RESPONSE OF SANDAL *(Santalum album* Linn.) SEEDLINGS TO SHADE AND MYCORRHIZAL ASSOCIATION

By

BINU. N. KAMALOLBHAVAN

THESIS

Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF TREE PHYSIOLOGY AND BREEDING
COLLEGE OF FORESTRY
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DECLARATION

I hereby declare that this thesis entitled **"Response of sandal (*Santalum album* Linn.) seedlings to shade and mycorrhizal association"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any University or Society.

Vellanikkara
31 -08-02

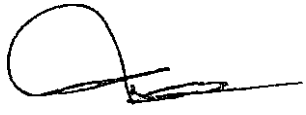
BINU.N.KAMALOLBHAVAN

Dr. P. K. Ashokan
Chariman, Advisory Committee
Associate Professor
Department of Tree Physiology and Breeding

College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur-680686

CERTIFICATE

Certified that this thesis, entitled **"Response of sandal (*Santalum album* Linn.) seedlings to shade and mycorrhizal association"** is a record of research work done independently by **Sri. Binu.N.Kamalolbhavan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



Vellanikkara,
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Dr. P. K. ASHOKAN

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sri. Binu.N. Kamalolbhavan**, a candidate for the degree of **Master of Science in Forestry**, agree that this thesis entitled **"Response of sandal (*Santalum album* Linn.) seedlings to shade and mycorrhizal association"** may be submitted by **Sri. Binu.N. Kamalolbhavan** in partial fulfilment of the requirement for the degree.

18/9/02

Dr. P. K. Ashokan
(Chairman, Advisory Committee)
Associate Professor
Dept. of Tree Physiology and Breeding
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

18/09/02

Dr. Luckin.C.Babu
(Member, Advisory Committee)
Associate Dean
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

18/9/02

Dr. N.K.Vijayakumar
(Member, Advisory Committee)
Professor and Head
Dept. of Tree Physiology and Breeding
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

18/9/02

Dr. M.Balasundaran
(Member, Advisory Committee)
Scientist-in-Charge
Div. of Plant Pathology
Kerala Forest Research Institute
Peechi, Thrissur

18/9/2002

External Examiner
(K-VANANGAMUDI)
PROFESSOR & HEAD
Dept. of Seed Science & Technology
T.N.A.U.
Coimbatore

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Finally, I bow my head before the **ALMIGHTY**

Binu. N. Kamalolbhavan

Dedicated
to
my beloved parents

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Introduction

INTRODUCTION

Sandal (*Santalum album* Linn.) is a precious tree well known for its fragrant heartwood (East Indian Sandalwood) and the scented essential oil derived from it (East Indian Sandalwood oil). The wood is used for expensive carving and is of considerable religious significance. As a result, the demand for the sandalwood is increasing and the price is going up. Current price (during 1999-2000) of heartwood of sandal is around Rs.6.5 lakhs per tonne and in the international market, it is around Rs.9 lakhs per tonne (Ananthapadmanabha, 2000).

Santalum album is mainly distributed in South India and the Indonesian islands. The other species of *Santalum* like *S. yasi, S. lanceolatum, S. austrocaledonium* occur in parts of Australia, Caledonia and Polynesia. In India, sandal forests are seen in the states of Karnataka, Tamil Nadu and Kerala, extending to about 9040 km^2. Sandal bearing forests in Kerala is mainly located on the drier parts of the Eastern side of the Western Ghats in the Anjanad valley of Marayoor Range in the Munnar Forest Division. On a limited scale, sandal is also found in Ariankavu range of Thenmalai Forest Division. Isolated patches of sandal are also found in Wayanad, Wadakancherry and Plamaram (Palaghat district) forest areas.

India enjoys a virtual monopoly of world sandal wood trade, meeting about 90 per cent of the demand and earning considerable foreign exchange (Hussain and Ponnuswamy, 1982). The monopoly, which India enjoys now, may not continue indefinitely, since many other countries are fast progressing in cultivating sandalwood with efficient management practices (Ananthapadmanabha, 2000).

Moreover, the production of sandal in India is decreasing annually at the rate of 20 per cent since 1995 (Ananthapadmanabha, 2000). The reasons include spike disease, illicit felling and failure in regeneration efforts, mainly due to the semi-parasitic nature of the tree and failure to standardize silvicultural techniques. To meet the demand of oil, about 1800 tonnes of sandalwood is needed. In addition to this, handicraft industry consumes around 2000 tonnes of wood. At present, the gap between supply and demand has come to about 1000 tonnes and is widening over time (Ananthapadmanabha, 2000).

The diminishing supplies of sandalwood from its natural habitat (forests) and its increasing demand, points to the need for expanding area not only in forest lands but also in farm lands. Its high economic value provides sufficient incentives to the farmers for growing sandal on a commercial scale. In Kerala, apart from the forest region of Marayoor, Wadakancherry and Plamaram, sandal is also observed as a component of the homesteads especially in North Kerala (Kumar *et al.*, 1994). The potential of growing sandal as a component in homesteads/ agroforestry systems were studied by Varghese (1996). Sandal, if planted as a component in homesteads/agroforestry systems may have to survive under varying levels of shade. The light intensity varies with the canopy levels and the composition of agroforestry systems. Therefore, an understanding of the responses of sandal to various shade levels is necessary in judging the suitability of sandal as an understorey species in agroforestry systems.

It has been well established that colonization by Arbuscular Mycorrhizal Fungi (AMF) assured good survival and growth of seedling on a variety of sites in many tree seedlings. Since sandal is a difficult species to establish and its growth is very slow, AMF association may help in the establishment and growth of sandal

seedlings. Literature on the effect of shade and AMF on the growth of sandal seedlings is meager. Hence, the present experiments were carried out with the following objectives:

1. To investigate the indigenous sandal-AMF association in sandal growing regions.
2. To study the influences of shade, host and Arbuscular Mycorrhizal Fungi (AMF) inoculation on nutrient absorption, water relations and growth of sandal seedlings.

Review of Literature

REVIEW OF LITERATURE

The sandal wood tree (*Santalum album* Linn.) is a small to medium sized, evergreen, semi parasite with slender, erect as well as drooping branches. Ordinarily, it attains a height of 12 to 15 m and 1 to 1.5 m girth, though larger specimens are sometimes met with.

Though initially shade tolerant, mature trees do not tolerate overhead shade. The most common form of soils on which sandal occurs is a red ferruginous loam (Rangaswamy *et al.*, 1986a), but sandal can be found in sandy, clayey laterite, loamy and even black cotton soils. The tree does not come up well in saline and calcareous soils.

The tree needs good drainage and does not withstand water logging. It is extremely sensitive to fire and frost. Root suckers are freely produced when the roots are exposed or injured. Sandal coppices well in the young stage only.

2.1 Host

Realising the importance of host species on growth of sandal, various researchers have classified the hosts of sandal. Iyengar (1965) published a list of all known host species of sandal until that time. Rangaswamy and Griffith (1939) observed that there is a great variation in the extent of haustorisation of sandal on different species of host plants. Ananthapadmanabha *et al.* (1988) classified the host plants into three categories such as poor, medium and good based on the growth of sandal. It is reported that *Casuarina equisetifolia* is the best host of sandal (Ananthapadmanabha *et al.*, 1988; Rangaswamy *et al.*, 1986b; Taide, 1991; Varghese, 1996). Srinivasan *et al.* (1992) has reported *Cajanus cajan* as the suitable primary host of sandal in the seedling stage.

Ananthapadmanabha *et al.* (1988) in a pot culture study observed that in most instances, sandal seedlings have drawn nutrients from hosts, but there are instances where some hosts derived benefit from sandal, by getting some amount of phosphorus, calcium, magnesium and nitrogen. Self-parasitism, a phenomenon in which a plant forms root connections with another plant of same species was also observed in sandal (Iyengar, 1965).

2.2 Influence of AMF on the growth of plants

The Arbuscular Mycorrhizal Fungi (AMF) are an ubiquitous symbiont in the world's ecosystems, probably occurring in over 90 per cent of vascular plant species (Hayman, 1975). The occurrence of AMF association has been reported from a number of crop plants (Mosse, 1953; Nicolson, 1959; Gerdemann, 1968). Arbuscular Mycorrhizal Fungi (AMF) colonization ensures good survival and growth of seedlings on a variety of soils. They play a significant role in improving the root surface area by virtue of their external mycelium (Atkinson, 1983), thus increasing the efficiency of roots in absorption of minerals (Stribly, 1987) and water (Bagyaraj *et al.*, 1979). AMF enhance the supply of hormones and nitrogen and increases the resistance to root disease (Gianinazzi and Gianinazzi, 1983).

In *Leucaena leucocephala*, it was observed that AMF inoculated plants out performed their non AMF counter parts in all respects, especially seedling establishment, plant height, root length, number of leaves and phosphorus intake (Koffa and De-La-cruz, 1995). Similar responses to AMF inoculation were observed for many tree species like *Acacia mearnsii* (Udaiyan *et al.*, 1997), *A. auriculiformis* (Sharma *et al.*, 1996), *Azadirachta indica* (Kalavathi *et al.*, 2000), *Dalbergia sisoo* (Singh *et al.*, 1998), *Pterocarpus marsupium* (Sharma *et al.*, 1996), *Pongamia pinnata* (Venkatesh *et al.*, 1998), *Tectona grandis* (Durga and Gupta, 1995;

Sugavanam *et al.*, 1998; Vijaya and Srivasuki, 2001). AMF association with two endemic trees of Western Ghats viz. *Gluta travancorica* and *Myristica malabarica* has also been recently reported (Vijayakumar and Abraham, 2001). In sandal also preliminary reports on AMF-association are available (Subbarao *et al.*, 1990; Thappar *et al.*, 1992). Investigations by Nagaveni *et al.* (1998) showed that the growth of sandal seedlings inoculated with *Glomus* spp. *viz. G. fasciculatum, G. aggregatum, G. caledonicum* and composite spores performed better than the uninoculated sandal seedlings. It was further observed that composite spore treatment (mixture of several *Glomus* and *Gigaspora* species) gave better results than any of individual species of inoculation, as well as uninoculated seedlings.

2.3 Soil types and AMF

The occurrence and composition of AMF are determined by many factors (Hayman, 1975). Among these, soil is a major factor that influences the performance of AMF (Mosse and Hayman, 1971). The increased occurrence of AMF has been reported for soils deficient in phosphorus (Russell, 1973). Several reports reveal the higher activity of AMF especially under low fertility levels. It has been observed that the development of AMF is discouraged by wet soil conditions and high concentrations of phosphate (Russell, 1977). The variations in the initiation of root colonization by AMF were also recorded in different soil samples. This was observed in species like *Casuarina equisetifolia* (Singh and Anjana, 1995). The intensity of root infection and number of spores in rhizosphere for tree species under all soil situations were found to vary. The spores extracted from the rhizosphere of sandal showed predominance of several *Glomus* and *Gigaspora* species (Subbarao *et al.*, 1990). It is essential to have knowledge of the appropriate host-fungus

combination for any species adapted to different soil types. This will help to get maximum benefit from the AMF association.

The effect of AMF on the host plant growth depends more on soil type than on host genotype. Several studies showed the presence of specific AMF species depending upon the soil types (Mosse and Hayman, 1971).

2.4 AMF and nutrient uptake

The symbiotic nature of AMF with higher plants plays an important role in plant nutrition (Harley and Smith, 1983). The beneficial effect of AMF is of special importance for those plants having a coarse and poorly branched root system, since the external hyphae can extend as much as 5 cm away from the roots (Rhodes and Gerdemann, 1978), absorbing nutrients from a much larger soil volume than the absorption zone surrounding a non-AMF root. The AMF inoculated roots increase the surface area within a given volume of soil (Atkinson, 1983). This is of particular importance for absorption of nutrients of low mobility in soil such as P, Zn and Cu. Except for a very few plant species, most plant roots form association with AMF (Mosse, 1981). However, a large difference exists between plant species in their dependence on AMF for P uptake and growth. It has been reported that AMF dependency of tree species varied and a direct correlation can be observed between the per cent of AMF infection and phosphorus content of the plants, though there may be few exceptions (Pavan *et al*, 2000). Stribly (1987) reported that phosphorus seemed to be the most important nutrient involved in absorption through AMF, while other nutrients such as N and K are translocated along with it.

In an experiment to find the influence of AMF on net photosynthesis and transpiration of *Ziziphus mauritiana*, it was found that AMF inoculation resulted in

significant increase in net photosynthesis, total chlorophyll, carotenoid, sugar and starch concentrations. They also increased stomatal resistance, thereby reducing the rate of transpiration (Mathur *et al.*, 1995). The ability of the AMF to colonize the roots and to promote plant growth differs with AMF species. This was observed in the roots of black pepper (Thomas and Ghai, 1987).

2.5 AMF and plant water relations

Relative turgidity of the leaves can be employed as a measure of water deficit in plants (Weatherley, 1950). Sinclair and Ludlow (1985), proposed relative water content (RWC) as an alternate measure of plant water status, which tells upon the metabolic process in tissues and lethal leaf water status. They reported that photosynthesis, protein synthesis, NO_3 reduction and leaf senescence are better correlated with changes in cell volume and RWC than with water potential in certain plants.

AMF significantly improved tolerance to moderate drought stress. It was also reported that AMF infected roots can apparently take up water (Bethlan Talvay *et al.*, 1988) by exploitation of a larger volume of soil water that is not available to the uninfected roots (Rao and Tarafdar, 1993).

Recovery from short drought stress is improved by AMF infection, as demonstrated by a faster recovery of leaf water potential and leaf turgor (Safer *et al.*, 1972). According to Shrestha *et al.* (1996), the tolerance of trees inoculated with *Gigaspora ramisporophora* to water stress treatment was greater than that of uninoculated trees. Similar results were obtained for citrus plant (Levy and Krikun, 1980). AMF association helped in the establishment of species *viz.*, *Acacia nilotica, A. senegal, A. tortilis* and *Prosopis cineraria* in arid and semi arid regions of India (Mohan *et al.*, 1996).

2.6 Shade and AMF

Shade influences not only the PAR availability but also the heat balance and the temperature in the plant micro-environment. The temperature optimum for the AMF development is around 30°C and the growth rate increases between infected and non-infected plants optimally at 30°C. Ectomycorrhizal fungi also depend to a large extent on the plant host for carbohydrates and it has been observed that the levels of AMF infection have been strongly linked to the amount of available light and therefore the production of photosynthate by the plant (Harley and Smith, 1983). However, little is known about the ecological role of different fungi at different shades (Ingleby *et al.*, 1998).

2.7 Influence of shade on seedling growth

The effect of shade on shoot growth varies from species to species. Saju (1992) found that *Grevillea robusta* and *Tectona grandis* performed well under full sunlight and *Ailanthus triphysa* under 75 per cent shade in terms of height and diameter growth. It was further observed that in *Grevillea robusta* and *Tectona grandis* seedlings, shade reduced the leaf area, leaf size and the leaf dry weight, while the root dry weight was found to be maximum in full sunlight. In *Ailanthus triphysa* root weight was more for seedlings grown under shade, for first three months. *Leucaena leucocephala* and *Azadirachta indica* showed maximum leaf weight under 25 per cent shade, while root dry weight was maximum in open and minimum under 75 per cent shade. Decrease in growth rate and biomass production under shade was also observed in *Pongamia pinnata* (Naidu and Swami, 1993), *Bridelia retusa, Holarrhena antidysentrica, Wrightia tinctoria* (Chaturvedi and Bajpai, 1999).

Barrett and Fox (1994) made a preliminary study on the shade response of sandal and observed that plant height, leaf number, crown width and stem diameter were not significantly influenced by shade levels, while leaf area was higher in sandal seedlings grown in shade, than in full sunlight.

The review reveals that much work has been done on the influence of AMF inoculation on the growth of tree seedlings and it has been proved that a correlation exists between the growth of the seedlings and AMF colonization, except in few cases. However, research work on tropical tree seedlings and the interactions of shade and AMF colonization are limited.

Materials and methods

MATERIALS AND METHODS

The first part of this project was to investigate the occurrence of sandal-Arbuscular Mycorrhizal Fungi associations in natural sandal growing forests, considering the poor success rate in establishing the sandal seedlings in non-traditional areas. For this purpose, two studies were conducted. In the first, the roots of the sandal seedlings collected from natural sandal growing regions were investigated for the presence of AMF. The same aspect was also verified by collecting the soil of these regions and growing sandal seedlings in them in a pot culture experiment at the laboratory in the College of Forestry, Vellanikkara. Two important sandal growing regions in the state, Marayoor (Idukki district) and Wadakacherry (Thrissur district) were selected for this study and a non-sandal growing area in Thrissur district was selected as a control for the soil studies. These experiments were conducted during February to June, 2001.

In the second part of the project the response of sandal seedlings to inoculation with commonly available cultures of AMF, shade levels and nature of hosts were investigated in a pot culture experiment in the green house of the College of Forestry, Kerala Agricultural University, Vellanikkara during the period from March to November, 2001. This location is in the Thrissur district of Kerala, India, at latitude 10° 32' N and 76° 10' E longitude with an altitude of 22.25 m from mean sea level. The minimum temperature varies from 22.2°C (December) to 24.7°C (May) and the maximum temperature from 28.6°C (July) to 36.2°C (March). The weather data for the experimental period is given in Appendix-I.

3.1 Experiment I - Survey of sandal-AMF associations

3.1.1 Survey of sandal-AMF associations in the seedlings collected from sandal growing regions

The occurrence of sandal-AMF associations was surveyed in natural sandal forest in Marayoor and Wadakancherry (Kerala). Root samples were collected from one - two- year old seedlings selected at random from the forests. The samples were washed thoroughly in running water and 1.0 cm long segments were cut from the extracted fine roots. These samples were cleared and stained in trypan blue following the methods of Phillips and Hayman (1970). The root segments were observed in a research microscope for presence of vesicles, arbuscules or hyphae which were taken as positive for infection of AMF. The total percentage of AMF associations was worked out by the following formula:

$$\text{Percentage AMF colonization} = \frac{\text{Number of positive root segments with AMF colonization}}{\text{Number of root segments observed}} \times 100$$

3.1.2 Investigation for native AMF in soils collected from sandal growing regions

Soils were collected from three different places *viz.*, Marayoor, Wadakancherry (sandal growing region) and Thrissur (region where sandal is not growing naturally – 'Control').

Fifteen polybags (12 x 15 cm) were filled with soil samples (approximately 1 kg) collected from each location. Sandal seedlings were grown in these soils for four months.

Observations on height, collar girth, number of leaves and leaf area of sandal seedlings were recorded monthly. Dry weight of shoot, root, root length and

percentage of AMF colonization were observed during the destructive sampling done four months after planting.

3.1.2.1 Soil analyses

The soil samples were analysed for the available nitrogen, phosphorus, potassium and organic carbon content.

Ten grams of the air dried soil was used for the estimation of available nitrogen by alkaline permanganate method. Bray's extraction method was used to determine the available phosphorus in the soil. Five grams of air dried soil passed through 0.2 mm sieve was mixed with 50 ml of Bray's solution in a glass stoppered bottle. One ml of this extract was used to read the phosphorus content by ascorbic acid blue colour method. To determine the potassium content of soil, five grams of air dried soil was extracted with neutral normal ammonium acetate and two ml of the extract was used to read the potassium content by flame photometer (Jackson, 1958).

The organic carbon content of the soil was estimated by Walkely and Black Method (Jackson, 1958).

3.1.2.2 Soil pH

Ten grams of soil was taken in a beaker to which 25 ml of distilled water was added and stirred thoroughly. The pH was read in a digital Elico pH meter (Jackson, 1958).

3.2 Experiment II – Response of sandal seedlings to AMF inoculation, shade and host species

The response of sandal seedlings to different shades and AMF associations when grown with two host species were studied in seedlings grown in polybags of size 21 x 15 cm. The influence of three selected species of AMF *viz.*

Glomus mosseae, G. fasciculatum and *G. intraradices*, four shade levels, *viz.*, 75 per cent, 50 per cent, 25 per cent, full sunlight and two host species *viz.*, casuarina (*Casuarina equisitefolia*) and red gram (*Cajanus cajan*) were compared in this experiment.

The different sandal + host + AMF combinations tested were as follows:

1) Sandal + Casuarina + *Glomus mosseae*

2) Sandal + Red gram + *Glomus mosseae*

3) Sandal + Casuarina + *Glomus fasciculatum*

4) Sandal + Red gram + *Glomus fasciculatum*

5) Sandal + Casuarina + *Glomus intraradices*

6) Sandal + Red gram + *Glomus intraradices*

7) Sandal + Red gram

8) Sandal + Casuarina

The seedlings under the above treatments were grown under four shade levels as given below for a period of eight months.

1. S_0 - 0 per cent relative shade (full sunlight)

2. S_{25} – 25 per cent relative shade

3. S_{50} – 50 per cent relative shade

4. S_{75} – 75 per cent relative shade

A factorial experiment was laid in CRD with seven replications. The required shade levels were created by erecting net houses covered from all sides using the appropriate shade nets. The shade levels were verified and regulated by measuring PAR using a Quantum Sensor (LICOR, U.S.A.) and adjusting the shade net.

Sandal seeds for this study were obtained from Marayoor Forest Range, which is identified as a seed stand of the Kerala Forest Department. Sandal seeds, after overnight soaking in 500 ppm GA_3 were sown in sterilized sand kept in plastic trays of 35 x 45 cm dimension. These were placed in green house and watered regularly with sterile water.

3.2.1 Preparation of potting media

Sieved soil and sand in the ratio of 1:1 were used as the potting media.

3.2.2 Solarization of the potting media

It has been demonstrated that the direct effect of high temperature induced during solarization is a significant factor in reducing the indigenous AMF (Chen *et al.*, 1991; Bendavid-val *et al.*, 1997). So the potting media used in this experiment were solarized before use.

A site without any over head shade was selected. The potting media were transferred to a polythene sheet and a raised bed of 20 cm height was made. The bed was levelled and watered with a rose can. The potting mixture was then mulched with 150 guage transparent polythene sheet as shown in Plate 1. The sides of the sheet were covered with soil to prevent formation of air pockets. The potting media was solarized for 45 days.

Soil temperature of the solarized soil at depths of 5 cm and 10 cm were recorded using thermometers at 8.30 am in the morning and 2.30 pm in the afternoon to confirm the solarizing temperature.

Depth (cm)	Soil temperatures (°C)	
	8.30 am	2.30 pm
5	34	51
10	32	48

The hole made for inserting the thermometer was perfectly sealed with cellophane tape, after the temperature measurement.

3.2.3 Multiplication of AMF spores

The two species of *Glomus viz.*, *Glomus fasciculatum* and *Glomus intraradices* were obtained from Tata Energy Research Institute, New Delhi. The spores of *Glomus mosseae* were obtained from the University of Agricultural Sciences, Bangalore.

All the three species of AMF were multiplied in sand and soil mixtures based medium in plastic pots, using sorghum seedlings (Plate 2).

Surface sterilized sorghum seeds (0.1 Per cent Mercuric chloride for 10 min) were placed in sterilized petridishes containing plain agar for pre-germination. The pre-germinated seeds were transferred to plastic basins containing sterilized, sieved sand and soil for mass multiplication of the AMF spores.

The pots were surface sterilized and three-fourth filled with sterilized soil + sand mixture. A layer of inoculum was spread over this and covered using a layer of potting media. The pre-germinated seeds were placed on this layer and covered by another thin layer of the media. The pots were incubated in the green house for three months with irrigation using sterile water.

3.2.4 Spore count

The spores were isolated from the soil samples by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Ten grams of soil was mixed with 100 ml of water in a beaker, stirred thoroughly, allowed to settle for a few minutes and sieved through a sieve assembly with mesh sizes ranging from 1 µm to 45 µm. The washings from 108 µm and 45 µm sieves were filtered through


Glomus mossae
Glomus Fasciculatum
Glomus intraradices

Whatman No.1 filter paper. The residue on the filter paper was observed under a microscope for AMF spores count.

3.2.5 Testing of root colonization by AMF

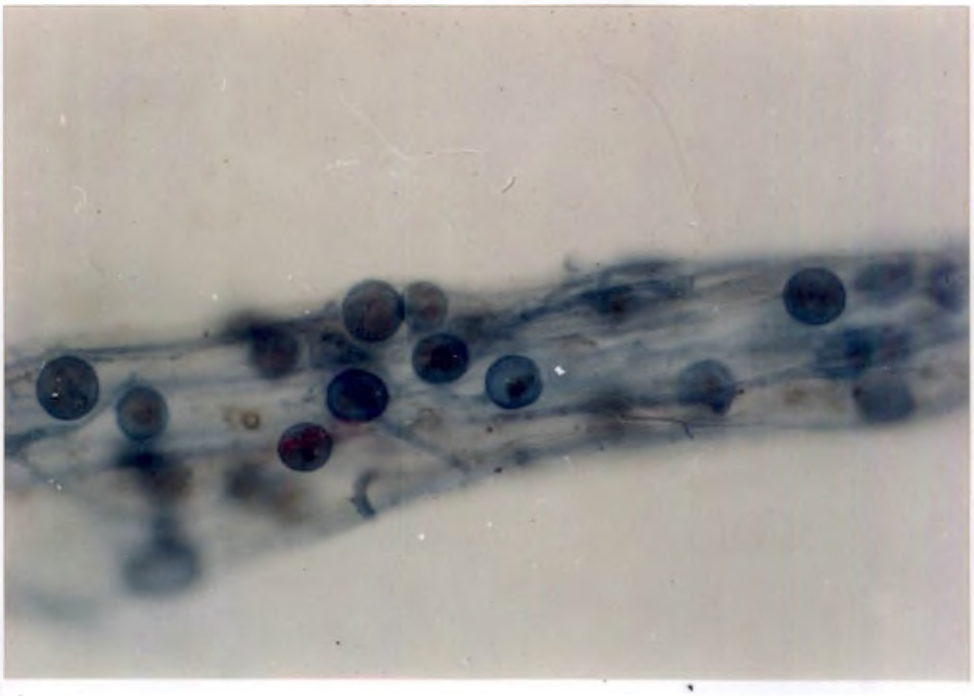
Infection by the three species of AMF on sorghum roots was tested separately by clearing and staining method (Philips and Hayman, 1970). Roots were washed thoroughly and cut into 1 cm bits. Root bits were kept in 10 per cent KOH for four days. After washing, the roots were acidified in 1 per cent HCl and stained with 0.05 per cent Trypan blue (Appendix II). If over stained, destaining was done by keeping the root bits in lactoglycerol (Appendix III). Roots of the sorghum plant infected with AMF were identified by observing through the microscope and taking micro-photographs (Plate 3a, 3b, 3c).

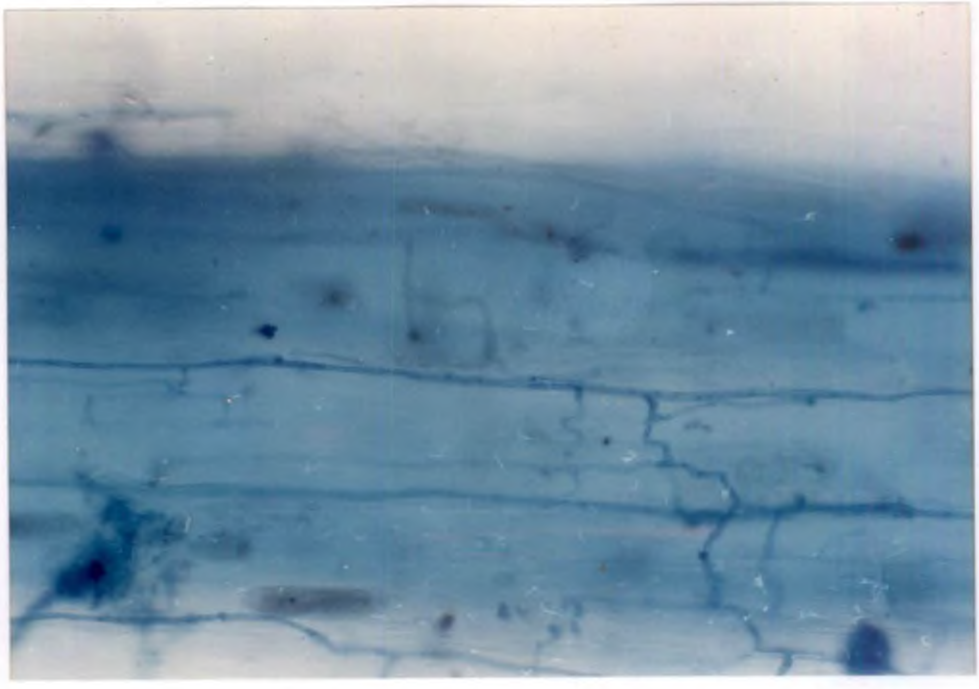
3.2.6 Inoculating the potting media with AMF

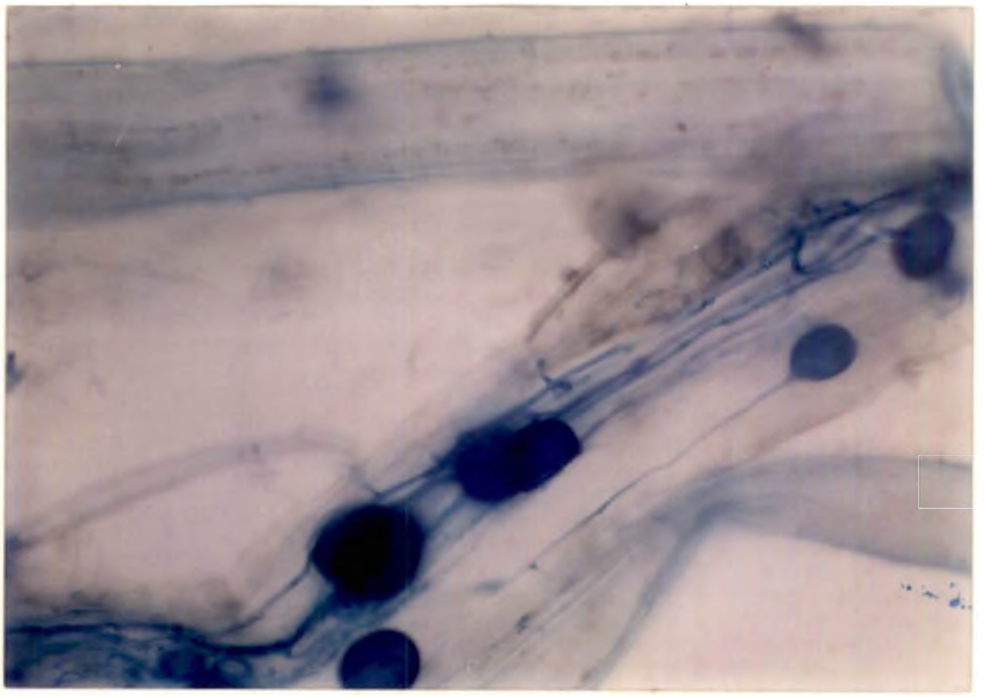
The solarized soil was immediately transferred to the green house and the polythene bags (21 x 15 cm) were filled with potting media (approximately 5 kg/bag) leaving 4 cm space at the top. Ten grams of AMF inoculum (approximately 250 spores) were placed in the poly bag as per the experimental treatment and covered with 1:1 mixture of sterilized sand and soil upto 2 cm above the inoculum. The pre-germinated sandal seeds were dibbled in these polybags to a depth of 2 cm. The pre-germinated seeds of the host species *viz.*, casuarina and redgram were dibbled away from the base of the sandal seedlings in the polybags, as per the treatments specified.

3.2.7 Transfer of sandal seedling to shade nets from green house

After inoculation with AMF the seedlings were transferred to shade houses as per treatments, allotted randomly under each shade level. The seedlings were irrigated regularly with sterile water.







3.2.8 Observations

Observations on height, collar diameter and number of leaves were recorded at monthly intervals. Total biomass, percentage of AMF colonizations, root length and leaf area were recorded during 6^{th}, 7^{th} and 8^{th} month after planting. The plant water potential, relative water content and the stomatal resistance were recorded after 8^{th} month of planting.

3.2.8.1 Height

The height of all seedlings was measured from collar region to the terminal bud at an interval of 30 days.

3.2.8.2 Collar diameter

The collar diameter was measured using a digital vernier calliper at an interval of 30 days.

3.2.8.3 Number of leaves

The number of leaves was counted individually for the seedlings at an interval of 30 days.

3.2.8.4 Shoot weight

Sandal seedlings from each treatment were sampled during 6^{th}, 7^{th} and 8^{th} month after planting. The stem portion was separated from the collar region and the shoot and root samples were dried in a hot air oven at 70-80°C for 48 hours, after drying for two days in shade. The dry weights of the shoot were estimated in a precision balance.

3.2.8.5 Root weight

The root weights were taken after drying the samples in hot air oven at 70-80°C for 48 hours as described above.

3.2.8.6 Root length

The length of roots from the collar region to the tip of the leading root was considered as the root length. This observation was made during 6^{th}, 7^{th} and 8^{th} month after planting.

3.2.8.7 Leaf area

The leaves of the seedling removed during destructive sampling were separated from the shoot. They were outlined on a graph paper and area found out by counting the squares.

3.2.8.8 Percentage of AMF association

The fine roots from the seedlings during destructive samplings were observed for estimating AMF infection. Staining procedure followed was same as mentioned earlier. The root bits were observed in a stereomicroscope and presence of vesicles, arbuscles or hyphae was taken as positive for infection of AMF and total percentage of AMF associations were worked out by the formula stated earlier in 3.1.

3.2.8.9 Physiological observations

3.2.8.9.1 Relative water content

Leaves were selected randomly from the sandal plants from two replications at each shade level. The relative water content was calculated using the formula (Barrs and Weatherley, 1962).

$$RWC = \frac{Fw - Dw}{Tw - Dw} \times 100$$

Where, Fw = Fresh weight of leaf disc

Dw = Dry weight of leaf disc

Tw = Turgid weight of leaf disc

3.2.8.9.2 Diffusive resistance

Diffusive resistance was measured at 8 am and 2 pm using a Steady State Porometer (Model LI-1600, LICOR, Nebraska, USA).

3.2.8.9.3 Plant water potential

The pre-dawn water potential of sandal seedlings were estimated during the 7^{th} and 8^{th} month after planting, using a Scholander's pressure bomb type plant water status console (Soil Moisture Equipment Corporation, Ohio, USA).

3.2.8.10 Plant nutrient analyses

The shoot portions of the samples at the end of the experiment were analyzed for the nutrient content. The samples from the three replications were dried, powdered and digested following the wet digestion using Sulphuric acid and 30 per cent hydrogen peroxide (Wolf, 1982). The digest was made upto 50 ml. The following nutrients were analysed:

3.2.8.10.1 Nitrogen

Nitrogen in the digest was determined calorimetrically using Nessler's reagent (Jackson, 1958). The colour intensity was read at a wavelength of 420 nm in a spectrophotometer.

3.2.8.10.2 Phosphorus

The phosphorus content in the digest was determined calorimetrically by the vanado-molybdo phosphoric yellow colour method (Jackson, 1958). The colour intensity was read at a wavelength of 470 nm in a spectrophotometer.

3.2.8.10.3 Potassium

A known quantity of aliquot from the extract was used to read potassium using flame photometer (Jackson, 1958).

3.3 Statistical analysis

The data were analysed statistically using the techniques for analysis of variance for CRD at each shade level separately at first. Then combined analysis of all observations at four shade levels was done using the technique of RCBD over locations (Panse and Sukhatme, 1978).

Results

Table 1. Height and number of leaves of sandal seedlings grown in soils collected from two sandal growing regions and a non-sandal growing region

Soils from	Height				Number of leaves			
	Month after planting							
	1	2	3	4	1	2	3	4
Marayoor	9.7	12.3[a]	14.7[a]	17.1[a]	10.3	13.3[a]	16.4[a]	18.5[a]
Wadakancherry	9.6	12.1[a]	14.2[a]	16.3[a]	8.3	10.5[b]	13.9[b]	16.2[ab]
Thrissur (Control)	8.2	9.5[b]	11.0[b]	11.9[b]	8.7	10.8[b]	12.8[b]	15.0[b]
LSD (0.05)	NS	1.58	2.09	2.17	NS	2.13	2.16	2.15
SEm±	0.49	0.55	0.72	0.75	0.64	0.74	0.75	0.74

* Values with similar superscript do not vary significantly

Table 2. Leaf area, root length, shoot and root weight of sandal seedlings grown in soils collected from two sandal growing regions and a non sandal growing region

Soils from	Leaf area (cm^2)	Root length (cm)	Shoot weight (g)	Root weight (g)
Marayoor	36.9[a]	11.7[a]	0.22[a]	0.05
Wadakancherry	34.5[b]	9.5[b]	0.19[b]	0.06
Thrissur (Control)	14.0[c]	7.4[b]	0.15[b]	0.03
LSD (0.05)	2.15	2.03	0.04	NS
SEm±	2.14	0.68	0.01	0.003

* Values with similar superscript do not vary significantly

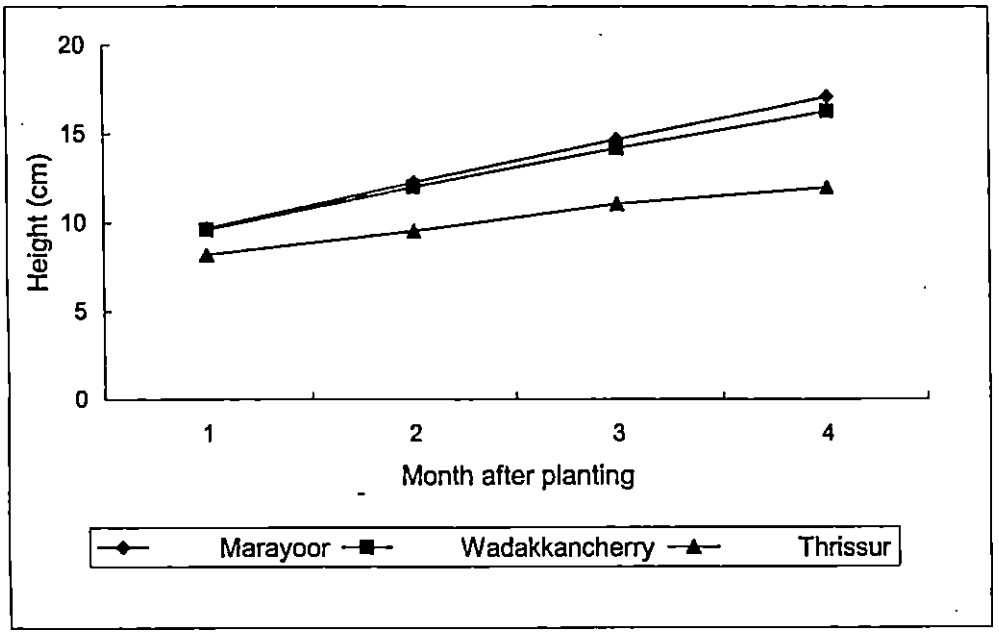


Fig.1. Variations in the height (cm) of sandal seedlings grown in soils from two sandal growing and one non-sandal region

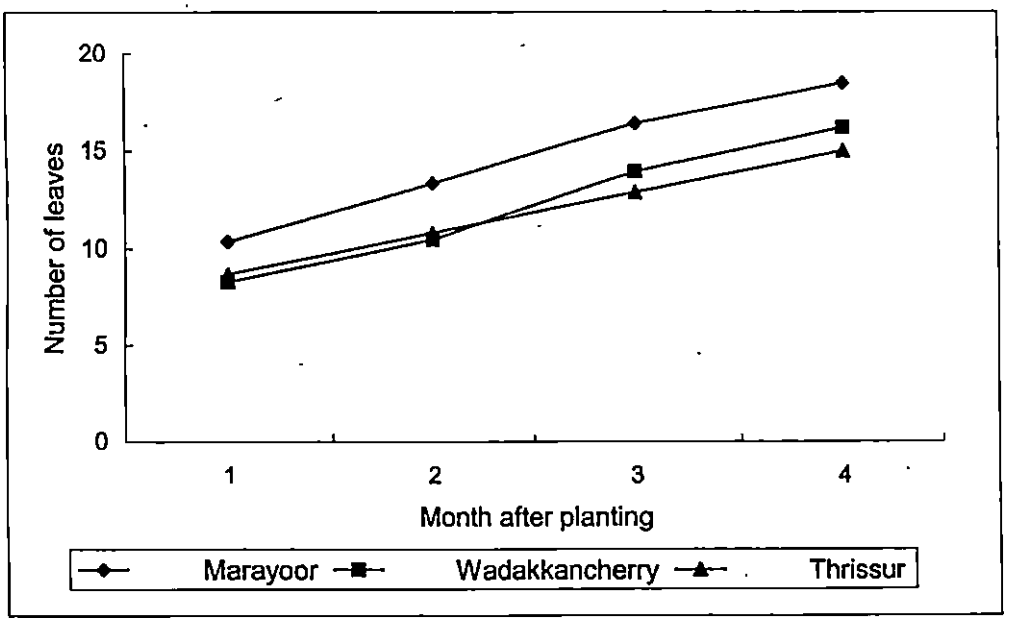


Fig.2. Variation in the number of leaves of sandal seedlings grown in soils from two sandal growing and one non-sandal region

The maximum leaf area was observed for the seedlings grown in soils, from Marayoor. However, it did not significantly differ from those grown in soils from Wadakancherry. Seedlings grown in soils from Thrissur showed the least leaf area.

Root lengths were maximum for the seedling grown in soils from Marayoor, followed by that from Wadakancherry. Root length was the least for the seedlings grown in the soil from Thrissur.

The shoot weight was also maximum for the seedling grown in Marayoor soils followed by the seedlings grown in Wadakancherry soils. Shoot weights were the least for the seedlings grown in Thrissur soils.

The root weight of the sandal seedlings did not vary significantly in response to the soils from different regions.

4.1.2.1 Soil analyses

The chemical analysis of the forest soils collected from Marayoor, Wadakancherry and also the agricultural lands in Thrissur is presented in the Table3.

The values for all the major nutrients N, P, K and C were higher for soils collected from Marayoor followed by that from Wadakancherry and least values were obtained for soils collected from Thrissur.

4.1.2.2 *Percentage of AMF colonization*

The results showed that there were no AMF colonization in the roots of seedlings grown in soils from Marayoor, Wadakancherry and Thrissur.

4.2 Experiment II-Response of sandal seedlings to AMF inoculation, *shade and host species*

4.2.1. Height

The influence of shade and AMF on the height of sandal seedlings grown with two separate host species is presented in Tables 4 to 8.

Table 3. Chemical properties of soils collected from Marayoor, Wadakancherry and Thrissur

Chemical properties	Soils from			Methodology used
	Marayoor (Sandal forest)	Wadakancherry (Sandal forest)	Thrissur (Agricultural land)	
Organic carbon (%)	2.14	1.56	0.54	Walkely and Black rapid titration method (Jackson, 1958)
Available nitrogen (%)	0.77	0.47	0.28	Kjeldhal's method (Jackson, 1958)
Available phosphorus (%)	0.060	0.005	0.003	Bray-1 Extract-Ascorbic reductant method (Jackson, 1958)
Available potassium (%)	0.66	0.45	0.24	Neutral normal ammonium acetate extractant-flame photometry (Jackson, 1958)
Soil reaction (pH)	6.3	5.2	4.8	1:2.5 soil : water suspension using pH meter (Jackson, 1958)

Table 4. Influence of AMF inoculation and host on the height (cm) of sandal seedlings grown under 75 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.2	10.2	12.9	13.3	13.7^{b}	14.2^{b}	14.6^{ab}	14.8^{ab}
G.intraradices	7.7	10.0	12.4	12.4	13.0^{b}	14.6^{b}	15.2^{ab}	16.2^{a}
G.mosseae	9.1	12.3	14.3	15.7	15.8^{a}	16.4^{a}	16.3^{a}	16.8^{a}
Uninoculated	9.2	11.8	12.1	12.6	12.8^{b}	12.9^{b}	13.1^{b}	13.5^{b}
LSD (0.05)	NS	NS	NS	NS	1.63	1.55	2.04	2.36
SEm±	0.62	0.72	0.64	0.72	0.57	0.55	0.63	0.79
Host								
Redgram	8.3	10.1	12.9	12.8	13.9	14.7	15.2	16.3
Casuarina	8.7	10.9	12.9	13.6	14.1	14.5	14.5	14.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.44	0.51	0.47	0.45	0.41	0.36	0.44	0.56

* Values with similar superscript do not vary significantly

Table 5. Influence of AMF inoculation and host on the height (cm) of sandal seedlings grown under 50 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	7.5	11.1	13.7	14.4	15.1	15.2	16.5^{ab}	17.0^{a}
G.intraradices	8.7	11.3	13.5	14.9	15.8	16.7	17.3^{a}	17.5^{a}
G.mosseae	8.1	10.6	13.2	13.9	14.10	14.8	15.7^{ab}	17.3^{a}
Uninoculated	7.6	10.7	12.5	13.3	13.6	14.1	14.9^{b}	13.5^{b}
LSD (0.05)	NS	NS	NS	NS	NS	NS	1.74	1.94
SEm±	0.67	0.68	0.72	0.64	0.64	0.60	0.61	0.69
Host								
Redgram	8.3	11.3	13.7	14.5	14.2	15.8	15.7	18.3
Casuarina	7.6	10.5	13.4	13.6	14.6	14.5	14.9	15.4
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.48	0.48	0.51	0.45	0.45	0.43	0.66	0.99

* Values with similar superscript do not vary significantly

Table 6. Influence of AMF inoculation and host on the height (cm) of sandal seedlings grown under 25 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.3	10.6	12.0	13.0	13.9	14.3	15.4[ab]	15.2[ab]
G.intraradices	6.2	9.1	11.6	12.6	13.2	14.1	15.0[ab]	15.7[ab]
G.mosseae	6.9	9.9	12.9	14.4	14.4	15.2	17.3[a]	17.2[a]
Uninoculated	6.9	9.1	10.5	12.2	12.2	13.3	13.5[b]	13.8[b]
LSD (0.05)	NS	NS -	NS	NS	NS	NS	1.99	2.48
SEm±	0.77	0.67	0.69	0.61	0.60	0.70	0.63	0.83
Host								
Redgram	7.4	10.1	13.7	13.3	13.3	14.8	15.7	16.3
Casuarina	6.8	9.7	13.4	12.8	12.8	14.1	14.9	15.2
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.54	0.47	0.49	0.43	0.43	0.49	0.44	0.55

* Values with similar superscript do not vary significantly

Table 7. Influence of AMF inoculation and host on the height (cm) of sandal seedlings grown in full sunlight

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.2	9.2	11.2	11.8	11.8	12.7	12.7	13.3
G.intraradices	5.3	7.6	10.0	10.9	10.9	11.4	13.0	13.5
G.mosseae	6.5	7.7	10.3	11.7	11.1	13.2	13.9	14.5
Uninoculated	6.7	7.7	11.4	11.3	11.3	12.4	12.8	13.2
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.62	0.61	0.53	0.46	0.65	0.71	0.73	1.09
Host								
Redgram	7.2	8.2	10.1	11.7	11.7	12.8	13.7	14.7
Casuarina	6.1	7.5	10.9	11.1	11.1	11.1	12.5	12.6
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.44	0.43	0.38	0.46	0.46	0.47	0.51	0.78

Table 8. Influence of different levels of shade on the height (cm) of sandal seedlings

Shade levels	Month after planting							
	1	2	3	4	5	6	7	8
75%	8.5^{a}	11.1^{a}	12.9^{b}	13.2^{b}	14.0^{b}	14.4^{b}	14.9^{b}	15.0^{b}
50%	7.9^{b}	10.9^{a}	13.5^{a}	14.0^{a}	14.7^{a}	15.8^{a}	16.4^{a}	16.9^{a}
25%	7.1^{c}	9.9^{b}	11.5^{c}	13.0^{b}	13.9^{b}	14.5^{b}	15.3^{ab}	15.8^{bc}
0%	6.7^{c}	7.9^{c}	10.7^{d}	11.4^{c}	12.1^{c}	12.5^{c}	13.3^{c}	13.6^{c}
LSD (0.05)	0.53	0.54	0.53	0.51	0.91	0.90	1.37	1.51
SEm±	0.32	0.33	0.32	0.31	0.32	0.35	0.38	0.53

* Values with similar superscript do not vary significantly

Sandal seedlings inoculated with AMF showed significant increase in height from 5-7 months after inoculation except in seedlings grown under full sunlight. However, the seedlings grown in soils inoculated with *G. mosseae* showed superior growth, under all levels of shade as well as in full sunlight.

The effect of the host species on the height growth of seedlings was not significant.

The seedlings grown under 75 per cent shade level showed significant increase in height for the first two months after planting. After eight months of planting, maximum height was observed for the seedlings raised under 50 per cent shade. This was followed by the seedlings grown under 25 per cent and 75 per cent shade levels respectively. Minimum height was observed for the seedlings grown in full sunlight.

4.2.2 Number of leaves

Influence of shade, AMF and host species on the number of leaves of sandal seedlings are shown in Tables 9 to 13 and Fig.3

Sandal seedlings grown in soils inoculated with *G. mosseae* under 50 per cent shade showed maximum number of leaves. A decrease in number of leaves was observed for seedlings grown under all shade levels and in full sunlight. The decrease in number of leaf was less in seedlings grown with *G. mosseae* inoculation.

The host species did not influence the number of leaves of sandal seedlings significantly.

For the first three months after planting, the seedlings grown in the shade showed a higher number of leaves when compared to the seedlings grown in full sunlight. By eight months after planting, the seedlings grown under 25 percent shade showed maximum number of leaves, followed by seedlings grown under 50 per cent

Table 9. Influence of AMF inoculation and host on the number of leaves of sandal seedlings grown under 75 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.4	11.0	9.9	13.6	9.9	7.0	5.6	3.3[b]
G.intraradices	5.9	8.5	9.1	12.5	10.8	7.9	6.1	4.5[b]
G.mosseae	7.6	10.1	10.6	12.7	11.2	8.4	7.3	7.5[a]
Uninoculated	6.1	8.6	9.6	11.4	9.6	6.7	5.9	3.7[b]
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	2.18
SEm±	0.48	0.68	0.72	0.86	0.82	0.75	0.72	0.77
Host								
Redgram	7.4	9.9	9.7	12.6	10.3	7.4	6.5	5.4
Casuarina	6.6	9.1	9.9	13.0	10.4	7.6	5.9	4.1
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.97	0.48	0.51	0.61	0.58	0.53	0.58	0.65

* Values with similar superscript do not vary significantly

Table 10. Influence of AMF inoculation and host on the number of leaves of sandal seedlings grown under 50 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	7.0	9.3	9.1	12.6	10.3[b]	10.5[b]	10.1[b]	8.2[b]
G.intraradices	7.5	9.9	11.3	13.3	12.3[b]	11.8[b]	10.6[b]	9.0[b]
G.mosseae	8.4	10.7	10.5	14.2	15.3[a]	14.8[a]	14.4[a]	13.3[a]
Uninoculated	9.0	10.3	8.9	13.6	12.2[b]	11.1[b]	7.3[c]	7.2[c]
LSD (0.05)	NS	NS	NS	NS	2.49	2.67	1.47	2.31
SEm±	0.73	0.71	0.64	0.98	0.88	0.89	0.59	0.77
Host								
Redgram	7.5	9.8	9.5	13.7	12.7	11.2	10.9	9.5
Casuarina	8.0	10.3	10.4	14.1	13.6	12.4	10.2	9.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.52	0.50	0.45	0.69	0.55	0.78	0.83	0.71

* Values with similar superscript do not vary significantly

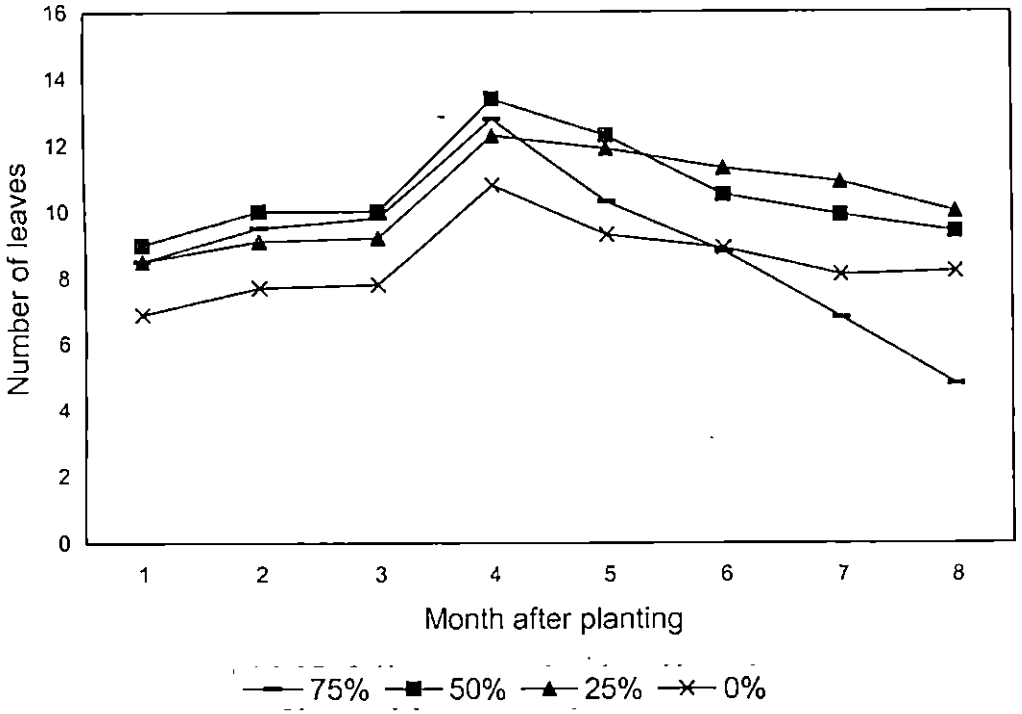


Fig. 3 Influence of different shade levels on the number of leaves of sandal seedlings

Table 11. Influence of AMF inoculation and host on the number of leaves of sandal seedlings grown under 25 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	7.0	9.2	8.5	11.9	12.0	12.8^{a}	12.5^{a}	11.8^{a}
G.intraradices	7.5	8.6	10.5	12.4	12.2	11.8^{ab}	11.2^{a}	9.8^{a}
G.mosseae	8.4	8.7	9.6	13.3	12.9	12.6^{ab}	12.2^{a}	12.3^{a}
Uninoculated	8.0	9.8	8.2	11.6	10.8	9.9^{b}	7.5^{b}	6.0^{b}
LSD (0.05)	NS	NS	NS	NS	NS	2.61	1.64	2.52
SEm±	0.73	0.82	0.62	1.01	0.77	0.91	0.93	0.84
Host								
Redgram	7.5	9.1	9.4	12.6	11.7	10.7	10.6	9.0
Casuarina	7.7	9.0	8.9	11.9	11.9	11.9	11.1	11.0
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.52	0.58	0.34	0.71	0.78	0.78	0.66	0.74

* Values with similar superscript do not vary significantly

Table 12. Influence of AMF inoculation and host on number of leaves of sandal seedlings in full sunlight

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	7.0	8.1	7.7	10.9	9.7	8.3^{ab}	7.1^{b}	8.0^{b}
G.intraradices	7.5	7.9	7.2	11.4	10.8	9.7^{ab}	7.3^{b}	7.1^{b}
G.mosseae	6.4	7.4	8.3	11.8	11.2	10.9^{a}	10.1^{a}	11.1^{a}
Uninoculated	6.5	7.4	8.1	9.1	9.6	8.6^{b}	6.9^{c}	6.5^{b}
LSD (0.05)	NS	NS	NS	NS	NS	2.37	0.99	1.84
SEm±	0.53	0.59	0.52	1.00	0.82	0.79	0.31	0.61
Host								
Redgram	7.1	7.6	8.0	10.4	10.3	8.3	8.3	8.1
Casuarina	6.9	7.7	7.6	11.2	10.4	9.7	7.4	7.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.52	0.42	0.37	0.71	0.58	0.48	0.39	0.52

* Values with similar superscript do not vary significantly

Table 13. Influence of different levels of shade on the number of leaves of sandal seedlings

Shade levels	Month after planting							
	1	2	3	4	5	6	7	8
75%	8.5[a]	9.5[a]	9.8[a]	12.8[ab]	10.3[b]	8.8[b]	6.8[c]	4.8[c]
50%	9.0[a]	10.0[a]	10.0[a]	13.4[a]	12.3[a]	10.5[ab]	9.9[ab]	9.4[ab]
25%	8.5[a]	9.1[a]	9.2[a]	12.3[b]	11.9[a]	11.3[a]	10.9[a]	10.0[a]
0%	6.9[b]	7.7[b]	7.8[b]	10.8[c]	9.3[c]	8.9[b]	8.1[ab]	8.2[b]
LSD (0.05)	0.68	1.04	0.77	0.93	0.97	1.95	2.10	1.23
SEm±	0.24	0.36	0.28	0.33	0.35	0.68	0.73	0.43

* Values with similar superscript do not vary significantly

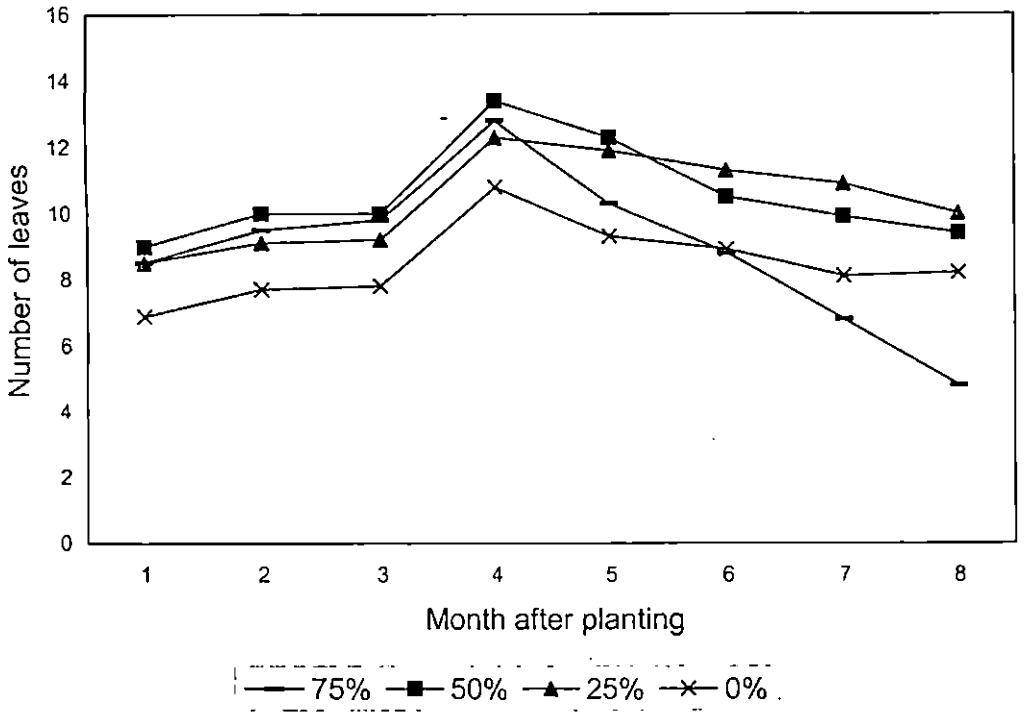


Fig. 3 Influence of different shade levels on the number of leaves of sandal seedlings

per cent shade and those grown in open. The number of leaves observed was least for seedlings grown under 75 per cent shade.

4.2.3 Leaf area

Influences of AMF and host species on the sandal seedlings grown under various shade levels are shown in Tables 14 and 15 and Fig.4 and 5.

The leaf area of sandal seedlings inoculated with *G. mosseae* and grown under 50 per cent shade was maximum. The seedlings grown in full sunlight also showed an increase in leaf area when inoculated with AMF.

The leaf area varied significantly depending on the shade levels under which the seedlings were grown. The seedlings grown under 75 and 50 per cent shade showed maximum leaf area, initially. However, by 8 months after planting, the leaf area under 75 per cent shade decreased considerably and was less than that for seedlings grown under full sunlight. The maximum leaf area was observed for the seedlings raised under 50 per cent shade.

4.2.4 Collar girth

The shade levels, AMF and host species did not show any significant influence on the collar girth of the sandal seedlings (Tables 16 to 20).

4.2.5 Shoot weight

Influence of shade levels, AMF and the host species on the shoot weight of sandal seedlings is presented in Tables 21 and 22 and Fig.6 and 7

Seedlings grown under 50 and 25 per cent shade showed significant variations in shoot weight. The maximum shoot weight was recorded in the seedlings inoculated with *G. mosseae* and grown under 50 or 25 per cent shade. The seedlings under 75 per cent shade and those in open did not show any significant variation in shoot weight.

Table 14. Influence of AMF inoculation and host on the leaf area (cm^2) of sandal seedlings

AMF	Shade levels											
	75%			50%			25%			0%		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
G.fasciculatum	27.5[b]	15.3[c]	9.3[b]	28.8[a]	28.8[a]	19.4[b]	22.4[b]	26.0[b]	23.5[b]	9.8[b]	12.4[b]	12.8[b]
G.intraradices	28.2[b]	15.9[b]	11.6[d]	28.8[b]	21.0[b]	20.2[b]	27.7[a]	25.2[b]	21.8[b]	9.3[b]	14.5[b]	11.2[b]
G.mosseae	38.5[a]	23.3[a]	21.1[a]	39.6[a]	40.9[a]	38.7[a]	29.4[a]	27.7[a]	26.6[a]	15.1[a]	17.0[a]	17.9[a]
Uninoculated	18.0[c]	10.5[c]	6.4[b]	14.0[b]	8.0[c]	8.2[c]	17.0[c]	11.3[c]	9.1[c]	10.3[b]	9.1[c]	7.5[c]
LSD (0.05)	3.29	3.20	3.24	3.72	3.53	3.72	2.61	3.61	3.84	2.81	3.60	3.66
SEm±	1.10	1.07	1.08	1.34	1.18	1.34	0.87	1.21	1.28	0.94	1.20	1.25
Host												
Redgram	31.54	13.75	8.48	17.54	22.88	17.54	25.4	18.12	15.26	12.45	13.25	12.48
Casuarina	32.51	13.59	8.63	17.25	23.34	17.25	26.9	16.81	16.16	11.86	13.28	12.18
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.98	0.82	0.82	0.64	0.98	0.64	0.58	0.87	1.25	0.77	0.55	0.58

* Values with similar superscript do not vary significantly

Table 15. Influence of different shade levels on the leaf area (cm^2) of sandal seedlings

Shade Levels	Month after planting		
	6	7	8
75%	26.8 [a]	16.2 [c]	12.1 [b]
50%	27.8 [a]	24.8 [a]	21.6 [a]
25%	24.1 [b]	22.5 [b]	20.2 [b]
0%	11.5 [c]	13.1 [c]	12.3 [c]
LSD (0.05)	2.00	2.43	1.98
SEm±	0.67	0.81	0.70

* Values with similar superscript do not vary significantly

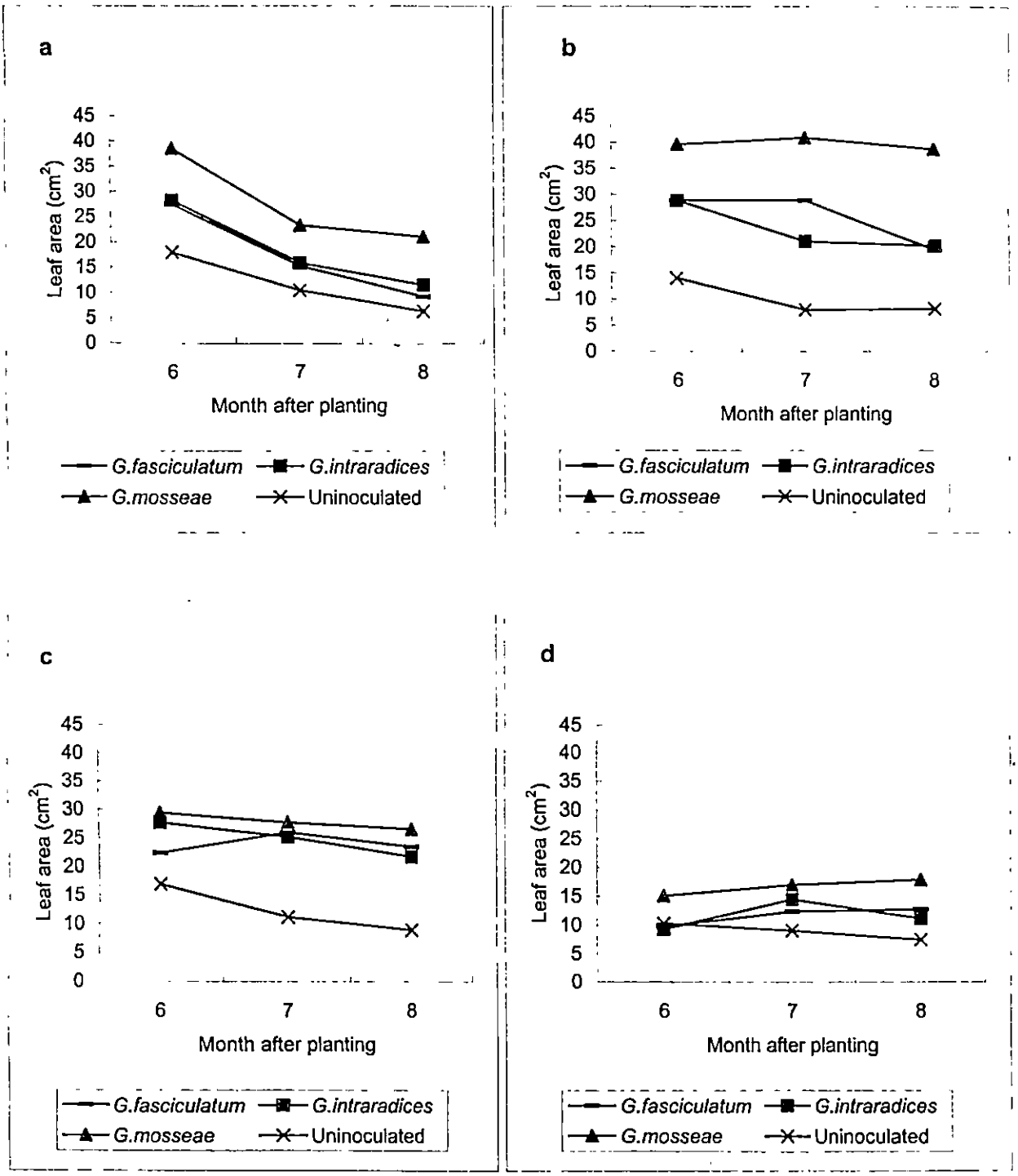


Fig.4 Influence of AMF inoculation on the leaf area (cm^2) of sandal seedlings grown under a. 75 per cent shade b. 50 per cent shade c. 25 per cent shade d. full sunlight

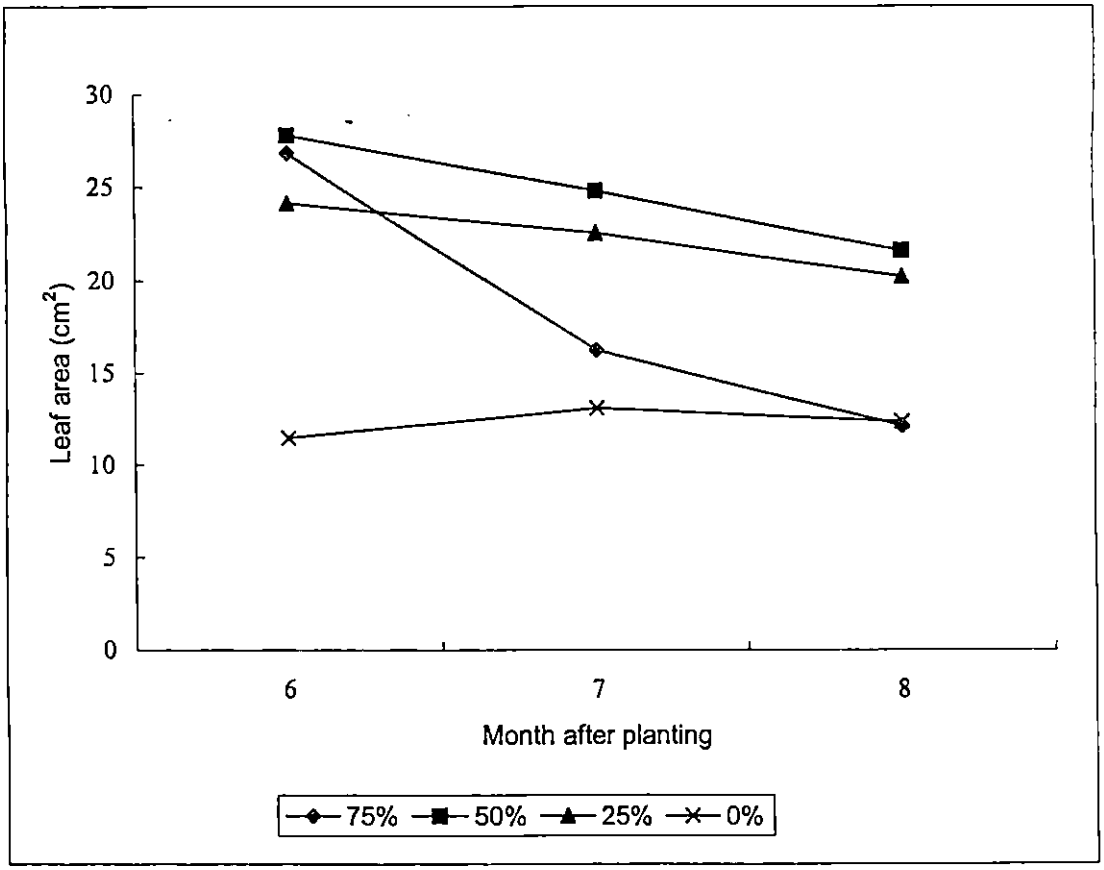


Fig.5. Influence of different shade levels on the leaf area (cm^2) of sandal seedlings

Table 16. Influence of AMF inoculation and host on the collar girth (cm) of sandal seedlings grown under 75 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.4	8.4	8.6	8.7	8.9	9.0	9.1	9.2
G.intraradices	7.2	7.2	7.3	7.6	7.9	8.0	8.1	8.1
G.mosseae	7.7	7.9	8.0	8.0	8.2	8.5	8.9	8.9
Uninoculated	7.1	7.5	8.0	8.2	8.5	8.2	8.9	8.9
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.57	0.46	0.45	0.56	0.55	0.59	0.62	0.64
Host								
Redgram	7.2	7.5	8.0	8.2	8.5	8.6	8.9	9.0
Casuarina	7.5	8.0	8.1	7.8	8.3	8.5	8.7	9.1
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.46	0.65	0.56	0.58	0.75	0.56	0.64	0.63

Table 17. Influence of AMF inoculation and host on the collar girth (cm) of sandal seedlings grown under 50 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.3	8.9	9.0	9.2	9.4	9.9	10.0	10.2
G.intraradices	7.8	7.9	8.1	8.2	8.4	8.6	8.7	7.8
G.mosseae	8.5	8.6	8.8	9.6	9.0	9.2	9.3	9.6
Uninoculated	8.4	8.6	8.6	8.9	9.0	9.1	9.3	9.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.43	0.54	0.65	0.55	0.49	0.56	0.52	0.49
Host								
Redgram	8.3	8.3	8.2	9.0	8.0	8.4	8.5	9.0
Casuarina	8.2	8.2	8.2	8.2	8.9	9.0	9.3	9.4
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.53	0.42	0.65	0.78	0.46	0.62	0.36	0.45

Table 18. Influence of AMF inoculation and host on the collar girth (cm) of sandal seedlings grown under 25 per cent shade

AMF	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	8.2	8.3	8.4	8.6	9.0	9.1	9.2	9.3
G.intraradices	8.4	8.4	8.5	8.9	9.0	9.2	9.4	9.7
G.mosseae	9.2	9.0	9.1	9.3	9.4	9.6	9.7	8.9
Uninoculated	8.5	8.6	8.7	8.9	9.0	9.1	9.1	9.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.55	0.58	0.52	0.46	0.56	0.42	0.60	0.63
Host								
Redgram	8.4	8.2	8.4	8.6	8.5	8.9	8.9	9.9
Casuarina	8.7	8.5	8.7	8.5	8.6	8.5	8.5	9.6
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.56	0.74	0.46	0.56	0.56	0.78	0.55	0.39

Table 19. Influence of AMF inoculation and host on collar girth (cm) of sandal seedlings grown in full sunlight

	Month after planting							
	1	2	3	4	5	6	7	8
G.fasciculatum	7.7	7.2	8.2	8.4	8.6	8.7	8.9	8.9
G.intraradices	8.1	8.3	8.4	8.6	8.8	9.0	9.2	9.3
G.mosseae	8.0	8.2	8.3	8.5	8.7	8.8	8.9	9.0
Uninoculated	8.4	8.5	8.7	8.7	8.9	9.1	9.2	9.4
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.53	0.45	0.57	0.46	0.36	0.56	0.46	0.45
Host								
Redgram	8.1	8.2	8.5	8.7	8.7	8.9	9.0	9.1
Casuarina	8.0	8.1	8.1	8.2	8.8	9.0	9.2	9.1
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.46	0.52	0.52	0.35	0.65	0.56	0.51	0.63

Table 20. Influence of different shade levels on the collar girth (cm) of sandal seedlings

Shade levels	Month after planting							
	1	2	3	4	5	6	7	8
75%	7.8	7.7	8.0	8.1	8.4	8.4	8.7	8.8
50%	8.2	8.5	8.6	9.0	9.0	9.2	9.3	9.2
25%	8.6	8.6	8.7	8.9	9.1	9.3	9.4	9.3
0%	8.0	8.0	8.4	8.6	8.8	8.9	9.0	9.1
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.22	0.32	0.33	0.21	0.24	0.25	0.37	0.24

Table 21. Influence of AMF inoculation and host on the shoot weight (g) of sandal seedlings.

AMF	Shade Levels											
	75%			50%			25%			0%		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
G.fasciculatum	0.28[ab]	0.25	0.20	0.31[b]	0.29[b]	0.27[b]	0.30[a]	0.28[a]	0.30[a]	0.13	0.16	0.17
G.intraradices	0.27[b]	0.24	0.22	0.25[d]	0.27[b]	0.29[b]	0.25[a]	0.26[ab]	0.28[a]	0.12	0.15	0.14
G.mosseae	0.33[a]	0.26	0.21	0.45[a]	0.46[a]	0.42[a]	0.30[a]	0.32[a]	0.31[a]	0.17	0.18	0.17
Uninoculated	0.20[c]	0.21	0.21	0.28[c]	0.27[b]	0.28[b]	0.14[b]	0.15[b]	0.17[b]	0.10	0.11	0.11
LSD (0.05)	0.52	NS	NS	0.07	0.07	0.02	0.05	0.05	0.05	NS	NS	NS
SEm±	0.01	0.01	0.01	0.01	0.15	0.02	0.02	0.01	0.01	0.02	0.02	0.02
Host												
Redgram	0.28	0.25	0.21	0.28	0.34	0.36	0.26	0.29	0.22	0.11	0.13	0.15
Casuarina	0.27	0.26	0.20	0.37	0.33	0.33	0.22	0.20	0.23	0.12	0.14	0.15
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.12	0.13	0.13

* Values with similar superscript do not vary significantly

Table 22. Influence of different shade levels on the shoot weight (g) of sandal seedlings

Shade Levels	Month after planting		
	6	7	8
75%	0.27^{b}	0.19^{c}	0.21^{bc}
50%	0.32^{a}	0.32^{a}	0.31^{a}
25%	0.23^{b}	0.25^{b}	0.27^{b}
0%	0.12^{c}	0.13^{c}	0.15^{c}
LSD (0.05)	0.023	0.040	0.061
SEm±	0.008	0.014	0.021

* Values with similar superscript do not vary significantly

The influences of host species on the shoot weight of sandal seedlings were not significant.

Shade levels showed significant effect on the shoot weight of the sandal seedlings and the maximum shoot weight was observed for the seedlings grown under 50 per cent shade. An increase in shoot weight was observed for seedlings under 50 and 25 per cent shade levels and in full sunlight. 75 per cent shade decreased the shoot weight considerably.

4.2.6 Root weight

Influences of AMF and host species on the root weight of sandal seedlings grown under various shade level are presented in Table 23.

The AMF and host species did not show any significant differences in the root weight of the sandal seedlings.

Influences of shade levels on the root weight of sandal seedlings are presented in Table 24.

Shade levels showed no significant influences on the root-weight of sandal seedlings.

4.2.7 Root length

The influence of shade levels, AMF and host species on the root length of sandal seedlings grown under various shade levels is presented in Tables 25 and 26.

The AMF, shade levels and host species did not show any significant effect on the root length of the sandal seedlings.

4.2.8 AMF colonization

Percentage colonization of AMF on sandal seedlings grown at four shade levels is shown in Table 27 and Fig.8 Microphotographs showing hyphae and

Table 23. Influence of AMF inoculation and host on the root weight (g) of sandal seedlings

AMF	Shade Levels											
	75%			50%			25%			0%		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
G.fasciculatum	0.07	0.08	0.08	0.07	0.09	0.08	0.05	0.06	0.07	0.04	0.05	0.05
G.intraradices	0.05	0.05	0.06	0.06	0.06	0.07	0.05	0.06	0.07	0.05	0.06	0.06
G.mosseae	0.08	0.08	0.09	0.08	0.09	0.10	0.06	0.07	0.08	0.05	0.05	0.06
Uninoculated	0.06	0.06	0.06	0.06	0.07	0.07	0.05	0.06	0.05	0.03	0.04	0.04
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.02	0.02	0.01	0.01	0.02	0.01	0.03	0.02	0.01	0.01	0.01	0.02
Host												
Redgram	0.04	0.06	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.05	0.05	0.06
Casuarina	0.05	0.07	0.06	0.06	0.07	0.08	0.06	0.06	0.06	0.05	0.05	0.06
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.01

* Values with similar superscript do not vary significantly

Table 25. Influence of AMF inoculation and host on the root length (cm) of sandal seedlings

AMF	Shade Levels											
	75%			50%			25%			0%		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
G.fasciculatum	11.6	13.5	14.7	11.3	12.6	13.3	10.3	11.6	13.3	9.9	11.6	12.8
G.intraradices	11.5	13.1	14.7	9.6	9.5	10.5	10.0	11.3	12.7	9.0	9.5	10.0
G.mosseae	10.5	11.6	13.0	10.3	11.3	12.7	10.3	11.3	12.8	9.5	9.6	10.7
Uninoculated	10.5	11.0	11.2	10.0	11.6	12.7	9.1	9.6	10.0	10.5	11.6	12.3
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	1.45	1.57	1.55	1.45	1.25	1.07	1.58	1.26	1.80	1.8	1.54	1.82
Host												
Redgram	9.6	10.7	12.6	11.6	11.5	11.9	10.3	11.3	13.3	10.6	11.6	12.4
Casuarina	10.5	11.5	11.8	10.6	12.4	13.4	10.6	10.5	13.5	10.6	10.5	12.0
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	1.35	1.54	1.09	1.07	1.08	0.76	1.85	1.54	1.27	1.87	1.45	0.54

Table 26. Influence of different shade levels on the root length (cm) of sandal seedlings

Shade Levels	Month after planting		
	6	7	8
75%	11.0	12.3	13.0
50%	10.3	11.2	12.6
25%	9.9	10.9	12.1
0%	9.7	10.6	11.5
LSD (0.05)	NS	NS	NS
SEm±	1.25	1.52	1.58

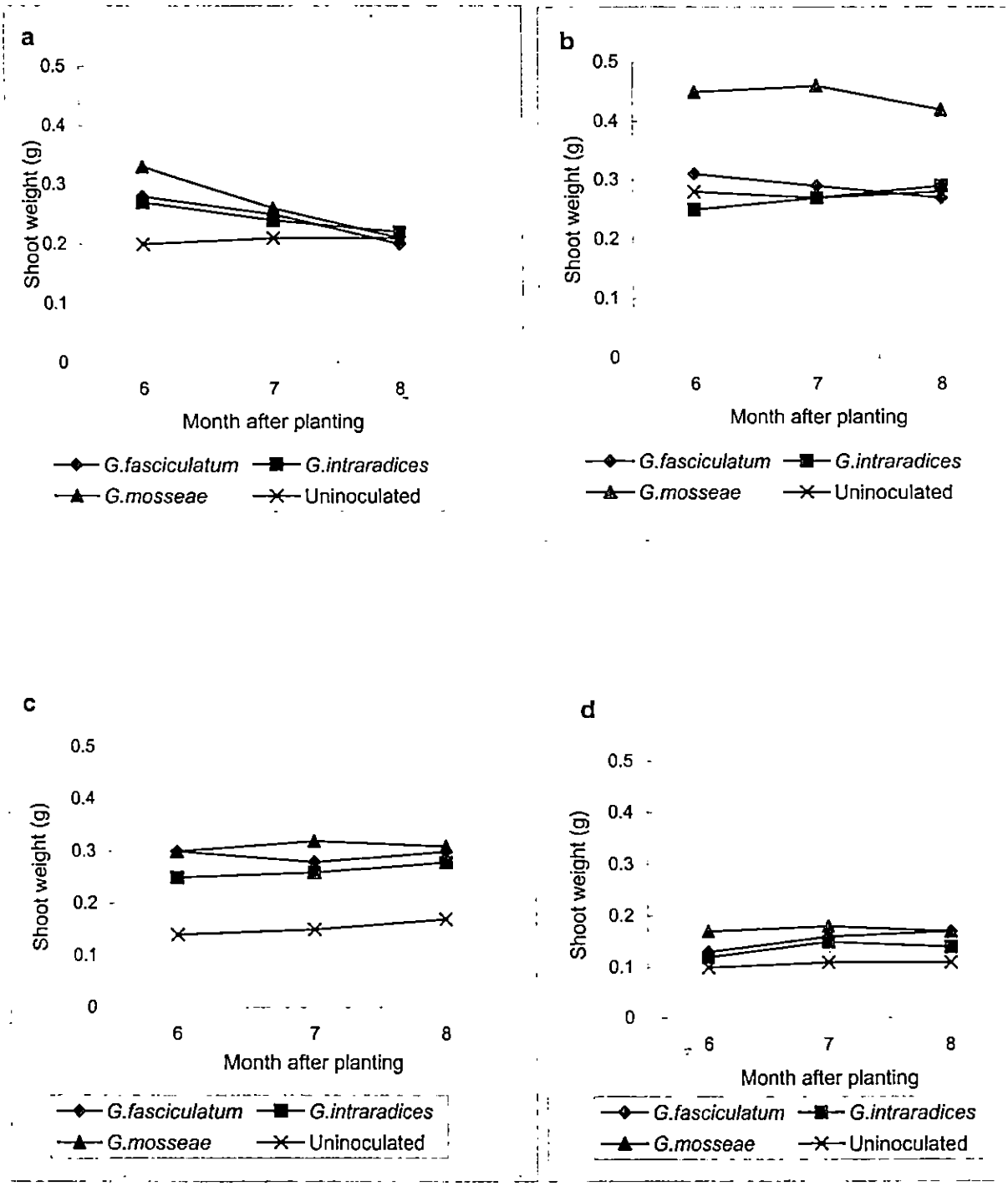


Fig. 6 Influence of AMF inoculation on the shoot weight (g) of sandal grown under a. 75 per cent shade b. 50 per cent shade c. 25 per cent shade d. full sunlight

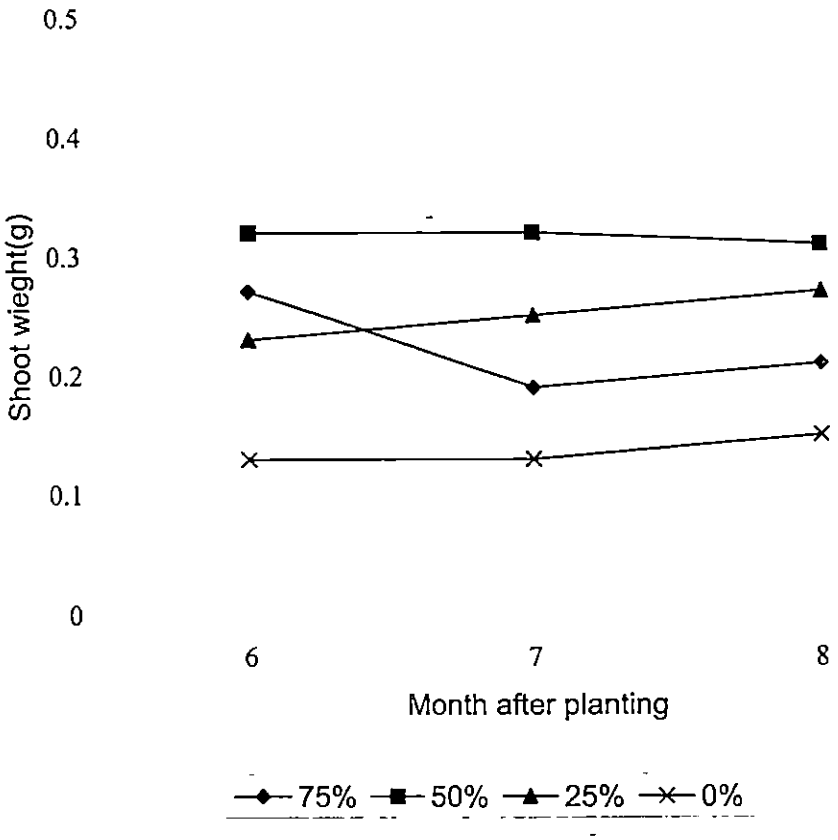


Fig. 7. Influence of different shade levels on the shoot weight (g) of sandal seedlings

Table 27. Percentage of colonization of various AMF inoculated sandal seedlings at different shade levels

AMF	Shade levels											
	75%			50%			25%			0%		
	Months of planting											
	6	7	8	6	7	8	6	7	8	6	7	8
G.fasciculatum	28	37	38	39	55	53	17	42	43	28	32	48
G.intraradices	44	53	57	40	48	53	20	46	48	25	35	51
G.mosseae	48	57	52	42	56	68	48	55	62	32	34	52
Uninoculated	2	4	4	5	8	6	4	6	8	6	11	14

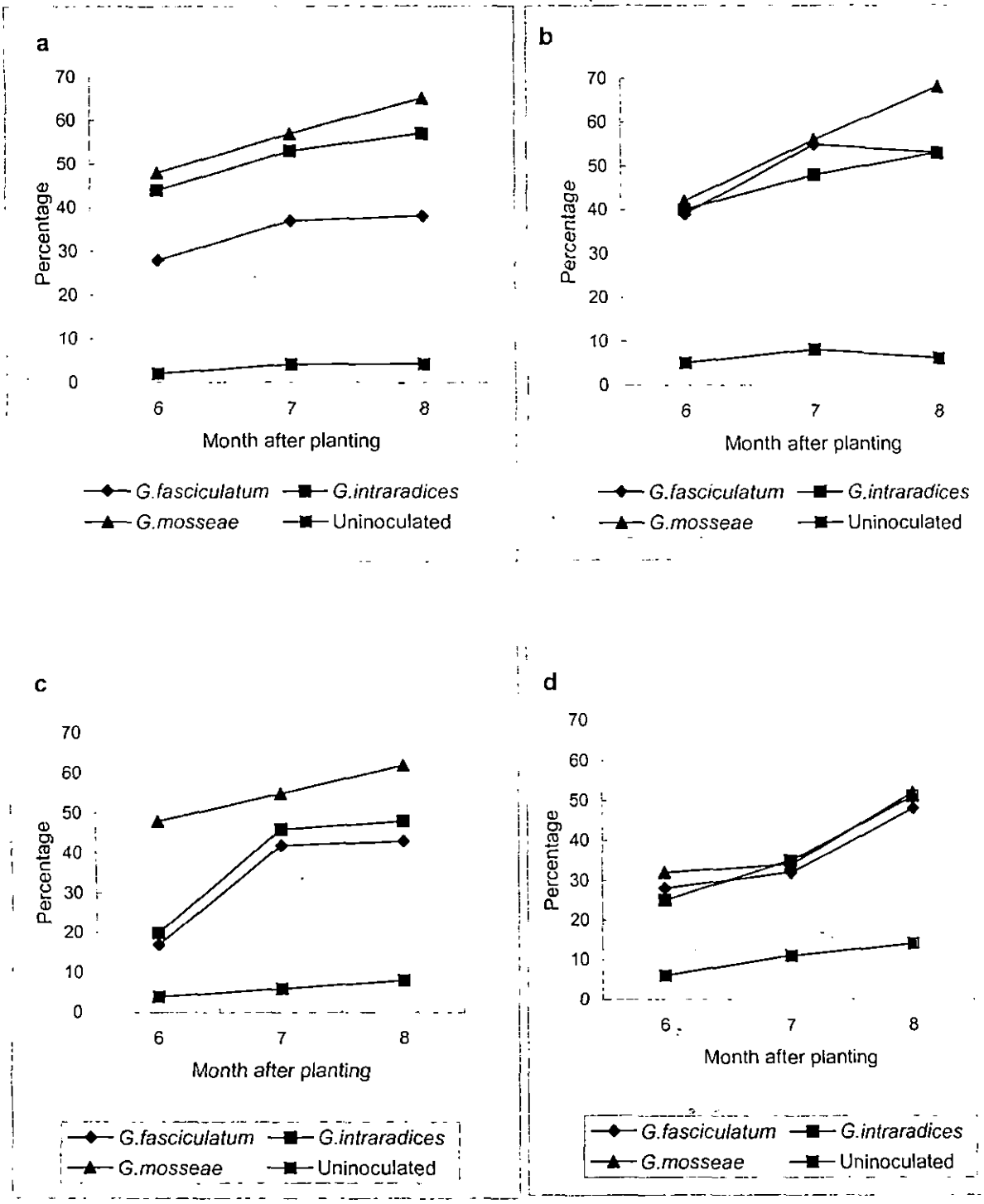


Fig.8 Percentage colonization of AMF on the roots of sandal seedlings grown under a. 75 per cent shade b. 50 per cent shade c. 25 per cent shade d. full sunlight

vesicles of Arbuscular Mycorrhizal Fungi in roots of sandal seedlings inoculated with three species of AMF viz., *Glomus fasciculatum, G. intraradices* and *G. mosseae* are given in Plates 4a, 4b and 4c respectively.

The inoculation with AMF showed increase in percentage of colonized root. The percentage of AMF colonization increased with time. This pattern of response was observed in all the shade levels.

The colonization was maximum for all the AMF species, when the seedlings were raised under 50 per cent shade.

Under all the shade levels as well as in full sunlight, the colonization was maximum in the seedlings inoculated with *G. mosseae*.

4.2.9 Stomatal resistance

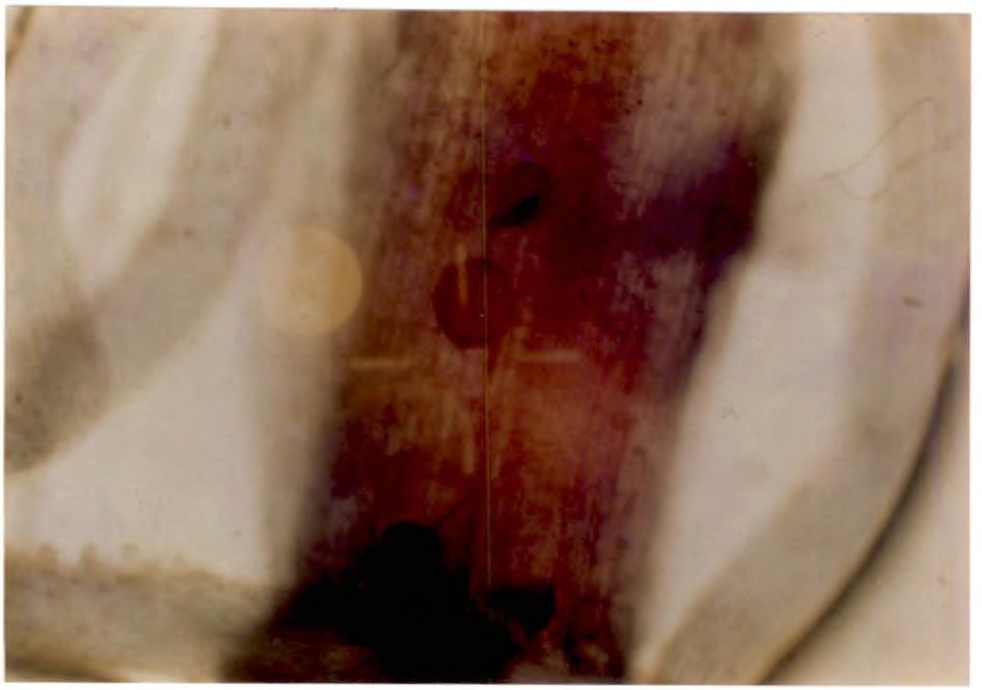
There was no significant influence of AMF and the host species on the stomatal resistance (Table 28), but shade had significant influence on the stomatal resistance of sandal seedlings (Table 29).

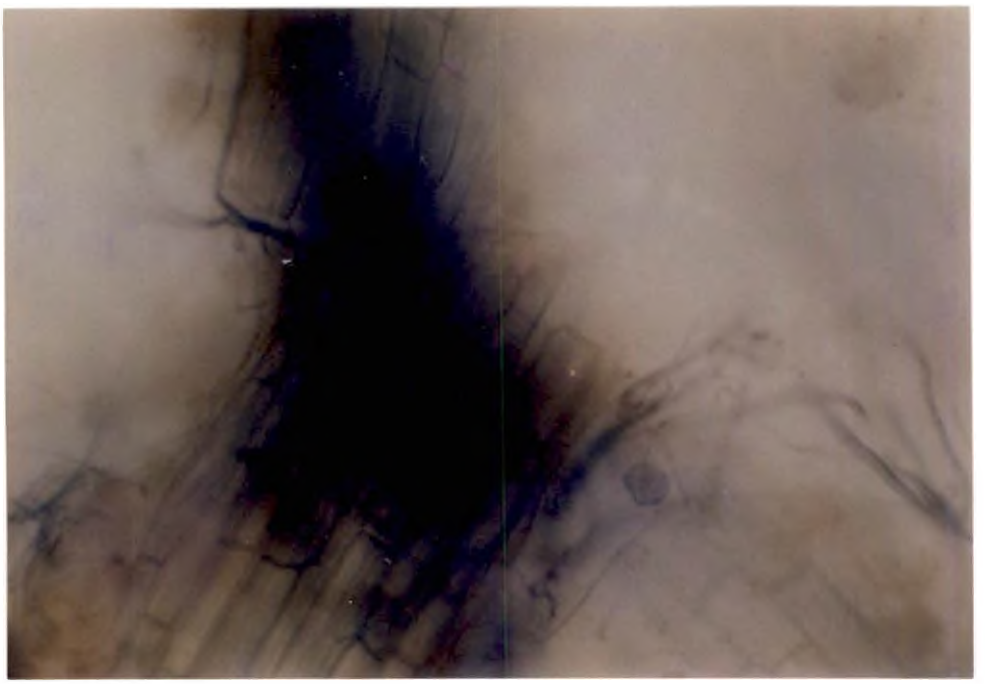
Maximum stomatal resistance was observed for the seedlings grown in full sunlight. This was followed by the seedlings grown at 50 per cent and 25 per cent shade levels. Stomatal resistance was least for seedlings grown under 75 per cent shade. The stomatal resistance showed a higher value in the afternoon hours.

4.2.10 Relative water content

Influences of AMF, shade and host species on the relative water content of sandal seedlings are shown in Table 30. There were significant influences of AMF and shade on the relative water content of the seedlings.

The leaves of the seedlings grown under 50 and 25 per cent shade had higher relative water content. This was followed by the seedlings grown in full





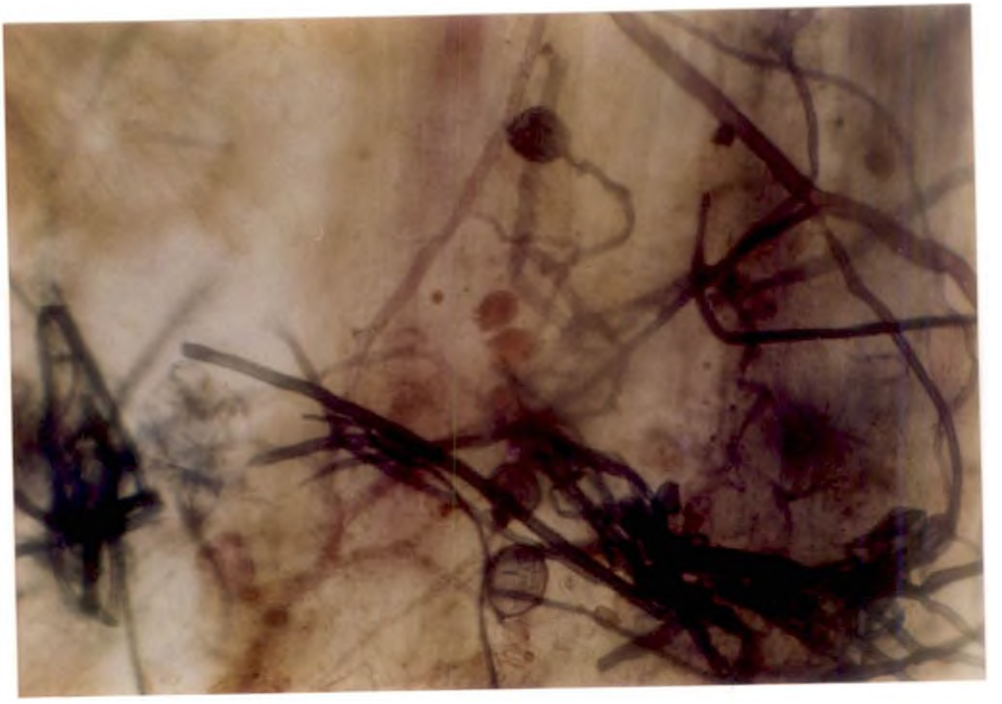


Table 28. Influence of AMF inoculation and host on the stomatal resistance (s. cm^{-1}) of sandal seedlings

AMF	Morning (8 am)				Afternoon (2 pm)			
	75%	50%	25%	0%	75%	50%	25%	0%
G.fasciculatum	0.06	0.06	0.08	0.18	0.10	0.17	0.19	0.21
G.intraradices	0.04	0.07	0.06	0.16	0.12	0.19	0.20	0.24
G.mosseae	0.07	0.08	0.06	0.18	0.10	0.22	0.20	0.25
Uninoculated	0.05	0.06	0.06	0.15	0.09	0.19	0.19	0.21
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.005	0.006	0.007	0.005	0.015	0.011	0.007	0.007
Host								
Redgram	0.06	0.07	0.07	0.16	0.12	0.20	0.19	0.24
Casuarina	0.06	0.07	0.07	0.17	0.12	0.18	0.19	0.22
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	0.004	0.004	0.005	0.004	0.011	0.011	0.005	0.005

Table 29. Influence of different shade levels on the stomatal resistance (s. cm^{-1}) of sandal seedlings

Shade levels	Morning (8 am)	Afternoon (2 pm)
75%	0.06^{b}	0.12^{c}
50%	0.07^{b}	0.19^{b}
25%	0.07^{b}	0.19^{b}
0%	0.16^{a}	0.23^{a}
LSD (0.05)	0.058	0.058
SEm±	0.003	0.005

* Values with similar superscript do not vary significantly

Table 30. Influence of AMF inoculation and shade on the relative water content of leaves of sandal seedlings

AMF	Relative water content (%)	Shade levels	Relative water content (%)
G.fasciculatum	87.6[a]	75%	85.2[c]
G.intraradices	87.9[a]	50%	89.5[a]
G.mòsseae	87.7[a]	25%	89.1[a]
Uninoculated	86.8[b]	0%	86.3[b]
LSD (0.05)	0.64		0.64
SEm±	0.14		0.14

* Values with similar superscript do not vary significantly

sunlight. The seedlings under 75 per cent shade showed minimum relative water content.

The relative water content of the leaves of sandal seedlings inoculated with AMF was higher and significant when compared to that of uninoculated seedlings.

4.2.11 Plant water potential

There was no significant influence of AMF and host species on the plant water potential of sandal seedlings (Table 31).

Shade had significant influence on the water potential of seedlings measured during the 7th month of planting (Table 32). The water potentials were high for the seedlings grown under 75 per cent shade level and for seedlings raised under full sunlight. In the seedlings grown under 50 and 25 per cent shade, the water potentials did not vary significantly.

The water potentials of seedlings, measured during the 8th month of planting, did not show significant variations.

4.2.12 Plant nutrient content

The influences of AMF on the nitrogen, phosphorus and potassium content in the shoot of the sandal seedlings were significant (Table 33, 34 and 35). Sandal seedlings inoculated with *G. mosseae* had higher N, P, and K content compared to other species of AMF. In all cases, uninoculated seedlings recorded minimum N, P and K. The host species did not show significant influence on the nutrient contents of the sandal seedlings. Shade had a significant influence on the nutrient contents of sandal seedlings. The N, P and K content of the seedlings increased with shade level and the maximum was observed for the seedlings grown under 75 per cent shade and minimum for the seedlings grown without shade.

Table 31. Influence of AMF inoculation and host on the water potential (Mpa) of sandal seedlings

AMF	Shade levels							
	75%		50%		25%		0%	
	Month after planting							
	7	8	7	8	7	8	7	8
G.fasciculatum	-1.3	-2.3	-1.0	-1.6	-1.1	-1.9	-1.3	-2.2
G.intraradices	-1.6	-2.3	-0.9	-1.6	-1.1	-2.0	-1.6	-2.2
G.mosseae	-1.3	-2.1	-0.9	-1.3	-0.9	-2.0	-1.2	-2.1
Uninoculated	-1.4	-2.3	-1.0	-1.4	-1.0	-1.9	-1.4	-2.2
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	-0.12	-0.90	-0.05	-0.11	-0.04	-0.79	-0.12	-0.15
Host								
Redgram	-1.52	-2.16	-0.96	-1.24	-0.99	-1.24	-1.52	-2.14
Casuarina	-1.26	-2.28	-0.98	-1.04	-1.02	-1.44	-1.2	-2.16
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
SEm±	-0.09	-0.77	-0.04	-0.99	-0.03	-0.56	-0.09	-0.46

Table 32. Influence of different shade levels on the water potential (Mpa) of sandal seedlings

Shade levels	Month after planting	
	7	8
75%	-1.4[a]	-2.2
50%	-0.9[b]	-1.5
25%	-1.0[b]	-1.9
0%	-1.4[a]	-2.2
LSD (0.05)	-0.21	NS
SEm±	-0.05	-0.68

* Values with similar superscript do not vary significantly

Table 33. Influence of AMF inoculation and host at different shade levels on the nitrogen content (%) of sandal seedlings

AMF	Shade levels				Mean
	75%	50%	25%	0%	
G.fasciculatum	2.297	1.472	0.593	0.428	1.198[b]
G.intraradices	2.322	1.443	0.537	0.362	1.166[b]
G.mosseae	2.732	1.813	0.877	0.647	1.517[a]
Uninoculated	1.848	0.102	0.468	0.197	0.648[c]
Mean	2.300[a]	1.207[b]	0.619[c]	0.409[d]	
Host					
Redgram	2.312	1.207	0.633	0.394	1.137
Casuarina	2.287	1.197	0.605	0.423	1.128
		LSD (0.05)	SEm±		
AMF		0.07	0.026		
Shade		0.07	0.026		
AMF x Shade		NS	0.037		
Host		NS	0.019		
Host x Shade		NS	0.037		

* Values with similar superscript do not vary significantly

Table 34. Influence of AMF inoculation and host at different shade levels on the phosphorous content (%) of sandal seedlings

AMF	Shade levels				Mean
	75%	50%	25%	0%	
G.fasciculatum	0.057	0.037	0.028	0.020	0.036[b]
G.intraradices	0.056	0.029	0.026	0.021	0.033[b]
G.mosseae	0.065	0.038	0.040	0.026	0.042[a]
Uninoculated	0.055	0.030	0.025	0.018	0.032[b]
Mean	0.058[a]	0.034[b]	0.030[b]	0.021[c]	
Host					
Redgram	0.065	0.035	0.032	0.024	0.039
Casuarina	0.051	0.032	0.025	0.019	0.032
		LSD (0.05)	SEm±		
AMF		0.007	0.020		
Shade		0.007	0.020		
AMF x Shade		NS	0.040		
Host		NS	0.012		
Host x Shade		NS	0.030		

* Values with similar superscript do not vary significantly

Table 35. Influence of AMF inoculation and host at different shade levels on the potassium content (%) of sandal seedlings

AMF	Shade levels				Mean
	75%	50%	25%	0%	
G.fasciculatum	0.365	0.212	0.227	0.153	0.239[b]
G.intraradices	0.433	0.162	0.165	0.148	0.227[b]
G.mosseae	0.515	0.237	0.233	0.173	0.289[a]
Uninoculated	0.277	0.170	0.188	0.152	0.197[b]
Mean	0.398[a]	0.195[c]	0.203[b]	0.157[c]	
Host					
Redgram	0.363	0.21	0.192	0.157	0.231
Casuarina	0.432	0.18	0.214	0.157	0.246
		LSD (0.05)	SEm±		
AMF		0.044	0.015		
Shade		0.044	0.015		
AMF x Shade		NS	0.036		
Host		NS	0.018		
Host x Shade		NS	0.024		

* Values with similar superscript do not vary significantly

Discussion

DISCUSSION

5.1 Experiment I-Survey of sandal-AMF associations

5.1.1 Survey of sandal-AMF associations in the seedlings collected from sandal growing regions

The roots of sandal seedlings from Marayoor showed no AMF colonization, while those from Wadakancherry showed 33 per cent colonization. The occurrence and composition of AMF fungi are determined by many factors (Hayman, 1975). Among these, chemical properties of the soil is a major factor that influences the performance of AMF (Mosse and Hayman, 1971). The soils from Marayoor had a higher nutrient content (Table 3) especially phosphorus when compared to the soils from Wadakancherry. The poor colonization might be due to the high nutrient content especially phosphorus. When phosphorus level in the soil was high, the AMF colonization was observed to be low in *Acacia auriculiformis* (Sankaran et *al.*, 1993). The relevance of soil nutrient concentration on the population of AMF is also evident from the increased occurrence of AMF reported for soils deficient in phosphorus (Russell, 1973). It has been observed that the development of AMF is discouraged by wet soil conditions and high concentrations of phosphate (Russell, 1977). Variation in root colonization by AMF was also recorded in different soil types for Casuarina. AMF colonization was maximum for soils collected from Jabalpur and were low for soils collected from Barrackpore (Singh and Anjana, 1995).

5.1.2 Investigation for native AMF from soils collected from sandal growing regions

The roots of the sandal seedlings when examined after four months of growth in soils from sandal-areas showed that there was no AMF colonization. So

the superior growth of sandal seedlings observed in Marayoor and Wadakancherry soils was due to the physical and chemical properties of the soils. It is also possible that the native AMF in the soils are not able to colonize the roots in four month period.

The soils from Marayoor had high N, P and K content (Table 3) followed by the soils from Wadakancherry, both sandal growing areas. The N, P and K contents were least for the soils from Thrissur. The increased shoot growth (Table 1) shown by the seedlings grown in soils from Marayoor followed by that from Wadakancherry might be due to the higher plant nutrient concentrations. Soils from Marayoor and Wadakancherry showed higher concentration of nitrogen when compared to the soils from Thrissur. Higher nitrogen increases the height, number of leaves, leaf area and biomass production.

Soil nutrient content especially that of phosphorus is a determinant factor for the AMF colonization. Though, the soils may have native AMF inoculations which may help symbiotic association, the quantity of inoculum may not be sufficient to reach an adequate mycotropy level to have a positive effect on the seedlings in early period of its growth. This has been made clear from the non-colonization of the sandal seedlings grown in soils from different sandal growing forests when seedlings were grown for four months. Further studies are needed to quantify AMF inoculum needed to have a positive effect on the sandal seedling growth.

The soil of Thrissur showed considerable acidity (pH 4.8) as compared to Marayoor (pH 6.3) and Wadakancherry (pH 5.2). This may be another reason for the difference observed in seedling growth.

Parameters like height, collar diameter, leaf production and leaf area were lower in seedlings of *Tectona grandis* (Varghese, 1996), cashew seedlings

(Gopikumar and Aravindakshan, 1988) and Ailanthus (Anoop, 1993), when they were deficient in N.

The root length was higher for the seedlings grown in Marayoor soils (Table 2). This might be due to the higher phosphorus content of the soil. According to Pandey and Sinha (1972) phosphorus promotes healthy root growth. The organic carbon content of Marayoor soils was also higher (Table 3). It has been reported that the maximum water holding capacity and volume expansion were closely related to the nature and content of organic matter (Elsy, 1989). It is also reported that various growth promoting compounds such as vitamins, aminoacids, auxins and gibberellins are formed as organic matter decays stimulating the higher plants and micro organisms (Brady, 1990). Soil organic carbon increases the cation exchange capacity and increases the supply and availability of plant nutrients.

The root weight of the sandal seedlings grown under the three soil types did not show any significant difference. Sandal is a slow growing species, so the root growth is slow (Srinivasan *et al.*, 1992). Due to this inherent character of the sandal seedlings, the favourable response observed in shoot growth was not reflected in root growth.

5.2 Experiment II-Response of sandal seedlings to AMF inoculation, shade and host species.

5.2.1 Growth parameters

Sandal seedlings showed improved growth in response to AMF inoculation. The height, leaf area and shoot weight of sandal seedlings increased due to the inoculation. The influence of AMF in increasing the root surface area by virtue of their external mycelium (Atkinson, 1983) and thereby increasing the

efficiency of roots in absorption of minerals (Stribly, 1987) and water (Bagyaraj *et al.*, 1979), hormone production, nitrogen production and resistance to root disease has been reported (Gianinazzi and Gianinazzi, 1983).

Sandal is a very slow growing species and the surface area of the root system is small as compared to many other species (Srinivasan *et al.*, 1992). The AMF might have helped in augmenting the root system surface area and increasing its absorption efficiency. Improved growth of forest tree seedlings in species like *Tectona grandis* (Durga and Gupta, 1995), *Dalbergia sisoo* (Singh *et al.*, 1998), *Pterocarpus marsupium* (Sharma *et al.*, 1996) etc., in response to AMF inoculation was reported earlier. In sandal seedlings, also *Glomus fasciculatum*, *G. aggregatum*, *G. caledonium* and composite spore mixture inoculated seedlings were reported to grow better than the uninoculated seedlings (Nagaveni *et al.*, 1998). It has been reported that the spores extracted from the rhizosphere of sandal growing areas showed preponderance of several *Glomus* and *Gigaspora* species (Subbarao *et al.*, 1990). However, there is no earlier report on the influence of AMF species like *G. mosseae*, *G. intraradices* and the influence of shade on AMF colonization and growth of sandal seedlings. Similarly, the sandal growing areas in Kerala were not surveyed for the presence of AMF earlier.

The maximum response in this study was observed for seedlings grown in soils inoculated with *G. mosseae*, under all shade levels and full sunlight. The AMF colonization was also higher for *G. mosseae*, as compared to other species. So the growth response observed in sandal seedlings inoculated with *G. mosseae* may be related to the higher colonization of the AMF (Table 27).

Though the number of leaves increased due to the inoculation with AMF until four months after inoculation, a decrease in the number of leaves was observed

under all shade levels and in full sunlight, after four months of planting. This might be due to heavy rains in the months of June and July and high humidity (Appendix1) during that period of the experimentation, which encouraged pathogenic fungal activity on leaves. Consequently, a decrease in the leaf area and shoot weight was observed for the seedlings grown under all the shade as well as in full sunlight. The decrease in the number of leaves was less in seedlings inoculated with *G. mosseae* especially under 50 per cent shade. This might be due to the better water relations of the sandal seedlings inoculated with *G. mosseae*, as evident from high relative water content (Table 30) and lower water potential (Table 32).

Reports on the interaction between AMF and shade on the growth of tree seedlings are limited. The present investigation revealed that there are considerable interactions between AMF and the shade levels. The growth of sandal seedlings and AMF colonization were best under 50 per cent shade level (Table 27 and Fig.8). Earlier reports indicate that the AMF colonization on the roots are strongly linked to the amount of sunlight, which in turn will decide the production of photosynthate by the plant, resulting in increased carbon allocation to the root system, stimulating AMF colonization (Harley and Smith, 1983). The overall effect of shade showed that the growth of sandal seedlings was best under 50 per cent shade. It has been observed that diffused sunlight is necessary for the growth of sandal seedlings especially during the first year of its growth (Troup, 1921). It is also possible that the colonization and growth of *G. mosseae* itself is benefited by shade. There is no report to confirm this probability. The improved growth of sandal seedlings inoculated with *G. mosseae* and grown under 50 per cent shade might be due to the higher photosynthate made available to the sandal root / AMF and/ or the beneficial effect of shade on AMF colonization and growth. The positive association between

shoot growth parameters and AMF colonization may also be related to the above mentioned reasons. These reasoning are further corroborated by the observations on the seedlings grown under full sunlight, which did not show significant response to AMF colonization (Table 27). This indicates the adverse effects of bright sunlight on the growth of AMF as well as sandal seedlings, resulting in poor carbon supply to AMF and suppressing its colonization.

Present study shows that for all the growth parameters, during the initial seedling phase, high shade (75 per cent) is needed but during the later stages of seedling growth, medium shade (50 per cent) is the best. Earlier studies made in six-month-old sandal seedlings, however, showed that characters like plant height, leaf numbers, crown width and stem diameters did not change significantly at various shade levels, while leaf area was higher for sandal seedlings grown under shade (Barrett and Fox, 1994).

Inoculating the seedlings with AMF did not affect characters such as collar girth, root weight and root length of the sandal seedlings. Similar results were reported for black pepper (Ashithraj, 2001) and for cowpea (Beena, 1999) where some of the biometric parameters like root weight and length were not significantly influenced, though all the other biometric parameters were higher for AMF-inoculated seedlings. The root weight of sandal seedlings inoculated with *G. mosseae* was higher under all the shade levels, even though it did not vary significantly (Table 24).

The influences of host species on the growth of sandal seedlings were not significant. Earlier reports also indicated that both *Casuarina equisetifolia* (Varghese, 1996) and *Cajanus cajan* (Srinivasan *et al*, 1992) were good hosts and improved the growth of sandal seedlings. However, red gram is considered as a short

term (temporary) host while casuarina as a long term host. The long term influences of casuarina on sandal are yet to be documented.

5.2.2 Plant water relations

The relative water content (RWC) was higher in seedlings inoculated with AMF (Table 30) when compared to the uninoculated seedlings. The RWC was higher for seedlings grown under 50 and 25 per cent shade (Table 30). The relative turgidity of the leaves can be employed as a measure of water deficit in plants (Weatherley, 1950). Sinclair and Ludlow (1985) proposed RWC as an alternate measure of plant water status, which tells upon the metabolic process in tissues. So, the higher RWC observed in seedlings inoculated with AMF and grown under 50 per cent shade indicate that the seedlings are in a better position with respect to plant water status, that the metabolic process and growth of the seedlings will be superior in these seedlings in the long run. The superiority in growth of sandal seedlings inoculated with AMF and grown under 50 per cent shade may be accounted for its better plant water status as deducted from the above observations. Similar influences of AMF colonization, i.e. higher leaf water potential and leaf turgor were also observed in soyabean seedlings (Safer *et al.*, 1972).

The seedlings grown in full sunlight and 75 per cent shade had lower RWC and lower leaf water potential (Table 30 and 32). In full sunlight, the seedlings were water stressed because of high transpiration loss. With high shade level the absorption of water might be less resulting in low RWC. Both these contrasting extreme environment are not suitable for sandal seedlings. The high intensity of light results in interception of light more than light saturation point for photosynthesis, which increases the leaf temperature. To dissipate this heat plant may transpire more (Landsberg, 1986). The absorption capacity of the root system of the plant is

reported to depend on the intensity of sunlight, which in turn decides the carbon allocation to roots and root system development. In high shade levels, the low carbon turn over and low carbon allotted to root system might have resulted in poor growth and absorption of water resulting in low RWC and poor growth of sandal seedlings. Shade had significant influence on the stomatal resistance of sandal seedlings (Table 26). The stomatal resistance was higher for seedlings grown in full sunlight. Partial closure of stomata in water deficit situation has been reported in many tree species (Pereira and Kozlowski, 1978; Kozlowski, 1982). The low RWC (Table 30) observed in the seedlings grown in full sunlight indicated that the plants were water stressed and partial or full closure of stomata was possible to regulate water loss and mortality of the seedlings. It has been reported that water stress becomes a factor for stomatal closure when the water potential falls quite low (Landsberg and Jarvis, 1976). It was also observed that the afternoon values of the stomatal resistance were higher when compared to the morning values. The stomatal opening and closing are mainly influenced by sunlight (photon-flux density) and air humidity (Whitehead *et al.*, 1981). Diurnal resistance patterns of stomata similar to this were observed by Whitehead *et al.* (1981) in teak. As the temperature in the afternoon hours were higher invariably, the higher values of stomatal resistance observed in the afternoon hours may be traced to the influence of this environmental parameter.

5.2.3 Plant nutrient content

The N, P and K content of the sandal seedlings inoculated with AMF were higher compared to the uninoculated seedlings. *Glomus mosseae* was most effective in improving the nutrient contents of the plant, compared to other species of AMF. The higher colonization of the AMF particularly by *G. mosseae* (Table 27)

might have increased the root system efficiency. It has been reported by many researchers that the AMF colonization play a significant role in improving the root surface area by virtue of their external mycelium (Atkinson, 1983), thus increasing the efficiency of roots in absorption of minerals (Stribly, 1987). It has been observed that a direct correlation exists between the percentage of colonization and phosphorus content of the plants (Pavan *et al.*, 2000). The higher nutrient concentration observed in sandal grown with AMF inoculation may also influence the plant water relation by its influence on osmotic potential of the plant cells there by influencing its establishment and growth under soil moisture stress. The significance of the influences of plant mineral nutrient concentration in plant water relations and water potential were reported in *Quercus robur* (Cater *et al.*, 1999).

The shade levels increased the plant nutrient contents of the seedlings. The highest N, P and K contents were observed in sandal seedlings grown under 75 per cent shade. Heavy shade leads to higher concentration of nutrients in the foliage, without being properly utilized, resulting in poor growth of the seedlings. The seedlings grown under 75 percent shade were poor in growth attributes like height and number of leaves (Tables 1 to 23). Similar observations were made by Robert (1971) in *Quercus alba*, while studying the effect of shade on the nutrient content of the seedlings.

The seedlings grown under 50 and 25 per cent shade utilized the absorbed nutrients more efficiently, resulting in improved growth (Table 4 to 26) and probably the lower nutrient content might be due to the dilution effect. Poor growth of sandal seedlings in full sunlight might have resulted in poor development of the root system (Table 24) and absorption of nutrients resulting in lower nutrient concentration.

Summary and conclusions

SUMMARY AND CONCLUSIONS

Experiments were conducted at the College of Forestry, Kerala Agricultural University, Vellanikkara during 2000-2001, to study the response of sandal (*Santalum album* Linn.) seedlings to different shade levels and AMF associations. Sandal-AMF association in sandal growing regions were also investigated. The salient features of the study are summarized below:

1. The sandal seedlings from Marayoor forest showed no AMF colonization, while those from Wadakancherry forest showed 33 per cent colonization.
2. Sandal seedlings showed improved growth in response to AMF inoculation. The height, number of leaves, leaf area and shoot weight of sandal seedlings increased due to the inoculation.
3. The maximum response for the parameters such as height, number of leaves, leaf area and shoot weight were observed for seedlings grown in soils inoculated with *G. mosseae*, under all shade levels and full sunlight.
4. The growth of sandal seedlings and AMF colonization were the best under 50 per cent shade level especially for those seedlings inoculated with *G. mosseae*.
5. For all the growth parameters, during the initial seedling phase, high shade (75 per cent) is needed but during the later stages of seedling growth, medium shade (50 per cent) is the best.
6. Inoculating the seedlings with AMF did not affect characters like collar girth, root weight and root length of the sandal seedlings.
7. The influence of host species casuarina and redgram on the growth of sandal seedlings was on par.

8. The relative water content (RWC) was higher in seedlings inoculated with AMF when compared to the uninoculated seedlings. The relative water content (RWC) and plant water potential were higher for seedlings grown under 50 and 25 per cent shade.
9. The stomatal resistance was lower for seedlings grown in shade.
10. The N, P, K content of the shoot of sandal seedlings inoculated with AMF did vary significantly. Maximum values were observed for sandal seedlings inoculated with *G. mosseae.*
11. Sandal seedlings grown in pots with soils from sandal-growing areas showed superior growth, but no AMF colonization was observed. The soils of sandal growing regions (Marayoor and Wadakancherry) showed higher pH and plant nutrient content.

Conclusions

Sandal - AMF associations has resulted in improved growth of sandal seedlings grown in polybags. Inoculation with AMF may be helpful in obtaining better establishment and growth of sandal seedlings in the field also. However, performance of AMF inoculated seedlings planted in the field has to be studied.

The influences of AMF species varied with the shade level under which the seedlings were grown. It can be concluded that 50 per cent shade is the most favourable for the growth of sandal as well as for the better colonization of AMF. The water relation of the plants was better when grown under moderate shade. Field evaluation of sandal as a component crop in the homesteads/agroforestry systems need to be taken up with appropriate management inputs for increasing the production of sandal in the country.

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Appendices

APPENDIX - I

Weather parameters during (January 2001 to December 2001)

Month	Temperature		Humidity(%)	Rainfall(mm)
	Minimum	Maximum		
JAN	23.2	32.6	71	0
FEB	22.9	34.5	86	12.2
MAR	24.0	34.9	84	4.4
APR	24.7	34.2	88	243.1
MAY	24.5	32.3	89	192.6
JUN	23.1	28.4	87	676.2
JUL	22.7	29	85	477.7
AUG	23.1	27.5	87	256.2
SEP	23.2	30.8	79	206.1
OCT	23.0	30.7	81	215
NOV	23.1	31.6	72	115
DEC	22.2	31.3	60	0

APPENDIX –II

Composition of Trypan blue

Trypan blue	-	50 mg
Lacto phenol	-	100 ml

APPENDIX –III

Lacto phenol

Lactic acid	-	10 ml
Phenol	-	10 ml
Glycerol	-	20 ml
water	-	20 ml

APPENDIX - IV

Abstracts of ANOVA tables for height and number of leaves of sandal seedlings for eight months

Source	df	Mean squares							
		Height				Number of leaves			
		Months after planting				Months after planting			
		1	2	3	4	1	2	3	4
Shade	3	40.72**	122.88**	97.39**	102.02**	65.73**	58.53**	70.22**	52.61**
AMF	3	11.06	14.18	12.64	13.01	16.02	5.03	6.445	8.20
Shade x AMF	9	10.50	11.06	10.61	10.85	10.21	8.98	13.504	9.18
Host	1	14.22	9.12	0.03	8.45	3.50	0.54	10.802	9.18
Host x AMF	3	5.96	3.16	2.58	4.12	6.29	4.18	13.504	0.11
Error	168	5.93	6.01	5.91	5.25	6.10	7.42	11.993	4.38

Source	df	Mean squares							
		Height				Number of leaves			
		Months after planting				Months after planting			
		5	6	7	8	5	6	7	8
Shade	3	62.21**	58.58**	65.94**	36.45**	75.44**	36.34**	177.36**	132.62**
AMF	3	12.61**	20.44**	35.95**	20.4**	34.58**	34.64**	115.64**	114.67**
Shade x AMF	9	6.64	7.83	7.11	3.36	8.01	7.78	5.63	8.83
Host	1	0.76	4.81	0.00	3.10	0.03	0.90	4.23	2.34
Host x AMF	3	6.72	7.94	2.85	1.24	1.88	2.78	3.38	4.01
Error	112 56	3.83	6.40	3.79	2.98	4.70	3.39	5.41	4.54

** Significant at 1% level

APPENDIX - V

Abstracts of ANOVA tables for root length, root weight, shoot weight and leaf area of sandal seedlings for sixth, seventh and eightth month after planting

Source	df	Mean squares			
		6th month after planting			
		Root length	Root weight	Shoot weight	Leaf area
Shade	3	5.10	0.000	0.14**	825.25**
AMF	3	8.93	0.001	0.04**	798.65**
Shade x AMF	9	1.60	0.000	0.01**	42.5
Host	1	37.52	0.000	0.00	32.5
Host x AMF	3	8.35	0.000	0.00	12.25
Error	28	13.85	0.001	0.00	17.75

Source	df	Mean squares			
		7th month after planting			
		Root length	Root weight	Shoot weight	Leaf area
Shade	3	8.89	0.000	0.12**	625.26**
AMF	3	30.52	0.000	0.05*	648.75**
Shade x AMF	9	5.57	0.000	0.01*	31.5
Host	1	26.27	0.001	0.00	28.75
Host x AMF	3	35.56	0.000	0.00	10.75
Error	28	16.97	0.001	0.01	15.75

Source	df	Mean squares			
		8th month after planting			
		Root length	Root weight	Shoot weight	Leaf area
Shade	3	13.26	0.000	0.12**	390.26**
AMF	3	36.51	0.001	0.06*	398.40**
Shade x AMF	9	0.88	0.001	0.02*	22.72
Host	1	7.50	0.000	0.00	8.76
Host x AMF	3	2.78	0.001	0.02	20.69
Error	56	14.10	0.001	0.01	14.38

* Significant at 5% level ** Significant at 1% level

APPENDIX - VI

Abstracts of ANOVA tables for the Relative Water Content (%) of leaves of sandal seedlings for eight month after planting

Source	df	Mean Square
Factor A	3	2.67*
Factor B	3	52.94**
AB	9	0.48
Error	32	0.24

APPENDIX - VII

Abstracts of ANOVA tables for the nutrient contents(%) of the shoot of sandal seedlings for eight month after planting

Source	df	Mean squares		
		N	P	K
Shade	3	17.24**	0.00**	0.28**
AMF	3	3.1**	0.00**	0.04**
Shade x AMF	9	0.01	0.00	0.01
Host	1	0.00	0.00	0.01
Host x AMF	3	0.00	0.00	0.01
Error	64	0.02	0.00	0.01

* Significant at 5% level ** Significant at 1% level

APPENDIX -VIII

Abstracts of ANOVA tables for the Stomatal resistance of the leaves of sandal seedlings during morning and afternoon

Source	df	Stomatal resistance	
		Morning	Afternoon
Shade	3	0.06**	0.05**
AMF	3	0.001**	0.003**
Shadex AMF	9	0.001**	0.002**
Host	1	0.00	0.00
Host x AMF	3	0.00	0.00
Error	56	0.00	0.00

APPENDIX - IX

Abstracts of ANOVA tables for the water potential of the sandal seedlings for seventh and eight month after planting

Source	df	Mean squares	
		Months after planting	
		7	8
Shade	3	7.65*	9.85*
AMF	3	1.69	2.05
Shadex AMF	9	1.63	2.25
Host	1	0.21	0.45
Host x AMF	3	1.62	2.25
Error	28	2.44	
	56		2.75

* Significant at 5% level ** Significant at 1% level

APPENDIX - X

Abstracts of ANOVA tables for height and number of leaves of sandal seedlings grown in soils from two sandal growing and one non-sandal growing region

Source	df	Mean squares							
		Height				Number of leaves			
		Months after planting				Months after planting			
		1	2	3	4	1	2	3	4
Soil	2	10.77	34.44**	58.17**	112.85**	18.02**	36.87**	49.27**	46.49**
Replication	14	3.20	1.56	3.03	4.56	5.55	4.13	5.68	5.94
Error	28	3.57	4.49	7.77	8.42	6.07	8.13	8.31	8.25

APPENDIX - XI

Abstracts of ANOVA tables for the leaf area, root weight, root length and shoot weight of sandal seedlings grown in soils from two sandal growing and one non-sandal growing region

Source	df	Mean Squares			
		Leaf area	Root-length	Shoot weight	Root-weight
Soil	2	1584.95**	46.23**	0.01**	0.00
Replication	14	45.47	4.09	0.00	0.00
Error	28	46.00	4.68	0.00	0.00

** Significant at 1% level

RESPONSE OF SANDAL *(Santalum album* Linn.) SEEDLINGS TO SHADE AND MYCORRHIZAL ASSOCIATION

By

BINU. N. KAMALOLBHAVAN

ABSTRACT OF THE THESIS

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Kerala Agricultural University

DEPARTMENT OF TREE PHYSIOLOGY AND BREEDING
COLLEGE OF FORESTRY
VELLANIKKARA, THRISSUR - 680656
KERALA, INDIA

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ABSTRACT

The occurrence of sandal- Arbuscular Mycorrhizal Fungi (AMF) associations in natural sandal growing forests and the response of sandal seedlings to inoculation with commonly available cultures of AMF, shade levels and nature of hosts were investigated in a pot culture experiment at the College of Forestry, Kerala Agricultural University, Vellanikkara. Two important sandal growing regions in the state, Marayoor (Idukki district) and Wadakancherry (Thrissur district) were selected for this study and a non-sandal growing area in Thrissur district was selected as a control for the soil studies.

The result showed that characters like height, number of leaves, leaf area and shoot weight of sandal seedlings increased due to the Arbuscular Mycorrhizal Fungi inoculation and maximum response was observed for seedlings grown in soils inoculated with *Glomus mosseae*. The characters like collar girth, root weight did not show any significant difference for the mycorrhizal inoculation. It was further observed that the interactions between shade and mycorrhizae were the best under 50 per cent shade level especially for those seedlings inoculated with *Glomus mosseae*.

During the initial seedling phase, high shade (75 per cent) is needed for sandal, but during the later stages of seedling growth, medium shade (50 per cent) resulted in best growth of seedlings. Growth of sandal seedlings with two host species, casuarina and redgram was on par. The relative water content and plant water potential were higher in seedlings inoculated with AMF. The N, P, K content of the shoot of sandal seedlings were maximum for seedlings inoculated with *Glomus mosseae*.

Sandal seedlings collected from natural sandal growing regions investigated for the presence of sandal-AMF association revealed that the roots of sandal seedlings from Wadakancherry forest showed 33 per cent of colonization, while the seedlings from Marayoor forest showed no colonization. Sandal seedlings grown for four months in the soils collected from these two regions showed superior growth when compared to those seedlings grown in soils from the agricultural lands of Thrissur, but no AMF colonization was observed in the roots of the sandal seedlings.