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SYMBIOSIS OF *RHIZOBIUM* AND VA MYCORRHIZA IN SUBABUL

By

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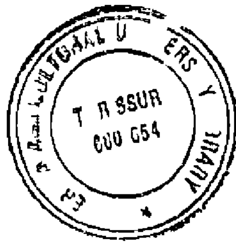

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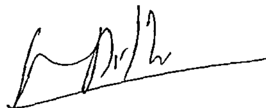

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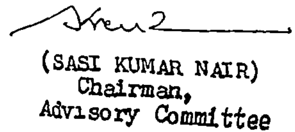

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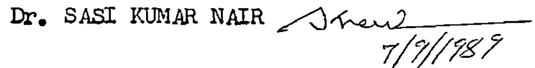

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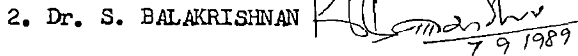

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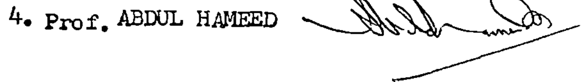

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INTRODUCTION

INTRODUCTION

In a recent article entitled 'challenges ahead - Time for new orientation', Dr. M.S. Swaminathan, eminent agricultural scientist has stressed the importance of accepting a new concept of 'Land saving Crop Husbandry and grain saving animal husbandry' for the future development of agriculture in India (The Hindu-Survey of Indian Agriculture, 1988). According to him, for an estimated live stock population of over 608 million animals in our country, about 700 million tonnes of fodder will have to be produced annually by the year 2000. Besides, 200 million tonnes of fuel wood per year will also be required. Hence, our need for fodder and fuel which will be substantial in the coming years will not be solely met by present sources or methods of production using traditional or improved plant varieties.

The situation regarding fodder and fuel requirement in Kerala is also not different from rest of the country. One of the major constraints in taking up large scale fodder cultivation in the State is the non availability of land exclusively for this purpose. Districts like Idukki, Calicut and Cannanore have comparatively larger grazing area but in other places, chances for cultivating fodder as a pure crop are very much limited.

The wide spread destruction of forest vegetation either for human habitation or for monoculture of trees for industrial purpose has led to the shortage of natural fuel. What we need today is a quick growing and efficient biomass producing plant. Subabul (Leucaena leucocephalla) appears to be a suitable crop for this purpose. In India, this plant is mainly popularised by Bharathiya Agro Industries Foundation, Pune and in Kerala by the Gandhi Smarak Nidhi, Trivandrum. It is a versatile tropical leguminous tree that can be used both as a protein rich fodder and a perennial source of natural fuel. Originally the plant is a native of Central America and later it spread to other countries.

Subabul belongs to family Leguminosae and sub family Mimosoideae. Its root system is fastgrowing and capable of penetrating even deeper layers of soil. However, for establishing this crop in acid soils, a condition prevailing in most part of Kerala, liming and inoculation with appropriate Rhizobium culture are often necessary. The use of VA mycorrhiza is also found beneficial in such soil because of the generally P fixing and P deficient nature of acidic soil and the lack of adequate root hairs for efficient absorption of nutrients in subabul (Munns and Mosse, 1980). But the development of a suitable strain of Rhizobium for this crop and a study on the nature and benefit of mycorrhizal

association are yet to be done in Kerala. It is therefore, with these objectives, the present investigation on 'Symbiosis by *Rhizobium* and VA mycorrhiza in subabul' was carried out at Kerala Agricultural University with following technical programme.

1. Survey for the occurrence of natural nodulation and VA mycorrhizal infection in subabul in four districts namely Idukki, Palghat, Trichur and Trivandrum.

2. Isolation, characterisation and screening of native *Rhizobium* and VA mycorrhiza for efficiency under pot culture conditions.

3. Fine structure studies of root nodule and VA mycorrhizal infection in subabul.

4. Development of a suitable technology for dual inoculation with *Rhizobium* and VA mycorrhiza for large scale propogation.

5. Study the combined effects of *Rhizobium*, VA mycorrhiza and fertilizer application on growth and forage yield under field conditions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Subabul (*Leucaena leucocephala* (Lam. de Wit) grows mainly in tropical and sub tropical countries upto an elevation of about 500m. It is capable of withstanding large variations in rainfall, temperature, wind and drought. However, maximum growth is obtained in deep and fertile soils with an annual rainfall of 600 to 1700 mm (Farinas, 1951). The plant is characterised by a well developed and deep tap root system capable of even breaking impervious layers of soil in the sub horizons (Gray, 1968). Neutral to slightly alkaline soils are generally considered as best suited for growth (National Academy of Sciences, 1977).

Rhizobium inoculation is often essential for an efficient symbiotic fixation of nitrogen by legumes especially in soils where efficient native *Rhizobium* strains are either absent or present only in low numbers (Alexander, 1961 and Date, 1970). Chhonkar and Negi (1971) evaluated the response of soybean to inoculation with different strains of *Rhizobium* in rhizobia free soil and discovered that seed inoculation increased ~~increased~~ the dry weight as well as the yield of plant. Sabu and Behera (1972) found that the application of *Rhizobium* to groundnut increased the grain yield as well as the nitrogen content of shoot, root and grains.

Bajpai et al. (1974) reported a 21 per cent increase in the yield of unshelled groundnut due to Rhizobium inoculation. Tripathi et al. (1975) found that Rhizobium inoculation alone increased the dry weight and yield in bengal gram by 18 and 25 per cent respectively. Rai et al. (1977) has also reported an yield increase in bengal gram due to Rhizobium inoculation. However, they did not get any correlation between yield and b number of nodules formed and the dry weight of nodules. Ramachandran(1979) screened 12 rhizobial strains for nodulation efficiency in cowpea and found that strain S10 and S17 were more effective than others. Nodule number, nodule dry weight and fresh and dry weights of shoot were significantly increased with these strains.

Santhanakrishnan et al. (1980) studied the relative performance of 11 strains of rhizobia from Leucaena leucocephala and found that the number of nodules produced by different strains varied considerably. Trinnick (1980) reported the fast growing nature of subabul Rhizobium and found that it could cross inoculate with other plant species that have fast growing rhizobia like Mimosa invisia, M. pudica, Acacia farnesiana, Sesbania grandiflora and Calliandra callothyrsus. Similar results

were also reported by Halliday and Somasegaran (1983). However, Basavaraju and Hegde (1983) reported that subabul rhizobia, were ineffective for seratro, leucerne, soybean, common bean, pea, groundnut and cowpea.

Moreno - Duiros et al. (1983) tested 24 rhizobial strains against subabul in Mexico. Among them, nine strains were found effective. Rest were found to be ineffective and few strains tested did not even form root nodules. Pahwa (1987) compared the relative efficiency of three rhizobial strains alone and in combination in subabul variety 28 and found that on 60 day, single strain inoculation increased the forage yield from 6 to 21 per cent while the multiple strain inoculation enhanced the yield by 24 to 37 per cent. Nodulation and fresh and dry matter yield were also higher in the above treatment.

Pathak and Patil (1980) reported a forage yield of 1.94 kg/tree from cunningham variety of subabul planted in single row with a spacing of 2m x 0.75m after four years of plant growth while the variety K8 planted with a density of 5000 trees/ha yielded 7.5 tonnes/ha after one and a half years of planting. The leaf forage usually contain 89.4 per cent dry matter, 24.2 per cent crude

protein, 13.3 per cent crude fibre, 10.8 per cent ash, 1.98 per cent calcium, 0.27 per cent phosphorus and 19.7 per cent digestible protein (International consultation on ipil-ipil research, 1976).

Role of VA mycorrhiza in host nutrition

VA mycorrhiza occur in many diverse ecological conditions such as sand dunes (Khan, 1974) Sutton and Sheppard, (1976), coal mines (Khan, 1978) and aquatic environments (Sondergaird and Laegard, 1977). The presence of this beneficial fungal association in a vide variety of cultivated crops and trees of Kerala has been reported by Potty (1978) in tuber crops, Sivaprasad *et al*. (1983) in cocoa, Sulochana and Nair, (1985) in cassava, Girija and Nair (1985) in many vegetables Girija and Nair (1988) in banana varities and Nair and Girija (1988) in many tree crops.

It is now well established that the uptake of phosphorus by host plant is greatly favoured by VA mycorrhizal infection. The uptake of phosphorus and other nutrient elements by VAM infected plants were first studied by Mosse (1957). She reported that mycorrhizal apple absorbed more P,K, Fe and Cu than non mycorrhizal plants. Work done by Baylis (1959) confirmed the above observations. In a study, Gray and Gerdemann (1969)

mycorrhiza in natural ecosystem were not regularly correlated with the abundance of VAM root infections. A positive correlation between spore number in soil and mycorrhizal development and between the extent of root infection and increased plant growth in maize was reported by Khan (1972).

Gray and Gerdemann (1973) studied the effect of VA mycorrhiza on S uptake and observed an increased uptake of S^{35} by mycorrhizal red clover and maize. In a comparative yield evaluation study of mycorrhizal soybean with non mycorrhizal soybean by Ross and Gilliam (1973) in P deficient soil fertilised with various P sources, the mycorrhizal plants recorded increased yield of the order of 79, 53 and 53 per cent when both the plants were fertilized with Al, Fe or rock phosphate respectively. More absorption of Zn and P from fumigated soil was noticed by La Rue et al. (1975) in mycorrhizal peach. Koucheki and Read (1976) reported greater accumulation of phosphorus in plants grown in irradiated soils with mycorrhizal association.

Sanni (1976) got a positive correlation between VA mycorrhizal infection and the amount of phosphorus and nitrogen in the tissues of cowpea, tomato and maize. A

higher percent of nitrogen and phosphorus in mycorrhizal *Medicago sativa* was also noticed by Smith and Daft (1977) at the time of harvest. Barrow *et al*. (1977) tested the ability of VA mycorrhiza in helping the plants to take up fixed soil phosphate using an *in vitro* technique. They found that inoculation with VAM endophytes more than doubled the availability of phosphate but the improvement was not more for firmly held phosphate when compared to freshly added phosphate. Powell and Jeannette (1978) demonstrated that naura rock phosphate was available to mycorrhizal clover and rye grass grown in sterlized soil but unavailable to nonmycorrhizal plants.

Increased concentration of P and Zn in mycorrhiza inoculated cowpea, cotton and finger millet was also reported by Bagyaraj and Manjunath (1980). However, the Mn content of these plants did not show any significant difference. Yost and Fox (1982) showed that cowpea and soybean grown on non-fumigated soils with soil P levels below 0.025 to 0.05 mg/lit contained higher K and Ca percent than plants grown in fumigated soil. A comparison was made by Jensen (1982) on the effectiveness of four mycorrhizal fungi on nutrient uptake by barley. Plants inoculated with *Glomus constrictum* and *G. fasciculatum*

showed an increased total uptake of P, Cu and Zn. In a study on drought tolerance of douglas fir seedlings, Parke et al. (1983) and Safir and Nelsen (1985) showed that water uptake was greatly increased in mycorrhizal plants and the plant's physiology was changed to reduce the stress due to soil drought. Meyer and Linderman (1986) reported an increase in VAM colonisation in subterranean clover as a result of dual inoculation with VAM fungus and a bacterium Pseudomonas putida.

Baltruschat (1987) applied expanded clay particles containing spores of Glomus etunicatum directly into or alongside the seed row at the time of sowing maize seeds with or without P fertilisation. He found that application of VAM inoculum directly into seed row with P fertilisation promoted better mycorrhizal infection and enhanced biomass production at 3-5 leaf stage. Hall (1987) stressed the importance of VA mycorrhizal inoculation in replacing costly fertilizer application to pastures. Smith (1988) reported that with few exceptions, the primary effects of mycorrhizal symbiosis on host pathogen relationship was intimately related to P nutrition. The multifold beneficial effects of VA mycorrhiza in red soil which is generally deficient in phosphorus has been recently stressed by Bagyaraj (1989).

pH tolerance of Rhizobium

Albrecht (1933) noted that nodulation in soybean failed at pH values of 5.0 and less. Rajagopalan (1938) in his study on the root nodule bacteria of groundnut, found that out of the six rhizobial isolates tested, two showed good growth between pH 4.0 and 9.0 while others showed growth only between pH 5.5 and 8.5. Muthusamy (1970) determined the pH tolerance of four isolates of *Rhizobium* of groundnut in YEM broth with varying pH ranging from 4.0 to 10.0. Among the four isolates, only one isolate showed good growth between pH 4.0 to pH 10.0. All other isolates were able to grow only between pH 4.5 to 10.0. Van Schreven (1972) studied the relation between tolerance to low pH and symbiotic effectiveness of *Rhizobium trifolii*. Three *R. trifolii* strains were repeatedly subcultured on agar medium of pH values ranging from 3.7 to 7.0. The symbiotic effectiveness of the isolates after frequent subculture on these pH was estimated by inoculation of asceptic seedlings of white clover in Jensen's medium. He found that the number of nodules formed, and the effectiveness of some strains seemed to be affected by growing, *Rhizobium* for a long period at low pH.

Yadav and Vyas (1973) found that the optimum pH for growth of groundnut *Rhizobium* was in the range of 5.0 to 7.0. None of the *Rhizobium* strains were inhibited at pH 10.0 while low pH of 3.0 and 4.0 were lethel for all the strains tested. A study involving five tropical legumes, five temperate legumes and *Medicago sativa* by Andrew (1976) revealed that nodulation and growth were strongly controlled by pH. *Macroptilium lathyroides* and *Lotononis bainesii* showed 100 percent nodulation at pH 4.0 where as *Medicago sativa*, *M. trunculata*, *M. scutellata* and *Glycine wightii* achieved this level of nodulation only at pH 6.0 in the presence of high amount of calcium. In general, the legumes exhibited a marked decline in the level of nodulation below pH 5.0.

Pandher and Kahlon (1978) reported an adverse effect of low pH on growth of *Rhizobium leguminosarum* from pea (*Pisum sativum* L). None of the strains tested showed any growth at pH 3.0. Good growth of the strains was noticed only between pH 6.5 to 8.0. Evans *et al.* (1980) conducted experiments to measure the effect of pH of the nutrient medium on symbiotic nitrogen fixation by *Rhizobium leguminosarum* and *Pisum sativum*. Both the bacterial growth and root development were adequate at all pH levels from 4.4 to 6.6. But efficient nodulation and nitrogen fixation did not occur at pH 4.8 and

below. Barber (1980) tested the growth of Rhizobium meliloti in media adjusted to pH values ranging from 4.5 to 6.5 and observed that the number of bacterial cells were greater with increase in pH of the nutrient medium. In an in vitro study on the effect of different levels of pH on the growth of various isolates of cowpea rhizobia from Kerala, Nair and Sivaprasad (1981) found that native isolates were more tolerant to low pH of 4.5 when compared to exotic isolates of rhizobia. Ahmad and Ng (1981) found that the critical pH level for the establishment of subabul was between 4.45 and 4.7 below which the species did not establish satisfactorly. Further they reported a growth variation in two year old subabul plants due to changes in soil pH and Rhizobium population. Plants were 'Good' at pH 4.85 with a rhizobial population of 5.8×10^2 and 'Bad' at pH 4.4 with a rhizobial population of 2.4×10^2.

Halliday (1981) made an important observation that unlike many tropical legumes, the primary reasons for the poor adaptation of subabul in acidic soil was its inability to tolerate such soil conditions. He further

stressed the importance to select base exuding rhizobia as a key to alleviate the acid soil stress on subabul-Rhizobium symbiosis. His experiments revealed that most Leucaena rhizobia were unable to multiply at acid pH eventhough some strains grew well at a wide pH range of 4.5 to 8.5. However, such strains usually constituted only less than 5 percent of the total isolates of Rhizobium from subabul.

Hu et al. (1981) studied the growth and nutrient levels of subabul in response to lime and phosphorus application in soils of pH 4.7, 6, 7 and 8. They found that the growth of subabul and nutrient uptake were increased with increasing soil pH upto 8. Dutt and Urmila Pathania (1983) added gypsum and sulphur to an alkaline soil of pH 8.4 at the rate of 5 g and 10 g per Kg of soil to reduce the pH. They reported that use of gypsum and sulphur to lower the soil pH increased nodulation and growth of subabul. The combined effects of liming and rhizobial inoculation in subabul was also tested by Young et al. (1983). They used Rhizobium strains TAL 82, TAL 1145 and TAL 582 and applied lime at the rate of 5, 10 and 15 kg/ha which increased the soil pH from 5.46 to 7.12, 7.14 and 7.33 respectively. It was found that liming considerably increased nodule weight and plant dry weight after 110 days. De la Garza

et al. (1987) evaluated the effect of Rhizobium strains, phosphorus and soil type on nodulation and growth of subabul. They found that it was advantageous to grow subabul with effective rhizobial strains under adequate phosphorus fertilisation but without added nitrogen in low and medium fertile soils.

Serological characterisation of Rhizobium

Zipfel (1912), first studied the serology of root nodule bacteria. He reported a relation between the Rhizobium of Pisum sativum and Phaseolus vulgaris. Later Klimmer and Kruger (1914) divided 18 cultures from different legums into nine serogroups using agglutination test and concluded that rhizobia isolated from different species of plants were serologically distinct but related strains. Stevens (1923) made a significant observation on 'Serogroups' within a species. Even more important was his finding that nodules from a single plant could contain rhizobia belonging to distinct serological groups which may be related or unrelated to each other. Bushnell and Sarles (1939) reported three distinct components of antigenic complex, first in heat washed cells, second with flagella and third with cell surface and or its associated polysaccharides. Vincent (1941) was first to make use of this information in grouping rhizobial isolates.

Koontz and Faber (1961) studied the somatic antigens of 23 strains of Rhizobium japonicum and classified them into six groups. Johnson and Means (1964) serotyped large number of nodules obtained from soybean plants grown in different soils from Iowa, Maryland, Mississipi and South Carolina. The results indicated that the kind and abundance of serogroups of Rhizobium japonicum in nodules differed in each of the four states.

Dudman (1964) employed the gel immuno-diffusion technique for rhizobial strain identification. He suggested that several antigens could be recognised simultaneously by this technique according to the position of precipitated bands. Employing a rapid agglutination test, Damirgi et al. (1967) distinguished several sero-groups of Rhizobium japonicum by using homogenised nodule suspension as antigens. Vincent and Humphrey (1970) found two internal antigens common to eighteen Rhizobium leguminosarum strains. However, they could not detect the same antigens in nine slow-growing cultures of Rhizobium. Charudattan and Hubbel (1973) using agar-gel double diffusion technique compared soluble antigents of three Rhizobium species with those of eight legumes representing compatible and non compatible hosts. Cross reactive antigens were found between all the legume hosts and three rhizobia.

Bohlool and Schmidt (1973) while studying the persistence and competition of a strain of Rhizobium japonicum in three non sterile soils using inmunoflo-uresent technique reported that competition of the added R. japonicum with indigenous soybean rhizobia depended on the number of rhizobia in the inoculum. Skrdleta (1973) determined the relationship between soybean cultivars and R. japonicum serotypes in single and multistrain inoculants and found that strain 311 B in the greater proportion of serotyped nodules out of all the nodules in soybean cultivars inoculated with the multistrain inoculum. Dadarwal et al (1976) found that rhizobia isolated from different varieties of chick pea (Cicer arietinum) grown in a locality were antigenically distinct. However, strain specific precipitation reactions were observed only with cicer rhizobia.

Brockwell et al. (1977) compared the immunogel-diffusion technique and the use of streptomycin resistant mutants for studying the competition and persistence of introduced Rhizobium over native rhizobia in soil and concluded that both the methods were equally sensitive for studying the competition of root nodule bacteria. Kremer and Wagner (1978) advocated a new

approach to the study of soil rhizobia by the use of direct diffusion of antigens from soil and development of specific bands. Using the technique of immuno-diffusion, they evaluated four strains of <u>Rhizobium japonicum</u> incubated in soil. Sheth (1979) investigated the antigenic relationship of inoculated strain CB 756 of rhizobia for groundnut with native rhizobia and revealed that the inoculated strains differed from the native strains. Trinnick (1980) studied the relationship among fast growing rhizobia from five plants genera and their affinities with other rhizobial groups by agglutination test. The test showed that isolates from each host shared antigens with one or more of the five <u>Rhizobium</u> strains of subabul.

<u>Fine structure studies of root nodules</u>

Bergersen and Briggs (1958) in a study on the fine structure of soybean root nodules reported that four to five bacteroids of <u>Rhizobium japonicum</u> were enclosed within the membrane of host cell origin. However, Dart and Marcer, (1963) could observe only one bacteroid in each of the bacteroidal membrane envelope of clover root nodules. Goodchild and Bergersen (1966) noticed more than one bacteroid per envelope. As the bacteria developed in the cytoplasmic membrane system, there was a loss of

fibrillar material from the nucleoid region and changes occurred in the distribution of ribosome like particles in both host and bacterial cells. When bacteroid was fully differentiated and presumbaly fixing nitrogen, the bacteriods formed the red zone of subterranean clover nodule and showed a well developed intra-cytoplasmic membrane system. Bergersen (1974) opined that almost in all legumes, the extent of nitrogen fixation was related to the amount and persistence of bacteroid containing tissue in the root nodule.

Dual inoculation studies with Rhizobium and VA mycorrhiza in legumes.

The presence of VA mycorrhiza in legumes was first reported by Jones (1924). Asai (1944) stated that nodulation in various legumes inoculated with appropriate *Rhizobium* and grown in sterilised soil depended on the addition of an inoculum of 5-10 g of unsterilized garden soil which favoured the formation of VA mycorrhiza in experimental plants.

Schenek and Hinson (1973) got increased growth of nodulating soybean line by 53 per cent by VAM inoculation. However, VA mycorrhiza had no effect on non nodulating

lines. Crush (1974) reported stimulated nodulation and growth of Centrosema pubscens, stylosanthes guyanensis, Trifolium repens and Lotus pedunculatus when VA mycorrhiza was inoculated along with Rhizobium. Daft and Giahni (1974) compared dual inoculation of Phaseolus with Endogone and Rhizobium and also with Rhizobium alone. The results revealed that dual inoculation increased the growth, number and weight of nodules, acetylene reduction, leghaemoghbin, phosphorus and total protein content.

Mosse et al. (1976) reported that added rock phosphate greatly improved nodulation and nitrogen fixation of mycorrhiza plants. A four fold increase of yield in barley to VA mycorrhizal inoculation was observed by Saif and Khan (1977). However, this difference between mycorrhizal and non mycorrhizal plants was almost eliminated by increased P application. Smith and Daft (1977) stressed the importance of time factor in the development of tripartite symbiosis between Medicago sativa, Rhizobium and mycorrhizal fungi. They could get both nodulation and mycorrhizal infection within two weeks of inoculation with appropriate endophytes. Mycorrhizal plants showed extensive nodulation and higher rates of nitrogenase activity from second week

In P deficient unsterile soils, Manjunath and Bagyaraj (1984) found that inoculation of Leucaena leucocephala with Glomus fasciculatus greatly improved nodulation by native Rhizobium and inoculation with Rhizobium alone improved colonisation of roots by native mycorrhizal fungi. Moreover, dual inoculation improved nodulation, mycorrhizal colonisation, dry weight, nitrogen and P content of plants when compared to single inoculation with either of the symbionts.

Mycorrhizal occurrence and their effect on certain forest plant species was studied by Kandasamy et al. (1988) Leucaena leucocephala and Acacia mellifera were inoculated with nodule bacteria and Glomus fasciculatus alone and in combination with Rhizobium. VA mycorrhizal inoculation gave 1.5 and 12.6 per cent increase in dry weight of subabul. Dual inoculation also increased VA mycorrhizal infection on 45th day.

Mimosine content in subabul

Mimosine the β - N (3-hydroxy-4-pyridone) - α - amino propionic acid was first reported from Mimosa pudica L. as mimosine and later from Leucaena leucocephala as as leucaenol. It is now well established that subabul leaves contain 3 to 5 per cent mimosine, which is toxic to animals. Rosas and Quintero (1980) reported that ensiling subabul leaf considerably reduced the mimosine

content. The rate of reduction was about 48.4 per cent in 3 weeks. They further reported that this reduction in mimosine content by ensiling might be due to some biochemical changes produced by intense fermentation during the ensiling process.

Brewbaker *et al.* (1981) laid down the procedure for mimosine estimation and tested the same in ten species of subabul. He found that large leaflet species had more mimosine content than small leaflet species. Joshi *et al.* (1983) worked out the positional effect of leaves on mimosine content and reported that the mimosine concentration was maximum in top leaves with 2.95 per cent followed by middle leaves with 0.95 per cent and bottom leaves with only 0.2 per cent. Krishnamurthy and Mune Gowda (1983) also conducted similar studies and found that maximum mimosine concentration was in growing tips. The lowest mimosine concentration was in young tender bud and stem.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study on 'Symbiosis of Rhizobium and VA mycorrhiza in subabul' was conducted during 1984-88 at College of Agriculture, Vellayani, Trivandrum and College of Horticulture, Vellanikkara, Trichur of the Kerala Agricultural University. The different methods adopted for this investigation are given below.

1. Survey for the occurrence of natural nodulation and VA mycorrhizal infection in subabul

A preliminary survey was conducted to find out the extent of natural nodulation and VA mycorrhizal infection in subabul (*Leucaena leucocephala* (lam. de Wit) in four districts of Kerala namely Idukki, Palghat, Trichur and Trivandrum. The districts were so selected as to have a reasonable representation of the variations in soil type and topography prevailing in the state (Table - 1).

Table - 1 General topography and soil characteristics of different districts selected for preliminary survey.

Districts	Topography	Soil pH	Soil type
Idukki	High ranges	Acidic	Forest soil
Palghat	Plains and hill tracts	Acidic to alkaline	Laterite and black soil
Trichur	Plains and hill tracts	Acidic	Laterite and sandy soil
Trivandrum	Plains and hill tracts	Acidic	Laterite and red loam

Four to five different locations in each district (Fig.1) where subabul was found to grow either naturally or cultivated on a regular basis by farmers or institutions were selected for this study on natural nodulation and VA mycorrhizal infection. Wherever possible, at each of these locations, the name of the cultivar grown was also recorded.

1.1 Nodulation status

The extent of natural nodulation in 45-60 day old seedlings near grown up subabul plants was recorded after carefully digging out each seedling without injuring its root system. They were initially washed in water to remove all adhering soil particles and then the number of nodules formed were recorded. A minimum number of five seedlings from three different places at each location were observed for this purpose. The relative number of healthy and efficient nodules formed in each seedling was determined by visual observation of each nodule for the presence of leghaemoglobin. Wherever necessary, such nodules were cut open to find out the presence of this haeme pigment within the nodular tissue. Five hundred gram of rhizosphere soil along with the seedlings examined for nodulation status at each location were also collected and brought to laboratory for further studies.

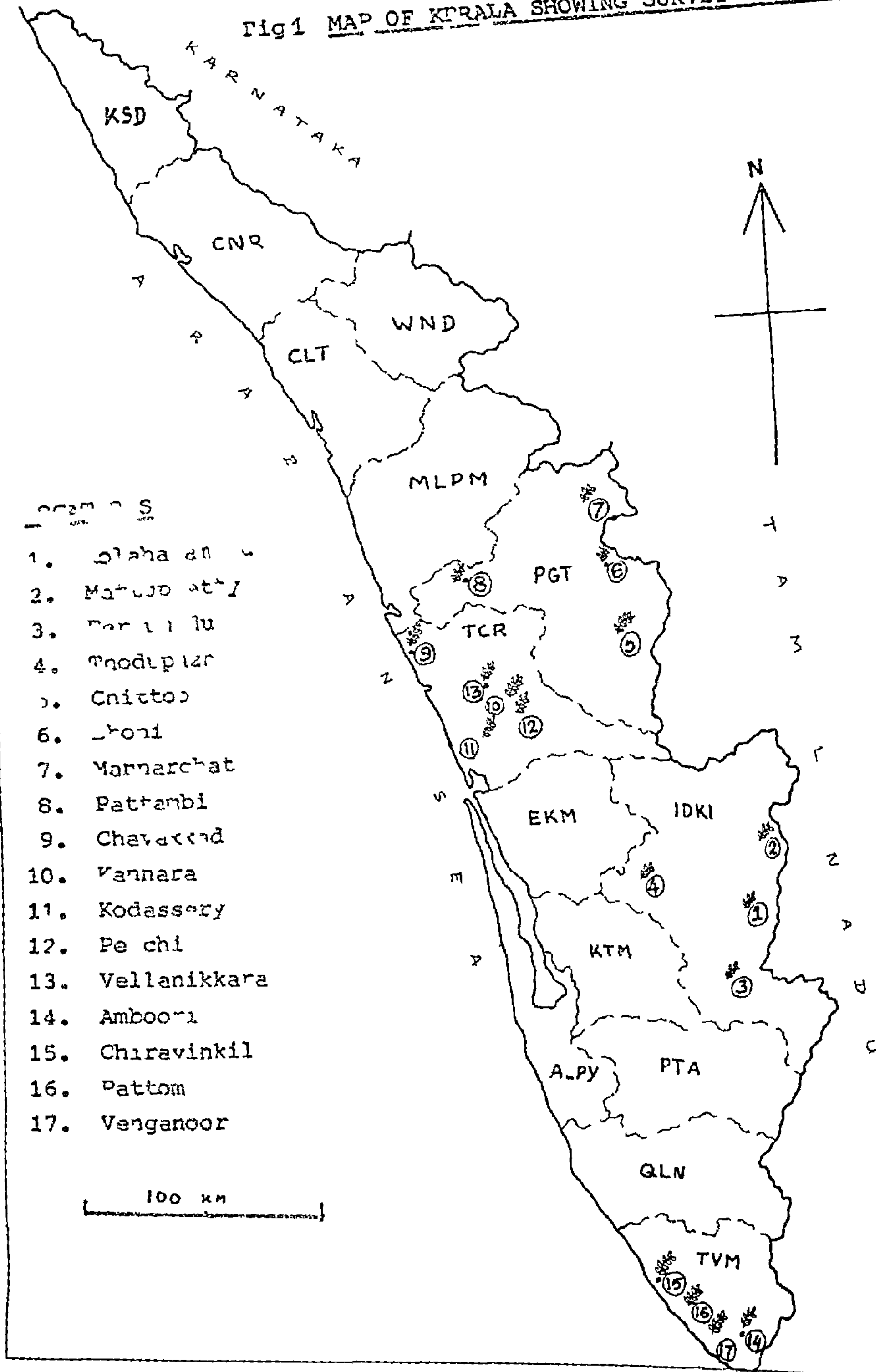

Fig 1 MAP OF KERALA SHOWING SURVEY LOCATIONS

1.2 Isolation of *Rhizobium*

Healthy pink coloured nodules were selected for the isolation of *Rhizobium* from each location by the method of Vincent (1970) on yeast extract mannitol agar medium of following composition.

Yeast extract mannitol agar (YEMA) (Allen, 1953)

Mannitol	10.0 g
$K_2H\ PO_4$	0.5 g
$Mg\ SO_4 \cdot 7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Yeast extract	1.0 g
Congo red	2.5 ml (1% aqueous solution)
Agar	15.0 g
Distilled water	1000.0 ml
pH	7.0

The nodules were initially transferred to clean test tubes and washed with water to remove all adhering soil particles. They were then surface sterilised with 70% ethanol for 30 seconds followed by 0.1% $HgCl_2$ solution for two minutes and washed thoroughly in five changes of sterilised tap water. The surface sterilised nodules were then crushed in few drops of sterilised water.

The residual milky suspension was streaked on YEMA medium in petri dishes and incubated at 28 ± 2°C in a B.O.D incubator for four days. Individual colonies showing the typical characteristics of *Rhizobium* were then selected, checked for purity by repeated streaking on YEMA and by Gram staining. Such cultures were transferred to sterilised YEMA slants for further studies. The different isolates were also given a particular serial number R1 to R17 which corresponded with the location number initially assigned for each specific site surveyed in Idukki, Palghat, Trichur and Trivandrum districts.

A. Idukki District

R1 - Isolate from Kolahalamedu

R2 - Isolate from Mattuppetty

R3 - Isolate from Peerumedu

R4 - Isolate from Thodupuzha

B. Palghat District

R5 - Isolate from Chittoor

R6 - Isolate from Dhoni

R7 - Isolate from Mannarghat

R8 - Isolate from Pattambi

C. Trichur District

R9 Isolate from Chavakkad

R10 Isolate from Kannara

R11 Isolate from Kodassery

R12 Isolate from Peechi

R13 Isolate from Vellanikkara

D. Trivandrum District

R14 Isolate from Amboori

R15 Isolate from Chirayinkil

R16 Isolate from Pattom

R17 Isolate from Venganur

and Hayman (1970). The root bits were first washed in tap water to remove all adhering soil particles and then cut into small bits of approximately one cm in length. They were transferred to clean labelled bottles and fixed with formalin: acetic acid: alcohol mixture of following composition.

Formalin (40%)	- 5 ml
Glacial acetic acid	- 5 ml
Ethanol (95%)	- 90 ml

For staining, the root bits were initially softened by simmering in 10 percent KOH at 90°C for one hour. After cooling, the excess of alkali was removed by repeated rinsing in tap water and then acidified with 2 percent HCl. They were stained with 0.05 percent trypan blue in lactophenol by boiling for three minutes.

Preparation of lactophenol

Lactic acid	10 ml
Phenol	10 ml
Glycerol	20 ml
Water	20 ml

Preparation of Trypan blue

Trypan blue (Romali)	50 mg
Lactophenol	100 ml

The excess stain from the root tissue was removed by clearing overnight in fresh lactophenol. The root bits were examined for the typical VA mycorrhizal infection under a light microscope. Each root bit was divided into four equal segments for recording the presence or absence of VA mycorrhiza in segment wise manner. Fifty root bits randomly selected from each location were observed for this purpose. The percentage of VAM infection was calculated by the following formula.

$$\text{Percent VAM infection} = \frac{\text{Number of positive root segment} \times 100}{\text{Number of root segment observed}}$$

1.7 Isolation of VAM mycorrhizal spores from soil

VA mycorrhizal spores were isolated from soil by the modified wet sieving and decanting method of Gerdemann and Nicolson (1963). For this, 100 g of rhizosphere soil collected from each location was initially suspended in 1000 ml of tap water in a measuring cylinder and after the heavier particles had settled, the supernatent was filtered through a set of sieves (Jayant test sieves,

Jambulwadi, Kalbadevi, Bombay) of B. S.S.No.60 (250 microns), 150 (150 microns) and 350 (45 microns). The residue left behind in the measuring cylinder was resuspended in 1000 ml tap water and passed through the same set of sieves. This procedure was repeated three to four times in order to collect maximum number of spores from the soil. The material finally present on each sieve was transferred to 100 ml beakers in small volume of water and filtered through Watman No. 1 filter paper. The contents of each filter paper were carefully examined under a stereo microscope for the typical spores of VA mycorrhiza. Spores of uniform size and shape which were predominent in number at a given location were selected and transferred to moistened filter paper in petri dishes with the help of fine capillary pipette for further studies. The number of these spores present in 100 g soil was also recorded.

1.8 Measurement of VAM spores

The size of the VAM spores in micrometer was measured with the help of an ocular micrometer precaliberated with a stage micrometer.

1.9 Identification of VA mycorrhizal spores

The spores of VA mycorrhiza were identified mainly on the basis of their size, shape, colour, surface texture,

spore contents and the nature of spore attachment by observing under a microscope. These characters were then compared with the key prepared by Gerdemann and Trappe (1974) for identification.

1.10 Mass production of VA mycorrhizal inoculum

Mass production of the standard as well as the local isolates of VA mycorrhiza was done by inoculating on guinea grass (*Panicum maximum* Jacq.) with fifty selected spores of each culture of VA mycorrhiza.

The plants were grown in steam sterilised sand in small pots of 11 x 14 cm size. The potting material consisted of washed sand which was initially steam sterilised in an autoclave for 2h at 1 kg/cm^2. The roots from these plants were harvested after 21 days for using as the starting material for the mass production of VA mycorrhiza inoculum. This was done in large pots of 35 x 35 cm size containing 10 kg of steam sterilised sand and soil in the ratio 1:1 (W/W) and seeded with fifty grams each of the appropriate mycorrhizal inoculum in the form of infected roots cut into small bits of approximately 1 cm size and soil from the pot where these plants were grown initially. The inoculum was placed in the centre of each pot at a depth of about 5 cm over which guinea grass was planted and grown for 60 days. The plants were irrigated regularly with sterilised water. Infected roots from these plants

along with 50 spores of the respective VA mycorrhiza were used as the mycorrhizal inoculum for various experiments conducted during this investigation.

The four local isolates of VA mycorrhiza selected were:

Isolate number	Location	District
M1	Mattuppetty	Idukki
M2	Dhoni	Palghat
M3	Peechi	Trichur
M4	Amboori	Trivandrum

1.11 Estimation of soil pH

pH of different soil samples from various survey locations was measured by preparing 1:2.5 soil and distilled water suspension. This was agitated vigorously and the pH was determined in an ELICO digital pH meter.

2. Preliminary screening of different isolates of *Rhizobium* and VA mycorrhiza for nodulation and root infection in subabul

A pot culture experiment was conducted under asceptic conditions to find out the efficiency of all the 17 isolates of *Rhizobium* and four isolates of VA mycorrhiza selected on the basis of maximum root infection. Along with local isolates of *Rhizobium*, three exotic strains were also used. The experiment was laid out as a factorial experiment in CRD with three replications.

The different treatments were as follows:

A. <u>Rhizobium inoculation</u>

RO	-	Control
R1-R17	-	Local isolates of *Rhizobium*
R18	-	TAL 82 culture from nif TAL, USA
R19	-	Culture from I.A.R.I. New Delhi
R20	-	Culture from I.C.R.I.S.A.T. Hyderabad

B. <u>Mycorrhizal inoculation</u>

MO	-	Control
M1-M4	-	Local isolates of VA mycorrhiza

The treatment combinations used for this preliminary screening trial are given in Fig.2.

The experiment was conducted in earthern pots of 25 cm diameter containing a potting mixture of sand and soil in the ratio of 1:2. This was initially steam sterilized in an autoclave for 2 h at 1kg/cm^2. Seed treatment with appropriate *Rhizobium* culture and inoculation with VA mycorrhiza were done wherever required by the methods described earlier. The plants were irrigated regularly with sterilised tap water.

Fig 2. TREATMENT COMBINATIONS FOR PRIMARY SCREENING OF *Rhizobium* AND VA MYCORRHIZA FOR NODULATION, VAM INFECTION AND PLANT GROWTH IN SUBABUL.

$R_0 M_0$	$R_0 M_1$	$R_0 M_2$	$R_0 M_3$	$R_0 M_4$
$R_1 M_0$	$R_1 M_1$	$R_1 M_2$	$R_1 M_3$	$R_1 M_4$
$R_2 M_0$	$R_2 M_1$	$R_2 M_2$	$R_2 M_3$	$R_2 M_4$
$R_3 M_0$	$R_3 M_1$	$R_3 M_2$	$R_3 M_3$	$R_3 M_4$
$R_4 M_0$	$R_4 M_1$	$R_4 M_2$	$R_4 M_3$	$R_4 M_4$
$R_5 M_0$	$R_5 M_1$	$R_5 M_2$	$R_5 M_3$	$R_5 M_4$
$R_6 M_0$	$R_6 M_1$	$R_6 M_2$	$R_6 M_3$	$R_6 M_4$
$R_7 M_0$	$R_7 M_1$	$R_7 M_2$	$R_7 M_3$	$R_7 M_4$
$R_8 M_0$	$R_8 M_1$	$R_8 M_2$	$R_8 M_3$	$R_8 M_4$
$R_9 M_0$	$R_9 M_1$	$R_9 M_2$	$R_9 M_3$	$R_9 M_4$
$R_{10} M_0$	$R_0 M_1$	$R_{10} M_2$	$R_0 M_3$	$R_0 M_4$
$R_{11} M_0$	$R_{11} M_1$	$R_1 M_2$	$R_1 M_3$	$R_{\cdot} M_4$
$R_{12} M_0$	$R_2 M_1$	$R_2 M_2$	$R_2 M_3$	$R_2 M_4$
$R_3 M_0$	$R_3 M_1$	$R_3 M_2$	$R_3 M_3$	$R_3 M_4$
$R_{14} M_0$	$R_{14} M_1$	$R_4 M_2$	$R_4 M_3$	$R_{14} M_4$
$R_{15} M_0$	$R_5 M_1$	$R_5 M_2$	$R_5 M_3$	$R_5 M_4$
$R_{16} M_0$	$R_{16} M_1$	$R_6 M_2$	$R_{16} M_3$	$R_{16} M_4$
$R_{18} M_0$	R [illegible] M_1	$R_{17} M_2$	$R_{17} M_3$	$R_{17} M_4$
$R_{18} M_0$	$R_8 M_1$	$R_8 M_2$	$R_8 M_3$	$R_{18} M_4$
$R_{19} M_0$	$R_{19} M_1$	$R_{19} M_2$	$R_9 M_3$	$R_{19} M_4$
$R_{20} M_0$	$R_{20} M_1$	$R_{20} M_2$	$R_{10} M_3$	$R_{20} M_4$

2.1 Subabul seeds

The cultivar Hawaiian giant (K-8) was used through out this investigation. The required quantity of seeds were obtained from Kerala Gandhi Smarak Nidhi, Trivandrum.

2.2 Observations

Observations on nodule number, number of pink nodules formed, nodule dry weight, percentage VAM infection, shoot height, shoot dry weight, percentage nitrogen and P_2O_5 content were recorded on 105 day of plant growth. This was done on the basis of a preliminary study which indicated that nearly three months are needed to complete the nodulation in subabul.

2.2.1 Dry weight of nodules and shoot

The dry weight of root nodules and shoot was determined after drying the samples to a constant weight at 60°C in a drying oven.

2.2.2 Estimation of total nitrogen (Jackson, 1967)

Five hundred mg of powdered leaf sample and 1 g of digesion mixture consisting of potassium sulphate, cupric sulphate and selenium powder in the ratio of 10:1:0.1 were initially digested with 10 ml of conc. H_2SO_4 for 2h in Kjeldahl's digesion flasks. These were cooled down to room temperature before the contents were carefully transferred to 100 ml volumetric flasks to make up the volume with distilled water. Ten mlof this sample along with 40percent NaOH solution were

then steam distilled till about 30 ml of the distillate was collected in a receiver flask containing 10 ml of 4 percent boric acid solution and a drop of mixed indicator.

Preparation of mixed indicator

Bromocresol green	-	0.5 g
Methyl red	-	0.1 g
Ethanol	-	100.0 ml

The ammoniacal nitrogen of the distillate was estimated by titration against 0.01 N HCl and from the titre value, the percentage nitrogen content was calculated as follows:

$$\text{Percentage nitrogen content} = \frac{V \times N \times V_1 \times 0.014 \times 100}{V_2 \times W}$$

Where, V = Titre value - blank value

V1 = total volume of sample made up

V2 = total volume of sample distilled

N = Normality of HCl and

W = Weight of sample used.

2.2.3 Estimation of phosphorus (Vanadomolybdo phospheric acid method of Jackson, 1967)

Preparation of Vanadate molybdate reagent

Solution A

Ammonium molybdate	-	25.0 g
Distilled water	-	400.0 ml

Solution B

Ammonium meta vanadate	-	1.25 g
Boiling water	-	300.0 ml
Conc. HNO_3	-	250.0 ml

Solution A was added to solution B before the final volume was made up to one litre with distilled water.

Five hundred mg of powdered leaf sample was digested with 10 ml. of conc. H_2SO_4 of specific gravity 1.84 for two hours in 100 ml Erlenmeyar flasks. These flasks were allowed to cool down to room temperature, before the contents were carefully transferred to 100 ml volumetric flasks for making up the volume with distilled water. Five ml of this solution was pipetted into a 50 ml volumetric flask. Two to four drops of 2,4 dinitrophenol indicator and 4 N Na_2CO_3 were added drop by drop to the above sample till an yellow colour was developed. This was decolourised with 6 N HCl in such a way that the final pH of the solution was 4.8. Ten ml of vanadate molybdate reagent was then added to the above solution before the final volume was made up to 50 ml with distilled water. After 30 mts, the intensity of yellow colour developed was measured in a spectronic - 20 spectrophotometer at 470 nm. A standard curve was also prepared in a similar manner using 0, 1, 2, 4, 6, 8 and 10 ppm solution of KH_2PO_4. The concentration of phosphorus in leaf extract was determined from the standard

curve. Percentage phosphorus content was calculated as follows:-

$$\text{Percentage phosphorus content} = \frac{X \times 50 \times V_1}{1000 \times V_2 \times W}$$

where,

X = ppm concentration of P from the standard curve

V_1 = Volume made up

V_2 = Volume taken for colour development

3. Effect of pH on growth of *Rhizobium* under *in vitro* conditions

An *in vitro* study was conducted to find that relative growth of different isolates of *Rhizobium* at different pH in yeast extract mannitol broth. The pH of the YEM broth was initially adjusted to 4,5,6,7 and 8 by adding appropriate quantity of either 1 percent HCl or 1 percent KOH prior to sterilisation. A loopful of each isolate was inoculated into appropriate broth in triplicate and incubated at 28 ± 2°C on a rotary shaker. The extent of growth was measured as optical density at 640nm in a spectronic 20 after 72 and 96 h of culture growth.

3.1 Combined effect of soil pH, *Rhizobium* and VA mycorrhizal inoculation on nodulation, VAM infection and growth in subabul

A 2 x 3 x 5 factorial experiment in pots was laid out in CRD to find out the relative performance of two selected isolates

of *Rhizobium* R5 and R8 along with mycorrhizal inoculation at two different soil pH 6 and 7.1. The treatment details are given below

A - Soil pH   S1 - pH 6.0

S2 - pH 7.1

B - *Rhizobium* treatment

R0 - Control

R5 - isolate R5

R8 - isolate R8

C - Mycorrhizal treatment

M0 - Control

M1 - isolate from Idukki district

M2 - isolate from Palghat district

M3 - isolate from Trichur district

M4 - isolate from Trivandrum district

Treatment combinations were as follows:

S1 R0 M0	S1 R5 M0	S1 R8 M0	S2 R0 M0	S2 R5 M0	S2 R8 M0
S1 R0 M1	S1 R5 M1	S1 R8 M1	S2 R0 M1	S2 R5 M1	S2 R8 M1
S1 R0 M2	S1 R5 M2	S1 R8 M2	S2 R0 M2	S2 R5 M2	S2 R8 M2
S1 R0 M3	S1 R5 M3	S1 R8 M3	S2 R0 M3	S2 R5 M3	S2 R8 M3
S1 R0 M4	S1 R5 M4	S1 R8 M1	S2 R0 M4	S2 R5 M4	S2 R8 M4

Soil pH of 6 and 7.1 were selected as to represent the pH of the soils from where the two cultures of *Rhizobium* were isolated. Lime and sulphur were used to adjust the soil pH.

Different quantities of lime or sulphur were added to a fixed quantity of soil and incubated for one month and the change in pH was noted. Based on this guideline, appropriate quantities of lime or sulphur were added to get the pH 6 and 7.1. The biometric observations of plants were recorded at 60 days of plant growth.

4. <u>Serological differentation of selected isolates of Rhizobium</u>

Strain differentiation of selected isolates of <u>Rhizobium</u> against the most efficient local isolate selected on the basis of preliminary screening trial was done by their serological properties.

4.1 <u>Preparation of antiserum</u>

A pure culture of the <u>Rhizobium</u> isolate was grown on YEMA in petri dishes at 28 ± 3°C for four days. The culture was harvested in physiological saline (0.85 percent NaCl) and centrifuged at 15000 rpm and a pellet was obtained. This pellet was suspended in minimum amount of physiological saline. This suspension served as antigen. One ml of this antigen was mixed with 0.5 ml of Freund's adjuvant of micro-biological grade and injected intramuscularly into a rabbit on the upper part of the hind legs. Injection was repeated three times at seven day intervals. On twentyeighth day after the first injection, one ml of the antigen was injected without adjuvant intravenously through the marginal vein

of the ear. After seven days of last injection, the rabbit was bled through the marginal ear vein to collect the blood in a beaker. It was allowed to coagulate at 35°C for an hour and stored in a refrigerator overnight to obtain a clear straw coloured supernatent. This was centrifuged at 3000 rpm for five minutes. This antiserum was stored in a deep freezer adding 0.025 percent sodium azide.

4.2 Preparation of antigen

96 h growth of selected isolates of *Rhizobium* in test tube slants were harvested in normal saline (0.85% NaCl) and centrifuged at 15000 rpm. A pellet was formed. This was suspended in minimum amount of saline. This suspension served as antigen.

4.3 Tube agglutination test

The titre of the serum prepared as above was estimated by the following procedure with both homologus and heterologus antigens described below.

Sl.No.	Antigen	Code No.	Nature
1.	Isolate R8	A	Homologus
2.	IARI strain	B	Heterologus
3.	ICRISAT strain	C	Heterologus
4.	Isolate R5	D	Heterologus
5.	A local isolate of *Mimosa indica*	E	Heterologus

From 0.2 ml of the antiserum a range of double fold serial dilutions from 1/5 to 1/2560 was prepared using physiological saline as detailed below.

1. Tube No.	1	2	3	4	5	6	7	8	9	10
2. Saline (ml)	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3. Serum (ml) from previous dilution	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4. Final dilution	5	10	20	40	80	160	320	640	1280	2560

From the last dilution 0.5 ml was discarded to make the volume uniform. A saline control was also kept. In each tube 0.5 ml aliquots of antigen suspension was added. The mixture in each tube was mixed with a Pasteur pipette and allowed to settle for four h at 37°C in a water bath and afterwards overnight in a refrigerator and observed for agglutination.

4.4 Immuno - diffusion test

A clean glass slide of 7.5 x 5 cm was precoated with 2 per cent agar in distilled water and kept overnight at 60°C for drying. 0.85 percent agarose containing 0.025 percent sodium azide was then prepared in normal saline by boiling. Ten ml of this agarose solution was poured on to the precoated slide and allowed to set. Seven wells of a diameter of 4 mm each were cut in a circular arrangement with one well at the centre. The central well was filled with the antiserum of isolate R8 and the surrounding six wells were filled with

antigens, A, B, C, D, E and F using separate capillary tubes. This was incubated in a humid chamber at room temperature and observed for the development of precipitation bands after 24h. The different bands formed were recorded photographically.

5. Fine structure studies of VAM mycorrhizal infection and nodulation in subabul

Subabul seeds inoculated with isolate R8 and M2 culture of VA mycorrhiza were raised asceptically in pots. The root samples were examined for mycorrhizal infection by the method described earlier.

5.1 Effect of age on root nodulation in subabul

The number of nodules formed if any were recorded at five days interval up to 95 days of plant growth.

5.2 Scanning electron microscopy of root nodules

Healthy root nodules were initially detached along with a bit of root and washed in tap water to remove all adhering soil particles. They were then fixed in 2.5 percent glutaraldehyde in phosphate buffer for 12h. Each nodule was then cut through the centre. These cut nodules were passed through an acetone series of 50, 60, 70, 80, 90 percent and absolute for 30, 30, 30, 45, 45 and 60 minutes respectively. The nodules were dried in a critical point dryer and the cut surface was coated with carbon. They were observed in Hitachi S-530 scanning electron microscope for the study of fine structure of root nodules.

6. Study on the efficiency of different methods of inoculation of *Rhizobium* and VA mycorrhiza in subabul

A field experiment was conducted to study the effectiveness of different methods of inoculation of *Rhizobium* and VAM on nodulation, mycorrhizal infection and plant growth in subabul. The experiment was laid out in RBD with seven treatments and four replications. The different treatments were as follows:

T_1 - Dual inoculation 10 days before sowing in polybag

T_2 - Dual inoculation at the time of sowing in polybag

T_3 - Dual inoculation 10 days after sowing in polybag

T_4 - Dual inoculation 10 days before sowing in nursery

T_5 - Dual inoculation at the time of sowing in nursery

T_6 - Dual inoculation 10 days after sowing in nursery

T_7 - Dual inoculation at the time of sowing in the main field.

Inoculation with *Rhizobium* (isolate R8) and VAM (isolate M2) in polybags of 40 cm length, nursery bed and field were done by the methods described earlier. For inoculation 10 days before sowing, the *Rhizobium* and mycorrhizal inocula were buried 5 cm below the soil in polybag and nursery bed. Soil around the stem was slightly removed and the inocula were added at about 5 cm depth for inoculation 10 days after sowing. The seedlings raised in polybags and in nursery bed were subsequently transplanted to main field on 20th day. The plot size of the main field

was 2 x 1 m and subabul was planted at a spacing of 40 x 20 cm. Observation on various growth parameters such as nodule number, nodule fresh weight, percentage of VAM infection, shoot height, shoot fresh weight, percentage nitrogen and P_2O_5 content were recorded on 60 days of plant growth by the methods described earlier.

7. Combined effect of VA mycorrhiza, *Rhizobium* and fertilizer application on nodulation, mycorrhizal infection and growth in subabul

A field experiment was laid out as a factorial experiment with following treatments.

A. Mycorrhiza

M- - Control

MS - *Glomus fasciculatus*

ML - Local isolate M_2

B. Rhizobium

R- - Control

R+ - Rhizobial isolate R8

C. Nitrogen

N- - Control

N+ - Nitrogen at 100 kg/ha

D. Phosphorus

P- - Control

P+ - Phosphorus at 100 kg/ha

The different treatment combination were as follows.

M-R-N-P-	MSR-N-P-	MLR-N-P-
M-R-N-P+	MSR-N-P+	MLR-N-P+
M-R-N-P-	MSR-N+P-	MLR-N-P-
M-R-N+P+	MSR-N+P+	MLR-N+P+
M-R+N-P-	MSR+N-P-	MLR+N-P-
M-R+N-P+	MSR+N-P+	MLR+N-P+
M-R+N+P-	MSR+N+P-	MLR+N+P-
M-R+N+P+	MSR+N+P+	MLR+N+P+

The experiment was conducted in plots of size 2 x 1 m with a spacing of 40 x 20 cm. Phosphorus was completely applied at the time of sowing as super phosphate. Nitrogen as ammonium sulphate was added in split doses of 25 kg at the time of sowing and 25 kg each at one month interval. Various biometric observations on nodule number, nodule fresh weight, percentage of VAM infection, plant height, forage yield, foliage colour, chlorophyll, nitrogen, protein and mimosine content of leaves were recorded at 105 days of plant growth.

7.1 Scoring of foliage colour

A standard colour chart was prepared with colour score ranging from 1 to 5. Randomly selected leaf samples were matched with the above chart for scoring the colour of foliage.

and 10 ml aliquot of the macerate was placed in a boiling bath tube. 15 ml of 0.1 N HCl with activated charocal was added to it and boiled for 15 minutes. This gave a 250 fold dilution of the sample. It was then filtered through Watman No.2 filter paper and 2 ml aliquot of the filterate was added to 5 ml of 0.4 percent Na_2 EDTA solution and 1 ml of 60 percent $FeCl_3$ solution in 0.1 N HCl. This was kept in dark for 15 minutes for colour development. Optical density was recorded at 535 nm in a spectronic 20. The minosine percentage was calculated as follows.

Mimosine percentage = OD valve x 250

8. Statistical analysis

All the data were analysed by the analysis of variance technique of Snedecor and Cochran (1967).

7.2 Estimation of chlorophyll

One gram of leaf sample collected from five plants chosen at random was ground in a mortar with a pinch of calcium carbonate and excess of acetone. After grinding, the contents were filtered through fresh 80 percent acetone until the washing was colourless. The extract and washings were made up to 250 ml in a volumetric flask and optical density of an aliquot was measured in spectronic - 20 at wavelengths 645 and 663 nm. chlorophyll-a, chlorophyll-b and total chlorophyll in the sample was computed in mg/g by the following method.

Chlorophyll - a = 12.72 A 663 - 2.58 A 645

Chlorophyll - b = 22.57 A 645 - 4.67 A 663

Total chlorophyll = 8.05 A 663 + 20.29 A 645

7.3 Estimation of protein content

Percentage of foliage nitrogen was estimated by the method described earlier. The protein content in percentage was then computed by multiplying, the percentage nitrogen content by a values 6.25.

7.4 Analysis of mimosine content

Mimosine content of leaf was estimated by the method of Brewbaker *et al*. (1981). Ten gram of leaf samples were dried at temperature below 40°C. One gram of the dried sample was then taken in a volumetric flask and the volume was made up to 100 ml with 0.1 N HCl. It was macerated in homogeniser

and 10 ml aliquot of the macerate was placed in a boiling bath tube. 15 ml of 0.1 N HCl with activated charocal was added to it and boiled for 15 minutes. This gave a 250 fold dilution of the sample. It was then filtered through Watman No.2 filter paper and 2 ml aliquot of the filterate was added to 5 ml of 0.4 percent Na_2 EDTA solution and 1 ml of 60 percent $FeCl_3$ solution in 0.1 N HCl. This was kept in dark for 15 minutes for colour development. Optical density was recorded at 535 nm in a spectronic 20, The minosine percentage was calculated as follows.

Mimosine percentage = OD valve x 250

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RESULTS

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1. Survey for the occurrence of natural nodulation and VA mycorrhizal infection in subabul

The survey for the occurrence of natural nodulation and VA mycorrhizal infection in subabul (Plate 1 a and 1 b) was conducted at 17 different locations of four districts of Kerala namely Idukki, Palghat, Trichur, and Trivandrum. Information regarding soil type and pH at each of these locations is given in table 2. The pH of most soil samples was acidic and it ranged between 5.0 at Kannara in Trichur district to 6.8 at Dhoni in Palghat district. But at Chittoor in Palghat district, the soil pH was 7.1.

There was no significant difference between seedlings in the mean number of root nodules formed per plant at different locations. The average number of 7.7 was maximum at Vellanikkara and Pattom while at other places such as Peerumedu and Kolahalamedu in Idukki district, the nodule number was only 1.5 and 1.7 respectively. There was also no significant difference between seedlings in the mean percentage of mycorrhizal infection. The mycorrhizal infection of 25.5 percent was maximum at Dhoni (Table 2). The extent

Plate 1 a Subabul plants growing at Dhoni, Palghat District

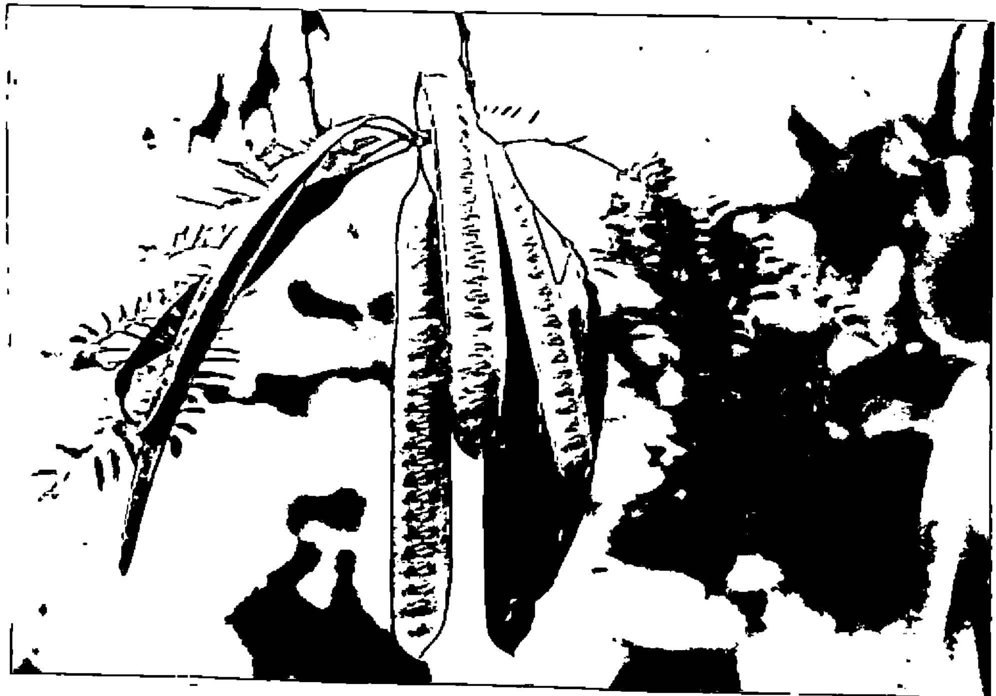


Plate 1 b A typical pod bearing branch of subabul

Table 2 Details of survey conducted for the occurrenceof natural nodulation and VAM infection in subabul.

District	Location	Soil type	pH	Name of cultivar	Nodule number*	Nodulation status@	VAM Spores/ 100g soil	%VAM infection	VAM status@
Idukki	1.Kolahalamedu	Forest Soil	6.0	K-8	1.7	Poor	21	10.0	Poor
	2.Mattuppetty	Forest Soil	6.0	K-8	3.0	Poor	6	20.5	Poor
	3.Peerumedu	Forest soil	6.2	Unknown	1.5	Poor	24	18.5	Poor
	4.Thodupuzha	Clay loam	5.6	K-8	4.2	Poor	18	6.0	Poor
Palghat	5.Chittoor	Black soil	7.1	K-8	7.2	Poor	18	8.5	Poor
	6.Dhoni	Clay loam	6.8	K-8	4.0	Poor	15	25.5	Poor
	7.Mannarghat	Clay loam	6.2	Unknown	3.0	Poor	18	12.0	Poor
	8.Pattambi	Laterite	5.5	K-8	2.5	Poor	14	14.0	Poor
Trichur	9.Chavakkad	Sandyloam	5.8	Unknown	2.5	Poor	7	4.5	Poor
	10.Kannara	Clay loam	5.0	Unknown	4.2	Poor	6	24.0	Poor
	11.Kodassery	Sandy loam	5.2	K-8	4.0	Poor	8	17.0	Poor
	12.Peechi	Laterite	5.6	K-8	5.5	Poor	25	24.5	Poor
	13.Vellanikara	Laterite	5.5	K-8	7.7	Poor	18	10.0	Poor
Trivandrum	14.Amboori	Forest soil	5.6	Unknown	6.0	Poor	12	19.5	Poor
	15. Chirayinkil	Laterite	5.8	K-8	4.2	Poor	5	13.0	Poor
	16.Pattom	Laterite	6.1	K-8	7.7	Poor	20	12.5	Poor
	17.Venganoor	Red loam	5.7	K-8	5.5	Poor	14	9.5	Poor

* Mean of three plants. @ In relation to inoculated plants.

Table 4 Primary characters of selected spore types of VA mycorrhiza associated with subabul.

Location	Spore size	spore shape	spore colour	surface texture	spore* contents	spore attach-ment	Identifi-cation
Mattuppetty	175	Globose	Brown	Smooth	Single large globule	Straight	*Glomus* sp.
Dhoni	140	Globose	Yellow	Smooth	Two large globules	Straight	*Glomus* sp.
Peechi	130	Globose	Yellow	Slightly rough	Single large globule	Straight	*Glomus* sp.
Amboori	145	Globose	Golden	Smooth	Many globules	Straight	*Glomus* sp.

* White to hyaline globules or granules.

of root infection at Peechi and Kannara were also higher. However, at places such as Chavakkad and Thodupuzha, the percentage of mycorrhizal infection was only 4.5 and 6.0 respectively. No significant difference between locations was also seen in the mean number of VA mycorrhizal spores present in 100 g of rhizosphere soil. The maximum number of 25 spores were isolated from soil samples collected at Peechi, in Trichur district. At other locations such as Chirayinkil, Mattuppetty, and Kannara this was as low as 5 and 6 respectively.

1.1 Isolation and characterisation of *Rhizobium*

In all, 17 cultures of rhizobia serially numbered R1 to R17 were initially isolated. All these isolates were rod shaped and Gram negative with a fast rate of growth of 48 to 72h on yeast extract mannitol agar medium (Table 3). On glucose peptone agar, the growth was scanty without any change in colour of the medium even after 7 days of incubation.

1.2 Identification of VA mycorrhiza

Spore samples initially isolated from rhizosphere of plants which had the maximum percentage of VA mycorrhizal infection during the initial survey in each district were identified on the basis of their size, shape, colour,

Table 3 Primary characterisation of *Rhizobium* isolates

Isolate No.	Gram reactions	Growth on YEMA	Growth on GPA	pH change on GPA
R1	- ve	F	±	-
R2	- ve	F	±	-
R3	- ve	F	±	-
R4	- ve	F	±	-
R5	- ve	F	±	-
R6	- ve	F	±	-
R7	- ve	F	±	-
R8	- ve	F	±	-
R9	- ve	F	±	-
R10	- ve	F	±	-
R11	- ve	F	±	-
R12	- ve	F	±	-
R13	- ve	F	±	-
R14	- ve	F	±	-
R15	- ve	F	±	-
R16	- ve	F	±	-
R17	- ve	F	±	-

- ve Gram negative ± Scanty growth

F Fast rate of growth - No pH change

surface texture, spore content and mode of hyphal attachment. Only those spore types which were dominant in the rhizosphere sample so selected were identified. The spores were typical of Glomus sp. with an average size of 130 to 175 µ (Table 4, Plate 2). They were globose with simple basal attachment. The colour ranged between yellow, brown or golden under incident light in a stereo microscope. The surface texture was usually smooth with the exception of spores collected at Peechi which had a slightly rough outer texture. The spore contents were characterised by the presence of one or more white to hyaline globules.

2 Screening for efficiency of microsymbionts: effect of combined inoculation of Rhizobium and VAM in subabul.

The result of the asceptic pot culture experiment conducted to study the efficiency of all the 17 native isolates of Rhizobium along with three exotic strains and the four selected cultures of VA mycorrhiza are presented below.

2.1 Nodule number

There were significant differences between treatments in the number of nodules formed per plant. The typical pattern of root nodulation in subabul is illustrated

Table 4 Primary characters of selected spore types of VA mycorrhiza associated with subabul.

Location	Spore size	spore shape	spore colour	surface texture	spore* contents	spore attachment	Identification
Mattuppetty	175	Globose	Brown	Smooth	Single large globule	Straight	*Glomus* sp.
Dhoni	140	Globose	Yellow	Smooth	Two large globules	Straight	*Glomus* sp.
Peechi	130	Globose	Yellow	Slightly rough	Single large globule	Straight	*Glomus* sp.
Amboori	145	Globose	Golden	Smooth	Many globules	Straight	*Glomus* sp.

* White to hyaline globules or granules.

Plate 2 Spore of <u>Glomus</u> sp.

in Fig. 3. The mean nodule number of 16.73 was maximum in plants inoculated with the native isolate of Rhizobium R8 (Table 5). This was significantly higher than control and all other Rhizobium treatments. The effect of mycorrhizal inoculation on nodulation was also significant. Thus, the use of various mycorrhizal cultures except M_4 along with seed treatment with Rhizobium resulted in significantly higher number of nodules when compared to control.

The interaction effect of both the microsymbionts on nodulation in subabul was significant. This was maximum in the R8 x M2 and R18 x M2 treatment combinations where an average number of 19 nodules were formed per plant (Table 5). Many other treatments were also significant and statistically on par with the above interactions.

2.2 Pink nodules

There were significant differences between treatments in the number of pink nodules formed per plant. The mean number of 8.53 pink nodules were maximum in plants inoculated with the native isolate of Rhizobium R8 (Table 6). This was significantly higher than control and other treatments except with R6, R13, R14, R18 and R19 where the number of pink nodules formed were

Fig 3. TYPICAL NODULE DISTRIBUTION PATTERN IN SUBABUL SEEDLING

Table 5 Screening for efficiency of microsymbionts - Effect of combined inoculation of Rhizobium and VA mycorrhiza on nodulenumber in Subabul

Rhizobium treatment	VA mycorrhiza treatment					
	M0	M1	M2	M3	M4	Mean
R0	0.00	0.00	0.33	0.67	0.33	0.27
R1	10.67	13.33	12.00	3.67	10.67	10.07
R2	8.33	8.67	11.67	14.00	3.67	9.27
R3	10.00	5.33	12.67	11.33	9.33	9.73
R4	14.67	11.33	9.33	14.33	10.67	12.07
R5	8.67	15.67	12.67	12.00	3.67	10.53
R6	8.00	14.67	13.00	14.00	11.67	12.27
R7	9.00	10.67	12.00	11.00	6.33	9.80
R8	16.00	14.67	19.00	16.67	17.33	16.73
R9	5.33	7.67	6.00	12.33	14.67	9.20
R10	10.00	15.00	9.33	14.00	11.00	11.87
R11	13.00	12.67	10.67	12.67	10.33	11.87
R12	9.00	10.67	9.67	10.67	9.67	9.93
R13	12.67	14.00	12.67	11.67	12.33	12.67
R14	11.67	15.00	12.33	7.67	13.00	11.93
R15	5.67	13.67	9.33	13.00	9.33	10.20
R16	6.33	12.33	7.67	16.00	8.33	10.13
R17	8.67	8.33	10.00	11.67	5.00	8.73
R18	12.00	10.67	19.00	13.67	10.33	13.13
R19	7.33	18.33	11.33	13.00	13.67	12.73
R20	10.67	15.33	13.33	11.33	8.33	11.80
Mean	9.41	11.81	11.14	11.68	9.51	10.71

CD for comparison		
	R (0.01)	3.42
	M (0.01)	1.67
	RXM (0.01)	7.64

Table 6 Screening for efficiency of microsymboints-Effect of combined inoculation of Rhizobium and VA mycorrhiza on number of pink nodules in subabul.

Rhizobium treatment	VA mycorrhiza treatment					Mean
	M0	M1	M2	M3	M4	
R0	0.00	0.00	0.00	0.00	0.00	0.00
R1	4.67	8.33	5.67	1.33	6.33	5.27
R2	4.33	4.00	8.00	8.33	0.67	5.07
R3	4.33	1.67	9.33	4.33	2.33	4.40
R4	6.33	6.67	5.00	4.67	3.33	5.20
R5	2.67	8.00	8.00	4.33	1.00	4.80
R6	4.67	8.67	8.33	8.00	6.33	7.20
R7	3.33	5.00	5.00	4.00	2.00	3.87
R8	9.33	7.00	11.67	7.33	7.33	8.53
R9	1.33	4.00	3.00	6.67	6.00	4.20
R10	6.00	8.00	5.00	6.33	3.67	5.80
R11	7.00	6.67	5.00	5.33	6.00	6.00
R12	3.00	5.67	6.00	5.33	6.00	5.20
R13	7.00	6.67	6.67	7.00	5.67	6.60
R14	8.67	9.00	6.33	3.00	7.67	6.93
R15	2.33	7.00	5.00	8.67	5.00	5.60
R16	1.67	3.33	3.67	8.00	4.00	4.13
R17	3.00	5.67	5.00	6.00	2.33	4.40
R18	6.33	5.67	12.33	7.33	4.67	7.27
R19	3.00	11.00	7.33	4.67	6.00	6.40
R20	6.00	8.33	8.33	4.00	3.67	3.07
Mean	4.52	6.21	6.41	5.46	4.29	5.38

CD for comparison

R	(0.01)	2.35
M	(0.01)	1.15
R x M	(0.01)	5.25

statistically on par with the above treatment. The effect of mycorrhizal inoculation on the number of pink nodules was also significant. Thus, the use of various mycorrhizal cultures except M4 along with seed treatment with Rhizobium resulted in significantly higher number of pink nodules when compared to control.

The interaction effect of both the microsymbionts on the number of pink nodules formed in subabul was significant. This was maximum in the R18 x M2 treatment combination where 12.33 pink nodules were formed per plant. Many other treatments were also significant and statistically on par with the above interaction.

2.3 Nodule dry weight

There were significant differences between treatments in the dry weight of nodules formed per plant. The mean dry weight of 0.48 g was maximum in plants inoculated with the native isolate of Rhizobium R8 (Table 7). This was significantly higher than control and other Rhizobium treatments except R18 and R19 where the dry weight of nodules formed was statistically on par with the above treatment. The effect of mycorrhizal inoculation on nodule dry weight was also significant. Thus, the use of various mycorrhizal cultures except M4 resulted in significantly higher dry weight of nodules when compared to control.

Table 7 Screening for efficiency of microsymbionts - Effect of combined inoculation of <u>Rhizobium</u> and VA mycorrhiza on dry weight (g) of nodules in subabul.

<u>Rhizobium</u> treatment	VA mycorrhiza treatment					Mean
	M0	M1	M2	M3	M4	
R0	0.00	0.00	0.01	0.17	0.03	0.04
R1	0.33	0.39	0.34	0.08	0.34	0.30
R2	0.25	0.20	0.30	0.36	0.07	0.24
R3	0.30	0.14	0.40	0.39	0.17	0.28
R4	0.44	0.25	0.22	0.45	0.20	0.31
R5	0.28	0.43	0.36	0.31	0.10	0.30
R6	0.25	0.35	0.32	0.43	0.40	0.35
R7	0.28	0.24	0.24	0.33	0.19	0.25
R8	0.48	0.34	0.57	0.49	0.52	0.48
R9	0.16	0.22	0.14	0.27	0.32	0.22
R10	0.32	0.37	0.29	0.33	0.20	0.30
R11	0.41	0.37	0.30	0.30	0.34	0.34
R12	0.29	0.28	0.24	0.36	0.26	0.29
R13	0.37	0.37	0.39	0.39	0.40	0.38
R14	0.35	0.34	0.34	0.14	0.29	0.29
R15	0.18	0.34	0.32	0.41	0.35	0.32
R16	0.19	0.28	0.23	0.46	0.16	0.26
R17	0.26	0.20	0.25	0.32	0.09	0.22
R18	0.36	0.34	0.58	0.50	0.28	0.41
R19	0.35	0.55	0.41	0.30	0.37	0.40
R20	0.23	0.37	0.40	0.32	0.25	0.31
Mean	0.29	0.30	0.32	0.34	0.25	0.30

CD for comparison		
	R (0.01)	0.09
	M (0.01)	0.04
	R x M (0.01)	0.19

The interaction effect of both the microsymbionts on dry weight of nodules formed in subabul was significant. This was maximum in the R18 x M2 treatment combination where the average dry weight of nodules formed was 0.58 g (Table 7). Many other treatments were also significant and statistically on par with the above interaction.

2.4 VA mycorrhizal infection

There were significant differences between treatments in the extent of root infection by VA mycorrhiza in subabul. The mean percentage root infection of 54.37 was maximum in plants inoculated with the M2 culture (Table 8). This was significantly higher than control and the remaining mycorrhizal treatments. The effect of *Rhizobium* inoculation on mycorrhizal infection was also significant. Thus, the use of R1 *Rhizobium* culture along with mycorrhizal inoculation resulted in significantly higher percentage of mycorrhizal infection when compared to control and other treatments.

The interaction effect of both the microsymbionts on root infection by VA mycorrhiza in subabul was significant. This was maximum in the M2 x R8 treatment combination where the percentage of mycorrhizal

Table 8 Screening for efficiency of microsymbionts - Effect of combined inoculation of Rhizobium and VA mycorrhiza on percentage VAM infection.

Rhizobium treatment	VA mycorrhiza treatment					
	M0	M1	M2	M3	M4	Mean
R0	0.00	53.33	56.67	30.00	51.67	38.33
R1	0.00	71.67	61.00	56.67	66.67	52.00
R2	0.00	50.00	55.00	33.33	33.33	34.33
R3	0.00	41.67	58.33	40.00	31.67	34.33
R4	1.67	16.67	48.33	31.67	58.33	31.33
R5	1.67	40.00	51.67	18.33	51.67	32.67
R6	0.00	46.67	48.33	25.00	58.33	35.67
R7	0.00	45.00	43.33	16.67	33.33	27.67
R8	0.00	50.00	76.67	21.67	26.67	35.00
R9	1.67	30.00	66.67	48.33	58.33	41.00
R10	0.00	15.00	51.67	51.67	41.67	32.00
R11	1.67	51.67	58.33	38.33	23.33	34.67
R12	0.00	56.67	51.67	10.00	38.33	31.33
R13	0.00	16.67	56.67	41.67	55.00	34.00
R14	1.67	51.67	48.33	31.67	45.00	35.67
R15	0.00	41.67	58.33	53.33	21.67	35.00
R16	0.00	46.67	65.00	60.00	36.67	41.67
R17	0.00	38.33	43.33	33.33	51.67	33.33
R18	1.67	43.33	61.67	25.00	36.67	33.67
R19	0.00	36.67	33.33	11.67	31.67	22.67
R20	1.67	41.67	43.33	13.33	23.33	24.67
Mean	0.56	42.14	54.37	32.94	41.67	34.33

CD for comparison

R	(0.01)	9.67
M	(0.01)	4.72
R x M	(0.01)	21.62

infection was as high as 76.67 (Table 8). Many other treatments were also significant and statistically on par with the above interactions.

2.5 Shoot height

There were significant differences between treatments in plant height of subabul. The mean shoot height of 94.2 cm was maximum in plants inoculated with the native isolate of Rhizobium R8 (Table 9). This was significantly higher than control and all other treatments. The effect of mycorrhiza inoculation on shoot height was also significant. Thus, the use of M1 culture resulted in significant increase in shoot height when compared to control and other mycorrhizal treatments.

The interaction of both the microsymbionts in increasing shoot height of subabul was significant (Plate 3a, 3b and 4). This was maximum in the R8 x M2 treatment combination where an average plant height of 46.67 cm was recorded. Many other treatments were also significant and statistically on par with the above interaction.

2.6 Shoot dry weight

There were significant differences between treatments in the dry weight of shoot in subabul. The mean dry

Table 9 Screening for efficiency of microsymbionts - Effect of combined inoculation of Rhizobium and VA mycorrhiza on shoot height (cm) of Subabul

Rhizobium treatment	VA mycorrhiza treatment					
	M0	M1	M2	M3	M4	Mean
R0	12.33	46.00	41.00	38.33	40.00	35.53
R1	32.67	32.33	29.33	14.33	23.67	26.47
R2	23.33	28.33	16.67	36.67	20.00	25.00
R3	20.00	29.33	19.67	29.00	27.33	25.07
R4	18.00	34.33	17.67	40.00	20.67	26.13
R5	24.00	36.33	33.67	27.33	16.33	27.53
R6	27.67	30.33	31.00	41.33	30.67	32.20
R7	34.00	30.00	17.00	23.67	15.67	24.07
R8	43.67	44.33	46.67	43.33	43.00	44.20
R9	15.33	24.00	20.33	33.00	30.67	24.67
R10	16.67	34.67	28.00	34.00	18.33	26.53
R11	29.00	31.33	29.67	24.00	28.33	28.47
R12	24.00	35.67	27.33	26.33	18.00	26.27
R13	21.00	28.00	36.33	28.67	38.33	30.47
R14	22.67	31.33	36.00	18.33	33.00	28.27
R15	17.67	33.67	23.00	27.00	34.33	27.13
R16	22.00	36.00	25.33	38.00	37.67	31.80
R17	31.00	24.33	17.33	37.67	19.33	25.93
R18	36.00	36.00	44.00	35.33	33.67	37.00
R19	39.67	42.33	25.00	18.67	30.00	31.13
R20	40.33	36.00	25.67	21.33	18.67	28.40
Mean	26.29	33.56	28.13	30.30	27.51	29.16

CD for comparison			
	R	(0.01)	1.93
	M	(0.01)	0.94
	R x M	(0.01)	4.32

Plate 3 a Effect of *Rhizobium* inoculation on plant growth in subabul

Plate 3 b Effect of VA mycorrhizal inoculation on plant growth in subabul

Plate 4 Effect of combined inoculation of *Rhizobium* and VA mycorrhiza on plant growth in subabul.

weight of 5.44 g was maximum in plants inoculated with the native isolate of R8 (Table 10). This was significantly higher than control and all other treatments. The effect of mycorrhizal inoculation on dry weight of shoot was also significant. Thus, the use of M1 culture resulted in significantly higher dry weight of shoot when compared to control and other treatments.

The interaction effect of both the microsymbionts on dry weight of subabul was significant. This was maximum in the R8 x M1 treatment combination where an average dry weight of shoot of 5.83 g was recorded. Many other treatments were also significant and statistically on par with the above interaction.

2.7 Nitrogen content of leaf

There were significant differences between treatments in the percentage nitrogen content of leaves. The mean nitrogen content of 4.24 percent was maximum in plants inoculated with the native isolate of *Rhizobium* R8 (Table 11). This was significantly higher than control and other treatments except R6 and R18 where the percentage nitrogen content of leaves estimated was statistically on par with the above treatment. The effect of mycorrhizal inoculation on nitrogen content

Table 10 Screening for efficiency of microsymbionts - Effect of combined inoculation of Rhizobium and VA mycorrhiza on shoot dry weight (g) of subabul

Rhizobium treatment	VA mycorrhiza treatment					
	M0	M1	M2	M3	M4	Mean
R0	2.77	4.77	5.00	4.93	5.10	4.51
R1	4.17	4.30	3.87	3.03	3.27	3.73
R2	3.77	3.73	3.13	4.30	3.20	3.63
R3	3.60	3.80	3.47	3.53	3.60	3.60
R4	3.33	4.50	3.43	4.90	3.20	3.87
R5	3.47	4.73	3.50	3.70	2.90	3.66
R6	3.90	3.57	4.23	5.17	4.10	4.19
R7	4.10	3.57	3.10	3.43	2.67	3.37
R8	5.27	5.33	5.83	5.43	5.33	5.44
R9	3.00	3.37	3.30	4.07	4.13	3.50
R10	3.67	4.43	4.10	4.17	3.13	3.90
R11	4.23	4.33	3.83	3.47	3.63	3.90
R12	3.57	4.40	3.63	3.63	2.80	3.61
R13	3.43	3.63	4.23	3.57	4.40	3.85
R14	3.50	4.27	4.53	3.37	4.40	3.93
R15	3.20	4.57	3.47	3.43	4.37	3.81
R16	3.50	4.70	3.63	3.83	4.83	4.10
R17	4.00	3.23	3.10	4.17	2.93	3.49
R18	4.33	4.87	5.73	4.30	4.20	4.69
R19	4.30	3.53	3.30	3.27	3.67	4.01
R20	4.03	4.83	3.53	3.43	3.03	3.77
Mean	3.77	4.31	3.90	3.96	3.74	3.94

CD for comparison

R	(0.01)	0.19
M	(0.01)	0.09
R x M	(0.01)	0.43

Table 11 Screening for efficiency of microsymbionts - Effect of combined inoculation of Rhizobium and VA mycorrhiza on nitrogen content (%) of leaf in subabul

Rhizobium treatment	VA mycorrhiza treatment					Mean
	M0	M1	M2	M3	M4	
R0	2.56	2.93	3.00	2.83	2.86	2.84
R1	3.93	3.73	4.10	4.06	4.00	3.96
R2	3.90	3.93	4.03	3.96	3.83	3.93
R3	4.03	3.86	4.06	3.93	3.86	3.95
R4	4.06	3.46	4.06	4.13	3.53	3.85
R5	4.16	3.90	4.16	3.73	3.93	3.98
R6	4.06	3.93	4.20	4.00	4.10	4.06
R7	4.06	3.70	4.20	3.86	3.66	3.89
R8	3.93	3.33	4.33	4.33	4.26	4.24
R9	3.63	3.70	3.83	3.86	3.76	3.76
R10	4.16	3.83	4.20	4.16	3.73	4.02
R11	4.20	3.66	4.13	3.73	3.93	3.93
R12	3.80	4.10	3.96	4.16	4.03	4.01
R13	3.93	3.70	4.10	3.96	3.56	3.85
R14	3.86	3.80	4.00	4.13	4.06	3.97
R15	3.56	3.76	3.96	4.30	3.56	3.83
R16	3.60	3.83	3.76	3.70	3.76	3.73
R17	3.93	4.00	4.20	4.13	3.56	3.96
R18	3.86	4.10	4.40	4.06	4.16	4.12
R19	3.63	3.90	3.93	4.00	4.00	3.89
R20	4.06	4.00	3.83	4.13	3.93	3.99
Mean	3.85	3.82	4.02	3.96	3.81	

CD for comparison			
	R	(0.01)	0.21
	M	(0.01)	0.09
	R x M	(0.05)	0.53

of leaves was also significant. Thus, the use of M2 and M3 cultures resulted in significantly higher percentage of nitrogen content of leaves when compared to control and other treatments.

The interaction effect of both the microsymbionts on nitrogen content of leaves in subabul was significant. This was maximum in the R18 x M2 treatment combination where a percentage nitrogen content of 4.4 was obtained (Table 11). Many other treatments were also significant and statistically on par with the above treatment combination.

2.8 Phosphorus content of leaves

There were significant differences between treatments in the percentage phosphorus content of leaves. The mean phosphorus content of 0.24 percent was maximum in plants inoculated with the M2 culture of VA mycorrhiza (Table 12). This was significantly higher than control and other treatments except M1 where the percentage phosphorus content was statistically on par with the above treatment. The effect of Rhizobium inoculation on phosphorus content of leaves was also significant. Thus, the use of R8 and R12 cultures along with mycorrhizal inoculation resulted in significantly higher percentage of phosphorus content when compared to control.

Table 12 Screening for efficiency of microsymbionts - Effect of combined inoculation of *Rhizobium* and VA mycorrhiza on phosphorus content (%) in subabul

Rhizobium treatment	VA Mycorrhiza treatment					Mean
	M0	M1	M2	M3	M4	
R0	0.16	0.21	0.23	0.21	0.20	0.20
R1	0.18	0.23	0.25	0.24	0.20	0.22
R2	0.19	0.19	0.22	0.20	0.20	0.20
R3	0.20	0.25	0.26	0.20	0.24	0.23
R4	0.18	0.26	0.24	0.19	0.22	0.22
R5	0.19	0.23	0.25	0.24	0.21	0.22
R6	0.17	0.23	0.23	0.20	0.25	0.22
R7	0.19	0.20	0.25	0.22	0.24	0.22
R8	0.19	0.27	0.29	0.22	0.25	0.24
R9	0.20	0.22	0.22	0.22	0.22	0.22
R10	0.17	0.27	0.24	0.23	0.18	0.22
R11	0.17	0.25	0.21	0.19	0.21	0.21
R12	0.20	0.25	0.25	0.25	0.24	0.24
R13	0.19	0.18	0.25	0.24	0.20	0.21
R14	0.17	0.25	0.20	0.20	0.25	0.22
R15	0.17	0.21	0.25	0.21	0.19	0.21
R16	0.19	0.21	0.22	0.20	0.19	0.20
R17	0.19	0.19	0.26	0.16	0.23	0.21
R18	0.18	0.24	0.28	0.20	0.24	0.23
R19	0.16	0.25	0.25	0.23	0.24	0.22
R20	0.19	0.20	0.22	0.22	0.24	0.21
Mean	0.18	0.23	0.24	0.21	0.22	0.22

CD for comparison R (0.01) 0.02

M (0.01) 0.01

R x M (0.01) 0.03

The interaction effect of both the microsymbionts on phosphorus content of leaves in subabul was significant. This was maximum in the R8 x M2 treatment combination where a percentage phosphorus content of 0.29 was obtained. Many other treatments were also significant and statistically on par with the above treatment combination.

3 Effect of pH on growth of different isolates of *Rhizobium*

The nature of growth and pH sensitivity were more apparent after 96 h of incubation (Table 13 and 14, Fig 4). In general, it was possible to group various cultures into two distinct catagories as those which had maximum growth at pH 6.0 and below (isolate numbers, R6, R8, R10, R11, R13, R14, R18 and R19) and those which preferred a slightly higher pH of 7.0 and above (isolate numbers R4, R5 and R20) for optimum growth under *in vitro* conditions.

3.1 Effect of soil pH, *Rhizobium* and VA mycorrhizal inoculation on nodulation, mycorrhizal infection and growth in subabul

Two *Rhizobium* isolates R5 and R8 which had maximum growth at pH 8.0 and 6.0 respectively under *in vitro*

Table 13 Effect of pH on growth* (optical density) of different isolates of <u>Rhizobium</u>

Isolate number	pH-4	pH-5	pH-6	pH-7	pH-8
R4	0.01	0.02	0.11	0.10	0.08
R5	0.01	0.12	0.17	0.21	0.19
R6	0.01	0.29	0.21	0.22	0.16
R8	0.04	0.28	0.28	0.28	0.15
R10	0.02	0.07	0.07	0.06	0.02
R11	0.02	0.06	0.09	0.05	0.05
R12	0.02	0.07	0.08	0.02	0.05
R14	0.02	0.06	0.08	0.06	0.01
R18	0.01	0.14	0.24	0.18	0.14
R19	0.02	0.19	0.22	0.21	0.17
R20	0.02	0.11	0.17	0.21	0.15

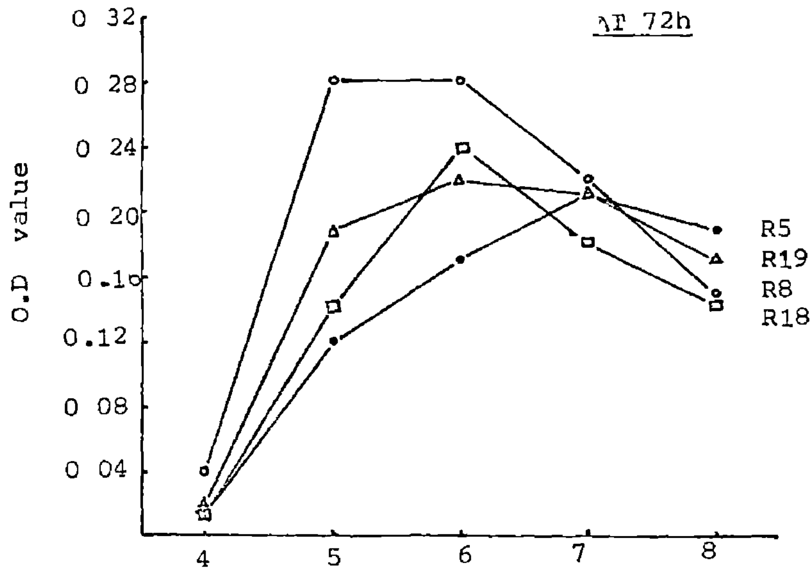
* After 72 h

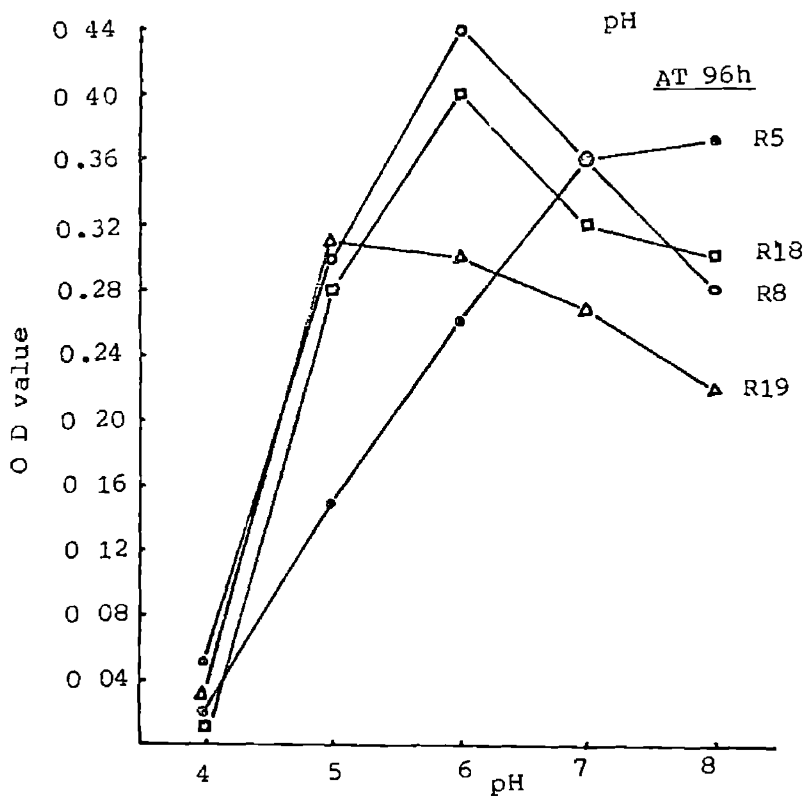
Table 14 Effectof pH on growth* (optical density) of different isolates of <u>Rhizobium</u>

Isolate number	pH-4	pH-5	pH-6	pH-7	pH-8
R4	0.02	0.03	0.12	0.15	0.14
R5	0.02	0.15	0.26	0.36	0.37
R6	0.03	0.30	0.38	0.29	0.28
R8	0.05	0.30	0.44	0.36	0.28
R10	0.03	0.12	0.08	0.09	0.09
R11	0.03	0.19	0.18	0.10	0.11
R13	0.02	0.15	0.12	0.07	0.11
R14	0.005	0.12	0.17	0.10	0.07
R18	0.01	0.28	0.40	0.32	0.30
R19	0.03	0.31	0.30	0.27	0.22
R20	0.005	0.19	0.19	0.22	0.27

* After 96h

Fig 4 GROWTH OF ISOLATES R8 R5 R18 and R19 AT DIFFERENT pH





conditions along with the four selected mycorrhizal cultures and two different levels of soil pH 6.0 and 7.1 were used for this investigation. Significant differences between treatments were there only in percentage of mycorrhizal infection, plant height and dry height of shoot. In general, the R5 isolate produced more number of nodules at pH 7.1 than at 6.0 while the isolate R8 produced more number of nodules at pH 6.0 instead of 7.1 (Table 15). In both the cases, the dry weight of nodules and the number of pink nodules formed followed a similar pattern.

The percentage of mycorrhizal infection was significantly high due to inoculation with the M2 culture. This was maximum in the treatment combination of S1 R8 M2 (Table 15). The increase in mycorrhizal infection in treatments such as S1 RO M2, S2 R5 M2, S1 R8 M2 were also significant and statistically on par with the S1 R8 M2 treatment. The plant height of 29.67 cm and dry weight of 4.13 g were also significantly high in the above treatment. The increase in plant height in the S2 R5 M1, treatment and plant dry weight in treatments like S1 R8 M1, S1 R8 M3, S1 R8 M4, S2 R5 MO, S2 R5 M2 were also significant and statistically on par with the S1 R8 M2 treatment.

Table 15 Combined effectof soil pH, Rhizobium and VA mycorrhizal inoculation on nodulation, VAM infection and growth in subabul

Treatment Combina-tions	Nodule number	Nodule dry weight (g)	No. of Pink nodules	% VAM infect-ion	Plant height (cm)	Plant dry weight (g)
S1 R0 M0	0.00	0.00	0.00	0.00	15.32	3.07
S1 R0 M1	0.00	0.00	0.00	3.67	19.67	3.03
S1 R0 M2	1.00	0.03	0.33	40.00	18.67	2.87
S1 R0 M3	0.33	0.01	0.33	18.33	18.33	2.83
S1 Ro M4	0.33	0.01	0.00	18.33	23.00	3.07
S1 R5 M0	5.00	0.12	2.00	0.00	17.33	2.97
S1 R5 M1	5.33	0.15	2.00	36.67	22.67	3.53
S1 R5 M2	8.33	0.22	5.00	46.67	19.67	3.10
S1 R5 M3	4.33	0.12	5.00	26.67	21.00	3.27
S1 R5 M4	6.67	0.15	4.67	21.67	15.67	3.17
S1 R8 M0	10.33	0.32	6.00	11.67	17.33	3.53
S1 R8 M1	11.67	0.41	4.67	35.00	21.33	4.00
S1 R8 M2	19.00	0.44	6.00	50.00	29.67	4.13
S1 R8 M3	7.67	0.26	3.33	23.33	18.33	3.83
S1 R8 M4	14.33	0.52	7.00	11.67	24.33	4.03
S2 R0 M0	0.33	0.01	0.00	1.67	18.33	2.53
S2 R0 M1	0.33	0.01	0.33	23.33	22.67	2.87
S2 R0 M2	0.00	0.00	0.00	36.57	19.67	2.60
S2 R0 M3	0.33	0.01	0.00	25.00	19.67	2.77
S2 R0 M4	0.67	0.01	0.00	38.33	19.67	2.63
S2 R5 M0	9.00	0.27	2.67	3.33	21.67	3.83
S2 R5 M1	9.33	0.31	5.00	13.33	29.33	3.87
S2 R5 M2	12.67	0.54	6.67	46.67	25.67	3.77
S2 R5 M3	11.67	0.33	6.67	28.33	19.67	3.50
S2 R5 M4	11.33	0.45	5.33	18.33	20.67	3.23
S2 R8 M0	3.00	0.10	0.67	0.00	22.67	2.77
S2 R8 M1	6.67	0.18	4.33	25.00	18.67	3.27
S2 R8 M2	3.00	0.10	1.00	41.67	20.67	3.13
S2 R8 M3	7.67	0.22	4.67	20.00	18.67	2.67
S2 R8 M4	3.33	0.08	2.00	35.00	19.67	2.77
CD (0.01)	NS	0.16	NS	NS	2.82	0.30
(0.05)	NS	NS	NS	10.18		

The detailed analysis of the data for the individual effect of above factors showed that with the isolates of *Rhizobium* and mycorrhizal cultures used during this investigation, the soil pH chosen did not have a significant effect on nodule number, nodule dry weight, number of pink nodules and percentage of VA mycorrhizal infection (Table 16). At the same time, the effect of *Rhizobium* inoculation was significant in increasing the nodule number, nodule dry weight, number of pink nodules formed per plant, plant height and plant dry weight. This effect was more pronounced with isolate R5 (Table 16). The effect of mycorrhizal inoculation was also significant in enhancing the nodule dry weight, percentage of mycorrhizal infection, plant height and plant dry weight (Table 16). In general such an effect was more with the M2 culture especially in significantly increasing the percentage of mycorrhizal infection.

4.1 Serological characterisation of selected isolates of *Rhizobium*

The results of the tube agglutination test with *Rhizobium* isolates R5, R8, R18, R19, R20 and R21 (*Mimosa indica* isolate) against antiserum of R8 at different dilutions ranging from 5 to 2560 is given in

Table 16 Individual effect of soil pH, Rhizobium and VA mycorrhizal inoculation on nodulation, VAM infection and growth in subabul.

Treatment	Nodule Number	Nodule dry weight (g)	No. of pink nodules	%VAM infection	Plant height (cm)	Plant dry weight (g)
Soil pH						
S1	5.69	0.18	2.82	24.11	20.16	3.36
S2	5.29	0.17	2.62	23.78	21.16	3.08
CD (0.01)	NS	NS	NS	NS	NS	0.08
CD (0.05)	NS	NS	NS	NS	0.78	
Rhizobium						
R0	0.33	0.01	0.10	23.33	19.50	2.83
R5	8.37	0.27	4.10	24.17	21.33	3.42
R8	7.77	0.26	3.97	24.33	21.13	3.41
CD (0.01)	1.56	0.05	1.16	NS	0.49	0.10
Mycorrhiza						
M0	4.61	0.14	0.89	1.11	18.78	3.12
M1	5.56	0.18	2.72	27.50	22.39	3.43
M2	5.83	0.22	3.17	43.61	22.33	3.27
M3	5.33	0.16	2.67	23.61	19.28	3.14
M4	6.11	0.21	3.17	23.89	20.50	3.15
CD (0.01)	NS	0.07	NS	5.23	3.65	0.12

Table 17. The homologus antigen R8 gave positive agglutination upto a serum dilution of 1:1280. A similar reaction with other antigens was observed only at a lesser dilution of the antiserum. These were 1:160 for R21, 1:80 for R18 and 1:40 for R20 isolates. However, the remaining two cultures, R5 and R19 showed no agglutination with the antiserum of R8.

4.2 Immuno diffusion test

The results of the tube agglutination test were more or less confirmed by the immuno-diffusion test except for the fact that no clear bands were formed with R5, R18 and R19 antigens (Plate 5). The remaining isolates tested produced a clear though non identical band of precipitation with the serum for isolate R8.

5 Fine structure studies of root infection by *Rhizobium* and VA mycorrhiza in subabul

5.1 Effect of age of plant on root nodulation in subabul

Any visible indication of nodulation was seen only from 15th day of plant growth (Table 18, Fig 5). This was followed by a period of gradual increase in nodule number till 70th day (Plate 6). Afterwards, there was no such variation in the number of nodules formed in subabul.

Table 17 Tube agglutination test against antiserum of isolate R 8

Antigen	Serum dilutions									
	5	10	20	40	80	160	320	640	1280	2560
A-(R-8)	+	+	+	+	+	+	+	+	+	-
B-(R-19)	-	-	-	-	-	-	-	-	-	-
C-(R-20)	+	+	+	+	-	-	-	-	-	-
D-(R-5)	-	-	-	-	-	-	-	-	-	-
E-(*Mimosa indica*)	+	+	+	+	+	+	-	-	-	-
F-(R-18)	+	+	+	+	+	-	-	-	-	-

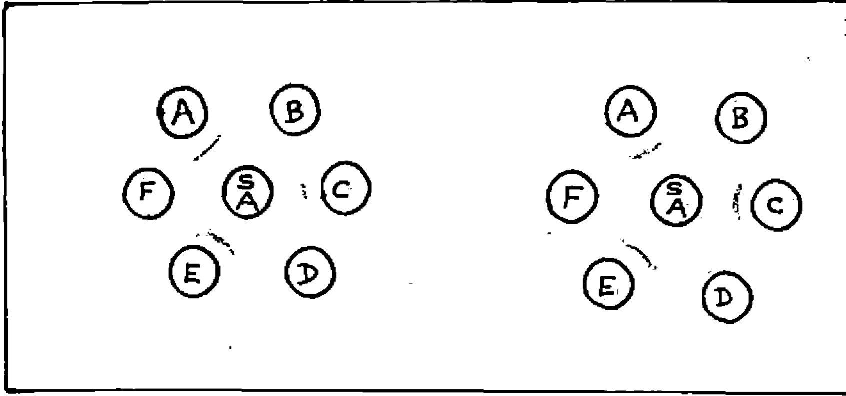


Plate 5 Immuno diffusion plate showing precipitation bands (A, B, C, D, E and F antigens, SA antiserum)

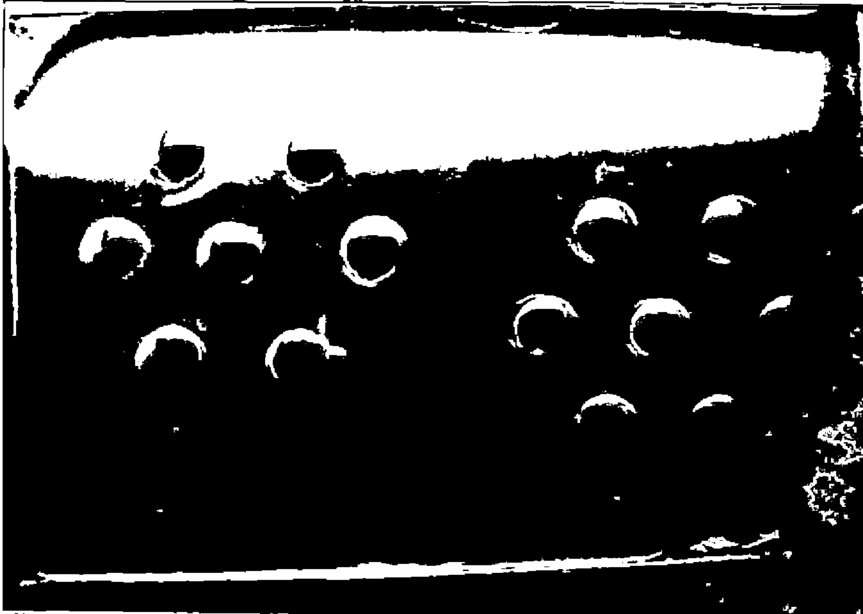


Table 18 Number of nodules formed per plant at different stages of growth in subabul

Days of growth	Nodule Number 1	2	3	Mean	Days of growth	Nodule number 1	2	3	Mean
5	0	0	0	0	50	14	12	18	14.6
10	0	0	0	0	55	17	16	21	18.0
15	1	0	0	0.3	60	20	18	23	20.3
20	1	0	1	0.6	65	22	20	25	22.3
25	3	1	2	2.0	70	22	23	27	24.0
30	4	2	3	3.0	75	21	23	27	23.6
35	6	4	4	4.6	80	21	24	25	23.3
40	7	5	7	6.3	85	22	25	26	24.3
45	10	8	12	10.0	90	20	26	25	23.6

Fig 5 NUMBER OF NODULES FORMED PER PLANT AT DIFFERENT STAGES OF GROWTH

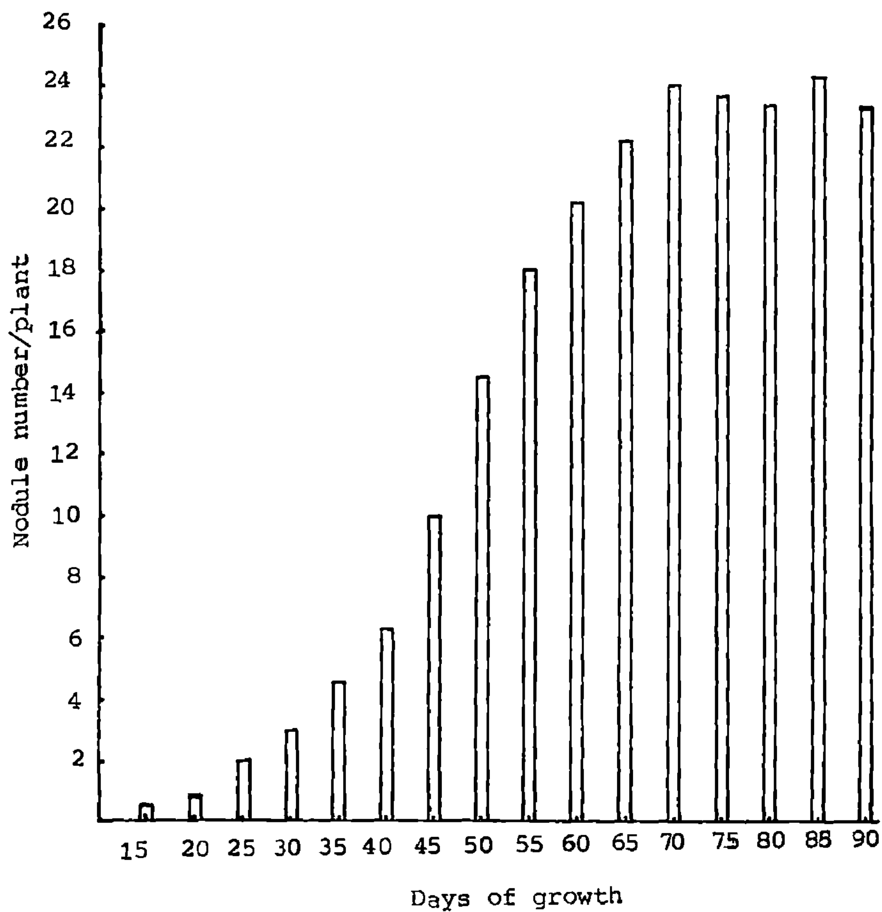


Plate 6 [illegible] Root nodulation on tap root of subabul.

5.2 Scanning Electron Microscopy of root nodules of subabul

During the early stages of root infection, *Rhizobium* was found closely associated with the root surface (Plate 7a). Later such cells were found between the cellulose fibrils prior to entry into the root tissue (Plate 7b). The cortical cells responded in a typical manner towards *Rhizobium* infection. In this process, it was found that the normal cells after bacterial infection got transformed into cells with an irregular shape (Plate 8a and 8b). The presence of bacteroid within such cells is shown in Plates 9a and 9b.

5.3 VA mycorrhizal infection

Observation of stained root samples under a light microscope showed the presence of VA mycorrhiza only in the root cortex. The distributive hyphae were found to grow almost parallel to the root axis, occupying much of the primary cortex depending on the degree of root infection (Plates 10 a and 10 b). The vesicles formed were typically terminal and oval in shape (Plate 11a and 11b). They did not show any intra cellular penetration of cortical cells.

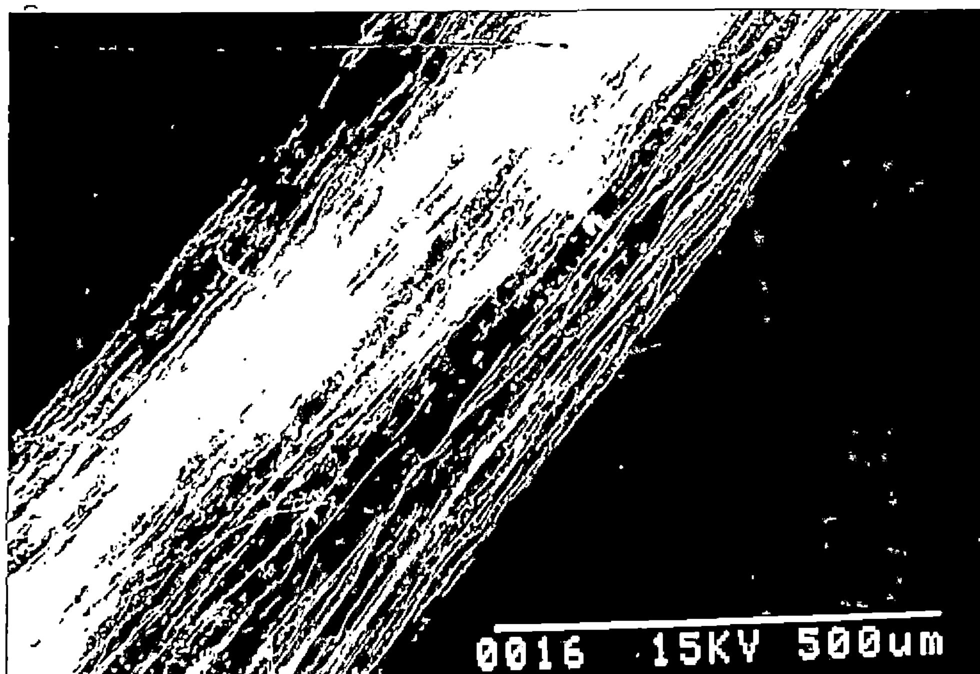


Plate 7 a SEM of a lateral root of subabul with *Rhizobium* association at lower magnification (150 X).

Plate 7 b SEM of a lateral root of subabul with *Rhizobium* association at higher magnification (600 X).

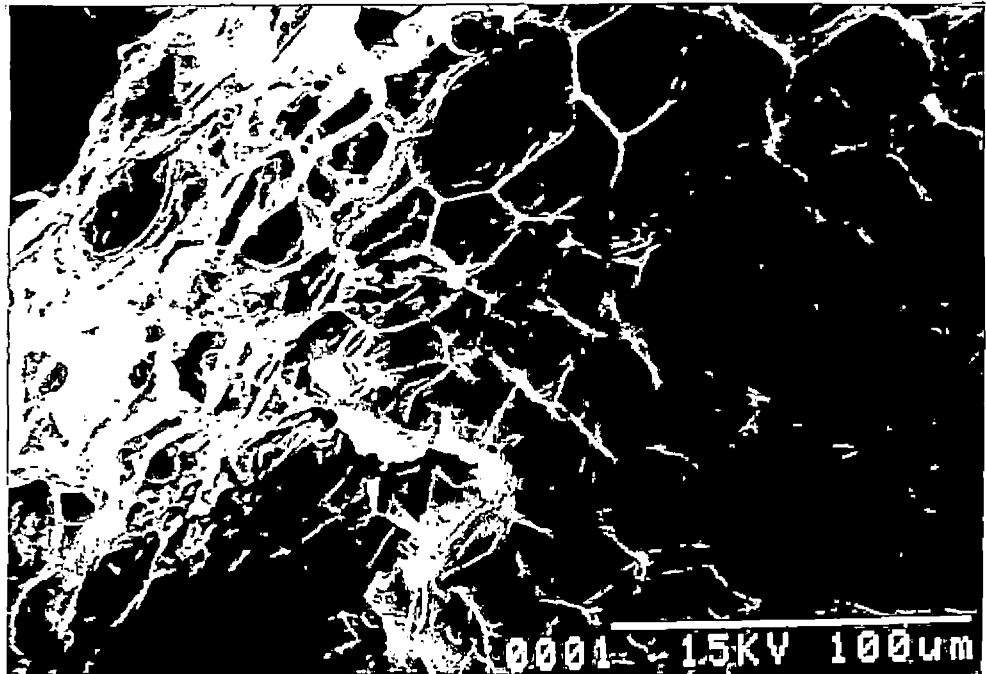


Plate 8 a SEM of normal cells of root cortex of subabul in the absence of Rhizobium infection (Mag.500 X).

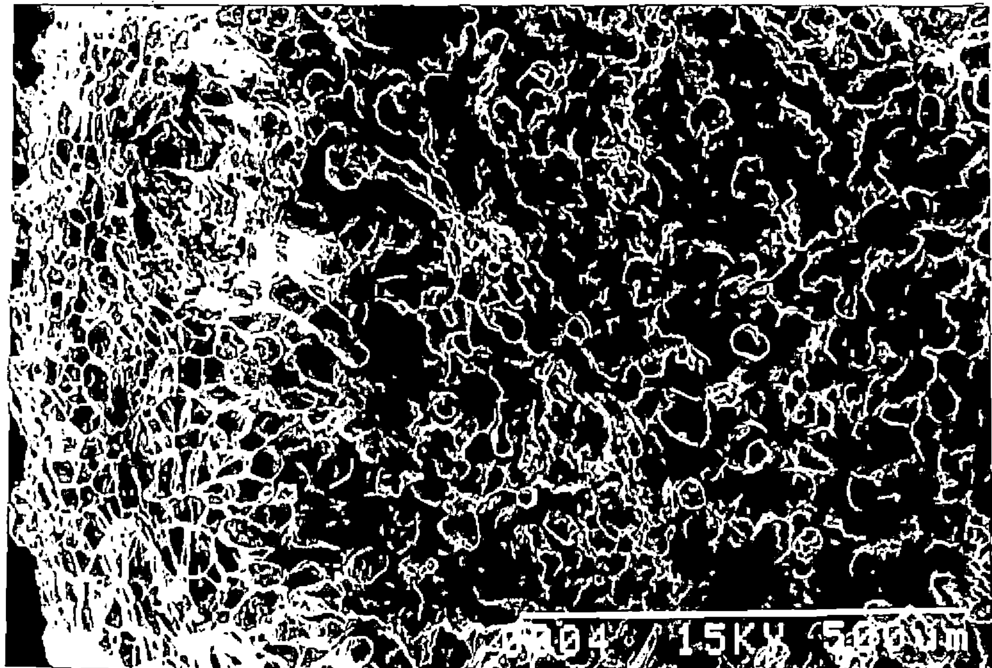


Plate 8 b SEM of transformed cells of root cortex of subabul due to Rhizobium infection (Mag. 500 X).

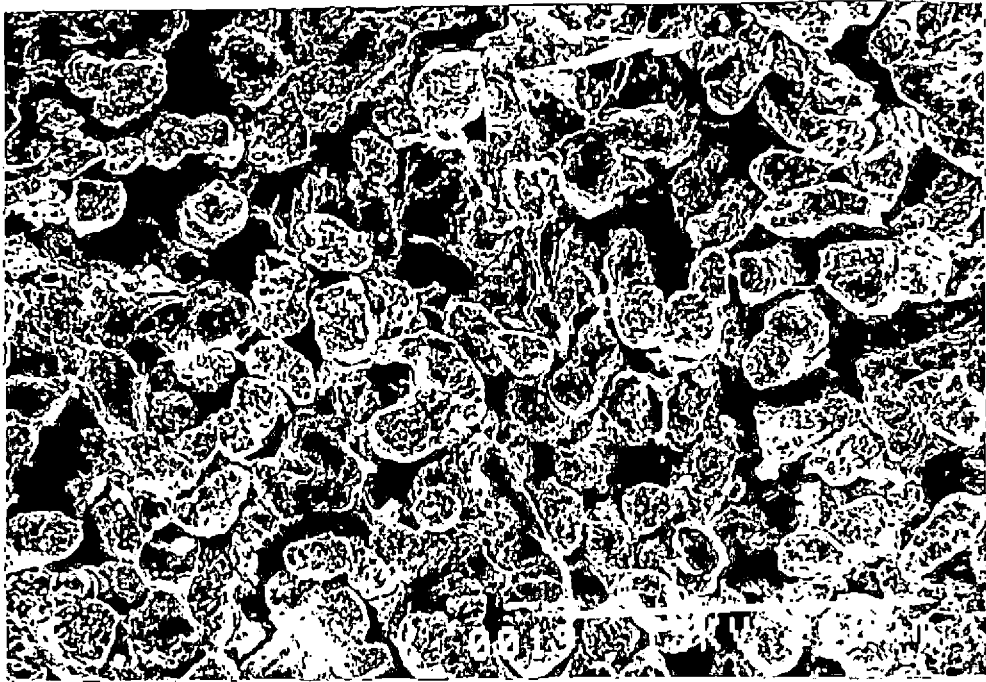


Plate 9 a SEM of nodule tissue with bacteroid at lower magnification (500 X).

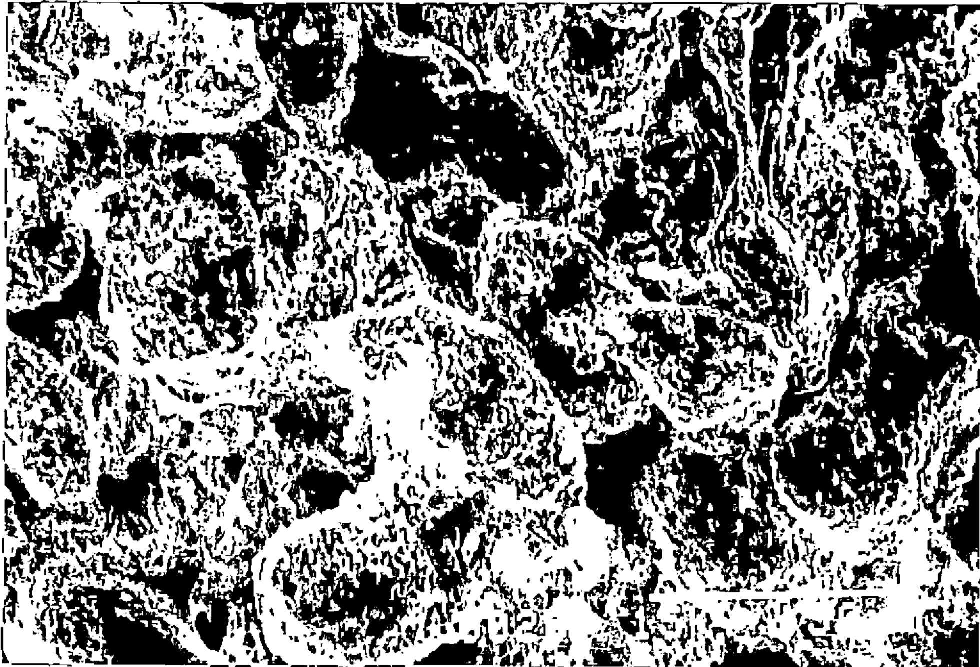


Plate 9 b SEM of nodule tissue with bacteroid at higher magnification (1500 X).

Plate 10 a A portion of lateral root of subabul without VA mycorrhizal association.

Plate 10 b A portion of the lateral root of subabul with low level of VA mycorrhizal infection.

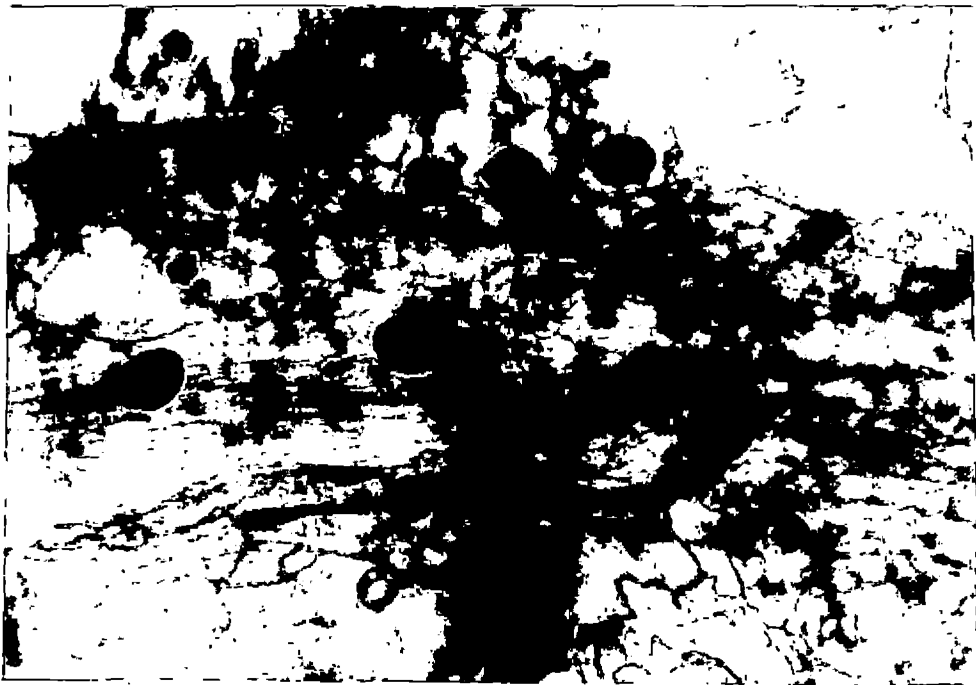


Plate 11 a A heavily infected portion of lateral root of subabul with VA mycorrhiza at lower magnification (100 X).

Plate 11 b A heavily infected portion of lateral root of subabul with VA mycorrhiza at higher magnification (450 X).

6 Effect of different methods of inoculation of Rhizobium and VA mycorrhiza on nodulation, mycorrhizal infection and growth in subabul

Significant differences between various methods of inoculation of microsymbionts were observed only in nodule fresh weight, percentage VAM infection, shoot height, shoot fresh weight and percentage phosphorus content of leaves. Between various treatments ,the beneficial effects were more pronounced with the use of microsymbionts either 10 days before sowing (T1) or at the time of sowing (T2) in polybag. The nodule number and nodule fresh weight of 22.50 and 0.805 g respectively were maximum in T1 treatment (Table 19). However, the extent of mycorrhizal infection of 80 percent, shoot height of 50.25 cm, shoot fresh weight of 5.825 g, percentage nitrogen and phosphorus content of 4.525 and 0.293 respectively were maximum in T2 treatment. The increase in mycorrhizal infection in T1 and T3 treatments, shoot height in treatments such as T1, T3 and T4 and percentage phosphorus content of leaves in T1 treatment were also significant and statistically on par with the T2 treatment.

Table 19 Effect of various methods of inoculation of microsymboints on nodulation VAM infection and growth in subabul.

Treatment	Nodule number	Nodule fresh weight (g)	% VAM infection	Shoot height (cm)	Shoot fresh weight (g)	%Leaf Nitrogen content	%Leaf phosphorus content
T_1	22.50	0.805	77.50	48.00	5.575	4.500	0.293
T_2	21.50	0.725	80.00	50.25	5.825	4.525	0.293
T_3	13.50	0.515	67.50	44.50	5.100	4.400	0.260
T_4	15.00	0.483	63.75	44.75	5.250	4.350	0.258
T_5	14.25	0.508	65.00	40.50	5.050	4.400	0.258
T_6	14.25	0.482	61.25	37.75	4.725	4.275	0.245
T_7	13.50	0.480	66.25	38.00	4.900	4.275	0.250
CD (0.01)	NS	NS	NS	6.26	0.560	NS	0.02
(0.05)	NS	0.230	12.75			NS	

T1 - Dual inoculation 10 days before sowing in polybag
T2 - Dual inoculation at the time of sowing in polybag
T3 - Dual inoculation 10 days after sowing in polybag
T4 - Dual inoculation 10 days before sowing in nursery
T5 - Dual inoculation at the time of sowing in nursery
T6 - Dual inoculation 10 days after sowing in nursery
T7 - Dual inoculation at the time of sowing in main field.

7 Combined effects of VA mycorrhiza, *Rhizobium* and fertiliser application on nodulation, VA mycorrhizal infection and forage yield in subabul

Significant differences between treatments were observed only in plant height and forage yield. The number of nodules formed (18.3), nodule fresh weight (0.587 g) and percentage of mycorrhizal infection (49.9) were maximum in the M-R+N+P-, ML R+ N+ R and ML R- N- P- treatments respectively (Table 20, Fig 6 and 7). The plant height of 54.3 cm was significantly high in the ML R+ N+ P- treatment when compared to control and other treatments except the MS R+ N- P- and MS R+ N+ P- treatments. The forage yield of 915 g per plot was maximum in the ML R+ N- P- treatment (Table 20, Fig.8). This was significantly higher than control and rest of the treatments except in the MS R+ N- P- and MS R+ N- P+ treatment combinations.

The individual effects of *Rhizobium*, VA mycorrhiza and fertiliser application on above observations under field conditions are given below.

7.1 Nodule number

It was observed that *Rhizobium*, nitrogen and phosphorus treatments alone had a significant effect on

Table - 20 Combined effect of VA mycorrhiza *Rhizobium*, and fertilizer application on nodulation, mycorrhizal infection and forage yield in subabul.

	Nodule number	Nodule fresh weight (g)	% VAM infection	Plant height (cm)	Forage yield/ plot (g)
M-R-N-P-	2.6	0.060	4.7	22.3	441
M-R-N-P+	2.3	0.063	00.5	27.3	526
M-R-N+P-	4.0	0.100	6.5	22.3	609
M-R-N+P+	1.7	0.043	8.8	30.0	651
M-R+N-P-	16.0	0.560	4.7	29.3	557
M-R+N-P+	10.3	0.306	6.5	40.0	684
M-R+N+P-	18.3	0.563	0.5	38.0	553
M-R+N+P+	15.6	0.463	4.7	38.6	592
MS R-N-P-	2.3	0.060	38.2	40.3	614
MS R-N-P+	1.3	0.023	41.1	35.3	356
MS R-N+P-	3.0	0.079	43.1	44.3	764
MS R-N+P+	2.61	0.093	30.7	47.3	720
MS R+N-P-	11.0	0.376	35.2	49.6	864
MS R+N-P-	11.3	0.349	42.1	51.3	848
MS R+N+P-	11.3	0.370	44.0	52.3	828
MS R+N+P+	14.3	0.470	40.9	51.6	795
ML R-N-P-	2.6	0.070	49.9	46.3	798
ML R-N-P+	2.6	0.086	46.9	42.0	700
ML R-N+P-	2.6	0.073	42.9	46.3	779
ML R-N+P+	2.6	0.080	44.0	48.6	747
ML R+N-P-	15.0	0.490	49.0	47.0	915
ML R+N-P+	8.3	0.330	45.9	44.0	691
ML R+N+P-	15.7	0.583	45.9	54.3	705
ML R+N+P+	12.0	0.406	46.9	49.6	774
CD (0.01)	NS	NS	NS	4.6	78

Fig 6 COMBINED EFFECT OF *Rhizobium*, VAM AND FERTILIZER APPLICATION ON NODULE NUMBER IN SUBABUL.

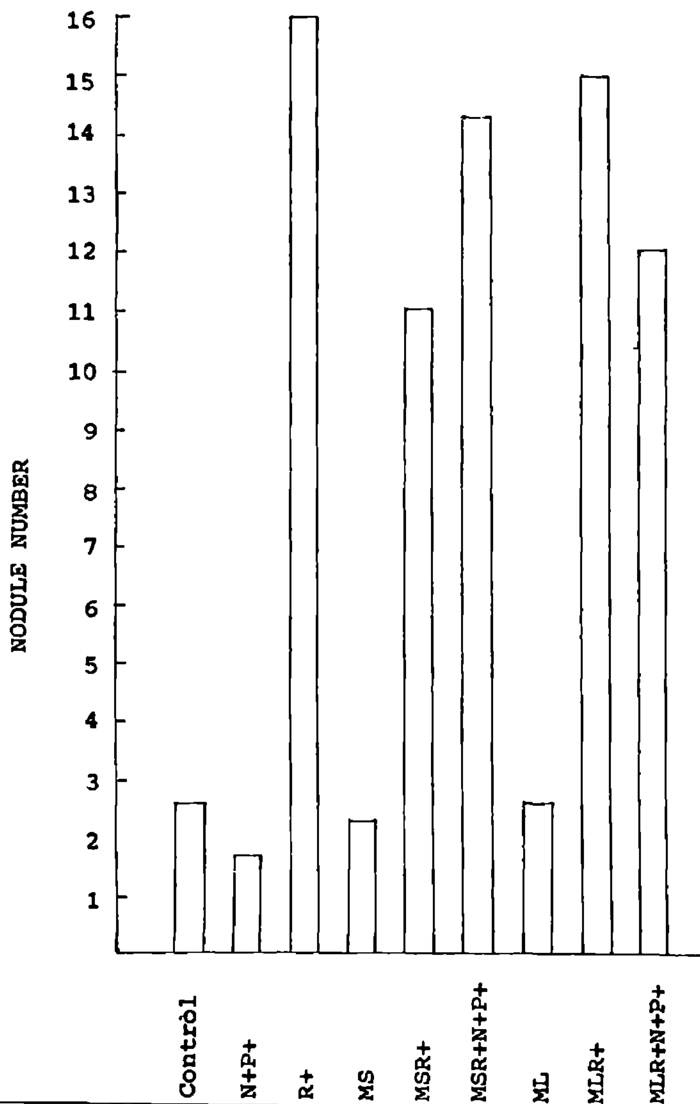


Fig 7 COMBINED EFFECT OF _Rhizobium_, VAM AND FERTILIZER APPLICATION ON PERCENTAGE VAM INFECTION IN SUBABUL.

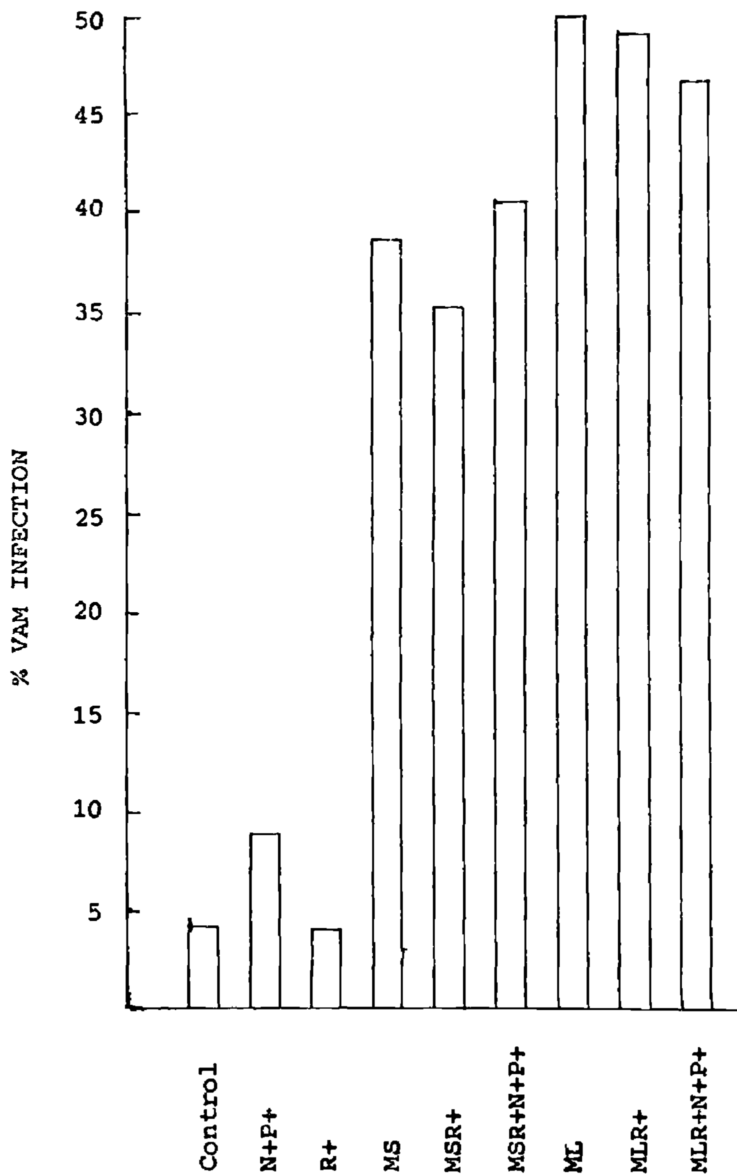
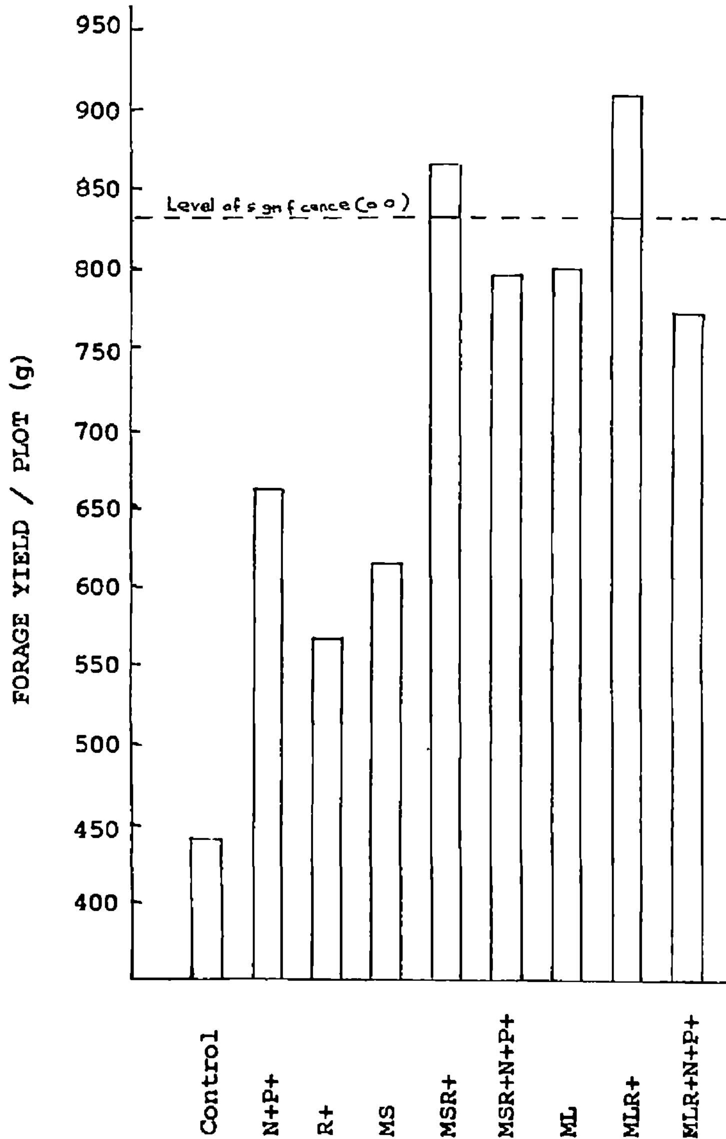


Fig 8 COMBINED EFFECT OF *Rhizobium*, VAM AND FERTILIZER APPLICATION ON FORAGE YIELD IN SUBABUL.



root nodulation in subabul. Thus, the increase in the number of nodules formed was significant due to *Rhizobium* inoculation and the application of nitrogenous fertiliser (Table 21). However, such an effect was also seen in the P- treatment.

7.2 Nodule fresh weight

As in the case of nodule number, *Rhizobium*, nitrogen and phosphorus treatments alone had a significant effect on nodule fresh weight. Thus, the increase in fresh weight of nodules was significant due to *Rhizobium* inoculation and the application of nitrogenous fertiliser (Table 22). However, such an effect was also seen in the P- treatment. The interaction between phosphorus and *Rhizobium* treatment was also significant.

7.3 VA mycorrhizal infection

Inoculation with appropriate VA mycorrhizal cultures alone had a significant effect on root infection in subabul. Thus, the increase in the percentage root infection was highly significant by the use of both the standard (*Glomus fasciculatum*) as well as the local (*Glomus* Sp.) cultures of VA mycorrhiza (Table 23). Further, between the two mycorrhizal treatments, the

Table - 21 Effect of VA mycorrhiza (M), Rhizobium (R), fertilizer nitrogen (N) and P_2O_5(P) application on nodule number under field conditions

	R-	R+	N-	N+	P-	P+	Mean of M-, MS, ML
M-	2.6	15.1	7.8	9.9	10.3	7.5	8.9
MS	2.3	12.0	6.5	7.8	6.9	7.4	7.2
ML	2.6	12.8	7.2	8.3	9.0	6.4	7.7
Mean	2.6	13.3	7.2	8.7	8.7	7.1	

	N-	N+	P-	P+
R-	2.3	2.8	2.9	2.2
R+	12.0	14.6	14.6	12.0

CD	(0.05)	(0.01)
R	-	1.62
N	1.2	-
P	1.2	-
Others	NS	

	P-	P+
N-	8.3	6.1
N+	9.2	8.2

Table: 22 Effect of VA mycorrhiza (M), <u>Rhizobium</u> (R), fertilizer nitrogen (N) and P_2O_5 (P) application on nodule fresh weight (g) of subabul under field conditions.

	R-	R+	N-	N+	P-	P+	Mean of M-, MS, ML
M-	0.066	0.473	0.248	0.292	0.321	0.219	0.270
MS	0.064	0.392	0.203	0.253	0.222	0.234	0.227
ML	0.078	0.452	0.244	0.286	0.304	0.225	0.265
Mean	0.069	0.439	0.231	0.277	0.282	0.226	

	N-	N+	P-	P+
R-	0.061	0.078	0.074	0.065
R+	0.402	0.476	0.491	0.388

	P-	P+
N-	0.269	0.193
N+	0.295	0.259

CD	(0.05)	(0.01)
R	-	0.061
N	0.046	-
PX R	0.065	-
P	0.046	-
Others	NS	

Table: 23 Effect of VA mycorrhiza (M), Rhizobium (R), fertilizer nitrogen (N) and P_2O_5 (P) application on percentage VAM infection in subabul under field conditions.

	R-	R+	N-	N+	P-	P+	Mean M-, MS, ML
M-	5.1	4.1	4.1	5.1	4.1	5.1	4.6
MS	38.2	40.6	39.1	39.7	40.1	38.7	39.4
ML	45.9	47.0	48.0	45.0	46.9	46.0	46.5
Mean	29.8	30.6	30.4	29.9	30.4	29.9	

	N-	N+	P-	P+
R-	30.2	29.3	30.9	28.7
R+	30.6	30.5	29.9	31.2

C.D	(0.01)
M	5.97
All others	NS

	P-	P+
N-	30.3	30.5
N+	30.5	29.3

application of the local culture was more beneficial in significantly enhancing the extent of root infection.

7.4 Plant height

All treatments involving microsymbionts and fertiliser application had a significant effect on plant height. Thus, the increase, in plant height was significant due to *Rhizobium* and mycorrhiza inoculation and the application of nitrogenous and phosphorus fertilisers (Table 24). A similar trend was also observed in the M x R and M x P interactions.

7.5 Forage yield

Rhizobium, mycorrhiza and nitrogen treatments alone had a significant effect on forage yield. Thus, the increase in yield was highly significant due to *Rhizobium* and mycorrhiza inoculation and the application of nitrogenous fertilizer (Table 25). A similar trend was also observed in the R x M, N x M, N x R and P x M interactions.

In addition to the above major growth parameters, following observations on foliage colour, chlorophyll content, percentage nitrogen, protein and mimosine content

is.

Table: 24 Effect of VA mycorrhiza (M), *Rhizobium* (R), fertilizer nitrogen (N) and P_2O_5 (P) application on plant height (cm) of subabul under field conditions.

	R-	R+	N-	N+	P-	P+	Mean of M-, MS, ML
M-	25.5	36.5	29.8	32.25	28.0	34.0	31.0
MS	41.8	51.3	44.2	48.9	46.7	46.4	46.5
ML	45.8	48.7	44.8	49.8	48.5	46.0	47.3
Mean	37.7	45.5	39.6	43.6	41.1	42.2	

	N-	N+	P-	P+
R-	35.6	39.8	37.0	38.4
R+	43.6	47.4	45.1	45.9
Mean				

	P-	P+
N-	39.2	40.0
N+	42.9	44.3

CD for comparing	(0.05)	(0.01)
M	-	1.64
MXR	-	2.32
R	-	1.34
N	-	1.34
MXP	-	2.32
P	1.0	-
Other	NS	

Table: 26 Combined effect of VA mycorrhiza, *Rhizobium* and fertilizer application on foliage colour and chlorophyll content of subabul under field conditions.

Treatment	Foliage Colour (Score)	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total Chlorophyll (mg/g)
M- R-N-P-	1.7	0.2181	0.3811	0.3383
M- R-N-P+	1.7	0.2681	0.3511	0.4245
M- R-N+P-	2.7	0.2781	0.7344	0.6911
M- R-N+P+	2.7	0.3018	0.9273	0.8611
M- R+N-P-	3.7	0.3635	0.9262	0.8780
M- R+N-P+	2.7	0.4820	0.5841	1.0959
M- R+N+P-	3.3	0.8359	0.8477	1.2849
M- R+N+P+	3.7	0.7612	0.9711	1.3849
Ms R-N-P-	1.7	0.4120	0.9145	0.5103
Ms R-N-P+	1.7	0.4395	0.7458	0.6478
Ms R-N+P-	2.7	0.5940	0.8897	0.9567
Ms R-N+P+	3.7	0.6357	0.9339	0.9279
Ms R+N-P-	4.3	0.8337	0.5485	1.0832
Ms R+N-P+	4.0	0.8490	0.7585	1.3222
Ms R+N+P-	3.7	0.7934	0.9428	0.9432
Ms R+N+P+	2.7	0.9156	1.0484	1.6391
ML R-N-P-	1.3	0.5344	0.4760	0.6291
ML R-N-P+	1.7	0.4920	0.5966	0.6343
ML R-N+P-	3.0	0.6664	0.6939	1.1449
ML R-N+P+	3.7	0.5836	0.6590	1.3822
ML R+N-P-	2.7	0.7917	0.6392	0.7426
ML R+N-P+	3.3	0.8956	0.7625	0.9137
ML R+N+P-	4.7	1.0512	0.9002	1.5537
ML R+N+P+	4.3	0.9266	0.9055	1.4880
CD (0.01)	NS	0.1474	0.2646	NS

Table 25. Effect of mycorrhiza (M), Rhizobium (R), fertiliser nitrogen (N) and P_2O_5(P) application on forage yield of subabul (g) under field conditions.

	R-	R+	N-	N+	P-	P+	Mean of N-, Ms, Ma
M-	558.4	596.8	552.2	601.6	540.8	616.0	579.2
Ms	689.6	833.6	745.6	777.6	768.0	755.2	761.6
ML	756.8	771.2	776.0	752.0	800.0	728.0	763.2
Mean	668.8	734.4	692.8	710.4	702.4	699.2	

	N-	N+	P-	P+
R-	625.6	710.4	668.8	668.8
R+	760	707.2	737.6	731.2

	P-	P+
N-	697.6	686.4
N+	707.2	713.6

CD	(0.05)	(0.01)
M	-	27.8
R x M	-	39.52
R	-	22.72
N x M	-	39.52
N x R	-	32.16
N	17.1	-
P x M	-	39.52
Others	NS	

of leaves were also taken during this investigation.

Significant differences between treatments were observed only in chlorophyll-a and chlorophyll-b content of leaves. In the score chart for foliage colour, the maximum score of 4.7 was obtained in the ML R+ N+ P- treatment (Table 26). The chlorophyll-a content of 1.0512 was also maximum in this treatment. However, chlorophyll-b content and total chlorophyll content (Table 26, Fig 9) was maximum in the MS R+ N + P+ treatment.

The individual effects of *Rhizobium*, VA mycorrhiza and fertiliser application on above observations are given below.

7.6 Foliage colour

It was observed that *Rhizobium* and nitrogen treatments alone had a significant effect on foliage colour. Thus, the positive increase in foliage colour was significant due to *Rhizobium* inoculation and the application of nitrogenous fertiliser (Table 27). A similar trend was also seen in the NxM and NxR interactions.

7.7 Chlorophyll-a content

Rhizobium, mycorrhiza and nitrogen treatments alone had a significant effect on chlorophyll-a content of leaves. Thus, the increase in chlorophyll-a content was

Table: 26 Combined effect of VA mycorrhiza, <u>Rhizobium</u> and fertilizer application on foliage colour and chlorophyll content of subabul under field conditions.

Treatment	Foliage Colour (Score)	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total Chlorophyll (mg/g)
M- R-N-P-	1.7	0.2181	0.3811	0.3383
M- R-N-P+	1.7	0.2681	0.3511	0.4245
M- R-N+P-	2.7	0.2781	0.7344	0.6911
M- R-N+P+	2.7	0.3018	0.9273	0.8611
M- R+N-P-	3.7	0.3635	0.9262	0.8780
M- R+N-P+	2.7	0.4820	0.5841	1.0959
M- R+N+P-	3.3	0.8359	0.8477	1.2849
M- R+N+P+	3.7	0.7612	0.9711	1.3849
Ms R-N-P-	1.7	0.4120	0.9145	0.5103
Ms R-N-P+	1.7	0.4395	0.7458	0.6478
Ms R-N+P-	2.7	0.5940	0.8897	0.9567
Ms R-N+P+	3.7	0.6357	0.9339	0.9279
Ms R+N-P-	4.3	0.8337	0.5485	1.0832
Ms R+N-P+	4.0	0.8490	0.7585	1.3222
Ms R+N+P-	3.7	0.7934	0.9428	0.9432
Ms R+N+P+	2.7	0.9156	1.0484	1.6391
ML R-N-P-	1.3	0.5344	0.4760	0.6291
ML R-N-P+	1.7	0.4920	0.5966	0.6343
ML R-N+P-	3.0	0.6664	0.6939	1.1449
ML R-N+P+	3.7	0.5836	0.6590	1.3822
ML R+N-P-	2.7	0.7917	0.6392	0.7426
ML R+N-P+	3.3	0.8956	0.7625	0.9137
ML R+N+P-	4.7	1.0512	0.9002	1.5537
ML R+N+P+	4.3	0.9266	0.9055	1.4880
CD (0.01)	NS	0.1474	0.2646	NS

Fig 9. COMBINED EFFECT OF *Rhizobium*, VAM AND FERTILIZER APPLICATION ON TOTAL CHLOROPHYLL CONTENT IN SUBABUL.

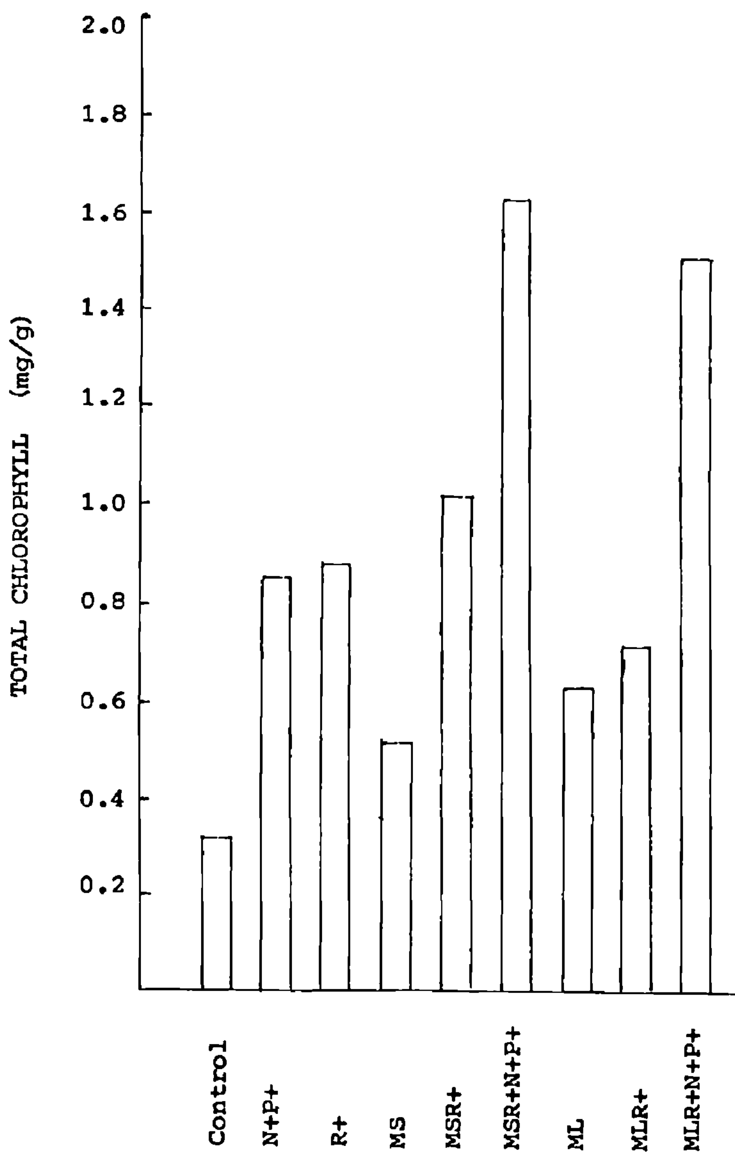


Table: 27 Effect VA mycorrhiza (M), *Rhizobium* (R), fertilizer nitrogen (N) and P_2O_5 (P) application on foliage colour of subabul

	R-	R+	N-	N+	P-	P+	Mean M-, MS, ML
M-	2.2	3.3	2.4	3.1	2.8	2.7	2.8
MS	2.4	3.7	2.9	3.2	3.1	3.0	3.0
ML	2.4	3.8	2.3	3.9	2.9	3.3	3.1
Mean	2.3	3.6	2.5	3.4	2.9	2.9	

	N-	N+	P-	P+
R-	1.6	3.1	2.2	2.5
R+	3.4	3.7	3.7	3.4

CD	(0.05)	(0.01)
R	-	0.48
NXM	-	0.83
NXR	-	0.68
N	-	0.48
Others	NS	

	P-	P+
N-	2.6	2.5
N+	3.3	3.4

significant due to Rhizobium and VA mycorrhiza inoculation and the application of nitrogenous fertiliser (Table 28). A similar trend was also observed in PxR, NxM, NxR and RxM interactions.

7.8 Chlorophyll-b content

Rhizobium, mycorrhiza and nitrogen treatments alone had a significant effect on chlorophyll-b content. Thus, the increase in chlorophyll-b content was significant due to Rhizobium and VA mycorrhiza inoculation and the application of nitrogenous fertiliser (Table 29). A similar trend was also observed in the RxM interaction.

7.9 Total chlorophyll content

All treatments involving inoculation of micro-symbionts and fertiliser application had a significant effect on total chlorophyll content. Thus, the increase in total chlorophyll content was significant due to Rhizobium and mycorrhiza inoculation and the application of nitrogenous and phosphorus fertilisers (Table 30). A similar trend was also observed in the RxM and NxM interactions.

Significant differences between treatments were also observed in the percentage nitrogen, protein and mimosine content of leaves. The percentage nitrogen and protein

Table: 28 Effect VA mycorrhiza (M) *Rhizobium* (R), fertilizer nitrogen (N) P_2O_5 (P) application on chlorophyll-a content (mg/g) of subabul

	R-	R+	N-	N+	P-	P+	Mean M-, MS, ML
M-	0.2665	0.6106	0.3330	0.5442	0.4239	0.4533	0.4386
MS	0.5203	0.8479	0.6335	0.7347	0.6583	0.7099	0.6841
ML	0.5691	0.8938	0.6559	0.8070	0.7610	0.7020	0.7315
Mean	0.4520	0.7841	0.5408	0.6953	0.6144	0.6217	

	N-	N+	P-	P+
R-	0.3940	0.5100	0.4505	0.4534
R+	0.6876	0.8807	0.7782	0.7900

	P-	P+
N-	0.5256	0.5560
N+	0.7032	0.6874

CD	(0.05)	(0.01)
M	-	0.0521
R	-	0.0425
NXM	0.0551	-
NXR	0.0450	-
N	-	0.0425
RXM	0.0551	-
PXR	-	0.0602
Others		NS

Table: 29 Effect VA mycorrhiza (M), Rhizobium (R), fertilizer nitrogen (N) P_2O_5 (P) application on chlorophyll-b content (mg/g) ofsubabul

	R-	R+	N-	N+	P-	P+	Mean M1 M2 M3
M-	0.5986	0.8323	0.5606	0.8701	0.7224	0.7084	0.7154
MS	0.8710	0.8246	0.7418	0.9537	0.8239	0.8717	0.8478
ML	0.6064	0.8019	0.6186	0.7897	0.6773	0.7309	0.7041
Mean	0.6920	0.8196	0.6403	0,8712	0.7412	0.7703	

	N-	N+	P-	P+
R-	0.5775	0.8064	0.6816	0.7023
R+	0.7032	0.9360	0.8007	0.8384

CD	(0.05)	(0.01)
M	-	0.0935
RXM	-	0.1323
R	-	0.0764
N	-	0.0764
Others		NS

	P-	P+
N-	0.6476	0.6331
N+	0.8348	0.9076

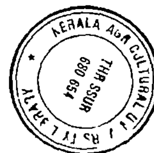
Table: 30 Effect VA mycorrhiza (M) *Rhizobium* (R), fertilizer nitrogen (N) and P_2O_5 (P) application on total Chlorophyll content (mg/g) of subabul.

	R-	R+	N-	N+	P-	P+	Mean of M-, MS, ML
M-	0.5787	1.1609	0.6842	1.0555	0.7981	0.9416	0.8698
MS	0.7607	1.2469	0.8909	1.1168	0.8734	1.1343	1.0038
ML	0.9476	1.1745	0.7299	1.3922	1.0176	1.1046	1.0611
Mean	0.7623	1.1941	0.7683	1.1881	0.8963	1.0602	

	N-	N+	P-	P+
R-	0.5307	0.9940	0.7117	0.8130
R+	1.0060	1.3823	1.0809	1.3073

	P-	P+
N-	0.6969	0.8398
N+	1.0957	1.2805

CD	(0.05)	(0.01)
M	-	0.1488
RXM	-	0.2105
R	-	0.1215
NXM	-	0.2105
N	-	0.1215
P	-	0.1215
Others	NS	



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content of 4.6 and 28.5 respectively were significantly high in the M-R+N+P+ treatment when compared to control and other treatments without Rhizobium inoculation and or the application of nitrogenous fertiliser (Table 31, Fig.10). The percentage phosphorus content of 0.28 was also maximum in the above treatment combination. The percentage mimosine content of 3.2 was significantly low in M- R- N- P-, MS R- N- P+, MS R+ N- P+ and ML R+ N- P- treatment combinations, when compared to most othe treatments.

The individual effects of Rhizobium VA mycorrhiza and fertiliser application on above observations are given below:

7.10 Nitrogen content of leaves

All treatments involving inoculation of microsymbionts and fertiliser application had a significant effect on the percentage nitrogen content of leaves. Thus, the increase in the nitrogen content was significant due to Rhizobium and mycorrhiza inoculation and the application of nitrogenous and phosphorus fertilisers (Table 32). A similar trend was also observed in the RxM, NxM, NxR and PxM interactions.

Table: 31 Combined effect of VA mycorrhiza, <u>Rhizobium</u> and fertilizer application on leaf nitrogen, protein, phosphorus and mimosine content of subabul under field conditions.

Treatment	% Leaf nitrogen content	% Leaf protein content	% Leaf Phosphorus content	% Mimosine content
M-R-N-P-	3.5	22.1	0.18	3.2
M-R-N-P+	3.7	22.9	0.28	3.4
M-R-N+P-	4.5	27.3	0.16	4.1
M-R-N+P+	4.5	28.4	0.26	3.9
M-R+N-P-	4.0	24.8	0.19	4.1
M-R+N-P+	4.4	27.1	0.27	3.9
M-R+N+P-	4.4	26.6	0.18	3.9
M-R+N+P+	4.6	28.5	0.28	3.5
MSR-N-P-	3.3	20.2	0.25	3.5
MSR-N-P+	3.5	21.9	0.25	3.2
MSR-N+P-	4.1	25.0	0.26	4.1
MSR-N+P+	4.2	26.9	0.26	3.8
MSR+N-P-	4.4	26.7	0.28	3.4
MSR+N-P+	4.5	27.8	0.24	3.2
MSR+N+P-	4.3	26.9	0.27	3.5
MSR+N+P+	4.2	26.3	0.27	3.6
MLR-N-P-	3.8	23.5	0.26	3.5
MLR-N-P+	3.7	23.2	0.26	3.5
MLR-N+P-	4.1	25.6	0.27	4.0
MLR-N+P+	4.1	25.6	0.28	3.7
MLR+N-P-	4.5	27.1	0.28	3.2
MLR+N-P+	4.5	27.1	0.26	3.6
MLR+N+P-	4.4	27.4	0.28	3.5
MLR+N+P+	4.3	26.8	0.28	3.8
CD (0.01)	0.3	1.8	NS	0.3

Fig10 COMBINED EFFECT OF *Rhizobium* /AM AND FERTILIZER APPLICATION ON LEAF PROTEIN CONTENT IN SUBABUL

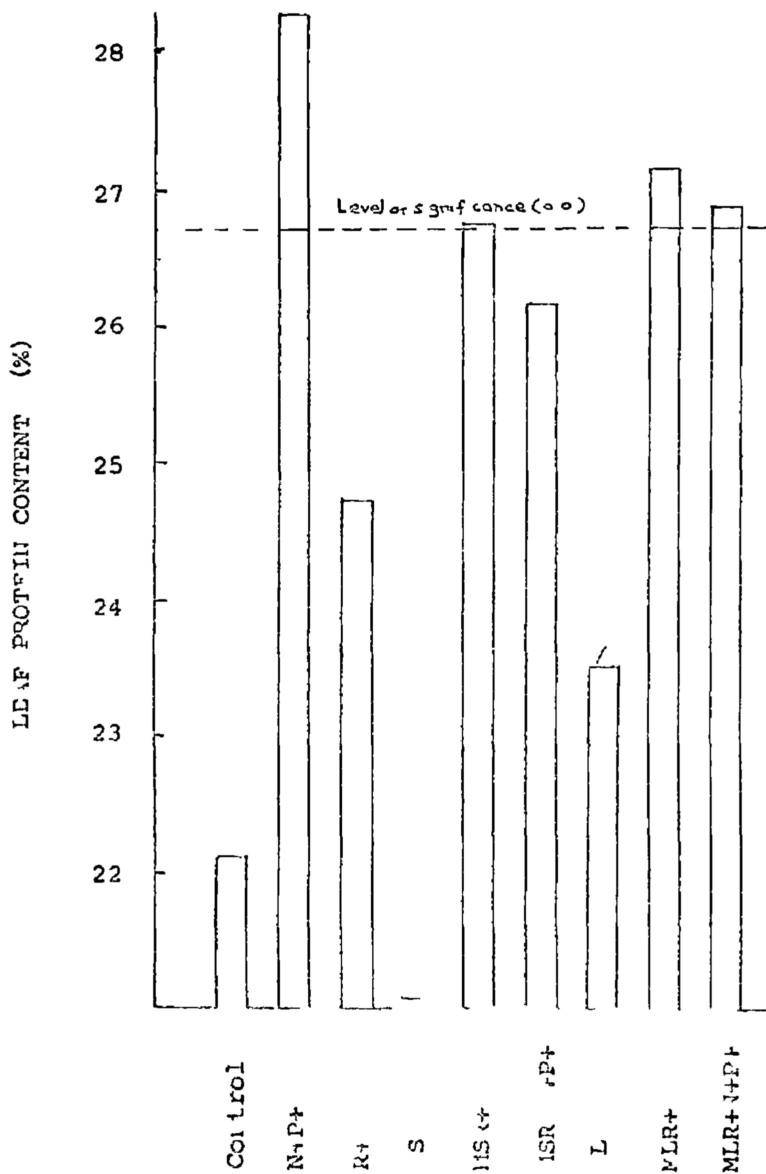


Table: 32 Effect VA mycorrhiza(M) Rhizobium (R), fertilizer nitrogen (N) and P_2O_5 (P) application on leaf N content (%) of subabul.

	R-	R+	N-	N+	P-	P+	Mean M MS ML
M-	4.05	4.33	3.90	4.49	4.08	4.31	4.20
MS	3.77	4.36	3.92	4.21	4.02	4.11	4.06
ML	3.93	4.41	4.12	4.23	4.18	4.16	4.17
Mean	3.91	4.37	3.98	4.31	4.09	4.19	

	N-	N+	P-	P+
R-	3.60	4.24	3.87	3.97
R+	4.36	4.37	4.32	4.42

	P-	P+
N-	3.90	4.06
N+	4.29	4.33

CD	(0.01)
M	0.1
RXM	0.13
R	0.08
NXM	0.13
NXR	0.11
N	0.08
PXM	0.13
P	0.08
Others	NS

7.11 Protein content of leaves

All treatments involving inoculation of micro-symbionts and fertiliser application had a significant effect on the percentage protein content of leaves. Thus, the increase in the protein content was significant due to Rhizobium and mycorrhiza inoculation and the application of nitrogenous and phosphorus fertilisers (Table 33). A similar trend was also observed in the RxM, NxM and PxM interactions.

7.12 Phosphorus content of leaves

Rhizobium mycorrhiza and phosphorus treatments alone had a significant effect on the percentage phosphorus content of leaves, Thus, the increase in phosphorus content was significant due to Rhizobium and mycorrhiza inoculation and the application of phosphorus fertiliser (Table 34). A similar trend was also observed in the NxM, PxM, PxN and PxR interactions.

7.13 Mimosine content

Mycorrhiza and nitrogen treatments alone had a significant effect on the percentage mimosine content of leaves. The decrease in mimosine content was highly significant due to mycorrhizal inoculation (Table 35, Fig.11). It was further observed that inoculation with the standard culture of VA mycorrhiza

Table: 33 Effect VA mycorrhiza (M), Rhizobium (R), fertilizer nitrogen (N) and P_2O_5 (P) application on percentage protein content of subabul.

	R-	R+	N-	N+	P-	P+	Mean of M- MS ML
M-	25.2	26.8	24.2	27.7	25.2	26.7	25.9
MS	23.5	26.9	24.2	26.3	24.7	25.7	25.2
ML	24.5	27.1	25.2	26.4	25.9	25.7	25.8
Mean	24.4	26.9	24.5	26.8	25.3	26.0	

	N-	N+	P-	P+
R-	22.3	26.5	23.9	24.8
R+	26.7	27.1	26.6	27.3

	P-	P+
N-	24.1	25.0
N+	26.5	27.1

CD	(0.05)	(0.01)
M	-	0.65
RXM	-	0.92
R	-	0.53
NXM	-	0.92
NXR	-	0.75
N	-	0.53
PXM	-	0.92
P	0.40	-

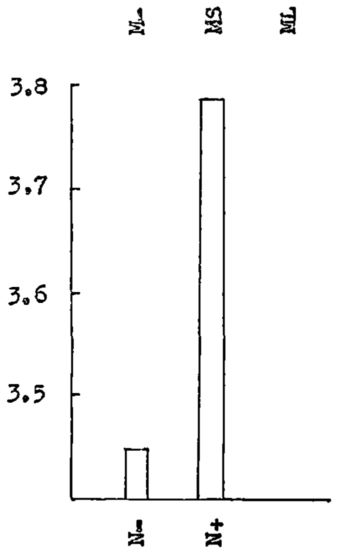
Table. 34 Effect VA mycorzhiza (M), *Rhizobium* (R), fertilizer nitrogen (N) and P_2O_5 (P) application on P_2O_5 Content (%) of subabul.

	R-	R+	N-	N+	P-	P+	Mean of M-, MS, ML
M-	0.22	0.23	0.23	0.22	0.18	0.27	0.22
MS	0.26	0.27	0.25	0.27	0.26	0.26	0.26
ML	0.28	0.27	0.27	0.28	0.28	0.27	0.27
Mean	0.25	0.26	0.25	0.25	0.24	0.26	

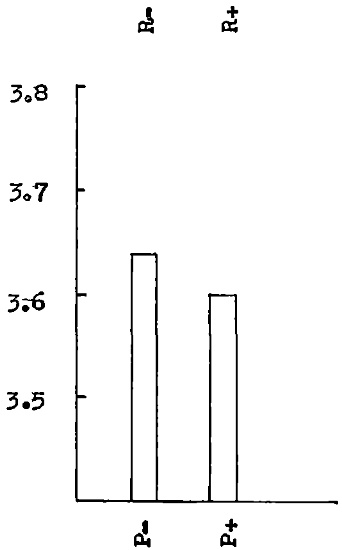
	N-	N+	P-	P+
R-	0.24	0.25	0.23	0.26
R+	0.26	0.26	0.25	0.27

	P-	P+
N-	0.24	0.26
N+	0.23	0.27

CD	(0.05)	(0.01)
M		0.0076
R		0.0061
NXM		0.0107
PXM		0.0107
PXR	0.0066	--
PXN		0.0087
P		0.0061
Others		NS.



% Mimosine content
3.8
3.7
3.6
3.5
N-
N+
M-
MS
ML



3.8
3.7
3.6
3.5
P-
P+
R-
R+

Fig. 11 Effect of Rhizobium, VA mycorrhiza and fertilizer application on mimosine content.

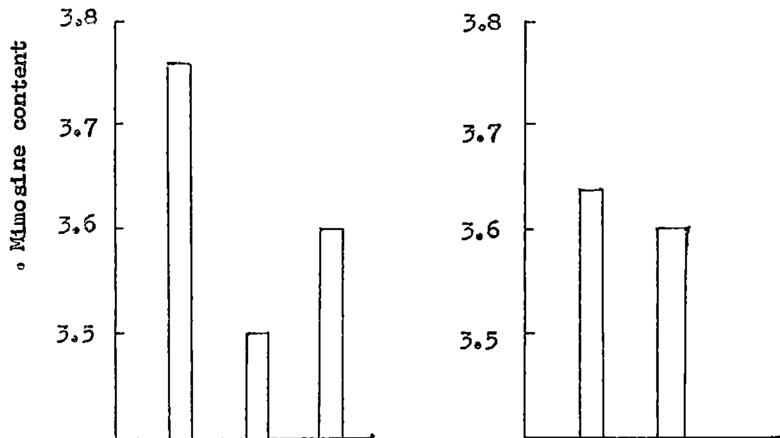


Table: 35 Effect of *Rhizobium* (R), VA mycorrhiza (M), fertilizer nitrogen (N) and P_2O_5 (P) application on mimosine content of subabul

	R-	R+	N-	N+	P-	P+	Mean MO, MS, ML
M-	3.68	3.84	3.66	3.87	3.83	3.70	3.76
MS	3.56	3.43	3.23	3.76	3.53	3.47	3.50
ML	3.67	3.52	3.45	3.74	3.56	3.63	3.60
Mean	3.64	3.60	3.45	3.79	3.64	3.60	

	N-	N+	P-	P+
R-	3.33	3.93	3.69	3.58
R+	3.56	3.63	3.59	3.61

	P-	P+
N-	3.43	3.47
N+	3.85	3.73

CD	(0.05)	(0.01)
M	-	0.11
R x M	-	0.15
N x M	-	0.15
N x R	-	0.12
N	-	0.09
P x M	-	0.15
P x N	0.09	-

(Glomus fasciculatus) resulted in maximum reduction in the mimosine content. The decrease in mimosine content was also significant in RxM, NxM, and PxM interactions. A reduction in mimosine content though nonsignificant was also obtained in the R+ and P+ treatments. However, a highly significant increase in mimosine content was observed due to application of nitrogenous fertiliser.

DISCUSSION

DISCUSSION

Agriculture began with the onset of human civilisation and since then the destiny of mankind has depended on food and fuel for himself and fodder for his domestic animals. In the beginning, land was cultivated for food purpose while the requirements of fuel and fodder were met mainly by forest vegetation and vast stretches of grazing lands within or outside human settlements. However, with the increase in population pressure the need for food grains, became so great that many of these exclusive areas for fuel and fodder got converted to agricultural land. This resulted in the elimination of vast stretches of our natural resources for fuel and fodder. It is in this context that the concept of an agricultural practice sustaining the ecology becomes important. How can we achieve this goal? We may succeed to some extent by growing high yielding varieties of crop plants and by cultivating wherever possible, plants like subabul that can be used both for fuel and feed purposes. In Kerala, the Gandhi Smarak Nidhi, Trivandrum, has been trying to popularise this crop for last few years. But probably due to the acidic nature of the soil in the State subabul has not come up well. One of the primary reasons cited for the non establishment of this plant in acidic soil is the lack of an efficient *Rhizobium*

symbiosis (Halliday, 1981). Besides the availability of some of the major plant nutrients like phosphorus will be low in such soil and for a plant without adequate root hairs, (Munns and Mosse, 1980) suitable VA mycorrhizal association will also be of great importance.

In the first part of this investigation, a survey was conducted at 17 different locations in four districts of Kerala namely, Idukki, Palghat, Trichur and Trivandrum (Fig. 1) to study the nature and intensity of nodulation and VA mycorrhizal infection in subabul. This showed that the extent of root nodulation by Rhizobium and root infection by mycorrhiza at all these locations were poor (Table 2). The average nodule number of 7.7 was maximum at Vellanikkara and Pattom while at other places such as Peerumedu and Kolahalamedu in Idukki district, the number of nodules formed per plant was as low as 1.5 and 1.7 respectively. Similarly the percentage of mycorrhizal infection was also relatively low at most of the survey locations. Maximum root infection of 25.5 percent was recorded at Dhoni in Palghat District. The reason for this poor nodulation and root infection by mycorrhiza at different survey locations can be the lack of sufficient number of suitable Rhizobium and VA mycorrhiza in the soil. It is under such circumstances, the need for inoculation of legumes with suitable Rhizobium

and mycorrhiza becomes important (Alexander 1961; Date, 1970 and Asai, 1944). The number of mycorrhizal spores present in the rhizosphere soil did not have any relation to root infection by VA mycorrhiza. Such lack of correlation between number of spores present in the soil and the extent of root infection has been reported earlier by Mosse and Bowen (1968).

As natural nodulation and root infection by Rhizobium and VA mycorrhiza was poor in all the four districts, rhizosphere samples from each survey location were collected and brought to laboratory for the isolation and characterisation of Rhizobium and VA mycorrhiza. In all 17 cultures of Rhizobium were isolated. They were all Gram negative with a fast rate of growth of 48-72 h on yeast extract mannitol agar medium (Table 3). The fast growing nature of subabul Rhizobium has been reported earlier by Trinnick (1980). Isolation of mycorrhizal spores was done only from one location in each district, Mattuppetty, Dhoni, Peechi and Amboori, where natural root infection by VA mycorrhiza was maximum. At these locations, the percentage of mycorrhizal infection was 20.5, 25.5, 24.5 and 19.5 respectively (Table 2). It was found that out of the four common genera of VA mycorrhiza commonly seen in soil, Glomus sp. was found associated with subabul at all the above locations. The identification was done mainly on the basis of spore

size, shape, colour, surface, texture, spore content and basal attachment (Table 4, Plate 2). The association of *Glomus* sp. with subabul has been observed earlier by Sivaprasad *et al*. (1983).

All the 17 isolates of rhizobia and the four selected VA mycorrhizal cultures were screened for nodulation and root infection efficiency under aseptic pot culture conditions using the K8 variety of subabul. The inoculation response of plants with the *Rhizobium* isolate R8 was superior to all other isolates including the three exotic strains from nif TAL, IARI and ICRISAT. Thus, the increase in nodule number, number of pink nodules formed per plant, nodule dry weight, shoot height, shoot dry weight percentage nitrogen and phosphorus content of leaves were significantly high in plants pretreated with this culture of *Rhizobium* (Table 5 - 12). Hence this isolate was selected for further studies under field conditions.

The type of variations recorded in the symbiotic efficiency of different isolates of rhizobia was similar to that observed earlier by Santhanakrishnan *et al*. (1980). It is for this reason, there is the need for conducting an initial screening of *Rhizobium* isolates before selecting an efficient culture for a particular crop. The beneficial effects of *Rhizobium* inoculation on nodulation and other

plant characters both under aseptic and field conditions are already well documented.

Among the four local cultures of VA mycorrhiza tested for efficiency, M1 and M2 cultures were found superior to the remaining two isolates. The percentage of mycorrhizal infection was maximum with M2 culture (Table 8). The interaction of this culture with _Rhizobium_ was also positive and more beneficial than other cultures of VA mycorrhiza tested. This was especially so in the interaction with the native isolate of _Rhizobium_ R8 and the exotic strain R18. In these beneficial interactions, the nodule number, number of pink nodules formed per plant, nodule dry weight, shoot height, shoot dry weight, percentage nitrogen, and phosphorus content of leaves were maximum (c f. Table 5-12). Hence this culture of VA mycorrhiza was selected for further studies under field conditions. As in the case of _Rhizobium_ inoculation the beneficial effects of VA mycorrhizal association in crop plants are also well documented.

Since one of the main objectives of this investigation was to develop an efficient native culture of _Rhizobium_ for subabul suitable for acidic soils, an _in vitro_ study for pH sensitivity using the R8 culture and seven other native and 3 exotic strains of _Rhizobium_ was conducted. The growth pattern of these cultures showed that they could

be grouped into two distinct categories as those which grew well at pH 6.0 and below such as the isolates, R6, R8, R10, R11, R13, R14, R18 and R19 and those which had maximum growth at pH 7.0 and above, like isolates R4, R5 and R20. (Table 13 and 14, Fig.4). The pH sensitivity of subabul *Rhizobium* has attracted considerable attention earlier also (Halliday, 1981, Ahamed and Ng, 1981). However, since most of these studies were conducted with *Rhizobium* isolated from plants growing in neutral to alkaline soil, the cultures were found to be more sensitive to acidic pH. But in the present investigation, it was observed that majority of the isolates tested including R8 culture could grow well at pH 6.0 and below. This may be due to the reason that they were initially isolated from plants growing in soil with a pH of 6.0 and below. This observation was further supported by the fact that isolate R5 from a neutral soil of pH of 7.1 did not grow well at low pH.

Rhizobium isolates R8 and R5 were further selected for studies on the effect of soil pH on root nodulation in subabul. The two levels of soil pH selected were 6.0 at 7.1 respectively which were identical to the pH of the soil from where the R8 and R5 cultures were initially isolated. The four mycorrhizal cultures, M1, M2, M3, and M4 were also used for this investigation. In general, it was observed that isolate R5 produced

more number of nodules at pH 7.1 while the other isolate R8 produced maximum number of nodules at pH 6.0 (Table 15). The effect of pH on root nodulation in subabul has been studied earlier also by Ahmad and Ng (1981). The two levels of pH chosen for this study had no significant effect on VA mycorrhiza. The root infection was maximum with M2 culture. Further, it was also observed that mycorrhizal inoculation had a favourable effect at both pH on nodule number, nodule dry weight, plant height and plant dry weight.

The individual effects of <u>Rhizobium</u>, VA mycorrhiza and pH on various plant characters were also studied. The effect of <u>Rhizobium</u> inoculation on nodulation, plant height and plant dry weight and that of VA mycorrhiza on nodule dry weight, mycorrhizal infection, plant height and plant dry weight were highly significant (Table 16). However, it was interesting to note that with the two <u>Rhizobium</u> isolates selected for this study, the variations in soil pH introduced, 6.0 and 7.1, were not critical in producing any significant difference in nodulation by these cultures. This in fact indicated that minor variations in soil pH are not going to affect root nodulation by <u>Rhizobium</u> in subabul even if their pH sensitivity under <u>in vitro</u> conditions are different. This can be due to a natural buffering action in the rhizosphere soil either by host mediated factors or by <u>Rhizobium</u> itself.

In this connection, it may be important to recollect the suggestion of Halliday (1981) that one should look for 'base exuding rhizobia to alleviate the acid soil stress on subabul Rhizobium symbiosis'.

The serological relation between isolate R8 and five other cultures, R5 (from neutral soil) R18, R19 and R20 (exotic strains) and R21 (isolate from Mimosa indica) was studied both by tube agglutination and immuno diffusion tests. Positive agglutination with heterologus isolates was obtained only with R18, R20 and R21 cultures (Table 17). This was further confirmed by immuno diffusion test except for the fact that no clear bands were visible with isolate R18 (Plate 5). It is likely that isolates R20 and R21 had more antigens in common with the R8 culture. However, from the nature of precipitation bands, they were only related but non identical strains. This type of sharing of common antigens by heterologus rhizobia with that of subabul is in agreement with the earlier work of Trinnick (1980). The remaining two cultures, R5 and R19 were apparently distinct strains as they did not show any positive reaction with the antiserum of R8 culture.

The Rhizobium culture R8 was also used to study the effect of age of host plant on root nodulation in subabul and nodule fine structure. It was observed that a visible indication of nodule formation was there only from 15th day of plant growth (Table 18 and Fig. 5). Afterwards, there was

an increase in nodule number till 70th day. This meant that in subabul, the initial nodulation phase was rather prolonged when compared to many other grain and other fodder legumes.

The fine structure studies of root nodules under a scanning electron microscope showed that during early stages of root infection by Rhizobium, bacteria was closely adhering to root surface probably between the cellulose macrofibrils (Plate 7a and 7b). It may be entering the root cortex for nodule initiation by this route as root hairs are virtually absent in the subabul. However, it must be pointed out that this is only an assumption made here and that it requires further confirmation. The response of cortical cells to Rhizobium infection was similar to that normally occur in other legumes. The bacterial infection resulted in the transformation of normal cortical cells to cells with irregular shape (Plate 8a and 8b). One could also visualise the presence of bacteroids within such cells (Plate 9a and 9b). The presence of bacteroids within nodular tissue indicated that the root nodules formed by isolate R8 was efficient and nitrogen fixing. The relation between the presence of bacteroid within nodule tissue and nitrogen fixation was first reported by Bergrson and Briggs (1958).

The nature of mycorrhizal infection was studied after inoculation with M2 culture. Photomicrographs clearly showed the typical nature of VA mycorrhizal infection by Glomus sp.

The distributive hyphae were more or less oriented towards the root cortex (Plate 11a). The vesicles were terminal and oval in shape. Besides they were intercellular.

In an attempt to standardise an efficient technique for inoculation of Rhizobium and VA mycorrhiza in subabul, seven different methods of dual inoculation, 10 days before sowing in polybag (T1), dual inoculation at sowing time in polybag (T2), dual inoculation 10 days after sowing in polybag (T3), dual inoculation 10 days before sowing in nursery (T4), dual inoculation at sowing time in nursary (T5), dual inoculation 10 days after sowing in nursary (T6) and dual inoculation at sowing time in main field (T7) were tried. Out of these, it was found that the number of nodules formed per plant and nodule dry weight were significantly high in T1 treatment while the extent of mycorrhizal infection, shoot height, shoot dry weight, percentage nitrogen and phosphorus content of leaves were maximum in T2 treatment (Table 19). The increase in mycorrhizal infection, shoot height and percentage phosphorus content of leaves in the T1 treatment were also significant and statistically on par with the T2 treatment. Therefore, both the above methods are equally effective for inoculation of subabul with Rhizobium and VA mycorrhiza. This can also ensure the 'incorporation' of efficient cultures of microsymbionts in subabul at seedling stage itself prior to transfer under field conditions.

However, the choice of the method may be left to organisations that are actively engaged in raising subabul seedlings in polybags for various purposes.

In the last part of the present investigation, the effects of VA mycorrhiza, Rhizobium and fertilizer application on nodulation, mycorrhizal infection and forage yield was studied under field conditions. Significant differences between treatments were observed in plant height, forage yield, chlorophyll a and b content, percentage nitrogen, protein and mimosine content of leaves (Table 20,26 and 31). However, a uniform treatment effect on various biometric observations taken was lacking. Hence the data is mainly interpreted on the basis of individual effects of different components used in various treatment combinations. Out of the 13 different observations taken, only those parameters related to nodule number, mycorrhizal infection, forage yield, total chlorophyll content, percentage nitrogen, protein, phosphorus and mimosine content of leaves which are commonly used to evaluate the beneficial effects of Rhizobium, VA mycorrhiza and fertilizer application and also the quality of fodder, are used for the above purpose.

It was observed that Rhizobium, nitrogen and phosphorus treatments alone had a significant effect on root nodulation in subabul. The increase in the number of nodules formed

was significant due to Rhizobium inoculation and application of nitrogenous fertilizer (Table 21). Such an effect was also seen in P- treatment. The positive effect of Rhizobium inoculation on root nodulation in legumes is already well established by many earlier workers like Chhonker and Negi (1971) in soybean, Ramachandran (1979) in cowpea, Santhanakrishnan et al.(1980), Moreno-Duiroz et al. (1983), Pahwa (1987) in subabul. However, the observed effect of nitrogen and ~~phosphate~~ treatment on nodulation was rather very unusual.

The increase in root infection by the use of both the standard (Glomus fasciculatum) as well as the local (Glomus sp.) cultures of VA mycorrhiza were significant with the effect being more pronounced in the application of local culture of VA mycorrhiza (Table 23). This may be due to the fact that the local isolate was more competitive in establishing an early root association in subabul.

Rhizobium, VA mycorrhiza and nitrogen treatments alone had a significant effect on forage yield (Table 25). The benefits of Rhizobium inoculation alone on plant growth is well documented. The positive effect of VA mycorrhizal association may be due to increased translocation of nutrients especially P from the soil. The role of mycorrhiza in enabling the host plant to absorb more nutrients like P, K, Fe and cu has been reported earlier by Mosse (1957) Baylis (1959) and Hayman and Mosse (1971).

Further, it has been observed that in many legumes such as soybean (Schenek and Hinson, 1973, Carling _et al._ 1978), _Centrosema pubesens_, _stylosanthes guanensis_, _Trifolim repens_, _Lotus pedunculatus_ (Crush, 1974), Phaseolus (Daft and Giahni, 1974), sweetvetch (Redente and Reeves, 1981), blackgram, green gram and cowpea (Ramraj and Shanmugom, 1986), the growth of the plant considerably improved due to combined inoculation of _Rhizobium_ and VA mycorrhiza. The beneficial effects of such dual inoculation of microsymbionts in subabul as observed here are almost similar to tnat reported earlier by Sivaprasad _et al._ (1983), Manjunath and Bagyraj (1984), and Kandaswamy _et al._ (1988).

The increase in the total chlorophyll content was significant due to _Rhizobium_ and mycorrhiza inoculation and the application nitrogen and phosphorus fertilizers (Table 30). Such an increase in chlorophyll content of leaf will have a positive effect on the over all photosynthetic efficiency which in fact is actually sustaining both the symbiotic organisms efficiently within the root system.

The increase in the nitrogen and protein content of leaves was significant due to _Rhizobium_ and mycorrhiza inoculation and the application of nitrogenous and phosphorus fertilizers (Table 32 and 33). Such an increase was also obtained in the percentage phosphorus content of leaves due

to *Rhizobium* and VA mycorrhiza inoculation and the application of phosphorus fertilizer. The increase in nitrogen, protein and phosphorus content of leaves are also indicative of the overall beneficial effects of *Rhizobium* and VA mycorrhiza inoculation and the application of fertilizer in subabul.

In the study on the effect of inoculation of both the microsymbionts and fertilizer application on mimosine content, it was observed that mycorrhiza and nitrogen treatments alone were significant on the percentage mimosine content of leaves. The decrease in mimosine content due to mycorrhizal inoculation was highly significant when compared to non mycorrhizal plants (Fig.35). This observation was further supported by the reduction in mimosine content obtained in various interactions such as R x M, N x M and P x M involving VA mycorrhiza. A slight reduction (though non significant) was also observed with *Rhizobium* inoculation and application of phosphorus fertilizer. But the increase in mimosine content was highly significant due to the application of nitrogenous fertilizer. The actual factor responsible for reduction in mimosine content due to mycorrhizal inoculation is to be identified. At the same time, it may be better to reduce the application of nitrogenous fertilizer for subabul cultivation. This means that an ideal package of practice for subabul will be dual inoculation with efficient cultures of *Rhizobium* and VA mycorrhiza in seedling stage along with adequate phosphate fertilization and need based application of nitrogenous fertilizer.

SUMMARY

SUMMARY

The survey for the occurrence of natural nodulation and VA mycorrhizal infection in subabul was conducted at 17 different locations of four districts of Kerala namely Idukki, Palghat, Trichur and Trivandrum. There was no significant difference between seedlings in the mean number of root nodules formed per plant at different locations. The average nodule number of 7.7 was maximum at Vellanikkara and Pattom while at other places such as Peerumedu and Kolahalamedu in Idukki district, the nodule number was only 1.5 and 1.7 respectively. There was also no significant difference between seedlings in the mean percentage of mycorrhizal infection. The mycorrhizal infection of 25.5 percent was maximum at Dhoni.

In all, 17 cultures of rhizobia serially numbered R1 to R17 were initially isolated. All these isolates were rod shaped and Gram negative with a fast rate of growth of 48 to 72 h on yeast extract mannitol agar medium. VA mycorrhizae were identified on the basis of their spore size, shape, colour, surface texture, spore content and mode of hyphal attachment. The spores were typical of <u>Glomus</u> sp. with an average size of 130 to 175 um. They were globose with simple basal attachment.

A pot culture experiment was conducted under aseptic conditions to study the efficiency of all the 17 native isolates of Rhizobium along with three exotic strains and the four selected cultures of VA mycorrhiza. There were significant differences between treatments in the number of nodules formed per plant. The mean nodule number of 16.73 was maximum in plants inoculated with the native isolate of Rhizobium R8. The effect of mycorrhizal inoculation on nodulation was also significant. Significant differences between treatments in the extent of root infection by VA mycorrhiza were also observed. The mean percentage root infection of 54.37 was maximum in plants inoculated with the M2 culture.

The mean dry weight of 5.44 g was maximum in plants inoculated with the native isolate of R8. This was significantly higher than control and all other treatments. The effect of mycorrhizal inoculation on dry weight of shoot was also significant. There were significant differences between treatments in the percentage nitrogen content of leaves. The mean nitrogen content of 4.24 percent was maximum in plants inoculated with the native isolate of Rhizobium R8. The mean phosphorus content of 0.24 percent was maximum in plants inoculated with the M2 culture of VA mycorrhiza.

In the study on pH sensitivity, it was possible to group various Rhizobium cultures into two district categories as

those which had maximum growth at pH 6.0 and below (isolate numbers, R6, R8, R10, R11, R13, R14, R18 and R19) and those which preferred a slightly higher pH of 7.0 and above (isolate numbers R4, R5 and R20) for optimum growth under in vitro conditions. The R5 isolate produced more number of nodules at pH 7.1 than at 6.0 while the isolate R8 produced more number of nodules at pH 6.0 instead of 7.1. In both the cases, the dry weight of nodules and the number of pink nodules formed followed a similar pattern. The percentage of mycorrhizal infection was significantly high due to inoculation with the M2 culture. This was maximum in the treatment combination of S1 R8 M2.

The serological characterisation of different isolates of Rhizobium showed that the homologus antigen R8 gave positive agglutination upto a serum dilution of 1:1280. A similar reaction with other antigens was observed only at a lesser dilution of the antiserum. These were 1:160 for R21, 1:80 for R18 and 1:40 for R20 isolates. However, the remaining two cultures, R5 and R19 showed no agglutination with the antiserum of R8. The results of the tube agglutination test were more or less confirmed by the immuno diffusion test except for the fact that no clear bands were formed with R5, R18 and R19 antigens.

The effect of age of plant on root nodulation in subabul indicated that any visible initiation of nodulation was there only from 15th day of plant growth. This was followed by a period of gradual increase in nodule number till 70th day. Afterwards, there was not much variation in the number of nodules formed in subabul. In the scanning electron microscopy of root nodules of subabul, it was found that during the early stages of root infection, Rhizobium was found closely associated with the root surface. Later such cells were found between the cellulose fibrils prior to entry into the root tissue. The cortical cells responded in a typical manner towards Rhizobium infection. In this process, the normal cells after bacterial infection got transformed into cells with an irregular shape. The presence of bacteroid within such cells is shown in plates 9a and 9b. Observation of stained root samples for VA mycorrhiza under a light microscope showed the presence of mycorrhiza only in the root cortex. The distributive hyphae were found to grow almost parallel to the root axis, occupying much of the primary cortex depending on the degree of root infection. The vesicles formed were typically terminal and oval in shape. They did not show any intra cellular penetration of cortical cells.

Significant differences between various methods of inoculation of microsymbionts were observed only in nodule fresh weight, percentage VAM infection, shoot height, shoot fresh weight and percentage phosphorus content of leaves. Between various treatments, the beneficial effects were more pronounced with the use of microsymbionts either 10days before sowing or at the time of sowing in polybag.

In the study on the combined effects of VA mycorrhiza, Rhizobium and fertilizer application on nodulation, VA mycorrhizal infection and forage yield in subabul, significant differences between treatments were observed in plant height and forage yield. The number of nodules formed, nodule fresh weight and percentage of mycorrhizal infection were maximum in the M - R + N + P -, ML R + N + R and ML R - N - P - treatments respectively. The plant height was significantly high in the ML R + N + P - treatment when compared to control and other treatments except the MS R + N - P - and MS R + N + P - treatments. The forage yield of 915 g per plot was maximum in the ML R + N - P - treatment. Significant differences between treatments were also observed in chlorophyll-a and chlorophyll-b content of leaves. Similarly, significant differences between treatments were also observed in the percentage nitrogen, protein and mimosine content of leaves. The percentage nitrogen and protein content were significantly high in the M - R + N + P + treatment when compared to control

and other treatments without *Rhizobium* inoculation and or the application of nitrogenous fertilizer. The percentage phosphorus content was also maximum in the above treatment combination.

Mycorrhiza and nitrogen treatments had significant effect on the percentage mimosine content of leaves. The decrease in mimosine content was highly significant due to mycorrhizal inoculation. It was further observed that inoculation with the standard culture of VA mycorrhiza (*Glomus fasciculatum*) resulted in maximum reduction in the mimosine content. A reduction in mimosine content though nonsignificant was also obtained in the R + and P + treatments. However, a highly significant increase in mimosine content was observed due to application of nitrogenous fertilizer.

REFERENCE

REFERENCES

Ahmad, N. and Ng, F.S.P. (1981). Growth of Lucaena leucocephala in relation to soil pH, nutrient levels and Rhizobium concentration. Leucaena Res. Rept., 2 : 5-9.

Albrecht, W.A. (1933). Inoculation of legumes as related to soil acidity. J. Amer. Soc. Agron., 25 : 512-522.

Alexander, M. (1961). In Introduction to Soil Microbiology, John Wiley and Sons, Inc., New York and London. pp 472.

Allen, O.N. (1953). Experiments in Soil Bacteriology. Burgees Publ. Co. Minneapolis Minn. USA., I Ed. pp.69-70.

Alvarez, Racelis, E. and Baugaloyos, A.P. (1977). Pre germination treatment for ipil-ipil. Canopy. 3 (10):11.

Andrew, C.S. (1976). Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate pasture legumes on nodulation and growth. Aust. J. Agric. Res., 29: 611-623.

* Asai.T, (1944), Uber die Mykorrhizen bildung der leguminosen Pflanzen, Jap. J. Bot., 13 : 463-485.

Bagyaraj, D.J. (1989). Role of VA mycorrhiza in red soils. Mycorrhiza News, 1 (1): 6-7.

Bajpai, P.D., Lehri, L.K. and Pathak, A.N. (1974). Effect of seed inoculation with Rhizobium strains on the yield of leguminous crops. Proc. Ind. Nat. Sci. Acad., 40 : 571-575.

Bagyaraj, D.J. and Manjunath, A. (1980). Response of crop plants to VA mycorrhizal inoculation in an unsterile Indian soil. New. Phytol. 85: 33-36.

Bagyaraj, D.J., Manjunath, A. and Patil, R.B. (1979). Interaction between vesicular-arbuscular mycorrhizae and Rhizobium and their effect on soybean in the field. New Phytol. 82 : 141-145.

*Baltruschat, H. (1987). Field inoculation of maize with vesicular-arbuscular mycorrhizal fungi by using expanded clay as carrier material for mycorrhiza. Zeitschrift fur Pflanzenkrankheifen und Pflanzenschutz., 94 (4) : 419-430.

Barber, L.E. (1980). Enumeration effectiveness and pH resistance of Rhizobium meliloti population in Oregon soils. Soil Sci. Soc. Amer.J., 44 : 3-4.

Barrow, N.J., Malajezuk, N. and Shaw, T.C. (1977). A direct best of the ability of vesicular-arbuscular mycorrhiza to help plants take up fixed soil phosphate. New Phytol., 78: 269-276.

Basavaraju, V. and Hegde, S.V. (1983). Native nodulation of Leucaena in Karnataka (INDIA). Leucaena Res. Rept., 4 : 17.

Baylis, G.T.S., (1959). The effect of vesicular-arbusecular mycorrhizas on growth of Griselinia littoralis (Cornaceae). New Phytol. 58 : 274-280.

Bergersen, F.J. (1974). Formation and function of bacteroid. In: Biology of nitrogen fixation. Ed. Quispel, A., North-Holland Publishing Company Amsterdam pp 473-498.

Bergersen, F.J. and Briggs, M.J. (1958). Studies on the bacterial component of soybean root nodules; cytology and organization in the host root tissue. J. Gen. Microbiol. 19: 482-490.

Bethlenflavay, G.J. Brown, M.S. and Pacovsky, R.S. (1982). Parasitic and mutualistic associations between a mycorrhizal fungus and soybean development of the host plant. Phytopathol. 72 : 889-893.

*Bohlool, B.B. and Schmidt, E.L. (1973). Persistance and competition aspects of Rhizobium japonicum observed in soil by immuno fluorescnce microscopy. Proc. Soil Sci. Amer., 37 : 561-564.

Brewbaker, James, L. and Stevekaye (1981). Mimosine variations in species of the genera Leucaena. Leucaena Res. Rept., 2 : 66-68.

Brockwell, J. Schwinghamer, E.A. and Goult, R.R. (1977). Ecological studies of root nodule bacteria introduced into field environments II. A critical examination of the suitability of antigenic and streptomycin markers for identifications of strains of Rhizobium trifolii. Soil Biol. Biochem., 9 : 19-24.

Bushnell, O.A. and Sarles, W.B. (1939). Investigation upon the antigenic relationships among the root nodule bacteria of soybean, cowpea and lupine cross inoculation group. J. Bact. 38 : 401-410.

Carling, D.E. Richle, W.O., Brown, N.F. and Johnson, D.R.(1978), Effects of vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nondulating soybeans. Phytopathology, 68 : 1590-1596.

*Charudattan, B. and Hubbel, D.H. (1973). The presence and possible significance of cross reactive antigens in Rhizobium legume associations. Antonie van Leewenhock. 39 : 619-627.

Chhonkar, P.K. and Negi. P.S. (1971). Response of soybean to rhizobial inoculation with different strains of Rhizobium japonicum. Indian J. agric. Sci., 41 (9) : 741-744.

Crush, J.R. (1974). Plant growth responses to vesicular-arbuscular mycorrhiza VII. Growth and nodulation of some herbage legumes. New Phytol., 73 : 743-749.

Dadarwal, K.R. Shashi Prabha and Tauro, P. (1976). Varietal differences with regard to Rhizobium compatibility and efficiency in nitrogen fixation of chick pea. Proceedings of National Symp. on Nitrogen Assimilation and Crop Productivity pp. 235-243.

Daft, M.J. and El-Giahmi, A.A. (1974). Effect of Glomus infection on three legumes. In Endomycorrhizas (ed). Sanders, F.E., Mosse, B. and Tinker, P.B., Academic Press, London, pp. 581-592.

Damirigi, S.M., Federick, L.R. and Andersen, J.C. (1967). Serogroups of Rhizobium japonicum in soybean nodules as affected by soil types. Agron.J., 59 : 10-12.

*Dart, P.J. and Mercer, F.V. (1963). Development of bacteriod in root nodule of Barrel medica (Medicago tribuloides) and subterraneum clover (Trifolium subterraneum L.) Arch. Microbiol., 46 . 382-401.

Date, R.A. (1970). Microbiological problems in the inoculation and nodulation of legumes. Pl. Soil., 32 : 703-725.

De la Garza, H., Valdas, M. and Aguirre, J.F. (1987). Effect of Rhizobium strains, phosphorus and soil type on nodulation and growth of Leucaena leucocephala. Leucaena Res. Rept., 8 : 42-43.

Dudman, W.F. (1964). Immuno-diffusion analysis of extra cellular soluble antigens of two strains of Rhizobium meliloti, J. Bact., 88 : 782-844.

Dutt, A.K. and Urmila Pathania (1983). Response of Leucaena to rhizobial inoculation. Leucaena Res. Rept., 4 : 18.

Evans, L.S., Levin, K.F. and Vella, F.A. (1980). Effect of nutrient medium pH on symbiotic nitrogen fixation by Rhizobium leguminosarum and Pisum sativum. Pl. Soil, 56 : 71-80.

Farinas, E.C. (1951). Ipil-ipil, the alfalfa of the tropics : its establishment, culture and utilisation as fodder and pasture crop. Phil. Jour. Anim. Ind. 12 : 65-85.

Gerdemann, J.W. (1975). Vesicular-arbuscular mucorrhizae. In Development and Function of Roots. (Ed.) J.G. Torrey and D.T. Clarkson, Academic Press, London, pp 575-591.

Gerdemann, J.W. and Trappe, J.M. (1974). Taxonomy of the endogonaceae. In : Endomycorrhizas (Ed. sanders, P.E., Mosse, B. and Tinker, P.B.). Academic Press, London. pp. 35-51.

Gilmore. A.E. (1971). The influence of endotropic mycorrhiza on the growth of peach seedlings. J. Am.Soc.Hort. Sci., 96 : 35-38.

Girija, V.K. and Nair, S.K. (1985). Occurrence of Vesicular-arbuscular mycorrhiza in certain crop plants of Kerala. Agric. Res. J. Kerala. 23 (2) : 185-188.

Girija, V.K. and Nair, S.K. (1988). Incidence of VAM in banana varieties. Indian J. Microbiol. 28 (4) : 294-295.

Goodchild, D.J. and Bergersen, F.J. (1966). Electron microscopy of the infection and subsequent development of soybean nodule cells. J. Bacteriol., 92 : 204-213.

Gray, S.G. (1968). A review of research on Leucaena leucocephala. Trop. Grasslands. 2 (1) : 19-30.

Gray, L.B. and Gerdemann, J.W. (1969). Uptake of ^{32}P by vesicular-arbuscular mycorrhiza. Pl.Soil. 80: 415-422.

Gray, L.B. and Gerdemann, J.W. (1973). Uptake of sulphur-35 by vasicular-arbuscular mycorrhiza. Pl. Soil, 39 : 687-689.

Guycox and Tinker, P.B. (1976). Translocation and transfer of nutrient in vesicular-arbuscular mycorrhizas. I. The arbuscles and phosphorus transfer. A. quantitative ultra structural study. New Phytol., 77 : 371-378.

Jackson, M.L. (1967). Soil Chemical Analysis. Prentice Hall of India (Pvt.) Ltd., New Delhi.

Jensen, H.L. (1982) Vesicular-arbuscular mycorrhiza in the field grown barley. J. Agron.Crop.Sci. 145 : 75-78.

Johnson, H.W. and Means, U.M. (1964). Selection of competitive strains of soybean nodulating bacteria. Agron. J., 56 : 60-62.

Jones, F.R. (1924). A mycorrhizal fungus in the roots of legumes and some other plants. J. Agril.Res. 29 : 459-480.

Joshi, U.N. Arorra, S.K., Paroda, and Saini, M.L. (1985). Positional effect on the chemical composition of Leucaena leaves. Leucaena Res. Rept. 4 : 24.

Kandasamy, D., Rangarajan, M., Oblisamy, G., Narayanan, R. and Ponniah, C. (1988). Mycorrhizal occurrence and their effect on certain forest plant species. In Proceedings on Mycorrhiza Round Table held at New Delhi - 13-15 March, 1987.

Khan, A.G. (1972). The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. I. Effect on maize growth. New Phytol. 71 : 613-619.

Khan, A.G. (1974). The occurrence of mycorrhizae in halophytes, hydrophytes and xerophytes and of endogone spores in adjacent soils. J. Gen. Microbiol. 81 : 7-14.

Khan, A.G. (1978). VA mycorrhizas in plants colonizing black wastes from bitaminous coal mines in the Illawara regions of New South Wales. New Phytol., 81 : 53-63.

* Klimmer, M. and Kruger, R. (1914). Sind debeiden verschiednen Legumi nosen geb unden knollehem - backlarien ant verechieden. Contbl. Backt. Abt., 240 : 256-265.

Koontz, P.J. and Faber, S. (1961). Detection of soluble Rhizobium japonicum antigens in soil by immuno - diffusion Soil. Biol. Biochem. 10 : 247-250.

Koucheki, H.K. and Read, D.J. (1976). Vesicular-arbuscular mycorrhiza in natural vegetation systems. II. The relationship between infection and growth in Festuca ovina L. New Phytol. 77 : 655-666.

Kremer, R.J. and Wagner, G.H. (1978). Detection of soluble Rhizobium japonicum antigens in soil by immuno-diffusion. Soil.Biol. Biochem. 10 : 247-250.

Krishnamurthy, K. and Mune Gowda, M.K. (1983). Mimosine concentration in Leucaena cultivars. Leucaena Res. Rept. 4 . 27.

La-Rue, J.H. Maclean, W.D. and Peakcock, W.L. (1975). Mycorrhizal fungi and peach nursery nutrition. Calif. Agric. 29 : 7.

Manjunath, A., Bagyaraj, D.J. (1984). Dual inoculation with VA mycorrhiza and Rhizobium is beneficial to Leucaena. Plant and Soil. 78 : 445-448.

Meyer, J.R. and Linderman, R.G. (1986). Response of Subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and plant growth promoting bacterium Pseudomonas putida. Soil Biol. Biochem., 18: 185-190.

Moreno- Duiroz, A.R., Ferrera - Cerrato and Nenez - Escobar. R. (1983). Effect of inoculation with different Rhizobium sp. strain in Leucaena leucocephala. Leucaena Res.Rept., 4 : 57-58.

Mosse, B. (1957). Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature 179 : 922-924.

Mosse, B. and Bowen, G. D. (1968). The distribution of Endogone spores in some Australian and New Zealand Soils and in an experimental field soil at Rothamsted. Trans. of British Mycological Society, 51 : 485-492.

Mosse, B., Powell, C.Ll and Hayman, D.S. (1976). Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interaction between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76 : 331-342.

Munns, D.N. and Mosse, B. (1980). Mineral nutrition of legume crops. In Advances in legume science Eds. R.J. Summer field and A.H. Bunting. Royal Botanic Garden, Kew, England.

Muthusamy, S. (1970). Studies of Rhizobium of groundnut. M.Sc. Thesis. TNAU, Coimbatore.

Nair, S.K. and Girija , V.K. (1988). Incidence of Vesicular-arbuscular mycorrhiza in certain tree crops of Kerala. Journal of Plantation Crops. 16 (1): 67-68.

Nair, S.K. and Sivaprasad, P. (1981). Preliminary study on nodulation in cowpea in acid soil. Agric. Res. J. Kerala, 19 : 60-62.

NAS, (1977). Leucaena Promising Forage and Tree Crop for the Tropics. National Academy of Sciences. Washington D.C. pp 1-115.

Pahwa, M.R. (1987). Comparative effects of dingle and multiple strains of Rhizobium on Leucaena leucocephala (Var.28). Leucaena Res.Rept., 8 : 33.

Pandher, M.S. and Kahlon, S.S. (1978). pH and salt tolerance of Rhizobium leguminosarum isolates from pea (Pisum sativum). Indian J. Microbiol., 18 : 81-84.

Parke, T.L., Linderman, R.G. and Black, C.H. (1983). The role of ectomycorrhiza in drought tolerance of Douglas fir seedlings. New Phytol. 95 : 83-95.

Pathak, P.S. and Patil, B.D. (1980). Fuel wood and forage production from Leucaena leucocephala. Leucaena Newsletter. 1 (11).

Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans.Br. Mycol.Soc. 55 : 158-160.

Potty, V.P. (1978). Occurrence of vesicular-arbuscular mycorrhiza in certain tuber crops. J. Root Crops 4 : 49-50.

Powell, C.Ll and Jeanette, D. (1978). Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from phosphate deficient soil. New Phytol. 80 : 351-358.

Rai, R., Singh, S.N. and Murtuza, M.D. (1977). Different responses of Rhizobium strains of bengal gram grain yield. Curr. Sci., 46 (6) : 572-573.

Rajagopalan, T. (1938). Studies on groundnut root nodule organism. I. Isolation of strain and study of culture. Indian J. agric. Sci. 8 : 331-348.

Ramachandran, K. (1979). Studies on cowpea Rhizobium with special reference to mass culture technique using indigenous carriers. M.Sc.(Ag.) thesis submitted to Kerala Agricultural University.pp. 85.

Ramaraj, B. and Shanmugam,N. (1986). Growth responses of vesicular-arbuscular mycorrhizae on pulses. Madras Agric. J., 73 (1) : 32-35.

Redente, E.F., Reeves, F.B. (1981). Interaction between vesicular-arbuscular mycorrhiza and Rhizobium and their effect on sweetvetch growth. Soil Sci., 132 : 410-415.

Rosas, H., Quintero, S.O., and Gomez, J. (1980). Mimosine disappearance in arboreus leucaena silage. Leucaena Newsletter. 1 (17).

Ross, J.P. and Gilliam, J.H. (1973). Division S-4 Soil fertility and plant nutrition. Effect of Endogone mycorrhiza on phosphorous uptake by soybean from inorganic phosphates. Soil.Sci.Soc.Am.Proc. 37 : 237-239.

*Safir, G.R. and Nelsen, C.E. (1985). VA mycorrhizas plant and fungal water relations. In Proceedings of 6th American Conference on Mycorrhizae, June 25-29, pp. 161-164.

Sahu, S.K. and Behera, B. (1972). Note on the effect of Rhizobium inoculation on cowpea groundnut and green gram. Indian. J. Agron., 17 : 359-360.

Saif, S.R. and Khan, A.G. (1977). The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. III. Effects on Barley growth. Pl. Soil. 47 : 17-26.

Sanders, F.E. and Tinker, P.B. (1971). Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature (Lond.) 233 : 278-279.

Sanni, S.O. (1976), Vesicular-arbuscular mycorrhiza in some Nigerian soils and their effect on the growth of cowpea (Vigna unguiculata) tomato (Lycopersicon esculentum) and maize (Zea mays). New Phytol. 77 : 667-671.

Santhanakrishnan, D., Vaidhyanathan, P. and Oblisami, G. (1980). Effect of Rhizobium strains on Leucaena seedlings. Abstract of papers presented in a National Symposium on Biological Nitrogen Fixation in relation to crop production held at TNAU, Coimbatore.

Schenek, N.C. and Hinson, K. (1973). Response of nodulating and non-nodulating soybeans to a species of Endogone mycorrhiza. Agron. J. 65 : 849-850.

Sheth, R.D. (1979). Studies on the development of Rhizobium inoculants for groundnut. Ph.D. thesis, UAS, Bangalore.

Sivaprasad, P., Hegde, S.V. and Rai, P.V. (1983). Effect of Rhizobium and mycorrhiza inoculation on growth of Leucaena. Leucaena Res. Rept., 4 : 42.

Sivaprasad, P. and Rai, P.V. (1987) Mechanism of enhanced nodulation in vesicular-arbuscular mycorrhial(VAM) pigeon pea (Cajanus cajan (L.). Agric.Res.J. Kerala, 25 (1) : 99-102.

*Skrdleta, V. (1973). Competition between inoculum strains of R. japonicum in the process of soybean nodulation during three planting period. Folia Microbiol., 18 : 341-47.

Smith, G.S. (1988). The role of phosphorus nutrition in interactions of Vesicular-arbuscular mycorrhizal fungi with soil borne nematodes and gunfi. Phytopathol., 78 (3) : 371-374.

Smith, S.E. and Daft, M.J. (1977). Interaction between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*. *Aust.J.Pl. Physiol.* **4** : 403-413.

Snedecor, W.G. and Cochran, G.W. (1967). *Statistical methods*. Oxford and IBH Publ. Co., New Delhi.

Sondergaird, M. and Laegard, S. (1977). VA mycorrhiza in some aquatic crop plants. *Nature* **268** : 232-233.

Stevens, J.W. (1923). Can all strains of a specific organism be recognised by agglutination ? *J. Infect. Dis.*, **33** : 557-566.

Sulochana, K.K. and Nair, S.K. (1985). Occurrence of VAM in some local cultivars of tapioca. In *Proceedings of the National Symposium on Tropical Tuber Crops production and utilization*, held at CTCRI, Trivandrum, Nov., 27-29, 1985.

Sutton, J.C. and Sheppard, B.R. (1976). Aggregation of sand dune soil by endomycorrhizal fungi, *Can.J.Bot.* **54**: 326-333.

Trinnick, M.J. (1980). Relationship amongst the fast growing rhizobia of *Lablab purpurens*, *Leucaena leucocephala*, *Mimosa* sp., *Acacia farnesiana* and *Sesbania grandiflora* and their affinities with other rhizobial groups. *J. Appli. Bacteriol.* **49** : 39-53.

Tripathi, R.S. Dubey, C.S. Khan, A.W. and Agarwal, K.B. (1975). Effect of application of *Rhizobium* inoculum on the yield of gram varieties in chambal command area of Rajasthan *Sci. Cult.* **41** (6) : 266-269.

Van Schreven, D.A. (1972), Some factors affecting the uptake of nitrogen by legumes. In Nutrition of the legumes (Ed.E.G.Hallsworth), p.137 London: Butterworth.

*Vincent, J.M.(1941). Serological studies of the root nodule bacteria I. strains of Rhizobium meliloti. Proc.Linn. Soc. (N.S.W), 66 : 145-154.

Vincent, J.M.(1970). A manual for the practical study of the Root-nodule Bacteria. IBP - Handbook No.15. Blackwell Scientific Publications, Oxford. pp.196.

Vincent, J.M. and Humphrey, B.A. (1970) Taxonomically significant group of antigens in Rhizobium. J. Gen. Microbiol. 63 : 78-82.

Yadav, N.K. and Vyas, S.R. (1973). Effect of salt and pH on the growth of Rhizobium strains. Indian J. Microbiol. 11 : 97-102.

Yost, R.S. and Fox, R.L. (1982). Influence of mycorrhizae on the mineral content of cowpea and soybean. Agron.J., 74 : 475-481.

Young, Chiu - Chung and Chen-Ching Chao (1983). Effect of liming and Rhizobial inoculant on seedling growth of Leucaena leucocephala in the field experiment. Leucaena Res. Rept., 4 : 73-74.

*Zipfel, H. (1912). Beilrange Eumorphologie and Biologieder Knollehen bakterein deteguminosen. Zent-11. Bakt. Parasit. Ked. Abst. 11. 32 : 97-137.

*Originals not seen

APPENDICES

APPENDIX - I

Summary of Analysis of Variance of preliminary screening of <u>Rhizobium</u> and VA mycorrhiza for nodulation, mycorrhizal infection and growth of subabul.

Source	df	Mean Square							
		Nodule number	No.of pink nodules	Dry weight of nodules	% VAM infection	Shoot height	Dry weight of plant	% Nitrogen content	% Phosphorus content
Mycorrhiza (M)	4	86.154**	58.059**	0.063**	26128.1**	514.74**	3.264**	3.358**	0.029**
<u>Rhizobium</u> (R)	20	135.342**	44.589**	0.115**	549.5**	360.74**	3.301**	3.412**	0.002**
M x R	80	22.499**	11.909**	0.023**	385.3**	159.84**	0.966**	0.891*	0.001**
Error	210	13.187	6.235	0.008	105.6	4.22	0.042	0.039	0.000

* Significant at 5% level

** Significant at 1% level

APPENDIX - II

Summary of Analysis of variance of interaction effect of soil pH, <u>Rhizobium</u> inoculation and mycorrhizal inoculation on growth of subabul.

Source	df	Mean Square					
		Nodule number	Nodule dry weight	No.of pink nodules	% VAM infection	Plant height	Plant dry weight
Soil pH (S)	1	3.60	0.003	0.90	2.50	22.50*	1.79**
Rhizobium (R)	2	600.74**	0.655**	154.84**	8.61	30.34**	3.50**
S x R	2	225.03**	0.434**	50.80**	110.83	73.63**	3.78**
Mycorrhiza (M)	4	5.87	0.022**	4.92	4144.03**	50.71**	0.30**
S x M	4	13.18*	0.007	13.21**	473.92**	22.19**	0.12**
R x M	8	7.61	0.010	3.98	82.57	33.21**	0.11**
S x R x M	8	7.28	0.019	4.11	95.21*	23.25**	0.09**
Error	60	5.13	0.006	2.84	38.89	3.38	

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

APPENDIX - III

Summary of Analysis of variance of the effect of different methods of inoculation of *Rhizobium* and VA mycorrhiza on growth of subabul.

Source	df	Mean Square						
		Nodule number	Dry weight of nodules	% VAM infection	Plant height	Weight of plant	% nitrogen content	% P_2O_5 content
Block	3	2.52	0.009	193.75	7.37	0.007	0.016	0.000
Treatment	6	60.82	0.073*	204.17*	93.74**	0.588**	0.039	0.002**
Error	18	27.99	0.025	73.61	9.45	0.076	0.015	0.000

* Significant at 5% level

** Significant at 1% level

APPENDIX - IV (a)

Summary of Analysis of variance of combined effect of Rhizobium, VA mycorrhiza and fertiliser application on nodulation, VAM infection and forage yield of subabul.

Source	df	Mean Square						
		Nodule number	Nodule fresh weight	% VAM infection	Plant height	Forage yield	Foliage colour score	Chlorophyll-a content
Block	2	8.17	0.0135	22.11	5.01	17.72	0.29	0.0079
M	2	18.29	0.0127	12041.70**	2030.10**	1060.45**	0.79	0.5932**
M x R	2	13.18	0.0095	17.17	110.09**	112.64**	4.17	0.0006
R	1	2069.38**	2.4605**	11.00	1088.88**	304.22**	28.13**	1.9856**
M x N	2	1.63	0.0001	29.54	10.93	33.22**	3.18**	0.0182*
R x N	1	20.05	0.0142	3.26	0.50	337.13**	6.13**	0.0267*
N	1	40.50*	0.0378*	4.15	296.05**	22.22*	13.35**	0.4297**
M x P	2	20.09	0.0218	10.22	114.59**	128.34**	0.43	0.0205*
R x P	1	16.05	0.0396*	54.69	2.00	0.84	1.68	0.0003**
N x P	1	6.72	0.0074	9.03	1.39	5.91	0.12	0.0096
P	1	46.72*	0.0561*	3.71	22.22*	0.56	1.39	0.0009
Interactions	9	7.30	0.0087	57.51	29.62**	39.56**	0.88	0.0262**
Error	46	6.51	0.0093	59.07	4.48	5.04	0.57	0.0045

* Significant at 5% level

** Significant at 1% level

APPENDIX - IV (b)

Summary of Analysis of variance of combined effect of *Rhizobium*, VA mycorrhiza and fertilizer application on nodulation, VAM infection and forage yield of subabul.

Source	df	Mean Square					
		Chlorophyll-b content	Total Chlorophyll	% nitrogen content	% Protein content	% P_2O_5	% Mimosine content
Block	2	0.0424	0.0441	0.027	1.92	0.00056	0.043
M	2	0.1531**	0.2312**	0.120**	3.64**	0.01404**	0.436**
M x R	2	0.1384**	0.2027**	0.154**	4.92	0.00008	0.170**
R	1	0.2932**	3.3560**	3.600**	115.29**	0.00190**	0.023
M x N	2	0.0303	0.2962**	0.357**	8.27**	0.00101**	0.162**
R x N	1	0.00007	0.0339	1.837**	65.99**	0.0000009	1.253**
N	1	0.9590**	3.1722**	1.967**	91.55**	0.000311	2.101**
M x P	2	0.0084	0.0472	0.094**	4.93**	0.02077**	0.072*
R x P	1	0.0013	0.0705	0.0	0.12	0.00061*	0.073
N x P	1	0.0343	0.0079	0.061	0.44	0.00151**	0.117*
P	1	0.0153	0.4830**	0.170**	11.11**	0.0159**	0.031
Interaction	9	0.0683	0.0611	0.045**	1.88*	0.00016	0.148**
Error	46	0.0145	0.0367	0.015	0.69	0.00009	0.019

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

SYMBIOSIS OF *RHIZOBIUM* AND VA MYCORRHIZA IN SUBABUL

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ABSTRACT

A survey was conducted at 17 locations in four districts of Kerala for natural nodulation and VA mycorrhizal infection in subabul. The survey revealed that natural nodulation and VA mycorrhizal infection were poor compared to inoculated plants. When all the 17 isolates of rhizobia and four cultures of VA mycorrhizal fungus were tested for effectiveness, the rhizobial isolate R8 and VAM fungus M2 were emerged as most efficient rhizobial and mycorrhizal cultures respectively.

An *in vitro* study conducted revealed that in an acid pH of 6, the rhizobial isolate R8 survived better than other cultures. At pH 8, growth of another isolate R5 was found maximum. However, in an *in vivo* study, there was not much significance for the soil pH ranging from 6 to 7.1 in influencing various biometric parameters of subabul. In both the pH of 6 and 7.1, the performance of rhizobial isolate R5 and mycorrhizal culture M2 was best.

Serological studies revealed that the exotic strains R18, R20 and and isolate from *Mimosa indica* had serological similarities with the best selected local isolate R8.

Fine structure studies of nodules clearly showed the morphological differences between the uninfected nodular tissues and the infected central nodular tissue. The rhizobial infection transformed the normal cells into irregularly shaped cells within which numerous rhizobial cells were visible.

In another observation, it was found that subabul plants starts to form nodules only from 15 days of sowing. Thereafter, the nodule number increased steadily attaining the peak at 70 days of growth and then remained more or less steady.

Among various methods of inoculation of the microsymbionts tested, inoculation of both the microsymbionts at the time of sowing in polybag was found good for the better establishment of the plants in the field.

In a field study, it was found that inoculation of the local isolate of Rhizobium R8 and mycorrhizal fungus M2 had great influence in increasing all the growth parameters. Standard mycorrhizal culture and local isolate performed equally well. Maximum forage yield was obtained when plants were inoculated with the selected local rhizobial isolate R8

and mycorrhizal fungus M2. Maximum mycorrhizal infection was also seen in the same treatment. Dual inoculation also had significant influence in increasing the leaf protein content. An important observation was that both rhizobial and mycorrhizal inoculation reduced the mimosine content of leaves. However, fertilizer nitrogen increased mimosine content.

In short, dual inoculation by *Rhizobium* and VA mycorrhiza was found necessary for better establishment, growth and low mimosine content of subabul.