GROWTH AND NODULATION CHARACTERISTICS OF SELECTED INDIGENOUS TREE LEGUMES

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Growth and nodulation characteristics of selected indigenous tree legumes" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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TO MY PARENTS

CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

INTRODUCTION

Tree legumes form an important group of Nitrogen Fixing Trees (NFTs), which is having symbiotic association with *Rhizobium*. These trees are an important component of forestry, especially farm and social forestry programmes. Cultivation of NFT legumes can be used beneficially to resolve economic, environmental and equity problems facing peoples of the tropical world.

More than 640 tree species are known to fix nitrogen (Brewbaker *et al.*, 1990), but this number may represent fewer than 20 per cent of all nitrogen fixing tree species. There are nitrogen fixing trees of 115 genera in the legume subfamilies *Caesalpiniaceae, Mimosaceae* and *Papilionaceae*, as well as in eight other families (*Betulaceae, Rhamnaceae, Coriariaceae, Eleagnaceae, Myricaceae, Rhamnaceae, Rosaceae* and *Ulmaceae*). Most of these genera are legumes.

In India, during the recent years much emphasis has been given for popularising some of the exotic trees like subabul (*Leucaena leucocephala*), gliricidia (*Gliricidia sepium*), etc. A lot of interest has been evinced on subabul and the various aspects of cultivation of this tree are documented (Balasundaran and Ali, 1987). Multipurpose NFTs such as *Leucaena leucocephala* and *Gliricidia sepium*

have shown promise as effective fallow improvement crops (Agboola *et al.*, 1982). *Gliricidia sepium* is a Nitrogen Fixing Tree species that has been widely tested and provides a good source of fuelwood and green manure while controlling weeds and stabilizing soil (IITA, 1986). There are a number of studies on growth and nodulation characteristics of these species in India and abroad. Dutt *et al.* (1983) reported that, in *Leucaena leucocephala* growth at 30 months was greater in inoculated trees than in uninoculated trees. The study by Ramprasad *et al.* (1984) indicated that inoculation enhanced nodulation and seedling height growth in *Leucaena leucocephala*. Green leaved seedlings produced higher nodule weight than pale-leaved seedlings in *Gliricidia sepium* (Atta-Krah, 1987).

There are a large number of indigenous/naturalized tree species like *Albizia lebbeck, Pterocarpus marsupium, Pongamia pinnata,* etc. which are popularly grown in Kerala in farmlands as well as in forests. In many parts of the state, indigenous NFT's are much more popular than the exotic leucaena and gliricidia. Some of these indigenous legumes are known for its superior timber, others yield fuelwood, green manure, fodder, etc. It is a paradox that the growth characteristics and N_2 fixing potential of the several indigenous/naturalized legume trees are not studied much. Hence the present investigation has been taken up to study the nodulation behaviour and N_2 fixation ability of selected indigenous/naturalized tree legume species grown in the state.

Review of Literature

REVIEW OF LITERATURE

Nitrogen-fixing trees have a special advantage over other tree species through symbiotic associations with *Rhizobium* or *Frankia* in their root nodules, fixing elemental nitrogen from the atmosphere. Next to nitrogen, phosphorus is essential for the growth of trees and many species have symbiotic mycorrhizal fungal colonizers in their root system which help in the absorption of this element from the root milieu and work synergistically with the nitrogen fixing symbiotic system. These attributes of symbioses with beneficial micro-organisms can naturally be harnessed in soils of any established forest ecosystem.

A number of studies have been conducted on symbiotic nitrogen fixation in herbaceous as well as tree species. The renowned Dutch Microbiologist, Beijerinck (1888) was the first to recognise the bacterium causing the formation of root nodules in legumes and demonstrated that the purified bacteria formed nodules in plants grown from sterilized seeds. The ability of the *Rhizobium* to establish a symbiotic relationship with legume host is dependent on two important characteristics of the microsymbiont viz., infectivity and effectiveness (Schwinghamer, 1964). It has been considered that each of the partners, *Rhizobium* and legume is unable by itself to fix substantial amount of nitrogen and the effective

nitrogen fixation is the phenotypic expression of the two associated genomes (Bergerson, 1971).

Thompson (1952) reported that the amount of nitrogen fixed and that added by a legume to the soil depend on its age, photosynthetic rate and presence of combined nitrogen in the soil. Mirchandani and Khan (1953) pointed out that, the amount of nitrogen fixed by a legume depend on its age, conditions of growth, type of legume and the stage at which it is ploughed into. They found that the quantity of N_2 added to the soil by a legume depend mainly on whether the legume was incorporated in the soil or not.

2.1 Nitrogen Fixing Trees

A survey on nodulation behaviour made among 16 species of Mimosaceae, 9 species of Caesalpiniaceae and 14 species of Papilionaceae in Pakistan showed that all but 3 Cassia species had nodulation characteristics (Athar and Mahmood, 1980). Much interest has been generated in the production and use of nitrogen fixing trees (NFTs) in farming systems due to their ability to fix nitrogen as it can improve tree crop and animal production in a sustainable manner (Brewbaker *et al.*, 1982; Nair *et al.*, 1984). More than 640 tree species, the majority of which are members of the Leguminosae (Fabaceae) are known to fix atmospheric nitrogen (Brewbaker *et al.*, 1990).

Nitrogen-fixing trees (NFTs) are an ideal class of trees for afforesting degraded sites (MacDicken, 1994).

2.2 Factors affecting Nodulation and Nitrogen fixation

2.2.1 Environmental factors

The effects of the supply of photosynthate on nitrogen fixation was reported in alder and peas by Wheeler and Laurie (1976). Dawson and Gordon (1979) demonstrated the influence of photosynthesis, as reflected by leaf area, on nitrogen fixation in *Alnus glutinosa*. Symbiotic N_2 probably is limited by any factor that reduces tree vigour and health. Fixation of nitrogen is limited by the biological yield of the host plant even if a suitable symbiont (ie. *Rhizobium* or *Frankia*) and appropriate soils are present, because, the host plant is both the source of energy for the symbiont and the sink for the fixed nitrogen (App *et al.*, 1980). Most of what is known about environmental influence on nodulation comes from research on herbaceous crops. Much of this work has focused on the effects of single factors (eg. combined nitrogen or temperature); very little has been reported on the effects of multiple factors on N_2 fixation.

2.2.1.1 Moisture

Soil moisture, both too low or too high may be a limiting factor for N_2 fixation in plants. Experiments with

alfalfa, soybean and *Acacia mellifera* have shown that the optimum level of soil moisture for N fixation is approximately 60 per cent to 75 per cent of soil water holding capacity (Fred *et al.*, 1932; Habish, 1970). Moisture contents above or below optimum level may adversely affect the number of nodules formed and their longevity. Under conditions of moisture stress in sandy soils, nodulation in NFTs may occur deeper in the soil (Beadle, 1964). Under moisture stress conditions, the wilting of lower leaves is usually a good indicator that nodules are operating at suboptimal rates (Sprent 1972). Huang *et al.* (1975) demonstrated that nitrogen fixation is reduced indirectly by the effects of moisture stress on photosynthesis.

The NFT *Alnus glutinosa* growing on permanently waterlogged soils has been found to nodulate primarily in the well-aerated, humid, upper soil horizons, but not on permanently submerged roots (Akkermans and Van Dijk, 1976). In a study of *Sesbania sesban* and *S. rostrata*, Ndoye *et al.* (1990) demonstrated a decrease in the amount of N_2 fixed per plant for *S. sesban*, but not for the stem-nodulated *S. rostrata* when both were grown under waterlogged conditions. Rao and Barrueco (1993) reported that waterlogging depresses nitrogen fixation, primarily due to low oxygen levels (anoxia) and lower rates of N - diffusion to the nodules.

2.2.1.2 Oxygen

An important factor which is necessary for the formation and functioning of nodules is oxygen. An abundant supply of oxygen is needed to ensure adequate respiration of the root and nodule tissue. McConell (1959) determined that nodule formation and nitrogen fixation in *Alnus glutinosa* are considerably reduced at oxygen levels below 0.21 atmospheres. This is consistent with results obtained for soybeans and clover (Ferguson and Bond, 1954).

2.2.1.3 pH of the soil

Plants that depend on nitrogen fixation have been found to be more sensitive to pH than plants of the same species supplied with mineral nitrogen (Andrews, 1976). Dixon and Wheeler (1983) stated that in general, the critical pH for nodulation of most legumes is above 4.5 to 5.5. Low soil pH generally inhibits nitrogen fixation by reducing the development of rhizobia, increasing the number of ineffective rhizobia or disrupting the infection process (Sprent and Sprent, 1990).

The most widely reported effect of soil acidity on symbiotic N-fixation is reduction in nodulation. This sensitivity is most evident in early infection stages (Carvahlo *et al.*, 1981). The specific nature of the

effects of soil acidity on symbiotic N-fixation are dependent on interactions between pH and nutrient availability (Munns, 1986).

Ahmad and Ng (1981) observed reduction in subabul growth with lower pH. Liming of acid soils to increase the pH to 6.5 improved the performance of the symbiosis of *Acacia mangium* (Umali-Garcia *et al.*, 1988).

2.2.1.4 Temperature

The studies on effect of temperature on N_2 fixation are rather scanty in tree species. Fyson and Sprent (1982) found that nitrogenase activity began at about the same physiological stage for *Vicia faba* plants grown at two different temperature conditions. Hawkins and Mcdonald (1994) studied the interaction of temperature and soil water status on the growth, photosynthesis, transpiration, plant water potential, foliar nutrient concentrations and nitrogen fixation rates of 2-month old red alder seedlings over a 9-week period. The greatest seedling growth occurred at 25°C day temperature, while 20°C and 25°C days produced the greatest nodule growth.

At temperatures of 11°C and 21°C, seedlings of the subalpine species *Alnus viridis* fixed significantly more N at higher temperature (Benecke, 1970). However, since growth rates were significantly lower at lower temperature

the total accumulated plant nitrogen as a percentage of total plant growth was greater at lower temperature. Perry *et al.* (1979) demonstrated the effect of temperature on two non-legume NFTs, *Alnus rubra* and *Cytisus scoparius*. Optimum temperature for nitrogenase activity of *C. scoparius* was approximately 22°C, while the optimum for *A. rubra* was approximately 35°C.

2.2.1.5 Salinity and Alkalinity

Problems with soil salinity and alkalinity tend to occur most frequently in arid and semi-arid environments (Lal, 1987). Saline conditions can depress N_2 fixation through osmotic withdrawal of moisture from nodules, reductions in bacterial colonization of roots and root hair curling. This depression can be due to a lack of adaptation to saline conditions by either the tree or symbiont. In fact, there is tremendous variation in the response of tree and *Rhizobium* to saline soils. NFT species such as *Prosopis tamarugo* survive and fix nitrogen in extremely saline soils where other NFTs or symbiont strains would not survive (MacDicken, 1994).

Balla *et al.* (1990) demonstrated the suitability of salt-tolerant *Rhizobium* strains in an experiment using 57 strains of *Rhizobium* and several NFT species. Salt tolerant strains showed better survival, nodulation and nitrogen fixation than salt sensitive strains under saline

conditions. A strain from each of the four NFTs, *Acacia nilotica, Dalbergia sissoo, Leucaena leucocephala* and *Prosopis juliflora* was tested, and all were found to be highly tolerant to saline conditions. Zou *et al.* (1995) compared the growth, nodulation and N content of *Acacia ampliceps* seedlings inoculated with salt tolerant *Rhizobium* PMA 63/1 and salt sensitive *Bradyrhizobium* PMA 37 and found that *Acacia ampliceps* inoculated with the former was less affected in growth, nodule number and N content per plant by 200 mM NaCl than plants inoculated with the latter. He concluded that inoculation with a salt tolerant *Rhizobium* strain may therefore, improve biological N_2 fixation under saline conditions.

Lakshmi-Kumari *et al.* (1974) demonstrated the existence of 'alkali sensitive steps' in early phases of nodulation and effects of alkalinity on root hair development. The mechanisms by which the effects are mediated are not known. Although some leguminous and actinorhizal plants and their associated microsymbionts are tolerant of alkaline environments, the effects of alkalinity on symbiotic N_2 fixation have received very little attention (Rao and Barrueco, 1993).

2.2.1.6 Fertilizer application

A study by Reinsvold and Pope (1987) on the effect of N and P supplements in *Rhizobium* inoculated

Robinia pseudoacacia revealed that application of phosphorus ranging from 25-100 mg/kg of soil increased the growth and acetylene reduction activity of the nodules while N above 50 mg/kg soil actually reduced the number of nodules and nodule dry weight. Mao *et al.*(1989) studied the effects of fertilizer on nodulation and nitrogen fixation of *Acacia mangium*. They observed that, biomass of nodules was two times more in fertilized plots than in plots with no fertilizer. Toky *et al.* (1994) studied the N_2 fixation in an Indian provenance of *Acacia nilotica* inoculated with *Rhizobium* and fertilized with urea (0, 15, 30 or 60 ppm) and found that increasing amounts of fertilizer did not significantly affect nodulation, but significantly reduced nitrogenase activity, although there was a significant increase in the growth of plants and their nitrogen content.

Addition of super phosphate in *Rhizobium* inoculated *Leucaena leucocephala* grown in a glass house showed an increase of 116 per cent of yield in infertile soil while only 46 per cent increase was observed in medium fertile soil (Garza *et al.*, 1987). Nitrogen-fixing plants need the same nutrients as other green plants, but they may need additional quantities of cobalt, iron and molybdenum to produce leghaemoglobin and nitrogenase (Sprent and Sprent, 1990).

High levels of combined soil N may reduce nodulation and nodule activity and accelerate senescence (Gibson and Nutman, 1960; Becana and Sprent, 1987). Zhang *et al.* (1990) found a marked decrease in the nodulation rate of *Casuarina cunninghamiana* seedlings as soil N increased. Soil nitrogen in excess of 10 ppm had the greatest effect in reducing the nodulation rate. Reductions of 35 per cent to 45 per cent in number and weight of *Glyricidia sepium* nodules were reported when treated with 25 kg N/ha to 100 kg N/ha in nursery studies (Umali-Garcia, 1990). The levels that inhibit N_2 fixation are not necessarily the same for different rhizobial strains (Murphy, 1988), NFT species (George and Singleton, 1989), or clones of the same species (Mackay *et al.*, 1987).

2.2.2 Inoculation

Inoculation of legume seeds with the appropriate cultures of *Rhizobium* was originally introduced as a means of ensuring the establishment of seedlings in nitrogen deficient soils which lacked adequate population of nodulating bacteria (Fred *et al.*, 1932). Nair *et al.* (1970) studied the effect of seed inoculation with *Rhizobium* on yield and nitrogen content of leguminous green manure crops and reported a fixation of 8-14 kg nitrogen per hectare and an increase in the dry matter production. Sahu and Bahara (1972) observed that inoculation increased

the number of nodules and nitrogen content of shoot and root in cowpea, groundnut, and green gram. The presence of suitable *Rhizobium* or *Frankia* is essential to nitrogen fixation, without which symbiotic biological nitrogen fixation cannot occur (MacDicken, 1994).

Pahwa (1988) observed that natural nodulation was poor in six local tree species of Bundelkhand (U.P.) and hence recommended inoculation of seeds of these species with specific rhizobia. Ntumbula *et al.* (1990) reported that *Albizia lebbeck* seeds inoculated with *Rhizobium* from a mature *A. lebbeck* tree produced more nodules in a sterilized media.

Sanginga *et al.* (1988) examined the effect of inoculation and N, P and trace elements on nodulation and growth of *Leucaena leucocephala*. In pot experiments all parameters measured except the percentage of N in shoots, were improved by inoculation. Establishment of uninoculated and unfertilized *L. leucocephala* was poor in the field. Siddiqui *et al.* (1993) studied the effect of inoculation (with Azotobacter) and fertilizer treatments on mulberry and found that all inoculated plants fixed more nitrogen than non-inoculated plant.

2.2.2.1 Characteristics of Rhizobia

The genus *Rhizobium* includes fast-growing rhizobia that produce acid on yeast mannitol agar and are most often of temperate origin (Wheeler *et al.*, 1991). The three species in this genus are: *Rhizobium leguminosarum*, *R. meliloti* and *R. loti*. The genus *Bradyrhizobium* contains slow-growing bacteria that do not produce acid on yeast mannitol agar. Bacteria of this genus commonly infect tropical legumes, although numerous exceptions make this a loose generalization (MacDicken, 1994).

Competition between strains of rhizobia can result in reduced or delayed nodulation (Lie *et al.*, 1988). The presence of both nodulating and non-nodulating rhizobial strains in the rooting zone can result infection sites being taken by non-nodulating bacteria, preventing or reducing the establishment of an effective symbiosis.

2.2.2.2 Indigenous Vs Exotic Rhizobia

The persistence of introduced *Rhizobium* strains also can affect the amount of nitrogen fixed. In a study of labelled *Rhizobium* and *L. leucocephala*, South (1982) found that at the time of transplanting, 46 per cent to 97 per cent of nodules contained labelled *Rhizobium*. While 10 weeks later 4 per cent or less contained the labelled *Rhizobium*. In this case, the indigenous *Rhizobium* was a better competitor than the inoculated strain for infection sites on the roots.

An indirect, competitive, enzyme-linked immunosorbent assay (ELISA) was developed to identify strains (SU 391, SU 303, WSM 937, NZM 5472) of *Rhizobium leguminosarum* bv. *viciae* in the nodules of *Pisum sativum* and *Vicia faba*. The effect of legume species and indigenous *Rhizobium leguminosarum* viciae on the nodulating competitiveness of these strains, applied as seed inoculants was also studied. It appears that indigenous *R. leguminosarum* viciae number of at least 500 g^{-1} soil at sowing may severely reduce the nodulating-competitiveness of seed applied inoculant strains with pea; and numbers much >500 g^{-1} soil, the nodulating competitiveness of inoculant strains applied to faba bean. No inoculant was remarkably better than another in competing with indigenous *R. leguminosarum* viciae. It is concluded that seed inoculation often may be more successful with *V. faba* than *P. sativum*, which is fortuitous because indigenous *R. leguminosarum viciae* are not likely to be less efficient for N_2 fixation with faba bean than that with pea (Evans *et al.*, 1996).

Renodulation and nitrogen fixation potential of indigenous (AB3 and AD4) and exotic isolates of *Rhizobium* (USDA 3325) were studied by Lal and Khanna (1993) in a field experiment with *Acacia nilotica* at Gwalpahari, India. The study revealed that the inoculum isolates belonged to different serotypes and did not show cross-reaction with the native population of *Rhizobium*. Maximum nodulation was

observed with isolate AB3 followed by AD4 and USDA 3325. Simanungkalit (1996) stated that proportion of nodules formed in soybean by the inoculated strain is an index of its competitiveness against the naturalized root-nodule bacteria and the relative competitiveness of the strain was site dependent.

Galiana (1991) reported that *Bradyrhizobium* samples isolated from indigenous *Acacia mangium* trees in Queensland (Australia) were more effective than strains from other origins like Hawaii and Senegal. Sanginga *et al.* (1994) found that, in 1992, ten years after *Rhizobium* introduction, uninoculated *L. leucocephala* fixed ~150 kg N/ha per year compared with 180 kg/ha per year as measured in 1982. ELISA and intrinsic resistance to the streptomycin tests showed that 96 per cent of nodules formed in 1992 were by *Rhizobium* strains IRC 1045 and IRC 1050 which were inoculated in 1982. Nodules were absent on uninoculated *L. leucocephala* grown on the adjacent field with no history of *L. leucocephala* cultivation. The study indicated that *Rhizobium* strains IRC. 1045 and IRC 1050 persisted for many years in the absence of *L. leucocephala.*

2.2.2.3 Specificity

The efficacy of pure culture of *Rhizobium* strains of different forest tree species viz. *Dalbergia sissoo, Leucaena leucocephala, Pongamia pinnata* and *Albizia lebbeck*

was tested on the growth of *Albizia lebbeck* seedlings by Jamaluddin *et al.* (1995). They observed that the shoot length, girth, number of nodules, fresh and dry weight and nitrogen content of *A. lebbeck* seedlings increased to a considerable extent upon inoculation with the *Rhizobium* strain isolated from *A. lebbeck* than with *Rhizobium* strains of other legume species.

Some evidence for co-evolution of host and symbiont has recently been reported (Lie *et al.*, 1987; Eardly *et al.*, 1990). The former authors cited a number of examples of wild pea genotypes for which compatible rhizobia are only found in the area of cultivation. For example, a pea-type found in Southern Turkey only nodulates with Rhizobia from the same area and not with strains from other parts of Turkey.

Girija (1982) conducted a study on host varietal specificity for *Rhizobium* for nodulation in groundnut. She used seven varieties of groundnut and isolates of the root nodule bacterium from each of the seven varieties of groundnut and reported a favourable response for all plant growth characters studied in response to inoculation with its respective homologous isolate of *Rhizobium*. *Rhizobium fredii* USDA 257 is a Gram-negative soil bacterium from China that fixes nitrogen in symbioses with primitive soybean (*Glycine max*) cultivars such as peking, but not

with advanced cultivars such as Mccall. Mutation of either of 2 bacterial loci, nol BTUVWX or nol C, removes this cultivar - specificity constraint, allowing this bacterium to nodulate advanced cultivars, (Krishnan and Pueppke, 1994).

2.2.2.4 Cross inoculation

Iqbal and Mahmood (1992) studied the response of *Leucaena leucocephala* to inoculation with rhizobia from tropical legumes like *Albizia lebbeck, Clitoria ternatea, Medicago sativa, Pithecellobium dulce, Sesbania sesban, Vigna ungiculata* and groundnut and found that with the exception of groundnut all isolates produced nodules on *L. leucocephala*. Isolates from *V. ungiculata, A. lebbeck* and *P. dulce* were most effective in N_2 fixation and significantly increased host plant N content.

Seven nitrogen fixing tree species (*Faidherbia albida, Acacia senegal, A. auriculiformis, Calliandra calothyrus, Gliricidia sepium, Leucaena leucocephala* and *Prosopis juliflora*) were grown in pots in a non-sterilized inceptisol either not inoculated, or inoculated with species - specific *Rhizobium* strains, *Rhizobium* strain TAL 1145, a Leucaena isolate, and *Bradyrhizobium japonicum* strain TAL 169. Significant dry matter yield increase in response to inoculation with species-specific rhizobia was observed in *G. sepium* genotypes and in *A. senegal*, while

increase in response to inoculation with TAL 1145 was obtained in *G. sepium* genotypes and in *C. calothyrus*. There was no response to inoculation with TAL 169. All the seedlings inoculated with species-specific rhizobia showed significant total N yield and Ndfa (nitrogen derived from the atmosphere) increases except for *F. albida* and *A. auriculiformis*. *G. sepium* and *L. leucocephala* genotypes and *P. juliflora* responded to inoculation with TAL 1145. However, no significant response to inoculation with TAL 169 was observed (Bekunda, 1993).

2.2.2.5 Dual inoculation

Dual inoculation of *Scuttellospora persica* + *Rhizobium*, *Gigaspora margarita* + *Rhizobium* and *Glomus fasciculatum* + *Rhizobium* were most effective for *Acacia auriculiformis* (Dela Cruz *et al.*, 1988). Two isolates of *Bradyrhizobium* viz, B-1 (Commercial *Bradyrhizobium* culture recommended for cowpea in Kerala) and B-2 (isolated from the nodules of cowpea grown in the vegetable garden of College of Horticulture, KAU, Trichur) were inoculated in combination with the four selected *Azospirillum* isolates to study their associative effect on nodulation and growth of cowpea (Menon, 1992). She observed significant interaction of *Azospirillum* and *Bradyrhizobium* only on the number of nodules, and that combined inoculation inhibited the nodulation by *Bradyrhizobium* B-1 whereas it enhanced the nodulation of B-2. Studies

conducted by Mehrotra (1996) on *Acacia catechu* and *Dalbergia sissoo* indicated that inoculation with Rhizobia will maximise seedling growth when co-inoculated with VAM (Vesicular-Arbuscular Mycorrhizae).

2.2.2.6 Selection of suitable strain

Substantial efforts in NFTS research are directed at identifying the most effective strains. Identification is crucial since the success of the host inoculation depends upon the ability of the organism to survive local conditions and carry out its functions (Balasundaran, 1996). One method adopted is preliminary screening of a large number of isolates, which obviates the need for laborious in vivo screening for selected traits, such as tolerance to acid - aluminium stress, and thus reducing the number of candidate isolates. This strategy has been employed by Ty (1995) and Sharma *et al.* (1996) to select *Rhizobium* strains suitable for acid soils.

Haydock *et al.* (1980) reported that the most effective *Rhizobium* strain could be selected on the basis of dry matter yield of whole plant or plant top only. Selection of a single isolate becomes problematic when the same isolate does not show the highest score for each selected parameter. Sharma *et al.* (1996) relied on cluster analysis which gives equal weight to several key attributes to select the two best isolates from several tested.

2.2.3 Genetic/Provenance

The role of host genotype in deciding the efficiency of nodulation and nitrogen fixation was reported by Caldwell and Vest (1979). They concluded that effective nodulation and nitrogen fixation were greatly influenced by genetic make up of both the symbionts. Anjily (1991) suggested that selection or development of improved rhizobial strains which are geographically and genetically suited to the legume trees in the arid zone should be conducted to derive maximum growth from the important multipurpose tree species of the Indian arid zone. A study on biological nitrogen fixation potential of three *Gliricidia sepium* provenances by Liyanage *et al.* (1994) revealed marked variation in dry matter, total N and nodulation among the three gliricidia genotypes. Differences in N_2 fixing abilities of 18 provenances (16 from India and 2 from Israel) of *A. nilotica* were studied by Beniwal *et al.* (1995). They found significant difference in growth and amount of N_2 fixed and stored in plants between provenances. The differences were attributed to genetic variability among provenances. Vadez *et al.* (1995) studied the comparative growth and symbiotic performance of four *Acacia mangium* provenances from Papua New Guinea in response to the supply of phosphorus at various concentrations. They found that the response of seedlings varied with provenances.

2.2.4 Other factors

Ramirez *et al.* (1990) found that pollarding *Erythrina* causes nodules to deteriorate rapidly but that once regrowth is well underway new nodules are formed. Any lopping treatment resulting in significant reduction in residual leaf area is likely to reduce nitrogen fixation and growth, (MacDicken, 1994).

Studies conducted by Wickliff and Evans (1980) revealed a strong negative correlation between nitrogenase activity and cadmium concentration in nodules. Studies on acetylene reduction activity associated with tree roots in Cypress wetland conducted by Dieberg and Brezonik (1981) indicated that nitrogenase activity was absent in roots from a sewage-enriched area.

Albizia lebbeck and *Leucaena leucocephala* seedlings were grown under field conditions at Dehra Dun, to observe the changes in nitrogenase activity during the period of their growth ie., March to August. A marked increase in nitrogenase activity was observed from March to July (Pokhriyal *et al.*, 1991). Sellestedt *et al.* (1991) found that highest cumulative nitrogen fixation was always associated with highest leg-haemoglobin concentration and highest rates of acetylene reduction activity in the case of six Casuarina symbioses-(*Casuarina cunninghamiana, C. equisetifolia* and *C. glauca* each inoculated with two different *Frankia* sources).

Nitrogen fixation should respond to plant density in a way similar to above-ground biomass production. The highest rates of fixation per tree should be found in open-grown widely spaced trees. Intermediate spacings should produce and sustain the greatest total nitrogen fixed per unit area, while the highest population densities initially should produce the greatest total nitrogen fixed per unit area but decline as competition increases (MacDicken, 1994).

2.3. Assessment of Nitrogen Fixation

2.3.1 Acetylene Reduction Assay

This assay is based on the fact that the nitrogenase enzyme can reduce acetylene to ethylene (Rao and Barrueco, 1993). The most commonly used conditions under which it is carried out are still those of the closed assay recommended by Hardy *et al.* (1968) despite the fact that research works have been published pointing out the potential errors involved. Temperature, humidity, gas pressure and composition, length of time of assay and plant disturbance have all been shown to affect the results. Acetylene itself may cause a decline in the enzyme activity (Criswell *et al.*,1977; Witty *et al.*, 1984; Minchin *et al.*, 1985).

Roskoski *et al.* (1982) in their study on nitrogen fixation rates based upon acetylene reduction obtained a value of 9-15 kg/ha/yr in the case of *Erythrina.* In

Leucaena leucocephala the N fixation rate was 80-140 kg/ha/yr using this method (Hogberg and Kvarnstrom, 1982). In *Acacia alata*, Hansen *et al.* (1987) obtained a value of 1.6 kg/ha/yr.

2.3.2. Natural ^{15}N abundance method/isotope dilution method

Recently a lot of investigators are using ^{15}N, a stable isotope of N as a tracer in nitrogen fixation research. There are basically two approaches. One is to observe the dilution of added ^{15}N (as gas or as soluble compounds) and the other is to observe the natural vâriations of the proportion of ^{15}N to ^{14}N. These two methods are known as ^{15}N dilution technique and ^{15}N natural abundance technique, respectively. The latter method rely on the differences between the N_2 in air and combined N in soil. Plants reliant on N_2 fixation will have a ^{15}N content similar to air. Those using only soil N will reflect the ^{15}N content of soil. Plants which fix both N_2 and use soil N (nitrate, ammonium, urea) will be intermediate (Rao and Barrueco, 1993).

Considering the high cost of ^{15}N and its estimation, in most laboratories, field fixation of N_2 has to be studied by observing the plant gain in nitrogen.

2.3.3 Xylem Sap Analysis

Some species synthesise ureides when fixing N_2 but not when assimilating mineral nitrogen. The proportion of the

total xylem sap nitrogen in the form of ureides gives a direct estimate of the proportion of the total nitrogen derived from the atmosphere (McClure and Israel, 1979 and Peoples *et al.*, 1990).

A woody genera which, based on nodule structure and taxonomic position, is likely to synthesise ureides when fixing N_2 is *Erythrina* (Rao and Barrueco, 1993). The xylem ureide method, based on analysis of xylem sap for N solutes associated with N_2 fixation (ureides) and soil N use ($NO3^-$, 2 amino -N) was used for estimating the proportion of plant N derived from N_2 fixation in *Calliandra calothyrus, Leucaena leucocephala, Gliricidia sepium, Sesbania grandiflora, Desmodium rensonii* and *Codariocalyx gyroides,* by Herridge *et al.* (1996). The results indicate that the xylem ureide technique is suitable for estimating N_2 fixation in *D. rensonii* and *C. gyroides* only.

It should also be remembered that synthesis of ureides, especially by woody plants is not necessarily associated with N_2 fixation (Van Kessel *et al.*, 1988).

2.3.4 Other methods

Some of the other methods are N-soluble method, N difference method, isotope dilution technique and ^{15}N enrichment method.

The N_2 fixing capacity of *Leucaena diversifolia* and *Sesbania sesban* seedlings inoculated after germination with 6 strains of *Rhizobium* was determined after 4 months growth in glass house. The potting media was a mixture of compost/grit/clay uniformly mixed with ^{15}N enriched ammonium sulfate, and nitrogen fixation was measured by calculating the isotope dilution in the total plant N. Seedling growth and N fixation varied between *Rhizobium* strains, with N fixation representing 31-54 per cent of plant N. Reference (non-inoculated) plants contained relatively more N from fertilizer and soil than inoculated plants (Rao and Giller, 1994).

Kadiata and Mulongoy (1995) studied the N fixation and utilization of *Albizia lebbeck, Leucaena leucocephala* and *Gliricidia sepium* using the ^{15}N dilution technique. The study revealed that *A. lebbeck* was the best N_2 fixer with 44 per cent nitrogen derived from atmosphere (Ndfa) equivalent to 533 mg N per plant. *G. sepium* followed with 28 per cent and *L. leucocephala* with 18 per cent Ndfa. However, the relatively higher N_2 fixation in *A. lebbeck* was not translated into higher N or dry matter yields.

Meneill *et al.* (1994) used a closed system flow-through enclosure apparatus for direct measurement of nitrogen fixation in *Trifolium repens* and *Alnus glutinosa*. A legume based system comprising 6-week old *T. repens* growing with *Lolium perenne* in an agricultural soil was

incubated 19 d in a ^{15}N enriched atmosphere. An actinorhizal based system comprising 1-year old *Alnus glutinosa* sapling, growing with *Festuca rubra* in open cast coal spoil was incubated for 21 d in a ^{15}N enriched atmosphere. Indirect estimates of N_2 fixation were carried out concurrently using N difference and ^{15}N isotope dilution techniques. In the legume based system, the simple N difference method gave similar values for pinc (The proportion of N increment during the measurement period that was derived from fixation) to those derived from more complicated isotope methodologies, both indirect and direct.

Various studies reviewed above throws light to the fact that, investigations on the nodulation characteristics of indigenous tree legumes are rather scanty, so the present study was undertaken to know the nodulation characters of selected indigenous/naturalized tree legumes which are common in the homesteads of Kerala, which inturn will help us in selecting suitable tree species for agroforestry systems.

Materials and Methods

MATERIALS AND METHODS

The investigation was carried out at the College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur district, Kerala, during the period from October 1994 to September 1996. The study involved a field survey for the collection of nodules and isolation of rhizobia from seven selected indigenous/naturalized trees viz., *Adenanthera pavonina* L., *Albizia lebbeck* L., *Dalbergia latifolia* Roxb., *Erythrina stricta* Roxb., *Pongamia pinnata* (L.) Pierre, *Pterocarpus marsupium* Roxb. and *Tamarindus indica* L. and evaluation of the strains by reinoculation on the same species.

3.1 Survey

The survey was conducted in Thrissur district which is located between 10° 10' and 10° 46' N latitude and 76° 10' and 76° 50' E longitude. The area enjoys a warm humid climate.

Five out of 98 Panchayats in Thrissur district was selected at random for the present work. These include Madakkathara, Nadathara, Ollukkara, Pananchery and Puthur panchayats. Grown up trees of above species were identified in farmers field in each panchayat. Their nodulation characteristics were studied using core sampling and

sieving technique (Sankaran *et al.*, 1993). In this method soil will be collected using Soil Core Sampler, 15 cm long and approximately 3.5 cm diameter. Soil samples was collected from a depth of 0-10 cm at a distance of 20-30 cm from the basal region of the tree using core sampler. Five samples were collected from the base of each tree from five points around the tree. Each soil sample was then sieved in a 2 mm sieve over water and the number of nodules was enumerated and their size and shape was also noted. After this, nodulation behaviour in the seedlings if any growing below the plant was recorded. Where core sampling failed to yield nodules, the root of the trees and seedlings was thoroughly searched for the presence of nodules. The nodules thus collected were taken to the laboratory for further investigation.

3.1.1 Tree species selected

3.1.1.1 *Adenanthera pavonina* L. (Manchadi)

It is popularly known as `Manchadi' and belongs to the family Mimosaceae. It often reaches a height of 18-25 m, and a girth of 2.0 - 2.4 m. Wood is used for building purpose and for cabinet making.

3.1.1.2 *Albizia lebbeck* L. (Nenmeni Vaka)

Belongs to the family Mimosaceae commonly known as `Nenmeni vaka'. *A. lebbeck* usually attains a height of 15 m to 20 m and a diameter of about 50 cm at maturity.

This medium sized tree of the tropics is having wood, fodder and ornamental uses and is common in our homesteads.

3.1.1.3 *Dalbergia latifolia* Roxb. (Rosewood/Veeti)

A very valuable timber tree of the tropics often attaining a height of 30-40 m. Belongs to the family Papilionaceae and is commonly known as `Rosewood'.

3.1.1.4 *Erythrina stricta* Roxb. (Mullumurukku)

Commonly found in the homesteads of Kerala. Belongs to the family Papilionaceae. Trees often reaches a height of 10-15 m and a girth of about 100 cm. It is a good matchwood tree and the leaves have good fodder value.

3.1.1.5 *Pongamia pinnata* (L.) Pierre (Ungu)

It is a medium sized tree that can reach a height of 15 m and a diameter of 50 - 70 cm. *P. pinnata* popularly known as `Ungu' belongs to the family Papilionaceae. The wood has a number of timber uses, and the leaves are a valuable fodder. Common in the homesteads of Kerala.

3.1.1.6 *Pterocarpus marsupium* Roxb. (Venga)

A member of the family Papilionaceae which attains a height of about 40 m and a diameter of 200 cm. `Venga' is its malayalam name, `Kinogum' is obtained from it. It is a good timber yielding tree.

3.1.1.7 *Tamarindus indica* L. (Tamarind)

Tamarind is a tree famous for its fruit and is common in the homesteads of Kerala. It attains a height of 25-30 m and a girth of 150-200 cm. It belongs to the family Caesalpiniaceae. Wood is having a high fuel wood value.

3.2 Isolation of *Rhizobium*

In those species which showed nodulation, *Rhizobium* was isolated following the method described by Vincent, 1970. The various steps in isolation are given below:

3.2.1 Preparation of media

3.2.1.1 Sterilization

The glasswares and media used for the investigation were sterilized by autoclaving at 15 lbs/inch^2 pressure for 20 minutes. A pressure cooker can also be made use of for this purpose ie., after the first whistle, the pressure cooker was kept for 20 minutes, under low flame.

3.2.1.2 Yeast Extract Mannitol Agar with Congo Red (YEMA)

Yeast Extract Mannitol agar medium with the following composition was used for the isolation of rhizobia.

Ingredients		g/litre
Mannitol	:	10.0
Dipotassium hydrogen phosphate (K_2HPO_4)	:	0.5
Magnesium sulphate ($MgSO_4.7H_2O$)	:	0.2
Sodium chloride (NaCl)	:	0.1
Yeast extract	:	1.0
Agar	:	20.0
Congo Red	:	0.025

The pH of the media was adjusted to 6.8-7.0 by using 0.01N HCl or NaOH

For the present investigation, the ready made media from M/s. Hi Media was used. The media was prepared by adding 1000 ml of distilled water to 31.8 g of the readymade media and then heated to boil to dissolve the medium completely. It is then sterilized by autoclaving (or using pressure cooker) for 20 minutes at 15 lbs. pressure (121°C).

3.2.1.3 Plating

The sterilized medium was poured into sterilized petriplates in an evenly manner. This was carried out in a contaminant free atmosphere which was ensured with the help of a `Laminar Flow Chamber'.

3.2.1.4 Slants

The medium was heated to dissolve completely. After that 4-5 ml of it was poured into culture tubes. These tubes were then properly plugged with cotton and sterilized. The tubes along with the media were then kept in a slanting position to solidify.

3.2.2 Isolation

Successful isolation depends very much on the quality of the nodule sample. Damaged and dried up nodules were discarded.

Good nodules collected from individual tree species from each panchayat were selected for isolation. They were washed in tap water to remove gross soil contamination. After that, the nodules were surface sterilized by dipping in 0.1 per cent mercuric chloride for 2-3 minutes and then freed of mercuric chloride by washing with sterilized water atleast 5 times. Then the nodules were then crushed using a glass rod to get a turbid suspension. With the help of a flamed loop a drop of the suspension was streaked on to the media surface in Petric plates. The loop was flamed after each streak. The above operations were carried out in a Laminar Flow Chamber.

The plates were incubated at room temperature and were observed at 24 hour interval for typical growth of

Rhizobium (as slimy white colonies). The number of days taken for growth was recorded.

From the plates small isolated colony of *Rhizobium* were identified and was transferred to slants with the help of a sterilized inoculation needle to obtain contamination free isolates (Plate 1).

3.2.3 Multiplication

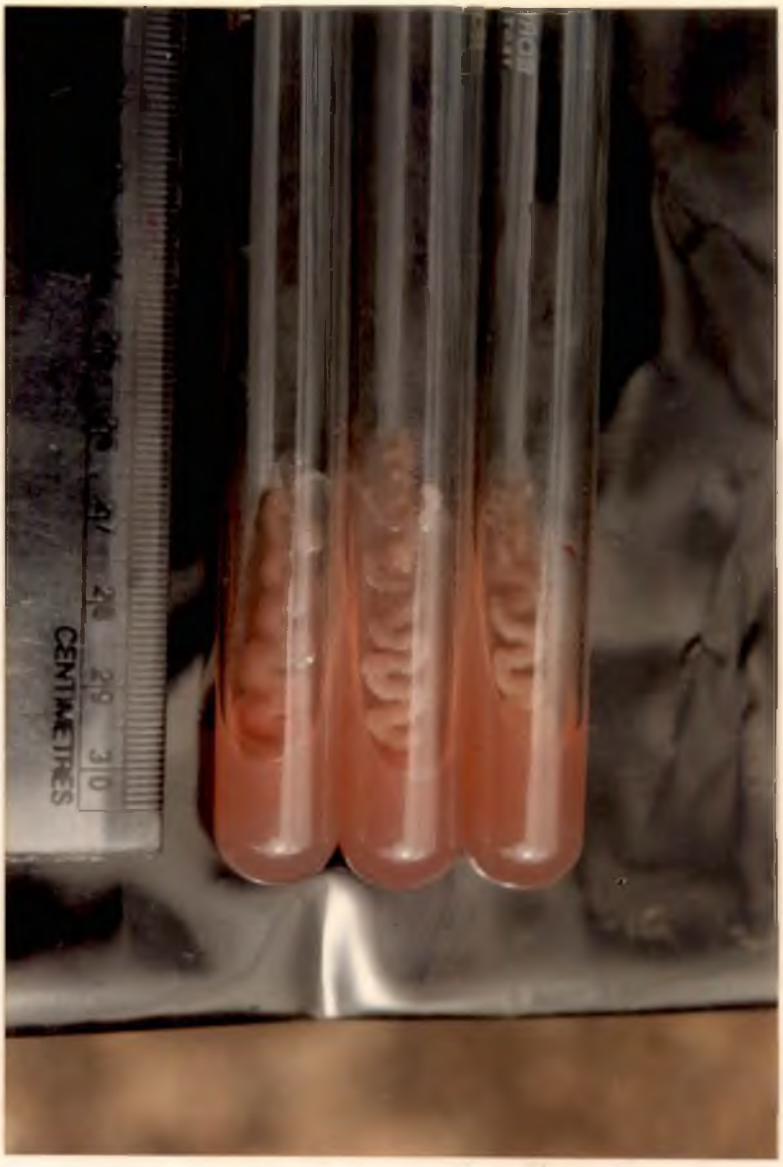
For the multiplication of rhizobia the medium used was Yeast Extract Mannitol Broth which is YEMA medium without agar. The multiplication of rhizobia were carried out only 7-8 days before pelleting of seeds.

The pH of the media was adjusted to 6.8-7 by using 0.01N HCl or NaOH.

For the multiplication purpose readymade media from M/s. Hi Media was used. In 100 ml of distilled water taken in a 250 ml conical flask, 1.28 g of the readymade medium was dissolved and sterilized. With the help of a sterilized inoculation needle, a loop full of rhizobia was transferred from slants into the sterilized broth in conical flask under aseptic conditions. These conical flasks were incubated at room temperature in a rotary shaker at 85 rpm for 5-6 days (Plate 2).

Plate 1 *Rhizobium* growing in yeast mannitol agar with congo red slants

Plate 2 Multiplied *Rhizobium* in the Broth medium


CENTIMETRES
29
30


ALB
N.TARO
3/4/96
MUR
MAD.
3/4/96

3.3 Commercial cultures

The following commercial cultures were also evaluated along with the isolates from different panchayats.

3.3.1 Peat inoculants from Niftal centre

TAL-1536

Multi-strain *Rhizobium* mix for *Albizia lebbeck*. This strain was used for `tamarind' and `manchadi' since there was no specific strain for them.

TAL-169 - strain specific to Rosewood and Pongamia

TAL-749 - strain specific to *Erythrina stricta*

TAL-990 - strain specific to *Pterocarpus* marsupium

3.3.2 Agroforester TM inoculants

Group `H' - meant for *Erythrina* species, was also used in `Pongamia', *Albizia lebbeck*, `tamarind' and `manchadi' since there was no specific strain for them.

Group `O' - it was a group meant for *Dalbergia* species

Group `PTER' - meant for *Pterocarpus* species

3.3.3 ABTECH *Rhizobium* culture

This was obtained from ABTECH Bio Tech Research Centre, Kottayam. This culture was actually meant for pulse crops like black gram, green gram, soybean etc.

3.3.4 NBL *Rhizobium* culture

This is commonly known as Rashtriya Jaiv Urvarak *Rhizobium*, manufactured by National Biofertilizer Development Centre, Government of India, Ministry of Agriculture, Ghaziabad (UP). This is meant for inoculating pulse crops.

3.4 Soil

Soil samples were collected from the root zone of each species in every selected panchayat. The soil samples were analysed for the chemical properties of the soil viz., total nitrogen, available phosphorus, available potassium, organic carbon and pH of the soil (Jackson, 1958). The data of the chemical analysis are presented in Table 1.

3.5 Inoculation

3.5.1 Inoculation with local isolates and commercial cultures of *Rhizobium*

Inoculation with isolates and commercial cultures were carried out by pelleting the seeds with the respective cultures and sowing them in sand. Pelleting is considered to be the most successful method of inoculation (Vincent, 1970).

3.5.1.1 Commercial cultures

First the seeds were coated with jaggery as a sticking agent. Then they were coated with the peat based commercial inoculants, and then with calcium carbonate o as to have a

Table 1 Properties of soil from the various Panchayats

Species	Panchayat	pH	% OC	% N	P kg ha^{-1}	K kg ha^{-1}
Rosewood (*Dalbergia latifolia*)	Pananchery	5.3	2.91	0.1878	6.7	372
	Madakkathara	5.4	2.01	0.1370	11.8	300
	Ollukkara	5.1	2.11	0.1651	71.7	364
	Nadathara	4.6	1.54	0.1327	38.6	174
	Puthur	4.6	3.48	0.2380	15.7	580
Venga (*Pterocarpus marsupium*)	Pananchery	5.9	2.44	0.1522	9.0	218
	Madakkathara	4.3	1.22	0.1432	3.9	260
	Ollukkara	6.5	2.80	0.1360	15.1	109
	Nadathara	6.0	1.91	0.1260	23.0	199
	Puthur	5.4	2.12	0.1467	10.1	342
Mullumurukku (*Erythrina stricta*)	Pananchery	4.7	1.24	0.1392	25.2	252
	Madakkathara	4.9	1.71	0.1107	24.1	454
	Ollukkara	5.1	2.00	0.1489	15.1	202
	Nadathara	5.5	1.39	0.1174	44.8	540
	Puthur	4.3	1.91	0.1200	20.1	370
Ungu (*Pongamia pinnata*)	Pananchery	4.5	1.55	0.1198	15.7	207
	Madakkathara	4.8	2.72	0.2169	12.3	241
	Ollukkara	4.8	2.17	0.2239	17.4	31
	Nadathara	5.3	2.45	0.1988	16.8	462
	Puthur	6.8	1.68	0.1619	121.0	563
Nenmeni Vaka (*Albizia lebbeck*)	Madakkathara	4.9	2.38	0.1846	11.1	543
	Nadathara	4.4	2.13	0.1510	10.6	151
Control (Sand)		5.5	0.42	0.0098	19.04	30

uniform coating on each and every seed (Plate 3). The quantity of cultures required varied according to size of seeds.

3.5.1.2 Isolates

The seeds were coated with jaggery in a beaker. To this the rhizobia-multiplied broth and a carrier medium (peat was used for the present study) was added. Thorough mixing was done. After that a coating of calcium carbonate was given. The amount of isolate and peat required varied according to size of seeds.

3.5.2 Inoculation with soil

This was carried out by dibbling the uninoculated seeds in poly bags containing soil collected from the root zone of each species in every selected panchayats.

3.6 Raising seedlings

The efficiency of the isolates and commercial cultures on inducing nodulation in the seedlings of the respective species was estimated by raising the pelleted seeds in polythene bags containing sand. In addition to this, a control ie. growing seeds in sand without inoculation, for comparison was also carried out. All these seedlings ie. those inoculated with commercial culture, isolate, soil, and the control were raised in a greenhouse.

Plate 3 Seeds of Venga (*Pterocarpus marsupium*) pelleted with commercial culture of *Rhizobium*

3.7 Observations

The experiment was laid out in completely randomised design. After four months of growth, the seedlings were uprooted and the following observations were recorded.

3.7.1 Number and weight of nodules

The number of nodules and their fresh weight in mg were recorded.

3.7.2 Number of effective nodules

This was found out by cutting each nodule into two equal halves and noting the pink colouration (due to the presence of leg-haemoglobin) in the central region. Those nodules which had pink colour were classified as effective and those without pink colour as ineffective.

3.7.3 Nitrogen content of the plant

Micro Kjeldhal method was used to find out the nitrogen content of the plant.

3.7.4 Nitrogen content of the soil in the polythene bag

Before growing seedlings the soils were analysed for total nitrogen.

3.7.5 Nitrogen fixed by *Rhizobium* in the soil

The total nitrogen in the soil after uprooting the plant was also estimated. The nitrogen fixed by symbiosis was estimated by difference method.

3.7.6 Height of the plant

The length of the plant from the base to the tip of the terminal bud was measured using a meter scale and expressed as height of the plant in cm.

3.7.7 Girth of the plant

The girth of the plant at the basal region was measured with the help of a twine and was expressed in mm.

3.7.8 Number of leaves

The number of leaves per plant in each treatment was recorded. In the case of compound leaved species the number of compound leaves was recorded.

3.7.9 Biomass of the plant

Dry weight of the plant was taken to get this.

3.8 Statistical analysis

The experimental data were tabulated and statistically analysed by applying the technique of analysis of variance for CRD, and significance was tested by the `F' test (Snedecor and Cochran, 1967).

Results and Discussion

RESULTS AND DISCUSSION

The results of the experiments on the growth and nodulation characteristics of selected indigenous/ naturalized tree legumes of Thrissur district are presented and discussed in this chapter.

4.1 Nodulation of the selected tree species in homesteads

The five Panchayats selected for the survey at random were Pananchery, Madakkathara, Nadathara, Ollukkara and Puthur. The core sampling and sieving technique used to study nodulation failed to yield nodules in soil samples from all the panchayats and hence the roots of the selected plants were thoroughly searched for collecting the nodules. The survey indicated that out of the seven selected tree species only five are nodulating and the rest two are non-nodulating type (Table 2).

4.1.1 Nodulating species

The species which showed nodulation were *Albizia lebbeck* L. (Nenmeni vaka), *Dalbergia latifolia* Roxb. (Rosewood/Veeti), *Erythrina stricta* Roxb. (Mullumurukku), *Pongamia pinnata* (L.) Pierre (Ungu) and *Pterocarpus marsupium* Roxb. (Venga).

Table 2 Nodulation of the various species in the selected panchayats and nature of growth of rhizobia

Tree species	Panchayats					Shape of nodules	Days taken for the rhizobia to grow in YEMA
	Pananchery	Madakkathara	Nadathara	Puthur	Ollukkara		
Rosewood/Veeti	+	+	+	-	-	Round	3-4
Venga	+	+	+	+	+	Round	3-4
Mullumurukku	+	+	+	+	+	Round	3-4
Ungu	+	+	+	+	+	Round	3-4
Nenmenivaka	-	+	+	-	-	Coralloid	3-4
Manchadi	-	-	-	-	-	-	-
Tamarind	-	-	-	-	-	-	-

(+) Nodules were obtained
(-) Nodules were not obtained

YEMA - Yeast Mannitol Agar Medium with Congo Red

Among the five panchayats surveyed, the nodules of *Dalbergia latifolia* were obtained only from three panchayats viz., Pananchery, Madakkathara and Nadathara (Table 2). The *Rhizobium* isolates from these three panchayats were designated as DTR_1, DTR_2 and DTR_3 respectively.

It can be seen from Table 1 that Puthur panchayat recorded high content of soil nitrogen (0.238 per cent) which might have hindered nodulation in *Dalbergia latifolia*. High levels of combined soil nitrogen may reduce nodulation and nodule activity and accelerate senescence (Becana and Sprent, 1987). The N levels that inhibit N fixation are not necessarily the same for different rhizobial strains (Murphy, 1988), nitrogen fixing tree (NFT) species (George and Singleton, 1989) or clones of the same species (Mackay *et al.*, 1987). Another probable reason for non-nodulation may be the absence of viable natural strain of *Rhizobium* in the soil for nodulating rosewood.

The percentage soil nitrogen was not that high in Ollukkara; however, nodules were not obtained from this area. This may be attributed to the absence of viable colonies for nodulating the species.

Nodulation in *Pterocarpus marsupium* was observed in all the five panchayats viz., Pananchery, Madakkathara, Nadathara, Puthur and Ollukkara (Table 2). The *Rhizobium* isolates obtained from these panchayats were designated as `$PTR_1$', `PTR_2', `$PTR_3$', `PTR_4' and `PTR_5' respectively.

In the case of *Erythrina stricta* also, nodules were obtained from all the five Panchayats. `$ETR_1$', `ETR_2', `$ETR_3$', `ETR_4' and `ETR_5' were the names given to the isolates extracted from the nodules of Pananchery, Madakkathara, Nadathara, Puthur and Ollukkara panchayat respectively.

In *Pongamia pinnata* nodulation was obtained from the homesteads of all the five panchayats. The isolates obtained were designated as P_0TR_1, P_0TR_2, P_0TR_3, P_0TR_4 and P_0TR_5 respectively, for Panancherry, Madakkathara, Nadathara, Puthur and Ollukkara panchayats.

Nodules were obtained only from Madakkathara and Nadathara in the case of *Albizia lebbeck*. This species was not encountered in the homesteads of the remaining three panchayats. The isolates were designated as ATR_1 (Madakkathara) and ATR_2 (Nadathara).

4.1.1.1 Size and shape of Nodules

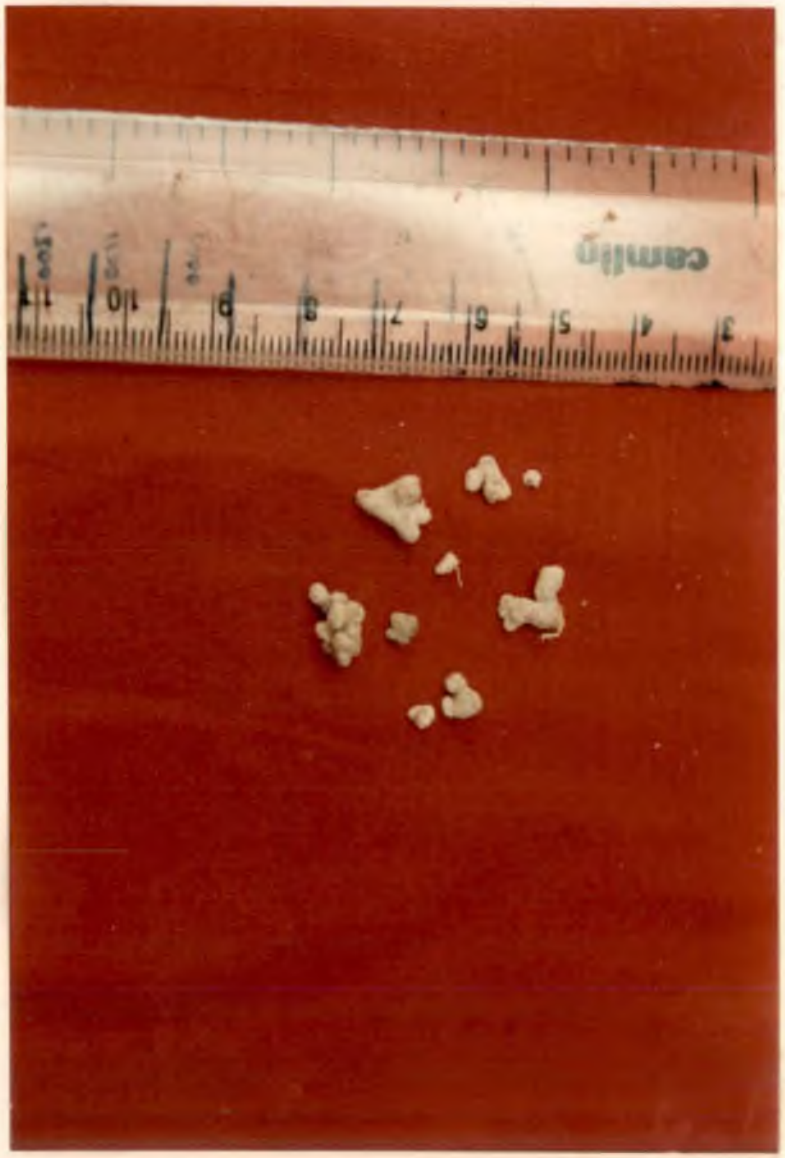
Nodules of the largest size were obtained in the case of *Erythrina stricta* (Plate 4) and the smallest in *Dalbergia latifolia*. Shape of nodules was round in all species except *Albizia lebbeck*, which was coralloid in nature (Plate 5 and Table 2). Xiangquan and Sufeng (1984) also obtained coralloid form of nodules in *Albizia chinensis*, *Albizia julibrissin* and *Albizia saman*. It seems that the plant usually determines the shape of nodule, eg., round nodules on *Amorpha fruticosa*, elongated, cylindrical, finger-forms nodules on *Wisteria sinensis* and coralloid nodules of *Albizia* are all caused by the identical rhizobial strain (Sufeng and Xiangquan, 1991).

4.1.1.2 Nature and growth of *Rhizobium* isolates

Rhizobium isolates of each species obtained (altogether twenty isolates were obtained) from the five panchayats of Trichur district showed growth within four days in the Yeast Mannitol Agar with Congo Red Medium (YEMA). Since the isolates showed growth within four days, these species of *Rhizobium* may be fast growing (Table 2). Sharma *et al.* (1996) in their study on *Rhizobium* isolates from *Pterocarpus marsupium*, classified those isolates that takes 3-5 days for growth on YEMA medium as fast growing and 5-9 days as slow growing. They found that isolates obtained from 8 localities in Kerala State were fast growing and only two were slow growing.

Plate 4 Root portion of Murukku (*Erythrina stricta*) showing nodules

Plate 5 Coralloid nodules obtained from Nenmeni Vaka (*Albizia lebbeck*)



The genus *Rhizobium* includes fast growing rhizobia that produce acid on yeast mannitol agar and are most often of temperate origin (Wheeler *et al.*, 1991). The genus *Bradyrhizobium* contains slow growing bacteria that do not produce acid on yeast mannitol agar. However, this was not found to be true always; it was reported that five strains of slow growers lowered the pH of YEMA medium (Ding Ming-mao *et al.*, 1994). Bacteria of this genus commonly infect tropical legumes, although numerous exceptions make this a loose generalization (MacDicken, 1994).

The taxonomic position of root nodule bacteria of tree legumes is controversial. Any taxonomic revision must await comprehensive comparative studies involving large number of bacterial strains from a wide variety of tree legumes. Only if considerably more data is obtained can this taxonomy be properly codified (Sufeng and Xiangquan, 1991). All the isolates obtained as per this study were fast growers. However, it was difficult to place them under any of the genera of rhizobia, since lot of controversies are going on regarding the various classifications of rhizobia. Generally the strains (irrespective of fast growers and slow growers) of rhizobia seen in tropical regions are designated as *Bradyrhizobium*. So we presume that the isolates obtained as per this study to be *Bradyrhizobium*.

4.1.2 Non-nodulating species

The survey indicated that two tree species namely *Adenanthera pavonina* L. (Manchadi) and *Tamarindus indica* L. (Tamarind) were non-nodulating type since these two species failed to yield nodules in any of the panchayats surveyed (Table 2 and Plate 6). This finding is in conformity with the study of Allen and Allen (1981). Another study by de Faria *et al.* (1989) revealed that *Adenanthera* a genus of about 8 species in tropical Asia and the Pacific are non-nodulating.

4.2 Effect of inoculation with local isolates and commercial cultures on the nodulating species

4.2.1 Rosewood/Veeti (*Dalbergia latifolia* Roxb.)

The effect of seed inoculation with the local isolates and commercial cultures on number of nodules, number of effective nodules, weight of nodules and dry weight per plant were found to be significant. However, characters like height, girth, root length, number of leaves per plant, nitrogen content of plant and that of soil showed no significant difference. All the inoculated plants except the plants inoculated with Abtech cultures showed better nodulation as compared to uninoculated control (Table 3a).

Inoculation with the strain TAL-169 resulted in highest value for characters like number of nodules, number

Plate 6 Seedlings of Tamarind (*Tamarindus indica*) and Manchadi (*Adenanthera pavonina*) - Note the absence of nodules on roots

Table 3a Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on nodulation of Rosewood (*Dalbergia latifolia*)

Isolate/ culture	Number of nodules per plant	Number of effective nodules per plant	Fresh weight of nodule (mg/plant)
DTR1	23.8	17.2	36.8
DTR2	16.0	12.6	27.3
DTR3	12.4	10.0	27.1
TAL-169	42.0	23.6	64.4
NBL	11.0	6.8	16.5
GR-O	15.6	9.6	16.5
Abtech	7.4	4.2	10.3
Control	8.2	2.6	34.2
SEM(±)	3.5	3.3	5.8
CD (0.05)	9.9	9.4	16.8

of effective nodules, weight of nodules and dry weight per plant and was significantly superior to all other cultures. In the case of number of effective nodules, inoculation with DTR_1 culture could produce similar results as that of TAL-169 inoculation. However, the percentage of effective nodules was highest in DTR3 and the least in control (Fig.1). The height, girth and number of leaves were also found to be high in TAL-169 though these characters were not significantly different (Table 3b). This points to the superiority of this strain over the rest. TAL-169 is a specific culture recommended by Niftal for rosewood.

Inoculation with the isolate DTR_1 stands second with respect to the number of nodules, effective nodules and the fresh weight of nodules produced. The mean value of 23.8 for number of nodules in DTR_1 treated plants was on par with that of DTR_2 and GR-0; however it significantly differed from DTR_3, NBL, Abtech and Control (Table 3a).

When the number of effective nodules was considered, DTR_1 formed a homogenous grouping with DTR_2, DTR_3 and GR-0 but significantly differed from NBL, Abtech and control. In the case of weight of nodules, DTR_1, was on par with control, DTR_2, and DTR_3 whereas it was significantly superior to NBL, GR-0 and Abtech.

Table 3b Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on growth characteristics of Rosewood (*Dalbergia latifolia*)

Isolate/ culture	Plant height (cm)	Root length (cm)	Girth (mm)	No. of leaves per plant	Dry weight (g/plant)	Nitrogen content (%)	
						Plant	Soil
DTR1	33.5	30.7	5.1	10.6	0.21	1.93	0.020
DTR2	37.6	40.3	5.3	13.0	0.38	2.27	0.018
DTR3	35.3	17.9	6.8	11.8	0.37	2.28	0.026
TAL-169	41.5	49.3	7.6	12.4	1.27	2.12	0.015
NBL	39.2	45.1	6.5	12.4	0.56	2.04	0.020
GR-O	34.9	49.9	5.7	12.2	0.62	2.23	0.020
Abtech	35.5	37.3	6.1	11.4	0.46	2.04	0.015
Control	33.7	37.9	5.8	12.2	0.53	1.86	0.018
SEM(±)	3.1	8.6	0.6	0.9	0.12	0.23	0.00
CD (0.05)	NS	NS	NS	NS	0.36	NS	NS

further evaluation in rosewood. No other culture has been found to be promising since their performance were not superior as compared to the uninoculated control.

Balasundaran and Ali (1987) in their study on nodulation characteristics of different isolates/commercial cultures on *Leucaena leucocephala* reported that three local *Rhizobium* isolates (NSC, ND_1, and CDS) and three exotic isolates (RCR-3817, TAL-582 and RCR-3878) were found to be promising for seed inoculation. In the present study on rosewood one exotic culture namely TAL-169 and one local isolate DTR_1 were found to be promising.

According to Turk and Keyser (1992), some species of nitrogen fixing trees are very specific in their requirements for rhizobial strains. *Rhizobium fredii* USDA-257 is a Gram -ve soil bacterium from China that fixes nitrogen in symbioses with primitive soybean (*Glycine max*) cultivars such as Peking, but not with advanced cultivars such as Mc Call (Krishnan and Pueppke, 1994). The specific strain of Rhizobia was found to give maximum nodules in many a case.

In the present trial rosewood treated with TAL-169 (a specific strain of rhizobia for *Dalbergia* species from Niftal), GR-0 (specific strain from agroforester tropical seeds) and the three isolates from the tree gave higher nodulation than NBL and Abtech cultures, which were common

biofertilizers for soybean, cowpea etc. This indicates that rosewood is specific in its requirement of rhizobial strains.

4.2.2 Venga (*Pterocarpus marsupium* Roxb.)

The treatment with the five isolates and the four commercial cultures were not found to influence nodulation and growth characteristics of the plant (Table 4a and Table 4b). Nodulation was very poor in all the treatments. The reason for this poor nodulation was not clear. This may be due to the delayed nodulation observed in certain tree species. In *Pentaclethra macrophylla*, a multipurpose tree of the humid lowlands of West Africa, Ladipo *et al.* (1993) reported that nodulation occurred only after six months in a glass house experiment. Similar to this Venga may nodulate at its full potential after some specified period. So further long term investigations are needed to confirm inoculation response of *Pterocarpus marsupium*.

4.2.3 Mullumurukku (*Erythrina stricta* Roxb.)

Inoculation with the local isolates and commercial cultures of *Rhizobium* influenced the number of nodules, number of effective nodules, weight of nodules, height of plant, root length and dry weight of the plants (Table 5a and Table 5b).

Table 4a Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on nodulation of Venga (*Pterocarpus marsupium*)

Isolate/ culture	Number of nodules per plant	Number of effective nodules per plant	Fresh weight of nodules (mg/plant)
PTR1	1.4	0.6	9.6
PTR2	3.4	2.0	15.0
PTR3	3.6	2.4	11.3
PTR4	8.0	4.4	28.3
PTR5	5.5	3.5	18.4
TAL-990	5.0	1.3	26.3
NBL	3.2	2.4	6.5
GR-PTER	8.0	2.2	3.6
Abtech	5.2	2.2	6.4
Control	4.3	0.0	4.0
SEM(±)	1.4	1.1	5.4
CD (0.05)	NS	NS	NS

Table 4b Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on growth characteristics of Venga (*Pterocarpus marsupium*)

Isolate/ culture	Plant height (cm)	Root length (cm)	Girth (mm)	No. of leaves per plant	Dry weight (g/plant)	Nitrogen content (%)	
						Plant	Soil
PTR1	18.5	28.4	5.3	7.0	0.41	1.44	0.020
PTR2	17.1	22.3	5.0	7.2	0.50	1.93	0.020
PTR3	23.0	17.4	6.3	8.0	0.46	1.60	0.018
PTR4	15.7	23.8	6.2	7.2	0.78	1.35	0.020
PTR5	18.6	17.8	5.5	7.8	0.55	2.01	0.020
TAL-990	18.9	22.3	4.8	8.3	0.72	1.56	0.020
NBL	17.7	21.0	5.6	7.6	0.68	1.77	0.013
GR-PTER	18.1	30.5	5.7	8.8	0.81	1.60	0.020
Abtech	20.8	26.0	6.9	9.2	0.89	1.93	0.018
Control	21.4	21.5	5.9	6.8	0.73	1.67	0.015
SEM(±)	1.7	3.6	0.7	0.7	0.14	0.14	0.00
CD (0.05)	NS	NS	NS	NS	NS	NS	NS

Table 5a Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on nodulation of Murukku (*Erythrina stricta*)

Isolate/ culture	Number of nodules per plant	Number of effective nodules per plant	Fresh weight of nodules (mg/plant)
ETR1	21.2	20.2	372.8
ETR2	18.6	13.0	182.2
ETR3	14.8	11.8	315.8
ETR4	14.0	11.0	233.4
ETR5	16.8	10.6	282.5
TAL-749	24.0	21.0	429.8
NBL	15.8	10.6	212.8
GR-H	15.6	12.4	363.3
Abtech	12.0	8.0	261.3
Control	16.5	9.8	305.3
SEM (±)	1.96	1.5	53.1
CD (0.05)	5.6	4.3	151.9

Table 5b Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on growth characteristics of Murukku (*Erythrina stricta*)

Isolate/ culture	Plant height (cm)	Root length (cm)	Girth (mm)	No. of leaves per plant	Dry weight (g)	Nitrogen content (%)	
						Plant	Soil
ETR1	44.8	32.7	19.8	9.4	4.81	1.79	0.020
ETR2	48.5	23.3	19.4	10.0	2.70	2.27	0.013
ETR3	50.9	26.6	19.1	10.0	3.07	2.19	0.020
ETR4	49.7	19.3	20.4	7.6	3.11	1.97	0.020
ETR5	43.3	22.4	21.2	10.2	3.23	2.56	0.013
TAL-749	43.0	22.6	18.5	9.6	2.79	2.53	0.013
NBL	46.5	30.0	20.7	8.6	4.78	2.00	0.020
GR-H	50.5	27.2	20.7	9.8	4.58	2.35	0.020
Abtech	46.8	18.6	21.4	8.2	3.56	2.37	0.020
Control	64.7	21.4	21.7	8.0	4.58	2.19	0.018
SEM(±)	3.2	2.6	1.5	0.7	0.54	0.17	0.00
CD (0.05)	9.1	7.3	NS	NS	1.6	NS	NS

Inoculation with the culture, TAL-749 resulted in highest number of nodules per plant and effective nodules followed by ETR_1 and ETR_2; nevertheless these three cultures were on par with each other. When the effective nodules were considered, both TAL-749 and ETR_1 were found to be significantly superior to the rest of the treatments including control. In this case of percentage of effective nodules, control had the least value (Fig.2). Weight of nodules was highest in TAL-749 followed by ETR_1 eventhough the differences were not significantly different from the control (Table 5a).

The tallest plant was in control which was significantly superior to all other treatments. There was no significant difference in plant height due to inoculation with isolate and the cultures. For root length the highest value was that of ETR_1 and it was superior to ETR_2, TAL-749, ETR_3, ETR_4, Abtech and control. ETR_1 inoculated plants recorded highest plant dry weight, eventhough it was not significantly different from control. The various inoculation treatments neither influenced the nitrogen content of plant nor that of the soil (Table 5b).

From the results it is deduced that only two cultures, namely ETR_1 and TAL-749 can be considered promising. Though TAL-749 excelled in nodulation characteristics, its

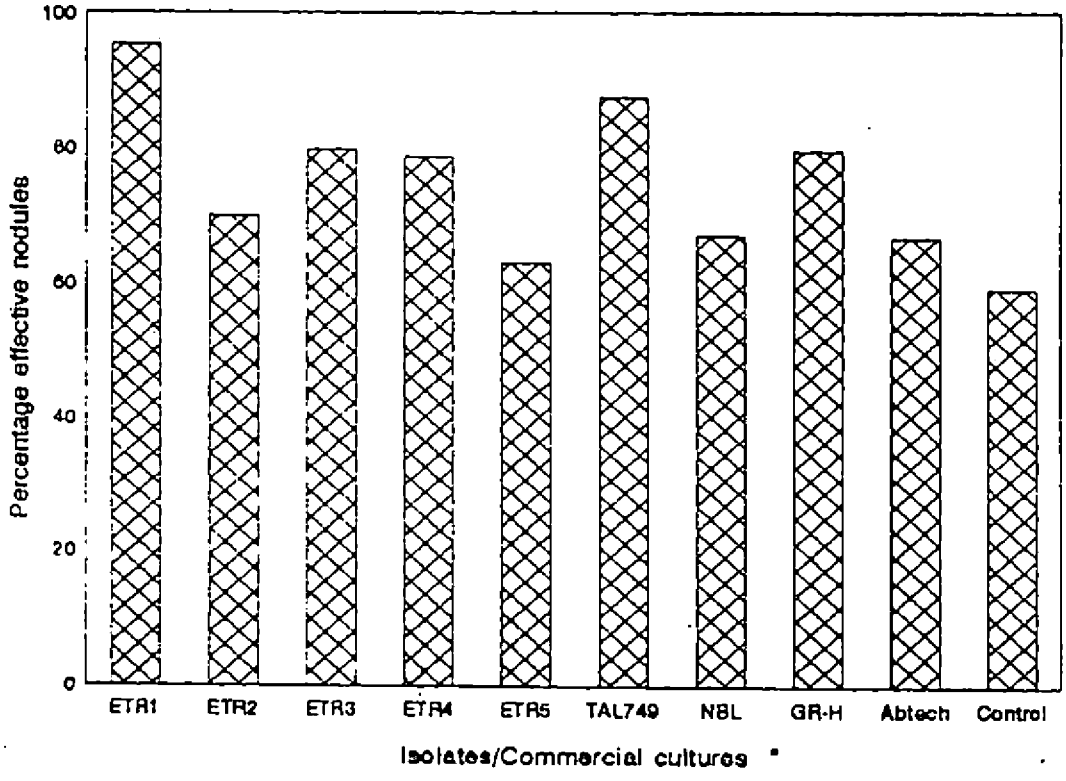


Fig.2 Percentage effective nodules in Mullumurukku(*Erythrina stricta*) as influenced by *Rhizobium* inoculation.

performance in growth aspects was dismal. This may be due to the diversion of more energy of the plant for nitrogen fixation. The metabolic cost of N assimilation via., N_2 fixation is higher than that for root uptake primarily due to the high energy requirement of the nitrogenase enzyme activity and auxiliary costs involved in maintaining and developing nodule tissue (Imsande, 1988; Lynch and Wood, 1988; Pate and Layzell, 1990). In fact, the minimum theoretical biological cost of N assimilation via., N_2 fixation has been estimated to be as much as 36 per cent greater than that for NO_3 uptake and reduction (Pate and Layzell, 1990). This additional energy requirement of symbiotic plants may then result in differences in developmental and growth rates due to the diversion of energy to fix nitrogen that might otherwise have been used for other growth processes. This could be a probable reason for high nodulation rates and reduced growth observed in *Erythrina* in response to inoculation with TAL-749.

As mentioned earlier (Sharma *et al.*, 1995) we cannot select a culture based on its performance on all growth characters. Among the cultures tested, TAL-749 performed better in nodulation characteristics (though its performance was not appreciable in other characters), and the culture ETR_1 performed above average in many of the

characters studied. So these two cultures may be selected for further field evaluation.

4.2.4 Ungu (*Pongamia pinnata* (L.) Pierre

The seed inoculation with the five isolate and four commercial cultures resulted in significant improvement for characters like number of nodules, number of effective nodules, fresh weight of nodules, root length and dry weight (Table 6a and Table 6b).

Inoculation with the isolates P_0TR_5 and P_0TR_3 showed the highest value for number of nodules. Effective nodules were also high in P_0TR_5 (41.0) followed by P_0TR_3 (32.7), however, only the former differed significantly. The control was found to have the least percentage of effective nodules (Fig.3). This indicates the incompetency of *Rhizobium* in sand to nodulate effectively. Weight of nodules was significantly superior over the rest in P_0TR_5 inoculated plants. All the other treatments were on par (Table 6a).

In the case of root length P_0TR_5 had the least value of 18.1 and was found to be inferior to control. P_0TR_1 had the highest value for this parameter and formed a homogenous grouping with P_0TR_3, Abtech, P_0TR_2, NBL, GR-H and control.

Table 6a Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on nodulation of Ungu (*Pongamia pinnata*)

Isolate/ culture	Number of nodules per plant	Number of effective nodules per plant	Fresh weight of nodules (mg/plant)
PoTR1	30.0	23.3	170.7
PoTR2	32.3	25.0	256.3
PoTR3	44.7	32.7	226.3
PoTR4	7.0	6.3	45.1
PoTR5	48.0	41.0	583.5
TAL-169	11.7	11.0	35.4
NBL	12.7	10.0	113.1
GR-H	27.7	25.3	185.5
Abtech	24.3	19.0	233.0
Control	21.7	13.0	293.5
SEM(±)	8.6	6.4	85.6
CD (0.05)	25.3	19.0	252.3

Table 6b Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on growth characteristics of Ungu (*Pongamia pinnata*)

Isolate/ culture	Plant height (cm)	Root length (cm)	Girth (mm)	No. of leaves per plant	Dry weight (g)	Nitrogen content (%)	
						Plant	Soil
PoTR1	35.4	46.7	12.3	7.0	2.77	2.56	0.020
PoTR2	55.3	41.8	16.8	10.0	4.63	2.55	0.020
PoTR3	46.5	46.5	16.7	8.0	4.19	2.18	0.026
PoTR4	29.2	23.2	9.3	6.7	1.91	3.33	0.020
PoTR5	46.0	18.1	16.2	8.7	5.48	2.41	0.020
TAL-169	26.2	26.1	10.8	5.7	1.04	3.45	0.020
NBL	27.1	35.2	10.8	6.3	1.58	2.83	0.020
GR-H	43.3	32.5	12.7	7.3	4.34	2.35	0.020
Abtech	41.7	44.8	11.9	8.3	3.16	2.49	0.020
Control	42.1	43.5	14.3	8.3	3.32	2.65	0.013
SEM(±)	7.8	5.6	1.8	1.5	0.66	0.24	0.00
CD (0.05)	NS	16.6	NS	NS	1.95	NS	NS

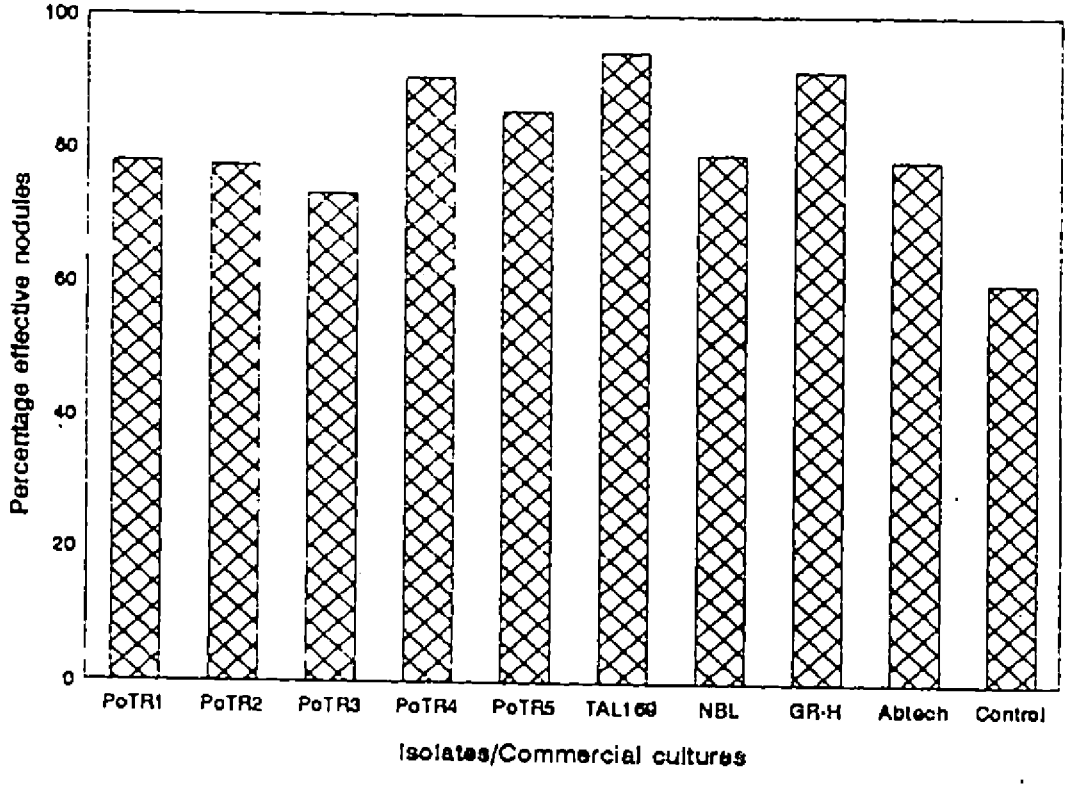


Fig.3 Percenatge effective nodules in Ungu (*Pongamia pinnata*) as influenced by *Rhizobium* inoculation

The isolate P_0TR_5 excelled in the case of dry weight also. It had the highest value of 5.5 and significantly differed from control, Abtech, P_0TR_1, P_0TR_4, NBL, and TAL-169. The various inoculation treatments did not affect the plant height, girth, number of leaves, nitrogen content of plant and that of soil (Table 6b).

A study by Basu and Kabi (1987) on *Pongamia glabra* indicated that *Rhizobium* inoculation enhanced the nodulation of this species. Sharma *et al.* (1996) in their study to select suitable strains of *Rhizobium* symbionts in acid soils for *Acacia auriculiformis* and *Pterocarpus marsupium* took the number of nodules, plant dry weight and total nitrogen content as indicators of the efficiency of the strain. They found that majority of the inoculated strains of *Rhizobium* were found to increase plant dry weight, nodule number and total nitrogen content. In the present study with *Pongamia pinnata* the same trend was observed except for the total nitrogen content in the case of P_0TR_5, P_0TR_2, P_0TR_3 and GR-H. The dismal performance of remaining cultures may be due to their inability to compete with the native rhizobia found in the sand.

In a study by Rohm and Werner (1992) on nodulation characteristics of *Robinia pseudoacacia*, it was found that the local isolate of black locust (*Robinia pseudoacacia*) 'R_1' was more promising than strains 3112, 3113 and 3115

obtained from USDA. The present study revealed that local isolate P_0TR_5 of *Pongamia pinnata* performed better than all other cultures including those from outside namely TAL-169 and GR-H.

From the results obtained it is quite evident that the isolate P_0TR_5 with an upper hand in most of the characters studied, is a promising one. However, the isolate P_0TR_3, though, did not differ significantly from control in many of the growth factors, had an edge over all others in the case of nodulation and plant dry weight. Hence these two isolates may be considered as promising ones and selected for further field evaluation.

4.2.5 Nenmeni Vaka (*Albizia lebbeck* L.)

Significant differences in number of nodules, effective nodules, height and number of leaves occurred among treatments. Inoculation did not influence other characters like fresh weight of nodule, root length, girth, dry weight, nitrogen of plant and that of soil (Table 7a and Table 7b).

Number of nodules and effective nodules were more in GR-H, NBL and ATR_2. GR-H had the highest value for both these parameters and was on par with the values of NBL and ATR_2, however, it was significantly superior to ATR_1, TAL-1536, Abtech and control. The control was the poorest

Table 7a Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on nodulation of Nenmeni Vaka (*Albizia lebbeck*)

Isolate/ culture	Number of nodules per plant	Number of effective nodules per plant	Fresh weight of nodules (mg/plant)
ATR1	22.0	19.4	141.9
ATR2	29.6	22.4	202.5
TAL-1536	20.6	19.6	231.0
NBL	30.8	27.8	176.2
GR-H	41.4	35.6	145.2
Abtech	16.0	11.0	117.5
Control	12.8	6.3	299.3
SEM(±)	4.9	4.5	43.0
CD (0.05)	14.4	13.1	NS

Table 7b Influence of inoculation with local isolates and commercial cultures of *Rhizobium* on growth characteristics of Nenmeni Vaka (*Albizia lebbeck*)

Isolate/ culture	Plant height (cm)	Root length (cm)	Girth (mm)	No. of leaves per plant	Dry weight (g)	Nitrogen content (%)	
						Plant	Soil
ATR1	29.2	40.0	7.6	10.2	2.13	2.82	0.020
ATR2	21.0	35.0	8.6	9.6	2.84	2.39	0.020
TAL-1536	28.9	37.8	8.2	11.4	2.57	2.53	0.020
NBL	28.6	31.4	7.5	12.4	2.22	2.37	0.020
GR-H	37.3	31.8	9.5	14.0	3.23	2.23	0.020
Abtech	24.0	34.4	9.0	8.8	2.63	2.42	0.020
Control	41.5	29.5	9.6	13.8	3.83	1.97	0.013
SEM(±)	3.1	3.9	0.7	1.1	0.5	0.1	0.00
CD (0.05)	9.0	NS	NS	3.1	NS	NS	NS

in number of nodules and effective nodules (Table 7a). As in the case of other species, the percentage of effective nodules in control showed the minimum value in the case of *A. lebbeck* also (Fig.4). This throws light to the fact that inoculation is needed for effective nodulation in *A. lebbeck*.

The uninoculated control was found to have the highest value for height, nevertheless, it was statistically comparable to GR-H and significantly superior to the rest of the treatments. For number of leaves again, GR-H showed its supremacy and was significantly different from ATR_1, ATR_2 and Abtech, however, it was statistically similar to control.

The dry matter yield, showed no significant difference due to the inoculation. In most of the other characters no definite trend was observed, nevertheless, the per cent nitrogen of plant was higher in inoculated ones than the uninoculated control (Table 7b).

Turk and Keyser (1992) reported that *Albizia lebbeck* can be nodulated by several strains of rhizobia. This may be true since NBL (meant for cowpea, soybean, etc.) treated plants had good nodulation in this species. Another study by Jamaluddin *et al.* (1995) on the efficacy of pure cultures of *Rhizobium* strains of different forest tree

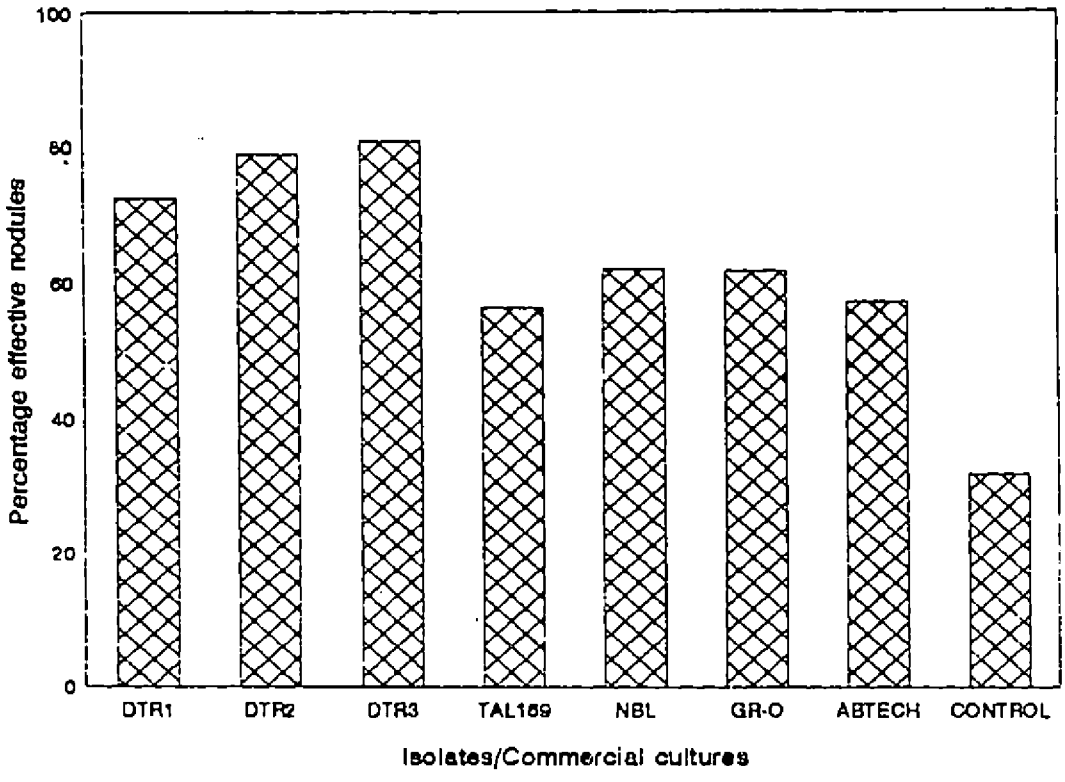


Fig.1 Percentage effective nodules in Rosewood (*Dalbergia latifolia*) as influenced by *Rhizobium* inoculation

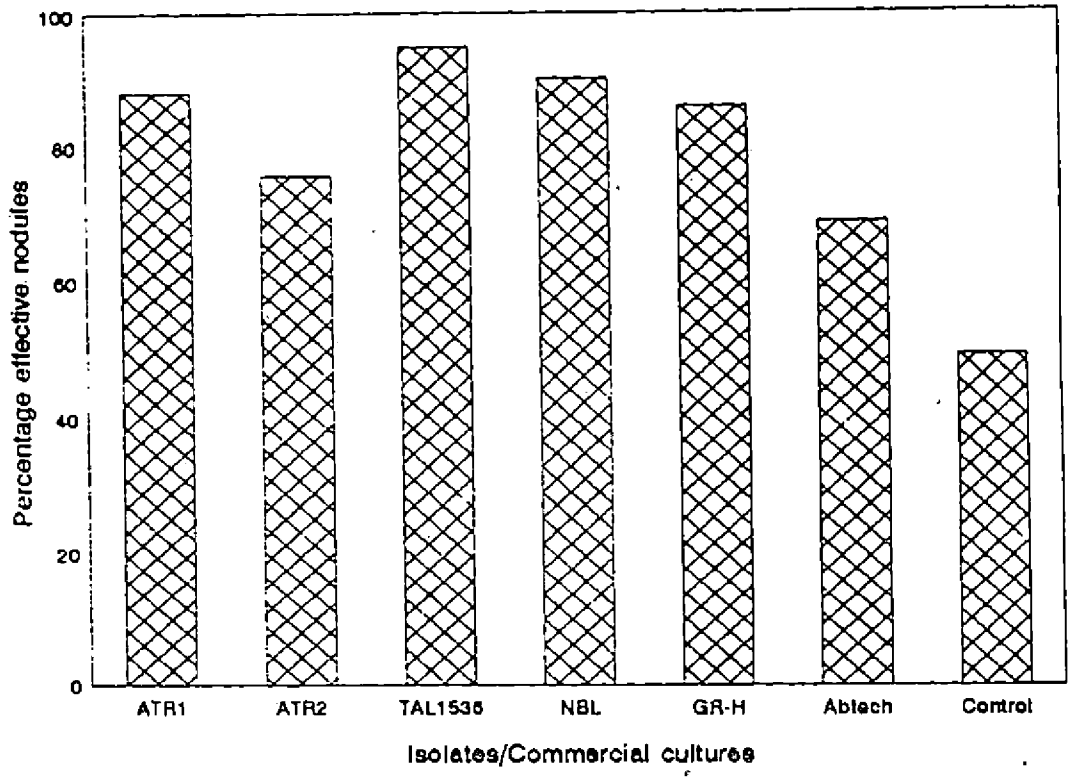


Fig.4 Percentage effective nodules in Nenmeni Vaka (*Albizia lebbeck*) as influenced by *Rhizobium* inoculation

species viz. *Dalbergia sissoo, Leucaena leucocephala, Pongamia pinnata* and *Albizia lebbeck* on the growth of *Albizia lebbeck* revealed that *Rhizobium* strain isolated from *A. lebbeck* was promising. This points to the fact that, though this species can be nodulated by different strains, when the specific strain is inoculated it performed better. In the present study the specific Rhizobia GR-H for *A. lebbeck* caused better nodulation in it. However, the local isolates ATR_1 and ATR_2 were found to be inferior in their competence. The specific rhizobia from Niftal TAL-1536 also performed dismally. This indicates that, even when the specific strain is inoculated, it must be able to compete with the native population of rhizobia and effectively nodulate the species. May be in this study the cultures ATR_1, ATR_2 and TAL-1536 failed to compete with native rhizobia present in sand.

The results indicates that only two cultures, namely GR-H and NBL can be considered as promising cultures for *A. lebbeck*. Though these two cultures didn't excel in many of the growth parameters, their better performance in nodulation characteristics makes them, probable promising cultures for future inoculation studies.

The dismal performance of GR-H in growth characters compared to control may be due to the diversion of energy for nitrogen fixation by the inoculated plants as discussed earlier. This may be a short term effect and will get nullified in due course.

The dismal performance of Abtech in nodulating *A. lebbeck*, as in the case of other four species conclusively proved its incompetence as a biofertilizer. This incompetence may be attributed to the reduced viable count of rhizobia in this biofertilizer. The serial dilution technique (Brockwell, 1963; Vincent, 1970) employed revealed that viable count was very less in this commercial biofertilizer.

4.3 Effect of inoculation with commercial cultures on the non-nodulating species

The commercial *Rhizobium* cultures TAL-1536, group `H', ABTECH and NBL applied in Tamarind (*Tamarindus indica* L.) and Manchadi (*Adenanthera pavonina* L.) failed to induce nodulation. This confirms that these species are non-nodulating type.

4.4 Effect of inoculation with soil on the selected Nitrogen Fixing Trees

Soil used as an inoculant to induce nodulation was found to be unsuccessful in all the selected

species (Table 8 to 12). In most of the treatments nodulation was absent and those in which nodulation was present, were found to be meagre.

The inability of the soils to nodulate the five species effectively can be attributed to various factors influencing biological nitrogen fixation. The presence of both nodulating and non-nodulating rhizobial strains in the rooting zone can result in infection sites being taken by non-nodulating bacteria, preventing or reducing the establishment of effective symbiosis (Lie *et al.*, 1988). This could be one probable reason for the reduced nodulation in the soil.

pH is an important factor, affecting nodulation. Low soil pH generally inhibits nitrogen fixation by reducing the development of Rhizobia, increasing the number of ineffective rhizobia or disrupting the infection process (Sprent and Sprent, 1990). In general, the critical pH for nodulation of most legumes is above 4.5 to 5.5 (Dixon and Wheeler, 1983).

The pH of the soils used in the present study ranged from 4.3-6.8 (Table 1). The soil with low pH (4.3) and high pH (6.8) have showed poor nodulation. The pH of sand

Table 8 Effect of soil used as an inoculant on Rosewood (*Dalbergia latifolia*)

Soil	Number of nodules per plant	No. of Effective nodules per plant	Fresh weight of nodule (mg/plant)
Pananchery	0.00	0.00	0.00
Madakkathara	0.60	0.00	0.55
Ollukkara	3.60	1.60	5.12
Nadathara	0.00	0.00	0.00
Puthur	0.00	0.00	0.00
Control	8.20	2.60	34.15
SEM(±)	0.98	0.65	1.19
CD (0.05)	2.86	1.892	3.46

Table 9 Effect of soil used as an inoculant on Venga (*Pterocarpus marsupium*)

Soil	Number of nodules per plant	No. of Effective nodules per plant	Fresh weight of nodule (mg/plant)
Pananchery**	0.0	0.0	0.0
Madakkathara**	1.6	0.8	16.26
Ollukkara**	0.0	0.0	0.0
Nadathara**	0.0	0.0	0.0
Puthur**	0.6	0.4	0.12
Control*	4.25	0.0	4.03
SEM5 (±)	0.74	0.29	6.27
SEM4 (±)	0.83	0.33	7.01
CD5 (0.05)	2.17	NS	NS
CD4,5 (0.05)	2.30		

** Treatments having 5 replications
* Treatments having 4 replications

Table 10 Effect of soil used as an inoculant on Murukku (*Erythrina stricta*)

Soil	Number of nodules per plant	No. of Effective nodules per plant	Fresh weight of nodule (mg/plant)
Pananchery**	0.0	0.0	0.0
Madakkathara**	0.0	0.0	0.0
Ollukkara**	0.4	0.4	4.48
Nadathara**	1.4	0.8	2.94
Puthur**	1.2	1.0	2.12
Control*	16.5	9.80	305.3
SEM5 (±)	0.84	0.58	24.48
SEM4 (±)	0.95	0.65	27.4
CD5 (0.05)	2.44	1.70	21.62
CD4,5 (0.05)	2.59	1.80	71.69

** Treatments having 5 replications
* Treatments having 4 replications

Table 11 Effect of soil used as an inoculant on Ungu (*Pongamia pinnata*)

Soil	Number of nodules per plant	No. of Effective nodules per plant	Fresh weight of nodule (mg/plant)
Pananchery**	3.0	3.0	3.5
Madakkathara**	0.7	0.7	0.4
Ollukkara*	0.0	0.0	0.0
Nadathara*	0.0	0.0	0.0
Puthur**	0.0	0.0	0.0
Control**	21.7	13.0	293.5
SEM3 (±)	3.57	2.52	33.54
SEM2 (±)	4.37	3.09	41.07
CD2 (0.05)	13.76	9.73	120.42
CD3 (0.05)	11.23	7.94	105.67
CD2,3 (0.05)	12.56	8.88	74.72

** Treatments having 3 replications
* Treatments having 2 replications

Table 12 Effect of soil used as an inoculant on Nenmeni Vaka (*Albizia lebbeck*)

Soil	Number of nodules per plant	No. of Effective nodules per plant	Fresh weight of nodule (mg/plant)
Madakkathara**	0.0	0.0	0.0
Nadathara**	2.2	4.9	5.4
Control*	12.8	3.3	299.3
SEM4 (±)	2.14	1.53	35.9
SEM5 (±)	2.39	1.72	40.1
CD5 (0.05)	6.66	NS	111.7
CD4.5 (0.05)	7.06		118.5

** Treatments having 5 replications
* Treatments having 4 replications

used in the control was 5.5, soils having pH near or above this value also showed poor nodulation. So we cannot attribute the reduced nodulation to pH.

The absence of viable rhizobial colonies in the soil may be the probable reason for the reduced nodulation. This can only be overcome by inoculating the seeds with specific rhizobia prior to sowing.

Summary

SUMMARY

Studies were conducted on the nodulation characteristics of seven indigenous/naturalized tree legumes of Thrissur district. A preliminary survey was conducted in five panchayats, selected at random, viz., Pananchery, Madakkathara, Nadathara, Puthur and Ollukkara to collect nodules of these species. During the second phase of the study, seeds of the tree legumes were inoculated with local isolates and commercial cultures of *Rhizobium*. These seeds were used to raise seedlings in polythene bags with sand in glass house. The nodulation behaviour, N content of the plant and soil, and the growth of the seedlings were observed. In a separate experiment the soil collected from the nodulating zone were also evaluated as an inoculant. Important findings from the experiments are summarised below:

Among the seven tree species investigated, five, viz., *Albizia lebbeck* L. (Nenmenivaka), *Dalbergia latifolia* Roxb. (Rosewood/Veeti), *Erythrina stricta* Roxb. (Mullumurukku), *Pongamia pinnata* (L.), Pierre (Ungu) and *Pterocarpus marsupium* Roxb. (Venga) in their natural stand in homesteads showed nodulation, whereas the other two viz., *Adenanthera pavonina* L. (Manchadi) and *Tamarindus indica* (Tamarind) did not show nodulation. Among the

species that nodulated, the nodules of largest size were found in *Erythrina stricta* and the smallest in rosewood. The shape of nodules was round in all species except *Albizia lebbeck* which was coralloid in nature.

All the species of trees investigated were encountered in all the five panchayats except for *A. lebbeck* which was encountered only in Madakkathara and Nadathara. Five *Rhizobium* isolates were obtained in the case of *E. stricta*, *P. pinnata* and *Pterocarpus marsupium*. The number of isolates obtained in the case of *D. latifolia* and *A. lebbeck* were three and two respectively as in some of the panchayats the trees were not found nodulating. In Yeast Mannitol Agar Medium all these twenty isolates showed growth within 3-4 days. This indicates that, the isolate belongs to the genus of fast growing *Rhizobium*.

The commercial culture TAL-169 (specific culture for rosewood from Niftal) and the isolate DTR_1 (isolate from Pananchery) was found to be promising in the case of rosewood. The commercial culture TAL-169 enhanced the nodulation and growth characters better than any other culture, whereas the isolate DTR_1 showed better performance only in nodulation characters. In general, the inoculated plants of rosewood showed a marginal superiority over the nitrogen content of the plant as compared to control.

The response of *Pterocarpus marsupium* to inoculation was dismal in all the characters studied. The five isolates and the four commercial cultures applied failed to nodulate *P. marsupium* effectively.

Only two cultures, ETR_1 (isolate from Pananchery) and TAL-749 (commercial culture from Niftal) were found to be effective in *Erythrina stricta*. The culture ETR_1 showed superior performance in number of nodules, effective nodules, weight of nodules, root length and plant dry weight. However the commercial culture TAL-749 showed its superiority mainly in nodulation characteristics. The performance of the remaining local isolates and commercial cultures was poor compared to control.

In *Pongamia pinnata*, the isolate from Ollukkara (P_0TR_5) showed superiority in nodulation and many of the growth characters including dry matter production. The isolate P_0TR_3 (isolate from Nadathara) was also found to increase nodulation and dry weight and hence considered as a promising culture. The performance of the remaining local isolates and commercial culture was not promising.

In *Albizia lebbeck* the inoculated plants showed better nodulation than control. The cultures, GR-H (From

Agroforester Tree Seeds) and NBL (Common commercial culture for cowpea, soybean, etc. from National Biofertilizer Development Centre) was found to be promising for *A. lebbeck*. Though, these two cultures did not excel in many of the growth parameters, their better performance in nodulation characteristics suggest the potential of these cultures, which may be further investigated.

Soil used as an inoculant to induce nodulation was found to be unsuccessful in all the selected species. In most of the treatments, nodulation was absent and in some eventhough observed, was negligible. This is an indication of absence of viable rhizobial colonies in the soil.

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GROWTH AND NODULATION CHARACTERISTICS OF SELECTED INDIGENOUS TREE LEGUMES

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ABSTRACT OF A THESIS

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ABSTRACT

An investigation was carried out to study the nodulation characteristics of seven indigenous/ naturalized tree legumes in Trichur district of Kerala State. The homesteads of five selected panchayats viz. Pananchery, Madakkathara, Nadathara, Puthur and Ollukkara of the district were surveyed to assess the natural nodulation and to collect nodules for isolating *Rhizobium*. The response of these tree legumes to inoculation with local isolates and commercial cultures of *Rhizobium* were done by seed inoculation and growing the seedlings in a glass house. in a separate experiment, the soil collected from the base of the nodulating tree were also evaluated as an inoculant. The inoculation studies were laid out in CRD with ten replications.

The study revealed that, among the seven tree species investigated, five, viz., *Albizia lebbeck* L. (Nenmeni vaka), *Dalbergia latifolia* Roxb. (Rosewood/Veeti), *Erythrina stricta* Roxb. (Mullumurukku), *Pongamia pinnata* (L.) Pierre (Ungu) and *Pterocarpus marsupium* Roxb. (Venga) were nodulating in their natural stand. In the other two viz., *Adenanthera pavonina* L. (Manchadi), *Tamarindus indica* L. (Tamarind) nodules were absent. The largest size nodules were found in the case of *E. stricta* and the smallest in *D. latifolia*. Regarding the shape, all nodulating species had round nodules except for *A. lebbeck* which had coralloid nodules. The *Rhizobium* isolates obtained from all the species showed growth in YEMA medium within 3-4 days, which indicates that, they are fast growing type.

The promising *Rhizobium* isolate/commercial cultures identified were TAL-169 and DTR1 for *D. latifolia*; TAL-749 and ETR_1 for *E. stricta;* P_0 TR_5 and $P_0 T_{1,3}$ for *P. pinnata;* GR-H and NBL for *A. lebbeck.* inoculation with the various isolates/commercial isolates showed no response in *P. marsupium.* Soil used as an inoculant to induce nodulation was found to be unsuccessful in all the selected tree legumes.