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USE OF MYCORRHIZAL AND NITROGEN FIXING SYMBIONTS IN REFORESTATION OF DEGRADED ACID SOILS OF KERALA

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1. SUMMARY

Several monosporal VAM cultures, raised from spores isolated from the rhizosphere soils under *Acacia auriculiformis, Casuarina equisetifolia* and *Pterocarpus marsupium* and other VAM cultures obtained from various sources were screened under glasshouse conditions to select the most efficient ones for enhancing the growth of each of the three test tree species. Inoculation with VAM fungi was found to benefit the growth and biomass production of *Acacia, Casuarina* and *Pterocarpus*. However, the response to inoculation varied with different isolates of VAM fungi. A significant increase in total P content was also observed in the inoculated plants. Based on overall performance and a cluster analysis of the data, the most efficient VAM selected for field studies were *Glomus calidonius* and *Glomus mosseae* for *Acacia*, culture M-23 and *G. fasciculatum* (V-10) for *Casuarina*, and *G. intraradices* and culture *G. mosseae* (V-8) for *Pterocarpus*.

Several of forty cultures of *Rhizobium* and *Frankia*, isolated from the nodules of *A. auriculiformis, P. marsupium* and *C. equisetifolia* were subjected to authentication. The *Rhizobium* isolates were further screened for acid/alkali production and tolerance to acid-aluminium stress. All the isolates were screened for their efficiency in the glasshouse and two best strains selected for each of the test species. The isolates A-19 and A-16 obtained from *A. auriculiformis*, P-8 and P-9 from *P. marsupium* and C-2, and C-3 from *C. equisetifolia* were found to be superior as compared to others in the ANOVA followed by a cluster analysis. These strains were selected for field trials.

In glasshouse trials on the effect of fertilizers on seedling growth and symbionts, significant differences (P=0.05) were observed between various treatments. The behaviour of all the three species was different in terms of influence of phosphorus and nitrogen on various growth parameters of plants and VAM and N2 fixing symbionts. In *A. auriculifomis* and *P. marsupium*, though significant difference was observed between treatments, no definite trend emerged on the seedlings biomass due to fertilizer application in combination with the symbionts. In *A. auriculiformis* highest VAM infection was recorded in dual inoculation treatments. Generally, in all the fertilizer treatments number of nodules was higher in seedlings with dual Inoculation. There was no evidence of pronounced influence of either phosphorus or nitrogen on nodulation. In *P. marsupium*, treatment with both the dosage of nitrogen i.e. N1 (20 ppm), N2 (40 ppm) resulted in higher VA mycorrhizal infection of roots; however, higher dosage of N and P (i.e.,N2P2) resulted in poor VAM colonization. Phosphorus did not show any significant impact on VAM colonization. Nodulation was poor in fertilizer treated seedlings, especially and N2; apparently P treatments had higher nodulation. In *C. equisetifolia*, only fertilizer treatments in combination with symbionts gave enhanced biomass. Low dose of phosphorus (15 ppm) in combination with nitrogen appeared to increase VAM colonization; at higher level

of P (30 ppm), the colonization decreased. Root nodulation was poor in all the fertilizer treatments.

Significant difference (P=0.0l) was observed between treatments (VAM, N2 fixing symbionts and fertilizer) in height and diameter of field planted l-year-old *A. auriculiformis*. All the treatments responded favourably to fertilizer application. The percent increase in growth (height and diameter) was more in symbiont-inoculated plants than control, especially in fertilized seedlings. Maximum percent increase in height was 57.4 *(G. calidonius)* and 56.2 *(G. calidonius* + *Rhizobium* A-19 Calicut) whereas for diameter growth the maximum increase was 84.5% percent *(Glomus intraradices* + *Rhizobium* A-16 Panamaram), followed by 70.0% *(G. calidonius)*. In control treatments the increase in height and diameter growth was 11.5percent and 41.4 percent.

2. INTRODUCTION

Scarcity of fuelwood, food resources, coupled with increased erosion and decreased soil fertility are the problems shared by many developing nations, especially those situated in tropics. Tropical forests are subject to natural and human disturbance which lead to their degradation (World Resources Institute, 1988). Degradation of forest soils results in laterization which renders it unfit for plant growth due to immobilization of phosphorus, absence of organic matter, low pH and absence or complete depletion of mycorrhiza (Varghese and Byju, 1993). When such a severely degraded site is to be reforested, a common objective is to stabilize the site to prevent fbrther erosion/degradation and to increase its productivity, ideally to the point when it yields a commercially viable product. This requires the diagnosis of limitations to plant growth, their correction as far as possible and introduction of tree species tolerant to the degraded site. In this situation the key issue becomes choice of suitable species. A number of exotic species, especially those fixing atmospheric nitrogen are the potential species for reforestation due to several positive attributes (NAS, 1979). In addition to their rapid growth and ability to symbiotically fix nitrogen they increase the nitrogen content of otherwise impoverished soils. *Acacia* spp. and *Cusuarina* spp. have been used successfully in a number of situations in tropics helping to stabilize site or improve the soil fertility (Anon, 1985; Prasad, 1991).

Mycorrhizal fungi are an integral part of practically all plant communities, natural or managed, and form the link by which mineral nutrients are transferred from the soil to the plant (Brundrett, 1991) while carbon compounds are transported in the opposite direction. Thus they have a fundamental role in determining plant productivity and in the fbnctioning of ecosystems. In many tropical soils, lack of availability of phosphorus is the most important constraint on plant growth (Vitousek, 1984). In degraded/disturbed sites mycorrhizal populations are adversely affected, reducing the amount of inoculum available to infect root systems during the period when trees are becoming established. Hence, in any reforestation programme, inoculation of trees with vesicular arbuscular mycorrhiza (VAM), may be needed to ensure that adequate inoculum is

present to support the growth. However, the benefit which individuals are likely to receive from the presence of VAM varies according to the plant species under consideration because plant species differ in the extent to which they depend on VAM fungi for survival and growth at a given soil fertility (Janos, 1980; 1987). Positive responses of VAM inoculation have been reported for seedlings of numerous leguminous tree species like *Acacia auriculiformis, A. mangum, Leucaena leucocephala* and *Casuarina equisetifolia,* many of which require VAM to supply P for nodulation and nitrogen fixation (Manjunath et al., 1984; Cruz et al., 1988; Aggangan et al. 1990; Vasanthakrishna et al., 1994).

Nitrogen fixing trees (NFTs) are considered as ideal tree species for afforesting degraded soils. This is not only because of their unique ability to fix atmospheric nitrogen in their root nodules with the help of symbiotic bacteria of the genus *Rhizobium* or actinomycetes of the genus *Frankia,* but also because of their tolerance to adverse soil conditions, rapid growth and ease of management (MacDicken, 1994). Although nitrogen fixing trees are able to establish and thrive in nitrogen deficient degraded soil, one of the most serious impediments for nitrogen fixation in degraded soil is the low pH which is an inherent character of degraded tropical soils. Low soil pH generally inhibits nitrogen fixation by reducing the development of *Rhizobia/Frankia,* increasing the number of ineffective strains of the organisms or disrupting the root infection process (Sprent and Sprent, 1990; Mengel and Shubert, 1983). However, it has been reported that there are strains of *Rhizobium* which can tolerate low pH and fix nitrogen efficiently in highly acidic soils (Keyser and Mums, 1979). This clearly demonstrates the ability of *Rhizobium* to select its strains for adverse conditions.

Considering that there is an increasing need to reforest the expanding areas of degraded land that occur in most parts of the Asia Pacific region, the present study focuses on the screening and use of promising VA mycorrhizal and nitrogen fixing symbionts for three nitrogen fixing tree species of high regional importance viz. *Acacia auriculiformis,* and *Casuarina equisetifolia,* (both exotics of Australian origin) and *Pterocarpus marsupium,* a hardy tree species indigenous to India, in reforesting degraded strongly acidic soils of Kerala. The specific objectives of the study were,

i. to identify suitable species/strains of mycorrhizal and *Rhizobium /Frankia* symbionts for the test species in acid soils.

ii. to examine the efficiency of different mycorrhizal species and nitrogen fixing symbionts, individually and in combination, in enhancing the survival, establishment and growth of seedlings of the best species.

iii. to attempt reforestation of degraded acid soils on a pilot scale using the best mycorrhizal and *Rhizobium/Frankia* symbionts.

3. MATERIALS AND METHODS

3.1. Selection of Species

For reforestation studies in highly acidic degraded soils of Kerala, ***Acacia auriculiformis, Casuarina equisetifolia*** and ***Pterocarpus marsupium*** were selected. Of these, the first two species have already been used widely for reforestation of disturbed sites in the tropics and the latter species is known to grow luxuriantly on poor sites in Western Ghats. Characteristics of these species are detailed below.

3.1.1. *Acacia auriculiforrnis* A. Cunn. ex Benth.

Acacia auriculiformis is a versatile tree growing naturally, mainly in the coastal lowlands of northern Australia, Papua New Guinea and a few islands in eastern Indonesia. It favours tropical, frost free, humid to sub-humid climate having annual rainfall of 1,000 to 1,500 mm with a monsoonal distribution pattern and a dry season of upto six months. It grows mainly at low altitudes (below 100 m) and has the ability to grow on harsh sites tolerating clayey, sandy, acidic, alkaline, saline or seasonally water-logged soils with long dry season.

Because of its spreading, densely-matted shallow root system ***A. auriculiformis*** is an excellent species for stabilizing eroded land and it competes effectively with dominant weeds. Rapid growth even on infertile site, and tolerance to both acidic and alkaline soil, have made it popular for stabilizing and revegetating the degraded soils (Anon, 1987) (Fig.1).

3.1.2 *Pterocarpus marsupium* Roxb.

P. marsupium, a leguminous tree indigenous to peninsular India and Sri lanka, is a hardy tree found growing equally well in various types of soils. It grows luxuriantly at an altitude of 100-120 m msl, though it is found at lower elevation also. It prefers tropical warm-humid climate with an annual rainfall ranging from 2500-4500 mm. Trees are common in moist deciduous forests (Fig.2), in and around grasslands, the sides of ravines, rocky forest fringes, and rocky and dry habitat.

3.1.3. *Casuarina equisetifolia* Forst & Forst (Syn.C *litorea)*

C. equisetifolia is the most widespread and well known member of the family Casuarinaceae indigenous to southeast pacific islands and Australia. Though, generally it is a lowland tree it grows upto an altitude of 600 m in a wide variety of soils under hot-humid conditions. ***C. equisetifolia*** tolerates a wide range of moisture regimes and calcareus, alkaline and acidic soils (Fig.3), it thrives in sandy to lateritic soils and grows poorly in clay soils, with some exceptions. It

Fig.1. A view of a plantation of *Acacia auriculiformis* (planted in 1987) in acidic soil at Ramavarmapuram, Kerala, India

Fig.2. A. Natural habit of *Pterocarpus marsupium* in a moist deciduous forest at Walayar, Kerala, India. B. Root nodules on six-month-old seedlings.

Fig.3. A view of a plantation of *Casuarina equisetifolia* raised in 1990 in acidic soil at Ramavarmapuram, Kerala, India.

cannot stand water logging for long as it inhibits nodule development. ***C. equisetijolia*** supports actinorhizae symbiont in their root nodules and fixes atmospheric nitrogen. Root nodules are prolific and of large size. It is also known to have VA mycorrhizal association (Anon, 1985, 1990).

3.2. Mycorrhiza

3.2.1. Collection of Rhizosphere soil

Rhizosphere soil samples were collected from plantations of ***Acacia auriculijormis*** and ***Casuarina equisetifolia*** and naturally occurring trees of ***Pterocarpus marsupium*** from different parts of Kerala, especially those having lateritic and acidic soil.

3.2.2. Isolation of VAM spores

VAM spores were isolated from the soil samples by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Ten gram of soil was mixed with 100 ml 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 mm to 45 µm. The washings from 108 µm and 45 µm sieves were filtered through filter paper. The particles remaining on the filter paper were observed under stereoscope and VAM spores were picked up carefblly and transferred to glass vials containing sterile water.

3.2.3. Sterilization of VAM spores

Spores were surface sterilized with sterilant mixture (5% chloramine T., 0.1% Tween 80 and 0.2% streptomycin sulphate in the ratio 9:9:2 by volume for one minute and washed in several changes of sterile water.

3.2.4. Raising monosporal VAM cultures

Monosporal VAM cultures were raised from surface sterilized spores employing funnel technique (Menge and Timmer, 1982) (Fig.4). Glass funnels were filled with 1:1 mixture of soil and sand after plugging the tail portion with cotton. The whole assembly was sterilized in autoclave. A part of the soil-sand mixture was transferred to sterile petridish and the spores were kept inside the funnel a few cm deep. The fbnnel was refilled with sterile soil sand mixture and sown with maize seeds, surface sterilized in 0.1% sodium hypochlorite (10 minutes). After 6 weeks, roots were examined for VAM infection. The inoculum was then mass multiplied using maize sown in soilrite mix in large plastic trays.

3.2.5. VAM cultures from other sources

VAM cultures were obtained from various sources such as International Crop Research Institute for Semi Arid Tropics (ICRISAT), Bharathiya Agro-

Fig.4. Funnel technique to raise single spore culture of VA mycorrhiza using maize seedlings as a host plant

Industries Foundation (BAIF), Tamil Nadu Agricultural University, Coimbatore (TNAU), University of Agricultural Sciences, Bangalore (UAS), Tata Energy Research Institute, New Delhi (TERI), Jawaharlal Nehru University, New Delhi (JNU). These were mass multiplied using maize grown in soilrite mix and maintained in glasshouse (Fig.5,6).

3.2.6. Testing of root colonization by VAM Fungi

Different VAM cultures thus obtained were tested periodically for their infection in maize roots by clearing and staining method (Philips and Hayman, 1970). Roots were washed thoroughly and cut into 1 cm bits. Root bits were cleared by boiling in 10% KOH. After washing, the roots were acidified in 1% HCI and stained with 0.05% Trypan blue. Destaining was done by keeping the root bits in lactoglycerol overnight. Roots were observed under the microscope for VAM colonization.

3.2.7. Selection of VAM cultures for glasshouse trial

Cultures which showed very poor colonization on maize roots were discarded. Number of spores per 100 g inoculum was determined by wet sieving and decanting method.

3.2.8. Screening of VAM cultures under glasshouse conditions

Seed treatment: Acacia seeds were scarified in hot water (80°C) for 3 minutes and soaked in sterile water overnight. ***Casuarina*** seeds were sown without any pretreatment. For ***Pterocarpus,*** the seeds were surface sterilized in 0.1% mercuric chloride for 10 minutes, washed and soaked in sterile water overnight. The ***Pterocarpus*** seeds were sown in sterilized sand, and the seedlings were transplanted after 2 weeks.

i. ***Inoculation:*** 250 g of inoculum was mixed with 250 g soilrite for filling one polythene bag. Superphosphate was applied as a basal dose to provide 10 ppm/bag. Seeds of Acacia and ***Casuarina*** were sown directly in polythene bags and thinning was done after one week, retaining only 6 seedlingshag. For ***Pterocarpus,*** 2-week-old seedlings were transplanted to polythene bags at the rate of 3 seedlingshag.

ii. ***Maintenance of seedlings:*** Seedlings were irrigated daily with filtered water and fertilized with Ruakura Nutrient Solution once in 15 days.

iii. ***Recording of observations:*** Hundred days after inoculation, the plants were uprooted and observation on plant height, root length and fresh and dry weight of roots/shoots were recorded. Roots were cleared and stained (120 root bits/culture) for recording % root colonization. Total P content of seedlings was estimated by Vanadomolybdate yellow colorimetric method (Jackson, 1973).

Figs.5-6. Fig.5. Multiplication of inoculum of VA mycorrhiza raised from single-spore using maize as host plant. Fig.6. Different single spore cultures of VA mycorrhizal fungi maintained in the glasshouse using maize as host plants.

iv. ***Analysis of data:*** The data were analysed using one way ANOVA test and means compared using DMRT. Most efficienct VAM cultures (2 each) for ***Acacia, Casuarina*** and ***Pterocarpus*** were selected based on complete linkage cluster analysis (Everitt, 1974) using the statistical package SPSS/PC.

3.3. *Rhizobium*

3.3.1. Collection of root nodules

The root nodules of ***A. auriculifomis*** (Fig.7,8) and ***P. marsupium*** (Fig.2B) were collected from different localities of Kerala (Table 11 and 12) and ***Rhizobium*** was isolated as described by Vincent (1970).

3.3.2. Isolation of *Rhizobium*

Large and healthy nodules were selected for isolation. The selected nodules were washed free of surface soil, surface sterilized in 95% ethyl alchohol for 5 to 10 seconds, then transferred to a solution of 0.01% mercuric chloride for three minutes. They were washed in several changes of sterile distilled water in petriplates The surface sterilized nodules were then asceptically transferred to test tubes containing one ml of sterilized distilled water. The nodules were crushed with a sterile glass rod and suspension was prepared. A loopful of the suspension was streaked on yeast extract mannitol agar medium (YMA) with Congo red to obtain isolated colonies. The plates were incubated at 26-28°C for four days; water clear or white colonies occurring on the media were selected and purified by repeated streak method. Pure colonies were maintained on yeast-extract mannitol agar slants and used for further work.

3.3.3. Authentication

The following tests were conducted to authenticate the rhizobial isolates obtained:

Growth on Congo red medium: The yeast extract mannitol agar medium with 2.5 ml of 1% Congo red per litre was prepared. The petriplates were poured with 15-20 ml of the media and allowed to solidify. Young cultures of rhizobia were streaked on these plates and incubated for 7 days at 26-27°C. Little or no absorption of Congo red by colonies was confirmatory to ***Rhizobium.*** Growth rate was recorded.

ii. ***Growth on YMA with Bromothymol Blue:*** Freshly prepared yeast extract mannitol agar plates containing bromothymol blue having a pH of 6.8-7.0 were streaked with rhizobial isolates. Slow growing rhizobia gave alkaline reaction in this medium turning the dye colour to blue. Fast growers gave acid reaction, turning the medium yellow from green colour.

Figs.7-8 Nitrogen fixing rhizobial nodules of *Acacia auriculiformis*. Fig.7. Natural habit of nodules attached to roots in the sub-surface layer of soil. Fig.8. Detached nodules to show the different shapes of nodules.

iii. ***Plant infection test:*** The isolates were tested for their nodulating ability on their respective host namely: ***Acacia auriculiformis*** and ***Pterocarpus marsupium*** in seedling agar tubes (Vincent, 1970).

a. ***Preparation of seedling agar tubes:*** Coming test tubes 2.9 cm x 19 cm size containing 40 ml of sterile Jensen's agar slope was used.

b. ***Development of seedlings:*** Healthy seeds of ***Acacia auriculiformis*** were taken in 250 ml sterile beaker and rinsed in 95% alcohol for 3 minutes with agitation. Then, the alcohol was poured off and seeds were immersed in 0.1% mercuric chloride for 3 minutes. The mercuric chloride was decanted and seeds were washed in several changes of sterile distilled water. The surface sterilized seeds were spread on 1.5% solidified agar on petriplates. The seeds were allowed to germinate and then placed on the Jensen's agar slants using sterile forceps.

c. ***Preparation of inoculant:*** Five ml of yeast-extract mannitol broth was dispensed in test tubes and sterilized by autoclaving at 121°C at 15 lb/in^{2}pressure for 15 minutes. Then a loopful of the rhizobial culture was inoculated to the broth. The inoculated broth was incubated at 26-28°C for seven days and aerated on a rotary shaker.

d. ***Inoculation:*** The seedlings were inoculated with two ml of the test isolates into the tubes. The lower portions of the tubes were covered with black paper. The seedling agar tubes were incubated in a light chamber for 16 hours and watered periodically with sterile distilled water. Five replicates were maintained for each test isolate. The seedlings were observed for nodules till six weeks.

3.3.4. Screening of different Rhizobium isolates for acid-tolerance (Ayanaba et al., 1983)

Two media containing the basal solution (Keyser and MUMS, 1979) were used to test the response of rhizobia to acid and aluminium stress. The control medium contained the basal medium with 0.005% bromocresol purple indicator. The pH was adjusted to 7.0 before autoclaving and fell to 6.3 after autoclaving. The stress medium contained the basal medium with 0.005% Bromocresol green and 50µ M Al as KAI $(SO_4)_2$ was filter sterilized. The pH was adjusted to 4.7 before and after autoclaving with HCl. The medium was poured into petriplates at the rate of 20 ml per plate and allowed to solidify. Each rhizobial culture broth of 0.04µ L was inoculated to the stress medium and control medium. The petridishes were incubated at 28-30°C for 9 days and growth was recorded for each isolate.

3.3.5. Screening of different rhizobial isolates under pot cultures

The efficiency of different ***Rhizobium*** isolates was studied in polypots using sterilised sandsoil-sand mixture.

Raising of seedlings: The ***Acacia auriculiformis*** seeds were put into flasks containing hot water at 80°C for 3 minutes and washed with sterile distilled water. The washed seeds were soaked overnight and sown on tray containing sterilized sand. The seeds of ***Pterocarpus marsupium*** were surface sterilized with 0.1% $HgCl_2$ and washed several times with distilled water. After soaking for 12 hours, the seeds were sown in trays containing sterile sand. The one- week-old-seedlings were transplanted in polythene bags containing sterilized sand.

ii. ***Preparation of inoculum:*** Yeast extract mannitol broth (25 ml) was dispensed into 100 ml conical flask and sterilized at 120°C for 15 minutes. A loopful of culture of each isolate was inoculated to the broth and incubated for 5 days at 26-28°C.

iii. ***Inoculation of seedlings:*** The ***Acacia auriculiformis*** and ***Pterocarpus marsupium*** seedlings were inoculated with 25ml (34×10^7 cells/ml and 47×10^7 cells/ml) of inoculum containing respective ***Rhizobium.*** Fifteen replications were maintained for each isolate.

iv. ***Maintenance and harvesting:*** The plants were maintained in the glasshouse and watered with sterilized N-free deionised solution. After 90 days, the seedlings were carefully removed with their root system intact. The following observations were recorded.

a. Fresh weight of root
b. Fresh weight of shoot
c. Fresh weight of plant
d. Fresh weight of nodules
e. Dry weight of root
f. Dry weight of shoot
g. Dry weight of plant
h. Number of nodules
i. Total nitrogen of plant

v. ***Analysis of data:*** The data obtained were analysed based on one way analysis of variance test (ANOVA) and the means compared using Duncan's multiple range test (DMRT).

vi. ***Selection of two best Rhizobium strains;*** The two best ***Rhizobium*** stains were selected based on dry weight of plant, fresh weight of nodules and total nitrogen content of plant using complete linkage cluster analysis. The best strains were used for field studies.

3.4. *Frankia*

3.4.1. Collection of root nodules

Freshly formed root nodules of ***Casuarina equisetifolia*** (Fig.9-12) were collected from eight localities in Kerala, including riverbed and seashore and transported to laboratory (Table 22). Culturing of ***Frankia*** was attempted using the following two methods.

i. ***Modified sucrose density gradient method (Baker and O'Keefe, 1984)***

A small portion of nodule tissue was surface sterilized with 0.1% $HgCl_2$ for 3 minutes. The nodule pieces were rinsed with sterile distilled water several times and crushed using a sterile pestle and mortar to form a crude nodule suspension. The suspension was inoculated with 0.7% phenol (V/V) and incubated for 10 minutes. After incubation, a small sample of the nodule suspension was applied to a sterile discontinuous sucrose density gradient composed of 3 layers with bottom layer (60%) middle layer (45%) and top layer (30%). The gradients were centrifbged to equilibrium using ultracentrifbge (SORVALL OTD 65B) equipped with swinging bucket rotor. On completion, the gradient tubes were removed and 0.1 ml of the inoculum was added to defined propionate minimal medium. The plates were incubated at 25-28°C for a period of 3 weeks and checked for the growth of ***Frankia.***

ii. Serial dilution method (Quispel and Tak, 1978)

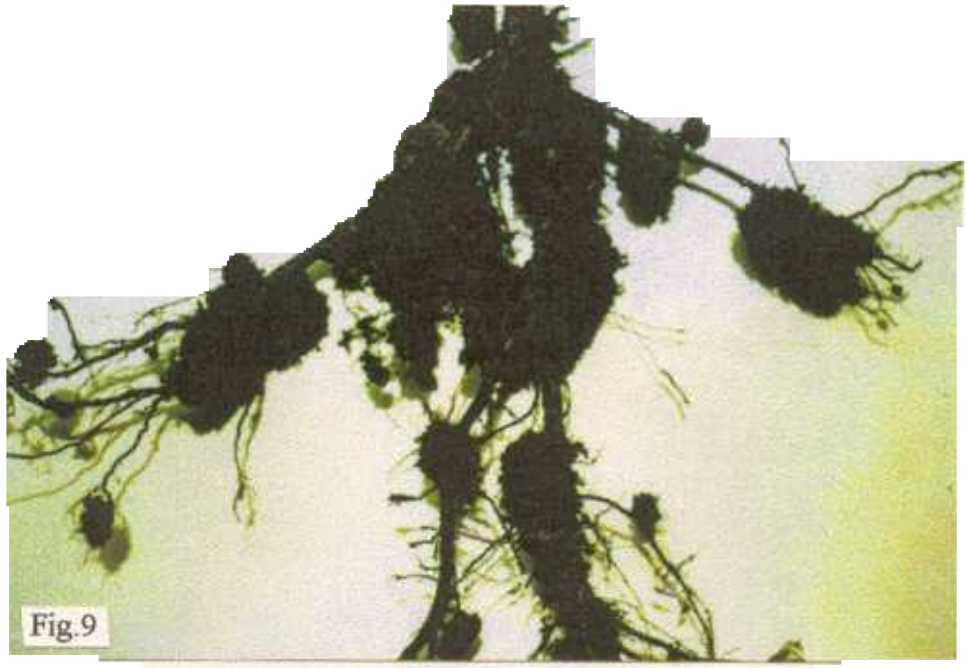
Young nodules, 1 g was taken and washed with sterile distilled water. The nodule was surface sterilized with 30% Hydrogen peroxide for 10 minutes and washed serially with several changes of sterile water. The nodule was crushed using a sterile pestle and mortar with 10 ml of sterile water. The nodule suspension was serially diluted and 0.1 ml was inoculated to Sodium propionate medium. Each isolate was replicated three times. The petriplates were incubated at 2530°C for 3 weeks and checked for ***Frankia*** growth.

3.4.2. Screening of different Frankia isolates under pot culture studies

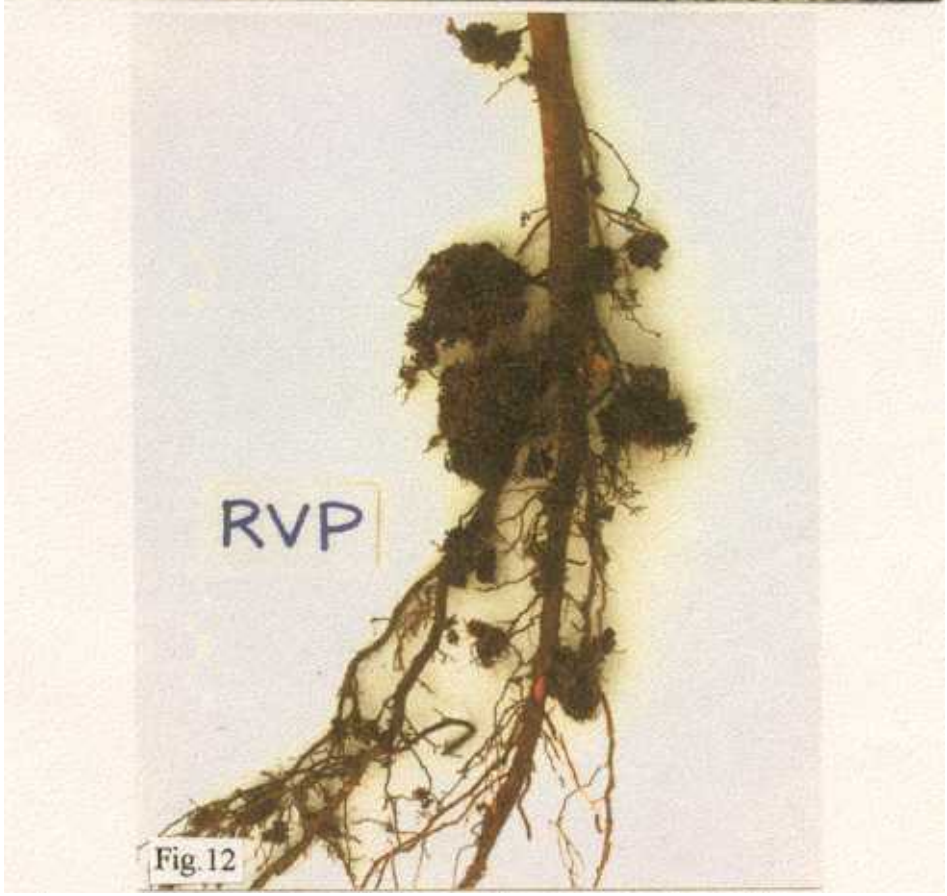
The ***Frankia*** strains were tested for their efficiency in glasshouse using crushed nodule suspension as the inoculum.

i. ***Raising of seedlings:*** The ***Casuarina*** seeds were washed with sterile water and sown in tray containing sterilized soil. Two-week-old seedlings were transplanted in poly bags (13 x 18 cm) filled with sterilized soil.

ii. ***Preparation of inoculum:*** The nodules (100 g) were washed in sterile water and then surface sterilized with 30% H_20_2 for 10 minutes. The nodules were washed with several changes of sterile water, crushed



Figs.9-10. Large actinorhizal root nodules produced on the roots of *Casuarina equisetifolia* growing at river banks in sandy soil.



Figs. 11-12. Actinorhizal root nodules of *Casuarina equisetifolia*. Natural habit of nodules attached to roots in acidic soil at Ramavarmapuram, Kerala, India.

using a sterile pestle and mortar with 250 ml of sterile distilled water. The nodule suspension was filtered with cheese cloth into a conical flask (1000 ml).

iii. ***Inoculation of seedlings;*** The seedlings were inoculated with 100 ml of nodule suspension and fifteen replications were maintained for each isolate.

Maintenance and Harvesting: The plants were maintained in the glasshouse for 6 months and then carefully removed with whole root system intact. The following observations were recorded.

a. Fresh weight of root b. Fresh weight of shoot
c. Fresh weight of plant d. Fresh weight of nodules
e. Dry weight of root f. Dry weight of shoot
g. Dry weight of plant h. No. of nodules

v. ***Analysis of data:*** The data obtained were analysed using one-way analysis of variance test and means compared adopting Duncan's Multiple Range Test.

vi ***Selection of two best Frankia strains:*** The two best ***Frankia*** strains were selected based on dry weight of plant, fresh weight of nodules and number of nodules using cluster analysis. The best strains were used for field studies.

3.5. Standardisation of fertilizer dosage in relation to seedling biomass and symbionts

Effect of fertilizers on seedling biomass, VA mycorrhizal infection and number of nodules per plant was studied in a glasshouse trial.

3.5.1. Raising of seedlings

Seedlings of ***Acacia auriculiformis, Pterocarpus marsupium*** and ***Casuarina equisetijolia*** were raised as described earlier.

3.5.2. Preparation of inoculum

Inoculum of best mycorrhiza in glasshouse trials i.e. ***Glomus calidonius (A. auriculiformis), G. intraradices, (P. marsupium)*** and M-23 **(C. *equisetifolia)*** was raised as described earlier. For ***Rhizobium,*** yeast extract mannitol broth (250 ml) was dispensed into 500 ml conical flask and sterilized at 121° C for 15 minutes. A loopful of the best ***Rhizobium*** culture A-19 (Calicut) for ***A. auriculiformis*** and P-19 (Kannara) for ***P. marsupium*** were inoculated to the broth and incubated for 5 days at 26-28°C. Inoculum of ***Frankia*** C-2 (Kappad) for ***C. equisetifolia*** was prepared by crushing fresh root nodules as described elsewhere.

3.5.3. Inoculation of seedlings

Ten gram of mycorrhizal inoculum was mixed with 400 g of sand:soil mixture and filled in polythene bags. Fifteen-day-old seedlings of ***Acacia, Casuarina*** and ***Pterocarpus*** were transplanted at the rate of two seedlings/bag and N and P fertilizers applied as urea and superphosphate to provide 20 and 40 ppm of N and 15 and 30 ppm of P respectively.

The ***A. auriculiformis, P. marsupium*** and ***C. equisetifolia*** seedlings, raised in sterilized sand: soil mixture (1:1) were inoculated with 2 ml of respective ***Rhizobium/Frankia*** culture appropriately. The nitrogen fertilizer (urea) was applied at the rate of 20 ppm (N1) (@ 8 kg Nha) and 40 ppm (N2) (@ 16 kg N/ha) and phosphorus fertilizer (Single superphosphate) at the rate of 15 ppm (Pl) (@ 2.25 kg Pha) and 30 ppm (P2) (@ 4.5 kg Pha) to each bag containing 400 g of sand: soil mixture. There were 36 treatment combinations, each replicated 12 times (6 replicate bags, each with 2 p1ants)as shown in Table 1 below.

Table 1. Treatment combinations in fertilizer trial conducted in the glasshouse. No of Treatments:36; No. of replications: 12

Treatment Combinations				
M	MN1	R/FN1	MR/FNl	CNl
R/F	MP1	R/FP1	MR/FP1	CPl
MR/F	MNlP1	R/FN1P1	MR/FNlPl	CNlP1
C	MN2	R/FN2	MR/FN2	CN2
	MP2	R/FP1	MR/FP1	CP1
	MNlP2	R/FN1P2	MR/FNlP2	CNlP2
	MN2P1	R/FN2P1	MR/FN2P1	CN2P1
	MN2P2	R/FN2P2	MR/FN2P2	CN2P2

M, Mycorrhiza R, ***Rhizobium;*** F, ***Frankia;*** **MR/F,** Mycorrhiza + ***Rhizobium/Frankia;*** C, Control

After 3 months, seedlings were uprooted gently and observations were recorded on their fresh and dry weight, percent VAM infection and number of root nodules. The data were analysed statistically by subjecting them to ANOVA and DMRT.

3.6. Field Trial

3.6.1. Experimental site

Many degraded forest areas were visited and soil collected for testing the pH. Of the few areas which had highly acidic soil pH, Ramavarmapuram in Trichur Kerala was selected for pilot-scale field experiment on the basis of workable terrain and logistics. The selected site had a gentle slope and it was earlier

planted with cashew and teak, but the plantations failed. The area was covered with grasses and other thorny weeds; it had some rocky outcrops. Two sides of the selected site had plantations of ***A. auriculiformis*** (planted in 1985) and ***Casuarina equisetifolia*** (planted in 1989) raised by the Social Forestry Wing of the Kerala Forest Department.

3.6.2. Climatic data

Monthly rainfall and average maximum and minimum temperature for the year 1994-95 near the experimental site are provided in Fig. 13.

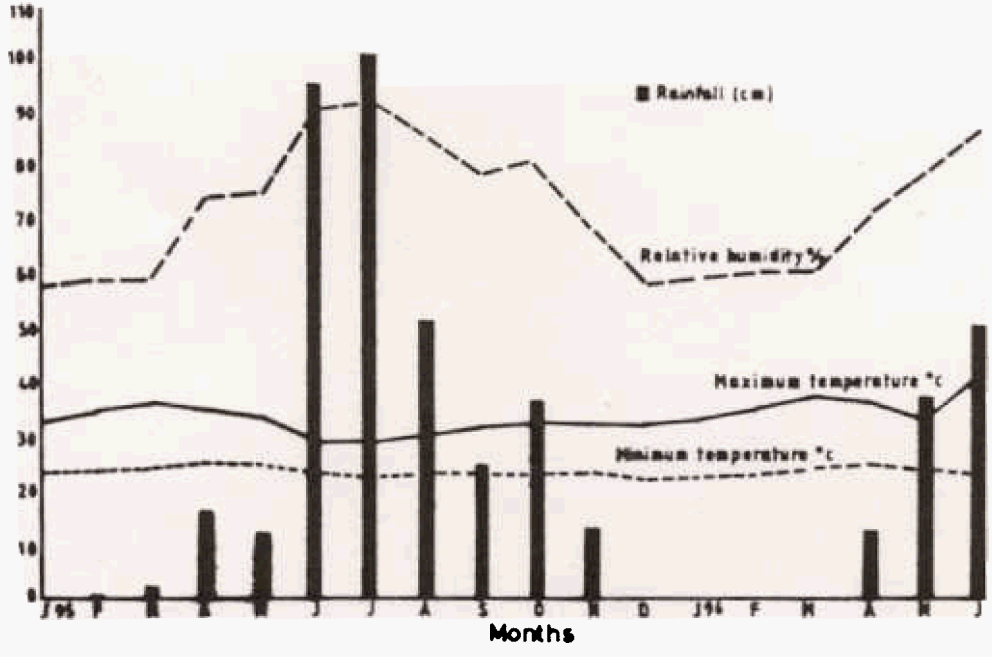


Fig.13. Climatic data (rainfall, Relative humidity and temperature) at the site of the Field Trial at Ramavarmapuram, Thrissur Dist., Kerala State, India.

3.6.3. Soil analysis

Soil samples were collected from each of the experimental plots belonging to ***Acacia auriculiformis*** and ***Pterocarpus marsupium.*** Four surface (00-20 cm) samples were taken from each plot and pooled into one. The soil material was brought to the laboratory, air dried and passed through a 2 mm sieve. The samples were analysed for texture, pH in water and KCl, organic carbon, exchange acidity and bases, exchangeable Ca and Mg and Bray II extractable P (Jackson, 1973).

Table 2. Physical and chemical properties of soil in the experimental trial plots of ***Acacia auriculiformis*** (AA) and ***Pterocarpus marsupium*** (PM)

No.	Sample Name	Gravel %	Sand %	Silt %	Clay %	pH H_2O	pH KCl	OC %	N%	P	Ca	Mg
								---------- Extractable (ppm)				
1	AAl	19	82	9	9	5.1	4.1	1.03	0.09	1.0	180	12
2	AA2	27	79	9	12	5.0	4.0	1.01	0.08	1.2	140	20
3	AA3	20	76	10	14	5.1	3.9	0.94	0.07	ND	120	36
4	AA4	25	75	11	14	5.0	4.0	1.15	0.10	1.1	120	24
6	AA6	30	80	10	10	5.1	4.0	0.91	0.09	2.0	160	12
1	PM1	6	77	11	12	5.0	3.9	0.85	0.08	2.2	120	30
2	PM2	8	79	11	10	5.0	3.9	0.69	0.07	0.6	100	30
3	PM3	4	78	10	12	4.9	3.9	0.65	0.07	ND	140	24
4	PM4	3	75	11	14	4.9	3.9	0.67	0.08	1.0	160	12
6	PM6	7	78	10	12	5.0	3.9	1.04	0.10	1.0	120	36

The results of the soil analysis are given in Table 2. The soils in the plots with ***Acacia auriculiformis*** and ***Pterocarpus marsupium*** are sandy loam in texture and highly acidic in reaction. There is a variation of over a point in pH in 1 M KCl than pH in H_2O_2 indicating high content of Al and Fe in the soil. The soil is very poor in organic carbon content and hence Nitrogen content also. The extractable (Bray 11) P in the soil is far below optimum range, the maximum being 3 ppm and minimum an undetectable level by the method. The Ca and Mg content are also very low. Thus, the soil in the experimental plot represents a highly degraded acidic ferrallitic one with very low levels of organic carbon, nitrogen and extractable phosphorus.

3.6.4 Site preparation

The planting site, about 1.5 ha, was weeded thoroughly before the onset of monsoon in June and fenced with barbed wire to afford protection against cattle. After proper alignment and staking pits of 30 cm x 30 cm x 30 cm were taken during June/July 1994 at an espacement of 1 m x 1 m as per the experimental design.

3.6.5 Experimental design

The experimental area was divided into three plots for planting the three species separately. Within each species, each of the following 18 treatment were replicated in three sub plots in randomised block design (RBD). Each treatment had 49 seedlings planted in a block in 7 x 7 pattern.

1. M1	5. M1 R1/F1	9. M1 R1/F1F	13. M1F	17. CF
2. M2	6. M1 R2/F2	10. MI R2/F2F	14. M2F	18. C
3. Rl/FI	7. M2 RI/Fl	11. M2 RI/FI F	15. R1 F	
4. R2E2	8. M 2R2/F2	12. M2/R2/F2 F	16. R2 F	

M1, M2 = Two isolates of VA mycorrhiza; RI/FI, R2/F2 = Two isolates of ***Rhizobium/Frankia;*** F= Fertilizer; C= Control (untreated).

3.6.6. Planting stock

Seedlings of ***Acacia auriculiformis, Casuarina equisetifolia*** and ***Pterocarpus marsupium*** were raised in the glasshouse. Seeds were surface sterilized and sown in sterile sand and field soil (2 mm mesh-sieved soil from Ramavarmapuram) mixture (1:1) in metallic trays. The trays were watered regularly with distilled water. Ten-day-old seedlings were transplanted into polythene containers (8 x 15 cm) filled with sand and field soil moisture (1 : 1).

3.6.7. Inoculation with symbionts

At the time of transplanting the seedlings into the containers, appropriate VAM culture (10 g), containing >l00 spores, was placed in the bag in direct contact with the roots of seedlings and container filled with planting medium. In the field, seedlings were inoculated by placing 25 g of VAM inoculum (containing >200 spores) of appropriate VAM fungus by spreading it into the pit and placing the seedling roots directly over it.

Seedlings were inoculated with ***Rhizobium/Frankia*** by pouring the inoculum solution at the root region at the time of transplanting, control seedlings did not receive any inoculation.

For preparing the inoculum of ***Frankia,*** fresh nodules were collected from field, washed thoroughly in running tap water to remove the adhering soil and fresh current year nodules, light pinkish in colour were removed. These nodules were washed in sterile tap water, ground into a solution using pestle and mortar in sterile water and filtered through a cheese cloth. Ten millilitre of this solution was added to each seedling at the root region.

The following table gives the details of the most efficient VAM fungi ***Rhizobium/Frankia*** symbionts in glasshouse studies which were used in field trial.

Table 3. Symbionts used in inoculation of outplanted seedlings

Symbionts	*Acacia auriculiformis*	*Pterocarpus marsupium*	*Casuarina equisetifolia*
VAM fungus	1.Glomus catidonius(M1)	1.Glomus intraradices (M1)	l.M-23 (Ml)
	2.G. mosseae(M2)	2.G. mosseae TNAU II(M2)	2.G.fasciculatum TNAU IV(M2)
Rhizobium	1. A-19 Calicut(R1)	1. P-8 Kannara(R1)	
	2. A-16 Panamaram(R2)	2. P-9 Mannuthy(R2)	
Frankia			1. C-2 Kappad(F1)
			2.C-3 Kuttipuram(F2)

3.6.8. Field operations

The seedlings were outplanted during August 1995. During October/November, weeding was done around (60 cm dia) the seedlings and the soil worked.

3.6.9. Fertilizer application

After one month of field planting urea and superphosphate were applied separately @ 10 g per seedling with a dosage of 40 kg N/ha and 15 kg P/ha, respectively. A circular trench, about 5 cm deep, was made 15 cm around the seedling to be fertilized and the measured quantity of fertilizer evenly applied in the trench; the fertilizer was covered with soil.

3.6.10. Observations and statistical analysis

Height growth and diameter were recorded at 25 cm height for all the surviving plants. The data were subjected to ANOVA and cluster analysis (Everitt, 1974) to find out the most suitable treatments.

4. RESULTS

4.1. Mycorrhiza

Eighteen monosporal VAM cultures were selected for mass multiplication (Table 4). Eighteen VAM cultures were obtained from other sources such as ICRISAT, BAIF, TNAU, UAS, TERI, JNU (Table 5). Out of these 36 VAM cultures, 26 cultures were selected for glasshouse studies on the basis of colonization of maize roots and number of spores produced in soilrite (Table 6) (Fig. 14-18).

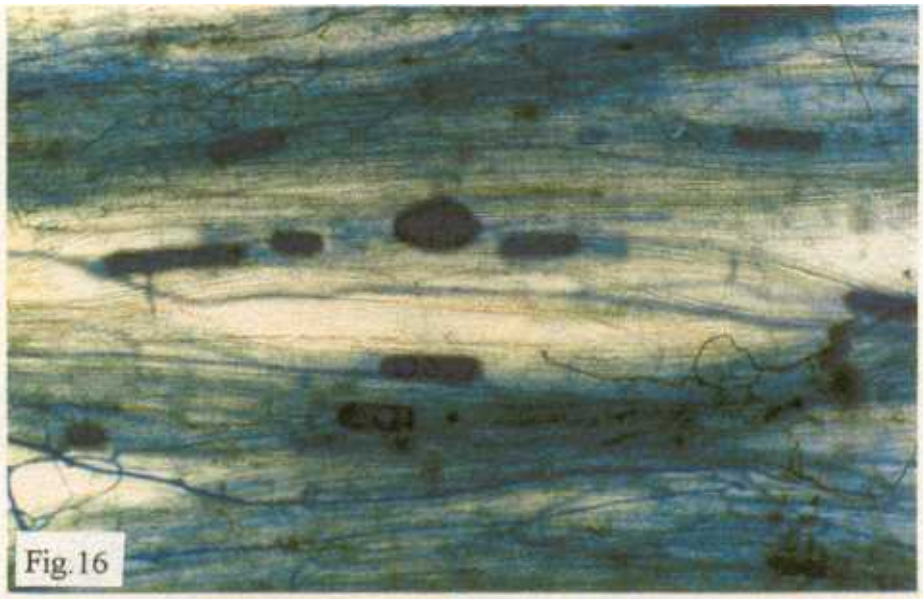
Table 4 List of monosporal VAM cultures isolated from Kerala soils under *Acacia auriculiformis*

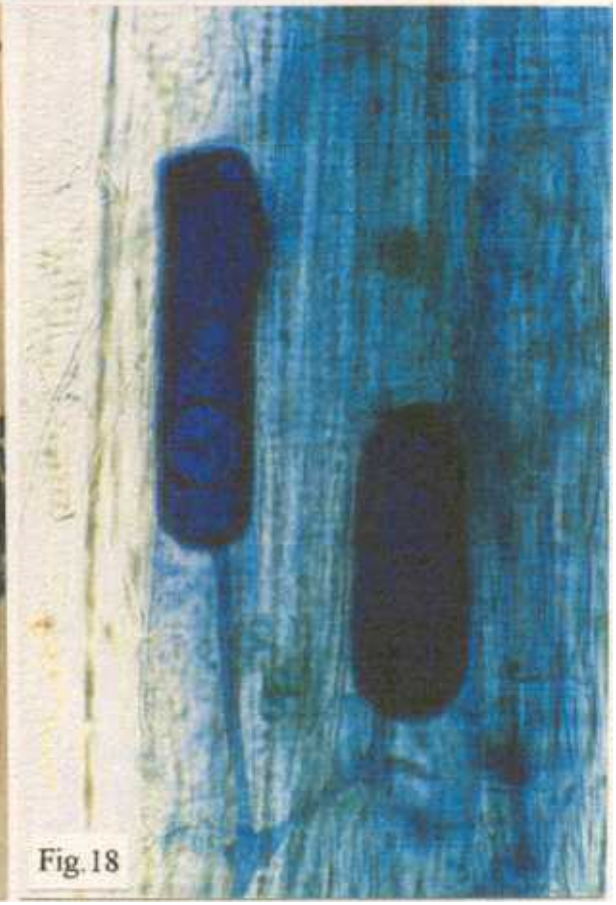
S1.No.	VAM Culture No.	Origin of culture	
		Locality	District
1	M-8	Chettikulam	Ernakulam
2	M-9	Chettikulam	Ernakulam
3	M-13	Ramavarmapuram	Trichur
4	M-18	Kuttipuram	Malappuram
5	M- 22	Peruvannamuzhi	Calicut
9	M-11	Kundukad	Trichur
10	M-12	Kuttipuram	Malappuram
11	M-16	Kanjikode	Palghat
12	M-17	Kuttipuram Hill	Malappuram
13	M-20	Ramavarmapuram	Trichur
14	M-25	Dharmadam	Cannanore
15	M-2	Chettikulam	Ernakulam
16	M- 21	KFRI	Trichur
18	M-31	KFRI	Trichur

Fig.

15

Figs Intact and broken spore *Glomus* (x510)





Figs.16-18. Colonization of maize roots showing extensive mycelium and vesicles of VA mycorrhizal fungus, *Glomus* sp. (Fig. 16-x80; Fig.17-x200; Fig. 18-x320).

Table 5. List of VAM cultures obtained from other sources

Sl.No.	VAM Species	Source
1.	*Glomus mosseae*	ICRISAT, Hyderabad
2.	*G.fasciculaturn*	ICRISAT, Hyderabad
3.	*G. monosporum*	ICRISAT, Hyderabad
4.	*G. constrictum*	ICRISAT, Hyderabad
5.	*G.fasciculatum* 1	BAIF, Pune
6.	*G.fasciculatum* 2	BAIF, Pune
7.	*G. aggregatum*	BAIF, Pune
8.	*G. fasciculatum* (TNAU I)	TNAU, from(1CRISAT)
9.	*G. mosseae* (TNAU II)	TNAU, Coimbatore
10.	*G. etunicatum* (TNAU III)	TNAU, Coimbatore
11.	*G. fasciculatum* (TNAU,IV)	TNAU, Coimbatore
12.	*G. intraradices*	UAS, Bangalore
13.	*G. velum*	UAS, Bangalore
14.	*G. mosseae*	TERI, Delhi
15.	*G.calidonius*	TERI, Delhi
16.	*G.fasciculatum*	TERI, Delhi
17.	*Gigaspora margarita*	TERI, Delhi
18.	*Glomusfasciculatum*	JNU, Delhi

Table 6. Percent infection of maize seedlings by different VAM cultures and their spore population in soilrite

Isolate No.	VAM culture	% colonization	No.of spored 100 g soilrite
V-1	*Glomus fasciculatum,* ICRISAT	83.3	60
V-2	*G. constrictum,* ICRISAT	91.6	65
V-3	*G. monosporum,* ICRISAT	58.3	110
V-4	*G.fasciculatum* BAIF, I	100.0	55
V-5	G. *mosseae,* ICRISAT	50.0	60
V-7	*G.fasciculatum* (TNAU I)	75.0	30
V-8	*G. mosseae* TNAU 11	100.0	50
V-9	*G. etunicatum T*NAU 111	100.0	20
V-10	*G.fasciculatum* TNAU IV	100.0	30
V-11	*Glomus velum,* UAS	100.0	70
V-12	*G. intraradices,* UAS	58.3	50
V-13-	*G. mosseae,* TERI	100.0	50
V-14	*G.fasciculatum,* TERI	100.0	90
V-15	*G. aggregatum,* BAIF	100.0	130
V-16	*Gigaspora margarita,* TERI	83.3	50
V-17	*Glomus calidonius,* TERI	100.0	60
v-18	*G.fasciculatum,* JNU	100.0	90
M-2	*Pterocarpus,* Chettikulam	66.7	50
M-11	*Casuarina,* Kundukad	100.0	20
M-12	*Casuarina,* Kuttipuram	100.0	30
M-21	*Pterocarpus,* Walayar	50.0	30
M-23	*Acacia,* Pariyaram	91.7	50
M-24	*Acacia,* Pariyaram	66.7	30
M-25	*Casuarina,* Dharmadam	83.3	50
M-27	*Acacia,* Peruvannamuzhi	83.0	60
M-31	*Pterocarpus,* KFRI	100.0	30

4.1.1. Glasshouse trial

i. *Biomass production:* In general, inoculation with VAM fungi had a positive influence on growth of *Acacia, Casuarina* and *Pterocarpus.* Different strains of mycorrhizal fungi varied in their abiity to enhance plant growth. Inoculation effects on height and dry matter yield were mostly significant.

In *Acacia,* maximum height (19.92 cm) was recorded in plants inoculated with *Glomus calidonius* (V-l7), maximum root length (28.4 cm) in those inoculated with *Glomus constrictum* (V-2) and maximum dry weight (0.54 g)in plants inoculated with *Glomus mosseae* (V-13) (Table 7). *Glomus monosporum* (V-3) gave the best colonization (94.2%) in *Acacia* (Figs. 19-20). However, selection

Fig. 19

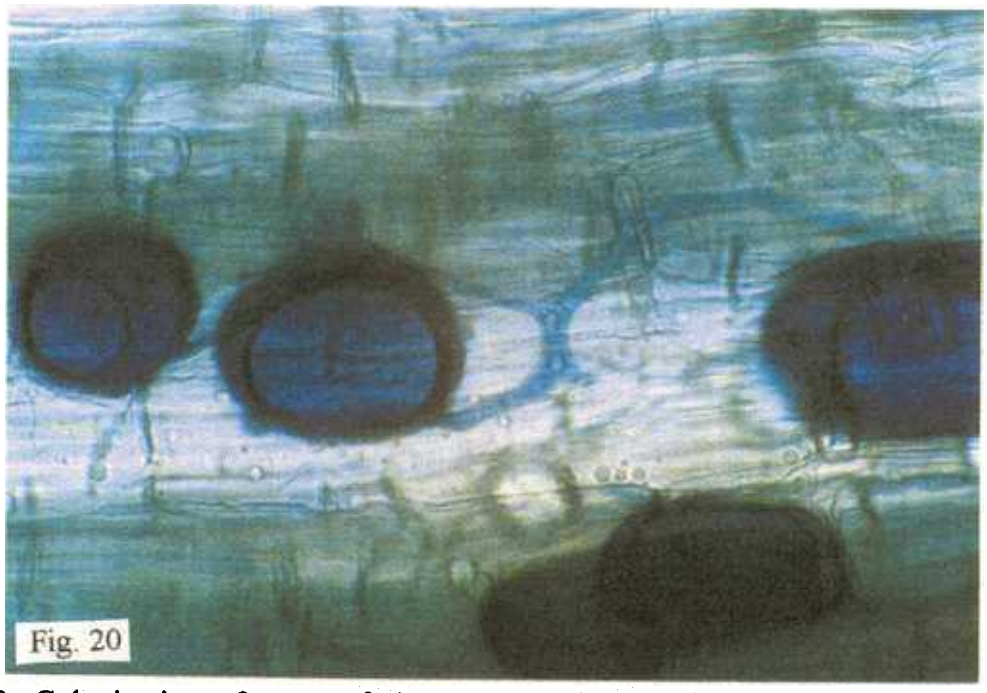


Fig. 20

Figs. 19-20. Colonization of roots of *Acacia auriculiformis* by VA mycorrhizal fungus, *Glomus* sp. Figures showing production of numerous vesicles (Fig. 19-x80; Fig. 20-x320).

of the best isolates was done based on overall performance using cluster analysis (Fig.21) and, thus, ***Glomus calidonius*** (V-17) and ***Glomus mosseae*** (V-13) were selected for field application studies (Figs. 24-25).

Table 7. Glasshouse screening for efficient VAM fungi: growth and percentage VAM colonization in ***Acacia*** seedlings

Isolate No.	Plant height* (cm)	Root length* (cm)	Dry weight* (g)	% increase over Control drywt.	% colonization (6 replications of 20 root bits each)
V-1	14.8	23.4	0.249hi	-12.63	50.5
v-2	16.3	28.4	0.376efg	31.92	40.8
v-3	17.6	23.1	0.406de	42.45	94.2
v-4	16.7	28.1	0.467bcd	63.85	70.0
v-5	16.4	28.3	0.390e	38.84	53.3
v-7	14.6	23.8	0.342gh	20.00	6.7
V-8	17.5	25.6	0.412def	44.58	72.3
v-9	17.9	27.1	0.43lcdef	51.22	58.3
v-10	13.6	23.5	0.304hi	6.66	30.8
v-11	18.6	23.9	0.475bc	66.66	93.3
v-12	19.8	27.3	0.466bcd	63.50	19.2
V-13	18.5	24.8	0.541a	89.82	71.2
V-14	17.7	26.2	0.463bcd	62.45	65.8
V-15	17.4	24.1	0.418cdef	45.96	78.3
V-16	16.1	26.1	0.379efg	32.98	24.2
V-17	19.9	26.5	0.520ab	82.45	64.2
V-18	18.3	27.2	0.416cdef	46.66	68.3
M-11	13.6	23.4	0.248h	-12.96	16.7
M-12	14.8	26.3	0.420cdef	47.38	89.2
M-23	13.4	25.9	0.343gh	20.35	73.3
M-24	14.7	24.5	0.366fg	28.42	28.3
M-25	12.4	23.9	0.207i	-27.36	15.0
M-27	12.3	26.0	0.281h	-1.40	33.0
Control	13.3	20.8	0.285hi		

* Mean of 20 replications

In ***Casuarina,*** eventhough the colonization of roots by different VAM cultures was better than other tree species, the growth response to inoculation varied (Table 8) (Figs. 26-27). Inoculation with M-21 gave the maximum plant height (24.7 cm). ***Glomus monosporum*** (V-3) gave maximum root length (26.7 cm), and maximum dry weight (0.12 g) was recorded in plants inoculated with M-23 and M-12. Maximum colonization (97.14%) was recorded in plants noculated with M-21. Based on best overall performance as revealed by cluster

analysis (Fig.23), monosporal cultures M-23 and ***G. fusciculutum*** (TNAU IV)(V 10) were selected for field trial (Figs. 28-29).

Table 8. Glasshouse screening for efficient VAM fungi: growth and percentage VAM colonization in ***Casuarina*** seedlings

Isolate No.	Plant Height* (cm)	Root length* (cm)	Dry weight* (g)	% increase over Control dry wt.	%colonization (6 replications of 20 root bits each)
V-1	16.8	6.5	0.024	-52	73.0
v-2	18.6	9.8	0.047	-6	48.0
v-3	17.6	26.7	0.053	6	78.5
v-4	20.1	11.9	0.040	-20	92.5
v-5	17.2	10.5	0.042	-16	83.2
v-7	20.4	18.5	0.085	70	84.8
V-8	21.1	11.4	0.050	0	72.9
v-9	19.5	9.8	0.053	6	94.8
v-10	23.6	19.1	0.091	82	85.1
v-11	22.8	9.8	0.040	-20	78.8
v-12	21.4	13.1	0.065	30	32.1
V-13	21.6	11.9	0.084	42	65.3
V-14	22.7	10.8	0.081	62	51.8
V-15	18.9	10.7	0.038	-24	74.9
V-16	22.3	11.1	0.084	68	78.0
V-17	20.6	12.0	0.049	-2	79.7
V-18	18.1	9.1	0.028	-48	87.5
M-11	21.0	13.9	0.054	8	31.0
M-12	21.7	16.4	0.122	144	54.0
M- 21	24.7	20.2	0.078	56	97.1
M-23	13.4	25.9	0.122	144	93.9
M-24	14.7	24.5	0.063	26	68.5
M-25	12.4	23.9	0,052	4	14.2
M-27	17.7	14. 1	0.048	-4	13.3
Control	17.3	14.8	0.050		

* Mean of 20 replications

Table 9. Glasshouse screening for efficient VAM fungi: Growth and percentage VAM colonization in ***Pterocarpus*** seedlings

Isolate No.	Plant height* (cm)	Root length* (cm)	Dry weight* (g)	% increase over Control dry wt.	%colonization (6 replications of 20 root bits each)
V-1	16.6	22.7	0.686f	-24.03	50.5
v-2	17.6	23.0	0.702f	-22.25	40.8
v-3	18.0	21.8	0.724f	-19.62	94.2
v-4	14.7	21.9	0.602f	-33.33	72.0
v-5	16.9	23.1	1.035cdef	14.95	59.7
v-7	17.1	22.7	1.197b	32.55	95.0
V-8	17.0	20.8	1.165bc	29.01	93.8
v-9	16.2	21.9	0.902de	-0.11	54.2
V-10	16.3	22.7	1.051c	16.38	12.5
V-11	16.7	23.0	1.133bc	25.47	60.0
V-12	18.5	21.7	1.322a	46.40	35.0
V-13	15.5	21.7	1.150bc	27.35	35.0
V-14	16.2	21.8	0.930de	0	43.3
V-15	15.4	20.5	0.865e	-4.20	29.2
V-36	13.5	23.2	0.653f	-27.65	70.0
V-17	16.1	21.3	1.119bc	23.92	65.8
V-18	16.0	22.1	0.774f	-14.28	70.8
M-2	16.2	21.4	1.014de	12.29	29.2
M-12	15.6	21.9	1.031de	14.17	70.8
M-21	17.3	21.7	0.996d	7.97	70.8
M-24	16.2	21.7	0.975de	7.97	45.0
M-27	16.7	18.3	0.941de	4.20	2.5
M-31	16.3	22.5	1.175bc	30.12	15.0
Control	15.8	21.8	0.903de		

* Mean of 20 replications

In ***Pterocarpus,*** inoculation with ***Glomus intraradices*** (V-12) resulted in maximum plant height and dry weight, even though %colonization was only nearly 35% (Table 9) (Figs. 30-33). None of the isolates significantly influenced the root length of ***Pterocarpus. Glomus fasciculatum*** gave the maximum root colonization (95%). ***Glomus intraradices*** (V-12) and ***G. mosseae*** (TNAU II) (V-8) were selected for field studies as most efficient cultures for ***Pterocarpus*** (Figs. 34-35) based on complete linkage cluster analysis (Fig. 22).

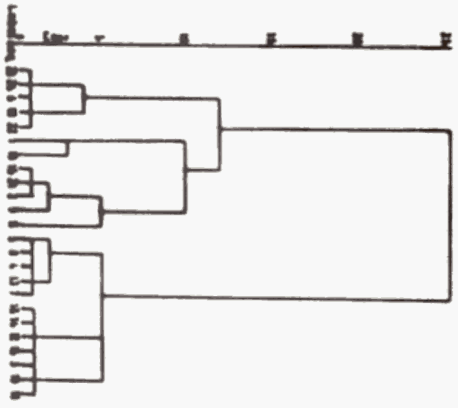


Fig.21 Cluster analysis using data on height, root length, dry weight and per cent VAM root colonization in ***Acacia.*** Dendrogram using Complete Linkage between Groups.

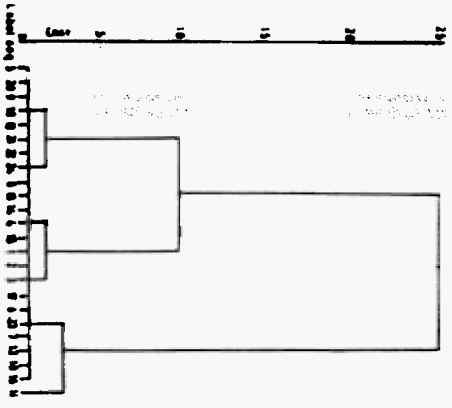


Fig. 22. Cluster analysis using data on height, root length, dry weight and per cent VAM root colonization in ***Pterocarpus.*** Dendrogram using Complete Linkage between Groups.

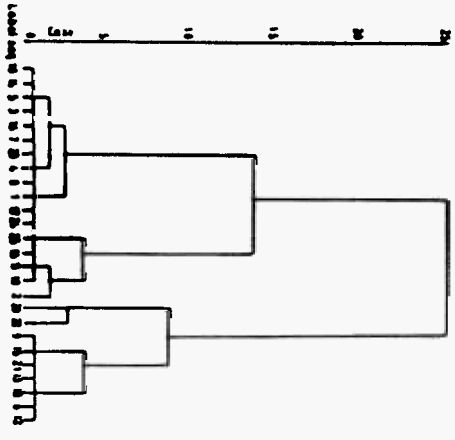
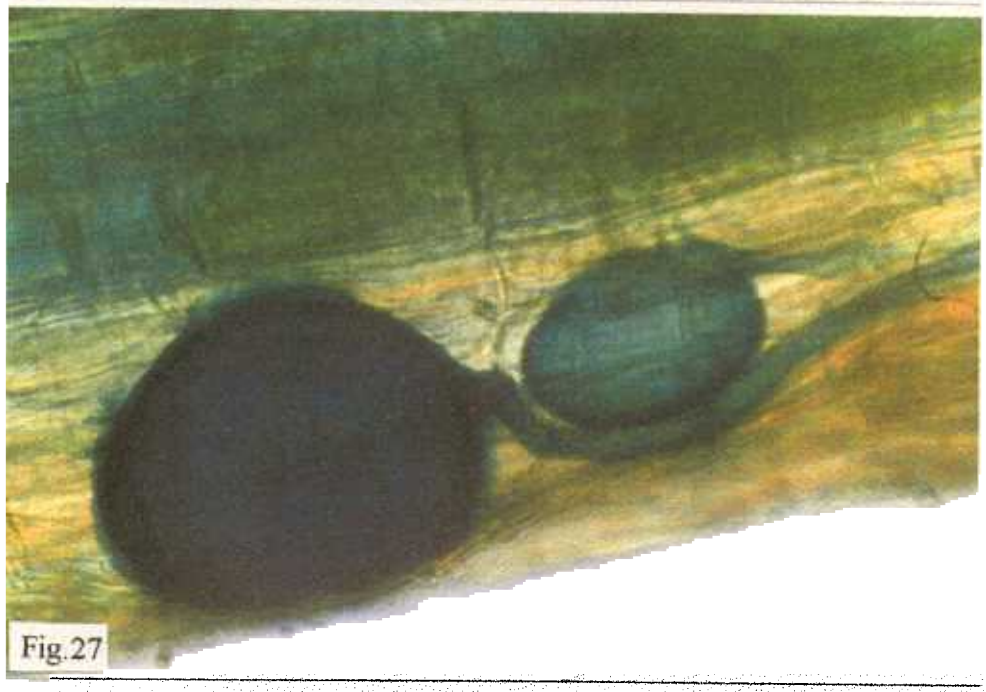
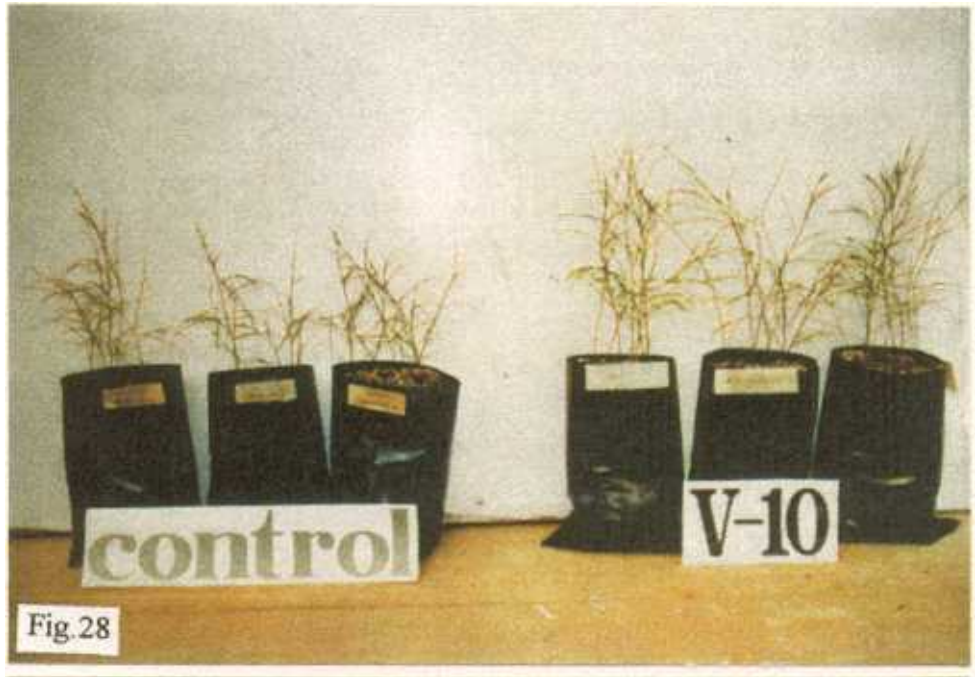


Fig.23. Cluster analysis using data on height, root length, dry weight and per cent VAM root colonization in ***Casuarina*** Dendrogram using Complete Linkage between Groups.

Figs.24-25. The most efficient VA mycorrhizal strains for *Acacia auriculiformis* in glasshouse trails; V-13, *Glomus mosseae*; V-17, *Glomus calidonius.*



Figs.26-27. Colonization of roots of *Casuarina equisetifolia* by VA mycorrhizal fungus, *Glomus* sp. Figures showing hyphae, vesicles and spores (Fig.26-x320; Fig.27-x510).



Figs.28-29. The most efficient VA mycorrhizal strains for *Casuarina equisetifolia* in glasshouse trials; V-10 (TNAU-14) *Glomus fasciculatum*; M-23.

ii. ***P content:*** The total P content of plants was estimated for selected VAM cultures which showed superior performance with respect to biomass production. All the inoculated plants had significantly higher P content compared to uninoculated ones (Table 10). The total P content of ***Acacia*** was found to increase upto 120% and 100% by inoculation of ***Glomus calidonius*** and ***G. mosseae*** respectively. In ***Casuarina,*** total P content increased by 45.8% and 55.9% as a result of inoculation of M-23 and ***G. fasciculatum*** (TNAU IV). In ***Pterocarpus*** both ***Glomus intraradices*** and ***G. mosseae*** (TNAU 11) resulted in 83.3% increase in total P content.

Table 10. Percentage of increase in Phosphorus content in VAM-inoculated seedlings of ***Acacia, Casuarina*** and ***Pterocarpus***

Sl. No.	Plant Species	VAM culture	% increase over Control
1.	***A. auriculiformis***	***G. calidonius***	120.0
		G. mosseae	100.0
2.	***C. equisetifolia***	M. 23	45.8
		G.fasciculatum (TNAU IV)	55.9
3.	***P. marsupium***	***G. intraradices***	83.3
		G. mosseae (TNAU 11)	83.3

4.2 *Rhizobium*

A total of 22 cultures of ***Rhizobium*** were isolated fiom root nodules of ***Acacia auriculiformis*** and 10 fiom nodules of ***Pterocarpus marsupium*** fiom different localities of Kerala.

4.2.1. Growth of Rhizobium isolates

The results indicated that the ***Rhizobium*** isolates obtained from root nodules of ***A. auriculiformis*** fiom 19 localities (namely A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-ll, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-21 were slow growers (Table 11). In case of ***P. marsupium,*** isolates fiom 8 localities (P-1, P-2, P-3, P-4, P-5, P-7, P-8, A-10) were fast growers and P-6, P-9 were slow growers (Table 12).

Fig.30

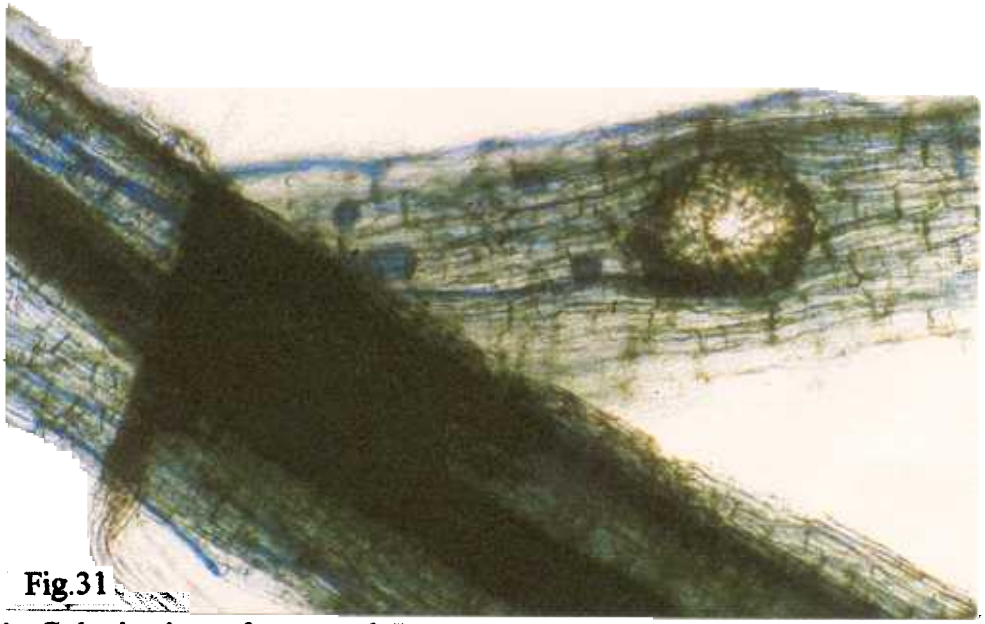
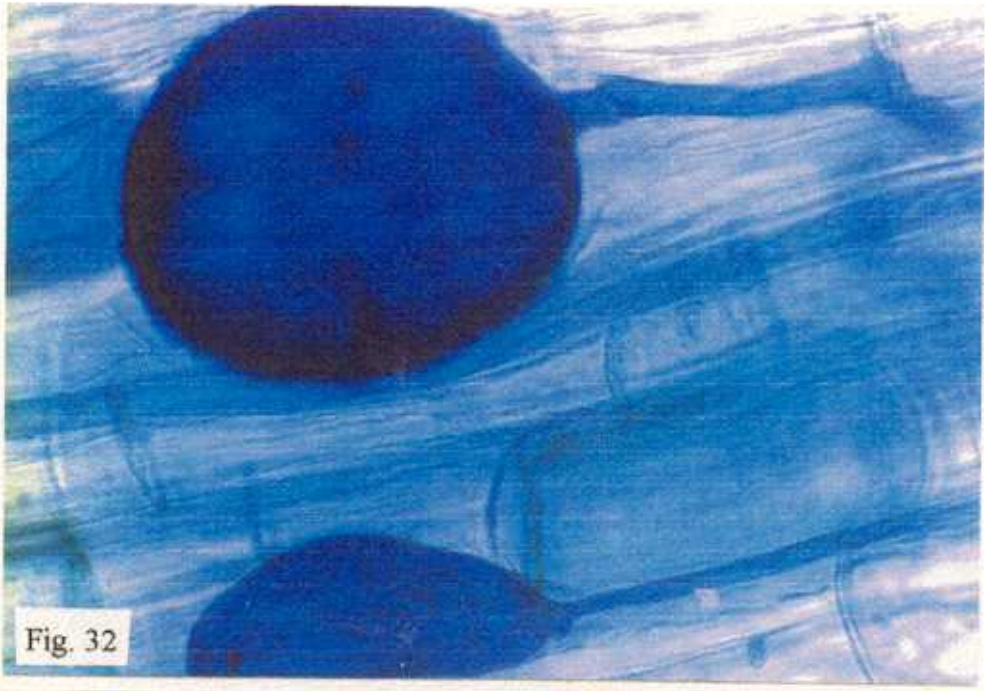
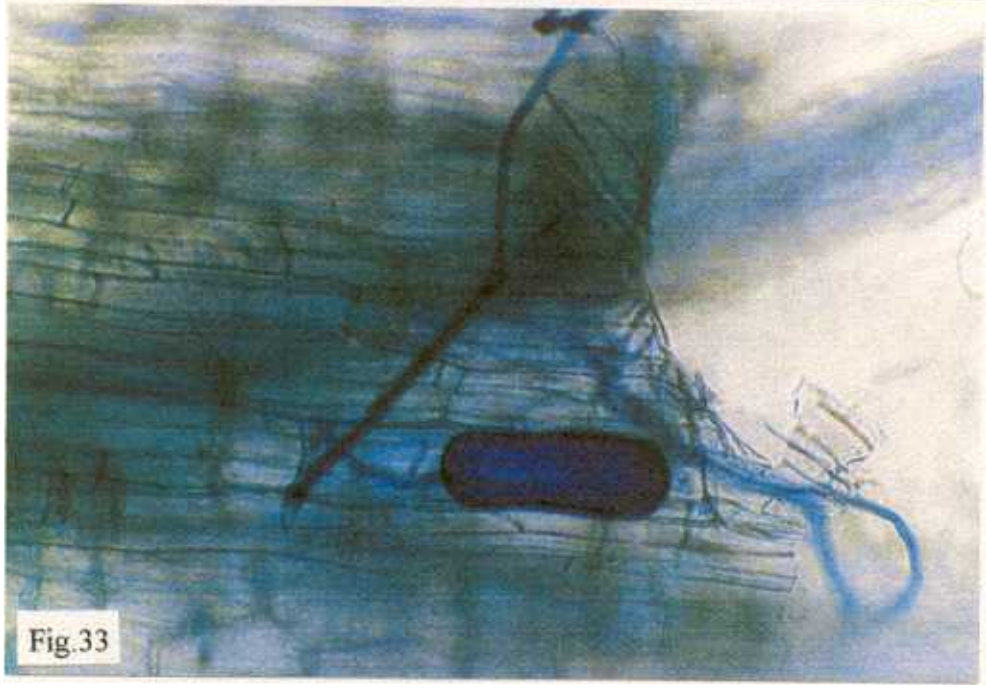


Fig.31

Figs.30-31. Colonization of roots of *Pterocarpus marsupium* by VA mycorrhizal fungus, *Glomus* sp. Figures showing mycelium and vesicles (Fig.30-x80; Fig.31-x200).





Figs.32-33. Colonization of roots of *Pterocarpus marsupium* by VA mycorrhizal fungus, *Glomus* sp. Figures showing mycelium and vesicles (Fig. 32-x510; Fig.33-x320).

Figs.34-35. The most efficient VA mycorrhizal strains for *Pterocarpus marsupium* in glasshouse trials; V-8, *Glomus mosseae* (TNAU II); V-12, *G. intraradices*.

Table 11. Growth of ***Rhizobium*** isolates from ***Acacia auriculiformis*** on yeast-extract mannitol agar medium

Sl. No.	***Rhizobium*** isolate	Origin of culture: Locality	Origin of culture: District	Growth performance
1.	A-1	Kotharmanakkad	Palghat	++
2.	A-2	Bangalore	Bangalore	++
3.	A-3	Peruvannamuzhy	Kozhikode	++
4.	A-4	Kottappara I	Eranakulam	++
5.	A-5	Kottappara II	Eranakulam	++
6.	A-6	Elanad	Trichur	++
7.	A-7	Kizhoor	Cannanore	++
8.	A-8	Kalladka	Trichur	++
9.	A-9	Alathur I	Palghat	++
10	A-10	Periyaram	Cannanore	++
11.	A-11	Vallarpattanam	Cannanore	++
12.	A-12	Thenhipalam	Malappuram	++
13	A-13	Kuttipuram	Malappuram	++
14	A-14	Veettoor	Eranakulam	++
15	A-15	Peechi	Trichur	++
16	A-16	Calicut	Calicut	++
17.	A-17	Palamaracoup	Palghat	+
18	A-18	Chandanathode	Wynad	+
19	A-19	Panammaram	Wynad	++
20	A-20	Alathur II	Palghat	++
21	A-21	Alathur III	Palghat	+
22	A-22	Kasargod	Kasargod	++

++, Fast grower, 3-5 days for growth; +, Slow grower, 5-9 days for growth

4.2.2. Growth on Yeast extract mannitol agar medium containing bromothymol blue

In the case of ***Rhizobium*** fiom ***A. auriculformis,*** 11 isolates (A-1, A-4, A-5, A-7, A-9, A-11, A-12, A-13, A-15, A-19, A-22) turned medium colour fiom green to yellow indicating acid production, while the other isolates turned medium colour to blue indicating alkali production (Table 13) (Fig. 36). In the case of ***P. marsupium,*** four isolates (P-4, P-7, P-8, P-10) were acid producers and others alkali producers (Table 14) (Fig. 37).

Figs. 36-37. Acid tolerance test for different Rhizobium isolates of E-3 (A-6) and Kalladka (A-8)obtained from *Acacia auriculiformis.*

Table 12. Growth of ***Rhizobium*** isolates from ***Pterocarpus marsupium*** on yeast-extract mannitol agar medium

Sl. No.	***Rhizobium*** Isolate	Origin of culture: Locality	District	Growth performance
1.	P-1	Kottappara	Ernakulam	++
2.	P-2	Nilambur	Malappuram	++
3.	P-3	Peechi 1	Trichur	++
4.	P-4	Peechi II	Trichur	++
5.	P-5	Walayar I	Palghat	++
6.	P-6	Thaliparamba	Cannanore	+
7.	P-7	Palappilly	Trichur	++
8.	P-8	Kannara	Trichur	++
9.	P-9	Mannuthy	Trichur	+
10.	P-10	Walayar II	Palghat	++

++, Fast grower, 3-5 days for growth; +, Slow grower, 5-9 days for growth.

Table 13 Screening of ***Rhizobium*** isolates from ***Acacia auriculiformis*** for acid/alkali production using bromothymol blue

Sl. No.	***Rhizobium*** isolates	Control (G)	Acid/alkali production (Y/B)	Sl. No.	***Rhizobium*** isolates	Control (G)	Acid/alkali production (Y/B)
1.	A-1	G	Y	12.	A-12	G	Y
2.	A-2	G	B	13.	A-13	G	Y
3.	A-3	G	B	14.	A-14	G	B
4.	A 4	G	Y	15.	A-15	G	Y
5.	A-5G	Y	1	6.	A-16	G	B
6.	A-6	G	B	17.	A-17	G	B
7.	A-7	G	Y	18.	A-18	G	B
8.	A-8	G	B	19.	A-19	G	Y
9.	A-9	G	Y	20.	A-20	G	B
10.	A-10	G	B	21.	A-21	G	B
11.	A-11	G	Y	22.	A-22	G	Y

G, Green - Neutral; B, Blue - Alkali producer; Y, Yellow Acid producer.

Table 14. Screening of ***Rhizobium*** isolates from ***Pterocarpus marsupium*** for acid/alkali production using bromothymol blue

Sl. No.	***Rhizobium*** isolates	Control (G)	Acid/alkali production (Y/B)	Sl. No.	***Rhizobium*** isolates	Control (G)	Acidalkali production (Y/B)
1.	P-1	G	B	6.	P-6	G	B
2.	P-2	G	B	7.	P-7	G	Y
3.	P-3	G	B	8.	P-8	G	Y
4.	P-4	G	Y	9.	P-9	G	B
5.	P-5	G	B	10.	P-10	G	Y

G,Green - Neutral; Y, Yellow - Acid producer; B, Blue- Alkali producer.

4.2.3. Effect of acid-aluminium stress on Rhizobium isolates

Except 6 isolates (A-12, A-15, A-18, A-20, A-21) others from ***A. auriculiformis*** showed good growth in the presence of acid-aluminium in growth medium indicating adequate tolerance of acid-aluminium stress by most of the ***Rhizobium*** strains, capable of nodulating ***A. auriculiformis*** (Table 15). In the case of ***P. marsupium,*** of the 10 isolates, only one was not capable of growing under acid-aluminium stress (Table 16).

Table 15. Screening of ***Rhizobium*** isolates from ***Acacia auriculiformis*** for acid-aluminium stress tolerance (observations recorded after nine days)

Sl. No.	***Rhizobium*** isolate	Growth: Control (pH 7.0)	Growth: Stress medium (pH 4.7)	Sl. No.	***Rhizobium*** isolate	Growth: Control (pH 7.0)	Growth: Stress medium (pH4.7)
1.	A-1	+	+	12.	A-12	+	
2.	A-2	+	+	13.	A-13	+	+
3.	A-3	+	+	14.	A-14	+	+
4.	A-4	+	+	15.	A-15	+	
5.	A-5	+	+	16.	A-16	+	+
6.	A-6	+	+	17.	A-17	+	+
7.	A-7	+	+	18.	A-18	+	-
8.	A-8	+	+	19.	A-19	+	+
9.	A-9	+	+	20.	A-20	+	
10.	A-10	+	+	21.	A-21	+	-
11.	A-11	+	+	22.	A-22	+	+

+, Growth; –,No

Table 16. Screening of ***Rhizobium*** isolates from ***Pterocarpus marsupium*** for acid-aluminium stress tolerance (observations recorded after nine days)

Sl. No.	***Rhizobium*** isolate	Growth: Control (pH 7.0)	Growth: Stress medium (pH 4.7)	Sl. No.	***Rhizobium*** isolate	Growth: Control (pH 7.0)	Growth: Stress medium (pH 4.7)
1.	P-1	+	+	6.	P-6	+	+
2.	P-2	+	+	7.	P-7	+	+
3.	P-3	+	+	8.	P-8	+	+
4.	P-4	+	+	9.	P-9	+	+
5.	P-5	+	+	10.	P-10	+	

+, Growth; -, No growth

4.2.4. Effect of different *Rhizobium* isolates on various growth parameters in glasshouse trial

A. auriculiformis seedlings inoculated with the isolate A-19 have the maximum biomass in terms of fiesh weight and dry weight and total nitrogen contendplant, while isolate A 16 gave the maximum fiesh weight of nodules. (Tables 17 and 18). In the case of seedlings of ***P. marsupium,*** isolate P-8 showed the maximum fresh weight and dry weight. However, the highest Nitrogen contendplant was shown by the isolate P-1 (Tables 19 and 20).

4.2.5. Selection of best *Rhizobium* strains

The recorded data were analysed by one-way analysis of variance test and means compared by Duncan's multiple range test followed by cluster analysis (Fig.38). The two best strains in ***A. auriculiformis*** were found to be isolates A-19 and A-16, based on plant biomass, nodule fiesh weight and total nitrogen content. The two best strains in ***P. marsupium*** were isolates P-8 and P-9 (Fig.39); these selected strains were used for field trial.

4.3. *Frankia*

Root nodules of ***C. equisetifolia*** were collected fiom eight localities in Kerala, including river bed and sea shore (Table 21).

Table 17. Effect of different ***Rhizobium*** isolates on fresh weight of ***Acacia auriculiformis*** in glasshouse trial

Sl. No.	Rhizobial Isolate	Fresh weight of Root (g/plant)	Fresh weight of Shoot (g/plant)	Fresh weight of plant (g/plant)	Fresh weight of Nodules (g/plant)
1.	A-1	0.4947bcde	0.6647	1.1593cdef	0.016%
2.	A-2	0.5040abcde	0.6307	1.1347cdef	0.0118c
3.	A-3	0.5273abcd	0.7733	1.3007cdef	0.0191c
4.	A-4	0.6380abcd	0.9920	1.3633abcde	0.0145c
5.	A-5	0.4867abcd	0.6513	1.1380cdef	0.0171c
6.	A-6	0.6833bcde	0.6713	1.3547abcde	0.0160c
7.	A-7	0.5967abc	0.7520	1.3600abcde	0.0074c
8.	A-8	0.7413abcd	0.7767	1.5187abc	0.0161c
9.	A-9	0.5120abcde	0.5753	1.0873cdef	0.0135c
10.	A-10	0.3627defg	0.5193	0.8820f	0.0181c
11.	A-11	0.3340fg	0.6160	0.9500ef	0.0211c
12.	A-12	0.2014fg	0.8000	1.0079def	0.0344bc
13.	A-13	0.2147fg	0.8460	1.0607cdef	0.0447bc
14.	A-14	0.4473bcdef	0.9107	1.2892cdef	0.0399bc
15.	A-15	0.4793bcde	0.8287	1.3073cdef	0.0069c
16.	A-16	0.3592defg	1.2058	1.6275ab	0.1023a
17.	A-17	0.5000abcd	1.0018	1.5018abc	0.0036c
18.	A-18	0.5250abcde	0.5563	1.0138cdef	0.0000
19.	A-19	0.7717a	0.9962	1.7833a	0.0071c
20.	A-20	0.4093cdefg	1.0857	1.4964abc	0.0206c
21.	A-21	0.3315efg	1.1169	1.4515cdef	0.0392bc
22.	A-22	0.18698	1.1231	1.3100a	0.0758a
23.	Control	0.3807defg	0.8772	1.2267abc	0.0000

Each value represents the average of fifteen replications

Table 18. Effect of different ***Rhizobium*** isolates on dry weight of ***Acacia*** and total nitrogen content in glasshouse trial

Sl. No.	***Rhizobium*** isolates	Dry weight of root (g/plant)	Dry weight of shoot (g/plant)	Dry weight of plant (g/plant)	Number of nodules/ plant	Total Nitrogen (g/plant)
1.	A-1	0.0533	0.3233	0.3767def	0.1693abc	0.6020bcd
2.	A-2	0.0887	0.2520	0.3407ef	0.0707def	0.5796cdef
3.	A-3	0.0687	0.3300	0.3987cdef	0.2227abc	0.4222cdef
4.	A-4	0.0660	0.3540	0.4193cde	0.1953abc	0.4072cdef
5.	A-5	0.0673	0.2927	0.3521ef	0.0327ef	0.2916def
6.	A-6	0.0713	0.3107	0.3820def	0.0440def	0.3148def
7.	A-7	0.0480	0.3340	0.3820def	0.1260cd	0.5294bcde
8.	A-8	0.0740	0.3753	0.4460cde	0.1107cde	0.4676cdef
9.	A-9	0.0713	0.2660	0.3380ef	0.0627def	0.2988def
10.	A-10	0.0367	0.2413	0.2780f	0.1l00cde	0.3130def
11.	A-11	0.1207	0.3440	0.4647cde	0.0240ef	0.1366f
12.	A-12	0.1754	0.3286	O.5000bcd	0.2414a	0.2710def
13.	A-13	0.2047	0.3440	0.5487abc	0.1140cde	0.2336ef
14.	A-14	0.2240	0.2927	0.5167bcd	0.1120cde	0.2874def
15.	A-15	0.1953	0.4247	0.6200ab	0.0193ef	0.5156cde
16.	A-16	0.1492	0.4908	0.6308ab	0.1438bcd	0.7498b
17.	A-17	0.2373	0.4000	0.6373ab	0.0045f	0.7130bc
18.	A-18	0.1038	0.3112	0.4I50cdef	0.0000	0.6712bc
19.	A-19	0.2358	0.4367	0.6725a	0.0083ef	1.2860a
20.	A-20	0.1721	0.4407	0.6129ab	0.1157cde	0.7640a
21.	A-21	0.1269	0.4500	0.5769abc	0.2500a	0.8742a
22.	A-22	0.1454	0.4969	0.6315ab	0.1953abc	0.7144b
23.	Control	0.1127	0.4593	0.5233bc	0.0000	0.4258cdef

Each value represents the average of fifteen replications

Table 19. Effect of different ***Rhizobium*** isolates on fresh weight of ***Pterocarpus marsupium*** in glasshouse trial

Sl. No.	Rhizobial Isolates	Fresh weight of root (g/plant)	Fresh weight of shoot (g/plant)	Fresh weight of plant (g/plant)	Fresh weight of nodules (g/plant)
1.	P-1	1.8315a	0.9515cde	2.7831abcd	0.0148b
2.	P-2	1.303lab	0.7392de	2.0423d	0.0052b
3.	P-3	1.0700b	0.8050de	1.8750d	0.0001b
4.	P-4	1.7553a	1.0227de	2.7807abcd	0.0182b
5.	P-5	1.4513ab	0.9927de	2.4440cd	0.0138b
6.	P-6	1.8947a	1.1847cd	3.0793abc	0.0214b
7.	P-7	1.0729b	1.3907bc	2.5679bcd	0.0747a
8.	P-8	1.7429a	1,7943a	3.5371a	0.0661a
9.	P-9	1.6264ab	1.5427ab	3.1691abc	0.0663a
10.	P-10	1.6600ab	1.5607ab	3.3540ab	0.0255b
11	Control	1.4500ab	1.1833cd	2.6327bcd	0.0000

Each value represents the average of fifteen replications

Table 20. Effect of different ***Rhizubium*** isolates on dry weight of ***Pterocarpus marsupium*** in glasshouse trial

Sl. No.	***Rhizobium*** isolates	Dry weight of root (g/plant)	Dry weight of shoot (g/plant)	Dry weight of plant (g/plant)	Number of nodules/ plant	Total Nitrogen (g/plant)
1.	P-1	0.4577abc	0.3792de	0.8369bcde	1.5385b	0.9400a
2.	P-2	0.2931c	0.3015f	0.5946e	0.3077b	0.8044a
3.	P-3	0.2820c	0.3060ef	0.5870	0.3000b	0.5266ab
4.	P-4	0.4013abc	0.4367cde	0.8100cde	2.8667 b	0.2896b
5.	P-5	0.3467bc	0.3627def	0.7093de	1.8667b	0.2876b
6.	P-6	0.4027abc	0.4793bcd	0.8820bcd	1.3333b	0.8024ab
7.	P-7	0.4514a	0.4786bcd	0.9300b	5.5000a	0.4586ab
8.	P-8	0.5636a	0.6507a	1.2114a	5.7857a	0.7724ab
9.	P-9	0.5145ab	0.5973ab	1.1173ab	7.1818a	0.8768a
10.	P-10	0.5080ab	0.5447abc	1.0527abc	0.4000b	0.4852ab
11.	Control	0.3767abc	0.4553cd	0.9033bcd	0.0000	

Each value represents the average of fifteen replications

Table 21. List of ***Frankia*** cultures isolated from root nodules of ***Casuarina equisetifolia*** in Kerala

Sl. No.	***Frankia*** Isolate	Origin of culture	
		Locality	District
1.	c-1	Ramavarmapuram	Trichur
2.	c-2	Kappad	Calicut
3.	c-3	Kuttipuram	Malappuram
4.	c-4	Dharmadam	Cannanore
5.	c-5	Nileshwar	Kasargod
6.	c-6	Thenhipalam	Malappuram
7.	c-7	Kasargod	Kasargod
8.	c-8	Chandanathode	Wynad

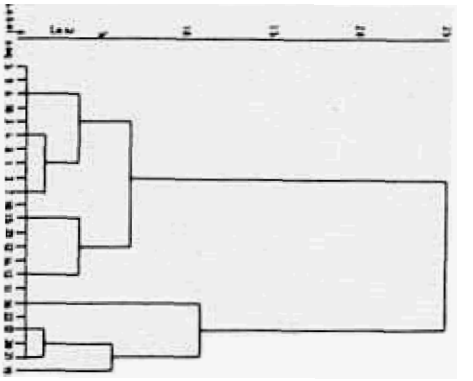


Fig.38. Cluster analysis of biomass, nodule fresh weight and total nitrogen content in seedlings of ***Acacia.*** Dendrogram using Complete Linkage between groups.

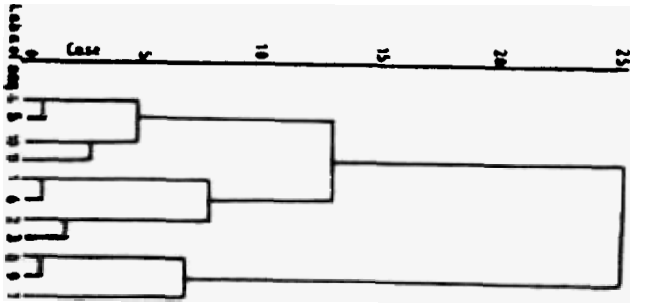


Fig.39. Cluster analysis of biomass, nodule fresh weight and total nitrogen content in seedlings of ***Pterocarpus.*** Dendrogram using Complete Linkage between groups.

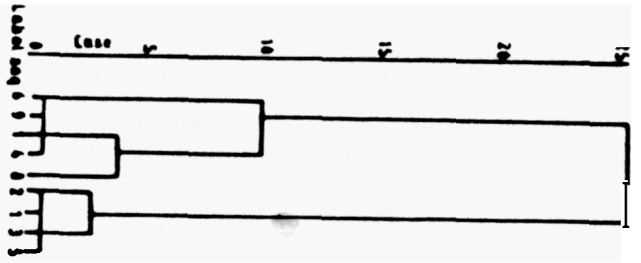


Fig.40. Cluster analysis of biomass, nodule fresh weight and total nitrogen content in seedlings of *Cusuarina.* Dendrogram using Complete Linkage between groups.

4.3.1. Isolation of *Fronkia*

Small ***Frankia*** colonies appeared by about 25 days on Na-propionate media by adopting serial dilution plate method. The colonies were white and showed vesicles (4-5 μ) under compound microscope). However, it was not possible to sub-culture them in Agar slants.

4.3.2. Effect of different *Fronkio* isolates on various growth parameters in glasshouse trial

The isolate C-5 fiom ***C. equisetifolia*** recorded maximum fiesh weight as compared to other isolates and control (uninoculated). However, the highest number and fiesh weight of nodules were given by C-3 which ranked second in dry weight of the plant (Table 22, 23).

4.3.3. Selection of best *Frankia* strains

The recorded data was analysed by one-way analysis of variance foIlowed by DMRT for comparison of means. The two best strains were found to be C-2 and C-3, based on cluster analysis of data on plant biomass and nodule weight (Fig.39). These two strains were selected for field trial.

4.4. Effect of fertilizer on seedling biomass, VA mycorrhiza and number of root nodules

Significant differences (P=0.05) were observed between various treatments in respect of biomass (dry weight) of seedlings, percentage of VA mycorrhiza infection and number of root nodules formed

4.4.1. *Acacia ouriculiformis*

The results in respect of biomass, % VA mycorrhiza infection and number of nodules per plant are presented in Table 24.

Biomass (dry weight): Though significant difference was observed between treatments no definite trend emerged on the seedling biomass due to fertilizer application in combination with the symbionts. Maximum biomass was obtained in R/N2P2/(422 mg) treatment followed by R/NlPl (393 mg). Generally, seedlings with dual inoculation had lower biomass in most of the fertilizer treatments than those of control without inoculation.

Table 22. Effect of different ***Frankia*** isolates on fresh weight of ***Casuarina equisetifolia*** in glasshouse trial

Sl. No.	***Frankia*** Isolate	Fresh weight of root (g/plant)	Fresh weight of shoot (g/plant)	Fresh weight of plant (g/plant)	Fresh weight of Nodules (g/plant)
1.	c-1	0.7575	2.2917	3.0567	0.0000
2.	c-2	1.1367	5.0420	6.1787	0.0129a
3.	c-3	1.4364	5.2450	6.5386	0.0169a
4.	c-4	0.8186	3.0714	3.8779	0.0000
5.	c-5	1.6971	6.0536	7.7507	0.0137a
6.	c-6	1.4886	4.6157	6.1043	0.0021b
7.	c-7	1.5987	5.3587	6.9573	0.0089ab
8.	c-8	0.3633	0.7625	1.1258	0.0007b
9.	Control	1.4362	4.5046	5.9446	0.0000

Each value represents the average of fifteen replications

Table 23 Effect of different ***Frankia*** isolates on dry weight of ***Casuarina equisetifolia*** in glasshouse trial

Sl. No.	***Frankia*** isolate	Dry Weight of Root (g/plant)	Dry weight of Shoot (g/plant)	Dry weight of plant (g/plant)	Number of Nodules/plant
1.	c-1	0.11c	0.5742cd	0.6875cd	0.0000
2.	c-2	0.32a	1.2973abc	1.5800ab	4.2667
3.	c-3	0.27ab	1.4421ab	1.7136ab	8.0714
4.	c–4	0.17bc	0.8457bcd	1.0186bcd	0.0000
5.	c-5	0.30ab	1.6750a	1.9793a	5.4286
6.	c-6	0.27ab	1.2271abc	1SO29abc	0.2857
7.	c-7	0.31ab	1.2533abc	1.5607ab	1.3333
8.	c-8	0.07c	0.2158d	0.2925d	1.7500
9.	Control	0.27ab	1.2608abc	0.4777	0.0000

Each value represents the average of fifteen replications

ii. ***VA mycorrhiza infection:*** Both the nitrogen treatments (N1 and N2) in VAM inoculated seedlings and low level of nitrogen with high level of phosphorus in VAM + Rhizobium inoculated plants had favourable effect on mycorrhizal infection. Highest VAM infection was recorded in dual inoculation treatment MR/N1 (75.5%) followed by MR/N1P2 (74%) M/N2 (73.3%) and M/Nl, MR/P2,M/C (70.0%).

iii. ***Number of nodules:*** Maximum number of nodules were observed in R/NlPl treatment (9.5) followed by MRNlPl(7.0). Except R/NlPl in all the fertilizer treatments, number of nodules was higher in seedlings with dual inoculation (MR); even the control (without fertilizer) seedlings with dual inoculation (MR) had higher number of nodules than other treatments. There was no evidence of any pronounced influence of either phosphorus or nitrogen on nodulation.

4.4.2. Pterocarpus marsupium

The results of biomass, % VA mycorrhiza infection and number of nodules are presented in Table 25.

Biomass (dry weight): No definite trend was observed in combinations in the utilization of fertilizers by the inoculated seedlings. Inoculated (M,R) controls (unfertilized) had higher biomass than those of fertilized. Highest biomass was recorded in M/N2 (585 mg) followed by M/Pl, (564 mg), M/C (562 mg), MRNl(531 mg).

ii. ***VA mycorrhiza infection:*** Treatments with both the dosage of nitrogen i.e. N1 and N2 had higher VA mycorrhizal infection. Phosphorus did not have any significant impact on VAM. However, in treatment with higher dosage N and P i.e. N2P2, VAM colonization of both M and MR inoculated seedlings was poor. Control seedlings (without fertilizer) had higher VAM colonization as compared to some of the fertilizer treatments.

iii. ***Number of root nodules:*** In general, the nodulation in all the fertilizer treatments was poor; the highest nodulation occurred in MR/C treatment (2.4/ plant) followed by R/NlP2 (2.0). Nodulation was extremely poor in N1 and N2 treatments; apparently phosphorus treatments had higher nodulation.

Table.24. Effect of fertilizer on seedling biomass (dry weight in mg), % VA mycorrhiza infection and number of root nodules (per plant) of ***Acacia auriculiformis***

Symbionts	Fertilizer combination								
	N1	N1P1	P1	N1P2	N2	N2P1	P2	N2P2	C
Biomass									
VAM (M)	333abcd	210de	270abcde	318abcd	212de	260bcde	210de	380abc	65bcde
Rhizobium (R)	218de	393a	106c	270abcde	302abcde	177de	262cde	422a	217de
VAM + ***Rhizobium*** (MR)	215de	200de	233cde	192de	250bcde	250bcde	228cde	145e	218de
Control (C)	252bcde	225cde	173de	240bcde	208de	312abcd	307abcd	187de	
% mycorrhizal infection									
VAM (M)	70.0	65.0	54.0	60.0	73.3	48.0	51.7	10.0	70.0
VAM+ ***Rhizobium*** (MR)	75.5	60.0	65.0	74.0	67.5	65.0	70.0	59.5	64.0
No. of root nodules/plant									
Rhizobium (R)	2.1	9.5	0.5	2.0	3.5	3.0	3.7	1.0	1.1
VAM+ ***Rhizobium***	2.1	7.0	6.1	5.0	3.7	3.7	5.0	5.8	5.7

Table.25. Effect of fertilizer on seedling biomass (dry weight in mg), % VA mycorrhiza infection and number of root nodules (per plant) of ***Pterocarpus marsupium***

Symbionts	Fertilizer combination								
	N1	N1P1	P1	N1P2	N2	N2P1	P2	N2P2	C
Biomass(mg)									
VAM(M)	490	300	564	215	585	453	382	376	562
Rhizobium (R)	340	432	487	462	255	375	363	433	516
VAM + ***Rhizobium*** (MR)	531	382	380	255	378	422	395	498	427
Control(C)	368	347	460	470	388	323	365	478	503
% VA mycorrhizal infection									
VAM(M)	83.5	71	70.6	62.4	65.0	75.0	72.5	43.4	71.7
VAM + ***Rhizobium*** (MR)	75.4	88.3	71.0	45.8	79.2	52.3	75.8	66.5	61.5
No. of root nodules/plant									
Rhizobium (R)	0.4	1.6	1.2	2.0	0.0	1.0	.4	1.8	0.6
VAM+ ***Rhizobium*** (MR)	0.6	1.5	0.8		0.0	1.4	1.8	0.8	2.4

4.4.3. Casuarina equisetifolia

Table 26 provides results on biomass, % VA mycorrhizal colonization and number of root nodules.

Table 26. Effect of fertilizer on seedling biomass (dry weight in mg), % VA mycorrizha infection and number of root nodules (per plant) of ***Casuarina equisetifolia***

Symbionts	Fertilizer combination								
	N1	N1P1	P1	N1P2	N2	N2P1	P2	N2P2	C
Biomass(mg)									
VAM(M)	152	198	130	85	160	222	204	154	112
Rhizobium (R)	124	152	95	157	80	140	87	162	80
VAM + *Rhizobium* (MR)	153	182	165	150	104	237	163	167	154
Control(C)	92	86	90	123	80	137	102	201	90
% VA mycorrhizal infection									
VAM(M)	58.3	88.2	72.9	46.1	64.6	69.6	66.2	57.0	59.7
VAM+ ***Rhizobium*** (M+R)	58.0	78.3	74.0	65.0	71.7	71.7	49.6	52.6	57.2
No. of root nodules/plant									
Rhizobium (R)	0.5	0.7	.4	.3	0.4	.0	1.3	1.8	0.5
VAM+ ***Rhizobium*** (M+R)	1.6	1.4	1.0	1.6	0.4	1.3	1.4	0.8	1.0

i. ***Biomass (dry weight):*** Both the control sets, either with fertilizer and without inoculation or without fertilizer and with inoculation had poor biomass except C/N2P2 (201 mg) indicating that in the absence of symbionts seedlings could not utilize the fertilizers. Fertilizer treatments with VAM or VAM+ Frankia were better than those of seedlings inoculated alone with ***Frankia.*** Generally biomass of seedlings decreased at higher dose of nitrogen i.e., N2. There was no clear trend with phosphorus application. Seedlings with dual inoculation did not show any appreciable differences among fertilizer treatments except with N2P1. The best treatment where highest biomass was recorded were MR/N2P1 (237 mg) followed by M/N2P1 (222 mg) M/P2 (204 mg) and M/NlPl (198 mg).

ii. ***VA mycorrhizal colonization:*** Low dose of phosphorus and increasing dose of nitrogen (N to N2) appeared to affect favourably VAM colonization as higher colonization occurred in M/NlP 1 (88.2%) followed by MR/NlP1(78.3), MR/P1 (74.0%) and M/P1 (72.9%); at higher level of phosphorus i.e., P2 the colonization decreased.

iii ***Number of nodules/plant:*** In general the nodulation was poor in all the fertilizer treatments and there was no trend reflecting the favourable response either of nitrogen or phosphorus on the number of root nodules of ***Frankia.*** Maximum number of nodules was recorded in R/N2P2 (1.8), followed by MR/N1 (1.6), MR/N1P2 (1.6), MR/P2(1.4) and RP1 (1.4).

4.5. Field trial

Observations on height and diameter at 25 cm height could be recorded only for ***A. auriculiformis.*** Plants of ***P.marsupium*** were repeatedly cut and destroyed by rabbits in spite of the fact that all measures to control their activity were taken such as fencing of the plot and sprinkling of Furadan around plants, an insecticide to repel the rabbits. Though <75% of the planting stock survived, most of them in the form of stubs, no observations could be recorded. Seedlings of ***C. equisetifolia*** failed to survive, partially due to rabbit problems and drought and, hence, the field planting was repeated during 1995.

Height and girth measurements recorded for ***A. auriculiformis*** after one year of outplanting are given in Table 27. Signficant differences (P=0.0l) were observed in respect of height and girth of plants observed between treatments. However, there was no significant difference (p=0.05) between various treatments for survival of plants. Curiously, control plants had the maximum height (135.9 cm) and diameter (9.9 mm) as compared to various treatment combinations of both the symbionts i.e. two isolates each of VA mycorrhiza and ***Rhizobium*** (without fertilizer). All the treatments responded favourably to fertilizer application by increasing their height and diameter as compared to treatments without fertilizer. The per cent increase in height and girth was more in symbiont inoculated plants than control.

The maximum height occurred in R2F (174.3 cm) followed by M2R2F (170.7 cm), R1F (161.0 cm) and MF (159.2 cm) whereas the maximum diameter was recorded in M2R2F (15.5 mm) MIF (15.5 mm), M1R2F(14.2 mm) and CF (14.0 mm). If we look at the percent increase in height and diameter due to fertilizer application, maximum height increase was in M1 (57.4%) followed by M1R1 (56.1%), R2 (46.9%) and M2R2 (46.7%). For diameter growth the maximum increase was in M2R2 (84.5%), followed by M1 (70.0%), M1R1 (61.%), R2 (68.8%) and M1R2 (67.0%). In control treatments the increase in height and diameter growth was 11.5% and 41.4%, respectively.

Dendrograms of the cluster analysis of height and diameter data are presented in Figs. 41 and 42. It is evident from the analysis that for height, eight out of nine treatments of the first cluster were non-fertilizer treatments while all the eight treatments with fertilizer were in the third cluster indicating that fertilizer treatments are significantly different from treatments which did not receive any fertilizer. Cluster analysis for diameter data showed that eight out of ten treatments in one cluster were non-fertilizer treatments and the second cluster comprised of all the six treatments with fertilizer application.

Table 27. Height and diameter of one year old field planted ***A. auriculiformis*** inoculated with VA mycorrhizae and N_2 fixing symbionts with and without fertilizer

Treatments	Mean Height (cm)			Mean diameter (mm)		
	Without fertilizer (WF)	With fertilizers (F)	% increase over WF	Without fertilizer (WF)	With fertilizer (F)	% increase over WF
M1	101.1(1)*	159.2 13)	57.4	8.0	13.6	70.0
M2	113.2(2)	131.4(14)	16.1	8.9	10.0	12.4
R1	120.3(3)	161.0(15)	33.8	9.5	13.8	45.3
R2	118.6(4)	174.3(16)	47.0	9.6	16.2	68.8
M1R1	94.9(5)	148.2(9)	56.2	7.2	11.6	61.1
M1R2	122.3(6)	163.2(10)	33.3	8.5	14.2	67.0
M2R1	122.4(7)	156.1(11)	27.5	9.1	12.8	40.5
M2R2	116.3(8)	170.7(12)	46.8	8.4	15.5	84.5
C	135.9(18)	151.6(17)	11.6	9.9	14.0	41.4

M1, M2 - Two isolates of VA mycorrhiza; R1, R2 - Two isolates of ***Rhizobium;*** WF - without fertilizer; F- Fertilizer

*Treatment Number referred to in cluster analysis

5. DISCUSSION

To prevent fbrther degradation of soils of waste or fallow lands it is imperative to reforest them at the earliest along with appropriate soil conservation measures, if required. But, irrespective of the tree species used, degraded sites, by definition are difficult sites to reforest. In most cases it is necessary to modify the site in some way to achieve success. Changing soil fertility is one of the important site modification option which will be critical for the successful establishment of the planting stock. In contrast to chemical fertilizers, increasing soil fertility through VA mycorrhiza and N_2 fixing symbionts is a long term proposition as once it is introduced into the soil it will be self sustaining. Although VAM fbngi and N_2 fixing symbionts (eg. ***Rhizobium/Frankia)*** are widely distributed, site degradation can adversely affect the population of the symbionts, either reducing the amount of inoculum available to intact root system during the period when trees are becoming established or eliminating the efficient strain altogether in which only the unproductive strains survive (Abbot and Robson, 1991; Sieverding, 1991). Hence, either conservation of indigenous VAM population in degraded soils or the inoculation of trees with VAM and N_2 fixing symbionts, will be needed to ensure that adequate inoculum of the appropriate strain is available to support higher tree growth. Although, VAM species differ in their abilities to enhance plant growth, there appears to be little evidence of plant specificity for particular VAM spores. While this fact and the ubiquitousness of VAM fungi suggests that infection should occur spontaneously, inoculated plants, heavily infected at the time of outplanting may better withstand the rigors of degraded soil environment.

5.1 Mycorrhiza

Generally, VA mycorrhizae have been shown to enhance growth and survival of plants (Harley and Smith, 1983), improve phosphorus, and micronutrient nutrition (Krikum and Levy, 1980; Manjunath et al., 1984; Kormanik et al., 1977; Schultz et al., 1979) and increase drought tolerance (Safir et al., 1971; Graham and Sylvertsen, 1984) especially those of mycorrhiza-dependent plants grown on P deficient soils (Punj and Gupta, 1988; Bagyaraj et al., 1989).

As evident from the results, VAM inoculation has positive effect on growth and biomass and Phosphorus uptake of all the three selected tree species i.e. ***Acacia auriculiformis, Pterocarpus marsupium*** and ***Casuarina equisetifolia.*** Different VAM strains/species significantly enhanced height, root length, dry weight and root colonization of the inoculated seedlings as compared to noninoculated ones. Increase in P content is highest in ***A. auriculiformis*** followed by ***P. marsupium*** and ***C. equisetifolia.***

Increase in biomass of ***Acacia*** seedlings due to inoculation with VAM fungi conform with the earlier findings of Aggangan et al. (1987) and Cruz et al. (1988)

from Philippines, Chang et al. (1986). Reena and Bagyaraj (1990), Sharma et al (1990) and Sankaran ct al (1993) from India In the present study, ***Glomus mosseae*** is the best VA mycorrhiza which produced maximum biomass (dry weight). However, in earlier reports, different VAM fungi have been found to support best growth. For example, Chang et al. (1986), Sharma et al. (1990), and Sankaran et al (1993) reported ***G. fasciculatum*** as the most efficient species Aggangan et al.(1987) found ***G. etunicatum*** and ***G. microcarpum*** more effective than ***G. mosseae*** and ***G. fasciculatum.*** Cruz et al. (1988) found ***Gigaspora peroica*** along with ***Rhizobium*** as the best VAM species for enhancing growth of ***Acacia.*** This is possibly because of the efficiency of a VAM fungus which depends upon soil factors. In this context, Mosse (1976) reported that one of the main factors affecting VAM specificity is soil pH and some VA mycorrhizae show marked pH preferences Selection of VAM isolates for soil of specific pH is more rationale and it increases the likelihood of successful establishment of an effective symbiotic association (Hayman and Tavares, 1985)

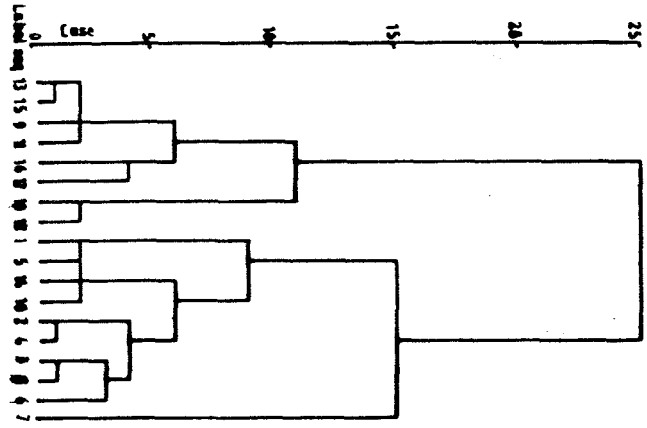


Fig.41. Cluster analysis of height of field planted 1-year-old ***Acacia.*** Dendrogram using average Linkage between groups.

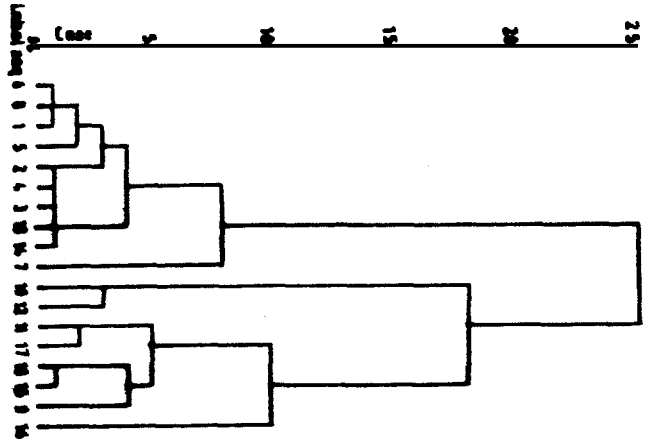


Fig.42. Cluster analysis of height of field planted 1-year-old ***Acacia.*** Dendrogram using average Linkage between groups.

Inoculation of ***C. equisetifolia*** with VAM fungi has been shown to improve growth and P uptake in P deficient tropical soils (Vasanthakrishna and Bagyaraj, 1991). Recently, Vasanthakrishna et al. (1994) reported that in dual inoculation of ***G. fasciculatum*** and ***Frankia,*** the effect of former was greater than those of the latter in P deficient soils. However, dual inoculation with both the symbionts enhanced the growth of ***C. equisetifolia*** more than single inoculation with either of these organisms. In the present study isolates M23 and M12 gave maximum dry weight, M-21 maximum plant height and root colonization, and ***G. monosporum*** maximum root length. Curiously, M12, M21 and M23 isolates, which were obtained from ***A. auriculiformis*** plantations in Kerala proved to be efficient for ***C. equisetifolia*** rather than ***A. auriculiformis.***

This is the first report on the occurrence of VA mycorrhiza in ***P. marsupium*** and also that efficient strains enhance the growth of seedlings. ***G. intraradices*** is the best strain for maximum dry weight, plant height and root length while ***G. monosporum*** gave maximum colonization in seedlings of ***P. marsupium.***

Higher root biomass due to VAM inoculation of all the three species is suggestive of that photosynthates of VAM seedlings are utilized in enhancing root biomass rather than above ground biomass production. This increase in root biomass associated with VAM can improve the outplanting performance of seedlings (Kormanik et al., 1982).

5.2. N_2 fixing symbionts - *Rhizobium/Frankia*

Effectiveness, competitiveness and persistence of inoculated ***Rhizobium/Frankia*** in soil is necessary for success of any inoculation programme, especially in problem soils. Even though seed or seedling inoculation is a simple process, identification of appropriate strain and its large scale multiplication for commercial exploitation is more complex. In the present study, in order to identify the ***Rhizobium*** strains efficient in degraded acidic soils, the isolates were screened for their ability for alkali production and acid-aluminium stress tolerance. These attributes are necessary for the success of inoculants in the degraded soils (Halliday, 1981; Ayanaba et al., 1983). Majority of the strains of ***Rhizobium*** and ***Frankia*** were capable of increasing the biomass (dry weight), nodule number, and total nitrogen. These findings are in agreement with the results of several other workers (Chang et al., 1986; Reddell, 1990). Since, number of nodules and total nitrogen content of plants were also considered as criteria for the efficiency of the inoculated strains, these attributes were also given equal weightage in identifing the best strains through cluster analysis.

5.3. Effect of fertilizer and symbiont(s) on seedling growth

This experiment was expected to give the most effective combination of the symbiont(s) and fertilizer for attaining higher biomass, VA mycorrhizal colonization and nodulation of the three tree species in the field. The glasshouse trial data on plant biomass (dry weight) of the three species indicate that there is no definite pattern on the effect of different combination(s) of fertilizer on plant

biomass in the presence of symbionts, except in the case of ***C. equisetifolia*** where biomass decreases at higher dose of nitrogen i.e., 40 ppm (M2). In all the three species both the levels of nitrogen, i.e., 20 and 40 ppm have favourable response in the presence of VAM. While in ***A. auriculiformis*** it is high level of P and in ***C. equisetifiolia*** low level of P which increases the VAM colonization, in ***P. marsupium*** phosphorus does not have any appreciable influence. VA mycorrhiza does not affect uptake of N from soil although it can enhance N_2 fixation by legumes in low P soils indirectly by increasing P uptake (Mosse et al., 1976).

In respect of root nodulation, phosphorus treatments resulted in higher nodulation in ***P. marsupium*** whereas in ***A. auriculiformis*** and ***C. equisetifolia*** there is no pronounced influence of either phosphorus or nitrogen on root nodulation. The reason for not emerging any definite trend in biomass, VA mycorrhizal colonization and root nodulation could be (i) the low dosage of fertilizer used and (ii) three month's duration of the experiment which might not have been sufficient to show any significant effect on symbionts and seedling growth.

As far as the levels of phosphorus and nitrogen used in the experiment are concerned, our results are in agreement with those of the earlier works of Nasr and Diem (1987), Sanginga et al. (1989) and Costa and Paulino (1990). The two levels of fertilizers applied are not low, but the results could have been more significant if two more levels, one higher and one lower were also taken. Nasr and Diem (1987) reported that after 4 months, dry weight and nitrogen fixation in seedlings of ***Acacia cyanophylla*** inoculated with ***G. mosseae*** increased upto 30 ppm of P input, but decreased at 50 ppm P. Similarly, frequency and intensity of VAM infection also increased upto 30 ppm of P but decreased at 50 ppm.

C. equisetifolia nodulated well by 12 weeks after transplanting and the number and weight of nodules increased markedly with P addition in acid soil. Growth of ***C. equisetifolia,*** dependent on symbiotically fixed nitrogen responded to low P (30 mg/kg soil) more readily than was growth of seedlings supplied with combined nitrogen; with high phosphorus, growth response is similar for both N fertilizer and inoculated plants. The interaction between P and N treatments demonstrates that there is a greater requirement of phosphorus for symbiotic nitrogen fixation than for plant growth when soil P is low (Sanginga et al., 1989). Costa and Paulino (1990) found in ***Leucaena Ieucocephala*** that all measured parameters such as dry matter, P and N content, N and P uptake, VAM infection and nodulation varied with both the P treatments i.e., with (22 kg/ha) or without P. The most effective VAM fungi varied with the parameters measured. In most of the earlier reports (Chang et al., 1986; Cruz et al., 1988 ; Badgi et al., 1989; Sharma et al., 1990), dual inoculation of seedlings with VA mycorrhizal fungus and ***Rhizobium/Frankia*** gave better growth response as compared to single inoculation. However, in our experiments there is no subtle difference between dual inoculation and single

inoculation either with VAM or N_2 fixing symbionts, except in the case of VA mycorrhizal colonization of A. ***auriculiformis*** and ***P. marsupium.***

Possibly the 3-month duration of the experiment was too short to obtain significant results from seedlings of perennial tree species. Probably some time will be required by the symbionts to establish and multiply and show their effect on various growth parameters of seedlings. Poor nodulation observed in all the tree species is also due to shorter duration of the experiment. It may be concluded that for tree species, especially slow growing ***P. marsupium,*** the duration of the pot-experiment involving symbiont(s) and fertilizer should be for at least six months. Furthermore, before attempting such studies the growth response curves of each species with respect to various levels of fertilizers should be worked out and that should form the basis for further studies incorporating symbionts and fertilizers.

5.4. Field trial

An information gap exists in the literature relating to performance of trees inoculated with symbionts after outplanting. Practically all studies have been restricted to short-term observations in the glasshouse or tree nurseries where inoculation is frequently beneficial. The present study provides substantial proof of the beneficial effects of symbionts in enhancing the growth of one-year-old field planted ***A. auriculiformis.*** Though, the survival rate of seedlings does not differ among the various treatments, fertilizer treated plants have shown better growth than non-fertilized ones. For degraded lands, the most important of the silvicultural indicators is the extent to which planted seedlings survive. This is commonly measured at a specific time after outplanting which takes account of the ability of seedlings to establish in the face of adverse edaphic conditions. Only when the survival rate is acceptable, attention shifts to growth rate, stocking levels etc. A field trial in Mexico with dual inoculation of ***A. pennatula*** with ***Rhizobium*** and VA mycorrhiza showed that inoculated plants exhibited greater growth than uninoculated controls (Roskoski et al., 1986). In Kenya, plants of dual inoculated tree species ***(Acacia tortiles, Prosopis juliflora, Terminalia brownii, T. prunioides,)*** grew and survived better than non inoculated ones (Wilson et al., 1991). However, while survival in the field was improved by inoculation, there was no substantial difference between treatments in the growth of surviving plants. An interesting observation is by Cornet et al. (1982) who observed that VAM inoculation improved the growth of ***Acacia*** spp. and the beneficial effects of inoculation diminished after a few years in the field.

Curiously, the uninoculated control out-performed the inoculated ones, both in height and girth. The only possible explanation for this is the existence of very efficient VA mycorrhiza and ***Rhizobium*** in the field soil which colonise the roots, and their beneficial effects are transferred to growth of plants. However, in fertilizer treatments, which are significantly different from non-fertilized ones, there is appreciable enhancement in growth; the percentage increase is more in treatments with symbionts(s) than non-inoculated control. This possibly indicate that the strains used as inoculants are more efficient than the field ones in

nutrient uptake. This is in agreement with the observations of Blal et al., (1990) who found that VAM inoculation increased fertilizer efficiency in oil palm grown in acid P fixing tropical soil and Jasper et al. (1989) who reported that several Australian ***Acacia*** spp. are strongly dependent on VA mycorrhizal fungi for phosphorus uptake. Furthermore, Hayman (1982) observed that if the soil is phosphorus fixing eg. like ferro-lateritic type in the tropics, somewhat similar to the field soil at Ramavarmapuram, a plant may respond to mycorrhizal inoculation even after appreciable amount of fertilizer has been added. This is because increased removal of plant-available P from soil by VA mycorrhiza rather than release of some of the vast bulk of inert insoluble P (Mayman and Mosse, 1972; Sanders and Tinkers, 1973) could lead to rapid exhaustion of the soil P reserves. Therefore, some fertilizer phosphate must be added for long-term maintenance of residual P in P deficient soils (Hayman, 1982).

Considering the performance of two strains.of VA mycorrhiza and ***Rhizobium*** in non-fertilized treatments, dual inoculated treatments M1R2 ***(Glomus calidonius*** + ***Rhizobium*** A-16 Panamaram) and M2R1 ***(G. mosseae*** + ***Rhizobium*** A-19 Calicut) are the best. Among the single inoculated treatments, ***Rhizobium*** inoculated ones show higher height and diameter. This is possibly because naturally occurring ***Rhizobium*** strain in the field soil is not efficient whereas VA mycorrhiza present in the soil appears to be very efficient as evident from the control treatment. Best growth obtained in dual inoculated treatment confirm several of the earlier observations on ***Acacia*** spp. (Chang et al., 1986; Cruz et al., 1988; Badji et al., 1989). Badji et al. (1989) studied the growth and nodulation of seedlings of ***Acacia laeta*** in degraded sandy soil, poor in mineral nitrogen and available phosphorus. The best growth was obtained in plants inoculated with ***Rhizobium*** + ***Glomus mosseae*** than in those with single inoculation, either of the two symbionts. Cruz et al. (1988) found that dual inoculation of ***A. auriculiformis*** with ***Scutellospora persica*** + ***Rhizobium*** (R), ***Gigaspora margarita*** + R and ***Glomus fasciculatum*** + R were more effective than inoculation with the single symbiont. Similarly Chang et al (1986) observed that dual inoculation of ***A. auriculiformis*** with ***Rhizobium*** + ***G.fasciculatum*** resulted in the greatest number of nodules, seedling height, uptake of N and P. In ***A. holosericea,*** Connet et al., (1984) also found that the dual inoculation of both ***Rhizobium*** and VA mycorrhizal fungi significantly increased growth of plants above single inoculation and controls.

6. CONCLUSION

Past experience shows that various nitrogen fixing tree species have an especially valuable role in the reforestation of degraded lands. For such situations the key issue is species choice. A number of exotic species are widely used in reforestation due to a number of positive attributes. However, ecological caution suggests that it is unwise to continue to rely on such a limited species mix for all future reforestation efforts (Lamb and Tomlinson, 1994). Hence, in this project two exotics, i.e., ***A. auriculiformis*** and ***C. equisetifolia*** and one indigenous tree species viz. ***P. marsupium*** were selected. But palatability of the

latter to wild animals as well as cattle make ***P. mursupium,*** a less suitable species for reforestation programme. There is a need to identify a few nitrogen fixing native tree species which can be effectively used in reforestation programmes, especially in degraded acid soils.

The study clearly brings out the beneficial role of VA mycorrhiza and nitrogen fixing symbionts ***(Rhizobium*** and ***Frankia)*** in enhancing growth of A. ***auriculiformis, C. equisetifolia*** and ***P. marsupium.*** Since inoculation requirement of leguminous trees may vary with site or species (Roskoski et al., 1986), the strains of symbionts for a particular tree species need to be selected to suit specific edaphic and climatic factors. Otherwise, their impact may not be always positive. Field trial also confirmed the suitability of ***A. auriculiformis*** in reforesting the degraded acidic soils.

Though, inoculation of seedlings with appropriate symbionts can be easily adopted by the forest managers as a regular plantation practice, the main constraint lies in the large-scale production of suitable inoculum of known strains effective in specific edaphic and climatic conditions. To overcome this the technology need to be transferred to industries which can raise the inoculum commercially and supply to forest managers.

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