commencement: November 2001
5. Scheduled date of completion: October 2004
6. Project Team:

   Principal Investigator: Dr. C. Mohanan

   Research Fellows : Ms. K.D. Priya (Jan. 2002-Aug. 2003); Mr. K. Girishmon (Sept. 2003-Oct. 2004).

   Technical Assistant : Mr. Shaju K Francis
7. Funding Agency : Department of Biotechnology, Ministry of Science & Technology, Govt. of India.

CONTENTS

ACKNOWLEDGEMENTS

The author expresses his sincere gratitude to the Department of Biotechnology, Ministry of Science & Technology, Government of India, New Delhi for financial assistance to this project. The author expresses his sincere appreciation and thanks to the members of the Task Force on Biofertilizer Biotechnology for their valuable guidance and suggestions throughout the project period.

The author expresses his sincere gratitude to Dr. Jyoti K. Sharma, Director, KFRI for encouragement and support during the entire project period and to Dr. R. Gnanaharan, Research Coordinator, KFRI for various suggestions during the course of work and also to improve the manuscript.

The whole hearted support and help rendered by the staff of Kerala Forest Department, especially, Mr. Nagesh Prabhu IFS, Conservator of Forests, Central Forest Circle, Mr. F. Martin, Forest Range Officer, Chettikulam Forest Nursery, and Mr. K. Kamaluddin, Forest Range Officer, Kalady Forest Range during the nursery and field trials are gratefully acknowledged.

Thanks are also due to Dr. Jose Kallarackal, KFRI for rendering help in chlorophyll fluorescence kinetic studies and Dr. K. Jayaraman and Dr. M. Sivaram for statistical analyses of data. Ms. K.D. Priya and Mr. K. Girishmon served as Research Fellows and their assistance in carrying out this project work is gratefully acknowledged. The author expresses his sincere thanks to Mr. Shaju K. Francis for providing technical assistance in the laboratory, glasshouse, nursery and field trials. Thanks are also due to Dr. M. Balasundaran and Dr. K.V. Sankaran, KFRI for their valuable editorial comments which helped to improve the presentation of this report.

ABSTRACT

Teak (*Tectona grandis* L. f.), the prime forestry species in India, is one of the most sought after hardwoods all over the world. Productivity of teak plantations in the southern States, especially Kerala State that has long history of teak cultivation, is alarmingly declining. Apart from silvicultural management measures, edaphic factors are considered as the most influencing ones. Improvement of soil nutrients status and their mobility by manipulating the soil biological characteristics are considered as most self-sustainable. Teak is reported to be a vesicular-arbuscular dependant forestry species. So far, no serious attempt has been made to improve the productivity of teak stands exploiting the mycorrhizal potential. Hence, the present study was taken up with the following objectives: i. to assess the mycorrhizal associations with teak and AM fungal inoculum density in teak plantation soils in various eco-climatic zones in southern India; ii. to evaluate the AM fungal dependency of teak and establish the efficiency of AM fungal species/strains in increasing the growth of teak plants in glasshouse and nursery trials; iii. to standardize technology for production and application of efficient AM fungi in root trainers; iv. to study the performance of potential AM fungi inoculated teak seedlings in degraded, stress-prone area in pilot-scale field trial.

Teak plantations of different age groups were surveyed in Kerala (28 plantations), Karnataka (17 plantations) and Andhra Pradesh (14 plantations) for collection of rhizosphere soil and feeder root samples. The soil and root samples collected from the plantations were processed and AM fungal root colonization, AM fungal spore density, species identity and diversity, and soil physical and chemical characteristics were studied. A total of 127 pot cultures of different AM fungi were prepared following funnel technique and maize seedlings and maintained for inoculum production. Artificial mycorrhization of teak seedlings employing selected AM fungal inoculum was carried out in glasshouse and efficacy of various AM fungi singly or in combination was screened. The most efficient and potential AM fungi were further screened under the forest nursery conditions. The field performance of mycorrhized and control planting stock was investigated in a pilot-scale field trial at Vembooram, Kodanad Forest Range, Kerala.

AM fungal root colonization was recorded in all the teak root samples collected from fifty nine plantations situated in different eco-climatic zones in the Kerala, Karnataka and Andhra Pradesh States. The intensity of colonization varied depending on the soil characteristics and age of the plants. All the AM fungal features like arbuscules, vesicles, intracellular hyphal coils, extra and intraradical hyphae within the feeder roots were occurred singly or in collectively. Among the root samples collected from 28 teak plantations in Kerala State, the overall extent of AM fungal root colonization was moderately high and ranged from 7-59%. From the Karnataka State, AM fungal root colonization was recorded from all the 17 teak plantations, which ranged from 1-43%; comparatively a very high percent AM fungal root colonization was recorded from teak plantations in the Andhra Pradesh State which ranged from 52-74%. A total of 86 Glomalean fungi belonging to six genera were retrieved from the teak rhizosphere soils from the Kerala, Karnataka and Andhra Pradesh States.

Among the Glomalean fungi, *Glomus* and *Acaulospora* were found widely distributed in teak rhizosphere soils in all the three States studied, while others were of limited distribution. Among 36 *Glomus* species recorded from the teak soils, *G. aggregatum, G. botryoides, G. fasciculatum, G. deserticola,* and *G. mosseae* were the most predominantly distributed ones. The AM fungal spore density in teak rhizosphere soils in the Kerala State ranged from 28 to 276/10 g soil, 50 to 193/10 g soil in the Karnataka State and 62 to 449/10 g soil in the Andhra Pradesh State. Soil samples from teak plantations in all the three States were moderately acidic to near neutral. The AM fungal spore density and species composition were found to be influenced by soil

characteristics such as soil pH, soil moisture contents and soil micronutrients. Moderately high to very high AM fungal root colonization as well as high AM fungal spore density and diversity of Glomalean species in teak rhizosphere soils in 4 to 50-year-old plantations belonging to different eco-climatic zones in the three States demonstrates the strong AM fungal association and mycorrhizal dependency of teak plants throughout its rotation age.

Glasshouse trial conducted during 2003 employing selected AM fungal inoculum prepared from native soils and applied on teak seedlings raised in root trainers with soil-sand as growing medium yielded improvement on seedling quality in terms of seedling height and seedling biomass production over control. However, no significant difference in chlorophyll fluorescence kinetics was recorded in AM fungi treated seedlings or in control sets. Inoculum of *G. fasciculatum, G. botryoides* and *A. appendicula* were the best ones among the 29 treatments tried. Promising mycorrhizal inoculation effect (MIE) was recorded in a few treatments with *G. fasciculatum, G. botryoides* and *A. appendicula*. Nursery trial carried out under forest nursery conditions during 2004 using weed compost as the growing medium in the root trainers, registered better growth performances in all the AM fungi treated seedlings than the control sets. All the artificially mycorrhized seedlings with different AM fungi, singly or in combinations, registered better height, collar diameter and biomass than the control (without AM fungal inoculation) seedlings. Among the 19 AM fungal treatments, *Glomus mosseae*2 treated seedlings yielded maximum seedling biomass (dry) and MIE%.

The results from glasshouse and nursery trials demonstrate the efficacy of mycorrhization of 10-15 day-old teak seedlings raised in weed compost medium in root trainers by application of inoculum of efficient native AM fungi. The nursery trial conducted during 2004 using inoculum of efficient AM fungi selected on the basis of their performance in the glasshouse trial confirmed the potential of AM fungi in boosting the seedling growth. The results also substantiates the improvement of quality of planting stock by application of inoculum of *Glomus botryoides, G. fasciculatum, G. macrocarpum, G. mosseae, Acaulospora appendicula, A. scorbiculata, Gigaspora gigantea* and *Scutellospora erythropa* singly or in combinations.

Pilot-scale field trials conducted employing the mycorrhized and non-mycorrhized (control) teak seedlings selected from 20 treatments from the nursery trial, registered highly significant differences in growth performances of mycorrhized seedlings in the field than control sets. In general, planting stock pre-colonized by efficient AM fungi in the nursery, exhibited better field performance than the control sets in terms of survival, height and collar diameter of plants and draught resistance. Among the 19 AM fungal treatments, inoculum of *G. fasciculatum* mixed with *A. appendicula* or *G. botryoides* recorded maximum plant height, collar diameter and vigour. Plants in *A. appendicula* 1 + *G. fasciculatum* treatment recorded maximum mean height of 123 cm with collar diameter of 2.89 cm within 9 months of field planting, while in non-mycorrhized plants (control plants), average plant height and collar diameter were only 86 cm 1.43 cm respectively. The nursery and field investigations employing various AM fungi signify that quality of teak planting stock can be improved by mycorrhization employing efficient AM fungi like *G. fasciculatum, G. botryoides, G. mosseae, A. appendicula* singly or in combinations. The pilot-scale field trial confirmed better field performance of artificially mycorrhized teak planting stock in terms of field survival, height and girth increment, and draught resistance. This boost in growth and vigour of artificially mycorrhized teak plants in the early establishment phase of the plantation may also reflect in the stand health and productivity. For further validating the beneficial effect on improvement of planting stock by mycorrhizal manipulations and thereby boosting the stand growth and productivity, large-scale nursery screening and multi-location field trials with long term monitoring are warranted.

1. INTRODUCTION

Teak (*Tectona grandis* L. f.), the prime forestry species in India, is one of the most favoured timbers all over the world. Teak is known for its strength, durability and attractive appearance. Obviously, it constitutes high-class furniture and one of the most sought after hardwoods in the international markets. The ever-increasing demand for teak timber has resulted in large-scale plantations both within and outside its range of natural distribution. It occurs in natural forests from 9^0 to 26^0 N latitude and from 73^0 to 104^0 E longitude, which includes southern and central India, Myanmar, Laos People's Democratic Republic and northern Thailand (White, 1991). Teak has been introduced in South-East Asia, Indonesia, Sri Lanka, Vietnam, Malaysia and Soloman Island as well as in Africa and Latin America. In India, teak has a wide, but discontinuous distribution. It grows in dry and moist-deciduous forests below 24^0 N latitude in the States of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Chattisgarh, Madhya Pradesh, Rajasthan, Uttar Pradesh, Manipur and Orissa. There are about 8.9 million ha teak bearing forests in India within the precipitation range of 800 to 2500 mm per annum (Tewari, 1991). It grows well from sea level to an elevation of 1200 m above sea level. Five sub-types of teak forests have been recognized: very dry, dry, semi-moist, moist and very moist (Seth and Khan, 1958). Teak shows poor growth in dry localities and thrives best in moist, warm and tropical climate.

The first teak plantation in India was established in 1846 at Nilambur, Kerala. At present about 1.5 million ha of teak plantations exist in India and around 50,000 ha teak plantations are raised annually (Subramanian *et al.*, 2000). Silvicultural practices like site matching, spacing, thinning methods, rotation age and harvesting have been refined, yet the productivity of plantations is low and is declining steadily in successive rotations. The rotation period of teak plantations in India differs according to site conditions, environmental factors and management and varies from 40 to 80 years; dry teak plantations in Madhya Pradesh have 80 years and moist teak plantations in Kerala have 50 to 60 years rotation. Stumps (prepared from 1-year-old bare root seedling) are used for planting at 2 x 2 m or 2.5 x 2.5 m spacing; however, recently, root trainer grown

seedlings (90-day-old) are employed for raising large-scale plantations in the Kerala State and elsewhere. Two mechanical and three silvicultural thinnings are performed in the plantation; the thinning is carried out at age of 5,10,15,20,30 for 60-year rotation in Kerala. After final thinning, 150 to 170 trees per ha are maintained in the plantation.

Teak is one of the most researched tropical hardwoods. Almost the entire century that followed the first planting of teak was spent on perfecting the technique of growing teak plantations. Nursery technique, choice of site, planting, weeding and maintenance of plantation, thinning, and fixing rotation age dominated the research priorities during the early years. More recently, emphasis has been given to standardize the nursery practices and production of quality planting stock (Chacko *et al.,* 2002) and also to improve the productivity of the stand by fertilizer application and irrigation.

Productivity of teak plantations is alarmingly declining in areas which have long history of teak cultivation. A meager 2.85 m^3 ha^{-1} on an average for a rotation of 53 years, where all thinning schedules were followed in Nilambur, Kerala State has been reported (Chundamannil, 1998). Even though, many factors such as silvicultural management measures, genetic make up of the plant and pests and diseases are partly responsible for the drastic reduction in stand productivity, edaphic factors are considered as the most critical ones. In general, soil under teak plantations in the Kerala State is reported to be problematic and nitrogen (N) and phosphorus (P) availability are the limiting factors (Balagopalan *et al.*, 1998). Application of fertilizers at the early phase of the plantations, soil and water conservation efforts to prevent leaching of nutrients and to increase moisture availability have been proposed as short-term strategy for problematic teak growing areas in the State (Balagopalan *et al.*, 1998). Many view improving the soil nutrient status and their mobility by mycorrhizal application in teak as a long-term strategy as well as most self-sustainable.

Mycorrhizae are symbiotic associations between the roots of a vast majority of plant species and a specific group of fungi. In these symbiotic associations, the host plant provides a source of fixed carbon to the fungal partner and in return receives inorganic

nutrients absorbed from soil and retrieved by the fungal partner. The dependency of fungal species on host plant ranges from obligate, where the fungal partner can obtain carbon only from the host, to facultative, where the energy requirement is met partly from carbon supplied from the host and partly from mineralization of organic carbon by the fungi itself (Brundrett, 1991). Mycorrhizal association varies widely in form and function depending upon the species involved in association. Arbuscular mycorrhyzae (AM) or Vesicular arbuscular mycorrhizae (VAM) are the most widely occurring associations followed by Ectomycorrhizae (ECM) which associate mostly with specific families of Gymnosperms and Angiosperms. Other mycorrhizal associations like Orchid and Ericoid mycorrhizae are confined to specific plant families.

Diversity of these root fungal associations has provided plants with strategies that maximize fitness (ability to grow and reproduce) across a gradient of climate, latitude (or altitude), and soil type. Mycorrhizal association shows a range of specificity in interaction as well as in the nature of association, in terms of degree of interdependence. More than one ecto- and endomycorrhizal species can coexist in one individual. However, coexistence of endo- and ectomycorrhizae is rare in forest plantation species (Mohanan, 2002,2003).

AM fungi are largely generalists, i.e., one AM fungal species is able to colonize a variety of host species. ECM fungi show a wider range of host specificities: some are highly host specific, some infect a wide range of host plants but intermediate host specificity in association is the most widespread.

Extraradical hyphae may act as extensions of the host plant root and solubulizing enzymes produced by the fungal hyphae may help in acquiring and absorbing nutrients from sources which may not be available to the plants. AM fungal hyphae have been shown to form mycelial linkages between root systems in natural forest ecosystems. Trees colonized and interconnected by a diverse fungal population in such a way have been recognized as functional guilds, where individuals are interlinked through transfer and exchange of resources (Read, 1991). It has been recently discovered that glomalin,

an iron containing glycoprotein and hydrophobic in its native state produced by hyphae of arbuscular mycorrhizal fungi plays an important role in structuring soil. Glomalin concentration is highly correlated with the percentage of water-stable aggregates in variety of soils. Plants show differential responses to mycorrhizal colonization depending on the species involved as well as at an individual level in respect of the growth stage of the plant. In effect, such non-uniform benefits of mycorrhizal symbiosis can bring about shifts in inter-specific plant competition and population structure of species and therefore influence community structure and control succession patterns in some plant communities.

Arbuscular mycorrhizal fungi have bimodal pattern of differentiation as they survive in two different habitats, the interior of root and the surrounding soil matrix. The vegetative phase of these fungi consists of intraradical appressoria, intra- and extraradical coenocytic hyphae, and dichotomously branched intraradical arbuscules. The reproductive phase of the fungi consists mainly of spores formed on the hyphae, inside or out side the root. Formation of sexual spores have also been reported recently only in one fungus, *Gigaspora decipiens* Hall & Abbot (Tommerup and Sivasithamparam, 1990). The AM fungi establish a compatible interaction with host plant cell and develop a biotrophical nutritional relationship of long duration with host plant, which normally results in increased growth of the host plant. Significant morphological and physiological differences between species exist in vegetative and reproductive structures, which have been used to differentiate taxa in AM fungi.

Recently, the potential for manipulating mycorrhizal association to increase productivity in plantation forestry is the focus of major research activities. There is also much interest in their potential utilization in agriculture and horticulture (Bagyaraj, 1992). Teak has been subjected to various investigations in India and abroad: on their mycorrhizal association and diversity (Coster, 1921; Chong, 1988; Dadwal *et al.*, 1986; Verma and Jamaluddin, 1995; Raman *et al.*, 1997; Talukdar and Thakuria, 2001; Dhar and Mirdha, 2003; Mohanan, 2003a), mycorrhizal dependency (Sugavanam *et al.*, 1998; Gurumurthy and Sreenivasa, 1988, 2000; Rajan *et al.*, 2000; Verma *et al.*, 2001a,b; Mohanan, 2003),

AM fungal population dynamics (Chandra and Jamaluddin, 1999), AM fungal interactions with soil microorganisms (Paroha *et al.*, 2000), nutrients and soil physical factors (Jamaluddin and Chandra,1997), influence in mineral nutrition (Durga and Gupta, 1995; Bhadraiah *et al.*, 2002), improvement of seedling growth and biomass (Ramanwong and Sangwaint, 2000; Vijaya and Srivasuki, 2001a,b; Chandra and Ujjaini, 2002; Gong *et al.*, 2002), and planting stock improvement (Mohanan and Sheeba, 2005).

All these studies have highlighted the benefits to the teak plant through mycorrhizal association/inoculation, which include: plant nutrients supply through mycorrhizal roots, antagonism against parasitic microorganisms, non-nutritional benefits due to water relations, nutrient cycling and conservation by soil mycelia, improving soil structure, and carbon transport from plant roots to other soil organisms. The functional diversity of mycorrhizal fungi includes variation between individual species in the following capacities: mobilizing of limiting soil nutrients, inorganic forms of phosphorus, nitrogen, and trace elements, amelioration of adverse soil conditions due to toxic concentration of metal ions, extremes in soil pH, high conductivity (salinity), nutrient imbalance such as high Mg : Ca ratios, responses to severe climatic conditions such as limited or excess water supply, temperature extremes, compatibility with different hosts, tolerance of adverse soil conditions such as disturbance and microbial competition.

Mycorrhiza dependent perennial species require a well-balanced functional mycorrhizal association for a sustainable soil-plant system. The functional diversity of the mycorrhizal fungi provides opportunities for selecting fungi adapted to specific combinations of host/environment/soil conditions in stands or plantations. The selected efficient AM fungal candidates can be employed as an effective biological tool for improving the planting stock and thereby increasing the stand productivity in a most environment friendly way by avoiding over usage of chemical fertilizers. So far, no serious attempt has been made to investigate the mycorrhizal association of teak in southern India and to exploit the AM fungi for improving the productivity of teak stands, hence, the present study has been taken up with the following objectives:

i. To assess the mycorrhizal associations with teak and AM fungal inoculum density in teak plantation soils in various eco-climatic zones in southern India.

ii. To evaluate the AM fungal dependency of teak and establish the efficiency of AM fungal species/strains in increasing the growth of teak plants in glasshouse and nursery trials.

iii. To standardize technology for production and application of efficient AM fungi in root trainers.

iv. To study the performance of potential AM fungi inoculated teak seedlings in degraded, stress-prone areas in pilot-scale field trial.

2. MATERIALS AND METHODS

2.1. Selection of teak plantations for rhizosphere soil and root sampling

A reconnaissance survey was made and teak plantations in the Kerala, Karnataka and Andhra Pradesh States. Twenty eight teak plantations belonging to different age groups (4 yr to 50 yr) were selected in different Forest Divisions in the Kerala State. From the Karnataka State, 17 teak plantations (18 yr to 44 yr) and from Andhra Pradesh State, 14 teak plantations (22 yr to 34 yr) in different Forest Divisions were selected (Figs. 1-3; Tables 1-3). Within the plantations, sampling was done following the line transect method; in line transect sampling, a distance of 50 m was given between each sample tree and five sample trees were selected in each plantation. Information on age of the plantation, cultural and management practices adopted and incidence of fire was collected from the concerned Forest Range Office/Forest Stations. Details on girth at breast height (gbh) and approximate height of the sampled trees were recorded.

Table 1: Teak plantations in the Kerala State selected for the study

Sl.No ..	Locality	Forest Range	Age (Yr)
1	Poolakkapara	Nilambur	22
2	Cherupuzha	Nilambur	45
3	Naduvathumuzhy	Konni	25
4	Malayattoor	Malayattoor	20
5	Kanimangalam	Malayattoor	26
6	Pandupara	Kalady	12
7	Mulamkuzhy	Malayattoor	10
8	Karimpani	Thundathil	20
9	Bhoothathankettu	Thundathil	26
10	Chakolathara	Pattikkad	40
11	Irumpupalam	Pattikkad	42
12	Dhoni	Olavakkod	4
13	Dhoni Qts	Olavakkod	50
14	Peruva	Kannavam	26
15	Mananthavady	Begur	25
16	Kattikulam	Begur	22
17	Tholpetty	Tholpetty	26
18	Kulathupuzha	Kulathupuzha	32
19	Arienkavu	Arienkavu	30
20	Achenkoil	Achenkoil	28
21	Achenkoil TP	Achenkoil	32

22	Achenkoil Thura	Achenkoil	20
23	Sungam	Sungam	25
24	Kannimara	Parambikulam	24
25	Peruvara Bl-51	Parambikulam	26
26	Poopara	Karimala	24
27	Karimala	Karimala	22
28	Parappa	Kasargode	20

Table 2: Teak plantations in the Karnataka State selected for the study

Sl.No	Locality	Forest Range	Age (yr)
1	Aletty West	Sullia	18
2	Kolchar (Aletty East)	Sullia	24
3	Medinadka West	Sullia	32
4	Mendekol	Sullia	14
5	Thodikana	Sampage	22
6	Sampage	Sampage	36
7	Chaparke	Kundupara	20
8	Kanchar	Ampar	25
9	Heigodemale	Ampar	26
10	Kollur	Kollur	21
11	Kodur	Hosnagar	18
12	Hirejene	Hosnagar	14
13	Arsalu	Shankar	48
14	Gajanur	Shakravelu	24
15	Mundagadde	Mundagadde	29
16	Balehonnur	Balehonnur	30
17	Belur	Belur	18

Table 3: Teak plantations in the Andhra Pradesh State selected for the study

Sl.No	Locality	Forest Range	Age (yr)
1	Narsipatnam	Narsipatnam	6
2	Krishnapuram	Chinthapally	15
3	Lothugedda	Lothugedda	20
4	Wangasara	Chinthapally	26
5	Wangasara SPA	Chinthapally	28
6	Rangthada SPA	Chinthapally	21
7	Mullametta	Chinthapally	18
8	Ebul SPA	RV Nagar	16
9	Chethalapadu SPA	Chinthapally	28
10	Valasagedda	Sileru	24
11	Bachlooru	Maredemella	29
12	Dharakonda	Sileru	32
13	Sileru	Sileru	18
14	Duppiawada	Sileru	16

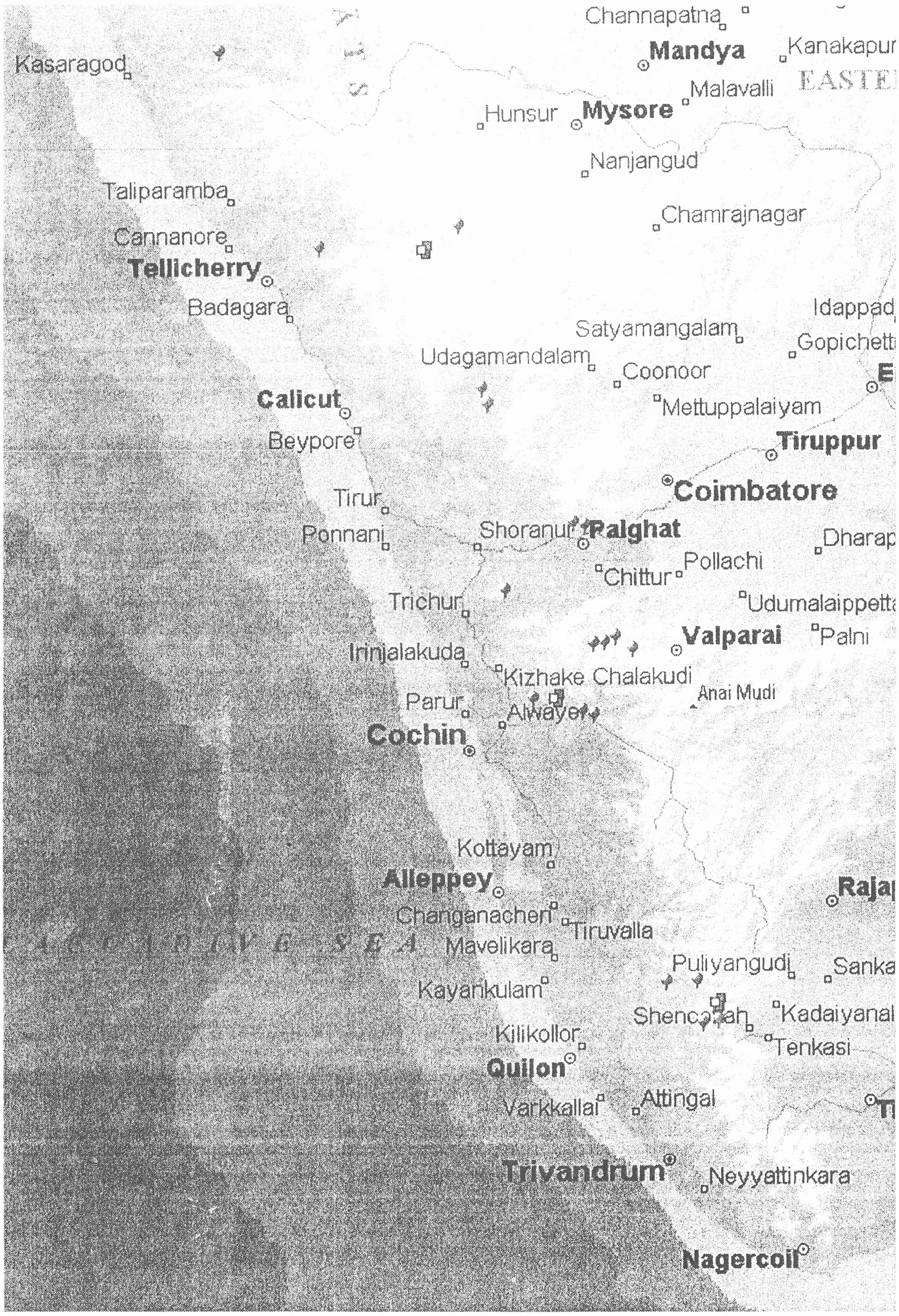


Fig. 1. Location of teak plantations in Kerala State selected for the study

Fig. 2. Location of teak plantations in Karnataka State selected for the study

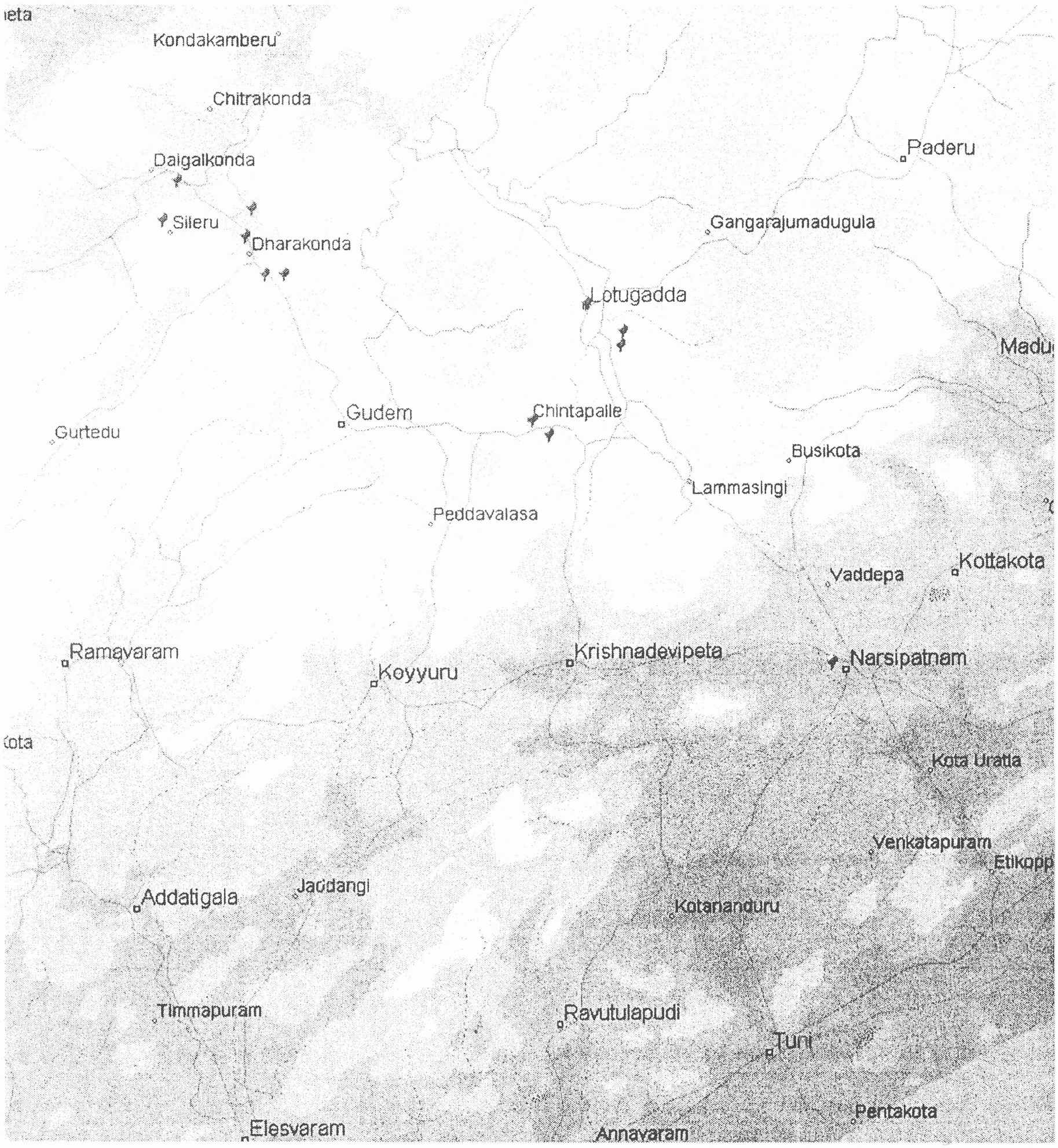


Fig. 3. Location of teak plantations in Andhra Pradesh State selected for the study

2.2. Collection of rhizosphere soil and root samples

About four kilogram of rhizosphere soil along with young feeder roots was collected from each selected tree from different teak plantations. Care was taken to ensure that fine feeder roots are well represented in the samples and to exclude the entangled roots of other plant species. The soil and root samples collected were transported to the laboratory, young feeder roots were separated using sieve (1mm) and processed.

2.3. Processing of mycorrhizal root samples

The root samples were cleaned, cleared and stained using standard procedures, mycorrhizal colonization quantified and types of associations established by careful microscopic examination. Roots were washed thoroughly in tap water over a 1-2 mm screen. After washing, the roots were kept moist in polythene bags and refrigerated (approax.5^0C). A working sample of the roots was taken by chopping the selected fine roots into about 1 cm in length and mixing. Then random sub-samples were drawn and kept in Petri dishes at 4^0C.

Clearing of mycorrhizal roots was required as structures produced by AM fungi are not visible when fresh roots are observed, as they are often obscured by the natural pigments and cell contents within roots. The root bits (1cm in length) were immersed in KOH 10% w/v solution in beakers and autoclaved for 45 min at 15 p.s.i. After clearing the roots, KOH solution was drained off and the roots were thoroughly washed with tap water for 3-4 times. Bleaching was done for moderately and highly pigmented roots by using alkaline H_2O_2. The roots were then neutralized with 1N HCl for 1-3 min. and then the roots were stained with Trypan blue (Phillips and Hayman, 1970; Kormanik and McGraw, 1982). The roots were immersed in Trypan blue (0.06%) and kept it for overnight. After staining, the roots were separated from staining solution and immersed in Lacto-glycerol, if necessary. The root bits were then observed under a light microscope for the presence of AM fungal structures, arbuscules, vesicles, internal hyphae and

spores. From each sub-samples, 100 root bits were observed and the percentage root colonization (RC) was calculated using the formula:

$$\%RC = \frac{\text{Number of root bits with vesicles and arbuscules}}{\text{Total number of root bits observed}} \times 100$$

2.4. Evaluation of physical and chemical properties of rhizosphere soils

Rhizosphere soil samples collected from various teak plantations were analyzed for their physical and chemical characteristics. Moisture content (%MC) of the soil was determined by oven dry method and soil pH was measured by a digital pH meter. Exchangeable cations Na, Mg, Ca, total Nitrogen (N), Phosphorus (P) and Organic Carbon were also analysed for a few selected samples (Keeney,1980; Hefferman, 1985; Rayment and Higginson, 1992).

2.5.Separating Arbuscular mycorrhizal spores from soil samples

Wet-sieving and decanting method (Gerdemann and Nicolson, 1963) with modification and wet-sieving and centrifuging methods were employed for retrieving the arbuscular mycorrhizal (AM) fungal spores from soil samples. For assessing the AM spore density and diversity in rhizosphere soil, ten gram of air-dried soil sample was used. Retrieval of AM fungal spores for inoculum preparation was carried out by using twenty five gram of air-dried soil sample. Soil sample was taken in a beaker (1000 ml), stirred thoroughly with tap water and kept for sometime for heavier particles to settle down. The supernatant was decanted through a series of test sieves ranging from 45μm to 750μm mesh. This process was repeated for 4 to 5 times until the soil solution becomes clear. The sievings from the three sieves, 45μm, 100 μm, and 250 μm were collected into a conical flask using a wash bottle, mixed thoroughly and kept for sometime. The supernatant was filtered through a filter paper (120 mm dia) and observed under a Stereo-binocular microscope. In the case of wet sieving and centrifugation method, the sievings collected on 45μm, 100 μm, and 250 μm sieves were suspended in 50% sucrose solution

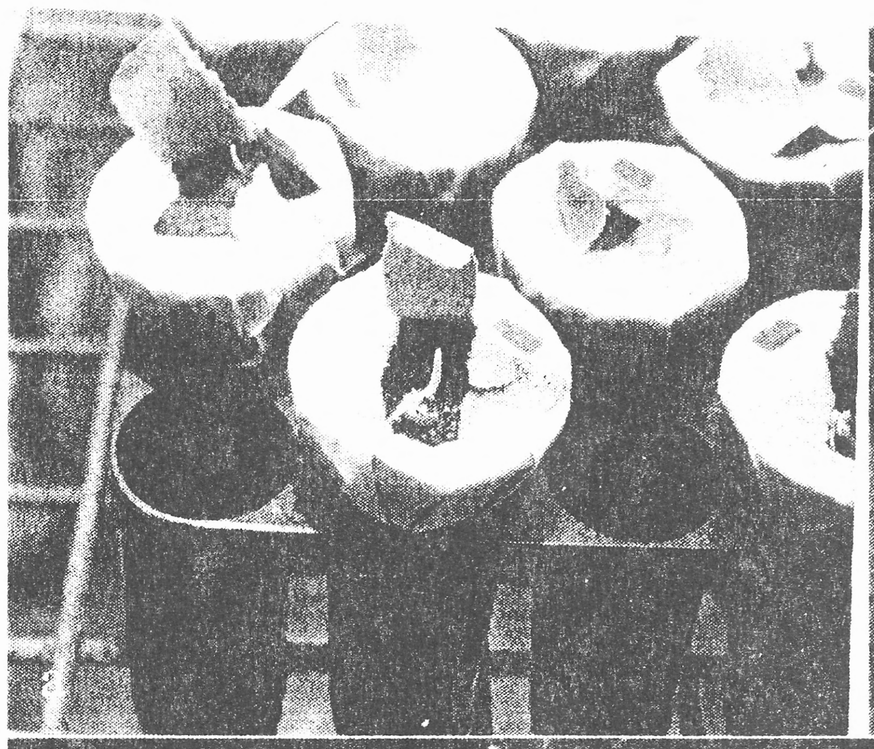
in centrifuge tubes separately and centrifuged for one minute at 2000 rpm. Immediately after the centrifugation, the spores in sucrose supernatant were poured onto a sieve (45μm mesh) and carefully washed with tap water to remove sucrose. After rinsing the spores, washed them onto a pre-wetted filter paper in a Buchner funnel before vacuum filtration. AM fungal spores from the filter paper surface were selected and transferred to a drop of polyvinyl-lacto-glycerol (PVLGA) or polyvinyl alcohol (PVA) mountant on microscope slide using a sharpened wooden dowel. Spore preparations with and without Melzer's reagent were made to reveal details on spore inner-wall layers. All the soil samples collected from the Kerala, Karnataka and Andhra Pradesh States were analysed for AM fungal density and diversity.

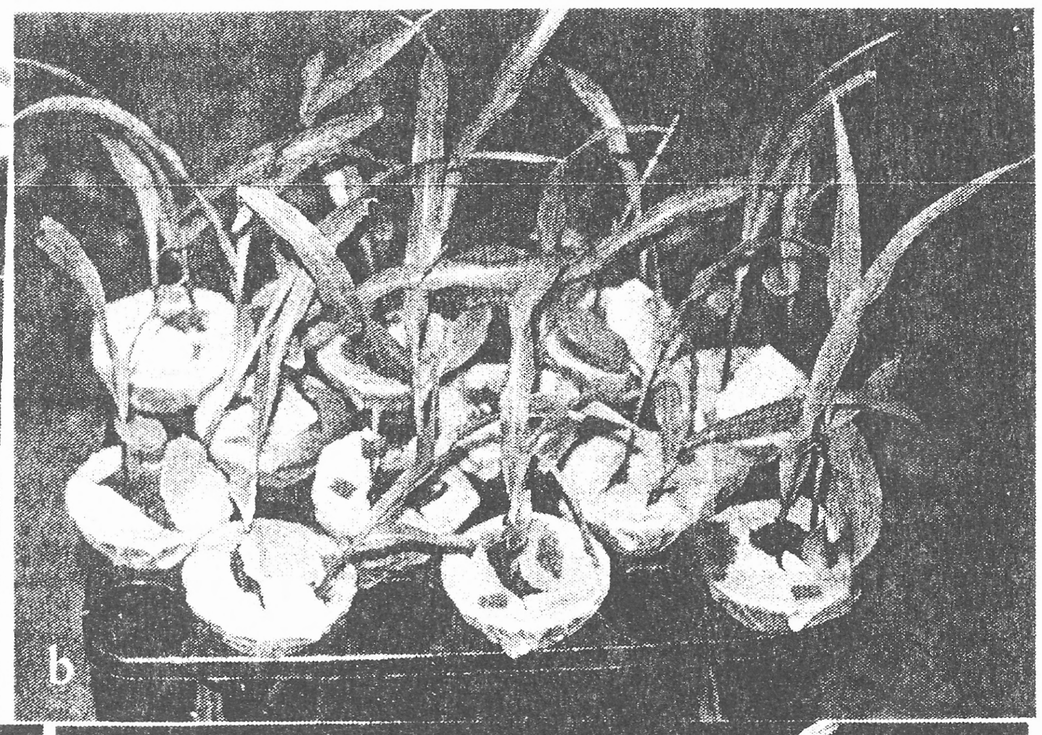
2.6. Identification of Arbuscular mycorrhizal fungi

Identification of the taxa was made by following the taxonomic descriptions of Schenck and Perez (1990) and Morton (1993). Spore characteristics such as spore color, shape, spore wall structure, subtending hypha, presence or absence of special structures like germination shield, suspensor and spore ornamentation were used for identification. Measurements on spores, spore wall layers, suspensor, subtending hypha, details on spore inclusion and spore wall ornamentation were also recorded. Details on spore wall characteristics were utilized for preparation of murographs. Sporocarp characteristics used for identification of species include: presence or absence of peridium, arrangement of spores, colour, shape and size. For arranging the taxa, Classification of Morton & Benny (1990) was followed.

2.7. Selection and propagation of inoculum of AM fungal inoculum

AM fungi were selected on the basis of their frequency of occurrence in the teak rhizosphere soil samples. A total of 127 pot cultures were established from single spore of different species of Glomalean fungi belonging to the genera, G*lomus, Acaulospora, Gigaspora* and *Scutellospora* employing funnel technique (Fig. 4; Table 4). The pot cultures were grown in non-draining buckets (20 cm height and 12 cm dia). Maize (*Zea*





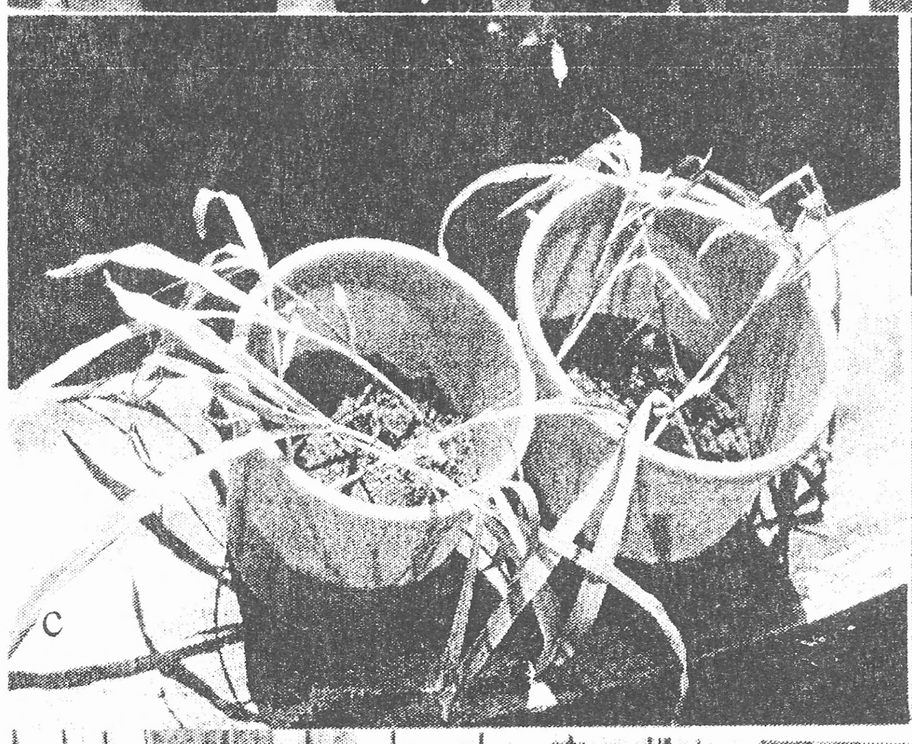


Fig.4: a-f: Pot and trap cultures of AM fungi prepared employing funnel technique (a, b) and maize seedlings and maintained in the glasshouse

mays) was used as host plants and the pot cultures were maintained in the glasshouse. Hoagland micronutrient solution was applied to the seedlings periodically. After six months of growth in pots, the maize plants were cut at collar region and left for two weeks. During this period, the soil was subjected to drying. AM fungal inoculum include spores, mycorrhizal root pieces, and organic matter containing hyphae and was prepared by chopping the roots and mixing them with the rhizosphere soil.

Table 4: Details on AM fungal pot cultures prepared and maintained in the glasshouse

Sl.No.	A M fungi	AM Pot culture No.
1	*Glomus botryoides*	P47, P51, P70, P78, DT118, DT134, DT150
2	*Glomus constrictum*	DT175
3	*Glomus deserticola*	P16, P39
4	*Glomus fasciculatum*	P18, P24, P32, P41, P48, DT65, P73, P79, DT86 P105, DT109, DT151, DT169, DT177
5	*Glomus globiferum*	DT162, DT186
6	*Glomus intraradices*	DT133
7	*Glomus macrocarpum*	P5, P66, DT71, P82, DT87, DT95, DT104, DT106, DT133, DT137, DT156, DT167, DT171, DT164, DT183, DT189
8	*Glomus maculosum*	P23, P36, P54, DT89, DT94, DT117, DT145, DT148, DT154, DT157, DT185
9	*Glomus mosseae*	P4, P11, P13, P19, P33, P43, P45, DT49, P62,DT81, DT97, DT122, DT126, DT138, DT141, DT142, DT153, DT166, DT170, DT173
10	*Glomus multisubtensum*	P58
11	*Glomus reticulatum*	DT178
12	*G. tortuosum*	DT197
13	*Acaulospora appendicula*	P10, P21, P29, P35, P52, P63, P67, DT69, DT115, DT26, DT187, DT179
14	*Acaulospora bireticulata*	P83
15	*Acaulospora delicata*	P85
16	*A.caulospora rehmi*	DT7, P59, DT91, DT102, DT111, DT140
17	*Acaulospora scorbiculata*	DT25, P75, DT130, DT131,
18	*Acaulospora.* spp	P15, P37, DT53, DT61, DT99, P107, DT125 DT129, DT160, DT181
19	*Scutellospora erythropa*	P74
20	*Scutellospora hetrogama*	P28
21	*Scutellaspora* spp	DT101
22	*Gigaspora albida*	DT146, DT147
23	*Gigaspora candida*	P40
24	*Gigaspora gigantia*	DT44
25	*Gigaspora* spp.	P9, P31, P55, DT135, DT158, DT161, DT174

Trap cultures were also prepared by using soil samples collected from the field. Rhizosphere soil samples collected from teak plantations at major teak growing areas, Nilambur, Malayattoor, Dhoni, Parambikulam, Wayanad and Konni (Kerala State) were used for trap pot culturing. Soil samples collected from teak plantations at Sullia, Mundagadde and Belur (Karnataka State) and at Chinthapally, Lothugedda and Sileru (Andhrapradesh State) were also used for trap culture preparation. Two kilogram of rhizosphere soil along with root bits was layered over sterile sand-soil mixture (1:1) half filled in plastic pots (non-draining). A thin layer of sterile soil-sand mixture was put over this. AM fungal consortium prepared from 5 kg of teak rhizosphere soil collected from the teak plantations in Wayanad and Nilambur (Kerala) was also used for preparation of large-scale inoculum. Germinated maize (*Zea mays*) seeds were aseptically transferred and planted in the pots containing soil-sand mixture and inoculum. The cultures were maintained in the glasshouse. The spores of different mycorrhizal fungi retrieved from these trap cultures were also used for preparation of pot cultures.

2.8. Glasshouse trial

A total of 127 pot cultures of different AM fungi were prepared and maintained (Table 4). A total of 12 trap cultures from soil samples collected from major teak growing areas in Kerala, Karnataka and Andhra Pradesh were also raised and maintained in the glasshouse. AM fungal inoculation experiments were conducted in the glasshouse. Preliminary trials using root trainers of different cell capacities (150 cc, 250 cc and 300 cc) were carried out; soil-sand (1:1 ratio) and coir pith-compost-sand were used as growing media for raising teak seedlings. Teak seeds collected from teak plantations at Nilambur were subjected to pre-treatments (alternate wetting and drying for 1 week) for getting high percent germination. Growth performance of teak seedlings in both the growing media was assessed. Soil-sand (1:1 ratio) was found supporting uniform growth of teak seedling in root trainers; root trainers with cell capacity of 150 cc were selected and styroform blocks with 24 root trainer cells were used for the trial.

Steam sterilized soil-sand medium (1:1) was used to fill the root trainer cells. Teak seeds obtained from KFRI Seed Centre (Seed lot 2003 from Nilambur) were utilized for this experiment. Teak seeds were subjected to wet and dry treatment for seven days in order to break the seed dormancy. Seed germination was recorded after 8 days of wet and dry treatment and germinated seedlings were aseptically transferred to the root trainer cells filled with growing medium. Sterilized tap water was used for watering the seedlings. After 15 days of growth in root trainers in the glasshouse, AM fungal inoculum was applied to the seedlings. Two gram of AM fungal inoculum was applied at the base of the seedlings in each root trainer cell and a thin layer of sterilized soil was put over the inoculum. A total of 28 AM fungal treatments were given and 3 replications were kept (Table 5). Control set was kept by adding sterilized soil (3 g) in place of AM fungal inoculum. Sterilized tap water was used for watering the treated seedlings during the entire course of the experiment. After 10 days of growth, the seedlings were transferred to the area provided with shade nets (50%shade). The shade nets were removed after 30 days of growth. The seedlings were watered regularly and observations on number of leaf pairs and seedling height were recorded at regular intervals of 15, 30, 45, 60, 75 and 90 days of inoculation.

2.8.1. Photosynthetic status of mycorrhized and control teak seedlings

Chlorophyll fluorescence kinetic was measured in teak seedlings treated with various AM fungi and untreated seedlings (control) using a Handy PEA (Hansatech Instruments, Norfolk, UK). The experiment was carried out to know the effect of mycorrhization on photosynthesis. Three seedlings from each of the 12 treatments were used for this experiment (Table 6). Healthy teak leaves kept under dark for a period of 30 minutes were subjected to sudden illumination and a time-dependent fluorescence induction (Kautsky Effect) was recorded. As time to reach the fluorescence peak is very short (500 m sec), hence it is useful to plot the fluorescence rise on a logarithmic scale to view the polyphasic kinetic. The polyphasic curve was then subjected to the OJIP analysis (Strasser *et al.*, 1995).

Table 5: Details on AM fungal inoculum used in the glasshouse trial

Pot Culture No	Treat. No.	VAM Fungus	Source	
			Locality	Forest Range
DT 97	1	*Glomus mosseae*	Kattikulam	Begur
DT 166	2	*Glomus mosseae*	Peruvara-block 51	Parambikulam
DT 13	3	*Glomus mosseae*	Kanimangalam	Malayatoor
DT 66	4	*G.macrocarpum*	Dhoni	Palakkad
DT171	5	*G.macrocarpum*	Peruvara-Block 51	Parambikulam
DT111	6	*A.rehmi*	Kulathupuzha	Kulathupuzha
DT21	7	*A.appendicula*	Pandupara	Kalady
DT 187	8	*A.appendicula*	Karimala	Karimala
DT16	9	*G.deserticola*	Pandupara	Kalady
DT 177	10	*G.fasciculatum*	Poopara	Karimala
DT 79	11	*G.fasciculatum*	Peruva	Kannavam
DT 24	12	*G.fasciculatum*	Mulankuzhy	Mlayatoor
DT 131	13	*A.scorbiculata*	Achenkovil	Achankovil
DT 154	14	*G.maculosum*	Sungum	Sungum
DT 78	15	*G.botryoides*	Peruva	Kannavam
DT 98	16	*G.tortuosum*	Kattikulam	Begur
DT133	17	*G.intraradices*	Achenkovil	Achenkovil
DT146	18	*Gigaspora albida*	Achenkovil-Thura	Achankovil
DT40	19	*Gigaspora candida*	Boothathankettu	Thundathil
DT44	20	*Gigaspora gigantia*	Boothathankettu	Thundahil
DT 142 + P 32	21	*G.fasciculatum + G.mosseae*	Karimpani,Achencoil	Thundathil, Achencoil
DT 173 + P 35	22	*G.mosseae + A.appendicula*	Peruvara, Karimpani	Parambikulam, Thundathil
P 10 + P41	23	*A.appendicula + G.fasciculatum*	Kanimangalm Boothathankettu	Malayattor Thundathil
*	24	Control		
*	25	DT 10 Consortium	Chakolathara	Pattikkad
*	26	DT13 Consortium	Dhoni	Palakkad
*	27	DT15 Consortium	Manathabvady	Begur
*	28	DT 20 Consortium	Achankovil	Achankovil
*	29	DT 27 Consortium	Karimala	Karimala

Table 6: Details on AM fungi treated and control seedlings subjected to chlorophyll fluorescence kinetic measurement

Sl.No.	Treat. No.	Treatments
1	2	*Glomus mosseae*
2	6	*Acaulospora rehmi*
3	7	*A. appendicula*
4	8	*A. rehmi*
5	10	*Glomus fasciculatum*
6	11	*G. fasciculatum*
7	12	*G. fasciculatum*
8	17	*G. intraradices*
9	23	*A.appendicula + G.fasciculatum*
10	24	Untreated - Control
11	25	AM fungi consortium DT 10
12	29	AM fungi consortium DT 27

2.8. 2. Seedling biomass

After 90 days of growth in root trainer, seedling biomass was recorded by destructive sampling method. Ten seedlings from each treatment were removed from the root trainer cells carefully and after removing the adhered soil particles on roots, wet weight was recorded; the samples were kept in oven at 60 ^{0}C overnight and dry weight was recorded. Mycorrhizal inoculation effect (MIE) was evaluated using the following formula:

$$\% \text{ MIE} = \frac{\text{Dry wt. of inoculated plants} - \text{Dry wt. of uninoculated plants}}{\text{Dry wt. of inoculated plant}} \times 100$$

2.8. 3. Seedling root colonization

The roots of inoculated and uninoculated (control) plants were collected after 90 days and processed as mentioned earlier (Section 2.3) and stained with Trypan blue. (0.06%). The root bits were observed under a light microscope for the presence of arbuscules, vesicles, internal hyphae and spores. From each treatment, 100 root bits were observed and the percentage root colonization was calculated.

2.8.4. Assessment of AM spore counts in growing medium in root trainer cells

After 90 days of growth, seedling from the root trainer cells were removed and the soil samples were taken for assessment of AM spore count. Ten gram soil from each treatment was taken and subjected to wet sieving and decanting method as described earlier and AM spore count was made.

2.9. Nursery trial

Nursery trial was carried out in Central Nursery at Chettikulam in 2004. Root trainer cells (150 cc) were used and compost made out of forest weeds mixed with soil, sand, coconut coir pith and burnt rice husk (70:10:10:5:5) was used as growing medium. Moisture

content and pH of the growing medium were determined. Styroform block containing 24 root trainer cells with 150cc capacity was used for raising the seedlings. The root trainer cells were filled with growing medium. Teak seeds obtained from the Seed Center, KFRI (Seed lot 2003, Nilambur) were used; seed pre-treatment was carried out (alternate wet and dry treatment for 7 days) to increase the seed germination. Germinated teak seedlings were transplanted in each root trainer cell carefully. A total of 100 styroform block containing 2400 teak seedlings were kept ready for mycorrhizal treatment.

2.9.1. Selection of AM fungi

AM fungi were selected on the basis of their performance in the glasshouse trial conducted during 2003. From the genus Glomus, *G. botryoides, G. fasciculatum* (pot culture Nos. 177, 79, 24), *G. macrocarpum, G. mossease* were selected. From the genus *Acaulospora, A. appendicula* (Pot culture Nos. 187, 21), *A. rehmii* and *A. scorbiculata* were selected. *Gigaspora candida* and *G. gigantea, Scutellospora heterogama* and *S. erythropa* were the other AM fungi selected for the study. Four treatments with combination of AM fungi were also included (Table 7).

Table 7: Details on AM fungal inocula used for nursery trials

Treatment No.	AM fungi	Pot culture No.
1	*G. botryoides*	78*
2	*G. fasciculatum*	177
3	*G. fasciculatum*	79
4	*G. fasciculatum*	24
5	*G. macrocarpum*	171
6	*G. mosseae*	97
7	*G. mosseae*	13
8	*A. appendiculata*	187
9	*A. appendicula*	21
10	*A. rehmii*	140
1	*A. scorbiculata*	25
12	*Giga spora candida*	40
13	*Gigaspora gigantea*	44
14	*S. heterogama*	28
15	*S. erythropa*	74

16	*A. appendi cula +G. fasciculatum*	187 + 24
17	*G.fasciculatum + G. mosseae*	169 + 97
18	*G. botryoides + G. fasciculatum*	78 + 18
19	*A. appendicula + G. mossease*	69 + 173
20	Control	-

* for details on pot cultures refer to Table 4

2.9.2. Preparation and application of AM inoculum

Pot cultures of respective AM fungi were mass multiplied using soil sand medium (1:1) and planted with maize seedlings. Maize seeds obtained from TNAU (Tamil Nadu Agricultural University, Coimbatore) were used for raising seedlings. The pot cultures were maintained in the glasshouse and after 60 days of growth of maize seedlings, they were cut just above the soil surface and the root zone was allowed to dry for about 20 days. The maize roots were cut into small bits and the soil-sand along with maize root bits was used as the inoculum. After 10 days of growth in root trainer cells, the teak seedlings were treated with various AM fungal inoculum. AM fungal inoculum prepared from the pot cultures of the respective Glomalean fungi was applied at the rate of 1g per seedling in the root zone of the teak seedlings. AM fungi were applied singly in 15 treatments; in two treatments, *Glomus* species and *Acaulospora* species were mixed, while in another two treatments, *Glomus* spp. were mixed. Control (untreated) seedlings were applied with sterilized soil-sand mixture. Five replicates of Styrofoam blocks containing a total of 120 seedlings were kept for each treatment.

2.9.3. Recording growth measurements

All the treated seedlings including the control sets were kept under shade nets (50% light) and watered three times per day regularly. After 30 days of growth under the shade nets, the seedlings in all the treatments were shifted to the hardening area without any shade regulation. Observations on seedling growth (height, leaf pairs) were recorded at 10 days interval, up to 60 days. Collar diameter of seedlings in all the treated and control sets was recorded using calipers; fifteen seedlings from each treatment were used for recording

seedling biomass. Root and shoot as well as whole seedling biomass were recorded separately.

2.9.4. Assessment of AM fungal root colonization in treated seedlings

Ten seedlings from each treatment were used for assessment of root colonization by AM fungi. The roots of inoculated and uninoculated (control) seedlings were collected after 90 days of growth and processed as mentioned earlier and stained with Trypan blue. (0.06%); the root bits were observed under a light microscope for the presence of arbuscules, vesicles, internal hyphae and spores. From each treatment, 100 root bits were observed and the percentage root colonization was calculated.

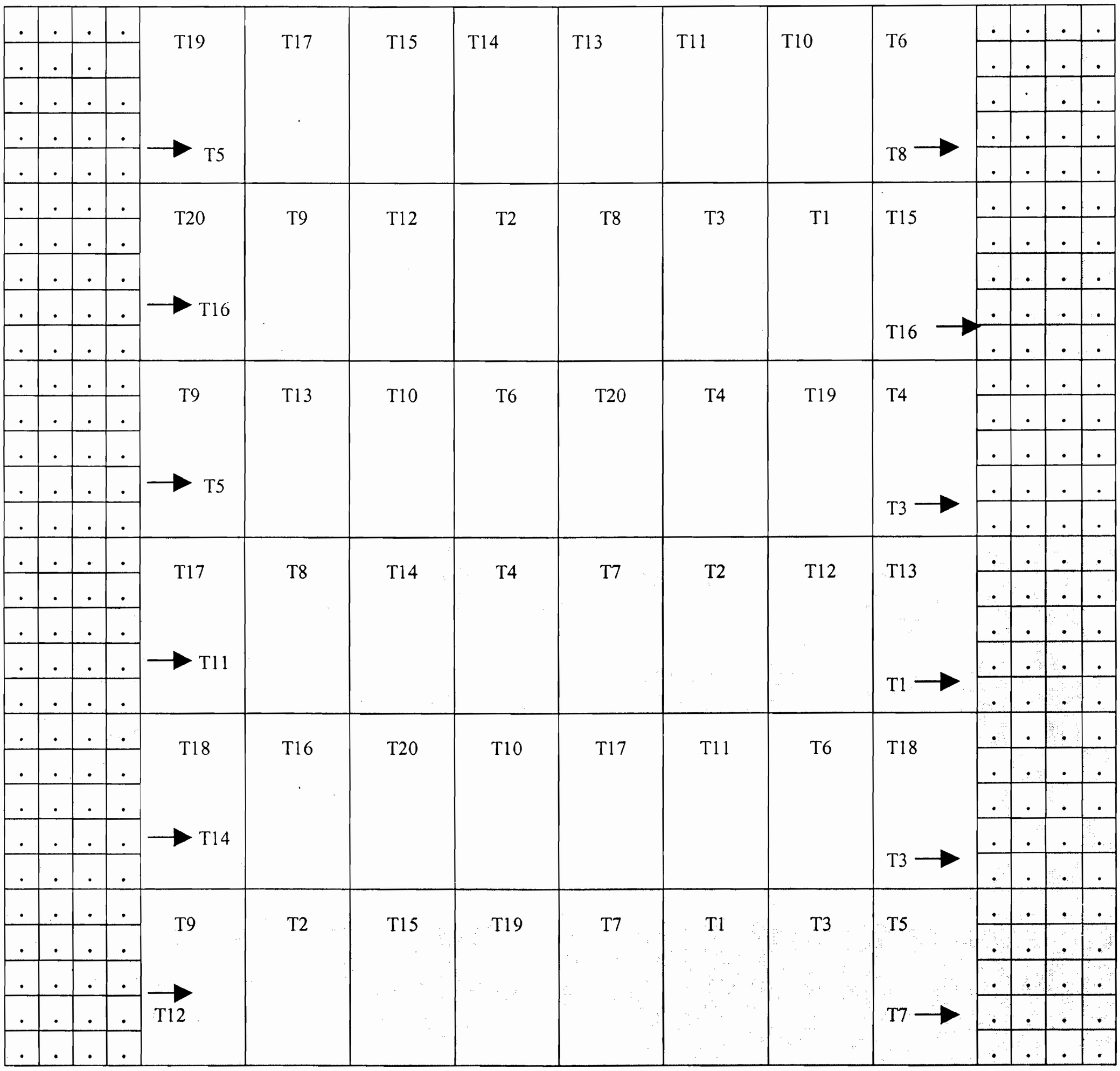
2.9.5. Selection and preparation of planting site

A planting site at Vembooram in 2003 clear-felled (First rotation) teak area in Kodanad Forest Range, Kerala was selected for the pilot-scale field trial. One hectare area was selected for the field trial and the area was cleared for planting; weeding and soil work were carried out. Trial plot was aligned and a total of 60 plots were marked with 20 stakes in each plot at 2.5 m spacement. Completely randomized block design was followed and for each treatment (total 20 treatments), 3 replications were provided (Fig.5). Soil pits of 30 x 30 x 30 cm were taken at 2.5 m spacement and reed bamboo splints were put at the center of each pit. Boundary of each plot was marked using paint marked poles. For each plot, paint-marked iron (T-shaped) labels were placed. Soil samples from three different locations in the selected area were collected and data on soil pH, soil moisture content and status of natural AM fungal flora in the soil were analysed.

2.9.6. Transportation of seedlings to the planting site

All the treated and control seedlings were transported to the planting site by truck. Before loading into the truck, the seedlings were treated (dipped in) with Bavistin (Carbendazim 0.1% a.i.) to protect against post-planting fungal infection in the field.

Fig. 5: Pilot-scale field trial with artificially mycorrhized teak seedlings - plot chart



Forest road

Locality: Vembooram, Kodanad Forest Range, Kerala State
Spacement: 2.5 x 2.5 m; Planting area: ca. I ha
Total number of treatments: 20; Replication : 3 ; Total number of seedlings used: 1200
Date of planting :4 June, 2004

N

Teak seedlings were carefully transported to the planting site with appropriate labeling of each seedlings using aluminium tags. Proper care was taken to avoid transportation shock and the transportation was carried out during early morning; the seedlings were unloaded from the truck and kept under shade overnight.

2.9.7. Planting of artificially mycorrhized and control seedlings

Planting of root trainer seedlings was carried out on 4th and 5th June 2004. A total of 1200 seedlings from the nursery trial were employed for pilot-scale planting. Sixty seedlings from each treatment were used and three replicate plots were kept. The seedlings were carefully removed from the root trainer cells and planted in the soil pit; soil was heaped around the plant to avoid water logging. After completing the planting operation, measurement on height, collar diameter of planted out seedlings were recorded.

2.9.8. Maintenance of trial plots

The trial plots were maintained properly and casualty replacement was made after ten days of planting with the seedlings from the respective treatment. Weeding (scrape weeding around the plant) was carried out after one month of planting. As insect (*Hyblaea puerea*) infestation was found severe in the area, inescticidal (Ekalux @ 0.1 % foliar spray) application was carried out. Another scrape weeding was carried out after 8 months of planting during February-March, 2005. The plots were protected from cattle damage.

2.9.9. Recording measurements and statistical analyses of data

Observations on plant height, collar diameter, leaf pairs, health and vigour of plants, defoliation, etc. were recorded from the trial plots after three months (August 2004) and nine months (March 2005) of planting. Plots were visited during June 2005, observations on growth performances were recorded and photographs of plants from different treatments taken. Data generated from the glasshouse, nursery and field trials were subjected to ANOVA and DMRT.

3. RESULTS AND DISCUSSION

3.1. AM fungal root colonization in teak in southern States

Young feeder root samples of teak collected from different eco-climatic zones in the Kerala, Karnataka and Andhra Pradesh States revealed that all the teak root samples, irrespective of variation in age of the plants, prevailing edaphic and climatic factors in the plantation area showed AM fungal colonization. AM fungal structures such as arbuscules (A), vesicles (V), intra-cellular hyphal coils (H), extra and intraradical hyphae (H) within the feeder roots were observed singly or collectively in most of the root samples studied. A very good percentage occurrence of intra-cellular hyphal coils/hyphae was recorded from most of the root samples studied. However, presence of intra-radical hyphae or hyphal coils (H) without vesicles / arbuscules alone was not considered for AM fungal colonization. The morphological diversity of fungal structures observed within the same root sample indicates that teak roots were colonized by several different AM fungal species.

In teak plantations in the Kerala State, the overall extent of AM fungal root colonization ranged from 7 to 59 per cent (Tables 8,9); the lowest AM fungal root colonization was recorded in root samples from 6-year-old teak plantation at Irumpupalam, Pattikkad Forest Range and highest root colonization of 59% was recorded in root samples from 20-year-old teak plantation at Karimpani, Thundathil Forest Range. Root samples from 4-year-old teak plantation at Dhoni, Olavakkod Forest Range also registered low percent (9%) AM fungal root colonization. Other samples yielded high AM fungal root colonization are from 22-year-old teak plantation at Mulamkuzhy, Malayattoor Forest Range, and 26-years-old teak plantation at Peruvara Bl.51, Parambikulam Forest Range (Fig. 6; Tables 8,9). The average AM fungal root colonization in teak plantations in Kerala registered was 33.17%

Teak root samples collected from 17 plantations in the Karnataka State showed a wide range of variation in AM fungal root colonization. Root samples from 18-year-old

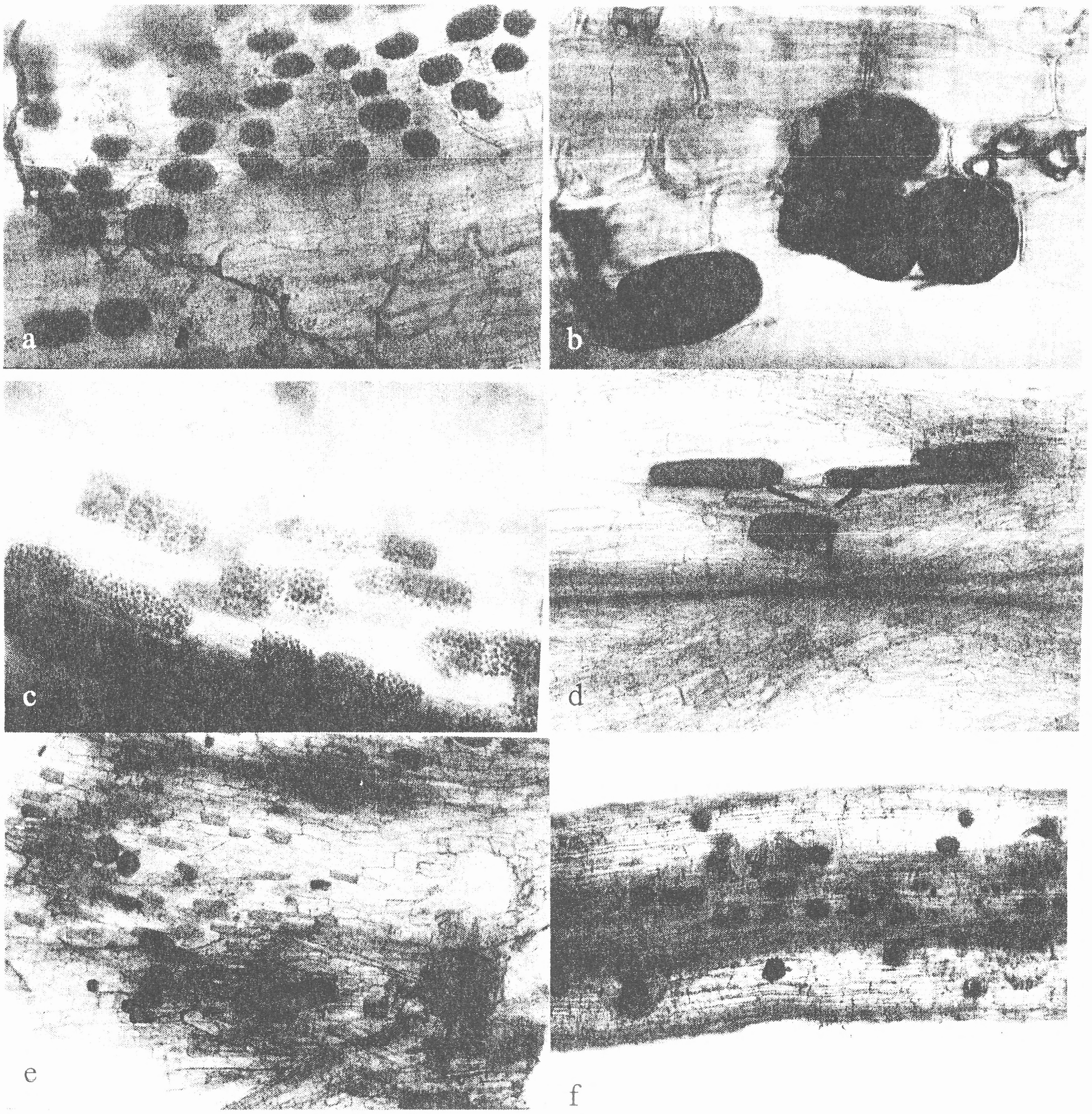


Fig. 6. AM fungal colonization in teak roots: a-f: vesicles, arbuscules and intraradical hyphae in infected teak roots.

plantation at Kodur, Hosnagar Forest Range showed the lowest AM fungal colonization of 1% among the root samples studied (Tables 10,11). Highest percent (43%) AM fungal root colonization was recorded in samples from 20-year-old plantation at Chaparke, Kundapura Forest Range. Samples from most of the plantations showed 20 to 30% AM fungal root colonization. Vesicles and arbuscules were comparatively less in the root samples than the intraradical hyphae. Even though, a very high percent of intraradical hyphae which ranged from 14 to 41% was recorded, this was not taken into account for AM fungal root colonization. Another interesting observation from the root samples collected from the Karnataka State was that presence of both vesicles and arbuscules in the same root sample was very less and it ranged from 0 to 5%. The mean AM fungal root colonization in teak plantations in the Karnataka State registered was 24.70% (Fig.7).

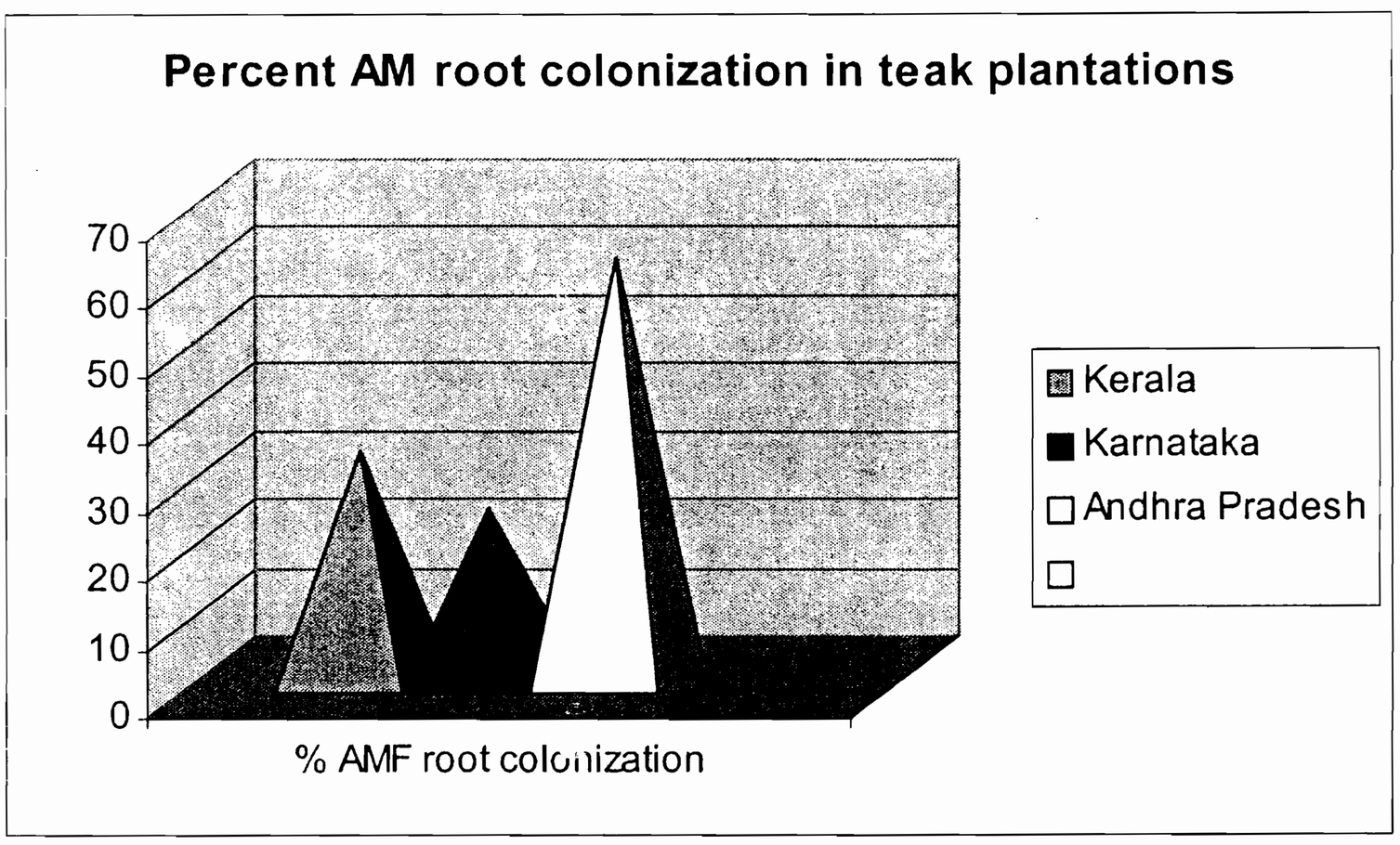


Fig. 7: Average AM fungal root colonization in teak plantations in three States

Comparatively a high percent AM fungal root colonization was recorded in teak plantations in different localities in the Andhra Pradesh State, which ranged from 52 to 74% (Tables 12,13). Mean AM fungal root colonization in teak plantations in Andhra Pradesh State registered was 61.42% (Fig.7). More than 70% AM fungal root colonization was recorded in root samples from 36-year-old teak plantation at Ebul, R.V. Nagar Forest Range, 32-year-old teak plantation at Dharakonda, Sileru Forest Range and

28-year-old plantation at Sileru, Sileru Forest Range. All the 14 samples from different localities in the State showed a high percent occurrence of vesicles in the root tissue; occurrence of both vesicles and arbuscules and arbuscules alone in the root samples was almost similar and that ranged from 10 to 24 % and 8 to 26% respectively. Intraradical hyphae ranged from 16 to 30% in the root samples, however, their presence was not considered for AM fungal colonization.

Table 8: Details on AM fungal root colonization, soil characteristics and AM fungal spore density in teak plants in different localities in the Kerala State

Sample No.	Locality	Forest Range	Soil pH	Soil MC (%)	AM root colonization (%)	AM spore count
DT1	Poolakkapara	Nilambur	5.71	3.30	37	135
DT2	Cherupuzha	Nilambur	5.82	6.58	40	67
DT3	Naduvathumuzhy	Konni	5.9	13.01	49	53
DT4	Malayattoor	Malayattoor	5.2	1.05	45	154
DT5	Kanimangalam	Malayattoor	5.3	4.68	39	71
DT6	Pandupara	Kalady	6.8	3.21	53	64
DT7	Mulamkuzhy	Malayattoor	4.7	9.26	58	52
DT8	Karimpani	Thundathil	5.5	4.96	59	128
DT9	Bhoothathankettu	Thundathil	5.9	1.84	53	61
DT10	Chakolathara	Pattikkad	5.9	11	33	130
DT11	Irumpupalam	Pattikkad	5.91	16.8	7	276
DT12	Dhoni	Olavakkod	6.52	8	9	111
DT13	Dhoni Qts	Olavakkod	6.70	8.40	26	76
DT14	Peruva	Kannavam	6.05	6.8	13	50
DT15	Mananthavady	Begur	5.90	15.2	13	106
DT16	Kattikulam	Begur	5.72	13	28	37
DT17	Tholpetty	Tholpetty	5.97	11.2	17	37
DT18	Kulathupuzha	Kulathupuzha	5.58	6.14	22	52
DT19	Arienkavu	Arienkavu	5.13	8.58	16	68
DT20	Achenkoil	Achenkoil	6.28	15.95	17	28
DT21	Achenkoil TP	Achenkoil	6.05	6.06	28	75
DT22	Achenkoil Thura	Achenkoil	5.72	11.60	32	116
DT23	Sungam	Sungam	6.83	15.67	31	61
DT24	Kannimara	Parambikulam	6.94	7.69	42	49
DT25	Peruvara Bl-51	Parambikulam	6.76	8.71	58	62
DT26	Poopara	Karimala	6.10	9.13	39	28
DT27	Karimala	Karimala	5.98	12.73	45	32
DT28	Parappa	Kasargode	5.89	3.20	20	86

Table 9: Teak root colonization by AM fungi in root samples collected from different localities in the Kerala State

Sample No.	No. of root bits examined	Vesicles (V)	Arbuscules (A)	V & A	Hyphae (H) only	Without V, A & H	% root colonization
DT1	100	15	9	13	31	32	37
DT2	100	10	18	12	18	42	40
DT3	100	22	13	14	18	33	49
DT4	100	7	27	11	34	21	45
DT5	100	5	29	5	30	31	39
DT6	100	18	9	26	25	20	53
DT7	100	28	10	21	16	25	59
DT8	100	26	9	24	23	18	59
DT9	100	29	12	12	24	23	53
DT10	100	11	13	9	25	42	33
DT11	100	6	1	0	6	87	7
DT12	100	1	8	0	35	56	9
DT13	100	3	17	6	33	41	26
DT14	100	9	4	0	20	67	13
DT15	100	7	2	4	15	72	13
DT16	100	12	10	6	25	47	28
DT17	100	7	8	2	30	53	17
DT18	100	10	8	4	26	52	22
DT19	100	12	3	1	26	58	16
DT20	100	6	11	0	44	39	17
DT21	100	9	12	7	31	28	28
DT22	100	8	18	6	23	45	32
DT23	100	11	15	5	36	33	31
DT24	100	27	7	8	22	36	42
DT25	100	23	20	15	22	20	58
DT26	100	20	12	7	26	35	39
DT27	100	9	30	6	29	26	45
DT28	100	3	4	13	32	48	20

Table 10: Details on AM fungal root colonization in teak plants, soil characteristics and AM spore density in different localities in the Karnataka State

Sl. No	Sample No.	Locality	Forest Range	Soil pH	Spore count (in 10g soil)	Root colonisation %	Moisture Content %
1	DT 29	Aletty West	Sullia	6.45	102	20	6.75
2	DT 30	Kolchar (Aletty East)	Sullia	6.30	168	31	3.44
3	DT 31	Medinadka West	Sullia	6.30	50	36	12.7
4	DT 32	Mendekol	Sullia	6.06	112	33	8.99
5	DT 33	Thodikana	Sampage section	5.82	110	27	6.63
6	DT 34	Sampage	Sampage section	5.73	127	25	4.66

7	DT 35	Chaparke	Kundupara	5.68	174	43	7.89
8	DT 36	Kanchar	Ampar	5.78	178	36	4.31
9	DT 37	Heigodemale	Ampar	5.69	107	32	9.58
10	DT 38	Kollur	Kollur	5.89	114	13	3.92
11	DT 39	Kodur	Hosnagar	6.25	193	1	4.77
12	DT 40	Hirejene	Hosnagar	5.68	124	20	9.34
13	DT 41	Arsalu	Shankar	6.15	86	13	0.6
14	DT 42	Gajanur	Shakravelu	6.49	57	18	2.56
15	DT 43	Mundagadde	Mundagadde	5.78	736	26	1.55
16	DT 44	Balehonnur	Balehonnur	6.18	62	23	5.07
17	DT 45	Belur	Belur	6.24	64	16	4.42

Table 11: Teak root colonization by AM fungi in root samples collected from different localities in the Karnataka State

Sample No.	No. of root bits examined	Vesicles (V)	Arbuscules (A)	V & A	Hyphae (H)only	Without V, A & H	% root colonization
DT29	100	14	16	1	26	43	31
DT30	100	19	15	2	18	46	36
DT31	100	15	13	5	37	30	33
DT32	100	10	10	0	24	56	20
DT33	100	14	13	0	32	41	27
DT34	100	7	16	2	29	46	25
DT35	100	4	35	4	30	27	43
DT36	100	6	27	3	31	33	36
DT37	100	4	25	3	36	32	32
DT38	100	3	10	0	18	69	13
DT39	100	1	0	0	22	77	1
DT40	100	4	16	0	25	55	20
DT141	100	3	10	0	41	46	13
DT42	100	7	11	0	22	60	18
DT43	100	8	18	0	29	45	26
DT44	100	11	12	0	14	53	23
DT45	100	5	11	0	27	57	16

Table 12: Details on AM fungal spore density, soil moisture content, pH and root colonization in samples collected from different teak plantations in the Andhra Pradesh State

Sl. No	Sample No.	Locality	Forest Range	Soil pH	Mean spore count (in 10g soil)	Root colonisation %	Moisture Content %
1	DT 46	Narsipatnam	Narsipatnam	5.50	62	54	0.86
2	DT 47	Krishnapuram	Chinthapally	5.69	85	62	0.65
3	DT 48	Lothugedda	Lothugedda	5.71	396	48	1.13
4	DT 49	Wangasara	Chinthapally	5.72	134	52	0.96
5	DT 50	Wangasara SPA	Chinthapally	5.83	224	64	0.87
6	DT 51	Rangthada SPA	Chinthapally	5.73	320	62	1.30
7	DT 52	Mullametta	Chinthapally	5.65	237	66	0.98
8	DT 53	Ebul SPA	RV Nagar	5.80	165	72	0.83

9	DT 54	Chethalapadu SPA	Chinthapally	6.03	159	64	0.96
10	DT 55	Valasagedda	Sileru	5.95	233	58	1.12
11	DT 56	Bachlooru	Maredemella	5.82	449	52	1.28
12	DT 57	Dharakonda	Sileru	5.99	346	74	1.36
13	DT 58	Sileru	Sileru	5.75	277	70	1.11
14	DT 59	Duppiawada	Sileru	5.69	251	62	1.32

Table 13: Teak root colonization by AM fungi in root samples collected from different localities in Andhra Pradesh

Sl.No.	No.of root bits examined	Vesicles (V)	Arbuscules (A)	V&A	Hyphae	Uninfected	% colonization
1	100	26	14	14	22	46	54
2	100	24	18	20	28	38	62
3	100	30	10	8	30	52	48
4	100	30	14	8	24	48	52
5	100	32	18	14	20	36	64
6	100	26	18	18	18	38	62
7	100	38	12	16	24	34	66
8	100	32	10	24	20	28	72
9	100	32	24	8	30	36	64
10	100	26	14	18	16	42	58
11	100	30	7	15	26	48	52
12	100	36	12	26	22	26	74
13	100	32	16	22	22	30	70
14	100	28	14	20	16	38	62

AM fungal root colonization is influenced by various factors including the age of the plants. Formation of vesicles and arbuscules inside the root tissue is governed by host and pathogen factors, especially nutrient status of the host tissues. Edaphic factors, especially soil pH, soil nutrient status and soil moisture content also affect the formation of arbuscules and vesicles. Earlier, an exhaustive study on AM fungal root colonization pattern in teak stands in the Kerala State (Mohanan, 2002, 2003) showed that 1-year-old plantation as well as > 90-year-old plantations exhibited AM fungal root colonization. Plantations at establishing phase (1 to 2-year-old) showed comparatively low percent AM fungal root colonization than 10 to 20-year-old plantations. Presence of extensive intraradical hyphae and hyphal coils in the root tissue was also reported in root samples from very young plantations (1 to 2-year-old), which indicates the need to consider these structures also for accounting AM fungal root colonization (Mohanan and Sheeba, 2005).

Soil pH in teak plantations in all the three States was found moderately acidic to highly acidic. Near neutral soils were also detected in plantations in Olavakkode and Parambikulam Forest Ranges in the Kerala State and in plantations in Shakravelu and Sullia Forest Ranges in the Karnataka State (Tables 8,10, 12). Teak thrives better in acidic soils, but good growth has also been recorded in near neutral to basic soils. AM fungal root infection in many forestry crops including teak has been reported to be influenced by soil physical and chemical characteristics (Sugavanam *et al.*, 1998; Mohanan and Sheeba, 2005). Among the various edaphic factors, soil pH was recorded as accounting for 35% of the total variability in AM fungal root colonization in teak in the Kerala State (Mohanan, 2003).

Soil moisture content in teak plantations varied from locality to locality in all the three States. Soil samples from 28 teak plantations in the Kerala State, all except, samples from Malayattoor, Malyattoor Forest Range and Bhoothathankettu, Thundathil Forest Range, showed very high soil moisture content; soil from plantations at Mananthavady, Begur Forest Range, Achencoil, Achencoil Forest Range, and Sungam in Sungam Forest Range showed > 15% MC (Table 8). Soil samples from the Karnataka State also showed high moisture content, except in plantations at Arsalu, Shankar Forest Range, and at Mundagadde, Mundagedde Forest Range, where the soil moisture content was 0.6% and 1.55% respectively (Table 10). Soils from all the 14 teak plantations in the Andhra Pradesh State were dry and moisture content was comparatively very low than the soil samples from the other two States; in these samples, soil moisture content ranged from 0.65% to 1.36% (Table 12). Soil moisture content depends on the locality, period of collection, whether during the rainy period or dry period and also the soil physical properties including water holding capacity. Prevailing moisture content of the soil in the root zone has been reported to be influencing the AM fungal root colonization to a great extent. In the present study, a correlation could be noticed in the frequency of occurrence of high percent of vesicles and arbuscules in the teak roots and soil with very low soil moisture content, especially the soil samples collected from the Andhra Pradesh State (Table 12).

3.2. AM fungal spores in teak rhizosphere soils in southern States

For retrieving the AM fungal spores from the soil samples, wet sieving-decanting as well as wet-sieving centrifugation techniques were employed. Both the techniques were equally effective in retrieving the AM fungal spores, however, fungal spores obtained by wet-sieving centrifugation method often lose hyphal attachment to the spores which are crucial for taxonomic investigation. The AM fungal spores obtained from different soil samples from the Kerala States ranged from 28 to 276/10 g soil, while much lower spore density was recorded in soil samples from the Karnataka State, which ranged from 50 to 193/10 g soil (Tables 8,10). AM fungal spore density was much higher in soil samples from the Andhra Pradesh State which ranged from 62 to 449 spores/10 g soil than the soil samples from other two States (Table 12). The lowest AM fungal spore count was recorded in soil samples collected from a 3-year-old plantation raised from tissue cultured teak plants (Table 12).

Production of asexual spores of AM fungi depends on the intrinsic characteristics of the AM fungal species, and influenced by the physical and chemical characteristics of the soil and also the host plant. Most AM fungi produce spores in large numbers, while a few species produce a limited number of spores in the substratum. Also, the available technology employed to assess the spores in the soil samples may be inefficient to record all the available spores. Hence, there is a limitation in assessing the spore density of AM fungi in soil and requires periodic assessment to get a clear picture on the AM fungal population dynamics. However, total spore density and species-wise frequency were taken into consideration to assign the most predominant fungal species in the population.

From the teak rhizosphere soils collected from different parts of Kerala, Karnataka and Andhra Pradesh States, a total of 73 species of Glomalean fungi belonging to six genera, viz., *Glomus, Gigaspora, Acaulospora, Scutellospora, Entrophospora* and *Sclerocystis* were encountered. Among these, *Glomus* species were the most predominant and widely distributed AM fungi in the rhizosphere soil of the teak plantations in all the three States and a total of 35 species were recorded (Table 14; Fig 8). *Acaulospora* (12

species) and *Scutellospora*(10 species) were the other genera most commonly found in teak rhizosphere soils (Table 15; Figs. 9,10). *Entrophospora* species have a very limited distribution in the teak rhizosphere soils in all the three States.

Table 14: Distribution of AM fungal species in teak plantations

Sl. No.	AM fungal genus	No. of species recorded	Remarks
1	*Glomus*	36	Widely distributed
2	*Acaulospora*	12	"
3	*Scutellospora*	10	Limited distribution
4	*Gigaspora*	6	"
5	*Sclerocystis*	7	Limited distribution
6	*Entrophospora*	2	Very limited distribution
	Total	73	

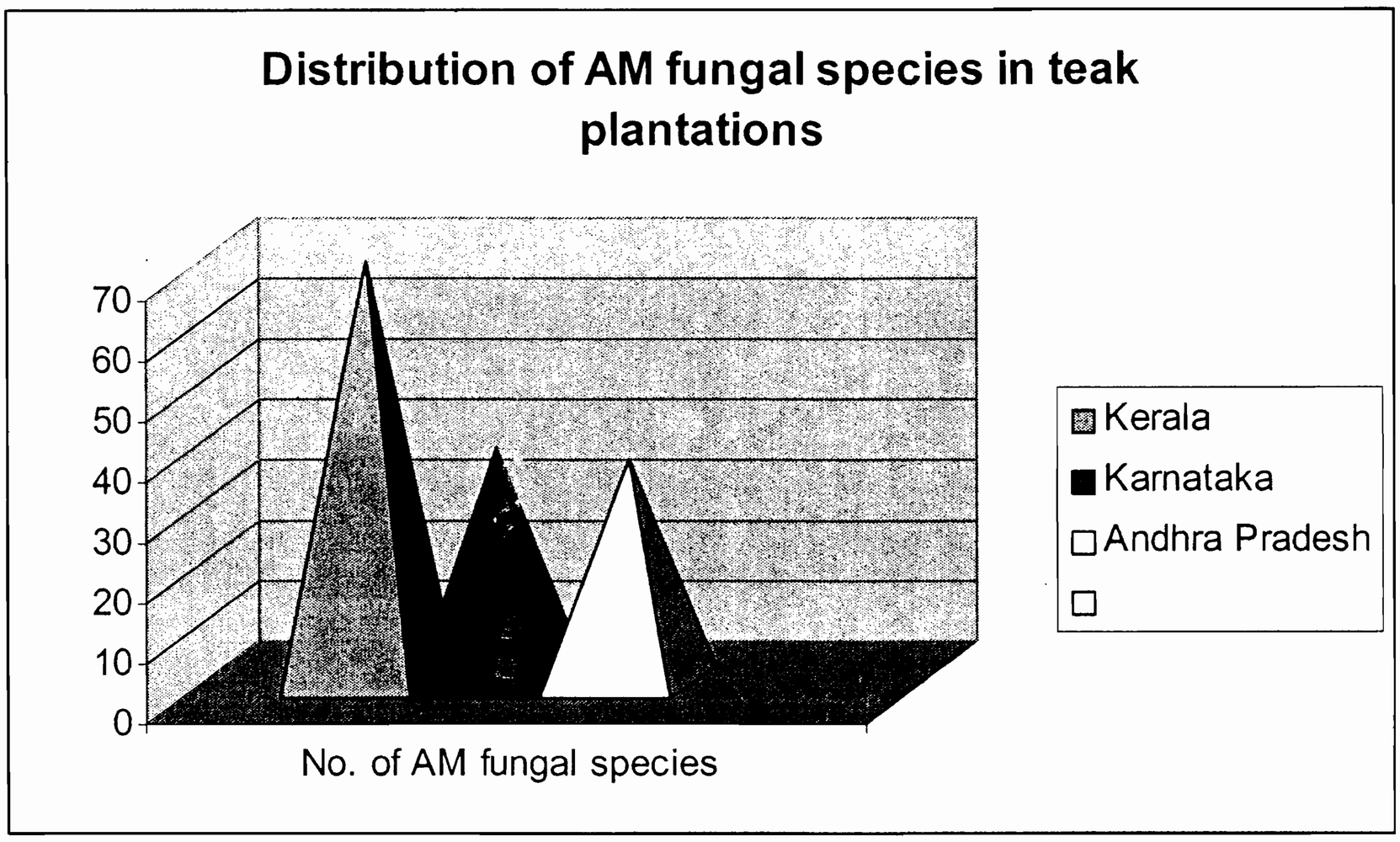


Fig.8: Distribution of AM fungal species in teak plantations in three States

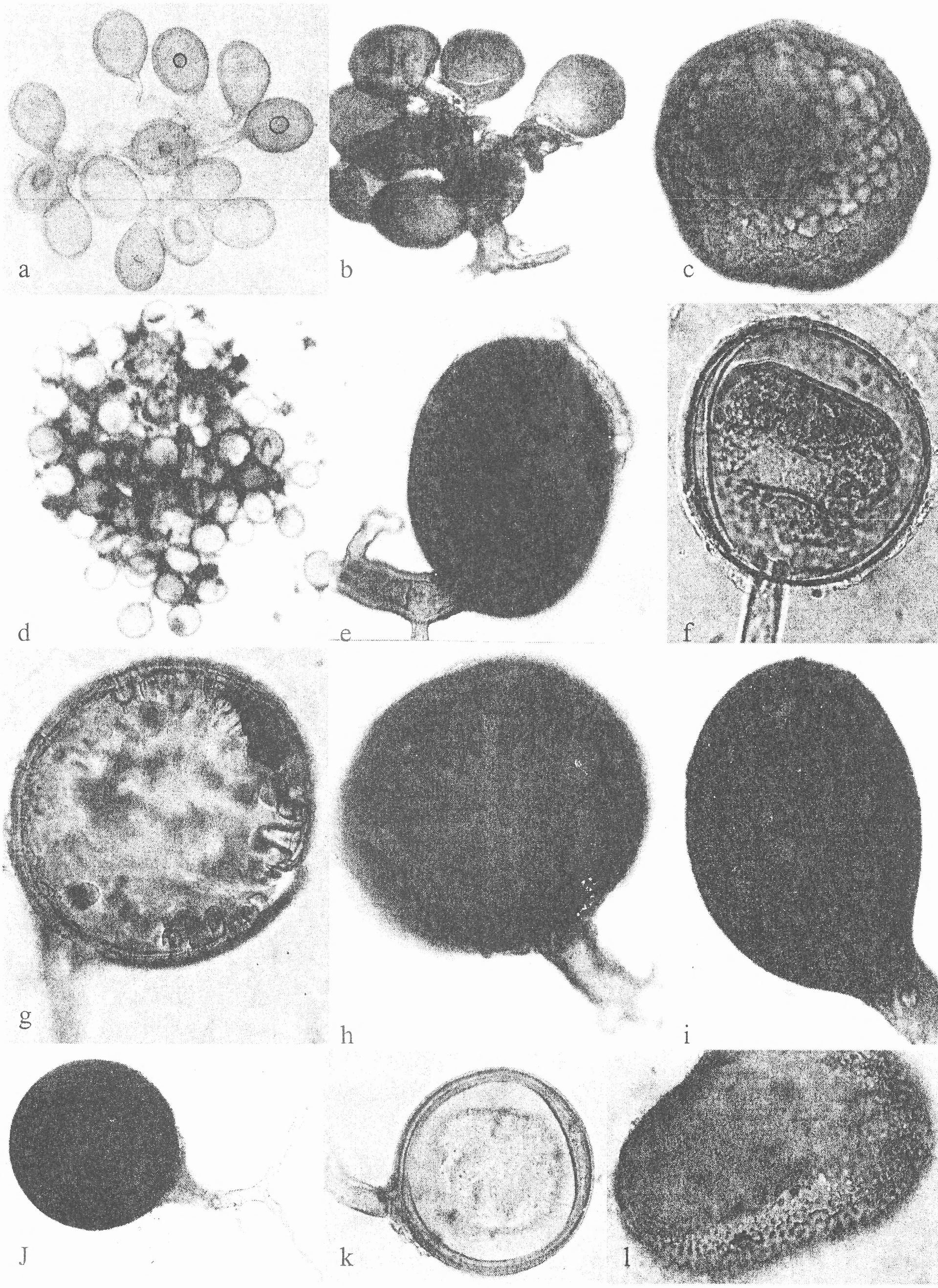


Fig.9: AM fungal spores: a:*Glomus fasciculatum*, b: *G.botryoides*,c: *G. reticulatum*, d: *G. aggregatum*, e: *G.multicaule,* f: *G. intraradices*, g: *G. maculosum*, h: *G. australe*, i: *G. mosseae*, j: *G. geosporum*, k: *G. intraradices* , l: *Acaulospora bireticulata.*

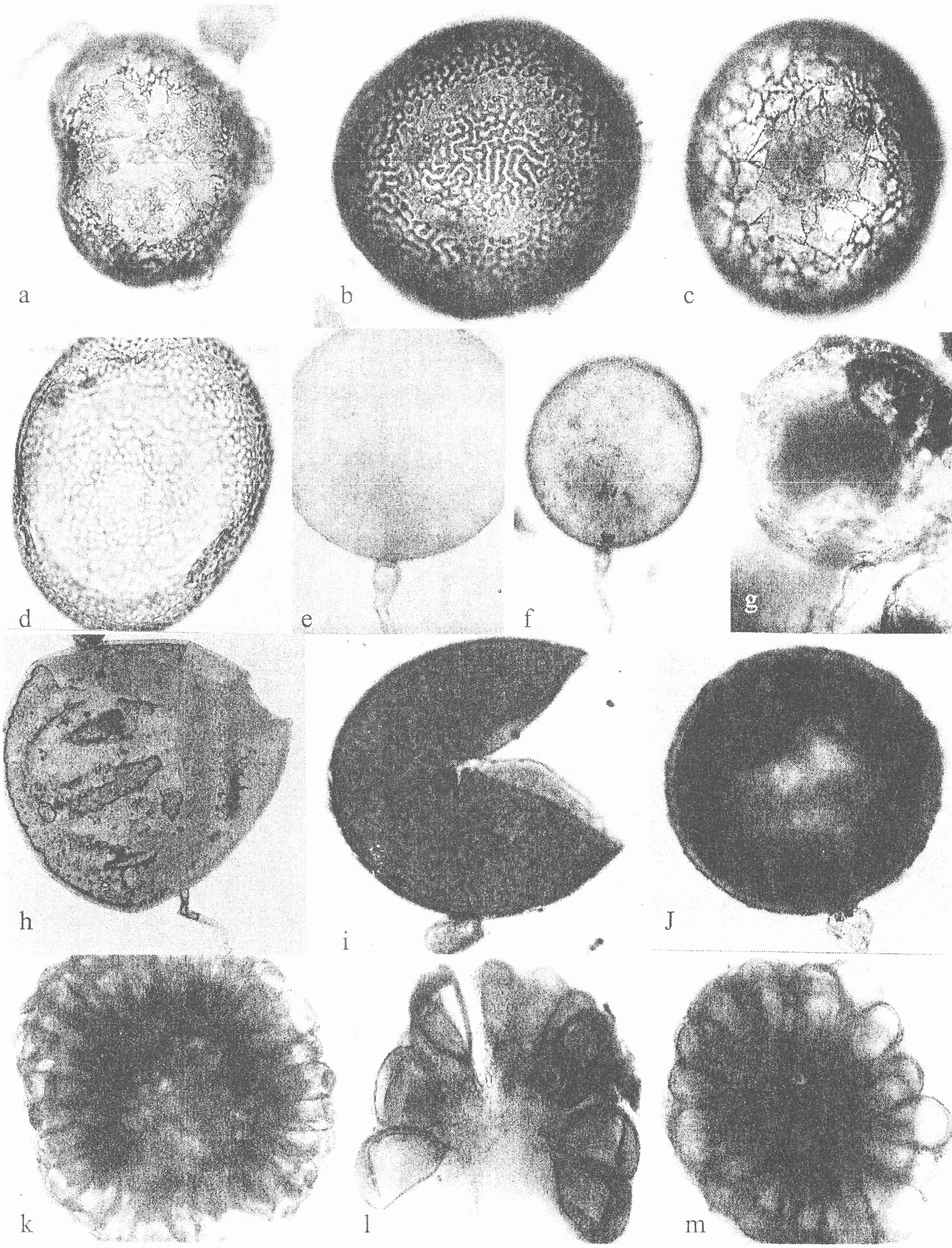


Fig.10: AM fungal spores: a: *Glomus tortuosum*, b: *A. rehmii*, c: *A.appendicula*, d: *A.scorbiculata*, e,f: *Gigaspora gigantea*,g: *G.rosea*, h: *Scutellospora gregaria*, I: *S. persica*, j: *S. nigra*,, k: *Sclerocystis clavispora*, l: *S.microcarpus* ,m: *S. dussii* .

Among the *Glomus* species, *G. fasciculatum, G. mosseae, G. macrocarpum, G. deserticola, G. botryoides* are the most widely distributed ones. In all the three States, *Acaulospora* and *Scutellospora* were the other genera most commonly encountered in teak rhizosphere soils. Among *Acaulospora, A. appendicula, A. rehmii* and *A. scorbiculata* were the most frequently encountered species. Among *Scutellospora, S. heterogama* and *S. erythropa* are the most widely distributed species in teak soils. Among *Gigaspora, G. gigantia, G. candida* and *G. albida* were the most predominant species. A total of 70 AM fungal species belonging to six genera were recorded from the teak rhizosphere soils from the Kerala State, while this figure was 39 and 37 in soil samples from the Karnataka and Andhra Pradesh States respectively. Glomalean fungal community in teak rhizosphere soils comprised of 10 to 24 AM fungal species and the species composition as well as their distribution varied depending on the host and edaphic factors.

Table 15: AM fungi recorded from the soil samples from teak plantations in the Kerala, Karnataka and Andhra Pradesh States

Sl. No.	AM fungi	Kerala	Karnataka	Andhra Pradesh
1	*Glomus aggregatum* Schenck & Smith	+	+	+
2	*Glomus albidum* Walker & Rhodes	+	-	+
3	*Glomus australe* (berk.) Berch	+	+	-
4	*Glomus botryoides* Rothwell & Victor	+	+	+
5	*Glomus citricolum* Tang & Zang	+	+	-
6	*Glomus caledonium* (Nicol. & Gerd.) Trappe	+	-	+
7	*Glomus canadebnse* Thaxter	+	-	+
8	*Glomus clarum* Nicholson & Schenck	+	+	-
9	*Glomus claroideum* Schenck & Smith	+	-	-
10	*Glomus constrictum* Trappe	+	-	-
11	*Glomus convolutum* Gerd. & Trappe	+	-	+
12	*Glomus deserticola* Trappe, bloss & Menge	+	+	+
13	*Glomus delhiense* Mukerji et al.	+	+	+
14	*G.etunicatum*	+	-	+
15	*Glomus fasciculatum* Gerd. & Trappe	+	+	+
16	*Glomus fulvum* (Berk. & Broome) Trappe & Gerd.	+	-	-
17	*Glomus geosporum* (Nicol. & Gerd.) Walker	+	+	+
18	*Glomus globiferum* Koske & Walker	+	-	+
19	*Glomus glomerulatum* Sieverding	+	+	-
20	*Glomus intraradices* Schenck & Smith	+	+	-
21	*Glomus invermaium* Hall	+	-	-
22	*Glomus lacteum* Rose & Trappe	+	+	-

23	*Glomus macrocarpum* Tul. & Tul.	+	+	+
24	*Glomus maculosum* Miller & Walker	+	+	-
25	*Glomus melanosporum* Gerd. & Trappe	+	+	-
26	*Glomus microaggregatum* Koske et al.	+	-	+
27	*Glomus microcarpum* Gerd. & Trappe	+	-	-
28	*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe	+	+	+
29	*Glomus multicaule* Gerd. & Bakshi	+	+	+
30	*Glomus multisubtensum* Mukerji et al.	+	-	-
31	*Glomus pulvinatum* (P. Henn.) Trappe & Gerd.	-	-	+
32	*Glomus radiatum* (Thaxter) Trappe & Gerd.	+	+	-
33	*Glomus reticulatum* Bhattacharjee & Mukerji	+	+	-
34	*Glomus tenuis* (Greenall) Hall	+	+	-
35	*Glomus tortuosum* Schenck & Smith	+	-	-
36	*Glomus vesiculiferum* (Thaxter) Gerd. & Trappe	+	-	-
37	*Gigaspora albida* Schenck & Smith	+	-	+
38	*Gigaspora candida* Bhattacharjee et al.	+	-	+
39	*Gigaspora decipiens* Hall & Abbott	+	+	+
40	*Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe	+	+	-
41	*Gigaspora margarita* Becker & Hall	+	-	+
42	*Gigaspora rosea* Nicol. & Schenck	+	+	-
43	*Acaulospora appendicula* Spain et al.	+	+	+
44	*Acaulospora bireticulata* Rothwell & Trappe	+	+	-
45	*Acaulospora delicata* Walker et al.	+	-	-
46	*Acaulospora denticulata* Sieverding & Toro	+	-	+
47	*Acaulospora elegans* Trappe & Gerd.	+	-	-
48	*Acaulospora foveata* Trappe & Janos	+	+	-
49	*Acaulospora morrowae* Spain & Schenck	+	-	+
50	*Acaulospora spinisa* Walker & Trappe	+	+	-
51	*Acaulospora tuberculata* Janos & Trappe	+	+	-
52	*Acaulospora rugosa* Morton	+	+	-
53	*Acaulospora rehmii* Sieverding & Toro	+	+	+
54	*Acaulospora scorbiculata* Trappe	+	+	+
55	*Scutellospora alborosea* (Ferr.&Herr.) Walker & Sanders	+	-	-
56	*Scutellospora aurigloba* (Hall) Walker & Sanders	+	-	+
57	*Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders	-	-	+
58	*Scutellospora erythropa* Walker & Sanders	+	+	+
59	*Scutellospora gilmorei* (Trappe & Gerd.) Walker & Sanders	-	-	+
60	*Scutellospora gregaria* (Sch. & Nicol.) Walker & Sanders	+	-	+
61	*Scutellospora heterogama* (Nicol.&Gerd.) Walker & Sanders	+	+	+
62	*Scutellospora nigra* (Redhead) Walker & Sanders	+	+	+
63	*Scutellospora persica* (Koske & Walk.) Walker& Sanders	+	+	+
64	*Scutellospora reticulata* (Koske et al.) Walker & Sanders	+	-	-
65	*Entrophospora colombiana* Spain & Schenck	+	-	+
66	*Entrophospora infrequens* (Hall) Ames & Schneider	-	+	-
67	*Sclerocystis clavispora* Trappe	+	+	+
68	*Sclerocystis coremioides* Berk.& Broome	+	-	-
69	*Sclerocystis dussii* (Pat.) von Hohn.	+	-	-
70	*Sclerocystis microcarpus* Iqbal & Bushra	+	+	+
71	*Sclerocystis pachycaulis* Wu & Chen	+	-	-
72	*Sclerocystis rubiformis* Gerd. & Trappe	+	-	-
73	*Sclerocystis sinuosa* Gerd. & Bakshi	+	+	+

Seasonal variation in AM fungal spores in various crop plants has been reported by various workers. In teak, rhizosphere soils collected from different localities in the Maharashtra State, Chandra and Jamaluddin (1999) reported maximum AM fungal spore counts during the summer months and least in spring and winter. In an earlier study, maximum number of AM fungal spores as well as species composition in AM fungal community have been reported in soil samples collected during the dry period than the wet period from the teak plantations in the Kerala State (Mohanan, 2003). From the teak stands in Assam, a large number of AM fungi have earlier been reported from the rhizosphere soils (Talukdar and Thakuria, 2001). From Tamil Nadu, Raman *et al.* (1997) have recorded 14 species of AM fungi belonging to *Glomus, Gigaspora* and *Sclerocystis* in teak rhizosphere soil. From the teak rhizosphere soils from 20 different localities in the Madhya Pradesh State 15 AM fungi belonging to *Glomus, Gigaspora, Sclerocystis* and *Acaulospora* have been reported and among these *Glomus etunicatum* and *Acaulospora scorbiculata* were the most widely encountered ones (Verma and Jamaluddin, 1995). However, in the present study, *Glomus etunicatum* was recorded only in soil samples from the Kerala State and Andhra Pradesh State and their frequency of occurrence was very less compared to other Glomalean species.

It is evident from the present study that among the various species of *Glomus* recorded from the three States, *Glomus botryoides, G. fasciculatum, G. macrocarpum, G. mosseae, G. deserticola* and *G. multicaule* are the most widely distributed AM fungi in teak rhizosphere soils. Of the several species of *Acaulospora* recorded in the present study, *A. appendicula, A. rehmii* and *A. scorbiculata* are the most widely distributed as well as associated AM fungi with teak plants. *Gigaspora decipiens, G. rosea* and *Scutellospra erythropa, S. heterogama, S. persica, S. nigra* are the other most widely distributed AM fungi in the teak rhizosphere soils. Among *Sclerocystis, S. clavispora, S. macrocarpum,* and *S. sinuosa* were the commonly encountered ones from teak rhizosphere soils in all the three States, however, their frequency of distribution is very low.

3.3. Nutrient status of teak rhizosphere soils

Rhizosphere soil samples from various teak plantations in the Kerala State were analyzed for their chemical characteristics like exchangeable cations Na, Mg, Ca, total Nitrogen (N), Phosphorus (P) and organic carbon. The result showed that organic carbon ranged from 1.48 % to 5.88 % with a mean of 2.667%. In most of the soil samples, the ratio of OC% to N% was found about 10:1 ratio indicating the nutrient richness of the soil. Exchangeable cations, Na, Mg, Ca, and K also showed high variation in the soil samples. Sodium (Na) ranged from 0.056 meq/100g to 0.98 meq/100g; potassium (K) ranged from 0.36 meq /100g to 0.108 meq/100g ; calcium (Ca) ranged from 0.166 meq/100g to 2.095 meq/100g ; magnesium (Mg) ranged from 0.042 meq/100g to 2.095 meq/100g . Total nitrogen (N) and phosphorus (P) ranged from 0.178% to 0.629% and 0.052% and 0.153% respectively (Table 16).

Table 16: Details on exchangeable cations, organic carbon, total N and P in soil samples from teak plantations in different localities in Kerala

Sample No.	Plantation locality	OC%	Na meq/ 100g	K meq/ 100g	Ca meq/ 100 g	Mg meq/ 100g	N%	P%
DT1	Poolakkapara	1.591	0.064	0.036	0.867	0.111	0.198	0.088
DT2	Cherupuzha	1.48	0.064	0.081	1.355	0.208	0.179	0.097
Dt3	Naduvathumuzhy	2.81	0.086	0.108	0.166	0.075	0.372	0.078
DT7	Mulamkuzhy	5.88	0.056	0.056	0.722	0.112	0.516	0.112
DT8	Karimpani	3.00	0.075	0.063	0.417	0.09	0.629	0.119
DT9	Bhoothathankettu	2.395	0.062	0.044	0.286	0.042	0.285	0.153
DT10	Chakolatharisu	2.349	0.082	0.093	1.631	0.262	0.339	0.145
DT11	Irumpupalam	2.55	0.067	0.068	0.661	0.114	0.292	0.124
DT12	Dhoni	2.046	0.084	0.095	2.095	2.095	0.232	0.101
DT17	Tholpetty	3.562	0.07	0.047	1.823	0.259	0.182	0.052
DT18	Kulathupuzha	1.683	0.098	0.062	0.372	0.134	0.178	0.073

The available percentage of phosphorus was also found in good percentage in most of the soil samples analysed. However, as most of the soils were moderately to highly acidic, the nutrient availability as well as mobility depend on interrelationships among the various chemical and physical factors of the soils. Under natural conditions, it is believed

that AM fungi play a major role in plant nutrient uptake and also stress tolerance mechanism. AM fungi increase the volume of soil explored by the plant roots (Bolan, 1991) by their network of hyphae. Root colonization by AM fungi often results in enhanced uptake of relatively immobile micro-nutrients, especially phosphorus (Faber *et al.*, 1990; Kothari *et al.*, 1990; Li *et al.*, 1991).

As mentioned earlier, among soil nutrients, availability of phosphorus in particular has been shown to play a major role in plant/microbial relations (Mosse, 1973; Hayman, 1983). Low phosphorus availability has been repeatedly shown to encourage AM fungal colonization, which in turn improves plant phosphorus nutrition (Daft and Nicolson, 1969; Hayman and Mosse, 1971).

3.4. Mycorrhization of teak seedlings with AM fungi – Glasshouse trial

AM fungal inoculation experiments were conducted in the glasshouse and nursery. As preliminary trial using root trainers of 150 cc cell capacity and soil-sand (1:1 ratio) growing medium gave uniform growth of teak seedlings, styroform blocks (root trainers) with 24 cells were used for the glasshouse trial (Figs. 11,12).

The seedling growth data collected from the trails showed highly significant differences in seedling height among the AM fungi inoculated teak seedlings and control seedlings (Tables 17,18). G*lomus fasciculatum* treated seedlings (Treatment No. 11 and 12) showed maximum mean seedling height of 15.36 cm and 16.14 cm respectively in 90 days (Table 19). Treatment Nos. 6 (*Acaulospora rehmii*) and 15 (*Glomus botryoides*) exhibited almost similar mean seedling height and fall in the homogeneous group in DMRT. Treatment No. 9 (*Glomus deserticola*), 3 (*Glomus mosseae*) and Treatment No.13 (*Acaulospora scorbiculata*) were the other best treatments which gave >14.38 cm of mean seedling height. While seedlings in control sets recorded mean height of 12.18 cm. Treatment No. 7 (*A. appendicula*) recorded mean height of 13.47 cm and was found different from other AM fungal treatments (Treatment Nos. 23 and 28).

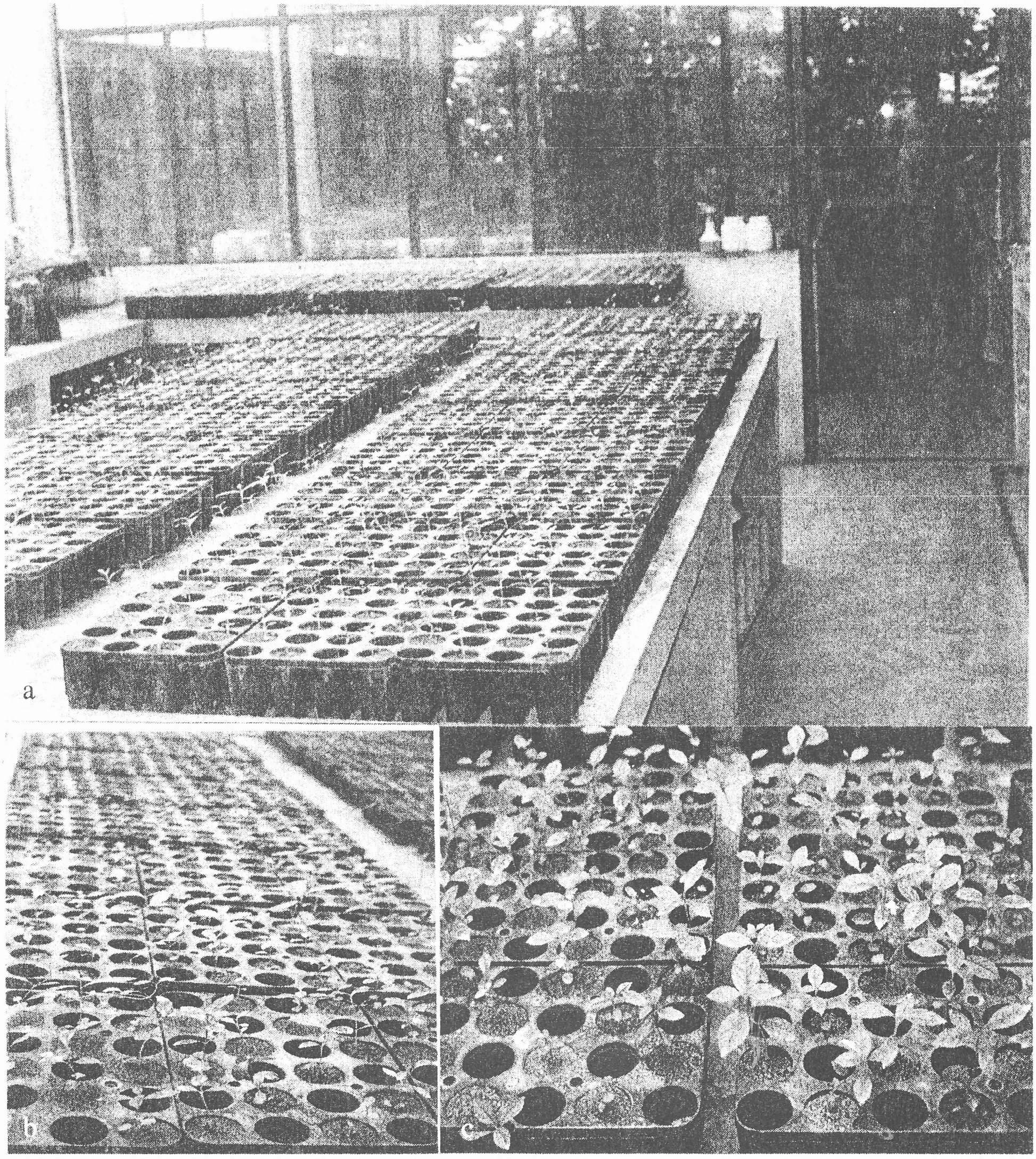


Fig.11: a-c: AM fungal inoculation of teak seedlings and screening of efficient AM fungi - glasshouse trial, c: 15-day old teak seedlings in soil-sand medium.

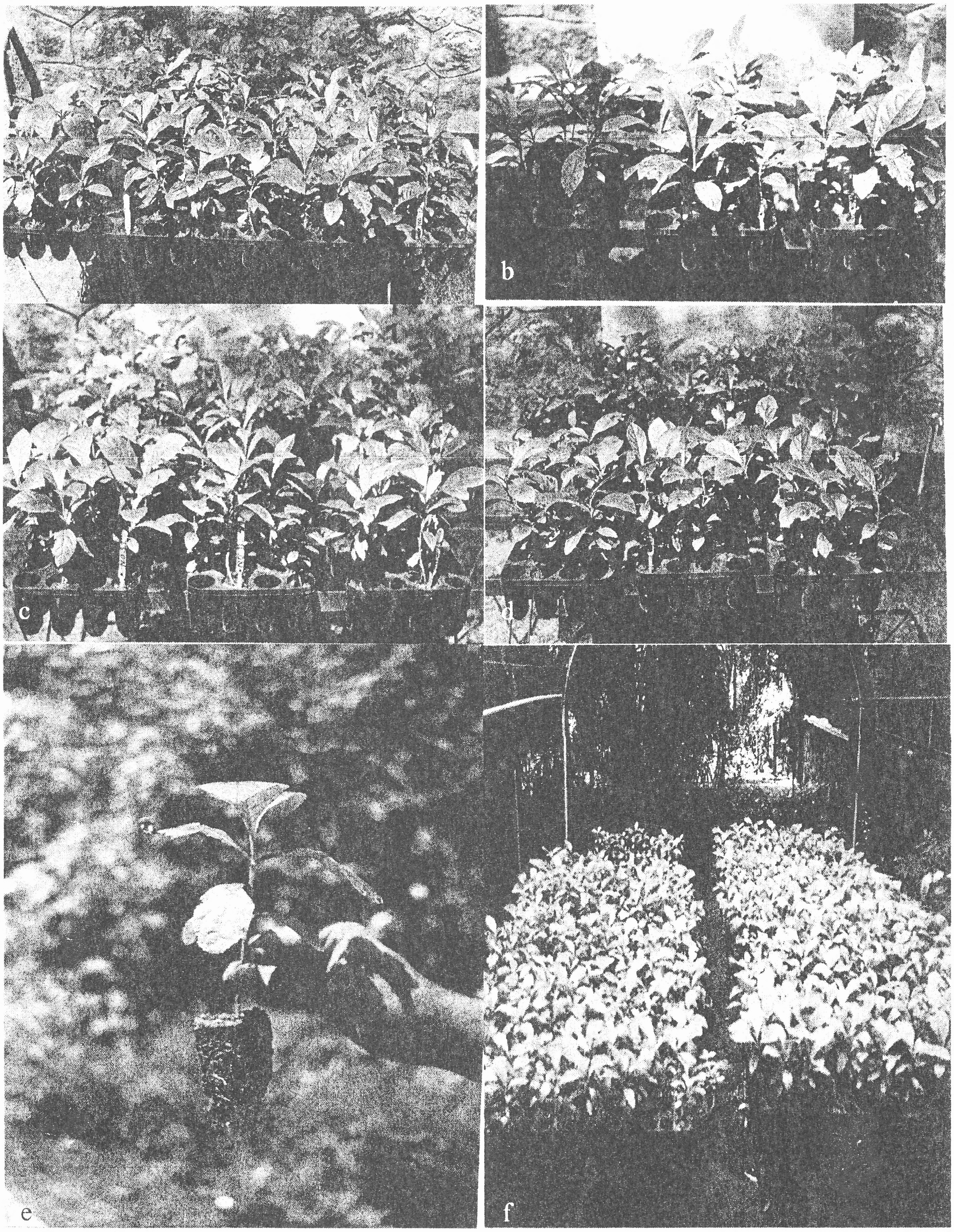


Fig.12: a-f: 90-day-old AM fungal inoculum applied teak root trainer seedlings- glasshouse trial; e: teak seedling removed from the root trainer cell.

Treatments Nos. 8 (*A. appendicula*), 10 (*G. fasciculatum*), and 16 (*G. tortuosum*), which recorded > 14.05 cm were found homogeneous group in DMRT and differ from other treatments. Observations recorded on number of leaf pairs in different treatments and control sets did not show any significant differences.

Seedling biomass recorded after 90 days of growth showed highly significant differences among the treatments (Table 18). Mean seedling biomass (dry weight) in different treatments ranged from 0.663 to 1.120g. Treatment No. 11 (*G. fasciculatum*) showed the highest mean biomass (1.12 g), whereas Treatment No. 21 (*Glomus fasciculatum* + *G. mosseae*) showed the least mean seedling biomass. Treatment No. 8 (*A. appendicula*) gave mean biomass of 1.10 g. Treatment No. 12 (*G. fasciculatum*) and 15 (*G. botryoides*) were the other best treatment as far as seedling biomass is concerned. Treatment No. 5 (*G. macrocarpum*), Treatment No. 9 (*Glomus deserticola*), Treatment No. 3 (*G. mosseae*), and Treatment No. 10 (*G. fasciculatum*) showed almost same mean seedling biomass and fall in the homogeneous group in DMRT (Table 20). Treatment No. 1 (*G. mosseae*) and Treatment No. 23 (*A. appendicula* + *G. fasciculatum*) yielded almost same mean seedling biomass and belonged to the homogeneous group in DMRT. Similarly Treatment No. 7 (*A. appendicula*) and Treatment No.19 (*Gigaspora candida*) belonged to the homogeneous group in DMRT.

Table 17: ANOVA of terminal data on seedling height in various treatments

	Sum of squares	Df	Mean square	F	Sig.
Between groups	101.498	28	3.625	3.257	<.001 **
Within groups					
Total	64.546	58	1.113		
	166.045	86			

Table 18: ANOVA of terminal data on seedling biomass in various treatments

	Sum of squares	Df	Mean square	F	Sig.
Between groups	1.431	28	0.051	37.555	<.001 **
Within groups					
Total	0.079	58	0.001		
	1.510	86			

Table 19: Growth (height) performance of AM fungi inoculated teak seedlings in root trainers

Treatment No	Mean height of seedlings (cm)*					
	15 days	30 days	45 days	60 days	75 days	90 days
	Observation date: 16-08-03	Observation date :1-9-03	Observation date: 16-09-03	Observation date :1/10/03	Observation date :16-10-03	Observation date: 31-10-03
1.	4.63	5.95	7.31	8.83	10.6	12.51
2	6.09	7.39	8.15	9.66	11.83	12.64
3	6.49	7.09	8.23	9.84	12.58	14.42
4	5.75	6.62	7.55	9.51	11.97	14.16
5	6.65	7.85	8.48	9.81	11.99	13.06
6	6.26	7.18	8.13	9.95	12.67	14.78
7	5.81	6.97	7.63	9.38	11.81	13.46
8	6.11	7.17	8.5	9.73	12.48	14.05
9	6.14	7.28	8.35	9.88	12.67	14.37
10	5.7	7.04	8.3	9.63	12.33	14.06
11	6.57	8.13	8.79	10.36	13.28	15.36
12	5.63	8.1	8.81	10.7	13.9	16.14
13	6.94	7.67	8.63	10.1	12.44	14.43
14	5.66	6.8	7.38	8.94	11.1	12.66
15	6.81	7.84	8.6	10.54	12.86	14.76
16	5.71	6.93	7.38	9.54	11.7	14.09
17	6.76	7.45	8.11	9.38	10.76	12.34
18	5.91	6.71	7.55	9.47	11.14	12.65
19	7.76	7.48	8.76	10.01	11.7	12.92
20	5.94	6.74	7.6	9.14	11.02	11.69
21	6.23	6.48	7.15	8.74	11.05	12.03
22	6.7	7.12	7.5	9.32	11.19	12.58
23	5.86	7.04	7.95	9.34	11.39	13.16
24	5.52	6.59	7.26	8.63	10.86	12.16
25	5.05	6.7	7.79	9.34	9.73	12.18
26	4.74	6.41	7.48	8.63	9.9	12.93
27	4.69	7.61	9.11	9.77	10.31	12.75
28	4.23	5.82	7.44	8.41	10.61	13.30
29	5.36	6.58	7.22	8.82	10.55	12.67

*Mean value obtained from 72 seedlings in 3 replications

Table 20: Effect of AM fungal treatments on growth (height and biomass) of teak seedlings in root trainers (terminal data)

Treatment No.	AM fungal inoculum	Mean seedling height (cm) at 90 days	Mean seedling biomass (dry wt.) at 90 days (g)
1	*Glomus mosseae*	12.5167 ^{abcd} (0.85163)	0.9600 ^{ijk} (0.01528)
2	*G. mosseae*	12.6400 ^{abcd} (0.69060)	0.8567 ^{ghi} (0.02404)
3	*G. mosseae*	14.4233 ^{defg} (0.56381)	0.9900 ^{jkl} (0.05568)
4	*G. macrocarpum*	14.1667 ^{cdefg} (0.17638)	0.9367 ^{ij} (0.00882)
5	*G. macrocarpum*	13.0633 ^{abcde} (0.63167)	0.9800 ^{ijkl} (0.00577)
6	*Acaulospora rehmi*	14.7833 ^{efg} (0.54949)	0.8400 ^{fg} (0.01732)
7	*A. appendicula*	13.4667 ^{abcdef} (0.83732)	0.9167 ^{hi} (0.01453)
8	*A. appendicula*	14.0500 ^{bcdef} (0.30414)	1.1000 ^{mn} (0.05774)
9	*Glomus deserticola*	14.3767 ^{defg} (0.33992)	0.9900 ^{jkl} (0.00577)
10	*G. fasciculatum*	14.0667 ^{bcdef} (0.38442)	1.0000 ^{jkl} (0.01528)
11	*G. fasciculatum*	15.3633 ^{fg} (0.14263)	1.1100 ^{n} (0.01155)
12	*G . fasciculatum*	16.1400 ^{g} (0.40857)	1.0100 ^{kl} (0.00577)
13	*Acaulospora scorbiculata*	14.4367 ^{defg} (0.43705)	0.6767 ^{a} (0.01453)
14	*Glomus maculosum*	12.6667 ^{abcd} (0.86859)	0.7300 ^{abc} (0.1155)
15	*G. botryoides*	14.7667 ^{efg} (0.39299)	1.0400 ^{lmn} (0.02309)
16	*G. tortuosum*	14.0900 ^{bcdef} (0.71234)	0.7667 ^{cde} (0.00882)
17	*G. intraradices*	12.3367 ^{abcd} (0.40908)	0.8300 ^{efg} (0.01155)
18	*Gigaspora albida*	12.6467 ^{abcd} (0.77299)	0.6900 ^{ab} (0.01155)
19	*Gigaspora candida*	12.9233 ^{abcde} (1.18679)	0.9167 ^{hi} (0.00882)
20	*Gigaspora gigantia*	11.6933 ^{a} (0.85560)	0.8200 ^{defg} (0.01155)
21	*Glomus fasciculatum + G. mosseae*	12.0300 ^{ab} (0.92154)	0.6633 ^{a} (0.00882)
22	*G. mosseae + A. appendicula*	12.5767 ^{abcd} (0.26181)	0.6700 ^{a} (0.01155)

23	*A. appendicula* + *G. fasciculatum*	13.1633 ^{abcde} (0.60001)	0.9500 ^{ijk} (0.01528)
24	Control	12.1567 ^{abc} (0.72998)	0. 7767 ^{cde} (0.02848)
25	Consortium of AM fungi	12.1767 ^{abc} (0.37711)	0.8400 ^{fg} (0.0155)
26	Consortium of AM fungi	12.9333 ^{abcde} (0.26034)	0.8300 ^{efg} (0.00577)
27	Consortium of AM fungi	12.7567 ^{abcde} (0.75291)	0.7900 ^{cdefg} (0.03786)
28	Consortium of AM fungi	13.3000 ^{abcde} (0.36056)	0.7600 ^{cd} (0.0155)
29	Consortium of AM fungi	12.6667 ^{abcd} (0.30551)	0.7500 ^{bc} (0.01421)
	Mean	13.3923 (0.14897)	0.8690 (0.01421)

* Figures given in parenthesis are SE; superscripts with same letters for means of seedling height and biomass in each treatment do not differ significantly

Mycorrhizal inoculation effect (MIE) of various treatments was also calculated following the formula proposed by Bagyaraj *et al.* (1988). Of the 29 treatments tried, Treatment No. 11 (*Glomus fasciculatum*) showed the highest percent MIE (30.65). The other two treatments with *G. fasciculatum* inocula (Treatment Nos. 10 and 12) also showed comparatively good percentage of MIE (Table 21). *Glomus botryoides* (Treatment No. 15) and *Acaulospora appendicula* (Treatment No. 8) were the other inocula which gave high per cent of MIE. The formula used here has the advantage over the one which proposed by Menge *et al.* (1978) for calculating mycorrhizal dependency of crop plants using inoculant VAM fungi in sterilized condition. Even though, the experiment was carried out under sterile condition in the glasshouse at the initial phase, later the inoculated seedlings were transferred to the nursery and grown under non-sterile condition. Hence, it was found most appropriate to use the formula for calculating the MIE under non-sterile conditions. MIE is very useful for the assessment of the extent to which introduced fungi compete with native VAM fungi to bring about plant growth response.

Table 21: Biomass (dry weight) of teak seedlings in various treatments and MIE

Treatment No.	VAM fungal inoculum	Mean seedling biomass (dry wt.) g	%MIE*
1	*Glomus mosseae*	0.9600 ^{ijk} (0.01528)	19.09
2	*Glomus mosseae*	0.8567 ^{ghi} (0.02404)	9.34
3	*Glomus mosseae*	0.9900 ^{jkl} (0.05568)	21.54
4	*G.macrocarpum*	0.9367 ^{ij} (0.00882)	17.08
5	*G.macrocarpum*	0.9800 ^{ijkl} (0.00577)	20.74
6	*A. rehmi*	0.8400 ^{fg} (0.01732)	7.53
7	*A. appendicula*	0.9167 ^{hi} (0.01453)	15.27
8	*A. appendicula*	1.1000 ^{mn} (0.05774)	29.39
9	*G. deserticola*	0.9900 ^{jkl} (0.00577)	21.54
10	*G. fasciculatum*	1.0000 ^{jkl} (0.01528)	22.33
11	*G. fasciculatum*	1.1100 ^{n} (0.01155)	30.65
12	*G. fasciculatum*	1.0100 ^{kl} (0.00577)	23.09
13	*A. scorbiculata*	0.6767 ^{a} (0.01453)	-14.77
14	*G. maculosum*	0.7300 ^{abc} (0.1155)	-6.39
15	*G. botryoides*	1.0400 ^{lmn} (0.02309)	25.31
16	*G. tortuosum*	0.7667 ^{cde} (0.00882)	-1.30
17	*G. intraradices*	0.8300 ^{efg} (0.01155)	6.42
18	*Gigaspora albida*	0.6900 ^{ab} (0.01155)	-12.56
19	*Gigaspora candida*	0.9167 ^{hi} (0.00882)	15.27
20	*Gigaspora gigantia*	0.8200 ^{defg} (0.01155)	5.28
21	*G. fasciculatum + G.mosseae*	0.6633 ^{a} (0.00882)	-17.09
22	*G.mosseae + A .appendicula*	0.6700 ^{a} (0.01155)	-15.92
23	*A.appendicula + G. fasciculatum*	0.9500 ^{ijk} (0.01528)	18.24
24	Control	0. 7767 ^{cde} (0.02848)	0

25	Consortium of AM fungi	0.8400 ^{fg} (0.0155)	7.53
26	Consortium of AM fungi	0.8300 ^{efg} (0.00577)	6.42
27	Consortium of AM fungi	0.7900 ^{cdefg} (0.03786)	1.68
28	Consortium of AM fungi	0.7600 ^{cd} (0.0155)	-2.19
29	Consortium of AM fungi	0.7500 ^{bc} (0.01421)	-3.56

* Figures given in parenthesis are SE; superscripts with same letters for means of seedling biomass in each treatment do not differ significantly

* MIE: Mycorrhizal inoculation effect

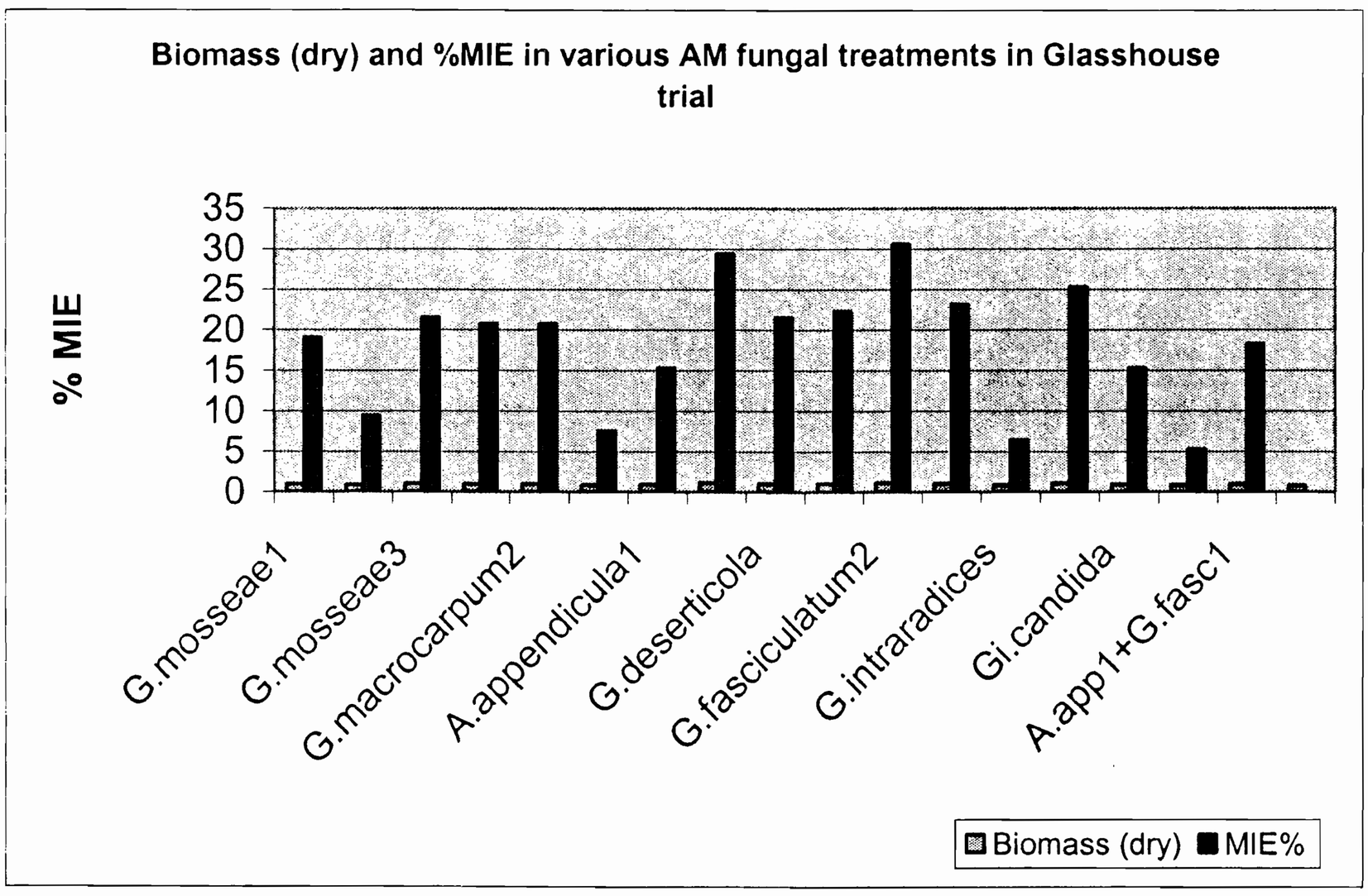


Fig. 13: Seedling biomass (dry wt.) and % MIE in selected treatments

The overall results on AM fungal inoculation trial showed that among various AM fungi and their combinations and consortium used for the mycorrhization of teak seedlings, fungi like *Glomus fasciculatum, G. botryoides, G. deserticola, G. macrocarpum, G. mosseae* and *Acaulospora appendicula* increased the seedling growth in terms of height and seedling biomass. All the AM fungi inoculated seedlings showed vesicles, arbuscules and intraradical hyphae in their roots, while in control sets, AM fungal features were almost absent. It was also evident that even though, all the inoculated AM fungi colonize the teak root efficiently, soil-sand medium is not suitable for

supporting the maximum growth of teak seedlings for more than two months; hence a nutrient rich growing medium is required for getting optimum growth of seedlings in the root trainers.

3.5. Photosynthetic status of AM fungi treated seedlings

Chlorophyll fluorescence kinetics was measured in AM fungi treated teak seedlings and control seedlings using a Handy PEA (Hansatech Instruments, Norfolk, UK). The experiment was carried out to know the effect of mycorrhizal fungi on photosynthesis. When a healthy leaf is suddenly illuminated after a period of darkness, a time-dependent fluorescence induction (Kautsky Effect) is observed, the amplitude of which is proportional to the incident light level. Typically, illumination of a healthy leaf after 10 - 30 minutes dark adaptation results in an immediate rise to level (O or Fo) followed by a rapid polyphasic rise to a peak level (P or Fp). If sufficient light is used, the primary electron acceptor from PSII (Qa) becomes fully reduced, transiently inhibiting photochemistry. In such circumstances the maximum fluorescence level is observed (Fm).

Table 22: Chlorophyll fluorescence parameter of AM treated and control seedlings

Sl.No.	Treatment No.	AM fungi	FV/FM (Mean)
1	11	*Glomus fasciculatum*	0.826
2	12	*Glomus fasciculatum*	0.827
3	24	control	0.83
4	7	*A. appendicula*	0.823
5	2	*Glomus mosseae*	0.827
6	23	*A.appendicula* + *G.fasciculatum*	0.826
7	8	*A. rehmi*	0.829
8	6	*A. rehmi*	0.829
9	10	*G.fasciculatum*	0.828
10	17	*G. intraradices*	0.829
11	25	Consortium	0.831
12	29	Consortium	0.826

The time to reach the fluorescence peak is very short (500 msec). Hence it is useful to plot the fluorescence rise on a logarithmic scale to view the polyphasic kinetic. The polyphasic curve was then subjected to the OJIP analysis according to Strasser *et al.* (1995) showed intermediate steps in the fluorescence induction transient such as the J step at approximately 2 milliseconds and the I step at approximately 30 milliseconds. It has also been reported that a K step at approximately 300 microseconds is observed in certain heat-stressed samples. Thus, in these circumstances the fluorescence induction kinetics can be characterized as O-K-J-I-P.

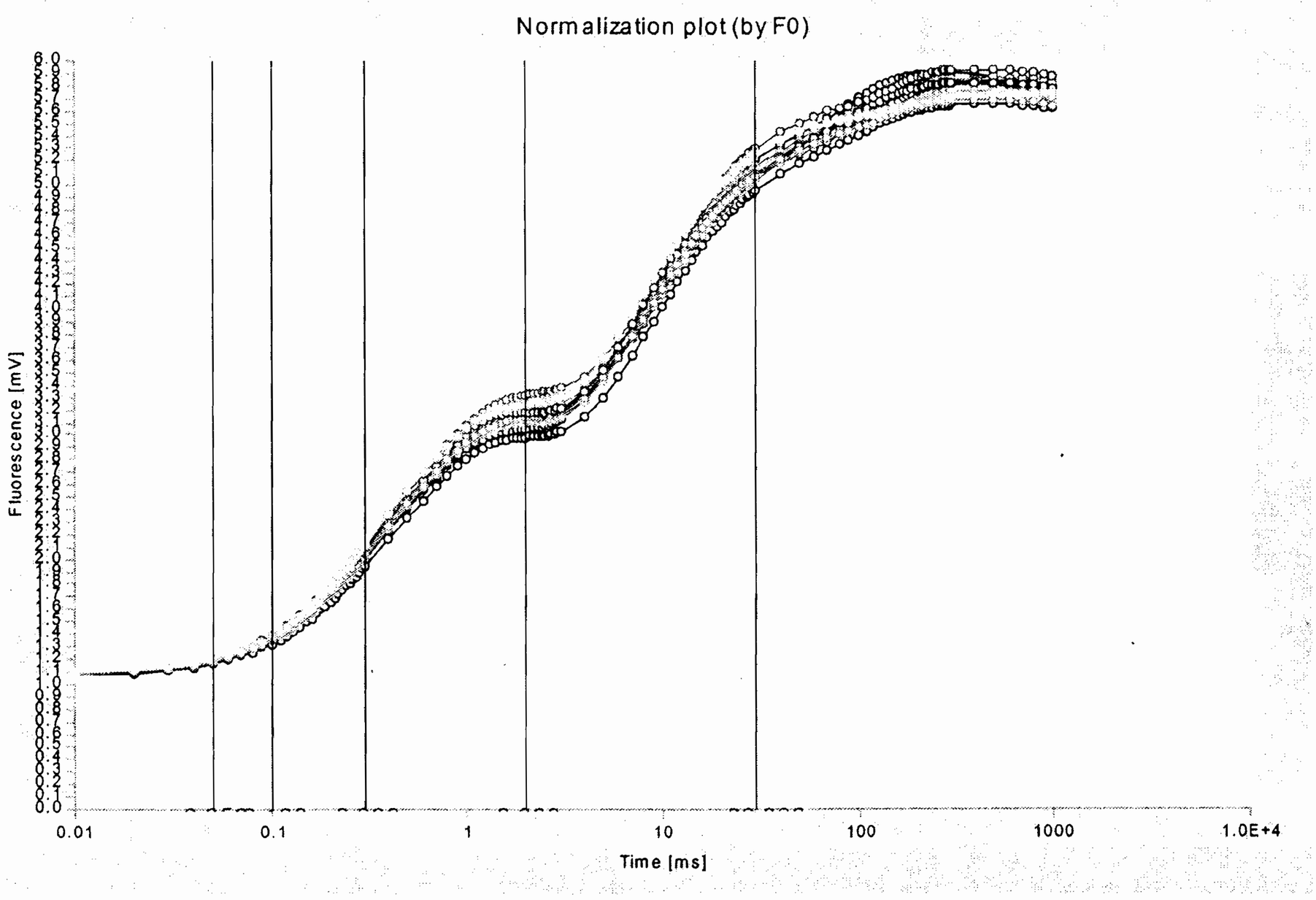


Fig. 14: A plot of the *Chlorophyll a* fluorescence induction kinetics in 90-day-old root trainer grown *Tectona grandis* seedlings

The fluorescence changes are plotted against the fraction of a second (OJIP). Each curve represents the average of six measurements in each treatment. Not much differences were observed between the different treatments and the control (Table 22; Figs. 14,15).

The PI is derived from the equation:

PI = Abs/CS * Tro/CS *Eto/CS

where,

Abs = absorption of quantum

Tr_o = Transfer energy of quantum

Et_o = Electron transport energy

CS = Cross sectional area of leaf

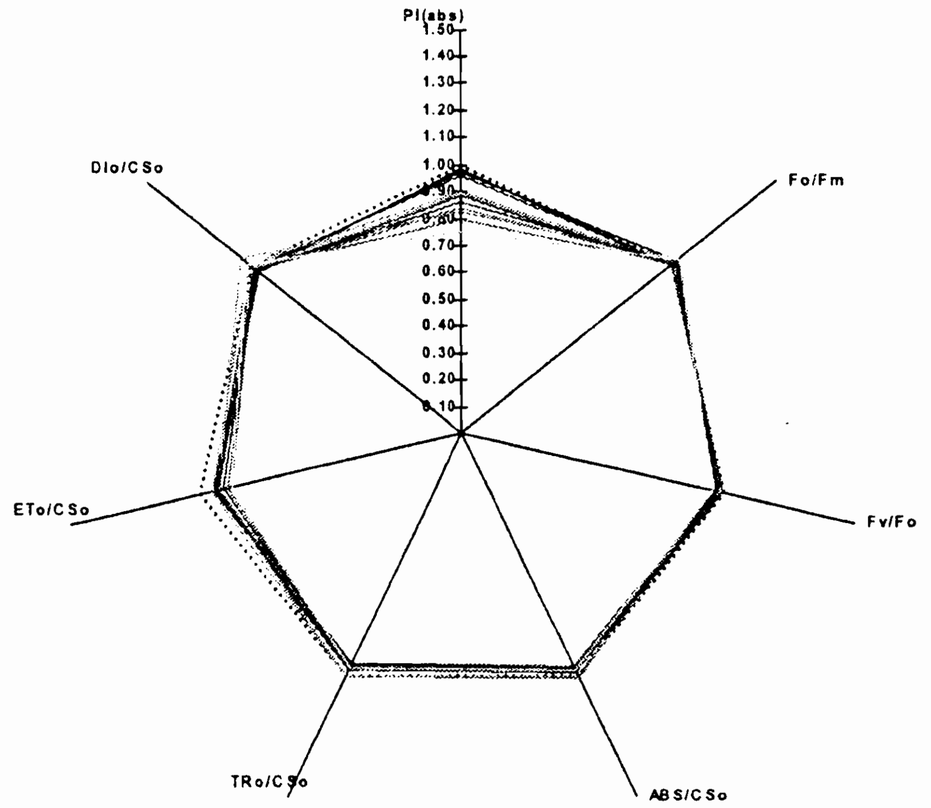


Fig. 15: A radar plot of the *chlorophyll a* fluorescence analysis showing the Performance Index

It was found that, there are only minor difference in the performance Index (PI) between the different AM fungal treatments in relation to the control set. The difference is mainly due to the variation in the electron transport capacity. The overall results indicate that the resent mycorrhization treatments do not affect the photosynthetic capacity of the plants positively or negatively.

3.6. Nursery trial

Nursery trial using various AM fungi for mycorhization of teak seedlings and weed compost as growing medium in the root trainer cells was carried out during 2004 under normal forest nursery conditions (Figs. 16-18). Observations on growth performance of seedlings in various treatments were recorded at 15 days intervals up to 90 days (Fig.19). Seedling height, leaf pairs and collar diameter are the parameters recorded. As number of leaf pairs produced in seedlings was found not differ among the treatments, statistical analysis was not carried out. However, the terminal data (data collected at days of growth) on seedling height, collar diameter and seedling biomass (dry weight) were subjected to ANOVA and followed by DMRT (Tables 23,24).

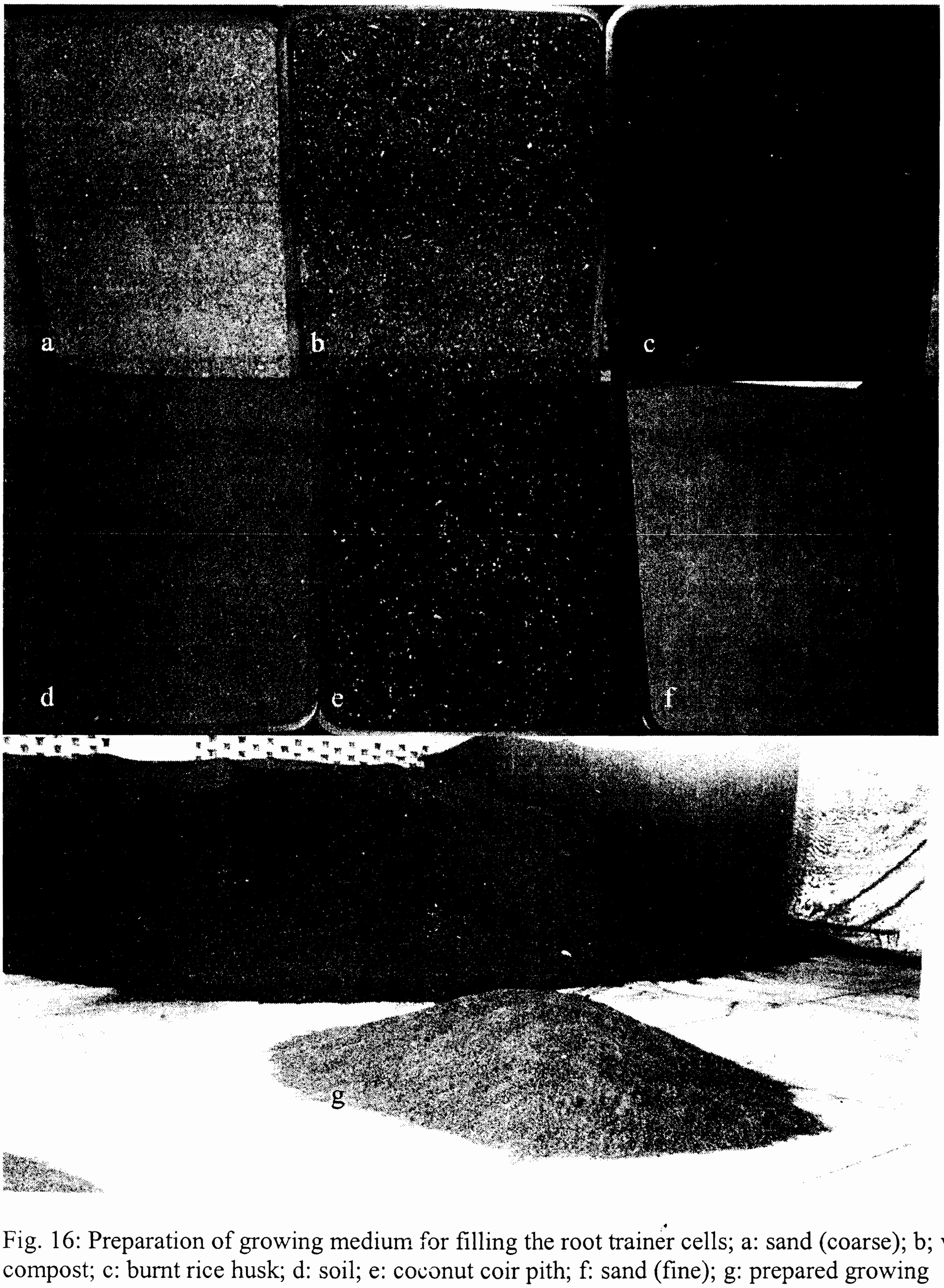


Fig. 16: Preparation of growing medium for filling the root trainer cells; a: sand (coarse); b; weed compost; c: burnt rice husk; d: soil; e: coconut coir pith; f: sand (fine); g: prepared growing medium

Fig.17: AM fungal application in teak seedlings in root trainers - nursery trial; 5-day-old teak seedlings; b: 10-day-old teak seedlings ready for AM fungal inoculum application; d: 20-day-old seedlings under shade nets.

Fig.18: AM fungal application – nursery trial; a-c: 30 to 90-day-old seedlings kept in open area (without shade) and recording the seedling growth measurements.

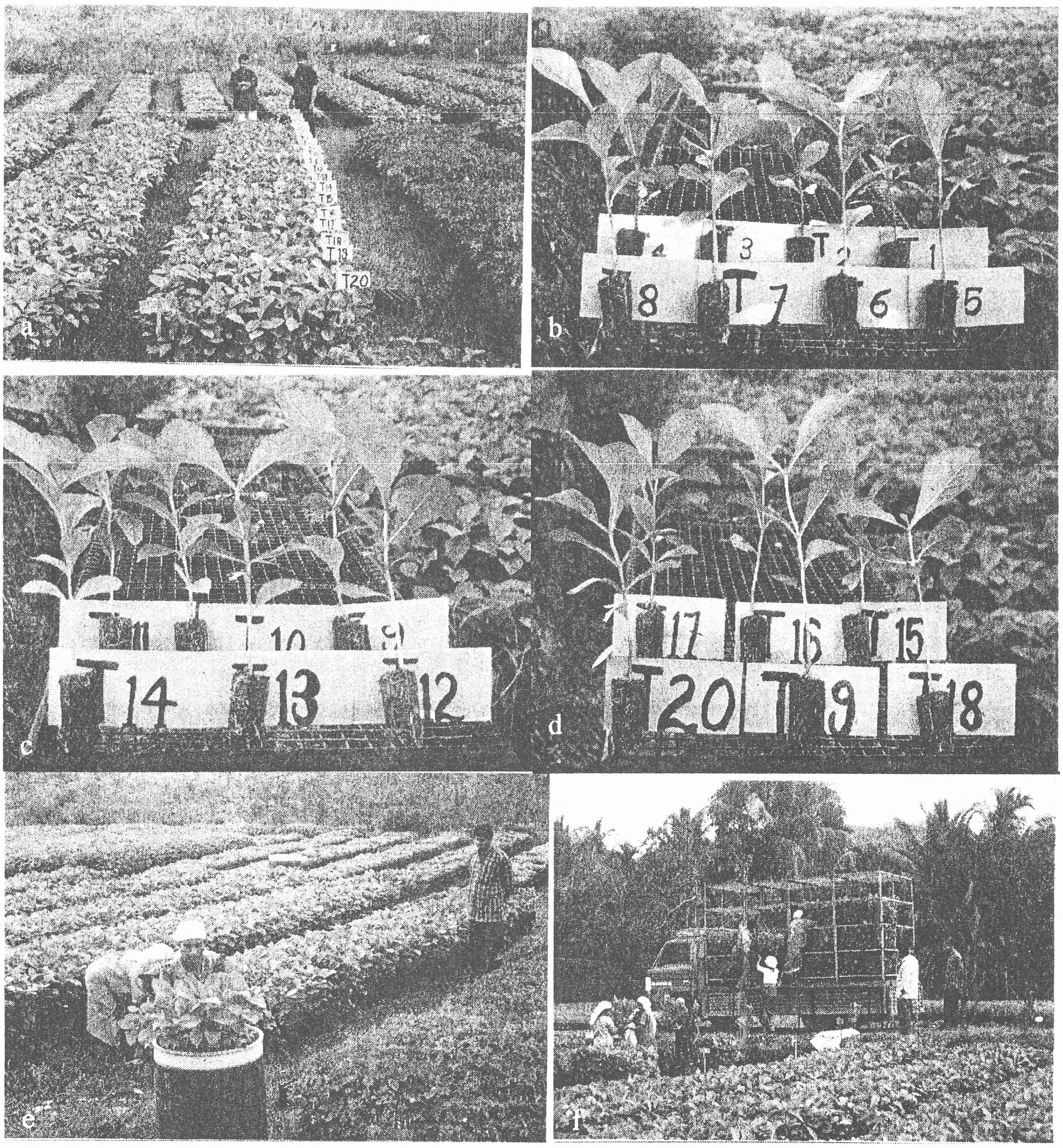


Fig. 19: AM fungal application - nursery trial; a: 90-day-old teak seedlings; b-d: representative seedling (removed from root trainer cells) from 20 different treatments; e: seedlings dipping in fungicide solution before transportation; f: seedlings ready for transportation to the planting site.

Mean seedling height and mean collar diameter recorded from the 20 treatments showed no significant differences among most of the treatments (Table 23). However, there is significant difference between control (Treatment No. 20) and most of the other AM fungal treatments.

The results showed that the mycorrhizal treatment has effects on the seedling growth performance and comparable with the control. The lowest mean seedling height of 23.92 cm was recorded for the Treatment No. 20 (control), while Treatment No. 8 with *Acaulospora appendicula* recorded the maximum mean height among various AM fungal treatments (Table 25). Treatment Nos. 2,3,4,5,11,13,14,17,18, and 19 are the homogeneous group in DMRT with mean seedling height of 26 cm to 28 cm. Treatment Nos. 6, 9, 12, 15, 16 belong to homogeneous group with seedling height ranged from 28 cm to 29 cm. Treatment No. 7 with *G. mosseae* and Treatment No. 8 with *A. appendicula* are the best treatment among the 19 treatments with AM fungi, with regard to the seedling height (Figs. 20,21,22).

Analysis of variance on data collected on seedling collar diameter after 90 days growth showed no significant differences among various treatments (Table 24). However, in DMRT specific homogeneous groups can be identified based on the collar diameter (Table 25). Treatment No. 20 (control) recorded the least collar diameter of 4.86 mm, while highest mean diameter of 5.15 mm was recorded for Tereatment No. 14 with *Scutellospora heterogama*. Treatment Nos. 8, 9, 10, 12, 13, and 14 belong to the homogeneous group in DMRT with >5.00 mm collar diameter (Table 25). The results showed that mycorrhization has affected the seedling growth performance considerably, however, within the short span of growth in the root trainers, no significant difference in seedling collar diameter among the various AM fungal treatments could be detected.

Table 23: ANOVA of terminal data on seedling height in various treatments

	Sum of squares	Df	Mean square	F	Sig.
Between groups	221.971	19	11.683	1.534	.096 ns
Within groups					
Total	609.269	80	7.616		
	831.241	99			

Table 24: ANOVA of terminal data on seedling collar diameter in various treatments

	Sum of squares	Df	Mean square	F	Sig.
Between groups	4.476	19	0.236	1.261	0.233 ns
Within groups					
Total	14.944	80	0.187		
	19.420	99			

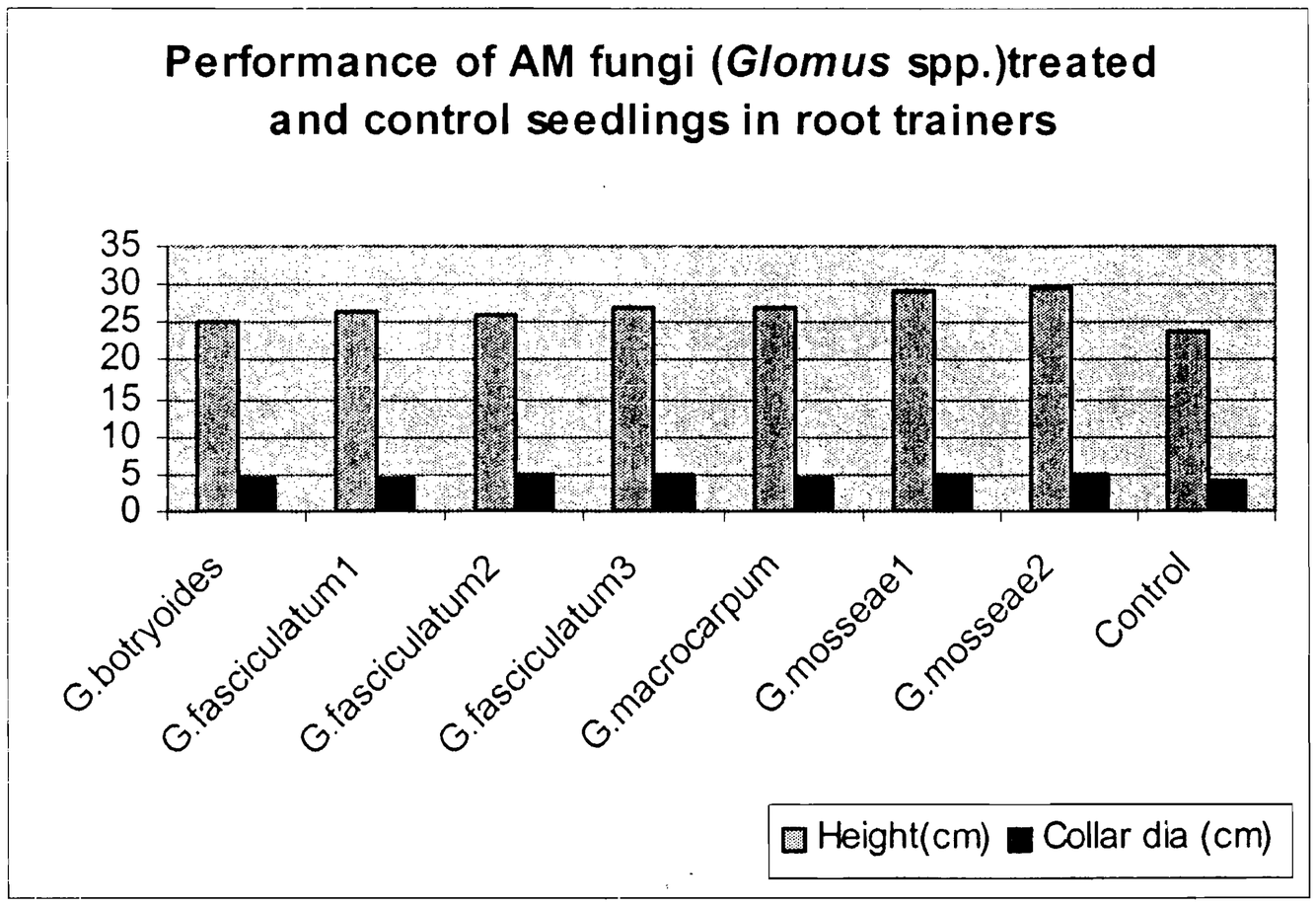


Fig. 20: Growth performance of *Glomus* species treated seedlings in the nursery

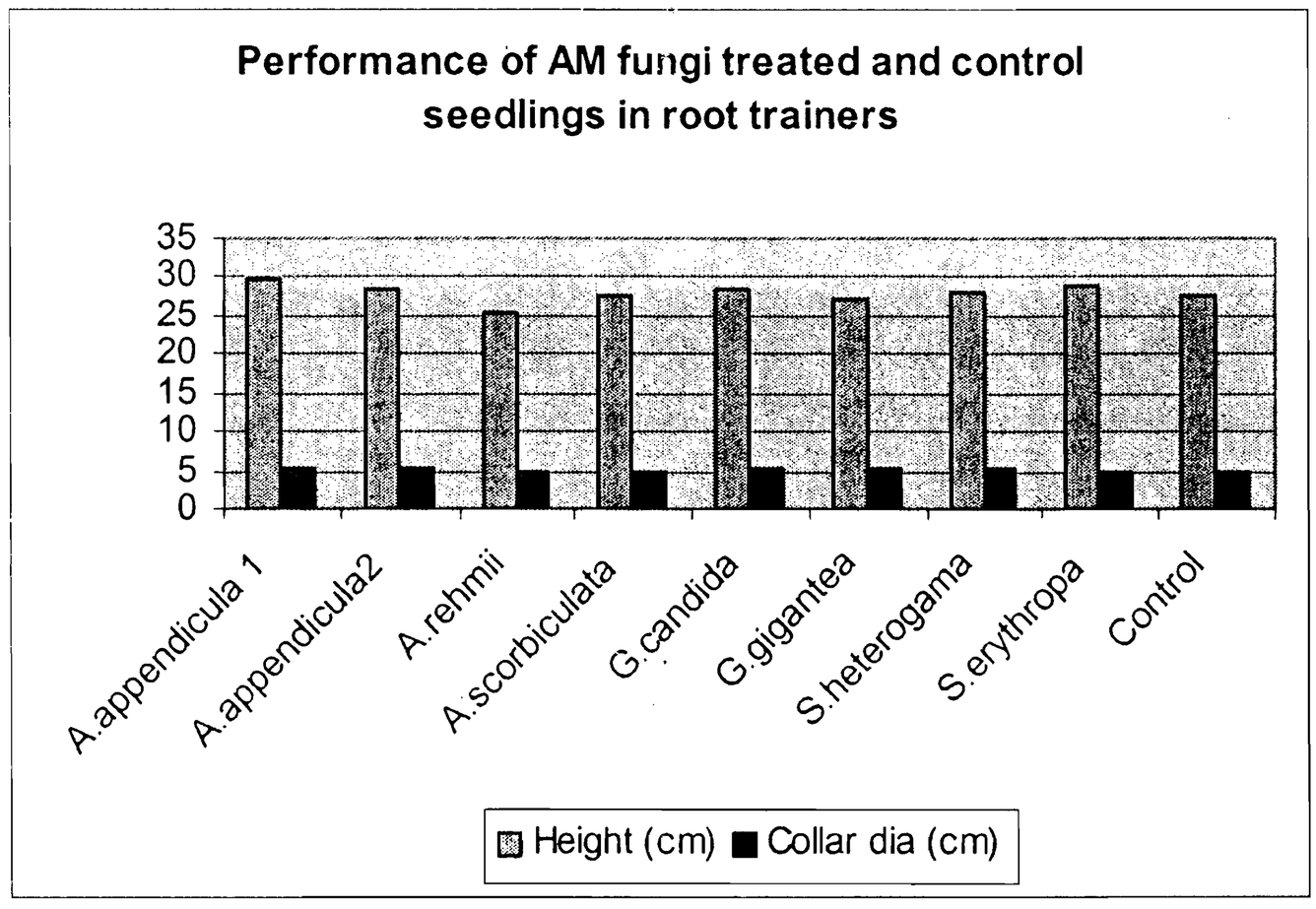


Fig. 21: Growth performance of *Acaulospora, Gigaspora* and *Scutellospora* species treated seedlings in the nursery trial

Table 25: Effect of AM fungal treatments on growth of teak seedlings

Treatment No.	AM inoculum	Mean seedling height (cm)	Mean seedling collar dia (mm)
T1	*G. botryoides*	25.0030 ^{ab} (1.1212)	4.6162 ^{ab} (0.2422)
T2	*G. fasciculatum*1	26.2598 ^{abcd} (1.3190)	4.6912 ^{ab} (0.2257)
T3	*G. fasciculatum*2	26.0628 ^{abcd} (1.1818)	4.7446 ^{ab} (0.2892)
T4	*G. fasciculatum*3	27.0370 ^{abcd} (1.2238)	4.7446 ^{ab} (0.2591)
T5	*G. macrocarpum*	27.0130 ^{abcd} (1.3253)	4.6988 ^{ab} (0.2406)
T6	*G. mòsseae*1	29.0648 ^{bcd} (2.1458)	4.8184 ^{ab} (0.3433)
T7	*G. mosseae*2	29.5260 ^{cd} (1.5833)	4.8466 ^{ab} (0.1609)
T8	*A. appendicula*1	29.8088 ^{d} (1.2213)	5.0548 ^{b} (0.1610)
T9	*A. appendicula*2	28.4398 ^{bcd} (1.1086)	5.0594 ^{b} (0.1456)
T10	*A. rehmii*	25.4702 ^{abc} (0.9289)	5.0074 ^{b} (0.2166)
T11	*A. scorbiculata*	27.3824 ^{abcd} (1.2370)	4.8010 ^{ab} (0.1209)
T12	*Giga spora candida*	28.3220 ^{bcd} (1.07758)	5.0914 ^{b} (0.0454)
T13	*Gigaspora gigantea*	27.1588 ^{abcd} (1.3422)	5.1132 ^{b} (0.0570)
T14	*S. heterogama*	28.0070 ^{abcd} (1.4608)	5.1502 ^{b} (0.2768)
T15	*S. erythropa*	28.8930 ^{bcd} (1.1314)	4.9748 ^{b} (0.1456)
T16	*A. appendicula*1 +*G. fasciculatum*2	28.5782 ^{bcd} (1.0378)	4.9430 ^{b} (0.1854)
T17	*G.fasciculatum* 3+ *G. mosseae*2	27.2410 ^{abcd} (1.2961)	4.9702 ^{b} (0.0827)
T18	*G. botryoides* + *G. fasciculatum*2	26.8770 ^{abcd} (0.6740)	4.9118 ^{b} (0.1033)
T19	*A. appendicula*2 + *G. mossease*1	27.6686 ^{abcd} (0.5984)	4.6990 ^{b} (0.1188)
T20	Control	23.9182 ^{a} (0.8019)	4.2378 ^{a} (0.0847
	Mean	27.3868 (0.2897)	4.8587 (0.0443)

* Figures given in parenthesis are SE; superscripts with same letters for means of seedling height and collar diameter in each treatment do not differ significantly

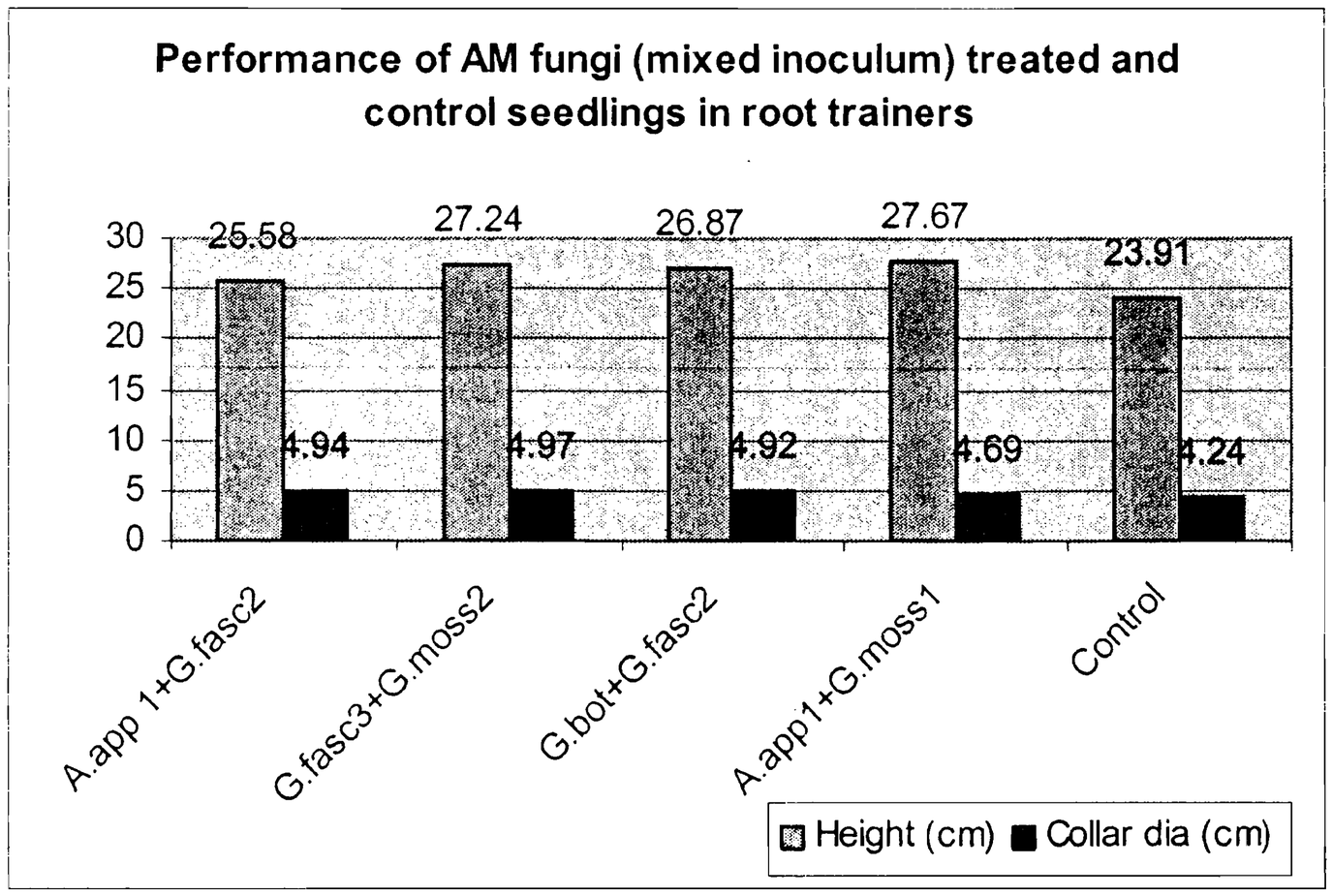


Fig. 22: Growth performance of AM fungi (mixed inoculum) treated seedlings

Data on seedling biomass (dry weight) recorded from 90 day-old AM fungi treated and control seedlings showed highly significant differences among the treatments (Tables 26, 27; Figs. 23-26). Results on seedling biomass and percent MIE obtained in *Glomus*, *Acaulospora, Gigaspora, Scutellospora* and mixed inoculum treatments are given in Figures 23-26. Lowest seedling biomass was recorded for the seedlings in control sets (1.75g), whereas Treatment No. 7 with *Glomus mosseae* exhibited the highest mean seedling biomass of 3.40 g. In DMRT, Treatments No. 20 (control), Treatment No. 14 (*S. heterogama*) and Treatment No. 7 (*G. mosseae*) stand out as separate homogeneous group, while all other 17 treatments belonged to the same homogeneous group with < 3.40 g or > 2.65 g seedling biomass (Table 27). Among the 19 AM fungal treatments, highest percent MIE of 48.53 was registered in seedlings treated with Glomus mosseae (Treatment No. 7) (Table 27; Fig.23).

Table 26: ANOVA of terminal data on seedling biomass in various treatments

	Sum of squares	Df	Mean square	F	Sig.
Between groups	9.281	19	0.488	2.233	0.007 **
Within groups					
Total	17.497	80	0.219		
	26.778	99			

Table 27: Effect of AM fungal treatments on seedling biomass (dry weight) in root trainers

Treatment No.	AM Fungal inoculum	Mean seedling boiomass (dry wt. g)	% MIE*
T1	*G. botryoides*	2.9710 ^{bc} (0.1342	41.09
T2	*G. fasciculatum*1	2.8190 ^{bc} (0.0672	37.92
T3	*G. fasciculatum*2	2.9180 ^{bc} (0.1284)	40.02
T4	*G. fasciculatum*3	2.8600 ^{bc} (0.0730)	38.81
T5	*G. macrocarpum*	2.9902 ^{bc} (0.2495)	41.48
T6	*G. mosseae*1	2.9530 ^{bc} (0.3589)	40.74
T7	*G. mosseae*2	3.4000 ^{c} (0.0584)	48.53
T8	*A. appendicula*1	2.8020 ^{bc} (0.2322)	37.50
T9	*A. appendicula*2	2.9800 ^{bc} (0.2979)	41.28
T10	*A. rehmii*	2.7520 ^{bc} (0.1890)	36.41
T11	*A. scorbiculata*	3.0880 ^{bc} (0.1889)	43.33
T12	*Gigaspora candida*	2.7940 ^{bc} (0.2114)	37.37
T13	*Gigaspora gigantea*	3.1360 ^{bc} (0.2483)	44.20
T14	*S. heterogama*	2.6460 ^{b} (0.1759)	33.64
T15	*S. erythropa*	2.9800 ^{bc} (0.3057)	41.28
T16	*A. appendicula*1 +*G. fasciculatum*2	2.9260 ^{bc} (0.0573)	40.19
T17	*G.fasciculatum* 3+ *G. mosseae*2	2.6940 ^{bc} (0.3208)	35.04
T18	*G. botryoides* + *G. fasciculatum*2	2.9680 ^{bc} (0.2125)	41.03
T19	*A. appendicula*1 + *G. mossease*1	3.0240 ^{bc} (0.1923)	42.13
T20	Control	1.7500 ^{a} (0.0537)	0
	Mean	2.8725 (0.0520)	

*Figures given in parenthesis are SE; superscripts with same letters for means of seedling height and collar diameter in each treatment do not differ significantly

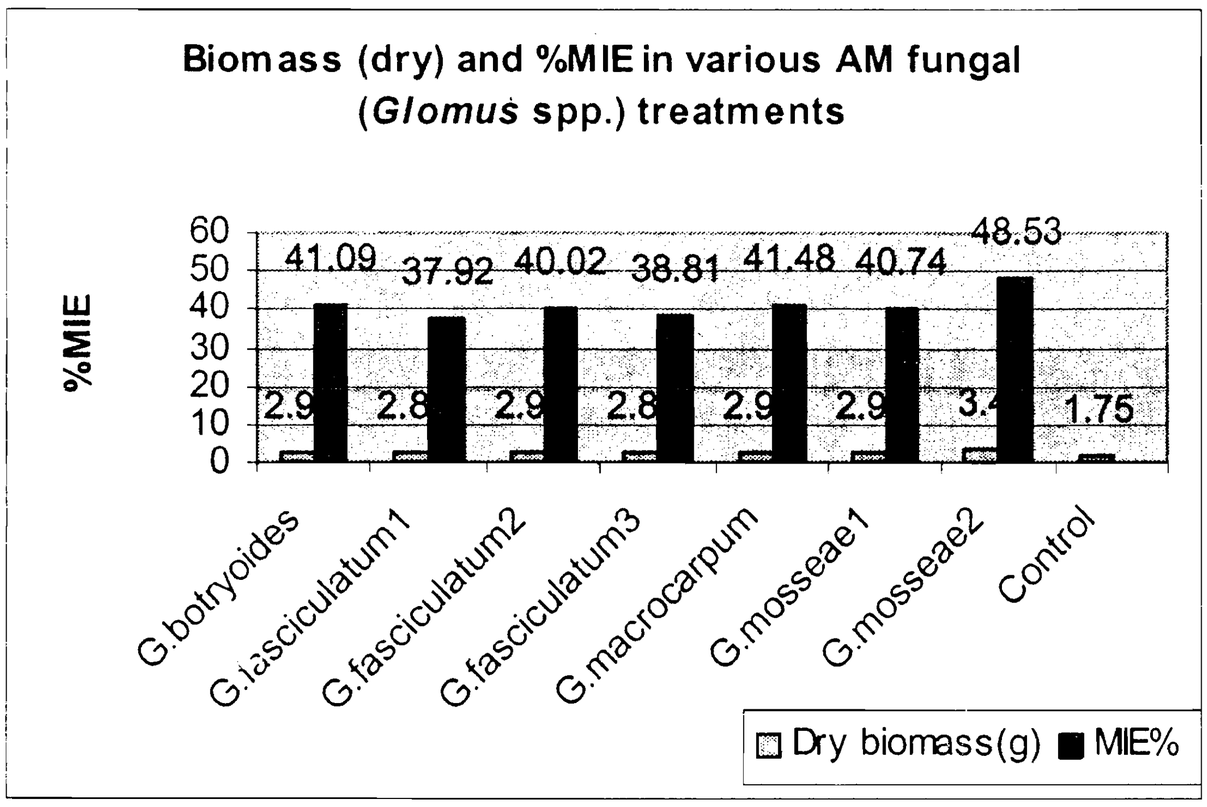


Fig. 23: Seedling biomass (dry wt.) and % MIE in various *Glomus* species treatments

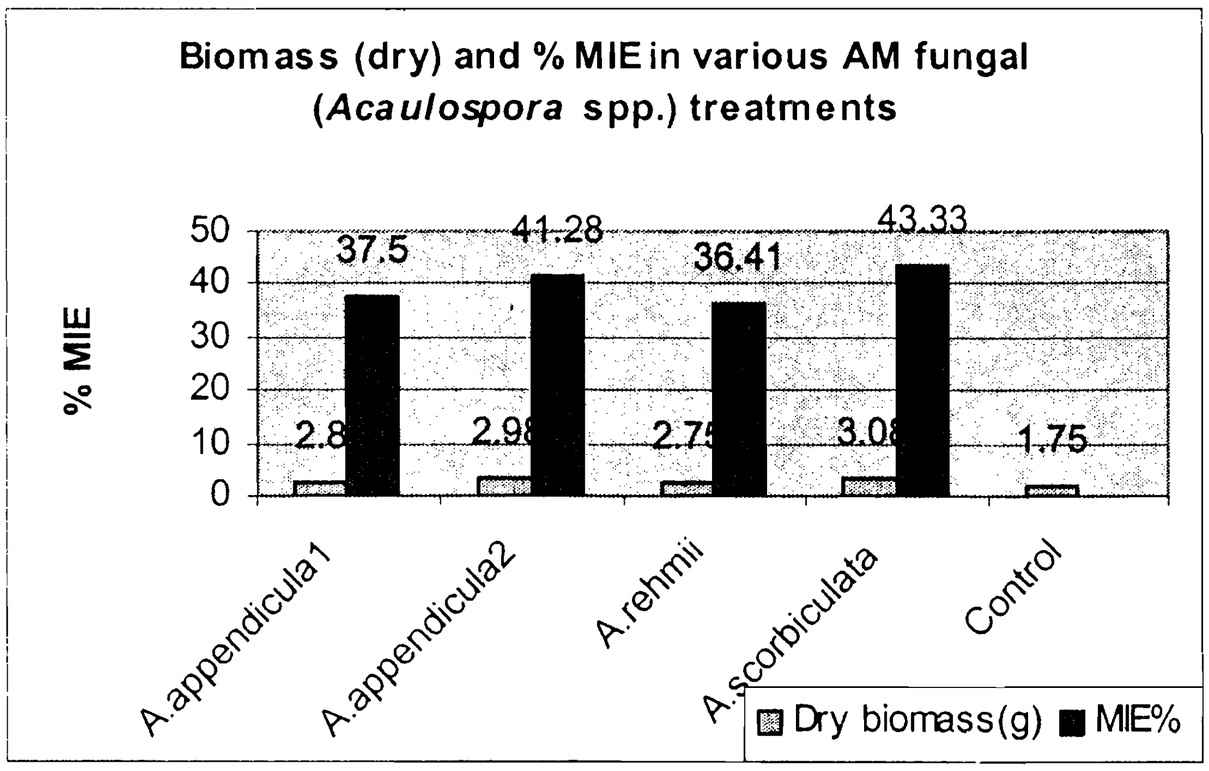


Fig. 24: Seedling biomass (dry wt.) and % MIE in various *Acaulospora* species treatments

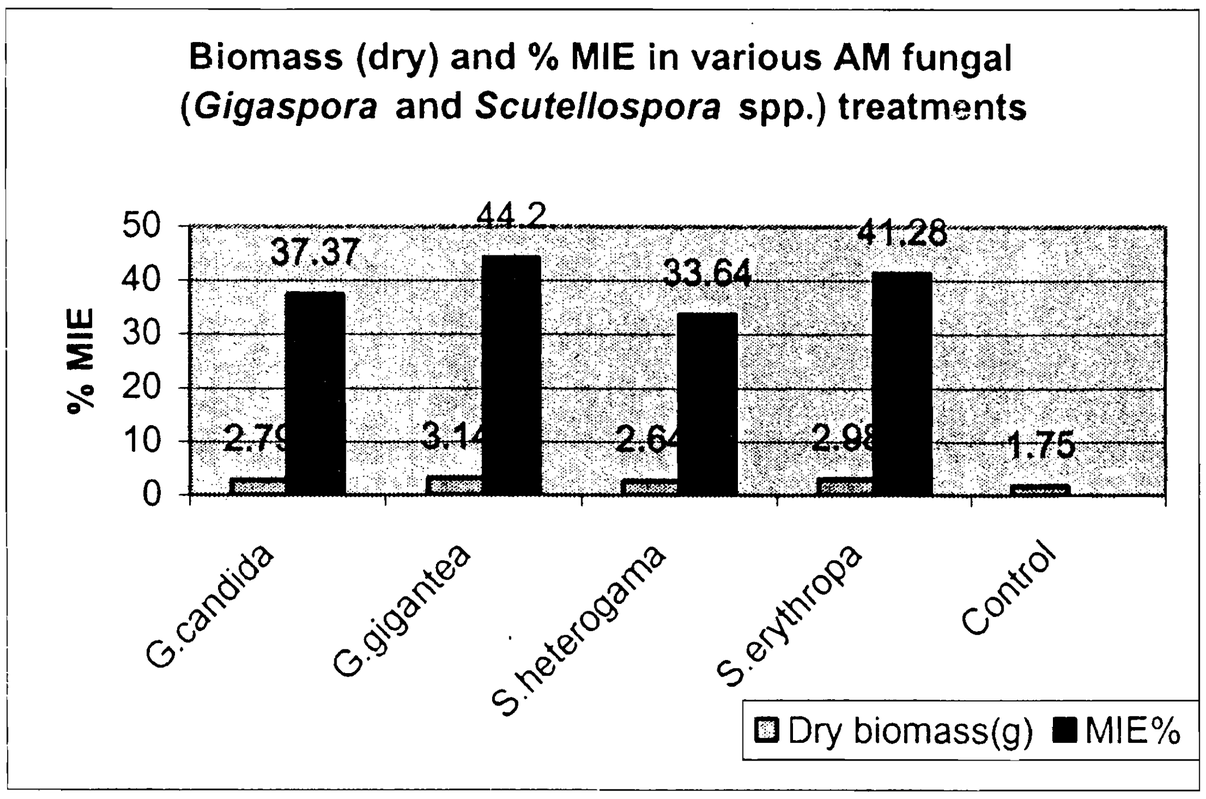


Fig. 25: Seedling biomass (dry wt.) and % MIE in *Gigaspora* and *Scutellospora* treatments

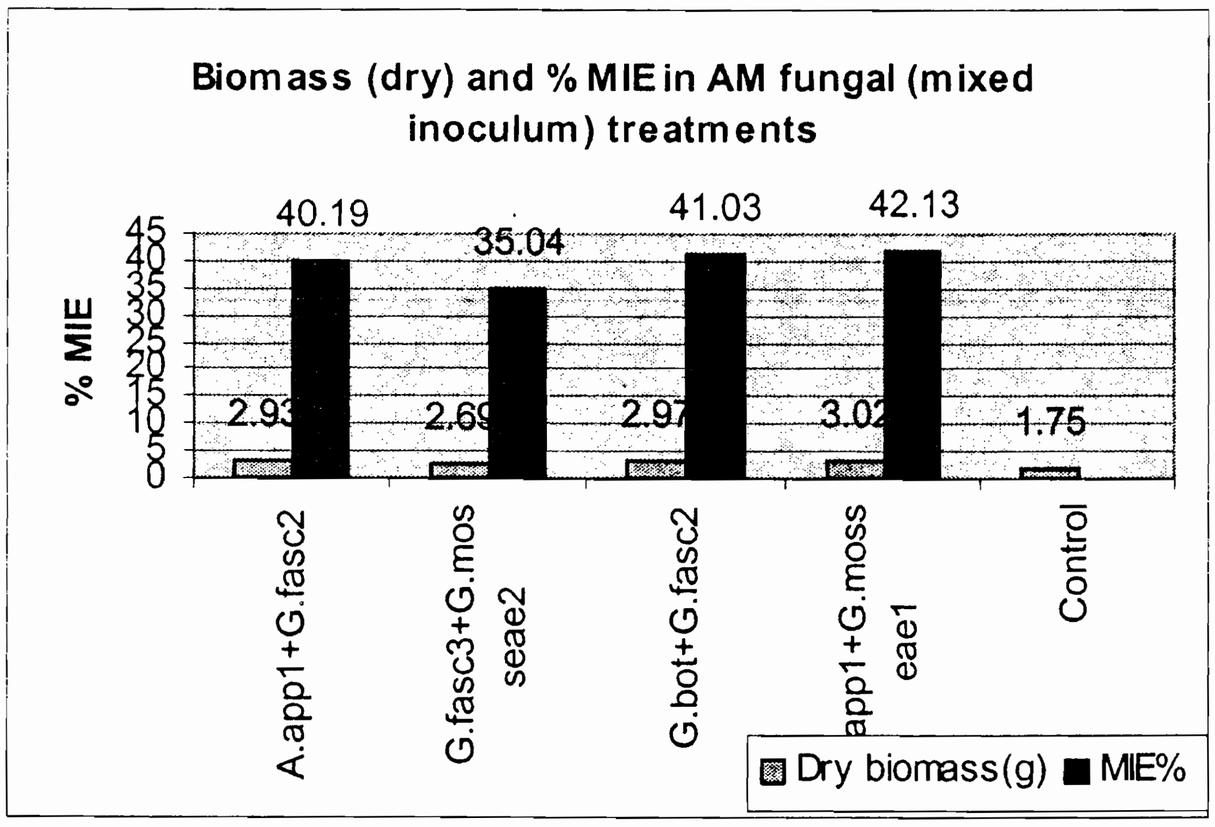


Fig. 26: Seedling biomass (dry wt.) and % MIE in various AM fungal mixed inoculum treatments

Root colonization of teak seedlings from various treatments was checked and found that seedlings in all the treatments were colonized by AM fungi in their feeder roots. Root colonization ranged from 14 to 65 per cent (Table 28). Seedlings applied with *Scutellospora heterogama* inoculum registered least root colonization (14%), while *Glomus fasciculatum, G. botryoides, G. macrocarpum* and *G. mossseae* inoculated plants showed highest root colonization which ranged from 54 to 65 per cent. Vesicles were found abundant in root segments than the arbuscules. The results showed that mycorrhization with different AM fungal inocula was effective and all the AM fungi treated seedlings were having the healthy mycorrhized root system.

Earlier, mycorrhization of teak seedlings using different AM fungi has been carried out by various workers and varying levels of enhancement of seedling growth has been achieved (Gong *et al*., 2002; Gurumurthy and Sreenivasa, 1998; Mohanan, 2002,2003; Rajan *et al*., 2000; Ramanwong and Sangwaint, 2000; Sugavanam *et al*., 1998; Verma *et al*., 2001; Verma and Jamaluddin, 1995). Recently, a study carried out under nursery conditions using nine different AM fungi on teak seedlings, an increase in plant growth and plant nutritional status over those grown with no soil inoculation of AM fungi has been reported (Rajan *et al*., 2000).

Table 28: Percent root colonization in AM fungi treated seedlings in the nursery trial

Treat ment No.	No.of root bits examined	Vesicles (V)	Arbuscules (A)	V&A	Hyphae	Uninfected	% root colonization
1	100	27	17	17	30	9	61
2	100	32	8	23	33	4	63
3	100	23	15	27	30	5	65
4	100	31	11	10	40	8	54
5	100	30	21	10	22	17	61
6	100	27	11	23	36	13	61
7	100	21	14	23	32	10	58
8	100	30	6	10	30	24	46
9	100	16	18	12	32	22	46
10	100	26	2	2	42	28	30
11	100	24	0	14	12	50	38
12	100	20	4	12	24	40	36
13	100	18	4	6	40	32	28
14	100	16	2	6	26	50	24
15	100	10	4	0	15	71	14
16	100	12	6	0	22	60	18
17	100	16	0	0	19	65	16
18	100	16	2	0	8	74	16
19	100	10	4	0	12	74	14
20	100	0	0	0	6	94	0

Recently, a similar results on AM fungal treatments in teak seedlings produced by tissue culture method have also been reported by Ramanwong and Sangwaint (2000). Teak seedlings inoculated with six species of AM fungi, *Acaulospora scorbiculata, Glomus aggregatum, G. deserticola, G. multicaulis*, and *Sclerocystis microcarpa*, exhibited greater seedling height, diameter at root collar, and seedling biomass than the control seedlings. Recently, improved growth and seedling biomass in teak seedlings inoculated with AM fungi (*Glomus fasciculatum, G. deserticola, G. mosseae, Acaulospora appendicula*) have been reported (Mohanan, 2002). Better growth and seedling performance in teak seedlings inoculated with AM fungi along with a strain of *Azotobacter chroococcum* have been reported (Paroha *et al.*, 2000). Maxiumum root development in seedlings has been recorded either with AM fungi or AM fungi with *Azotobacter*. A similar result has also been recorded by Sugavanam *et al.* (1998) using AM fungi and *Azospirillum* sp. Growth enhancement in teak seedlings in potting mixes inoculated with various combinations of AM fungus (*Glomus macrocarpum*), *Bacillus*

megaterium and *Aspergillus niger* has been reported by Vijaya and Srivasuki (2001a, b). Earlier, Verma and Jamaluddin (1995) have also reported improvement of seedling growth and biomass production by mycorrhization of seedlings using *Glomus fasciculatum* and a mixture of AM fungi. Mixed inoculum of AM fungi has been reported as more effective in boosting the teak seedling growth than the inoculum constituting a single AM fungus. Various researchers have used different AM fungi for enhancement of growth in teak seedlings, however, the selection of AM fungal species for the experiment was mostly based on the recorded performance of the particular AM fungi in other host plants. In the present study, under glasshouse conditions, AM fungi like, *Glomus fasciculatum* and *G. mossease* gave very good results on seedling growth and biomass production. Under usual nursery conditions using weed compost as the growing medium in root trainers, all the AM fungi and AM fungal combinations used as inoculum gave good results. Among these *Glomus mosseae* was the most efficient inoculum which gave maximum seedling height and MIE%. AM fungal mixed inoculum was also found equally effective in boosting the seedling growth as well as improving the seedling quality.

3.7. Pilot-scale field trial - 2004

A pilot-scale field planting of teak root trainer seedlings from different AM fungal treatments was carried out at Vembooram, Kodanad Forest Range, during the first week of June 2004. Sixty seedlings from each of the twenty treatments were planted out in three replicate plots (Fig. 27). A total of 1200 seedlings were planted in 60 sub-plots at 2.5 m x 2.5 m spacement. After completion of the planting operation, measurements on plant height and collar diameter were recorded from all the treatments. Weeding around the plants was carried out after one month of planting. Measurements on growth parameters like height, collar diameter and number of leaf pairs were recorded after three months (August 2004) and nine months (March 2005) of planting.

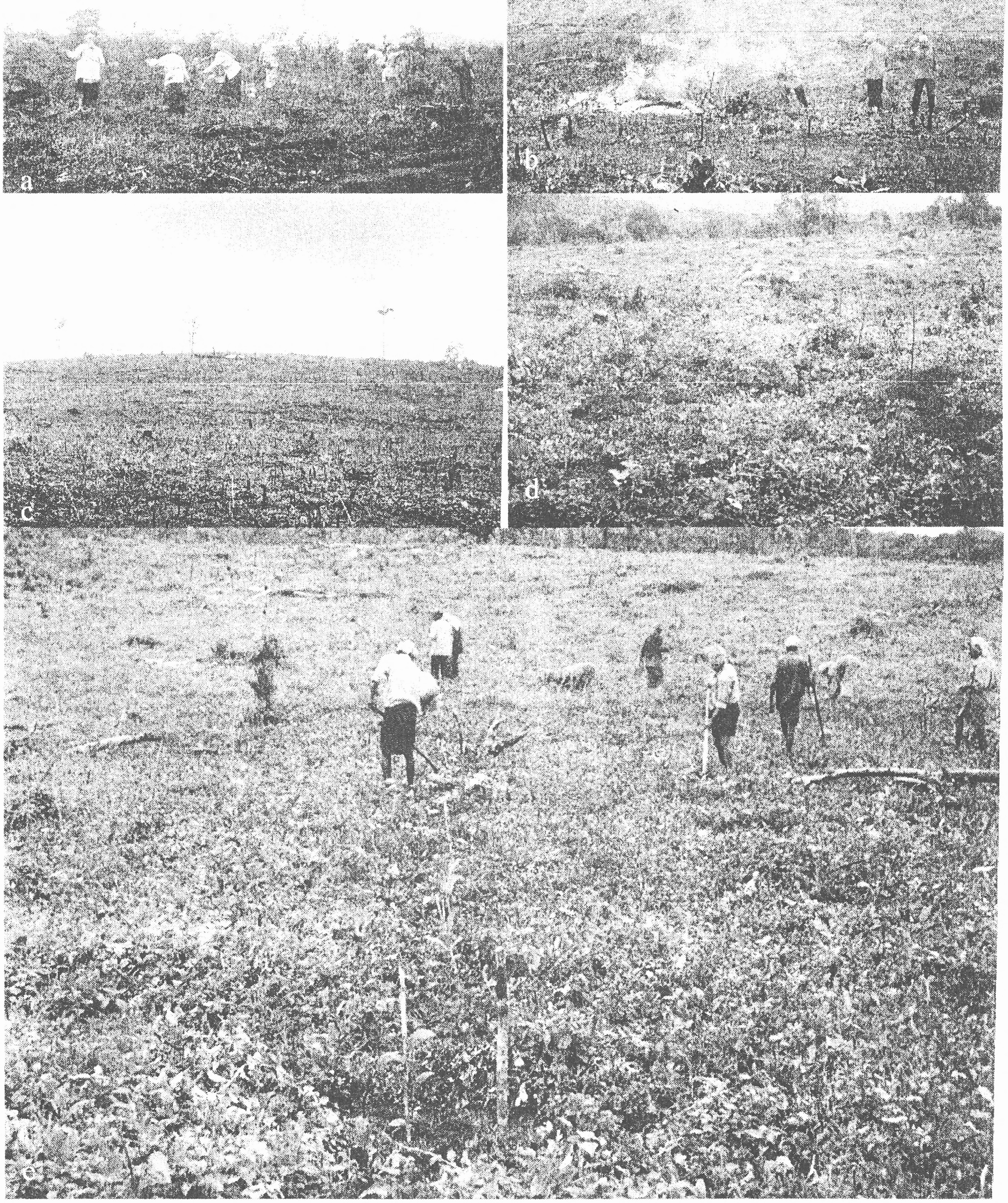


Fig.27: Preparation of the planting site at Vembooram, Kodanad. a: weeding , b: burning and site preparation, c: alignment of the plot and preparation of planting pits, d: planting operation in progress.

During the early field establishment phase (after 3 months of field planting), no significant difference in plant height and collar diameter among the treatments was observed (Tables 29,30; Fig. 28). Height growth was almost uniform except in Treatment Nos.2, 3, and 17; maximum height was recorded in plants mycorrhized with *Glomus fasciculatum* and in Treatment with *G. fasciculatum* + *G. mosseae*; lowest height was recorded in treatment No. 18 (*G. botryoides* + *G. fasciculatum*). Collar diameter of plants from various treatments also showed similar trend; highest collar diameter was recorded in Treatment No. 18 with *Glomus botryoides* + *G. fasciculatum*2 and lowest collar diameter was recorded in Treatment No. 11 with *Acaulospora scorbiculata*. Full stock of plants in all the 60 sub-plots in the trial plot was recorded during this period.

Table 29: ANOVA of data on collar diameter of plants (3 months after planting) in various treatments in the trial plots

	Type III Sum of squares	df	Mean square	F	Sig.
Corrected model	0.297 [a]	21	1.415E-02	1.157	0.339
Intercept	69.738	1	69.738	5700.765	0.000
Treatment	0.289	19	1.520E-03	1.243	0.277 [ns]
Replication	8.355E-03	2	4.178E-03	0.341	0.713 [ns]
Error	0.465	38	6.582E-02		
Total	70.500	60			
Corrected total	0.762	59			

*R squared = 0.390 (Adjusted R squared = 0.053)

Table 30: ANOVA of data on height of plants (3 months after planting) in various treatments in the trial plots

	Type IIISum of squares	df	Mean square	F	Sig.
Corrected model	1038.554[a]	21	49.455	2.037	0.028 [ns]
Intercept	121003.798.23	1	121003.798	4985.134	0< 0.001
Treatment	842.116	19	44.322	1.826	0.056 [ns]
Replication	196.437	2	98.219	4.046	0.026
Error	922.371	38	24.273		
Total	122964.723.17	60			
Corrected total	1960.925	59			

*R squared = 0.530 (Adjusted R squared = 0.270)

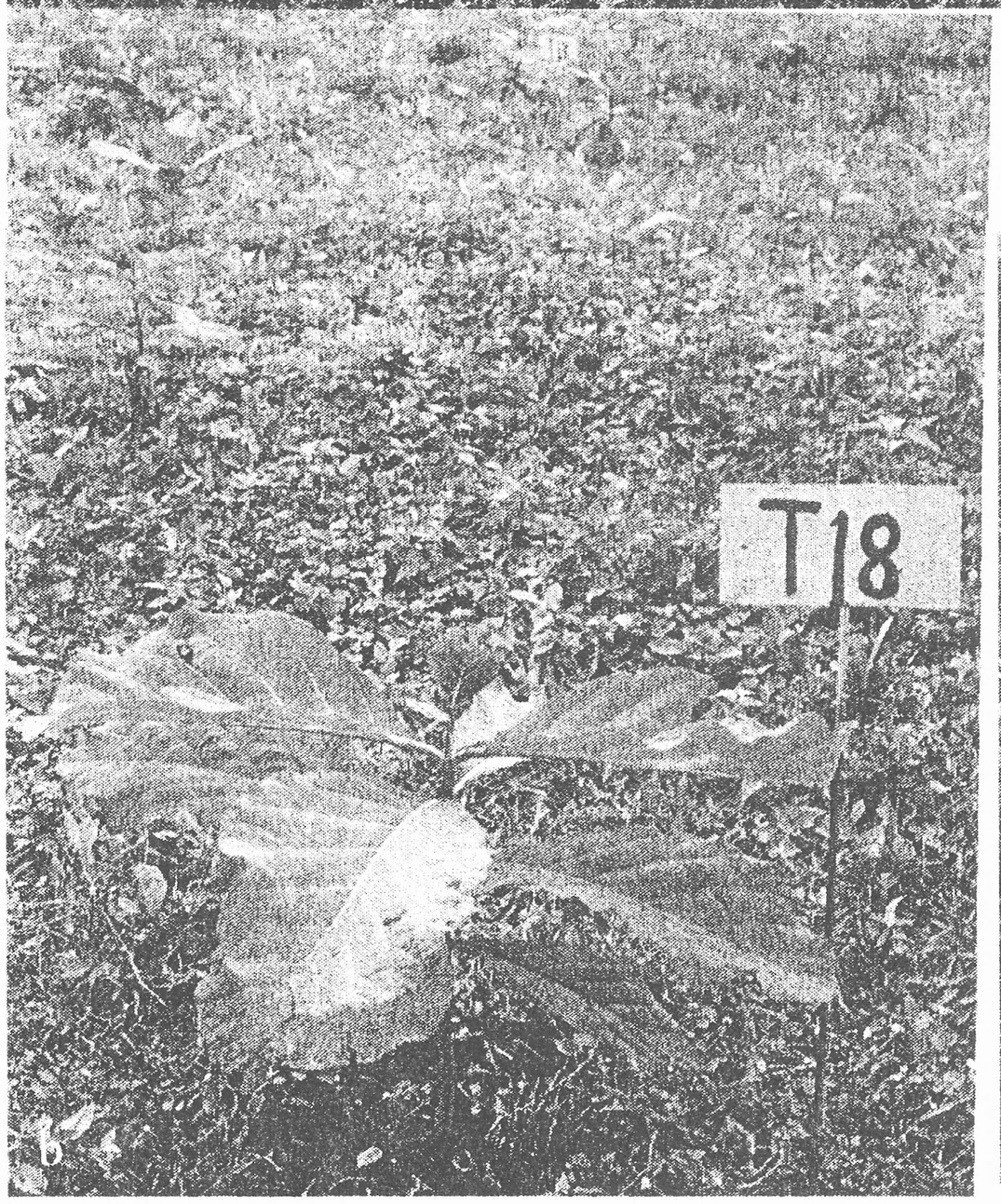


Fig.28: a - c: A general view of the pilot-scale trial plot three months after planting.

Growth measurements recorded after 9 months of field planting showed significant differences among various treatments (Tables 231,32; Figs. 29,30). Treatment No. 16 with *Acaulospora appendicula*1 + *G. fasciculatum*2 showed the maximum height growth of 122.76 cm followed by Treatment No. 7 with *G. mosseae2*. Treatments Nos. 11,12,13, 14,15 and 17 exhibited lower mean height than the control plants (Treatment No. 20). Lowest mean height was recorded in Treatment No. 14 inoculated with *S. heterogama*. Plants in Treatment Nos. 5 and 18 showed mean height of 113.33 cm and 114.02 cm respectively (Table 33). Among the 20 treatments including control, five treatments, *viz*. Treatment Nos. 1,3,7,16 and 18 showed better height growth than the other treatments (Table 33; Figs. 31-35).

Collar diameter of plants taken after 9 months of field planting also showed similar results. Highly significant difference among the treatment was observed (Table 33; Figs. 31-35). Maximum collar diameter of 2.673 cm was recorded in Treatment No. 18 with *G. botryoides* + *G. fasciculatum*2 inoculum. Treatment Nos. 1 (*G. botryoides*), 3 (*G. fasciculatum*2), and 16 (*A. appendiculata*1 + *G. fasciculatum*2) exhibited almost same mean collar diameter and fall in the same group in DMRT. Treatment No. 7 (*G. mosseae*2) with mean collar diameter of 2.407 cm was also found one of the promising treatments. Another interesting observation recorded was on the occurrence of casualty during the draught period (February-March). Treatment No. 20 (control) recorded maximum percent of casualty (2%) among the treatments.

Many plants in the control blocks showed more or less complete defoliation during the draught period (February-March). However, plants in all the other treatments exhibited resistance against draught and defoliation registered was negligible. Even plants in treatments with lower height and collar diameter exhibited comparatively better resistance against draught and defoliation.

The analyses of data on growth parameters and performance of plants in the field during the past nine months revealed that mycorrhization of seedlings has improved the growth of plants in the field and facilitated in reducing the casualty during the dry period.

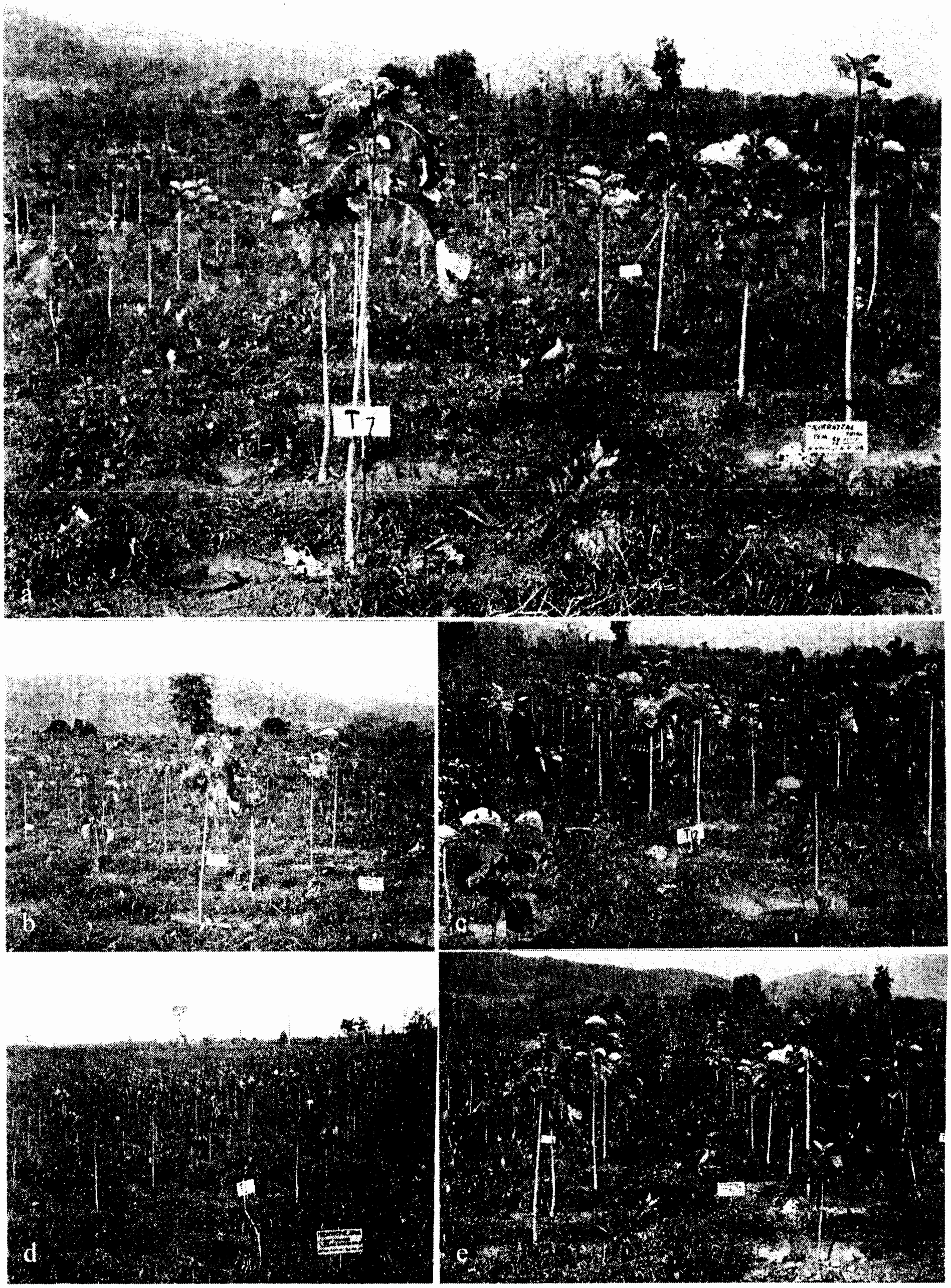


Fig.29: A view of trial plot during dry period (March 2005); recording height and girth measurements of teak plants (9 months after planting).

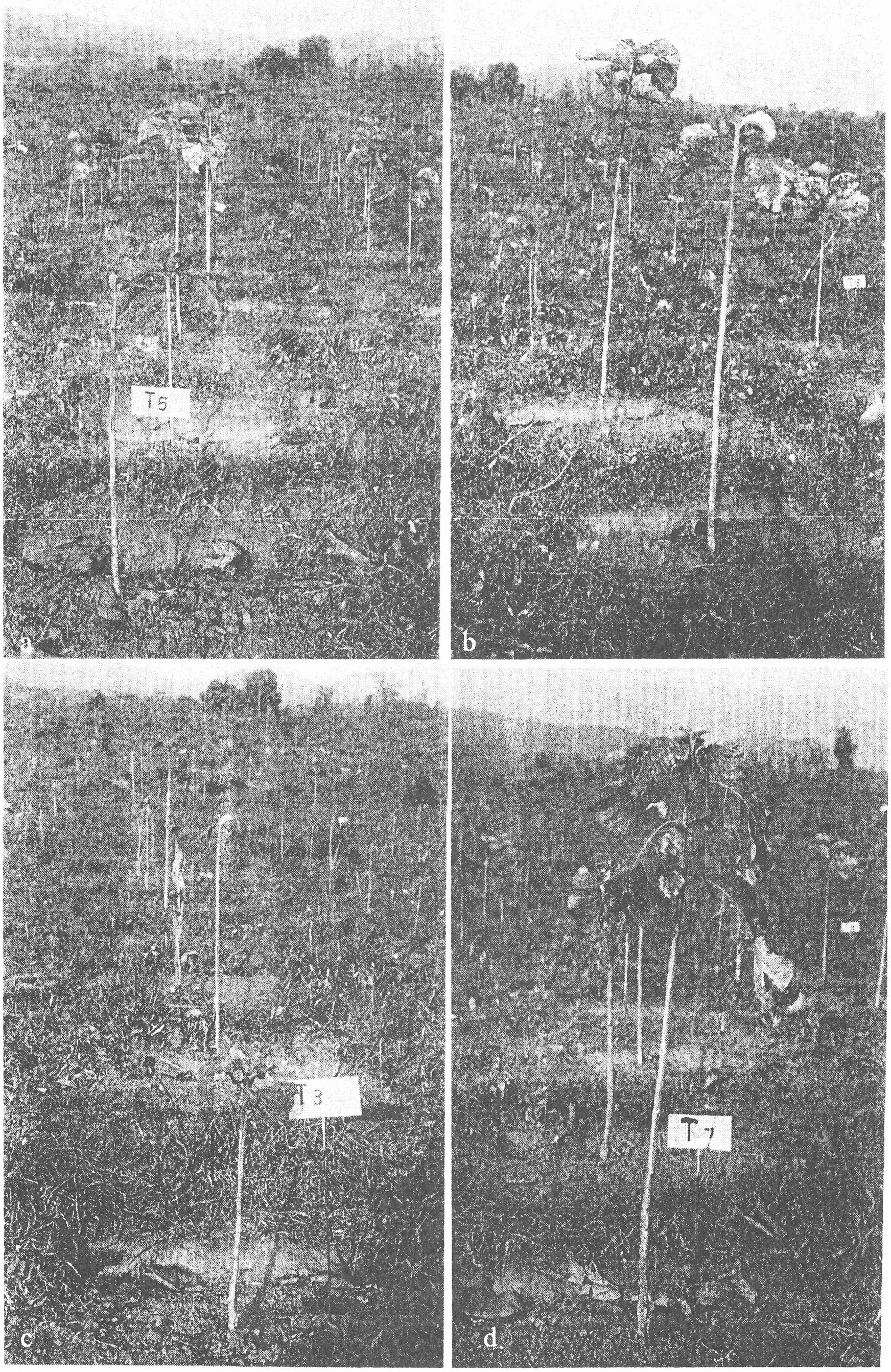


Fig.30: AM fungi treated teak plants after nine months of field planting (photo taken during the dry period-March 2005).

Among various AM inocula tried, *Glomus fasciculatum* mixed with *Acaulospora appendicula* (Treatment No. 16) and *Glomus fasciculatum* mixed with *Glomus botryoides* (Treatment No. 18) gave maximum height growth and collar diameter in plants. Inoculum of *Glomus fasciculatum*2 (Treatment No. 3) and *Glomus botryoides* (Treatment No. 1) and *Glomus mosseae*2 (Treatment No. 7) applied singly also yielded better growth in treated plants than in control. Observations from the trial plantation was recorded during the first week of June 2005 (wet period) and found that all the plants in AM fungi treated plots are performing better than the control plots in terms of plant height and vigour (Figs. 36,37).

Table 31: ANOVA of data on collar diameter of plants in various treatments in the trial plots (9 months after planting)

	Sum of squares	df	Mean square	F	Sig.
Corrected model	8.753 [a]	21	0.417	6.333	< 0.001 **
Intercept	263.132	1	263.132	3998.050	< 0.001 **
Treatment	7.415	19	0.390	5.929	< 0.001 **
Replication	1.338	2	0.669	10.166	< 0.001 **
Error	2.501	38	6.582E-02		
Total	274.386	60			
Corrected total	11.254	59			

* R squared = 0.778 (Adjusted R squared = 0.655)

Table 32: ANOVA of terminal data on height of plants in various treatments in the trial plots

	Sum of squares	df	Mean square	F	Sig.
Corrected model	16731.028[a]	21	796.716	4.265	< 0.001 **
Intercept	544424.237	1	544424.237	2914.678	< 0.001 **
Treatment	15275.332	19	803.965	4.304	< 0.001 **
Replication	1455.697	2	727.848	3.897	0.029 **
Error	7097.909	38	186.787		
Total	568253.175	60			
Corrected total	23828.937	59			

* R squared = 0.702 (Adjusted R squared = 0.538)

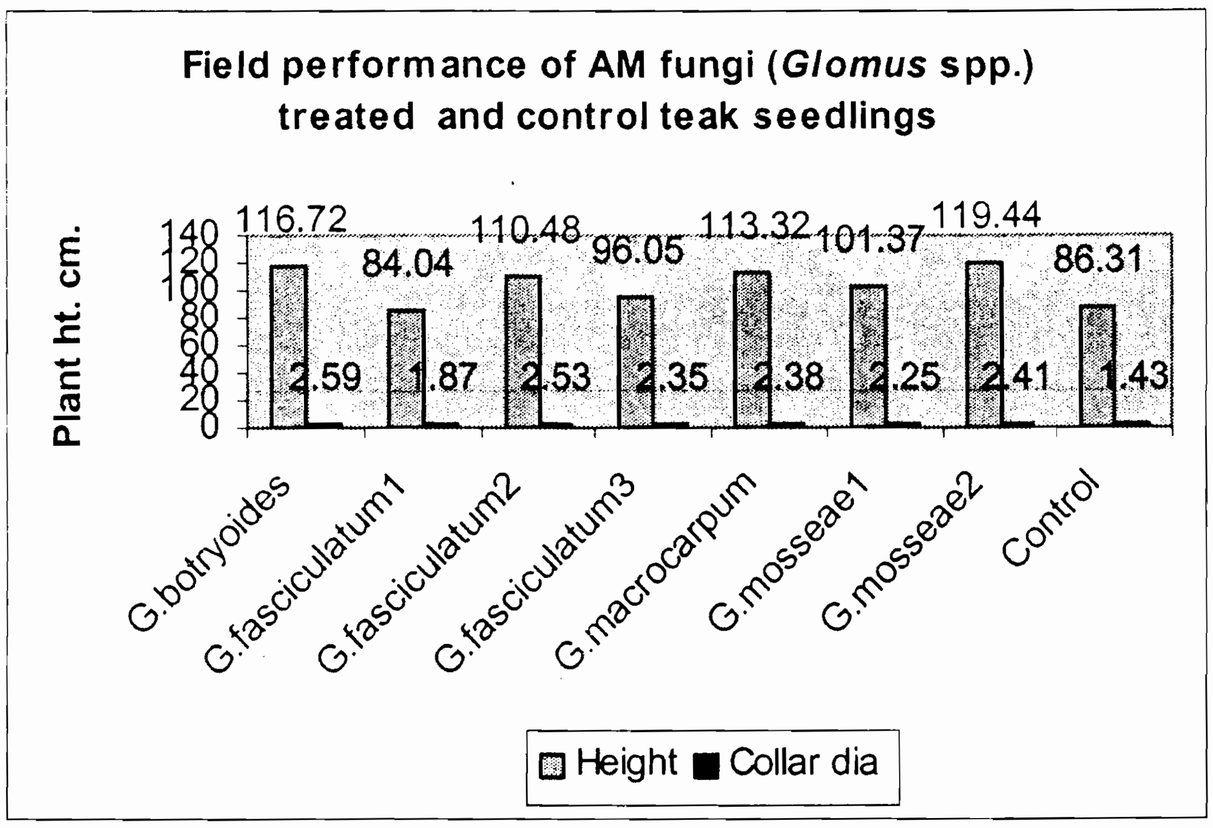


Fig. 31: Field performance (height and collar dia) of different *Glomus* spp. treated planting stock

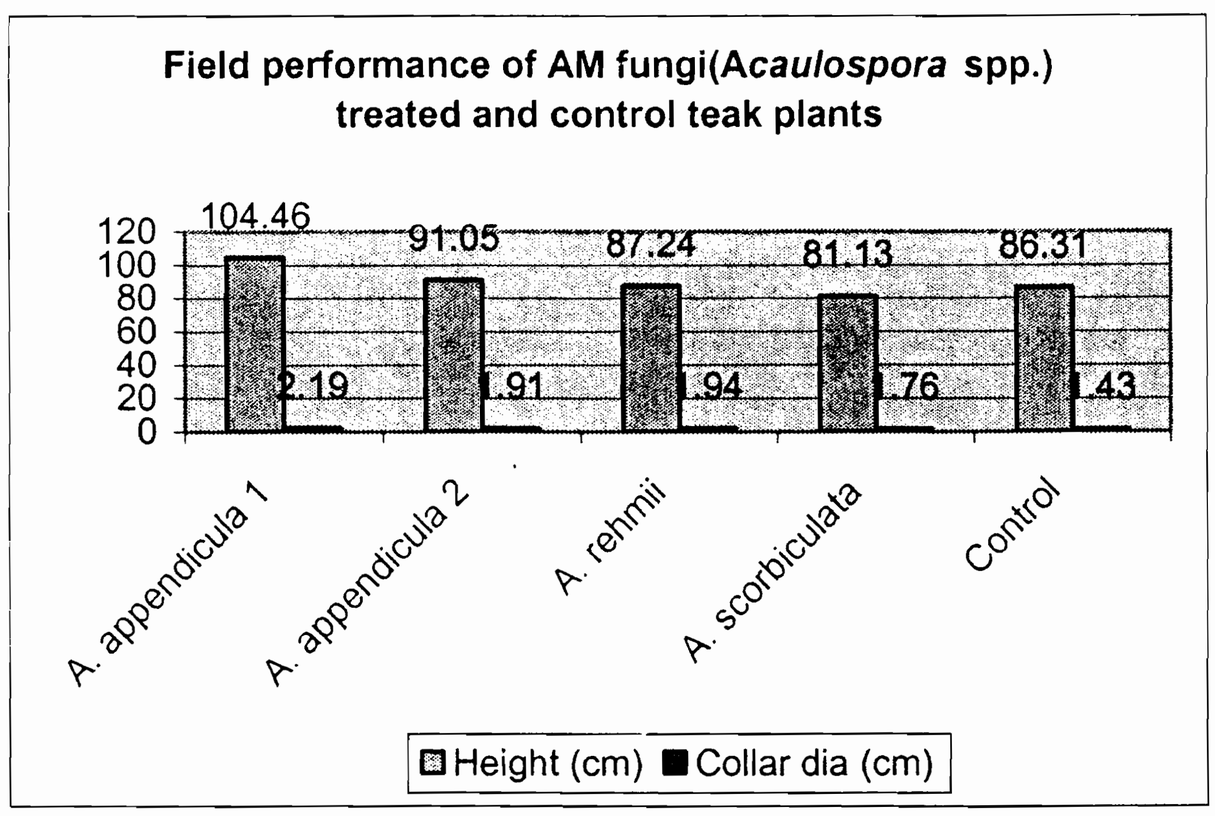


Fig. 32: Field performance (height and collar dia) of different *Acaulospora* treated planting stock

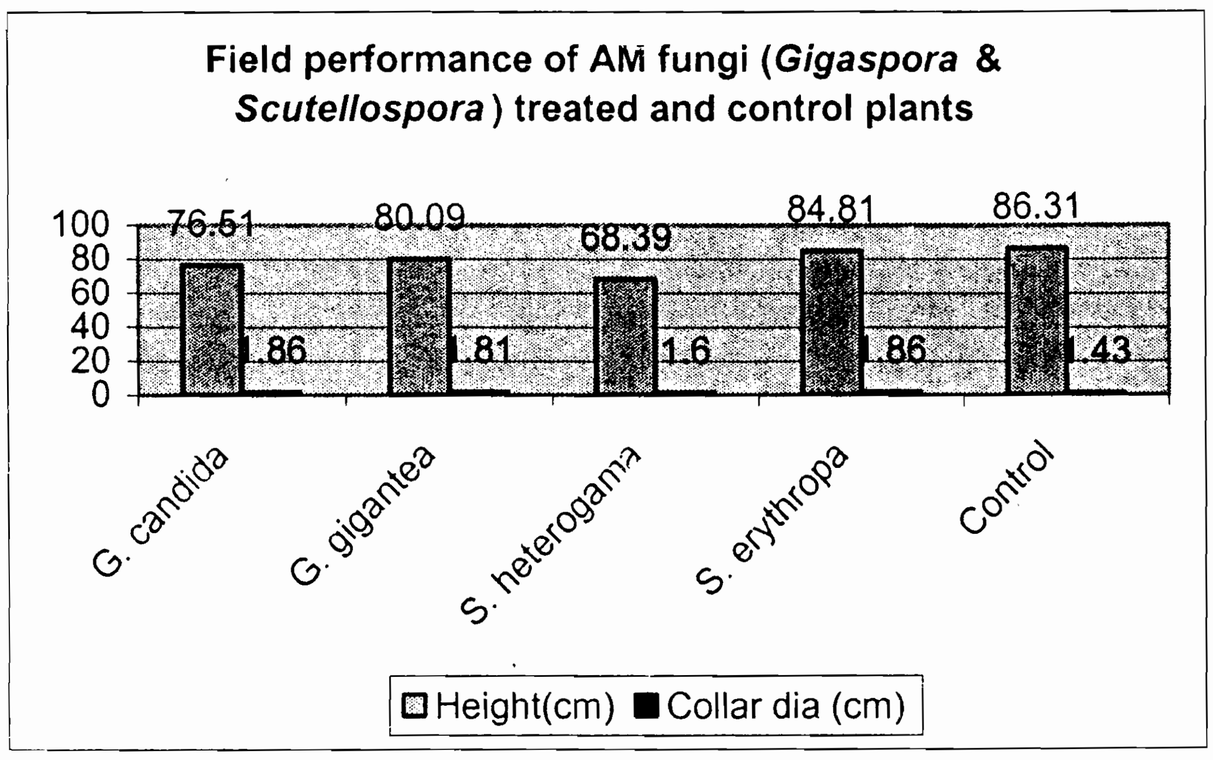


Fig. 33: Field performance of different *Gigaspora* and *Scutellospra* treated planting stock

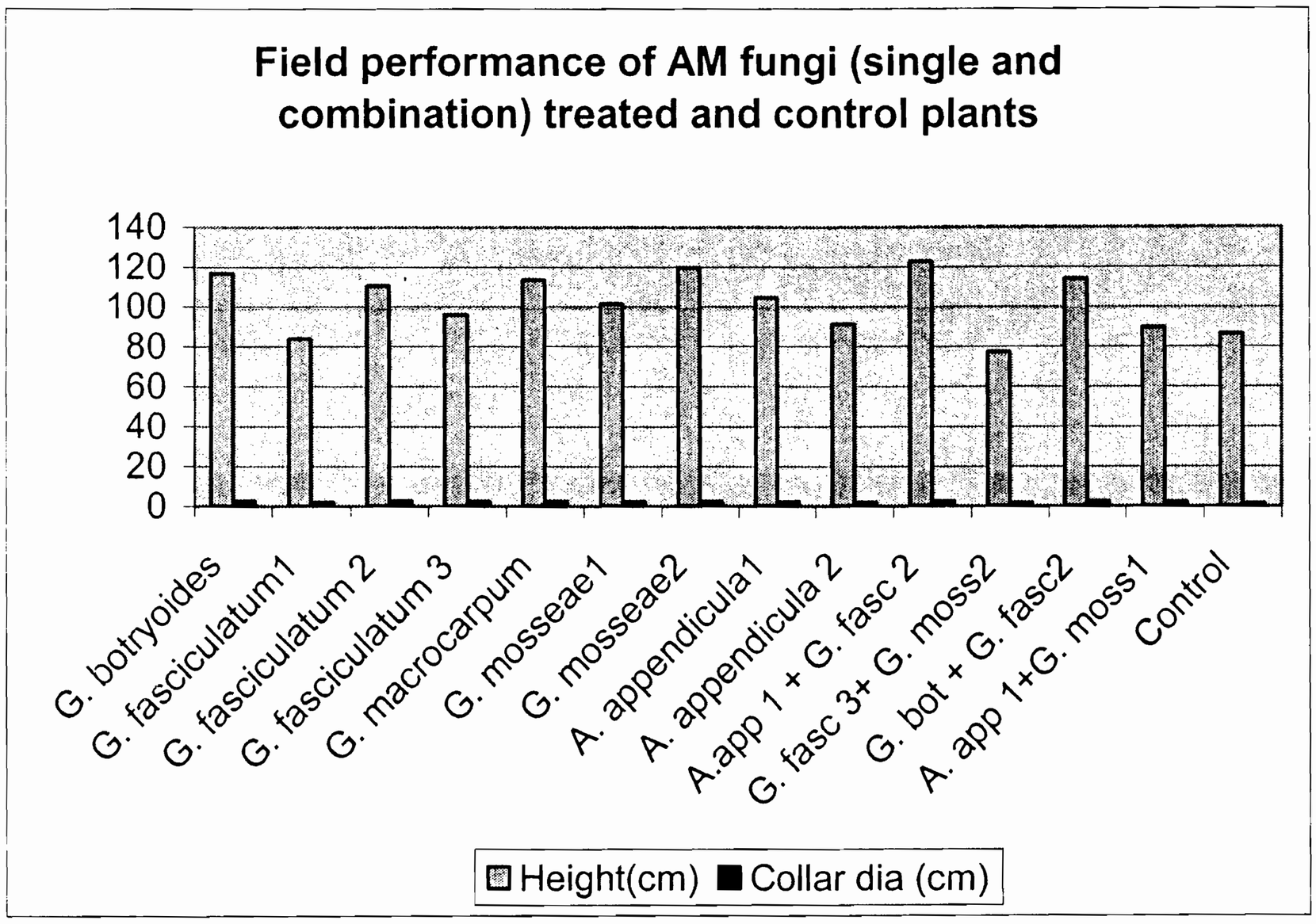


Fig. 34: Field performance of teak plants treated with AM fungi singly or in combination

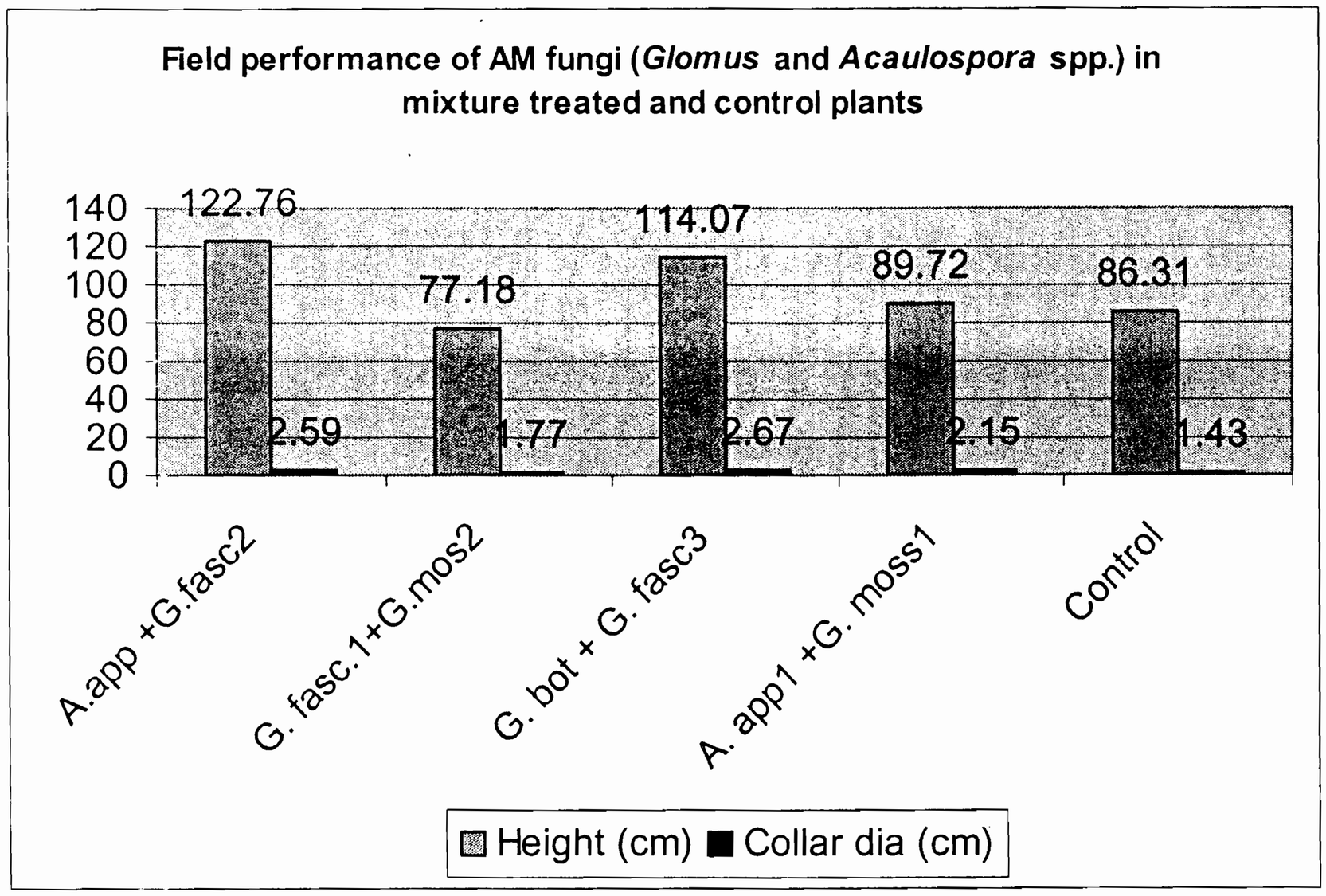


Fig. 35: Field performance of *Glomus* and *Acaulospora* mixed inoculum treated planting stock

Table 33: Height and collar diameter of plants 9 months after field planting

Treatment No.	AM Fungal inoculum	Mean height of plants (cm)*	Mean collar dia of plants(cm)*
T1	*G. botryoides*	116.723 ^{fgh} (7.891)	2.587 ^{gh} (0.148)
T2	*G. fasciculatum*1	84.040 ^{abcd} (7.891)	1.870 ^{abcd} (0.148)
T3	*G. fasciculatum*2	110.480 ^{defgh} (7.891)	2.533 ^{gh} (0.148)
T4	*G. fasciculatum*3	96.053 ^{bcdefg} (7.891)	2.347 ^{defgh} (0.148)
T5	*G. macrocarpum*	113.327 ^{efgh} (7.891)	2.373 ^{efgh} (0.148)
T6	*G. mosseae*1	101.370 ^{bcdefgh} (7.891)	2.250 ^{cdefgh} (0.148)
T7	*G. mosseae*2	119.443 ^{gh} (7.891)	2.407 ^{fgh} (0.148)
T8	*A. appendicula*1	104.457 ^{cdefgh} (7.891)	2.197 ^{cdefgh} (0.148)
T9	*A. appendicula*2	91.050 ^{abcde} (7.891)	1.910 ^{abcde} (0.148)
T10	*A. rehmii*	87.243 ^{abcd} (7.891)	1.937 ^{bcdef} (0.148)
T11	*A. scorbiculata*	81.133 ^{abc} (7.891)	1.757 ^{abc} (0.148)
T12	*Gigaspora candida*	76.510 ^{ab} (7.891)	1.857 ^{abcd} (0.148)
T13	*Gigaspora gigantea*	80.097 ^{abc} (7.891)	1.813 ^{abc} (0.148)
T14	*S. heterogama*	68.397 ^{a} (7.891)	1.603 ^{ab} (0.148)
T15	*S. erythropa*	84.810 ^{abcd} (7.891)	1.857 ^{abcd} (0.148)
T16	*A. appendicula*1 +*G. fasciculatum*2	122.763 ^{h} (7.891)	2.587 ^{gh} (0.148)
T17	*G.fasciculatum*3 + *G. mosseae*2	77.180 ^{ab} (7.891)	1.773 ^{abc} (0.148)
T18	*G. botryoides* + *G. fasciculatum*2	114.017 ^{efgh} (7.891)	2.673 ^{h} (0.148)
T19	*A. appendicula*1 + *G. mossease*1	89.717 ^{abcde} (7.891)	2.150 ^{cdefg} (0.148)
T20	Control	86.313 ^{abcd} (7.891)	1.433 ^{a} (0.148)

*Mean value of observations from 60 plants in three replications

*Figures given in parenthesis are SE; means of height and collar diameter in each column with superscripts of same letters do not differ significantly

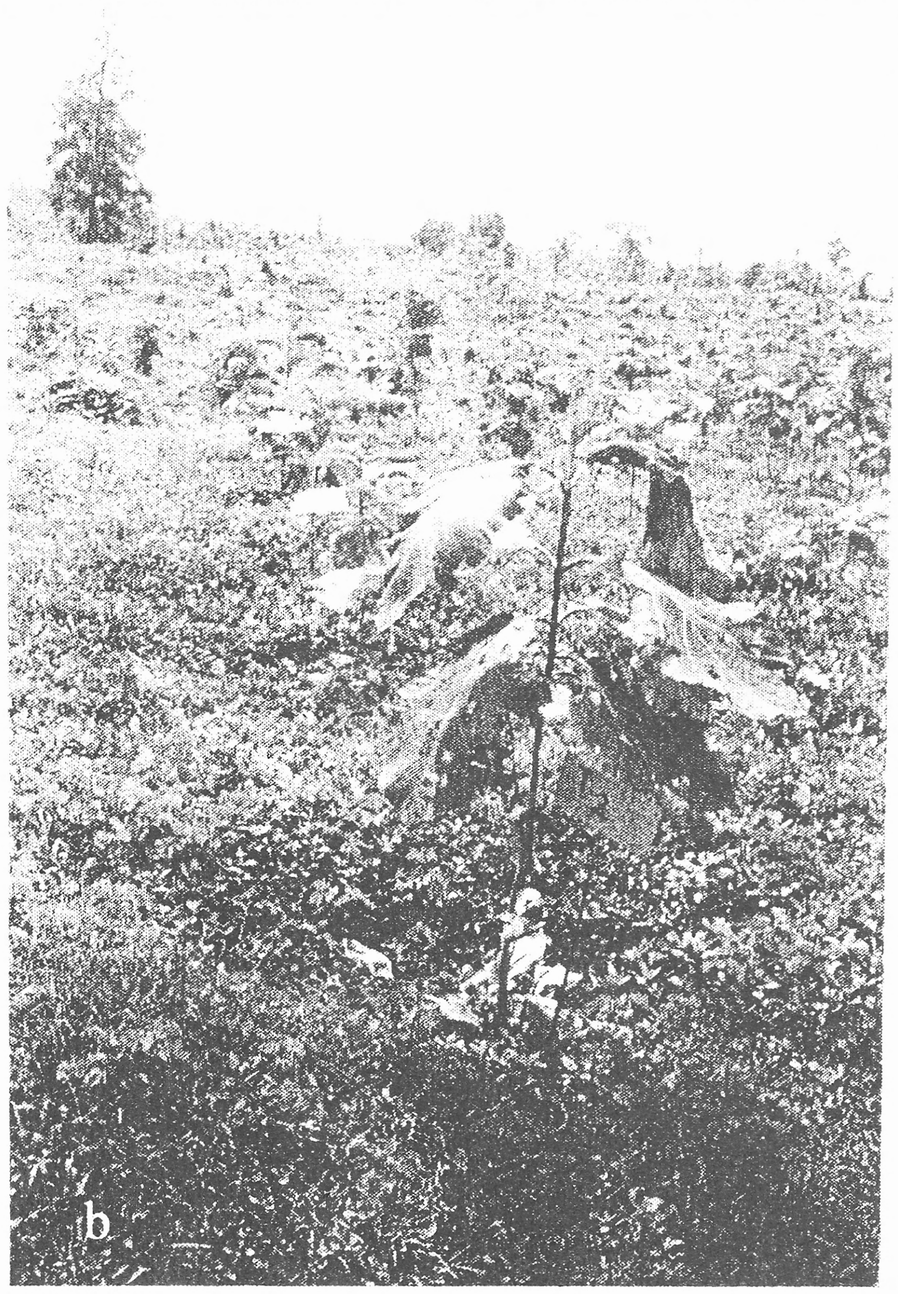
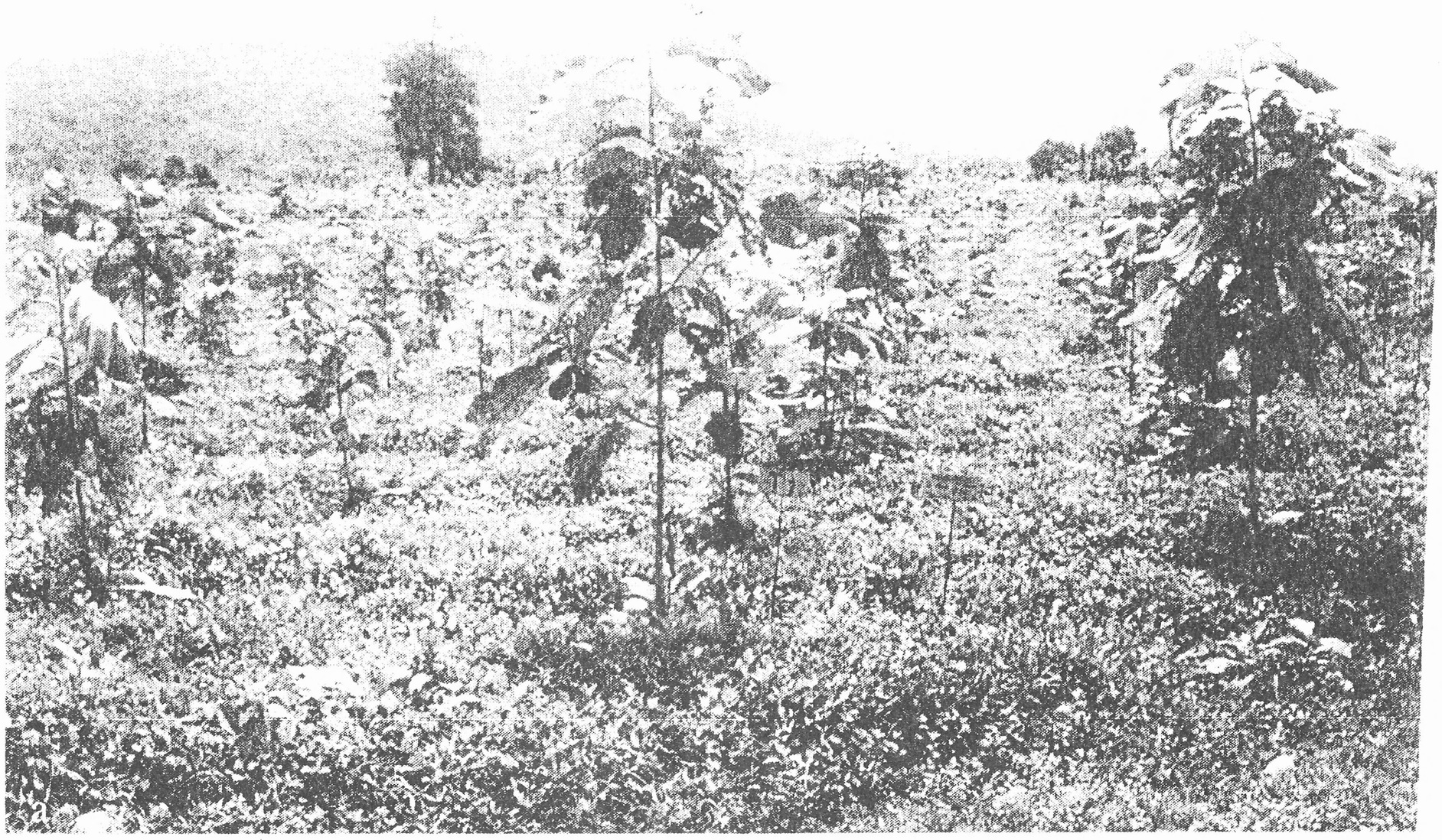
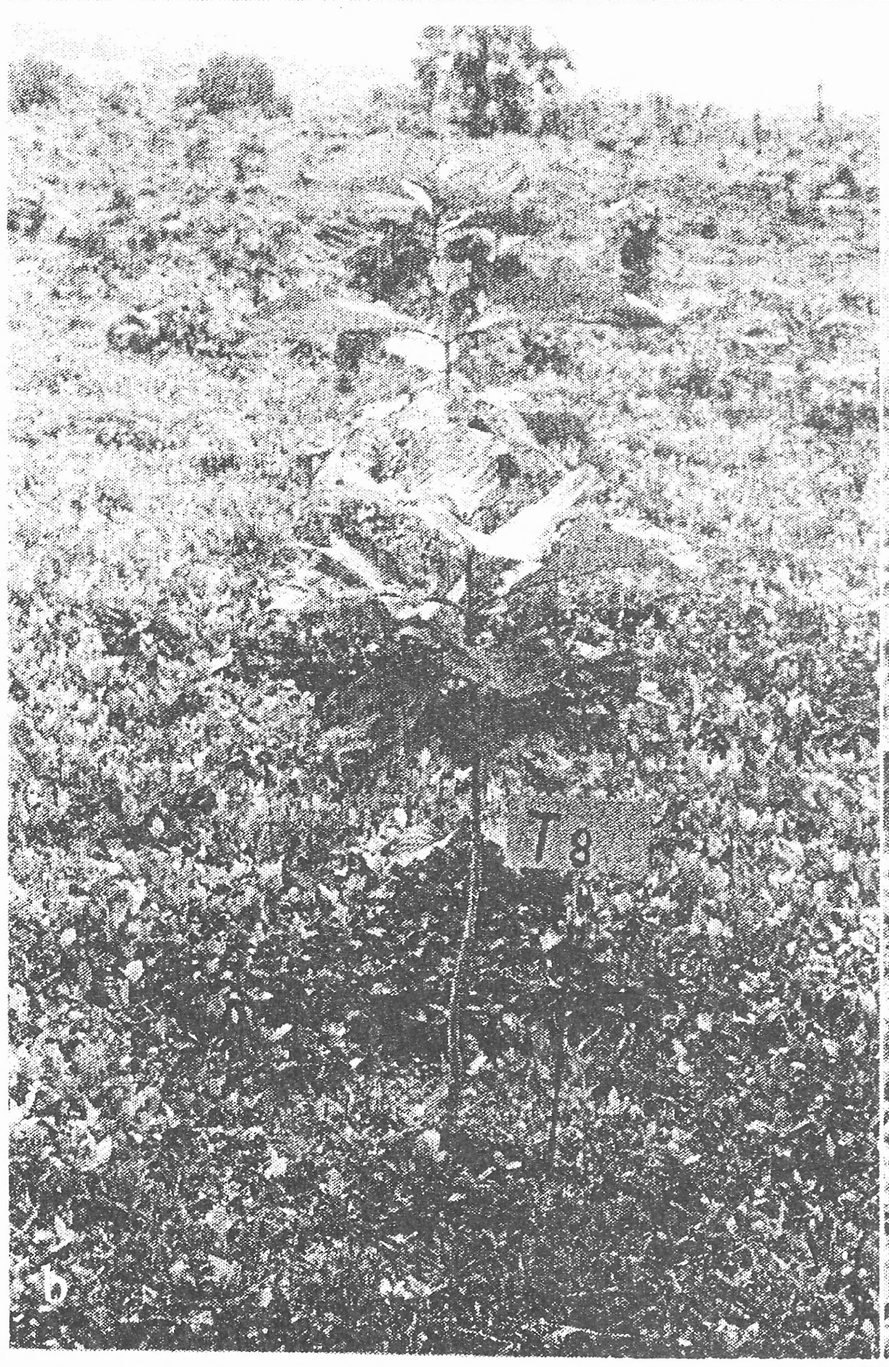


Fig. 36: AM fungal treatments trial plot during wet period: Teak plants after one year of field planting (photograph taken on 2 June 2005).



a



b

Fig.37: AM fungal treatments trial plot during wet period: Teak plants after one year of field planting (photograph taken on 2 June 2005).

4. CONCLUSIONS

Teak root samples collected from fifty nine plantations situated in different eco-climatic zones in the Kerala, Karnataka and Andhra Pradesh States exhibited AM fungal root colonization. The intensity of colonization varied depending on the soil characteristics and age of the trees. Rhizosphere soils collected from the teak plantations in Kerala, Karnataka and Andhra Pradesh States registered a total of 86 Glomalean fungi belonging to six genera. AM fungi belonging to the genera *Glomus* and *Acaulospora* were found widely distributed in teak rhizosphere soils in all the three States studied, while others were of limited distribution. Among 36 *Glomus* species recorded from the teak soils, *G. aggregatum, G. botryoides, G. fasciculatum, G. deserticola,* and *G. mosseae* were the most predominantly distributed ones. The AM fungal spore density in teak rhizosphere soils in the Kerala State ranged from 28 to 276/10 g soil, 50 to 193/10 g soil in the Karnataka State and 62 to 449/10 g soil in the Andhra Pradesh State. Soil samples from teak plantations in all the three States were moderately acidic to near neutral. AM fungal spore density and species composition were found to be influenced by soil characteristics such as soil pH, soil moisture contents and soil micronutrients. Moderately high to very high AM fungal root colonization as well as high AM fungal spore density and diversity of Glomalean species in teak rhizosphere soils in 4 to 50-year-old plantations belonging to different eco-climatic zones in the three States demonstrates the strong AM fungal association and mycorrhizal dependency of teak plants throughout its rotation age.

Glasshouse trial conducted during 2003 employing selected AM fungal inoculum prepared from native soils and applied on teak seedlings raised in root trainers with soil-sand as growing medium yielded improvement on seedling quality in terms of seedling height and seedling biomass production over control. However, no significant difference in chlorophyll fluorescence kinetics was recorded in AM fungi treated seedlings or in control sets. Inoculum of *G. fasciculatum, G. botryoides* and *A. appendicula* were the efficient ones among the 29 treatments tried. Promising mycorrhizal inoculation effect (MIE) was recorded in a few treatments with *G. fasciculatum, G. botryoides* and *A. appendicula*.

Nursery trial carried out under usual forest nursery conditions using weed compost as the growing medium in the root trainers, registered better growth performances in all the AM fungi treated seedlings than the control sets. Treatments with AM fungi singly or in combinations registered better seedling height, seedling collar diameter and seedling biomass than the control seedlings. Among the 19 AM fungal treatments, *Glomus mosseae*2 registered maximum seedling biomass (dry) and MIE%.

The results from glasshouse and nursery trials demonstrate the efficacy of mycorrhization of 10-15 day-old teak seedlings raised in weed compost medium in root trainers by application of inoculum of efficient native AM fungi. The results also substantiates the improvement of quality of teak seedlings within 90 days of their growth in the root trainer nursery by application of inoculum of *Glomus botryoides, G. fasciculatum, G. macrocarpum, G. mosseae, Acaulospora appendicula, A. scorbiculata, Gigaspora gigantea* and *Scutellospora erythropa* singly or in combinations.

Pilot-scale field trial carried out employing the mycorrhized and non-mycorrhized (control) teak seedlings selected from 20 treatments from the nursery trial, registered highly significant differences in growth performances of mycorrhized seedlings in the field than control plants. In general, planting stock pre-colonized by efficient AM fungi in the nursery, exhibited better field performance than the control plants in terms of survival, increment in height and collar diameter of plants and also resistance to draught. Among the 19 AM fungal treatments, inoculum of *G. fasciculatum* mixed with *A. appendicula* or *G. botryoides* recorded maximum plant height, collar diameter and plant vigour. Plants in *A. appendicula* 1 + *G. fasciculatum* treatment recorded maximum mean height of 123 cm with collar diameter of 2.89 cm within 9 months of field planting, while in non-mycorrhized plants (control plants), average plant height and collar diameter were only 86 cm and 1.43 cm respectively.

The nursery and field investigations employing various AM fungi signify that quality of teak planting stock can be improved by mycorrhization employing efficient AM fungi like *G. fasciculatum, G. botryoides, G. mosseae, A. appendicula* singly or in

combinations. The pilot-scale field trial confirmed better field performance of artificially mycorrhized teak planting stock. This boost in growth and vigour of artificially mycorrhized teak plants in the early establishment phase of the plantation may also reflect in health and productivity of the stands in the ensuing years. For further validating the beneficial effect on improvement of planting stock by mycorrhizal manipulations and thereby boosting the stand growth and productivity, large-scale nursery screening and multi-location field trials with long term monitoring are warranted.

5. REFERENCES

Abbott, L.K., Robson, A.D. and Gazey, C. 1992: Selection of inoculant vesicular-arbuscular mycorrhizal fungi. *In*: Methods in Microbiology Vol. 24, J.R. Norris, D.J. Read, A.K. Varma (Eds.), pp. 1-21, Academic Press, London, New York, Tokyo, Toronto.

Bagyaraj, D.J. 1992: Vesicular-arbuscular mycorrhiza: Application in agriculture. *In*: Methods in Microbiology Vol. 24, J.R. Norris, D.J. Read, A.K. Varma (Eds.), pp. 359-373, Academic Press, London, New York, Tokyo, Toronto.

Bagyaraj, D.J.; Reddy, M.S.B.; Nalini, P.A. 1988: Selection of an efficient inoculant VA mycorrhizal fungus for *Leucaena*. Forest *Ecology* and Management 27:791-801.

Balagopalan, M.; Rugmini, M.; Chandbasha, S., 1998: Soil nutrition management for teak plantations of Kerala. KFRI Research Report No. 138, Kerala Forest Research Institute, Peechi, Kerala.

Bhadraiah, B.; Kanakadurga, V.N.; Ramarao, P.; Manoharachary, C., 2002: Effect of AM fungi and rock phosphate on phosphatase activities in *Tectona grandis* L.f. *In:* Frontiers in Microbial Technology and Plant Pathology. C. Manoharachary; D.K. Purohit; S.R. Reddy; M.A.S. Charya; S. Girisham (eds.), pp.259-262, Scientific Publishers, Jodhpur.

Bolan, N.S. 1991: A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil. 134: 169-207.

Bundrett, M.C. 1991: Mycorrhizas in natural ecosystems. *In*: Advances in Ecological Research. A. Macfayden; M. Begon; A.H. Fitter (eds.), pp. 171-313, Academic Press, London.

Chacko, K.C.; Pandalai, R.C.; Balasundaran, M.; Mohanan, C.; Varma, R.V.; Jose Kallarackal; Sujatha, M.P.; Kumaraswamy, S. 2002: Standardization of root trainer technology for *Tectona grandis, Eucalyptus tereticornis, E. grandis, E. globulus, E. camaldulensis, Acacia auriculiformis, A. mangium* and *Paraserianthes falcataria*. KFRI Research Report No. 229. Kerala Forest Research Institute, Peechi, Kerala.

Chandra, K.K.; Jamaluddin 1999. Dynamics of VAM infectivity and their population under different seasons in teak and bamboo plantations of Chandrapur (M.S.). Vaniki Sandesh 23 (1):16-20.

Chandra, K.K.; Ujjaini, M.M., 2002: Interactions of AMF with three levels of soil organic matter and their influence on seedling biomass and root infection of six forest species. Myforest 38(2):155-161.

Chong, L., 1988: Occurrence of mycorrhizae in seedlings of some tree species in Sarawak. *In*: Mycorrhiza for Green Asia. Proc. 1st Asian Conference on Mycorrhizae, Madras, 29-31, January 1988. A. Mahadevan; N. Raman; K. Natarajan (eds.), pp. 70-72. University of Madras, Chennai, Tamil Nadu.

Chundamannil, M. 1998: Teak plantations in Nilambur: An economic review. KFRI Research Report No. 144, Kerala Forest Research Institute, Peechi, Kerala.

Coster, C., 1921: Mycorrhiza in some of our trees, particularly that of teak. Tectona 14: 563-575.

Dadwal, V.S.; Soni, K.K.; Jamaluddin, 1986: Rhizosphere microflora of teak (*Tectona grandis*). Indian Journal of Forestry 9(1): 59-62.

Daft, M.J.; Nicolson, T.H.1969: Effect of Endogone mycorrhiza on plant growth II. Influence of soil phosphate on endophyte and host in maize. New Phytol. 68: 945-952.

Dhar, P.P.; Mirdha, M.A.U., 2005: Status of colonization and spore population of Arbuscular mycorrhizal fungi in *Tectona grandis* L.f. from Bangladesh. *In*: Quality Timber Products of Teak from Sustainable Forest Management. K.M.

'Bhat; K.K.N. Nair; K.V. Bhat; E.M. Muralidharan; J.K. Sharma (eds.), pp. 485-90, Kerala Forest Research Institute, Peechi, Kerala, India.

Durga, V.V.K. and Gupta, S. 1995. Effect of vesicular arbuscular mycorrhizae on the growth and mineral nutrition of teak *(Tectona grandis* Linn. F.). Indian Forester 121 (6): 518-527.

Faber, B.A.; Zasoski, R.J.; Barau, R.G.; Uriu, K. 1990: Zinc uptake by corn affected by vesicular arbuscular mycorrhizae. Plant and Soil. 129: 121-131.

Feldmann, F. and Idczak, E. 1992. Inoculum production of vesicular-arbuscular mycorrhizal fungi for use in tropical nurseries. *In:* Methods in Microbiology Vol. 24, J.R. Norris, D.J. Read, A.K. Verma (Eds.), pp. 339-357, Academic Press, London, New York, Tokyo, Toronto.

Gerdemann, J.W.; Nicolson, T.H.1963: Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46:235-244.

Gong, M.Q.; Wang, F.Z.; Chen, Y.; Liang, K.N., 2002: Research on mycorrhiza of *Tectona grandis* and its effect on seedling growth. Forest Research 15(5):515-520.

Gurumurthy, S.B.; Sreenivasa, M.N., 1998: Screening and selection of efficient VA mycorrhizal fungus for teak *(Tectona grandis* L.f.). Karnataka Journal of Agricultural Sciences 11(4):956-960.

Gurumurthy, S.B.; Sreenivasa, M.N., 2000: Occurrence and distribution of VAM fungi in the rhizosphere of five tree species grown under agroforestry system. Environment and Ecology 18(2): 500-502.

Hayman, D.S. 1983: The physiology of vesicular arbuscular endomycorhizal symbiosis. Canad. J. Bot. 61:944-963.

Hayman, D.S.; Mosse, B. 1971: Plant growth responses to vesicular-arbuscular mycorrhizae. I. Growth of *Endogone* inoculated plants in phosphate deficient soils. New Phytol. 70:19-27.

Hefferman, B. 1985: A Handbook of Methods of Inorganic Chemical Analysis for Forest Soil, Foliage and Water. CSIRO Division of Forestry Research, Canbera, Australia, 94p.

Hefferman, B. 1985: A Handbook of Methods of Inorganic Chemical Analysis for Forest Soil, Foliage and Water. CSIRO Division of Forestry Research, Canbera, Australia, 94p.

Jamaluddin; Chandra, K.K., 1997: Distribution of VAM fungi in bauxite mine overburden plantation of Amarkantak (Madhya Pradesh). Indian Forester 123(5):412-418.

Keeney, D.R. 1980: Prediction of soil nitrogen availability in forest ecosystem: A literature review. Forest Science 26: 159-171.

Kormanik, P.P.; Mc Graw, A.C. 1982: Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In:* Methods and Principles of Mycorrhizal Research. N.C. Schenck (ed.), pp. 37-45, The American Phytopathological Society, St. Paul.

Kothari, S.K.; Marschner, H.; Romheld, V. 1990: Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize in calacarious soil. New Phytol. 116: 637-645.

Li, X.L.; Marschner, H.; Romheld, V. 1991: Acquisition of phosphorus and copper by VA mycorrhizal hyphae and root to shoot transport in white clover. Plant and Soil. 136: 49-57.

Menge, J.A.; Johnson, E.L.V.; Plat, R.G. 1978: New Phytol. 81: 553-559.

Mohanan, C. 2002: Mycorrhizal application to root trainer seedlings. *In*: Standardization of root trainer technology for *Tectona grandis, Eucalyptus tereticornis, E. grandis, E. globulus, E. camaldulensis, Acacia auriculiformis, A. mangium* and *Paraserianthes falcataria*. KFRI Research Report No. 229, pp. 189-226. Kerala Forest Research Institute, Peechi, Kerala.

Mohanan, C., 2003: Mycorrhizae in forest plantations: Association, diversity and exploitation in planting stock improvement. KFRI Research Report No. 252, Kerala Forest Research Institute, Peechi, Kerala, 133p.

Mohanan, C.; Sheeba, K.K., 2005: Productivity of teak stands in Kerala: Role of arbuscular mycorrhizal association and diversity of AM fungi. *In*: Quality Timber Products of Teak from Sustainable Forest Management. K.M. Bhat; K.K.N. Nair; K.V. Bhat; E.M. Muralidharan; J.K. Sharma (eds.), pp. 472-484. Kerala Forest Research Institute, Peechi, Kerala, India.

Morton, J.B. 1993: Problems and solutions for integration of Glomalean taxonomy, systematic biology and study of endomycorrhizal phenomena. Mycorrhiza 2:97-109.

Morton, J.B.; Benny, G.L. 1990: Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new Order, Glomales, two new Sub-Orders, Glomineae and Gigasporineae and two new families Acaulosporaceae, Gigasporaceae, with emendation of Glomaceae. Mycotaxon 37: 471-491.

Mosse, B. 1973: Advances in the study of vesicular arbuscular mycorrhizae. Annual Review of Phytopathology 11:171.

Paroha, S.; Chandra, K.K.; Tiwari, K.P., 2000: Synergistic role of VAM and *Azotobacter* inoculation on growth and biomass production in forestry species. Journal of Tropical Forestry 16(1):13-21.

Philips, J.M. and Hayman, D.S. 1970: Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. mycol. Soc. 55: 158-161.

Rajan, S.K.; Reddy, B.J.D.; Bagyaraj, D.J., 2000: Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecology and Management 126(2):91-95.

Raman, N.; Nagarajan, N.; Sambanandan,K.; Gopinathan, S., 1997: Vesicular arbuscular mycorrhizal association with teak plantations in Yercaud Hills, Tamil Nadu, India. In: Teak. S. Chand Basha; C. Mohanan; S. Sankar (eds.), Kerala Forest Department, Thiruvananthapuram; Kerala Forest Research Institute, Peechi, Kerala, India.

Ramanwong, K.; Sangwaint, U., 2000: Effect of vesicular arbuscular mycorrhizal fungi on the growth of teak seedlings. *In*: Biotechnology Applications for Reforestation and Biodiversity Conservation. M.S. Bista; B.B. Joshi; S.M. Amatya; A.V. Parajuli; M.K. Adhikari; H.K. Saju; R. Thakur; K. Suzuki; K. Ishii (eds.).pp.119-122. BIORIFOR, Ministry of Forests and Soil conservation, Kathmandu, Nepal,

Rayment, G.I.; Higginson, F.R. 1992: Australian Laboratory Handbook of Soil and Water chemical Methods. Intake Press, Melbourne, Sydney, Australia, 268p.

Read, D.J. 1991: Mycorrhizas in ecosystems. Experimenta 46: 367-390.

Schenck, N.C. and Perez, Y. 1990. Manual for Identification of VAM Fungi. 3rd. Edn. Synergistic Publ., Gainesville, Florida.

Seth, S.K.; Khan, M.A.W., 1958: Regeneration of teak forests. Indian Forester 84:455-466.

Subramanian, K.; Mandal, A.K.; Rambabu, N.; Chundamannil, M.; Nagarajan, B., 2000: Site, technology and productivity of teak plantations in India. *In*: Site, Technology and Productivity of Teak Plantations. T. Enters, C.T.S. Nair (eds.), pp.51-68. FORSPA Publication No. 124/2000, FAO, Bangkok. .

Sugavanam, V.; Udaiyan, K.; Devraj, P., 1998: Selection of an efficient vesicular arbuscular mycorrhizal fungi and *Azospirillum* sp., for inoculating Tectona grandis. Indian Journal of Forestry 21(4):281-284.

Talukdar, N.C.; Thakuria, D., 2001: Diversity and importance of vesicular arbuscular mycorrhizal fungi in teak (*Tectona grandis*) and gomar (*Gmelina arborea*) plantations of Assam. *In*: Tropical Forestry Research : Challenges in New Millenium. R.V. Varma;K.V.Bhat; E.M. Muralidharan; J.K. Sharma (eds.), pp. 177-182. Kerala Forest Research Institute, Peechi, Kerala, India.

Tewari, D.N., 1991: A Monograph on Teak (*Tectona grandis* L.f.). International Book Distributers, Dehra Dun, India, 479p.

Tommerup, I.C.; Sivasithambaran, K. 1990: Zygospores and asexual spores of *Gigaspora decipiens* – an arbuscular mycorrhizal fungus. Mycological Research 94: 897-900.

Verma, P.K.; Kumar, P.; Ansari, S.A., 2001: Comparative physiomorphological performance of half-sib seedlings of ten teak clones under suboptimal and optimal arbuscular mycorrhizal colonization. Journal of Tropical Forest Science 13(3): 423-433.

Verma, R.K.; Jamaluddin, 1995. Association and activity of arbuscular mycorrhizae of teak (*Tectona grandis*) in central India. Indian Forester 121 (6): 533-539.

Vijaya, T.; Srivasuki, K.P., 2001a: Growth enhancement of *Tectona grandis* inoculated with *Glomus macrocarpus* and *Aspergillus niger* and associated effects on microbial population and phosphatase activity in potting mixes. Indian Journal of Forestry 24(3):279-283.

Vijaya, T.; Srivasuki, K.P., 2001b: Response of micropropagated Tectona grandis to dual inoculation with *Glomus macrocarpus* and *Bacillus megatherium*. Indian Journal of Forestry 24(1): 43-47.

White, K.J., 1991: Teak: Some aspects of research and development. Publication 1991/17, FAO. Regional Office for Asia and Pacific (RAPA), Bangkok.