NODULATION BEHAVIOUR OF *Acacia mangium* Willd. IN RESPONSE TO *Rhizobium* INOCULATION

By
DHANESHKUMAR, P.

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Forestry

Faculty of Agriculture
Kerala Agricultural University

Department of Tree Physiology and Breeding
COLLEGE OF FORESTRY
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DECLARATION

I hereby declare that this thesis entitled **"Nodulation behaviour of *Acacia mangium* Willd. in response to *Rhizobium* inoculation"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Place : *Vellanikkara*
Date : 26-12-98

P. Dhaneshkumar

DHANESHKUMAR, P.

DR. P.K. ASHOKAN
Associate Professor
Department of Tree Physiology & Breeding

College of Forestry
Kerala Agrl. University
Vellanikkara, Thrissur

CERTIFICATE

Certified that this thesis entitled **"Nodulation behaviour of *Acacia mangium* Willd. in response to *Rhizobium* inoculation"** is a record of research work done by Sri. **DHANESHKUMAR, P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Place : *Vellanikkara*
Date : 26-12-'18

DR. P.K. ASHOKAN
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of Sri. **DHANESHKUMAR, P.** a candidate for the Degree of **Master of Science in Forestry,** agree that this thesis entitled **"Nodulation behaviour of *Acacia mangium* Willd. in response to *Rhizobium* inoculation"** may be submitted by Sri. **DHANESHKUMAR, P.** in partial fulfilment of the requirement for the Degree.

DR. P.K. ASHOKAN
(Chairman, Advisory Committee)
Associate Professor
Department of Tree Physiology and Breeding
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

DR. LUCKINS C. BABU
(Member, Advisory Committee)
Associate Dean
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

DR. N.K. VIJAYAKUMAR
(Member, Advisory Committee)
Associate Professor & Head
Dept. of Wood Science
College of Forestry
Kerala Agricultural. University
Vellanikkara, Thrissur

DR. M. BALASUNDARAN
(Member, Advisory Committee)
Scientist
Division of Pathology
Kerala Forest Research Institute
Peechi, Thrissur

EXTERNAL EXAMINER
INDU CHAUDAN. N-C.
SCIENTIST-IN-CHARGE
K F R I
Chand... ... ...ssur

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DHANESHKUMAR, P.

To
my parents

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Introduction

INTRODUCTION

Acacia mangium Willd. (mangium) is an important nitrogen fixing tree (NFT) suitable for reforestation/afforestation of degraded lands. It has excellent ability to survive on eroded, rocky, shallow, infertile and acidic soils. The tree is also tolerant to impeded drainage and some water logging. (Turnbull, 1986). Mangium has shown promise as a timber, pulp, furniture, veneer and fuel wood species. The wood is also suitable for manufacture of high-quality particle board. Mangium is indigenous to North Eastern Australia, Papua New Guinea and Eastern Indonesia. It has proved to be successful in a number of countries in the humid tropics, due to its versatility and ability to survive under degraded lands (Awang and Taylor, 1993). There are a few reports of mangium being used in integrated planting systems like agroforestry (Nair *et al.*,1984). Recently, interest in this species has increased dramatically in Kerala also and a number of large and small scale mangium plantations are coming up in the State.

An effective symbiotic association between the N_2-fixing bacteria, *Rhizobium* and mangium is necessary for deciding the success of the species in degraded sites. NFTs, unlike crop legumes were believed to be promiscuous and nodulated by any strain of rhizobia. However, recent studies indicate that, there is specificity among many NFTs and there is wide variation among rhizobial strains in their N_2-fixing efficiency. Mangium is reported to be more specific in its rhizobial associations than other Acacias (Dart *et al.*, 1991). Eventhough rhizobia are generally found in all types of soil, symbiotic associations may not be established automatically by plants introduced to a new site (Sanginga *et al.*, 1987). This is usually attributed to the absence of effective strains of the symbionts in the particular soil or unfavourable

soil pH, one of the serious impediments for N_2- fixation. However, it has been reported that, there are strains of rhizobia which can tolerate low pH and fix N_2 efficiently in high acidic soils (Keyser and Munns, 1979). Deficiencies of soil mineral nutrients also is a major constraint on symbiotic N_2-fixation in many tropical legumes. Hence identification of suitable strains of rhizobia for a particular ecological niche holds the key for achieving proper establishment and growth of the tree.

In Kerala, inspite of the recent boom in area planted with mangium, no attempt has been made to identify effective rhizobial strains suited to the species. Isolation and screening of rhizobia, effective on mangium are the first step to produce rhizobial cultures which may help to improve establishment, growth and productivity of mangium in the state.

Considering these, investigations were carried out to isolate rhizobia from mangium growing in different agroclimatic regions of Kerala and to compare their efficiency in nodulation and N_2-fixation. The relative efficiency of selected rhizobial isolates under varying soil fertility and soil acidity were also investigated.

REVIEW OF LITERATURE

Nitrogen is an essential plant nutrient abundant in atmosphere, but limiting in soil. Although all plants need nitrogen, the amount available for the plant uptake are limited (Stevenson, 1982). Fixation of otherwise unavailable but abundant, atmospheric nitrogen by micro-organisms has an important role in agro-ecosystems as well as forest ecosystems. Global biological N_2-fixation is estimated to be between 139-170 million t yr^{-1} while the nitrogen fertilizer produced annually is only about 65 million t (Paul, 1988). Thus biological process represents the major contributor to the ecological nitrogen cycle (Newton and Burgess, 1983).

The importance of legumes in building and conserving soil fertility has been recognized since the beginning of agriculture. However, scientific demonstration of the value of legumes in contributing to the nitrogen nutrition of plants was done only in the latter half of 19 century (Fred et al., 1932), when it was conclusively established that, nodules on legume roots were responsible for fixing atmospheric N through the bacterium *Rhizobium.*

Biological fixation of atmospheric N (BNF) is a "free" source of nitrogen for all ecosystems. High cost and environmental problems arising out of the use of inorganic fertilizer have sparked a renewed interest in BNF. Compared to using inorganic nitrogen fertilizers, BNF can provide a more regular supply of N (Henzell, 1977).

BNF contributes to productivity both directly, where the fixed N is harvested in grain or other food for human or animal consumption or indirectly, by contributing to the maintenance or enhancement of soil fertility in the agricultural system by

addition of N to the soil (Giller, *et al.* 1994).

2.1. Biological N_2- fixation in woody perennial

Although systematic BNF investigations on perennial species began much later than annual crops, the literature on them has been growing rapidly. In the recent years, more emphasis is being given to fertility maintenance and improvement of indigenous systems, in which trees and shrubs which dominate the bush fallows have come in to focus as effective soil fertility restorers (Wilson and Kang 1980). A major reason for this upsurge in interest is that, Nitrogen Fixing Trees (NFTs) are key components of forestry and agroforestry programmes in the tropics (Daniel, 1994). Like legumes in agriculture, legume trees play important roles in terms of N-input to forest lands and also in nitrogen cycling (Garcia *et al.*, 1988). Kabi *et al.* (1982) investigated the effects of rhizobial inoculation on the growth and nodulation of some important forest legumes and concluded that, use of BNF in forestry has a tremendous potential for enhanced N turnover from the atmosphere in to the biosphere.

Since last two decades many attempts have been made to study the growth and biomass production of many indigenous multipurpose tree species under diverse soil and climatic environments. A large number of woody legume species used for a wide range of purpose have been studied (Sprent and Sutherland, 1990).

2.2. Use of Nitrogen Fixing Trees in reforestation of degraded and marginal sites

The problem of salinization / alkalization occurs in varying intensities in more than 120 countries, extending to all the continents, and is more prominently witnessed in arid and semiarid areas. The nitrogen status of these soils is poor and

the most appropriate solution to this situation is to cultivate NFTs that are able to fix atmospheric nitrogen through symbiotic systems (Sane, 1987).

NFTs are an ideal group of trees for afforesting degraded sites (Mac Dicken, 1994) and play an important role in land reclamation and soil enrichment (Punj *et al.*, 1988). The capacity to fix atmospheric nitrogen allows NFTs to establish and grow in ecosystems with little soil nitrogen (Rundel *et al.*, 1982). This may explain their predominance in nitrogen poor natural ecosystems, such as rain forests and deserts. To minimize or eliminate the need for nitrogen fertilization of marginal grassland areas, the planting of tree legumes seems ideal (Manguiat *et al.*, 1984). These trees can also help to control erosion when properly used and some of NFTs which are tolerant to acid, alkaline or saline soils and may provide ground cover for soils which might other wise remain worthless wasteland (Mac Dicken, 1988).

Tree species that has attracted a lot of attention during the past is *Leucaena leucocephala*. According to National Academy of Sciences (1977), It is known to be the best forest tree for its quick growth and multiple uses.

The ability of many acacias to increase the N content of soil through their interaction with symbiotic bacteria augments their value for planting for soil stabilization and for other purposes (Longkamp *et al.* 1982). According to Delacruz and Garcia (1992), *Acacia mangium*'s popularity is partly due to its ability to establish on adverse sites. Miller (1989) reported that, mangium has the ability to survive on eroded, rock, shallow, infertile, acidic soils. It is also tolerant of impeded drainage and can tolerate some water logging. It grow well in deeply weathered or alluvial soils and has the ability to compete and grow reasonably well where competition is severe, such as imperata grass lands (Awang *et al.* , 1993).

Several studies have prompted use of nodulated and mycorrhizal legume trees to revegetate poor or depleted soils with the goal of restoring their fertility. Experiments with indigenous and introduced legume tree species have been successful in revegetating exposed sub soil, open mining areas and acidic residues from bauxite mining without addition of organic soil (Franco *et al.*, 1997).

2.3. NFTs in agroforestry

One of the advantages commonly attributed to agroforestry technologies is the potential for soil fertility improvement by more efficient cycling of nutrients, and it is often recommended that, nitrogen fixing trees and shrubs should be included in such technologies (Roskoski, 1981, Dommergues, 1987). According to Brewbaker *et al.* (1982) and Nair *et al.* (1984), much interest has been generated in the production and use of NFTs in farming systems and that is due to their ability to fix N that can improve tree, crop and animal production in a sustainable manner. Realising the limited potential to improve the production of food crop annuals in areas with soil of low fertility without high capital inputs, and greater awareness of the ecological fragility of such areas, increased attention has been paid primarily to fast growing leguminous trees such as acacias (Benge, 1989).

Twenty NFTs are studied by Liyanage *et al.* (1989) and among them, six are identified as promising for coconut based farming systems on the basis of green manure and wood yields. They are *Acacia auriculiformis*, *A. mangium*, *Leucaena leucocephala*, *Gliricidia sepium*, *Enterolobium cyclocarpum* and *Calliandra calothyrsus*.

Handawela *et al.* (1989) found that intercropping sesame and maize with

Glyricidia sepium and malching between the crop rows with tree loppings, increased crop yields and soil fertility.

Alley cropping is being widely tested in the tropics for its potential to sustain adequate food production with low agricultural inputs, while conserving the resource base. Fast growth and N yield of most trees used as hedgerows in alley cropping is greatly due to their ability to fix N symbiotically. Most suitable tree species for alley cropping are *L. leucocephala*, *A. mangium* and *G. sepium* which can derive between 100 and 300 kg N ha^{-1} yr^{-1} (Sanginga *et al.*, 1995). Kadiata *et al.* (1996) identified *L. leucocephala*, *L. diversifolia*, *Albizia lebbeck*, *Tephrosia candida*, *G. sepium* and *Cajanus cajan* as new candidate species for alley cropping on the acid Ultisol and non - acid Alfisol.

2.4. Rhizobial characteristics

Nitrogen fixation in tree species require the formation of root nodules in association with a symbiotic micro-organism. The micro-organism responsible for nodulation and nitrogen fixation is *Rhizobium* in Leguminosae and Ulmaceae families and an actinomycete of the genus *Frankia* in all other nodulating families (Brewbaker *et al.* 1990 ; Aspiras and Cruz, 1988).

Tropical legumes may nodulate with either of the two types of rhizobia. One is fast growing strains which belong to the genus *Rhizobium* and the other slow growing strains of the cowpea miscellany type, designated *Bradyrhizobium* (Elkan, 1984).

Dommergues (1987) identified three categories of tropical legumes. Among these two of categories which nodulate specifically with one of the two rhizobia

types, and the third category nodulate with both types. Both *Acacia auriculiformis* and *A. mangium* form effective nitrogen fixing nodules only with *Bradyrhizobium* strains (Galiana *et al.*, 1990), but *Leucaena* requires fast growing strains (Halliday, 1981). Species differences have been reported in their nodulation ability with the two rhizobia types (Dreyfus and Dommergues, 1981).

Awonaike *et al.* (1992) suggested that, the BNF of *G. sepium* in tropical soils in which *Bradyrhizobium* predominate can further be enhanced by inoculation with appropriate fast growing *Rhizobium* strains.

Three genera of rhizobia have now been characterised, *Rhizobium, Bradyrhizobium* (Jordan, 1984) and *Azorhizobium* (Drefus *et al.,* 1988). The former two genera were root nodulating and the later stem nodulating. In *Rhizobium* the major species recognized are *R. leguminosarum* with three biovars (phaseoli, trifolii and vicieae), *R. loti, R. meliloti* and *R. galegae* (Lindstrom and Lehtomaki, 1988). New species of *Rhizobium* later identified were *R. huacki* nodulating *Astragalus sinicus* and *R. tropici* (Miller and Wilson, 1991).

According to Allen and Allen (1950), the term "fast growers" commonly designated the rhizobia associated with alfalfa, clover, bean and pea, because in culture these grow much faster (less than one half the generation time) than the "slow growers" exemplified by soyabean, cowpea and lupine rhizobia. Ayanaba (1977) reported that, in most tropical soils *Bradyrhizobium* predominate. Fast growers show visible growth in yeast Mannitol Agar in 2-3 days while slow growers require 6-8 days for similar growth.

Vincent (1977) surveyed the literature and included some members of the

cowpea miscellany in the fast growers group. The slow growers include two reasonably well-defined subgroups, *R. lupini* and *R. japonicum* and a large number of relatively poorly defined members of the cowpea miscellany.

On the basis of a series of studies involving numerical taxonomy, DNA base ratio, DNA hybridization, Cistron similarities, serology, composition of extracellular gum, carbohydrate utilization and metabolism, bacteriophage sensitivity, antibiotic sensitivity, protein composition, and type of bacteroid inclusion bodies, all of the slow growing, non acid - producing root nodule bacteria have been placed in a new genus, *Bradyrhizobium,* seperate from the fast growing, acid producing root nodule bacteria (Jordan, 1982). The genus *Bradyrhizobium* represents an exceedingly heterogenous group of nodule bacteria within which the taxonomic relationships are not well understood (Zhou *et al.*, 1987). Barnet *et al.* (1991) assign four extremely slow growers to the genus *Bradyrhizobium.* Several reports have described an intermediate group between *Rhizobium* and *Bradyrhizobium* (Broughton *et al.*, 1984) but Padmanabhan *et al.* (1990) continue to assign these variant strains to either *Rhizobium* or *Bradyrhizobium.*

The taxonomic position of root nodule bacteria from tree legume is uncertain. Strains of rhizobia from members of tree legumes were usually cross infective (Zhou and Han, 1984). Rhizobia from woody species have been described as fast, moderately, slow, very slow and extremely slow growers (Barnet and Catt, 1991;Jordan, 1984). Many leguminous NFTs are nodulated by bacteria of the species *Rhizobium loti*, which contains a group of bacteria whose host range are not fully known (Singleton *et al.*, 1992). Great variation exists among *Rhizobium* species from different regions that nodulate *Robina psuedoacacia* (Batzli *et al.*, 1992).

Odee *et al.* (1997) isolated more than 48 rhizobia from root nodules of woody legume and herbaceous host species grown in soils collected from 12 different Kenyan sites. They showed a wide range of growth rates, very fast growing (mean generation time 1.6 - 2.5 h), fast growing (2.8-4.8h), intermediate between fast and slow growing (5.6 - 5.7h) and slow and very slow growing (6.4 - 8.8 h). The isolates were tentatively grouped in to *Rhizobium spp.*, to include very fast, fast and intermediate (acid producing) types, and *Bradyrhizobium* spp., to include very slow, slow and intermediate (alkali producing) types.

2.5. Host specificity of rhizobia to nitrogen fixation

Root nodule bacteria are often specific to a certain plant genus or a group of related genera. Infection and nodule formation are host specific phenomena. Legume species or cultivars which are nodulated by some rhizobial isolates are not nodulated by other isolates. Burton (1967) has attributed the host specificity of rhizobia and occurrence of strains. The difference in N_2-fixation due to the varietal differences has been reported by several workers. Due to strain specificity only a few per cent of the infected root hairs may give rise to nodules.

It has been reported recently that, host-bacterium symbiosis may be determined by the binding of host legume lectins (glyceroproteins) to characteristic carbohydrate receptors on the *Rhizobium* cell surface (Tilak, 1993). The lectin present on root hair serves as target cells for infection (Stacey *et al.*, 1980). But not all legume seed lectins specifically recognize the corresponding rhizobial symbiont (Pueppke *et al.*, 1980).

NFTs, unlike crop legumes were believed to be promiscuous and nodulated

by any strain. However, recent studies indicate that, there is specificity among many NFTs. Sutherland *et al.* (1993) have reported that, tree-*Rhizobium* associations can be highly specific and range of specifications found will be similar to that found in herbaceous plants. Habish and Khairi (1970) found that, of 10 Acacia species occuring in Sudan, some are nodulated by *Bradyrhizobium* others by *Rhizobium* species. Some species nodulated freely with rhizobial isolates from other species, others only nodulated with a very restricted range of isolates. Allen and Allen (1981) found that, in *Desmanthus virgatus*, effective nodulation was obtained with rhizobia isolated from *Prosopis chilensis, Leucaena leucocephala* and *Mimosa invisa*, in effective nodulation with isolates from *Mimosa pudica* and no nodulation with isolates from soybean.

Drefus and Dommergues (1981) observed that some African species nodulated effectively only with slow growing *Bradyrhizobium* strains (e.g. *Acacia albida*) whereas others only nodulated with fast-growing *Rhizobium* strains (e.g. *A. nilotica*, *A. raddiana, A. senegal*). *Acacia seyal* was effectively nodulated by both type of rhizobia.

Duchateau *et al.* (1989) conducted tests of 13 *Rhizobium* strains isolated from various nitrogen fixing tree species and observed that the *Rhizobium* / *L. leucocephala* reaction was not specific. Even among the strains that nodulate a particular tree species, their effectiveness varies. Studies of Sanginga *et al.* (1991) with *Gliricidia sepium* showed that, in the same provenance, depending upon the *Rhizobium* strain, the amount of N fixed by a plant varied between 20 mg per plant to 129 mg per plant, 14 weeks after planting. Turk and Keyser (1992) performed a series of inoculation experiments under growth room and green house conditions to

delineate rhizobial specificity of a variety of tree legumes. *Gliricidia sepium*, *Calliandra calothyrus* and *Leucaena leucocephala* nodulated effectively with rhizobia isolated from each of the three genera. With a few exceptions, *Sesbania grandiflora* and *Robinia psuedoacacia* nodulated effectively, only with rhizobial strains isolated from each genus respectively. A range of specificity was found among species that nodulate with *Bradyrhizoboium. Acacia mearnsii* nodulated with most strains but fixed N effectively with relatively few. *Acacia mangium* was specific for both nodulation and effectiveness.

Specificity among Australian Acacia species was comprehensively studied by Thompson *et al.* (1985), reported by Roughley (1987), who inoculated 63 species of Acacia with 20 strains of *Rhizobium* and 18 strains of *Bradyrhizobium*. The majority (70 per cent) of Acacia species nodulated with 75-100 per cent of *Rhizobium* strains. *Bradyrhizobium* strains were even more promiscuous. There were however, species differences.

Using eight *Bradyrhizobium* and two *Rhizobium* strains, Galiana *et al.* (1990) found that *A. mangium* and *A. auriculiformis* could nodulate effectively only with the former, with *A. mangium* having the more specific requirement. Miller *et al.* (1991) found that some Australian soils contained a varied populations of strains of *Rhizobium. A. mangium* nodulated and fixed nitrogen poorly with two of the four strain's tested, only three out of 33 isolates from different locations were effective. Jisheng Sun *et al.* (1991) demonstrated interactions between *Rhizobium* inoculant strains and *A. mangium* provenance, particularly with varying levels of phosphorus application.

2.5.1. Cross-inoculation

The leguminous species develop preference for certain kinds of bacteria and vice versa. The earlier theories on the interspecificity of rhizobia to nodulate the species were soon tested and found incorrect. By 1900, it became apparent that, there were groups of legumes each of which were nodulated by same type of *Rhizobium*. Each of these groups of legumes and their specific rhizobia constituted a "cross-inoculation group" and it was generally held (i) that with each cross-incoulation group, a *Rhizobium* isolated for one legume member of the group would nodulate all other members of the group, and (ii) that rhizobia isolated from one plant in cross-inoculation group would not nodulate plants from other groups. Such observations led to the concept that plants mutually susceptible to nodulation by a particular kind of rhizobia constituted a cross-inoculation group (Fred *et al.*, 1932). They recognised eight major cross-inoculation groups (alfaalfa, *R. meliloti*; clover, *R. trifoli*; Pea, *R. leguminosarum*; bean *R. phaseoli;* Lupin *R. lupini;* soybean, *R. japonicum;* cowpea, *Rhizobium sp.;* Lotus, *Rhizobium sp.;* and eight minor groups.

Habish *et al.* (1970) describes cross inoculation tests on 21 species. Results showed that, they may be grouped by the accepted system of classification, but there were inconsistencies in the cowpea group. Cross-inoculation tests among 10 *Acacia* spp. and cowpea (*Vigna Sinensis*) showed that the nodulation of cowpea with rhizobia from 8 of the *Acacia spp,* though non-reciprocal in the same cases, supported the placement of *Acacia* in the cowpea cross inoculation group. The cross inoculation grouping system is not perfect, since rhizobia have often been found to cross-infect between groups. Gaur *et al.* (1974) have reported existence of double promiscuity in groundnut rhizobia symbiosis. Promiscuity between cowpea

rhizobia and *R. japoricum* has been observed by Barua and Bhaduri (1967). They reported that, rhizobia isolated from soybean usually nodulate soybean and cowpea, whereas most cowpea rhizobia do not nodulate soybean.

Han *et al.* (1991); Zhou and Han, (1984) have reported that, strains of rhizobia from members of tree legumes tested were usually cross infective with low host specificity, cross inoculation was readily attained with established members of the so called cowpea group. A cross-inoculation study with eight strains of indigenous rhizobia was carried out on just germinated seedlings of the seven host legume tree species from which they had originally been isolated. The legumes were *Acacia albida*, *A. mearnsii*, *Calliandra calothyrsus*, *Leucaena leucocephala*, *Prosopis juliflora*, *Sesbania grandiflora* and *S. sesban*. After two weeks seedlings were visually rated, and shoot dry weights measured, in order to determine the effectiveness of the test combinations. The species varied in their promiscuousness with their rhizobial associates. The *Sesbania spp.* only nodulated and grew well with their own isolates, while *P. juliflora* produced a partially effective association only with the isolates from *C. calothyrsus*. The remaining species produced effective or partially effective combinations with several isolates and in effective ones (same not even nodulated) with others (Odee, 1989). Iqbal and Mahmood (1992) studied the response of *Leucaena leucocephala* to *inoculation* with rhizobia from tropical legumes like *Albizia lebbeck*, *Clitoria ternatea*, *Medicago sativa*, *Pithecellobium dulce*, *Sesbania sesban*, *Vigna ungiculata* and groundnut and found that with the exception of groundnut all isolates produced nodules on *L. leucocephala*. Isolates from *V. ungiculata*, *A. lebbeck* and P. dulce were most effective in N. fixation and significantly increased host plant N content. Study conducted by Bekunda (1993) in seven NFTs (*Faiderbia albida*, *Acacia senegal*, *A avriculiformis*, *Calliandra*

calothyrus, Glyricidia sepium, Leucaena leucocephala, and *Prosopis juliflova*) revealed that, all the seedlings inoculated with species - specific rhizobia showed significant total N yield and nitrogen derived from the atmosphere increased except for *F. albida* and *A. auriculiformis, G. sepium* and *L. leucocephala* genotypes. Jamaluddin *et al.* (1995) tested the effects of pure cultures of *Rhizobium* strains from the nodules of different forest tree species (*Dalbergia sisoo, Leucaena leucocephala, Pongamia pinnata* and *Albizia lebbek* on the growth of *A. lebbek* seedlings in the nursery. Shoot length and girth, fresh and dry weight and nitrogen content of seedlings, and number of nodules were all greater in inoculated seedlings than in non-inoculated seedlings, but inoculation with the *Rhizobium* strain from *A. lebbek* was more effective than with *Rhizobium* strains from the other legume species.

2.6. Competition with native rhizobia

Success of an inoculated symbiont in the soil depends upon the competitiveness of the strain. Inoculated strain should have intrinsic competitive ability to form nodules, to be able to colonize root systems readily, and move along root system as it growns so that, strain is present in the zone of the root susceptible to nodulation. Biological nitrogen fixation is dependent on the survival and successful competition of the introduced *Rhizobium* strains against the native *Bradyrhizobium* strains (Danso *et al.*, 1988). There is every chance that a less aggressive strain gets eliminated in the soil during establishment phase or later due to its poor competitive ability not only with the native symbionts but also with the general microbial flora existing in the soil. This is one of the most serious limitations of inoculation of perennial crops, as sometimes efforts seldom yield the desired

results (Balasundaran, 1995).

The degree of establishment and persistence of an inoculant strain generally decreased with increase in population density of the native rhizobia (ICRISAT, 1981). However, some inoculant strains have succeeded in forming more nodules even in the presence of active indigenous competing rhizobia (Nambiar *et al.*, 1984). Little is known of the factors controlling competitiveness but host cultivar, soil properties, soil microflora, environmental factors and the origin of the competing strains influence the success of inoculant strains in nodule formation (Alexander, 1982).

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

Evaluation of isolates from *Samanea saman*, *Gliricidia sepium* and *Leucaena leucocephala* has shown that only one isolate from *Gliricidia* was more competitive than native rhizobia. Results also indicated that, the isolates from each host legume differed in competitive ability and their competitiveness was affected by soil type also (Monsalud, *et al.*, 1989).

It is generally difficult to displace indigenous rhizobia with inoculate strains, Rupela and Sudarshana (1990) successfully displaced inefficient strains with an efficient inoculant strain through soil solarization and inoculation.

According to Thies *et al.* (1992), the most significant environmental variable controlling their competitive success was the size of the indigenous rhizobial population. In his study it was found that for each legume, one of the three inoculant strains was a poor competitor across sites. Competition between the other two strains varied between sites but was infrequently related to environmental variables. Results indicated that, competitive strains that perform well across a range of environments could be selected. Simanungkalit (1996) stated that, proportion of nodules formed in soybean by the inoculated strain is an index of its competitiveness against the naturalized root nodule bacteria and relative competitiveness of the strain was site dependent.

Swelim *et al.* (1996) examined the competitiveness of wild type - strains of *Rhizobium spp* and their genetically marked double mutants. Four wildtype-strains and their genetically marked derivatives were evaluated using double reciprocal pairs. The results indicated that, strain DS 65 and DS 78 as being more competitive than strain DS 144/2; that only strain DS 78 was more competitive than DS 158; and that strains DS 158 and DS 65 were equally competitive. There was no correlation between nodule number and competitiveness.

Galiana (1991) reported that *Bradyrhizobium* samples isolated from indigenous *Acacia mangium* trees in Queensland were more effective than strains from other origins like Hawaii and senegal.

2.7. Response to inoculation

Inoculation of legume seeds with the appropriate cultures of *Rhizobium* was originally introduced as means of ensuring the establishment of seedlings in N

deficient soils which lacked adequate populations of nodulating bacteria (Fred *et al.*, 1932).

Nair *et al.* (1970) studied the effect of seed inoculation with *Rhizobium* on yield and N content of leguminous green manure crops and reported a fixation of 8-14 kg N per hectare and an increase in the dry matter production. Badji *et al.* (1987) observed that, inoculated *Acacia senegal* had greater N content in above ground plant parts than uninoculated controls. Punj and Gupta (1988 a) observed that, rhizobial inoculation increased nodule-number, nodule dry weight and plant height of *Leucaena leucocephala*. They concluded that, rhizobial inoculation increased the N_2-fixing capacity of *Leucaena* and consequently lead to the better establishment of plants.

Snaging *et al.* (1988 a) examined the effect of inoculation and N, P and trace elments on nodulation and growth of *Leucaena leucocephala*. In pot experiments all parameters measured, except the percentage of N in shoots were improved by inoculation. Study conducted by Sanginga *et al.* (1988 b) at the International Institute of Tropical Agriculture and at South Western Nigeria found that, shoot dry weight and N, P contents of inoculated plants were 55% higher than in uninoculated plants. Positive initial growth response to inoculation was observed in *Leucaena* although by 64 or 72 weeks after sowing, there was no significant difference between inoculated and uninoculated treatments (Homchan *et al.*, 1989).

Positive effects of *Rhizobium* or *Bradyrhizobium* on the growth of *Acacia mangium* is demonstrated by Brunck *et al.* (1990). Galiana *et al.* (1990) inoculated seven day old *A. mangium* and *A. auriculiformis* seedlings with eight strains of *Bradyrhizobium* and two of *Rhizobium* . Observation after five months showed that,

the *Rhizobium* strains nodulated both acacia species but did not fix N. The infectivity of *Bradyrhizobium* strains differed between the acacia species. The number of nodules were generally higher on *A. mangium.* For *A. auriculiformis*, nodule dry weight, shoot dry weight and N_2-fixation were not significantly different between strains, whereas there were significant difference for *A. mangium.* Nodule efficiency, as expressed by the ratio of N fixed to nodule dry weight, appeared to be higher in *A. auriculiformis* than *A. mangium.* Cali (1991) reported that, inoculation independently improved height, shoot biomass, nodule weight and N content and uptake in mangium.

2.7.1. Need to inoculate

Eventhough *Rhizobium* are able to survive in the soil as free organisms for several generations, but it disappears along with the disappearance of its host. Generally microbial diversity is lost along with the extinction of their associate species. Hence, introduction of appropriate strains of symbionts are necessary in such areas when reforestation is attempted, with a new legume tree species.

Inoculation, the process of adding inoculant to seed or soil can introduce enough bacteria to infect roots. Determining whether or not to inoculate should be based on whether there will be a response to inoculation under specific field conditions. To determine the response to inoculation, two approaches can be taken. The first is to use crop history as a guide. The second is to conduct a field test (Mac Dicken, 1994).

Crop history suggest that, if the NFTs has never before been planted on site, inoculation is probably necessary. An example from two soils in Nigeria

demonstrates the importance of inoculating NFTs on new planting site. Most probable - number analysis for these and other soils in Nigeria indicated that *Leucaena*-rhizobia were few or absent in soils without prior *Lecaena* cultivation and were substantially higher on sites with a history of *Leucaena* cultivation (Sanginga *et al.*, 1987). The same pattern is true for other species, particularly *A. mangium* which seems to be very specific in its rhizobial requirements (Sovannavong and Galiana 1991). At the same time, other species of NFTs can nodulate with the native rhizobia in a site but do not fix much N. These species are known as promiscuous ineffective species (Date, 1982). Their capacity to fix N can be markedly increased by providing them with rhizobia that are more effective N_2-fixers than the native rhizobia.

Crop history however, can be a very imprecise means of predicting nodulation and response to nodulation. The more reliable approach to determining response to inoculation is to conduct a field test to determine the need for inoculation (FAO, 1984). Field experiment is a simple, short and relatively inexpensive means of determining whether or not to inoculate.

2.8. Factors affecting nodulation and nitrogen fixation

For successful establishment of the symbiotic association between trees and *Rhizobium*, the behaviour of the legume host and microsymbiont, rhizobia during each of the stage and the various factors, both environmental and genetic must be understood. Fixation of N is limited by the biological yield of the host plant even if a suitable symbiont and appropriate soils are present, because the host plant is both the source of energy for the symbiont and the sink for the fixed N (App *et al.*, 1980).

2.8.1. Environmental factors

N_2-fixation was positively correlated with temperature and soils moisture by (Jaegar and Werner, 1981). According to them N_2-fixation is inhibited by dry conditions and extreme cold. Environmental factors influencing symbiotic N_2-fixation have been reviewed by Havelka *et al.* (1982) and Dixon and Wheeler (1983).

Little has been published on the effects of different environmental variables on symbiotic N fixation by NFTs. While the following generalities are based primarily on information generated by studies on leguminous field crops, they should also apply to tree legumes. Much of this work has focused on the effect of single factors (e.g. fertilised N or temperature), very little has been reported on the effect of multiple factors on N_2-fixation.

2.8.1.1. Temperature

The effects of temperature on N_2-fixation have not been well established, but its effects on herbaceous legumes suggest that species differences, plant age and environmental factors influences the degree to which temperature affects N_2-fixation (Gibson, 1976). In the field, temperature affects may be most closely related to development of the host plant.

Different strains of *Rhizobium* also may respond differently to temperature variations. Working with several tropical grain legumes, Dart *et al.* (1976) reported little difference in nitrogen fixation between strains at 21°C, but found significant differences at air temperatures of 27°C and 33°C. In various parts of the world, temperature may be a major factor limiting the extent of the N_2-fixing period: in some cases low temperature are limiting, in other cases, high temperatures. The

symbiotic system is generally more temperature sensitive (at both extremes) than growth of plants (Sprent, 1979). Unfavourable high soil temperature is a major constraint to N_2-fixation in tropics. The optimum temperature regime for N_2-fixation is 25- 30 °C (NifTAL, 1990).

The slow growing strains of rhizobia dominate at higher temperatures in comparison with the fast growing ones as evidenced from the abundance of further group of cowpea type of rhizobia in the rhizosphere at 30°C and that of the latter group at 25°C (Trinick *et al.*, 1983).

2.8.2. Soil moisture

Soil moisture can be limiting, both when too low or too high. Absence or significant reduction in nodulation under both excessively dry and wet conditions have been reported by Diatloff (1967). However, little is known about the irrigation strategy to improve the nodulation behaviour of NFTs.

Water stress can induce a rapid inactivation of nitrogen fixation activity (Sprent, 1971). The inhibitory effects of water deficit on symbiotic N_2-fixation are well studied in *Rhizobium* inoculated nodulated plants (Sprent, 1976). Gallacher and Sprent (1978) reported that, the nodule development initiated prior to the imposition of stress is reduced by low water potential and further renewed when the stress is alleviated.

Jaeger and Werner (1981) observed that, nodule growth and nitrogenase activity in *Acacia nilotica* seedlings were significantly reduced by water stress. Sundstrom and Huss-Danell (1987) also reported that at moderate water stress, acetylene reduction was reduced by half and at more severe stress, acetylene

reduction was nearly zero in *Alnus incana.* Pokhriyal *et al.* (1989) already reported a considerable reduction in biomass and nitrogenase activity due to water stress treatments in *Albizia procera*. Nodule number, weight and nitrogenase activity were more severely affected due to water stress in *A. nilotica* (Pokhriyal *et al.*, 1997). Water deficits affect symbiotic N_2-fixation through influences on host plant metabolism, infection, nodule development and nodule function (Reddel, 1993).

2.8.3. Soil pH

Soils of low pH usually are low in phosphorous, calcium, magnesium and molybdenum and may have toxic levels of aluminium and manganese, all of which can affect NFT vigour and health. Plants that depend on N_2-fixation have been found to be more sensitive to pH than plants of the same species supplied with mineral N (Andrews, 1976).

Legumes usually need a natural to slightly alkaline soil for nodulation. Root hair infection and deformation and consequently the formation of nodules are inhibited by soil acidity around pH 4.5 as shown for peas (Mulder *et al.*, 1977) and lucerne (Munns, 1977). In general critical pH for nodulation of most legumes is above pH 4.5 to 5.5 (Dixon and Wheeler, 1983). Besides low pH, the presence of high concentration of aluminium in acid soils can impair nodulation even in *Rhizobium* strains that are acid tolerant (Munns and Keyser, 1979). The most widely reported effect of soil acidity on symbiotic N_2-fixation is reduction in nodulation. This sensitivity is most evident in early infection stages (Carvahlo *et al.,* 1981).

The impact of acidity is supposed to be most severe in *Leucaena leucocephala* which shows marked gradient in growth as the soil pH decreases, the

critical value is found to be around pH 4.4 - 4.7 (Norani and Ng, 1981).

Soil acidity and related factors (Al and / or Mn toxicity and Ca and Mo deficiencies) which affect many tropical soils (Franco, 1984), influence N_2-fixation by the direct or indirect affects they have on the host plant and the symbiotic micro-organizms. A typical example is *Acacia meansii*, which does not nodulate in highlands of Barundi, where soils have a low pH and a high content of exchangeable Al. Dowling and Broughton (1986) have reported that, low pH of lateritic soil limits the availability of phosphorus and increase the levels of aluminium and manganese, hence affect nodulation and N_2-fixation. According to Munns (1986), the specific nature of the effects of soil acidity on symbiotic N_2-fixation are dependent on interactions between pH and nutrient availability.

In any given situation, the impacts of soil acidity on symbiotic N_2-fixation reflect the relative and interactive effects of (i) low availability of P, Ca and / or Mo for plant growth and symbiotic N_2-fixation and (ii) high concentrations of H ions, Al and Mn (which are released from cation exchange sites in the soil) and may reach levels inhibitory or toxic to plant and root nodule bacteria. These individual factors may operate on different phases of the symbiosis and hence explain the markedly differing influences of soil acidity on plant growth and N_2-fixation (Coventry and Evans, 1989). There are also report that Al many interfere with multiplication of invading cells of the microsymbiont, but this nodule development phase appears less sensitive than either root or shoot growth (Franco and Munns, 1982).

Sprent and Sprent (1990) reported that, low soil pH generally inhibits N_2-fixation by reducing the development of rhizobia, increasing the numbers of ineffective rhizobia or disrupting the infection process.

2.8.3.1. Rhizobia tolerant to acidity

The detrimental effects of soil acidity can be overcome by selecting acid - tolerant host plants and symbiotic micro-organisms, an approach that has been adopted with *Leucaena leucocephala* (Hutton, 1984) and its competent *Rhizobium* (Halliday and somasegaran, 1982). In Southern Vietnam, *Acacia auriculiformis* grown in acid sulphate soils of pH less than 4.0 nodulated profusely in the undecomposed litter layer but not in the highly acid mineral soil (MacDicken, 1991). This demonstrates the ability of the symbiosis to adapt to strongly acid soils where effective nodulation would not be possible in the mineral soil.

Lesueur *et al* (1993) reported that the *A. mangium* - *Bradyrhizobium* symbiosis is tolerant of acidity and aluminium (Al) toxicity. Lesueur and Diem (1997) have reported that, *A. mangium* - *Bradyrhizobium* symbiosis is adapted to the constraints encountered in acid soils, i.e., low pH, low P and Mo contents, and excessive levels of Al and Fe.

Probert (1995) conducted two glasshouse experiments to refine screening procedures and to rank 36 native Acacia species for soil acidity tolerance. The first experiment showed that N_2-fixation was more sensitive to soil acidity than plant growth. This suggests that in screening experiments where acid tolerant rhizobia are not available, inorganic N should be supplied in determining intrinsic ability of the species to tolerate soil acidity and to avoid confounding sensitivity to acid soils with ineffectiveness of *Rhizobium* / *Bradyrhizobium*. The second experiment enabled the ranking of 36 species in to 4 classes of differing tolerance to acid soil. This experiment also indicated that, the ranking order of species differed with the soil type, suggesting the need to use more than one soil type in screening experiments

in order to obtain a better and more reliable ranking of species for tolerance to acid soil.

2.8.3.2. Control of soil acidity

It is also possible to control the effects of soil acidity by directly applying proper amendments to the soil or by pelleting the seeds in the case of direct sowing in the field. Different types of amendments such as lime or organic materials can be used. The acidity generated by N_2-fixing plants in the long run may lower the pH of weekly buffered soils, and periodic liming may be necessary to maintain high productivity (Franco, 1984). Addition of lime to the soil may alleviate the problem of soil acidity on symbiotic N_2-fixation but may also induce nutritional disorders like deficiencies of P, Zn, Cu and Fe (Franco and Munns, 1982).

The competitiveness of *Bradyrhizobium spp.* in an acid soil was evaluated by growing legume tree seedlings in a Red-Yellow Latisol with pH 4.5, 4.9 and 5.4 (Ribeiro *et al.* 1986). They have observed that, liming did not affect competitiveness significantly, however interactions between liming and strains were observed. Favourable effect of lime pelleting on growth of legumes was also observed by Basu *et al.* (1987). Umali - Garcia (1988) found that liming of acid soils improved the performance of the symbiosis in *Acacia mangium* seedlings in the nursery. Techniques shown by Xu *et al.* (1992) to improve growth of *Leucaena leucocephala* seedlings grown in acid soil from China, under glass house conditions, included inoculation with rhizobia and liming the soil or pelleting seeds with lime.

Lime is relatively expensive input and thus may not be affordable in many low-input production systems used in the tropics. The symbiotic micro-organism can

be protected against acidity by pelleting the seeds to be inoculated with calcium carbonate or rock phosphate. This technique developed in Australia and now used throughout the world, has indeed proved to be a high-value alternative for liming during the introduction and establishment of forage legumes in pastures (Williams, 1984). It could also be used successfully in agroforestry. However, in very acid soils with Al or Mn toxicity, pelleting the seeds alone cannot overcome the effects of acidity regardless of cropping system (Dommergues, 1987).

If the mechanisms by which soil acidity influence tree growth and symbiotic N_2-fixation are defined, selection of plant and bacterial genotypes tolerant of particular sets of soil conditions may be a more appropriate management strategy (Reddel, 1993).

2.8.4. Salinity and alkalinity

Although saline and alkaline soils have markedly different properties, in both groups of soils depression of plant growth and N_2-fixation can be caused by the same general effects: osmotic effects on water uptake, nutritional imbalances (Fe, Mn, Cu, Zn deficiencies), toxicity caused by specific ions (e.g. Na, Cl, HCO_3) and / or adverse physical properties of the soil (e.g. surface crusting, reduced permeability to water, lack of subsoil structure and increased mechanical resistance to root penetration). The effects of saline and alkaline soil conditions on nodulation and N_2-fixation in trees growing on these sites are poorly documented despite significant rates of N_2-fixation that can potentially occur in arid environments (Skujins, 1984). Surange *et al.* (1997) reported that, soil degradation through salinization or alkalization is one of the serious forms of land degradation affecting approximately 10% of the total land surface of the world.

Balla *et al.* (1990) demonstrated the suitability of salt-tolerant *Rhizobium* strains in an experiment using 57 strains and several NFT species. Salt tolerant strains showed better survival, nodulation and N_2-fixation than salt sensitive strains under saline conditions. A strain from each of the four NFTs, *Acacia nilotica, Dalbergia sissoo, Leucaena leucocephala* and *Prosopis juliflora* was tested, and all were found to be highly tolerant to saline conditions.

Generally, the rhizobia are more tolerant to osmotic stress than their leguminous hosts. The different *Rhizobium* species vary in their sensitivity to salt. *Rhizobium meliloti* strains are salt tolerant (Zhang *et al.*, 1991). In contrast, *Rhizobium leguminosarum* strains are salt sensitive (Zhang *et al.*, 1991).

Infact, there is a tremendous variation in the response of tree and *Rhizobium* to saline soils. NFT species such as *Prosopis tamarugo* survive and fix N extremely saline soils where other NFTs or symbiont strains would not survive (MacDicken, 1994). Zahran *et al.* (1994) have reported tolerance to 10% NaCl by rhizobia from *Acacia* and *Prosopis*. Zou *et al.* (1995) compared the growth, nodulation and N content of *Acacia ampliceps* seedlings inoculated with salt tolerant *Rhizobium* PMA 63/1 and salt sensitive *Bradyrhizobium* PMA 37 and found that, *Acacia ampliceps* inoculated with the former was less affected in growth, nodule number and N content per plant by 200 mM NaCl than plants inoculated with the latter. He concluded that, inoculation with a salt tolerant *Rhizobium* strain may therefore, improve biological N_2-fixation under saline conditions.

Lekshmi -kumari *et al.* (1974) demonstrated the existence of 'alkali, sensitive steps' in early phase of nodulation and effects of alkalinity on root hair development. The mechanisms by which the effects are mediated are not known. *Sesbania*

sesban was evaluated by Swift *et al.* (1987), in pot culture and laboratory studies for nodulation and N_2-fixation in alkaline soil. He observed that, native rhizobia produced good noduation and actively competed with inoculated strains. The species could tolerate EC (electrical conductivity) II mmho / cm (0.7% salts) and pH 10 at germination. Shenbagarathai (1993) has reported that *Rhizobium* SBS - R100 isolated from *Sesbania procumbens* is capable of growing at pH 11.0. Noor *et al.* (1994) has reported the pH tolerance up to 10 by *Rhizobium* isolated from chick - pea (*Cicer arietinum* L.). Surange *et al.* (1997) conducted a study to determine the variability among rhizobial strains isolated from different NFTs in growth response to pH, and salt concentrations. Rhizobial strains isolated from *Sesbania formosa, Acacia farnesiana* and *Dalbergia sisoo* were well adapted to grow on pH 12.0. All the rhizobial strains tolerated salt concentrations up to 5.0%.

2.8.5. Mineral nutrients

Nutrient deficiencies are wide spread in tropical soils (Sanchez, 1976) and place particular constraints on symbiotic N_2-fixation for many tropical grain legumes (Franco, 1977). Mineral nutrient deficiencies limit N_2-fixation by the legume - *Rhizobium* symbiosis in many agricultural soils and as a result seriously depress legume yields below their maximum potential (O' Hara *et al.*, 1988).

According to Arnon and Stout (1939), the following chemical elements are known to be essential for the legume - *Rhizobium* symbiosis: C, H, O, N, P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, Mo, B, Cl, Ni and Co. Each essential nutrient has specific physiological and biochemical roles and there are minimal nutrient concentrations required within both legumes and rhizobia to sustain metabolic function at rates which do not limit growth.

Reddell (1990) have reported that, nutrient deficiencies may affect symbiotic N_2-fixation in trees either via direct impacts on the component stages of symbiotic N_2-fixation or by limiting the growth of host tree.

2.8.5.1. Effect of fertilized N

The harmful effect of fertilized N in soil on nodulation and N_2-fixation by trees is attributed to different mechanizms reviewed by Duhoux and Dommergues (1985). The higher rates of N - fertilizer may have produced a nutrient imbalance thus rendering the nonavailability of other elements such as Cu, Zn Fe and Mn (Belen, 1987). This had also been reported by Powell and Webb (1972) and Umali - Garcia (1984). It is well established that, high rates of N application reduce root hair infection (Dazzo and Drill, 1978) and N_2-fixation (Michin *et al.*, 1981) of legumes.

Kang (1975) observed a reduction in nodule number and N_2-fixation of soyabean grown in Nigeria with large amounts of applied N. In Senegal the absence of nodules of *Acacia senegal* may be due to active nitrification of organic N (Bernard - Reversat and Poupon, 1979). Adverse effect of N - fertilization on nodulation of *Albizia procera* had been reported by Hussain *et al.* (1986). Maloney *et al.* (1986) observed that, *A. falcataria* nodulated in both N - fertilized and unfertilized pots but effective and bigger nodules were observed in the latter treatment. Becana and Sprent (1987) have reported that, high levels of fertilized N may reduce nodulation and nodule activity and accelerate senescence. Umali - Garcia *et al.* (1988) determined the effect of N - fertilizer application on the symbioses of the five *rhizobial* isolates with *A. falcataria* and *A. mangium*. They observed that, application of fertilized N suppressed nodulation of *A. falcataria.* Nodulation of unfertilized trees exceeded nodulation of those fertilized with fertilized

N by 114 to 237%. The application of fertilized N at the rate of 100 kg N ha^{-1} did not suppress nodulation in *A. mangium.* Sanginga *et al.* (1988 b) examined the effects of inoculation, N_2-fixation and growth of *Leucaena leucocephala* in Ibadan, Nigeria. He observed that, N fertilizer application depressed N_2-fixation and *Leucaena* responded to both inoculation and / or N application. The shoot growth and total N and P of inoculated plants were comparable to those of the highest N treatment, and the values were about 55% greater than those of uninoculated ones. Field data indicated that, total N yields of inoculated *Leucaena* seedlings were increased by 50% with 40 or 80 kg ha^{-1} of N fertilizer.

Sanginga *et al.* (1988 b) stated that, tree - *Rhizobium* symbioses can be more affected by fertilized N than annual crops because of their large variation in N_2-fixation that can occur throughout the life and the redistribution of N in the plant and the soil profile due to litter fall and its mineralization. According to them, two approaches have been suggested to improve N_2-fixation by trees in the presence of fertilized N: (i) develop specific partnerships of *Rhizobium* hosts that are more tolerant of combined N than others or, (ii) develop plants that can simultaneously absorb soil N and fix considerable atmospheric N.

The process of N_2-fixation does not begin immediately upon germination, and use of a small amounts of starter N fertilizer for legume establishment has often been suggested (Eaglesham *et al.,* 1977; Harper, 1974; Kang, 1975). In *Leucaena leucocephala,* N_2-fixation was delayed for about eights weeks in the plots without N (Sanginga *et al.*, 1988b). He also suggested the application of small amounts of N (20 ppm) as starter dose. The theory behind supplemented N fertilizer application is that, it will meet the plant's requirement for N during early period while nodules are

developing. In addition it should produce a large young plant, which will maintain a high growth rate (Mahon and Child, 1979; Michin *et al.*, 1981). This may be important under lowland tropical conditions, because many soils have low amounts of available N, which may be inadequate to satisfy the need during the N hunger period.

Cabahug (1991) studying the early growth of *A. mangium* in a grassland soil found that increasing levels of N gave a general trend of improved growth performance and nodulation. In the presence of N at the rate of 100 kg ha^{-1}, inoculation improved nodulation by 193.6 per cent over the unfertilized inoculation treatment. Application of 300 kg N ha^{-1} decreased nodulation but did not affect nodule weight. The soil used in this study had high populations of native rhizobia, and applications of N at the rate of 30 - 100 kg ha^{-1} improved infection of roots by the native rhizobia.

Goi *et al.* (1997) observed that, in *Mimosa caesalpiniaefolia*, a small amount of N can stimulate nodulation shortly after germination. He studied the effect of different sources of N on nodule structure and plant growth. Applied - nitrate drastically affected the nodule structure, and reduced nodule number and plant growth. Plant growth parameters were clearly superior in the presence of NH_4^+ and this had no deleterious effect on nodule structure.

2.8.5.2. Effect of P

Legumes require adequate supply of phosphorous for satisfactory nodule production and N_2-fixation (Mosse, 1977). Rhizobial inoculation of leguminous reforestation species coupled with phosphorus pelleting was thus a potentially

promising strategy for circumventing N and P constraints under these specified conditions (Manguiat and Padilla, 1982).

Addition of superphosphate in *Rhizobium* inoculated *Leucaena leucocephala* grown in a glass house showed an increase of 116 per cent of yield in infertile soil while only 46 per cent increase was observed in medium fertile soil (Garza *et al.*, 1987). *Leucaena* needs P for vigorous growth and N_2-fixation (National Academy of Sciences, 1977). The addition of P to soil usually causes an increase in the content of P and N in *Leucaena* (Sanginga *et al.*, 1988 a) and tropical pasture legumes (Andrew, 1978). Application of P on the productivity of *Desmodium introtum* on an Ultisol and *Sesbania sesban* on a Vertisol, Alfisol and Ultisol were studied by Haque *et al.* (1995). In *Desmodium*, inoculation had no significant effect on nodule weight, but application of P was necessary for nodulation. Because of previous P applications, P had no significant effect on *Sesbania* plants grown on the Vertisol. On the Alfisol and Ultisol, *Sesbania* responded differently to P applied in the nursery or field. On the Alfisol P applied in the nursery increased number of nodule, DM and N yields of shoot. However, P applied in the main field increased only nodule dry matter, the reverse occured on the Ultisol.

Stamford *et al.* (1997) studied the response of the tropical tree legume *Mimosa caesalpiniaefolia* to phosphogypsum as a P-fertilizer compared with soluble phosphorus fertilizer. They also evaluated the interaction of P-fertilization with *Bradyrhizobium spp* and vesicular arbuscular mycorrhizal fungi inoculations. It was reported that, in unsterilized acid soil without P addition, *Bradyrhizobium* strain NFB 577 seemed to be more effective, but when P-fertilizer was used both strains were similarly effective.

A. mangium can grow in soils with a phosphate content as low as 0.2 mg kg^{-1} of soil (National Research Council, 1983). Although P is essential for both nodulation and N_2-fixation in legumes, Vadez *et al.* (1995) have reported that, *A. mangium* does not have high requirement of P for growth and N_2-fixation compared with grain legumes.

2.8.5.3. Interaction of N and P

Reinsvold and Pope (1987) observed that, in *Robinia psuedoacacia* application of P ranging from 25 - 100 mg kg^{-1} of soil increased the growth and acetylene reduction activity of the nodules while N above 50 mg kg^{-1} soil actually reduced the number of nodules and nodule dry weight. Mao *et al.* (1989) studied the effect of fertilizer on nodulation and N_2-fixation of *A. mangium.* They observed that, biomass of nodules was two times more in fertilized plots than in plots with no fertilizer.

Masuka (1995) conducted a nursery experiment using sterilized soil with *Faidherbia albida*, two rhizobial strains, phosphate and ammonium nitrate fertilizers. Five months following inoculation, all the three factors *Rhizobium*, phosphate and N had effected mean nodule number per plant. There were significant interactions of *Rhizobium* with P and N. N consistently had a negative effect on both nodule number and weight. Neither phosphate nor N effected plant height. Mean shoot weight per plant was greater in the presence of *Rhizobium* and phosphate, but root weight was reduced by N.

Studies on the effect of liming and inoculation with *Rhizobium* in *A. mangium* in the presence or absence of NPK fertilizer, showed that, inoculation independently

improved height, shoot biomass, nodule weight and N content and uptake of *A. mangium* (Cali, 1991). This relationship between shoot biomass and nodule number, nodule weight, and N uptake was highly significant. The seedlings grew well in both soils when fertilized with 200 kg N ha^{-1} + 100 kg PK ha^{-1}, the second best treatment was inoculation with *Rhizobium* in the presence of only P and K. Weight, diameter, and nodule weight were significantly affected by the interaction of lime, inoculation and fertilizer treatments.

Materials & Methods

MATERIALS AND METHODS

Investigations were carried out at the College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur district, Kerala during the period from April1996 to December 1997. The studies involved a field survey for the collection of nodules and isolation of rhizobia from *Acacia mangium* Willd. (mangium) and *A. auriculiformis* A. Cunn. ex Benth. (Acacia) and evaluation of the efficiency of strains and its mutual nodulation efficiency by reinoculation on *A. mangium* seedlings.

3.1. Collection of nodules, soil samples and measurement of GBH

In order to assess the status of root nodulation, mangium plantations were identified from different districts of Kerala. One plantation each was selected at random from each district, except Kasargod (Fig. 1).

The nodulation characteristics of mangium in these plantions were studied using core sampling and sieving techniques proposed by Sankaran *et al.* (1993). In this method, soil was collected using Soil Core Sampler, 15 cm long and approximately 3.5 cm diameter. Soil samples were collected from a depth of 0 - 10 cm at a distance of 20 - 30 cm from the basal region of the tree using core sampler. Five samples were collected from the base of each tree from five points, selected at random, around the tree. Each soil sample was then wet sieved in a two mm sieve and the number of nodules were enumerated. Their size and shape were also noted. Where, ever core sampling failed to yield nodules, the roots of the trees and seedlings were thoroughly searched for the presence of nodules. The soil samples and the nodules collected were taken to the laboratory for further investigation. From each plant 20-25 nodules were collected. Girth at breast height (GBH) of 10

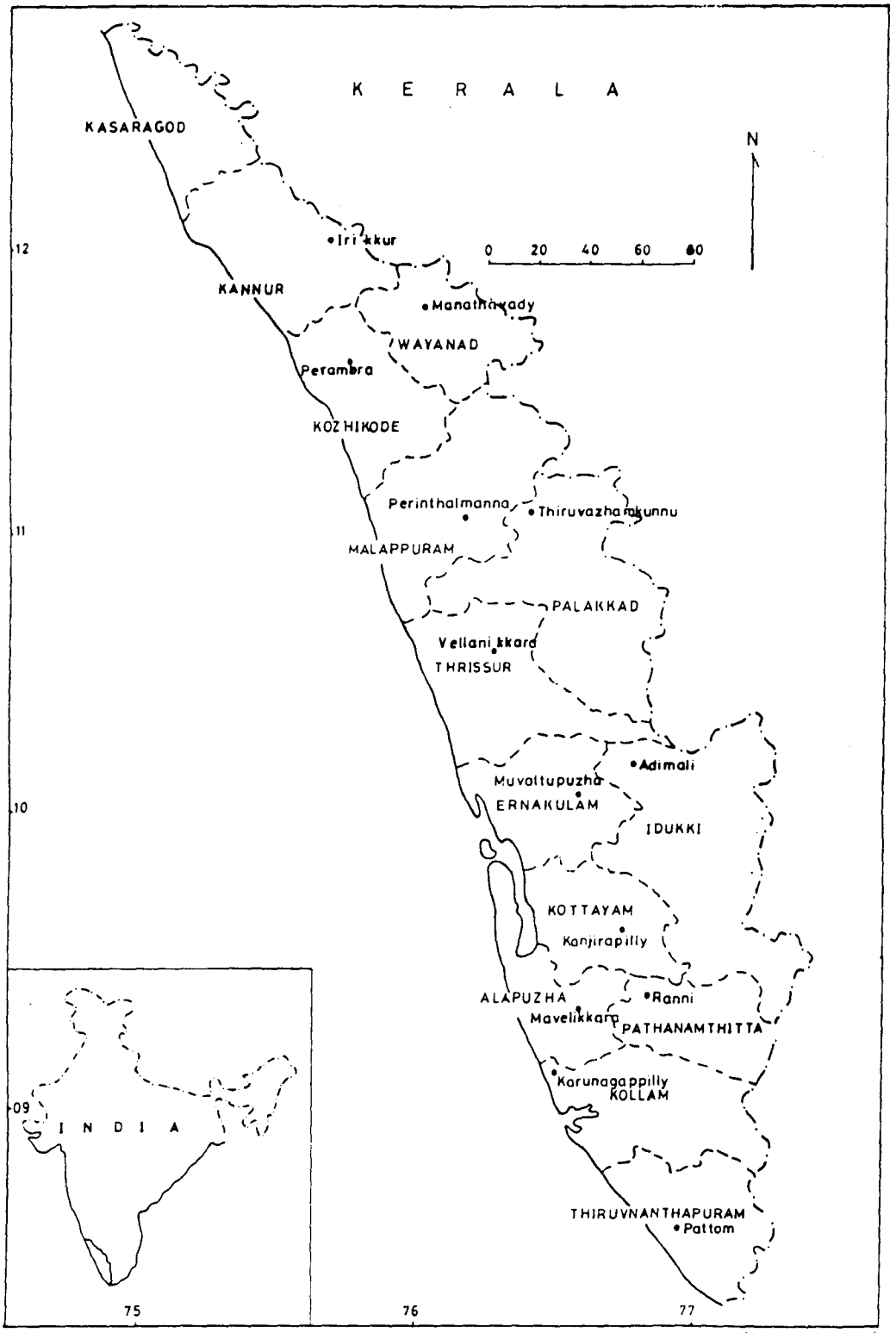


Fig. 1: Locations of mangium plantations from where, rhizobia were isolated

trees at the sampling site selected at random was measured. While transporting, the nodules were stored inside a flask with cotton wool plug and ice cubes. This technique helped to preserve nodules against decomposition and soil micro-organisms.

3.2. Isolation techniques

Isolation of *Rhizobium* was made based on the standard procedure of Vincent (1970).

3.2.1. Preparation of medium

3.2.1.1. Sterilization

The glasswares and medium used for the investigations were sterilized by autoclaving at 1.06 kg cm^{-2} pressure for 20 minutes, at a temperature of 121°C

3.2.1.2. Yeast extract mannitol agar with Congo Red (YMA)

Yeast extract mannitol agar medium with the following composition was used for the isolation of rhizobia

Ingredients		g $litre^{-1}$
Mannitol	:	10.0
Dipotassium hydrogen phosphate (K_2HPO_4)	:	0.5
Magnesium sulphate ($MgSO_4$ $7H_2O$)	:	0.2
Sodium chloride (NaCl)	:	0.1
Yeast extract	:	1.0
Agar	:	20.0
Congo Red	:	0.025

The pH of the medium was adjusted to 6.8 - 7.0.

Here the ready made medium from M/s. Hi Media was also used. The medium was prepared by adding 31.8 g of the ready-made medium to1000 ml of distilled water, and then heated to boil to dissolve the medium completely. The medium was then sterilized by autoclaving for 20 minutes at 1.06 kg cm^{-2} pressure (121°C)

3.2.1.3. Plating

The sterilized medium was poured into sterilized petriplates in an evenly manner. This was carried out in a 'Laminar Flow Chamber', to avoid contamination.

3.2.1.4. Slants

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

3.2.2. Isolation of *Rhizobium*

Large and healthy nodules collected during the survey were selected for isolation of *Rhizobium*. They were washed in tap water and transferred to tissue paper to dry. The nodules were then immersed in 95% ethanol for 5 - 10 sec and dipped in 0.1 per cent mercuric chloride for 2-3 minutes for surface sterilization. Sterilant was removed by washing the nodules at least five times in sterilized distilled water. The surface sterilized nodules were then asceptically transferred to test tubes containing one millilitre of sterilized distilled water. Then, the nodules were crushed with a sterile glassrod and a suspension was prepared. With the help

of a flamed loop, a drop of the suspension was streaked on YMA with congo red in petri plates. The loop was flamed after each streak. The above operations were carried out in a Laminar Flow Chamber. The plates were incubated at room temperature and were observed at 24 hour interval for the typical growth of *Rhizobium* (Plate 2). Slimy white or milky white colonies occurring on the media were selected and purified by repeated streak method. From the plates, small isolated colonies of *Rhizobium* were identified and was transferred to YMA slants with the help of a sterilized inoculation needle to obtain contamination free isolates (Plate 3).

3.3. Multiplication

For the multiplication of rhizobia, the medium used was Yeast Extract Mannitol Broth without agar, the composition of which is given below.

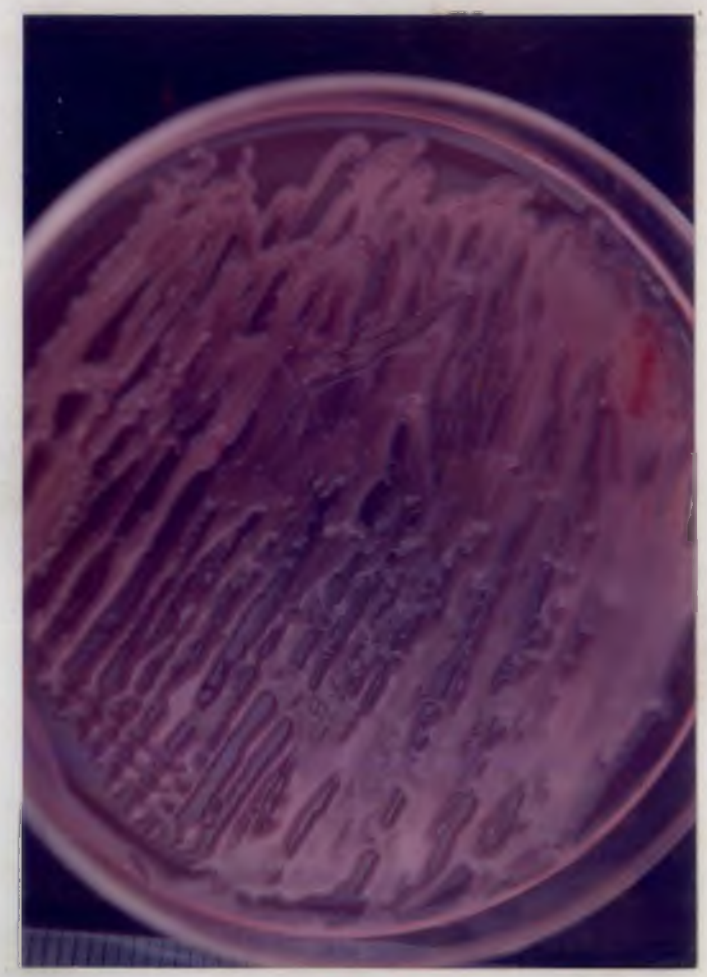
Ingredients	**g litre^{-1}**
K_2HPO_4	1.0
NaCl	0.1
$MgSO_4.7H_2O$	0.5
$CaCO_3$	1.0
Mannitol	10.0
Yeast extract	1.5

The pH of the media was adjusted to 6.8 -7.

For the multiplication purpose 1.28 g of ready-made media of M/s. Hi media was dissolved in 100 ml of distilled water taken in a 250 ml conical flask and sterilized. With the help of a sterilized inoculation needle, a loop full of rhizobia was

Plate 1. Detached nodules to show the different shapes of nodules

Plate 2. *Rhizobium* growing in Yeast mannitol agar with Congo red -plates




10
9
8
7
6

transferred from agar slants in to the sterilized broth in conical flask under aseptic conditions. These conical flasks were incubated at room temperature in a rotary shaker at 85 rpm for 5 - 6 days (Plate 4). The multiplication of rhizobia were carried out only 7-8 days before pelleting of seeds.

3.4. Authentication

Rhizobial isolates can be described according to their growth characteristics in the solid and liquid media (Vincent, 1970). The following tests were conducted to authenticate the isolates obtained

3.4.1. Growth on Congo red medium

The yeast extract mannitol agar medium with 2.5 ml of 1% congo red per litre was prepared. The petriplates were poured with 15-20 ml of the medium and allowed to solidify. Isolates of rhizobia were streaked on these plates and incubated for 7 days at 26 - 27°C. Little or no absorption of congo red by colonies was confirmatory to the presence of *Rhizobium*. The shape, colour and generation time were also recorded.

3.4.2. Plant infection test

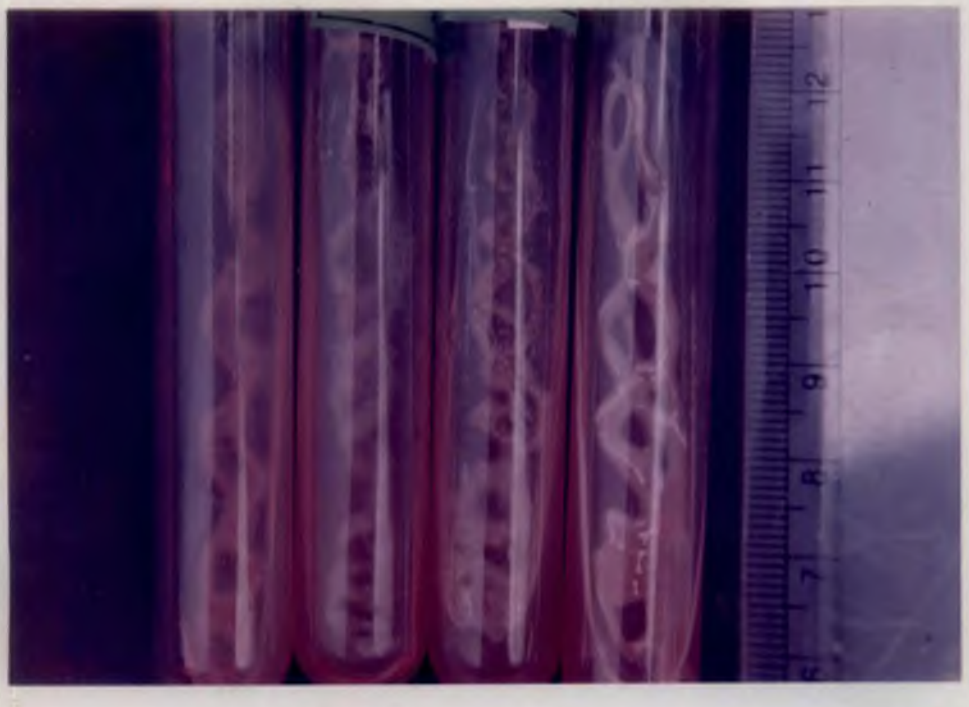
Isolates were tested for their nodulating ability on the host, *A. mangium* in seedling agar tubes (Vincent, 1970).

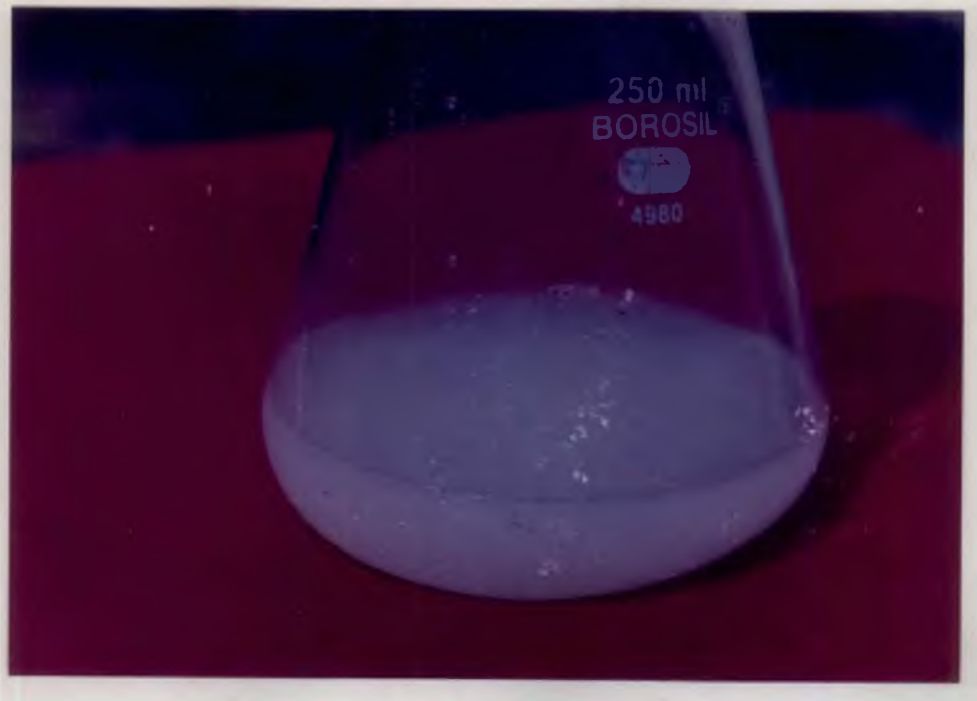
3.4.2.1. Preparation of seedling agar tubes

In order to prepare agar tubes, 1.67 g of Jensen seedling agar was suspended in 100 ml distilled water and boiled to dissolve completely. Sterilization

Plate 3. *Rhizobium* growing in Yeast mannitol agar with Congo red-slants

Plate 4. *Rhizobium* in Yeast mannitol broth




250 ml
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4980

was done in an autoclave at 1.06 $kg\ cm^{-2}$ pressure for 20 minutes. Forty millilitre of it was poured in to test tubes, 2.9 × 19 cm size in a Laminar flow chamber and Jensen's agar slope was prepared. The composition of Jensen's agar medium is given below (Jensen, 1942).

Ingredients	**g $litre^{-1}$**
$CaHPO_4$	1.0
K_2HPO_4	0.2
$MgSO_4.\ 7H_2O$	0.2
NaCl	0.2
$FeCl_3$	0.1

3.4.2.2 Preparation of seedlings

Healthy seeds were pre-treated in boiling water for 30 seconds and soaked overnight in cold water. These seeds were taken in 25 ml sterile beaker and rinsed in 95% alcohol for 3 minutes with agitation. Then the alcohol was poured off and seeds were immersed in 0.1% mercuric chloride for 3 minutes. The mercuric chloride was decanted and seeds were washed in several changes of sterile distilled water. The surface sterilized seeds were spread on 1.5% solidified agar on petriplates. The seeds were allowed to germinate and then placed on the Jensen's agar slants using sterile forceps.

3.4.2.3. Preparation of inoculant

Five millilitre of yeast extract mannitol broth was dispensed in test tubes and sterilized by autoclaving at 1.06 $kg\ cm^{-2}$ pressure for 15 minutes. Then a loopful of the rhizobial culture was inoculated to the broth. The inoculated broth was

incubated at 26-28°C for seven days and on a rotary shaker.

3.4.2.4. Inoculation

The seedlings were inoculated with two milliliter of the test isolates in to the tubes. The lower portions of the tubes were covered with black paper to cut off light. The seedling agar tubes were incubated in a light chamber for 16 hours. The seedlings were watered periodically with sterile distilled water. Five replicates were maintained for each test isolate. The seedlings were observed for nodules till six weeks (Plate 5).

3.4.3. Counts of viable rhizobia

Pure cultures of rhizobia were enumerated using plate counting procedure (Vincent, 1970). This has been done by preparing a set of serial dilutions.

Dilution bottles (99 ml and 9 ml) were set out according to desired range and steps. Six pipettes calibrated for the quick delivery of 1 ml was used with clean rubber bulbs. The pipettes used to take 1 ml from dilutions to be plated are used to transfer further 1 ml aliquots to petridishes. From the initial suspension, 1 ml sample is taken to the first bottle in the series. Using a fresh pipette, the suspension is drawn in to the pipette and expelled five times before transferring 1 ml to the next bottle in the series and the pipette used was discarded. This procedure is repeated until the suspension is transferred to the fifth bottle.

One millilitre of this dilution was taken in to labelled petridishes and within a few minutes, covered the sample with 15 ml of liquified and cooled Yeast extract Mannitol Agar and mixed by rotation several times clock wise, counter – clock wise,

Plate 5. Nodulated mangium seedlings in Jensen agar slope

from left to right and to and fro. Allowed the agar to solidify, inverted the dish and incubated at 26 - 28°C, for 5 – 6 days.

Rhizobial colonies in plates were viewed with indirect illumination against a non-reflecting black background and counted. Counts were multiplied by the dilution factor.

3.5. Isolates

The following isolates were obtained from the nodules collected from the mangium plantations of different districts of Kerala

Isolates	Locality of isolation	Isolates	Locality of isolation
MRh - 1	Irikkur (Kannur Dist.)	MRh - 8	Adimali (Idukki Dist.)
MRh - 2	Perambra (Kozhikode Dist.)	MRh – 9	Kanjirappalli (Kottayam Dist.)
MRh – 3	Mananthavadi (Wynad Dist.)	MRh – 10	Mavelikkara (Alapuzha Dist.)
MRh – 4	Perinthalmanna (Malappuram Dist.)	MRh – 11	Ranni (Pathanamthitta Dist.)
MRh – 5	Thiruvazhamkunnu (Palghat Dist.)	MRh – 12	Karunagappalli (Quilon Dist.)
MRh – 6	Vellanikkara (Trichur Dist.)	MRh - 13	Pattam (Trivandrum Dist.)
MRh - 7	Muvatupuzha (Ernakulam Dist.)		

The isolates collected from different districts of Kerala were divided in to five groups based on the agroclimatic regions of Kerala (NARP, 1989). These zones were Northern, Southern, Central, Highrange and Special zone of problem areas. From each group, an isolate showing better growth rate was selected for the evaluation of the efficiency of strains.

Isolate selected	Agroclimatic zone
MRh – 2	Northern zone
MRh – 13	Southern zone
MRh – 6	Central zone
MRh – 3	Highrange zone
MRh – 10	Special zone of problem areas

For evaluating mutual nodulation efficiency, rhizobial cultures of *A. auriculiformis* isolated from different agroclimatic regions of Kerala as done for *A. mangium* were also used.

Isolate	Agroclimatic region
ARh –1	Northern zone
ARh –2	Southern zone
ARh –3	Central zone
ARh –4	Highrange zone
ARh –5	Special zone of problem areas

3.6. Commercial cultures

The following strains of *Rhizobium* obtained as peat – based inoculants from Agroforester TM Tropical Seeds, Holualoa, Hawaii, USA, were also evaluated for mangium in Kerala

Agroforester-Group A – Strain of *Rhizobium* specific to *Acacia mangium*

Agroforester-Group C – Strain of *Rhizobium* for *A. auriculiformis, A. albida, A. bivenosa, A. holosericea, A. koa, A. siberiana*

3.7. Evaluation of isolates

The efficiencies of different rhizobial isolates under varying site conditions of soil fertility and soil acidity were studied in three potculture experiments, using sterilized soil-sand mixture (2:1), for six months under natural conditions. The evaluation was done during the periods of May to December,1997.

3.7.1. Experiment I. Competition from native ineffective rhizobia

To study the influence of ineffective native strains of rhizobia which may compete with the inoculated strains, on the efficiency of the strains under evaluation a potculture experiment was conducted under sterilized and unsterilized soil conditions (Plate 7). The isolates and inoculants evaluated in this experiment were:

1. Isolates of *Acacia mangium* from five agroclimatic regions of Kerala
2. Isolates of *A. auriculiformis* from five agroclimatic regions of Kerala
3. Agroforester TM inoculants of *A. mangium* (Agroforester-Group A)
4. Agroforester TM inoculants of *A. auriculiformis* (Agroforester-Group C)
5. Uninoculated-control

Treatment combinations	:	2 (soil) × 13 cultures = 26
Design	:	CRD
Replications	:	3

3.7.2. Experiment II: Efficiency of the isolates under different soil acidity

To evaluate the performance of the above isolates under different conditions of soil Acidity, a potculture experiment was conducted with four lime levels and ten isolates and /or cultures (Plate 8). The isolates / strains evaluated were :

1. Isolates of *A. mangium* from five agroclimatic regions of Kerala

2. *Two isolates (*ARh-1 and ARh-2) *of A. auriculiformis* (observed acid tolerant in preliminary trails-personal communication)
3. Agroforester TM inoculants of *A. mangium* (Agroforester-Group A)
4. Agroforester TM inoculants of *A. auriculiformis* (Agroforester-Group C)
5. Uninoculated-control

The lime levels were as follows.

Levels		g 1000^{-1} g of soil	kg ha^{-1}	Appr. Soil pH
L_0	=	0	0	5.0
L_1	=	0.75	500	5.5
L_2	=	1.25	1000	6.0
L_3	=	2.50	1500	6.5

Actual dose of lime was fixed based on incubation study of soil with lime to get the desired pH. Soil from College of Forestry campus which was not cultivated earlier was collected, sieved using a two mm sieve and thoroughly mixed with appropriate quantity of lime to get the desired pH level.

Treatment combinations	:	10 cultures × 4 Lime levels = 40
Design	:	Two factor CRD
Replications	:	3

3.7.3. Experiment III. Efficiency of the isolates under different soil fertility

To evaluate the efficiency of the isolates under different soil fertility conditions, they were evaluated with three levels each of N and P in factorial combinations (Plate 9). The isolates evaluated were:

1. Isolates of mangium from five agroclimate regions of Kerala
2. Uninoculated-control

The levels of N and P_2O_5 were as follows

N Levels	N (g 1000^{-1} g of soil)	kg ha^{-1}
N_0	0	0
N_1	0.25	100
N_2	0.50	200

For nitrogen, urea was applied by dissolving appropriate quantity in water.

P_2O_5 Levels	P (g 1000^{-1} g of soil)	kg ha^{-1}
P_0	0	0
P_1	0.125	50
P_2	0.250	100

For phosphorus, single superphosphate was applied by spreading appropriate quantity evenly over the surface soil in the polypots.

Design: Three factor C R D
Replications: 3

3.8. Sterilization of soil – sand mixture

Uncultivated soil collected from College of Forestry campus was , mixed with sand (2:1) and sterilized in an autoclave at 121°C at 1.06 kg cm^{-2} pressure for 15 minutes. The sterilized soil was filled in black polybags of size 22 × 15.2 cms. The approximate weight of soil in each bag was 1 kg.

3.9. Inoculation

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

3.9.1. Preparation of inoculation

Yeast extract mannitol broth (25 ml) was dispensed in to 100 ml conical flask and sterilized at 121°C for 15 minutes. A loopful of culture of each isolate was inoculated to the broth and incubated at 26-28 °C for seven days on a rotary shaker.

3.9.2. Pretreatment of seeds

Mangium seeds were pretreated in boiling water for 30 seconds and soaked overnight in sterile cold water.

3.9.3. Preparation of adhesive as a sticking agent

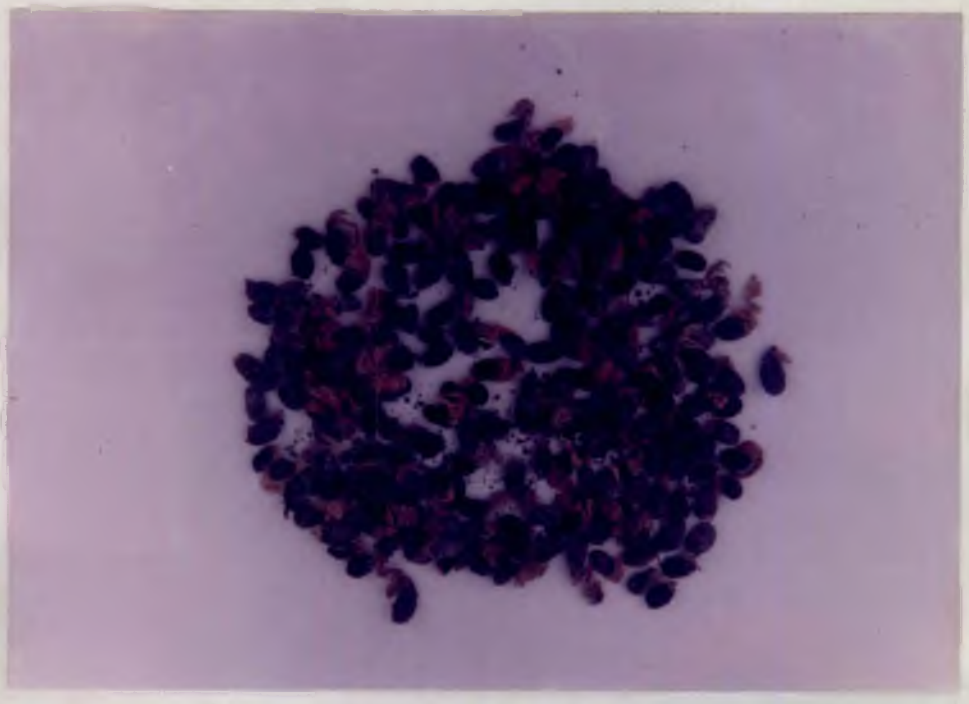
100g gum arabic was dissolved in 230 ml distilled water. The solution was heated to dissolve the gum more quickly.

3.9.4. Inoculation with local isolates

Mangium seeds were coated with small quantity of adhesive in a beaker. To this rhizobia inoculated broth and carrier medium (peat) were added, and mixed thoroughly. Finally small quantity of calcium carbonate ($CaCO_3$) powder was added and mixed rapidly but smoothly for 1 – 2 minutes until all seeds are evenly coated and separated (Plate 6).

Plate 6. Seeds of mangium pelleted with isolates of *Rhizobium*

Plate7. General view of the seedlings in trial "Competition from native ineffective rhizobia"



3.9.5. Inoculation with commercial cultures

First seeds were coated with small quantity of adhesive in a beaker. Then they were coated with peat based commercial inoculants, and mixed thoroughly. Finally small quantity of $CaCO_3$ powder was added and mixed for 1 – 2 minutes, until all seeds are evenly coated and separated.

3.10. Raising of seedlings

The pelleted seeds were sown in the black polybags containing sterilized soil – sand mixture, so that sunlight may not inhibit nodulation. In control pots seeds were sown in polybags without inoculation with any *Rhizobium*. The polybags were kept over plastic sheets and the sides of the plot were covered with polythene sheets to avoid contamination.

After the establishment, the excess seedlings were removed and only one seedling per bag was retained. Fertilizer and lime was applied after three weeks as per the treatments in different experiments .

3.11. Maintenance and harvesting

The plants were maintained in the polybags for six months. Proper care has been taken to prevent contamination. After six months, the seedlings were carefully removed with their root system intact and observations were recorded.

3.12. Observations

3.12.1. Nodulation characterstics

3.12.1.1. Number and weight of nodules

The root portion of the seedlings were washed, nodules were separated from the roots carefully and counted. Fresh weight was recorded on an electronic

Plate 8. General view of the seedlings in trial "Efficiency of the isolates under different soil acidity"

Plate 9. General view of the seedlings in trial "Efficiency of the isolates under different soil fertility"


ACIDITY TRIALS


FERTILIZER TRIALS

balance. Nodules were dried in a hot air oven at 60 - 80°C for 48 hours after initial drying for few days in shade and dry weight (g) was also estimated.

3.12.1.2. Number and weight of effective nodules

The nodules were separated in to effective and ineffective nodules and counted. This was found out by cutting each nodule in to two equal halves and noting the pink colouration (due to the presence of leghaemoglobin) in the central region. Those nodules which had pink colour were classified as effective and those without pink colour as ineffective (Vincent,1970). Fresh weight (g) of the effective nodules was recorded and then dried it in an oven at 60 - 80°C for 48 hours, after drying for few days in shade. The dry weight of the nodules was recorded using an electronic balance.

3.12.2. Growth Characterstics

3.12.2.1. Height

Height was measured from the collar to the tip of the growing point using a meter scale and expressed in centimetre.

3.12.2.2. Collar diameter

The collar diameter was measured with the help of a vernier calliper and expressed in millimetre.

3.12.2.3. Number of leaves

At the time of destructive sampling, the number of compound leaves and number of phyllodes of the seedlings were also counted.

3.12.2.4. Leaf area

The leaf area of individual seedlings were measured with a Portable Area meter (Model LICOR Li-3000A) and was expressed in cm^2.

3.12.2.5. Depth of tap root

Plants were carefully uprooted and length of the longest root was measured from base of the plant to the tip of the root and expressed in cm.

3.12.2.6. Number of lateral roots

The number of lateral roots of each seedlings was also counted.

3.12.2.7. Dry weight of the seedlings

The seedlings were destructively sampled, the shoot and root portions were separated from the collar. The fresh weights (g) of the stem, leaf and root were recorded separately with an electronic balance. Then the samples were dried in a hot air oven at 60 - 80°C for 48 hours, after initial drying for a few days in shade. The dry weights of the stem, leaves and root were recorded separately.

3.12.2.8. Above ground biomass

Above ground biomass was calculated by summing the weights of the leaf and stem of each plant.

3.12.2.9. Total biomass

Total biomass of the plant was obtained by summing the shoot weight and root weight of each plant.

3.12.3. Plant Nitrogen content and uptake

Nitrogen content of the plant were estimated by micro-Kjeldahl digestion and distillation method (Jackson, 1958). The uptake of N was calculated from the data on plant N content and dry weight of the plants.

3.12.4. Total nitrogen of the soil

Total N content of the soil before and after the experiment was estimated by micro-Kjeldahl digestion and distillation method (Jackson, 1958).

3.13. Statistical analysis

The data were subjected to analysis of variance for Completely Randomised Design. The means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

4.1. Nodulation characteristics of *Acacia mangium* plantations

4.1.1. Nodule characteristics

Nodules were observed in all the mangium plantations surveyed. The size of nodules ranged from 2 to 10 mm. The shape varied from round to finger shaped and coralloid to astragalloid (Plate1). Some of the nodules were too small and lacked the typical pink pigmentation of leghaemoglobin. Most of the nodules were found attached to fine roots, generally with in 0-5 cm layer of soil. The number of nodules per 100 cm^3 of soil ranged between 6.3 and 24.3 (Table 1). The highest number of nodules was recorded from a plantation at Muvatupuzha in Ernakulam district. This was followed by the plantation at Thiruvazhamkunnu in Palakkad district.

4.1.2 Growth characteristics of the rhizobial isolates

4.1.2.1. Growth on YEM with Congo red

The rhizobial isolates from different plantations varied in their growth rates. Eleven isolates were slow growing and showed growth on YEM only after five days (Table1), while two isolates were fast growing and from Mananthavadi (MRh-3) and Muvatupuzha (MRh-7) showed growth within 3-5 days. Altogether 13 isolates were identified from different districts of Kerala. These isolates were addressed as MRh-1 to MRh-13.

The rhizobial colonies of the isolates were circular, opaque, white and convex .The two isolates from Mananthavadi and Muvatupuzha were circular, convex, raised and mucilaginous. The isolates from Adimali and Trivandrum were circular, flat and mucilaginous. The isolate from Ranni was circular, milky, soft and gummy.

4.1.2.2. Plant infection test

All the isolates showed nodulation on the Jensen's agar slope 5-6 weeks after inoculation (Plate 5).

4.1.2.3. The plate count

The plate counts showed significant differences between the isolates in number of cells per ml. The plate count was highest for the isolate MRh-3 (172×10^6) followed by the isolate MRh-2 (94×10^6). The isolates MRh-1 and MRh-8 showed relatively low count (Table 1).

4.2. Evaluation of competition from native ineffective rhizobia

The performance of the rhizobial isolates showed considerable variation in sterilized and unsterilized soils.

4.2.1. Number of nodules and effective nodules

Seedlings grown in unsterilized soil showed a general superiority in number of nodules as compared to seedlings in sterilized soil (Table 2). The rhizobial isolate from *A. auriculiformis* (ARh-4, ARh-3 and ARh-1) dominated in unsterilized soil. While in sterilized soil, inoculation with isolates MRh-13 followed by MRh-10 and "Agroforester-Group A" were significantly superior to all other isolates. Among MRh-isolates, MRh-6 recorded lowest number of nodules. In sterilized soil, inoculation with ARh isolates showed no significant differences from uninoculated seedlings and were significantly inferior to seedlings inoculated with MRh isolates.

In unsterilized soil the isolate MRh-6 recorded maximum number of effective

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In unsterilized soil the isolate MRh-6 recorded maximum number of effective

Table 1: Characteristics of mangium plantations and rhizobia isolated from different parts of Kerala

Sl. No.	Origin of isolate		Soil pH	Age of the tree (year)	GBH of the tree (cm)	Number of Nodules per 100 cm^3 of soil	*Rhizobium* isolate	Rhizobial Growth	Viable count of bacteria per ml
	District	Locality							
1	Kannur	Irikkur	4.9	2	35.0	08.0	MRh-1	+	09.7×10^6
2	Kozhikode	Perambra	5.2	3	73.3	12.3	MRh-2	+	94.0×10^6
3	Wynad	Mananthavadi	4.9	3	30.0	16.3	MRh-3	++	172.0×10^6
4	Malappuram	Perinthalmanna	5.0	9	56.0	08.7	MRh-4	+	28.0×10^6
5	Palghat	Thiruvazamkunnu	6.2	3	95.7	23.0	MRh-5	+	23.3×10^6
6	Trichur	Vellanikkara	5.1	2	55.0	19.7	MRh-6	+	36.3×10^6
7	Ernakulam	Muvatupuzha	5.7	3	64.7	24.3	MRh-7	++	84.0×10^6
8	Idukki	Adimali	5.0	2	15.7	07.0	MRh-8	+	14.3×10^6
9	Kottayam	Kanjirappalli	4.9	2	26.3	15.3	MRh-9	+	55.6×10^6
10	Alappuzha	Mavelikkara	5.0	2	57.7	05.7	MRh-10	+	80.3×10^6
11	Pathanamthitta	Ranni	5.1	3	22.3	06.3	MRh-11	+	45.0×10^6
12	Quilon	Karunagappalli	4.9	2	34.7	11.0	MRh-12	+	22.3×10^6
13	Trivandrum	Pattam	5.0	9	110.8	06.3	MRh-13	+	61.7×10^6

++ Fast grower (3-5 days for growth) + slow grower (5 -9 days for growth)

Table 2: Influence of rhizobial inoculation on nodulation characteristics of *A. mangium* seedlings in sterilized and unsterilized soils

Sl. No.	*Rhizobium* isolate	Sterilized soil (g plant^{-1})						Unsterilized soil (g plant^{-1})					
		Number of nodules	Number of effective nodules	Fresh weight of nodules	Fresh weight of effective nodules	Dry weight of nodules	Dry weight of effective nodules	Number of nodules	Number of effective nodules	Fresh weight of nodules	Fresh weight of effective nodules	Dry weight of nodules	Dry weight of effective nodules
1	MRh-2	23.3	16.3	0.130	0.102	0.028	0.022	16.7	13.3	0.302	0.171	0.066	0.037
2	MRh-13	41.0	17.0	0.313	0.296	0.068	0.064	24.0	15.0	0.289	0.289	0.063	0.063
3	MRh-6	12.0	12.0	0.147	0.120	0.032	0.026	46.3	31.7	0.267	0.254	0.059	0.055
4	MRh-3	20.0	12.0	0.227	0.200	0.050	0.043	34.0	10.7	0.275	0.193	0.060	0.042
5	MRh-10	31.7	20.0	0.320	0.276	0.070	0.060	12.7	07.0	0.106	0.108	0.023	0.023
6	ARh-1	09.0	03.7	0.055	0.041	0.012	0.009	56.3	25.3	0.320	0.241	0.070	0.052
7	ARh-2	08.0	03.0	0.032	0.025	0.007	0.005	52.3	15.7	0.394	0.295	0.086	0.064
8	ARh-3	08.7	03.3	0.039	0.030	0.009	0.007	60.7	24.0	0.305	0.264	0.067	0.057
9	ARh-4	11.0	03.3	0.034	0.027	0.007	0.006	70.7	25.0	0.262	0.153	0.057	0.033
10	ARh-5	03.7	03.0	0.053	0.044	0.011	0.010	42.0	14.0	0.376	0.245	0.082	0.053
11	Commercial culture – Agroforester group A	24.3	21.3	0.281	0.151	0.062	0.033	24.0	11.0	0.299	0.207	0.066	0.045
12	Commercial culture – Agroforester group C	13.0	01.7	0.069	0.050	0.015	0.011	21.3	13.3	0.352	0.284	0.077	0.061
13	Uninoculated	04.3	03.0	0.041	0.035	0.009	0.008	36.7	12.7	0.230	0.220	0.050	0.048
	C.D (0.05)	04.3	02.3	0.016	0.016	0.005	0.005	04.3	2.30	0.016	0.016	0.005	0.005
	SEM (±)	01.5	0.80	0.006	0.005	0.002	0.002	01.5	0.80	0.006	0.005	0.002	0.002

nodules followed by the isolates ARh-1, ARh-4 and ARh-3 (Table 2). Eventhough the above isolates were significantly superior in unsterilized soil, the MRh-isolates except MRh-6 and MRh-3 showed significant increase in number of effective nodules in sterilized soil. In sterilized soil, seedlings inoculated with ARh-isolates showed no significant differences from uninoculated seedlings, which showed lowest number of effective nodules.

4.2.2. Weight of nodules

The fresh and dry weights of nodules and effective nodules were influenced by rhizobial inoculation to seedlings in both, sterilized and unsterilized soils (Table2). Most of the isolates showed superiority in unsterilized soil as compared to its performance in sterilized soil. The inoculation with isolates ARh-2 and ARh-5 showed maximum fresh and dry weight of nodules in unsterilized soil. The isolates MRh-13 and MRh-10 showed higher nodule fresh and dry weight in sterilized soil. While MRh-2 recorded lowest value followed by MRh-6. Seedlings inoculated with ARh isolates showed no significant difference from uninoculated seedlings and their values were significantly lower than that of MRh isolates.

In unsterilized soil seedlings inoculated with isolates, ARh-2, MRh-13, and "Agroforester-Group C" recorded maximum fresh and dry weights of effective nodules, while in sterilized soil MRh-13 and MRh-10 were superior. Seedlings inoculated with ARh isolates were on par with uninoculated seedlings.

4.2.3. Collar diameter

Collar diameter of *A. mangium* seedlings in sterilized and unsterilized soils showed significant difference due to inoculation (Table 3). Inoculation with

Table 3: Influence of rhizobial inoculation on growth characteristics of *A. mangium* seedlings grown in sterilized and unsterilized soils

Sl. No.	*Rhizobium* Isolate	Sterilized soil					Unsterilized soil				
		Collar diameter (mm)	Shoot height (cm)	Number of phyllodes	Number of lateral roots	Root depth (cm)	Collar diameter (mm)	Shoot height (cm)	Number of phyllodes	Number of lateral roots	Root depth (cm)
1	MRh-2	1.71	15.29	5.6	3.7	27.50	1.98	13.67	9.0	10.7	30.47
2	MRh-13	1.80	16.99	5.0	9.3	30.33	2.17	14.97	8.0	11.7	40.00
3	MRh-6	1.53	11.76	5.0	7.3	27.13	1.98	14.90	8.3	12.7	32.33
4	MRh-3	1.60	15.53	3.3	8.0	25.63	2.23	14.63	8.0	13.0	26.47
5	MRh-10	1.74	15.10	5.7	9.0	27.43	1.60	8.93	6.6	08.0	18.33
6	ARh-1	1.45	10.10	2.7	5.7	17.23	2.05	10.83	8.3	12.7	29.53
7	ARh-2	1.51	11.41	4.0	8.7	20.77	1.88	12.20	7.3	10.3	25.30
8	ARh-3	1.51	09.93	4.3	3.7	23.80	2.17	15.53	7.0	12.0	28.33
9	ARh-4	1.41	09.98	2.7	5.3	20.27	2.00	13.63	8.0	09.0	24.27
10	ARh-5	1.46	11.22	3.7	3.7	23.00	1.77	10.87	7.3	08.7	28.83
11	Commercial culture - Agroforester group A	1.73	13.50	5.3	4.7	29.80	2.56	15.57	8.0	10.7	38.67
12	Commercial culture - Agroforester group C	1.41	13.13	4.3	7.7	20.00	1.93	12.27	7.0	11.7	31.67
13	Uninoculated	1.34	09.76	3.0	7.0	22.67	1.82	13.07	5.7	08.3	24.13
	C.D (0.05)	0.279	02.42	1.3	1.0	01.48	0.279	2.42	1.3	01.0	01.48
	SEM (±)	0.098	0.854	0.48	0.36	0.520	0.098	0.854	0.48	0.36	0.520

"Agroforester-Group A" in unsterilized soil recorded maximum collar diameter. There were no significant differences between other MRh-isolates and ARh-isolates in unsterilized soil but MRh isolates showed superiority in sterilized soil. Except MRh-6 and MRh-3, all the MRh isolates showed superiority over the ARh isolates and uninoculated control. There were no significant differences between the seedlings inoculated with ARh isolates and uninoculated seedling.

4.2.4. Height of seedlings

Inoculation with rhizobial isolates showed significant differences in plant height but between sterilized and unsterilized soil, differences were not significant (Table3). In sterilized soil, seedlings inoculated with ARh-isolates and uninoculated-seedlings showed no significant differences, but were inferior to seedlings inoculated with all the MRh isolates except MRh-6.

4.2.5. Number of phyllodes

The number of phyllodes (Table 3) showed no significant difference between inoculated and uninnoculated seedlings in unsterilized soil. The seedlings inoculated with MRh isolates except MRh-3 and "Agroforester - Group A" showed more number of phyllodes than seedlings innoculated with Arh-isolates and uninoculated seedlings.

4.2.6. Depth of tap root and number of lateral roots

In general, seedlings in unsterilized soil showed an increase in root depth (Table 3). Inoculation with "Agroforester-Group A" and MRh-13 in unsterilized soil increased the root depth of seedlings. In sterilized soil also inoculation with MRh-13

and "Agroforester - Group A" were superior. In sterilized soil inoculation with other MRh isolate also resulted in significant increase in root depth over the seedlings inoculated with ARh isolates and uninnoculated seedlings.

Though seedlings in unsterilized soil did not differ significantly due to the influence of *Rhizobium*, the seedlings showed significant increase in number of lateral roots over seedlings in sterilized soil. Seedlings inoculated with isolates MRh-13 and MRh-10 recorded maximum number of lateral roots while innoculation with MRh-2, "Agroforester - Group A", ARh-5 and ARh-3 resulted in low number of lateral roots. Other MRh isolates and ARh isolates did not show significant difference from uninoculated control.

4.2.7. Leaf area

There were significant differences in leaf area of the seedlings due to rhizobial inoculation both in sterilized and unsterilized soil (Table 5). In unsterilized soil the range of increase in leaf area was much higher than that in sterilized soil. The seedlings innoculated with the isolate ARh-3 recorded highest value of leaf areain unsterilized soil. In sterilized soil, innoculation with MRh-10 showed more leaf area which was followed by MRh-3. Among MRh isolates, MRh-6 recorded relatively low leaf area. All the seedling inoculated with MRh isolates, in sterilized soil showed significant increase in leaf area over seedlings inoculated with ARh isolates and uninoculated seedlings.

4.2.8. Weight of leaf, stem and root

Significant differences in leaf-fresh weight and dry weight were observed due to the influence of inoculation in sterilized as well as in unsterilized soil (Table4).

Table 4: Influence of rhizobial inoculation on weight of *A. mangium* seedlings grown in sterilized and unsterilized soils

Sl. No.	Rhizobium isolate	Sterilized soil (g plant^{-1})						Unsterilized soil (g plant^{-1})					
		Fresh weight of leaves	Dry weight of leaves	Fresh weight of stem	Dry weight of stem	Fresh weight of roots	Dry weight of roots	Fresh weight of leaves	Dry weight of leaves	Fresh weight of stem	Dry weight of stem	Fresh weight of roots	Dry weight of roots
1	MRh-2	0.698	0.153	0.537	0.101	0.424	0.104	1.80	0.226	0.604	0.216	0.997	0.244
2	MRh-13	0.764	0.181	0.569	0.148	0.565	0.138	1.73	1.05	0.697	0.283	1.11	0.272
3	MRh-6	0.510	0.112	0.376	0.097	0.371	0.091	1.01	0.309	0.681	0.242	0.949	0.233
4	MRh-3	0.777	0.147	0.466	0.139	0.552	0.135	2.21	0.631	0.749	0.303	1.07	0.263
5	MRh-10	1.530	0.317	0.465	0.154	0.568	0.139	1.78	0.465	0.459	0.132	0.683	0.168
6	ARh-1	0.405	0.063	0.128	0.039	0.316	0.077	1.71	0.473	0.483	0.221	1.13	0.276
7	ARh-2	0.421	0.089	0.180	0.035	0.276	0.067	1.67	0.592	0.549	0.256	1.26	0.310
8	ARh-3	0.397	0.090	0.150	0.033	0.255	0.062	2.33	0.791	0.783	0.296	2.06	0.505
9	ARh-4	0.284	0.095	0.226	0.042	0.194	0.047	1.90	1.100	0.583	0.240	1.25	0.306
10	ARh-5	0.284	0.083	0.210	0.038	0.181	0.044	1.66	0.389	0.657	0.277	2.03	0.498
11	Commercial culture - Agroforester group A	0.711	0.158	0.571	0.183	0.722	0.177	1.44	0.435	0.457	0.200	1.03	0.252
12	Commercial culture - Agroforester group C	0.249	0.083	0.178	0.064	0.287	0.070	1.71	0.435	0.670	0.289	0.792	0.194
13	Uninoculated	0.271	0.059	0.185	0.038	0.280	0.069	1.11	0.285	0.777	0.282	1.22	0.300
	C.D (0.05)	0.137	0.073	0.016	0.005	0.052	0.016	0.137	0.073	0.016	0.005	0.052	0.016
	SEM (±)	0.048	0.026	0.006	0.002	0.018	0.006	0.048	0.026	0.006	0.002	0.018	0.006

However, the magnitude of increase in fresh weight and dry weight of seedlings were much higher in unsterilized soil. Inoculation with isolates ARh-3 and MRh-3 in unsterilized soil showed maximum leaf fresh weight while isolates ARh-4 and MRh-13 showed maximum leaf dry weights. In sterilized soil inoculation with MRh-10 showed maximum leaf fresh and dry weights. There were no significant variations between other MRh-isolates except MRh-6 which recorded very low leaf weights. In sterilized soil, seedlings inoculated with ARh isolates were not significantly different from uninoculated seedlings and were inferior to seedlings inoculated with MRh isolates.

The stem weight of inoculated seedlings in sterilized and unsterilized soils were significantly different (Table 4). In unsterilized soil uninoculated seedlings and seedlings inoculated with the isolate ARh-3 were not significantly different. The dry weight of stem was maximum in seedlings inoculated with the isolate MRh-3 in unsterilized soil. In sterilized soil, the isolate "Agroforester Group A" and MRh-13 were on par and was superior to other isolates. The highest stem dry weight was observed due to inoculation with "Agroforester - Group A" and MRh-10 in sterilized soil, followed by inoculation with MRh-13. The influence of the isolate MRh-6 was not significant. The ARh isolates and uninoculated control were inferior to MRh isolates.

The root weight of the seedlings in unsterilized soil was superior to that in sterilized soils (Table 4). Inoculation with isolates ARh-3 and ARh-5 recorded highest root weights in unsterilized soil. In sterilized soil, "Agroforester - Group A", MRh-10 and MRh-13 showed superiority. Among MRh isolates, MRh-6 recorded significantly low root weight. The influence of ARh isolates were significantly inferior

to MRh isolates, in sterilized soil.

4.2.9. Above-ground-biomass and total biomass

The biomass of seedlings inoculated with rhizobial isolates differed significantly in sterilized and unsterilized soils (Table 5). The magnitude of increases in above ground biomass and total biomass were observed in unsterilized soil was higher as compared to sterilized soil. The isolates ARh-4, MRh-13 and ARh-3 in unsterilized soil recorded maximum values for both parameters. In sterilized soil, inoculation with MRh isolates showed a clear superiority in biomass production over inoculation with ARh isolates and uninoculated control. The seedlings inoculated with the isolates MRh-10, "Agroforester - Group A" and MRh-13 recorded maximum biomass while the seedlings inoculated with MRh-2 and MRh-6 showed minimum biomass production among seedlings inoculated with MRh isolates. There were no significant differences between "Agroforester - Group A" and MRh-13; MRh-13 and MRh-3; MRh-2 and MRh-6.

4.2.10. Plant nitrogen content

There were significant differences in plant N content due to inoculation with rhizobial isolates (Table 5). The isolate MRh-2 in unsterilized soil and the isolates MRh-10 and MRh-2 in sterilized soil showed maximum percentage of plant N. In sterilized soil, seedlings inoculated with MRh isolates showed significant increase in plant N percentage compared to seedlings inoculated with ARh isolates and uninoculated seedlings.

Table 5: Influence of rhizobial inoculation on leaf area, biomass, nitrogen content and uptake in *A. mangium* seedlings and nitrogen content of the soil

Sl. No.	*Rhizobium* isolate	Sterilized soil						Unsterilized soil					
		Leaf area (cm^2)	Above ground biomass (g $plant^{-1}$)	Total biomass (g $plant^{-1}$)	Plant nitrogen (%)	N uptake (g $plant^{-1}$)	Soil nitrogen (%)	Leaf area (cm^2	Above ground biomass (g $plant^{-1}$)	Total biomass (g $plant^{-1}$)	Plant nitrogen (%)	N uptake (g $plant^{-1}$)	Soil nitrogen (%)
1	MRh-2	22.09	0.254	0.379	0.084	0.009	2.35	60.65	0.442	0.723	0.098	0.018	2.430
2	MRh-13	28.06	0.329	0.532	0.084	0.011	2.00	78.14	1.329	1.660	0.084	0.039	2.330
3	MRh-6	15.87	0.209	0.306	0.084	0.007	2.17	36.60	0.551	0.839	0.107	0.016	1.900
4	MRh-3	30.12	0.287	0.465	0.093	0.011	2.28	66.45	0.934	1.240	0.093	0.024	1.920
5	MRh-10	59.39	0.471	0.670	0.089	0.016	2.39	56.21	0.597	0.788	0.089	0.018	2.300
6	ARh-1	14.14	0.102	0.188	0.079	0.003	1.66	59.62	0.694	1.020	0.084	0.024	1.720
7	ARh-2	15.13	0.124	0.197	0.075	0.003	1.77	60.92	0.847	1.220	0.098	0.023	1.940
8	ARh-3	13.76	0.123	0.193	0.075	0.003	1.40	100.50	1.087	1.650	0.093	0.034	1.420
9	ARh-4	11.03	0.137	0.190	0.075	0.002	1.53	66.90	1.340	1.680	0.084	0.016	2.040
10	ARh-5	09.44	0.120	0.175	0.075	0.003	1.33	89.47	0.666	1.220	0.093	0.021	1.310
11	Commercial culture - Agroforester group A	24.75	0.341	0.550	0.084	0.012	2.24	54.23	0.635	0.932	0.084	0.019	1.830
12	Commercial culture - Agroforester group C	12.75	0.147	0.229	0.079	0.003	1.53	93.91	0.724	0.980	0.084	0.016	2.110
13	Uninoculated	13.13	0.097	0.173	0.075	0.002	1.33	85.82	0.567	0.915	0.084	0.006	2.110
	C.D (0.05)	0.500	0.073	0.073	0.016	0.016	0.073	0.500	0.073	0.073	0.016	0.017	0.073
	SEM (±	0.176	0.026	0.026	0.006	0.006	0.026	0.176	0.026	0.026	0.006	0.017	0.026

4.2.11. Plant nitrogen uptake

The plant N uptake significantly increased due to inoculation of the seedlings (Table 5). In sterilized soil, all isolates, except MRh-10 were on par with each other. In unsterilized soils, the seedlings inoculated with the isolate MRh-13 alone showed superiority with respect to N uptake. All the seedlings inoculated with ARh isolates and the uninoculated seedlings were on par and showed relatively low N uptake.

4.2.12. Soil nitrogen

There were significant variations in N contents of soils due to rhizobial inoculation (Table 5). The isolates MRh-6, MRh-2, ARh-2, ARh-5, ARh-3, MRh-3 and MRh-10 in unsterilized soil as well as MRh-3 and MRh-10 in sterilized soil showed maximum percentage of soil N. Other rhizobial isolates did not show significant differences. In sterilized soil, inoculation with ARh isolates and uninoculated control did not show significant differences and recorded low soil N.

4.3. Efficiency of a rhizobial isolates under different soil acidity

4.3.1. Number of nodules and effective nodules

Inoculation with different rhizobial isolates and moderating the soil acidity by application of lime had significant effect on number of nodules and effective nodules per seedlings (Table 6 and 7). Inoculation with rhizobial isolates increased the number of nodules and effective nodules. The seedlings inoculated with the isolate MRh-13 and MRh-10 recorded highest number of nodules followed by the seedling inoculated with the culture "Agroforester - Group A". Inoculation with the isolate MRh-10 resulted in highest number of effective nodules followed by seedlings inoculated with "Agroforester - Group A". Uninoculated seedlings produced only very

Table 6: Influence of liming and inoculation of *Rhizobium* on number of nodules in *A. mangium* seedlings

Rhizobium isolate	Number of nodules				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	23.3	13.0	23.3	24.0	20.9
MRh – 13	41.0	34.0	29.3	19.3	30.9
MRh – 6	12.0	18.7	14.0	19.0	15.9
MRh – 3	20.0	17.0	22.7	18.7	19.6
MRh – 10	31.7	19.0	37.3	40.3	32.1
ARh –1	09.0	10.0	12.3	09.7	10.3
ARh – 2	11.0	09.0	12.7	11.3	11.0
Commercial culture - Agroforester group A	24.3	30.0	42.7	21.0	29.5
Commercial culture - Agroforester group C	13.0	16.0	16.7	08.3	13.5
Uninoculated	04.3	04.0	03.7	03.0	03.8
Mean	19.0	17.0	21.5	17.5	

C.D (0.05) for isolates = 1.30 SEM (±) = 0.47
C.D (0.05) for Lime = 0.84 SEM (±) = 0.30
C.D (0.05) for isolates × Lime = 2.65 SEM (±) = 0.94

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 7: Influence of liming and inoculation of *Rhizobium* on number of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Number of effective nodules				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	16.3	04.3	10.0	13.3	11.00
MRh – 13	17.0	13.0	13.0	10.7	13.42
MRh – 6	12.0	12.3	06.3	12.3	10.75
MRh – 3	12.0	08.3	10.0	08.3	09.67
MRh – 10	20.0	10.3	20.0	22.7	18.25
ARh –1	03.7	06.0	09.7	04.0	05.83
ARh – 2	03.3	05.3	10.3	05.7	06.17
Commercial culture - Agroforester group A	21.3	11.0	16.7	13.7	15.67
Commercial culture - Agroforester group C	01.7	04.7	06.0	05.3	4.42
Uninoculated	03.0	03.0	02.7	02.0	2.67
Mean	11.0	07.8	10.5	09.8	

C.D (0.05) for isolates = 1.20 SEM (±) = 0.42
C.D (0.05) for Lime = 0.75 SEM (±) = 0.27
C.D (0.05) for isolates × Lime = 2.30 SEM (±) = 0.84

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

few number of nodules and effective nodules.

Lime at L_2 level recorded maximum number of nodules and effective nodules. Interactions between rhizobial inoculation and lime application significantly influenced the number of nodules and effective nodules. Inoculation with the isolates MRh-13 without lime, "Agroforester - Group A" with L_2 level of lime and MRh-10 with L_3 level of lime had shown maximum number of nodules. The rhizobial isolate MRh-10 with L_3 level of lime and culture "Agroforester- Group A" without lime (L_0) recorded maximum number of effective nodules.

4.3.2. Weight of nodules and effective nodules

The inoculation with all rhizobial isolates except ARh-1 and ARh-2 significantly increased the fresh and dry weight of nodules and effective nodules (Table 8,9,10 and 11). The seedlings inoculated with the isolate MRh-10 followed by "Agroforester - Group A" showed heavier nodules and effective nodules. There were no significant differences between seedlings inoculated with ARh-1, ARh-2 and uninoculated seedlings.

Interactions between rhizobial inoculation and lime application had significantly influenced the fresh and dry weights of nodules and effective nodules. Inoculation with the isolate MRh-10 with L_2 level of lime showed highest value of fresh and dry weight of nodules and effective nodules followed by the same isolate and MRh-13 without lime. The isolate MRh-10 with lime levels L_1 or L_3 was superior to all other isolates.

Table 8: Influence of liming and inoculation of *Rhizobium* on fresh weight of nodules in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of nodules (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.13	0.06	0.08	0.06	0.08
MRh – 13	0.31	0.11	0.10	0.09	0.15
MRh – 6	0.15	0.08	0.13	0.24	0.15
MRh – 3	0.23	0.17	0.17	0.17	0.18
MRh – 10	0.32	0.23	0.40	0.30	0.31
ARh –1	0.06	0.04	0.06	0.03	0.05
ARh – 2	0.03	0.04	0.05	0.03	0.04
Commercial culture - Agroforester group A	0.28	0.14	0.17	0.35	0.23
Commercial culture - Agroforester group C	0.07	0.09	0.09	0.05	0.07
Uninoculated	0.04	0 05	0.05	0.04	0.05
Mean	0.16	0.10	0.13	0.14	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 9: Influence of liming and inoculation of *Rhizobium* on dry weight of nodules in *A. mangium* seedlings

Rhizobium isolate	Dry weight of nodules (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.031	0.015	0.019	0.015	0.020
MRh – 13	0.074	0.026	0.023	0.021	0.036
MRh – 6	0.035	0.018	0.031	0.058	0.035
MRh – 3	0.054	0.040	0.041	0.039	0.044
MRh – 10	0.076	0.055	0.094	0.070	0.074
ARh –1	0.013	0.009	0.014	0.007	0.011
ARh – 2	0.008	0.010	0.012	0.008	0.010
Commercial culture - Agroforester group A	0.066	0.032	0.039	0.082	0.055
Commercial culture - Agroforester group C	0.016	0.021	0.022	0.011	0.018
Uninoculated	0.010	0.012	0.012	0.010	0.011
Mean	0.038	0.024	0.031	0.032	

C.D (0.05) for isolates = 0.003 SEM (±) = 0.001
C.D (0.05) for Lime = 0.002 SEM (±) = 0.001
C.D (0.05) for isolates × Lime = 0.005 SEM (±) = 0.002

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 10: Influence of liming and inoculation of *Rhizobium* on fresh weight of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of effective nodules (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.102	0.043	0.060	0.033	0.059
MRh – 13	0.296	0.086	0.086	0.074	0.135
MRh – 6	0.120	0.061	0.110	0.200	0.123
MRh – 3	0.200	0.143	0.136	0.141	0.155
MRh – 10	0.276	0.211	0.319	0.230	0.259
ARh –1	0.041	0.032	0.046	0.026	0.036
ARh – 2	0.027	0.035	0.043	0.022	0.032
Commercial culture - Agroforester group A	0.151	0.107	0.117	0.245	0.155
Commercial culture - Agroforester group C	0.050	0.069	0.079	0.035	0.058
Uninoculated	0.035	0.040	0.042	0.028	0.036
Mean	0.130	0.083	0.104	0.103	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 11: Influence of liming and inoculation of *Rhizobium* on dry weight of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Dry weight of effective nodules (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.022	0.010	0.013	0.007	0.013
MRh – 13	0.066	0.019	0.019	0.016	0.030
MRh – 6	0.027	0.014	0.025	0.044	0.027
MRh – 3	0.044	0.032	0.030	0.031	0.034
MRh – 10	0.061	0.047	0.070	0.051	0.057
ARh –1	0.009	0.007	0.010	0.006	0.008
ARh – 2	0.006	0.008	0.010	0.005	0.007
Commercial culture - Agroforester group A	0.033	0.024	0.026	0.054	0.034
Commercial culture - Agroforester group C	0.011	0.015	0.017	0.008	0.013
Uninoculated	0.008	0.009	0.009	0.006	0.008
Mean	0.029	0.018	0.023	0.023	

C.D (0.05) for isolates = 0.003 SEM (±) = 0.001
C.D (0.05) for Lime = 0.002 SEM (±) = 0.001
C.D (0.05) for isolates × Lime = 0.005 SEM (±) = 0.002

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

4.3.3. Collar diameter

Inoculation with different rhizobial isolates and application of lime showed significant effect on collar diameter of seedlings (Table12). An increase in collar diameter was observed for inoculated seedlings as compared to uninoculated seedlings. The isolates MRh-10 and "Agroforester - Group A" which did not show significant variation between them, were superior to all other isolates. The other isolates MRh-2, MRh-13, MRh-3, ARh-1, ARh-2 and "Agroforester - Group C" did not differ significantly. There were no significant differences between "Agroforester - Group C" and uninoculated control.

The interactions between rhizobial inoculation and lime application were also significant. Rhizobial isolates "Agroforester - Group A", MRh-10, ARh-2, MRh-3 at L_2 level of lime and "Agroforester - Group A", ARh-1 with L_3 level of lime were not significantly different, but recorded higher collar diameter over other isolates. At the lower level of lime (L_1) seedlings inoculated with the isolate MRh-10 recorded higher collar diameter. The seedlings inoculated with MRh-13 showed higher collar diameter without lime application.

4.3.4. Height of seedlings

Inoculation with different rhizobial isolates and application of lime showed significant effect on height of seedlings (Table13). The seedlings inoculated with the isolate MRh-10 were taller than other inoculated seedlings. This was followed by seedlings inoculated with the culture "Agroforester - Group A". All the inoculated seedlings except the seedlings inoculated with "Agroforester - Group C" were superior to uninoculated - control.

Table 12: Influence of liming and inoculation of *Rhizobium* on collar diameter of *A. mangium* seedlings

Rhizobium isolate	Collar diameter (mm)				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	1.71	1.83	2.19	2.02	1.94
MRh – 13	1.80	1.92	2.10	1.93	1.94
MRh – 6	1.53	1.64	2.01	1.77	1.74
MRh – 3	1.60	2.11	2.47	1.75	1.99
MRh – 10	1.74	2.19	2.68	2.00	2.15
ARh –1	1.45	1.84	1.91	2.41	1.90
ARh – 2	1.41	2.01	2.48	2.17	2.02
Commercial culture - Agroforester group A	1.73	2.16	2.72	2.45	2.27
Commercial culture - Agroforester group C	1.41	1.86	2.05	2.11	1.86
Uninoculated	1.34	1.99	2.03	1.57	1.73
Mean	1.57	1.96	2.27	2.02	

C.D (0.05) for isolates = 0.143 SEM (±) = 0.051
C.D (0.05) for Lime = 0.091 SEM (±) = 0.032
C.D (0.05) for isolates × Lime = 0.286 SEM (±) = 0.102

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 =1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 13: Influence of liming and inoculation of *Rhizobium* on shoot height of *A. mangium* seedlings

Rhizobium isolate	Shoot height (cm)				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	15.29	14.40	15.17	12.62	14.37
MRh - 13	16.99	17.84	19.08	12.33	16.56
MRh - 6	11.76	11.90	12.13	11.25	11.76
MRh - 3	15.53	17.00	18.33	12.55	15.85
MRh - 10	15.10	16.05	23.50	21.63	19.07
ARh -1	10.10	14.12	13.73	17.83	13.95
ARh - 2	09.98	15.93	17.57	15.17	14.66
Commercial culture - Agroforester group A	13.83	16.97	23.02	16.88	17.67
Commercial culture - Agroforester group C	13.13	12.77	13.67	15.03	13.65
Uninoculated	09.76	11.63	14.20	17.50	13.27
Mean	13.15	14.86	17.04	15.28	

C.D (0.05) for isolates = 0.423 SEM (±) = 0.150
C.D (0.05) for Lime = 0.268 SEM (±) = 0.095
C.D (0.05) for isolates × Lime = 0.846 SEM (±) = 0.301

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 =1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Application of lime showed significant influence on the height of the seedlings. Increasing levels of lime increased height of seedlings. The interactions between the rhizobial inoculation and lime application had significant influence on seedling-height. Inoculation with the isolates MRh-10 and "Agroforester - Group A" with application of lime at L_2 level resulted in tallest seedlings. The isolate MRh-13 showed superior influence at L_0 and L_1 levels of lime while MRh-10 showed superior influence at L_3 level of lime. Seedlings inoculated with isolates ARh-1 and ARh-2 were shorter without lime application.

4.3.5. Number of phyllodes

Rhizobial inoculation and lime application significantly influenced the number of phyllodes per seedlings (Table14). Inoculation with isolates MRh-10 and "Agroforester - Group A" showed significant increase in number of phyllodes over other isolates. The isolates ARh-1, ARh-2 and "Agroforester - Group C" were on par with uninoculated - control.

Lime application had significant influence on number of phyllodes. Increasing levels of lime increased the number of phyllodes. The levels L_2 and L_3 were not significantly different but were superior to L_0 and L_1 levels. Interactions between rhizobial inoculation and lime application were significant. The seedlings inoculated with the isolate MRh-10 at L_3 levels of lime and "Agroforester - Group A" with L_2 and L_3 levels recorded more number of phyllodes over others.

4.3.6. Depth of tap root and number of lateral roots

The influences of rhizobial inoculation and application of lime were significant on the root depth of the seedlings (Table15). Inoculated seedlings showed

Table 14: Influence of liming and inoculation of *Rhizobium* on number of phyllodes of *A. mangium* seedlings

Rhizobium isolate	Number of Phyllodes				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	5.6	3.3	4.6	3.6	4.3
MRh - 13	5.0	5.3	5.0	5.3	5.2
MRh - 6	5.6	3.6	5.3	5.0	4.9
MRh - 3	3.3	5.6	5.6	4.6	4.8
MRh - 10	5.6	5.6	6.3	7.6	6.3
ARh -1	2.6	4.0	4.3	4.3	3.8
ARh - 2	2.6	3.6	4.0	4.3	3.7
Commercial culture - Agroforester group A	5.3	5.6	7.0	7.0	6.3
Commercial culture - Agroforester group C	4.3	4.3	4.6	4.3	4.4
Uninoculated	3.0	4.3	4.3	5.0	4.2
Mean	4.3	4.6	5.1	5.1	

C.D (0.05) for isolates = 0.46 SEM (±) = 0.16
C.D (0.05) for Lime = 0.29 SEM (±) = 0.10
C.D (0.05) for isolates × Lime = 0.90 SEM (±) = 0.32

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 =1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 15: Influence of liming and inoculation of *Rhizobium* on root depth of *A. mangium* seedlings

Rhizobium isolate	Root depth (cm)				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	27.5	25.7	28.9	28.7	27.7
MRh – 13	30.3	25.7	19.4	304	26.4
MRh – 6	27.1	25.5	26.0	28.8	26.9
MRh – 3	25.6	31.1	29.7	29.0	28.9
MRh – 10	27.4	31.8	32.0	27.7	29.7
ARh –1	17.2	19.4	20.5	23.0	20.1
ARh – 2	20.3	22.4	25.0	30.5	24.5
Commercial culture - Agroforester group A	29.8	37.2	28.1	35.1	32.5
Commercial culture - Agroforester group C	20.0	25.2	29.2	21.9	24.1
Uninoculated	22.7	22.1	19.2	21.2	21.3
Mean	24.8	26.6	25.8	27.6	

C.D (0.05) for isolates = 0.81 SEM (±) = 0.29
C.D (0.05) for Lime = 0.52 SEM (±) = 0.18
C.D (0.05) for isolates × Lime = 1.63 SEM (±) = 0.58

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 =1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

significant increase in root depth over uninoculated seedlings. Seedlings inoculated with the culture "Agroforester - Group A" followed by seedlings inoculated with MRh-10 recorded maximum depth of roots.

Lime application significantly improved the root depth of the seedlings. The lime level L_3 resulted in maximum depth of roots followed by L_1 level. Interactions between rhizobial inoculation and lime application were significant. The culture "Agroforester - Group A" with L_1 level of lime recorded maximum depth. The seedlings inoculated with MRh-13 and "Agroforester - Group A" showed higher depth without lime application. The seedlings inoculated with MRh-10 showed deeper roots with L_2 level of lime. A shallow root system was observed in seedlings inoculated with the isolate ARh-1 without lime application.

Rhizobial inoculation and application of lime showed significant influence on number of lateral roots (Table16). Inoculation with the isolate MRh-13 followed by "Agroforester - Group A" recorded maximum number of lateral roots. Seedlings inoculated with MRh-2 and uninoculated seedlings were not significantly different and recorded the lowest number of lateral roots.

Higher dose of lime significantly increased the number of lateral roots. L_3 level of lime was superior to lower levels of lime. Seedlings without lime application produced less number of lateral roots. Interactions between rhizobial inoculation and lime application were significant. Inoculation of the isolate MRh-13 with L_3 level of lime resulted in highest number of lateral roots followed by inoculation with "Agroforester - Group A" with L_3 level of lime. The lowest number of lateral roots was recorded by the seedlings inoculated with "Agroforester Group A" without lime.

Table 16: Influence of liming and inoculation of *Rhizobium* on number of lateral roots of *A. mangium* seedlings

Rhizobium isolate	Number of lateral roots				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	3.6	6.0	6.7	11.0	6.8
MRh - 13	9.3	11.0	13.3	16.3	12.5
MRh - 6	7.3	8.0	7.3	9.6	8.0
MRh - 3	8.0	9.7	10.0	12.0	9.9
MRh - 10	9.0	9.0	8.0	11.7	9.4
ARh -1	5.7	6.7	7.3	8.7	7.0
ARh - 2	5.3	9.3	9.3	7.7	7.9
Commercial culture - Agroforester group A	4.7	12.0	12.3	15.0	11.0
Commercial culture - Agroforester group C	7.7	8.7	10.3	9.3	9.0
Uninoculated	7.0	6.3	6.7	5.7	6.4
Mean	6.7	8.6	9.1	10.7	

C.D (0.05) for isolates = 0.55 SEM (±) = 0.19
C.D (0.05) for Lime = 0.35 SEM (±) = 0.12
C.D (0.05) for isolates × Lime = 1.1 SEM (±) = 0.39

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 =1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

4.3.7. Leaf area

Inoculation with different rhizobial isolates and application of lime had significant effect on leaf area of the seedlings (Table17). Inoculation with rhizobial isolates, except ARh-2 significantly increased the leaf area as compared to uninoculated - control. Inoculation with the culture "Agroforester - Group A" resulted in highest leaf area of the seedlings. This was followed by the seedlings inoculated with the isolate MRh-10.

Increasing levels of lime increased leaf area of seedlings. Generally seedlings with L_2 leaf of lime recorded maximum leaf area. Seedlings applied with L_3 level of lime showed significant reduction in leaf area over the level L_2. Lowest leaf area was recorded by the seedlings without liming. Significant interactions were observed between rhizobial inoculation and lime application. Inoculation with the culture "Agroforester - Group A" with L_3 level of lime recorded maximum leaf area followed by MRh-10 with L_2 level of lime. The isolate MRh-10 showed superior performance without lime application also. The seedlings inoculated with isolate ARh-2 with L_3 level of lime and "Agroforester - Group C" without lime were on par with uninoculated seedlings which recorded the lowest leaf area.

4.3.8. Weight of leaf, stem and root

The fresh and dry weight of stem, leaves and root showed significant differences due to inoculation with rhizobial isolates and application of lime. All the inoculated seedlings were superior to uninoculated control. Seedlings inoculated with MRh-10 recorded highest fresh and dry weight of leaves followed by seedlings inoculated with "Agroforester - Group A (Table 18 and 19). Maximum values of fresh

Table 17: Influence of liming and inoculation of *Rhizobium* on leaf area in *A. mangium* seedlings

Rhizobium isolate	Leaf area (cm^2 $plant^{-1}$)				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	22.1	25.1	27.0	18.8	23.3
MRh – 13	28.1	37.1	60.1	49.5	43.7
MRh – 6	15.9	26.8	52.9	47.2	35.7
MRh – 3	30.1	32.0	36.3	35.1	33.4
MRh – 10	59.4	70.2	99.5	74.2	75.8
ARh –1	14.1	26.3	22.6	17.0	20.1
ARh – 2	11.0	14.9	19.7	13.2	14.7
Commercial culture - Agroforester group A	24.7	36.8	61.5	101.0	56.0
Commercial culture - Agroforester group C	12.7	22.0	35.0	22.2	22.7
Uninoculated	13.1	19.1	25.7	14.7	18.2
Mean	23.1	31.0	43.9	39.3	

C.D (0.05) for isolates = 0.60 SEM (±) = 0.21
C.D (0.05) for Lime = 0.38 SEM (±) = 0.13
C.D (0.05) for isolates × Lime = 1.20 SEM (±) = 0.43

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 18: Influence of liming and inoculation of *Rhizobium* on fresh weight of leaves in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of leaves (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.70	0.78	1.01	0.83	0.83
MRh – 13	0.76	0.99	1.51	1.40	1.17
MRh – 6	0.51	0.66	0.77	1.07	0.75
MRh – 3	0.78	0.97	1.05	0.99	0.95
MRh – 10	1.53	1.67	2.24	1.70	1.79
ARh –1	0.40	0.73	0.64	0.48	0.57
ARh – 2	0.35	0.42	0.56	0.37	0.42
Commercial culture - Agroforester group A	0.71	1.10	1.57	2.28	1.42
Commercial culture - Agroforester group C	0.25	0.57	0.80	0.49	0.53
Uninoculated	0.27	0.43	0.54	0.31	0.39
Mean	0.63	0.83	1.07	0.99	

C.D (0.05) for isolates = 0.036 SEM (±) = 0.013
C.D (0.05) for Lime = 0.023 SEM (±) = 0.008
C.D (0.05) for isolates × Lime = 0.073 SEM (±) = 0.026

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 19: Influence of liming and inoculation of *Rhizobium* on dry weight of leaves in *A. mangium* seedlings

Rhizobium isolate	Dry weight of leaves (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.153	0.166	0.244	0.182	0.186
MRh – 13	0.181	0.200	0.267	0.242	0.223
MRh – 6	0.112	0.124	0.208	0.173	0.154
MRh – 3	0.147	0.202	0.228	0.219	0.199
MRh – 10	0.317	0.337	0.483	0.502	0.410
ARh –1	0.063	0.150	0.122	0.098	0.108
ARh – 2	0.094	0.079	0.139	0.076	0.097
Commercial culture - Agroforester group A	0.158	0.244	0.399	0.669	0.367
Commercial culture - Agroforester group C	0.083	0.091	0.127	0.084	0.096
Uninoculated	0.059	0.090	0.115	0.064	0.082
Mean	0.137	0.168	0.233	0.231	

C.D (0.05) for isolates = 0.003 SEM (±) = 0.001
C.D (0.05) for Lime = 0.002 SEM (±) = 0.001
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

and dry weight of stem and root were observed in seedlings inoculated with "Agroforester - Group A" followed by seedlings inoculated with the isolate MRh-10 (Table 20,21,22 and 23).

Increasing levels of lime increased the fresh and dry weights of leaf, stem and root. The seedlings recorded maximum fresh and dry weights of leaf, stem and root with a lime levels of L_2. Interactions between rhizobial inoculation and lime application significantly influenced the fresh and dry weight of leaf, stem and roots. Seedlings inoculated with "Agroforester - Group A" with L_3 level of lime and MRh-10 with L_2 level of lime recorded maximum values for these parameters. MRh-10 performed well at L_1 levels of lime also. Seedlings inoculated with ARh isolates were inferior and not significantly different from uninoculated seedlings.

4.3.9. Above-ground-biomass and total biomass

Rhizobial inoculation and lime application significantly influenced the above ground biomass and total biomass of the seedlings (Table 24 and 25). Inoculated seedlings were superior to uninoculated seedlings. Inoculation with the isolate "Agroforester - Group A" showed maximum biomass followed by seedlings inoculated with MRh-10.

Increasing levels of lime increased the above ground biomass and total biomass. However, at L_3 level of lime, the seedlings showed lower biomass than at L_2 level. Seedlings without lime application recorded lowest values of plant biomass. Significant interactions were observed between rhizobial inoculation and lime application. The highest above-ground-biomass and total biomass were observed in the seedlings inoculated with the culture "Agroforester - Group A" at a lime level of

Table 20: Influence of liming and inoculation of *Rhizobium* on fresh weight of stem in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of stem (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.54	0.57	0.71	0.65	0.62
MRh – 13	0.57	0.64	0.66	0.62	0.62
MRh – 6	0.38	0.51	0.60	0.59	0.52
MRh – 3	0.47	0.56	0.68	0.59	0.57
MRh – 10	0.46	0.59	0.90	0.75	0.68
ARh –1	0.12	0.19	0.25	0.30	0.22
ARh – 2	0.23	0.17	0.29	0.16	0.21
Commercial culture - Agroforester group A	0.57	0.65	0.86	1.06	0.79
Commercial culture - Agroforester group C	0.18	0.25	0.38	0.27	0.27
Uninoculated	0.18	0.21	0.32	0.18	0.22
Mean	0.37	0.43	0.56	0.52	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 21: Influence of liming of inoculation of *Rhizobium* on dry weight of stem in *A. mangium* seedlings

Rhizobium isolate	Dry weight of stem (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.101	0.119	0.240	0.243	0.176
MRh – 13	0.148	0.217	0.241	0.223	0.207
MRh – 6	0.097	0.127	0.277	0.257	0.190
MRh – 3	0.139	0.154	0.268	0.173	0.183
MRh – 10	0.154	0.189	0.367	0.292	0.250
ARh –1	0.039	0.079	0.106	0.125	0.087
ARh – 2	0.042	0.066	0.125	0.070	0.076
Commercial culture - Agroforester group A	0.183	0.230	0.418	0.465	0.324
Commercial culture - Agroforester group C	0.063	0.111	0.174	0.128	0.119
Uninoculated	0.038	0.075	0.135	0.057	0.076
Mean	0.100	0.137	0.235	0.203	

C.D (0.05) for isolates = 0.003 SEM (±) = 0.001
C.D (0.05) for Lime = 0.002 SEM (±) = 0.001
C.D (0.05) for isolates × Lime = 0.005 SEM (±) = 0.002

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 22: Influence of liming and inoculation of *Rhizobium* on fresh weight of roots in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of roots (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.42	0.63	0.78	0.63	0.62
MRh – 13	0.56	0.69	0.78	0.73	0.69
MRh – 6	0.37	0.40	0.74	0.47	0.50
MRh – 3	0.55	0.58	0.82	0.60	0.64
MRh – 10	0.57	0.84	1.16	1.21	0.95
ARh –1	0.32	0.46	0.40	0.47	0.41
ARh – 2	0.19	0.30	0.49	0.32	0.32
Commercial culture - Agroforester group A	0.72	0.97	1.14	1.41	1.06
Commercial culture - Agroforester group C	0.29	0.42	0.64	0.38	0.43
Uninoculated	0.28	0.41	0.19	0.21	0.27
Mean	0.43	0.57	0.71	0.64	

C.D (0.05) for isolates = 0.026 SEM (±) = 0.009
C.D (0.05) for Lime = 0.016 SEM (±) = 0.006
C.D (0.05) for isolates × Lime = 0.051 SEM (±) = 0.018

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 23: Influence of liming and inoculation of *Rhizobium* on dry weight of roots in *A. mangium* seedlings

Rhizobium isolate	Dry weight of roots (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.112	0.167	0.206	0.167	0.163
MRh – 13	0.150	0.182	0.207	0.193	0.183
MRh – 6	0.098	0.107	0.200	0.124	0.131
MRh – 3	0.146	0.153	0.217	0.158	0.169
MRh – 10	0.151	0.223	0.308	0.320	0.250
ARh –1	0.084	0.123	0.107	0.123	0.109
ARh – 2	0.051	0.079	0.129	0.085	0.086
Commercial culture - Agroforester group A	0.191	0.257	0.301	0.374	0.281
Commercial culture - Agroforester group C	0.076	0.112	0.169	0.101	0.114
Uninoculated	0.074	0.107	0.049	0.055	0.071
Mean	0.113	0.151	0.189	0.170	

C.D (0.05) for isolates = 0.003 SEM (±) = 0.001
C.D (0.05) for Lime = 0.002 SEM (±) = 0.001
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 24: Influence of liming and inoculation of *Rhizobium* on above ground biomass of *A. mangium* seedlings

Rhizobium isolate	Above ground biomass (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh – 2	0.254	0.285	0.484	0.425	0.362
MRh – 13	0.329	0.417	0.509	0.464	0.430
MRh – 6	0.209	0.252	0.485	0.430	0.344
MRh – 3	0.287	0.356	0.495	0.392	0.382
MRh – 10	0.471	0.525	0.850	0.794	0.660
ARh –1	0.102	0.229	0.228	0.224	0.196
ARh – 2	0.136	0.145	0.264	0.147	0.173
Commercial culture - Agroforester group A	0.341	0.474	0.817	1.134	0.692
Commercial culture - Agroforester group C	0.146	0.202	0.301	0.212	0.215
Uninoculated	0.097	0.165	0.250	0.121	0.158
Mean	0.237	0.305	0.468	0.434	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 25: Influence of liming and inoculation of *Rhizobium* on total biomass of *A. mangium* seedlings

Rhizobium isolate	Total biomass (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	0.389	0.461	0.703	0.599	0.538
MRh - 13	0.544	0.618	0.734	0.674	0.643
MRh - 6	0.334	0.372	0.705	0.598	0.502
MRh - 3	0.477	0.541	0.743	0.581	0.585
MRh - 10	0.682	0.795	1.230	1.170	0.968
ARh -1	0.195	0.359	0.345	0.353	0.313
ARh - 2	0.193	0.231	0.403	0.237	0.266
Commercial culture - Agroforester group A	0.565	0.755	1.140	1.560	1.007
Commercial culture - Agroforester group C	0.234	0.330	0.487	0.321	0.343
Uninoculated	0.179	0.281	0.308	0.182	0.238
Mean	0.379	0.475	0.680	0.627	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

L_3 followed by the seedlings inoculated with the isolate MRh-10 at a lime level of L_2. The rhizobia, MRh-10 and "Agroforester - Group A" showed superior performance at L_0 and L_1 levels of lime as well. The ARh-isolates performed poorly and showed no significant differences from uninoculated seedlings.

4.3.10. Plant nitrogen content

There were significant differences in plant N content due to inoculation with rhizobial isolates and application of lime (Table 26). Seedlings inoculated with the isolate MRh-2 or MRh-3 recorded highest percentage of N. There was no significant difference between rhizobial isolate MRh-6 and MRh-10. Similarly MRh-13 and "Agroforester - Group A" were on par.

Increasing the lime levels from L_0 to L_2 reduced the plant N content while at L_3 level, the seedlings showed significant increase in N. The interactions between rhizobial inoculation and lime application showed significant influence on plant N percentage. Inoculation with the isolate MRh-6 and lime level of L_3 resulted in highest percentage of N in seedlings, followed by the seedlings inoculated with the isolates MRh-3 and MRh-13 with L_3 level of lime. This was followed by seedlings inoculated with the isolate MRh-10 with a lime level of L_1. Seedlings inoculated with ARh isolates showed very low plant nitrogen content.

4.3.11. Plant nitrogen uptake

The nitrogen uptake by the seedlings inoculated with MRh-isolates and "Agroforester - Group A" was significantly higher than the seedlings inoculated with ARh isolates, "Agroforester - Group C" and uninoculated seedlings (Table27). The ARh-isolates and the culture "Agroforester - Group C" were on par with uninoculated

Table 26: Influence of liming and inoculation of *Rhizobium* on plant nitrogen content of *A. mangium* seedlings

Rhizobium isolate	Plant nitrogen (%)				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	4.20	3.60	3.97	4.07	3.96
MRh - 13	3.57	3.23	2.83	4.37	3.50
MRh - 6	3.87	3.37	3.27	4.63	3.78
MRh - 3	4.07	3.83	3.47	4.47	3.96
MRh - 10	4.28	4.37	3.47	3.10	3.80
ARh -1	2.97	2.63	2.87	3.10	2.89
ARh - 2	2.73	2.10	2.33	3.97	2.78
Commercial culture - Agroforester group A	4.00	3.57	3.23	3.37	3.54
Commercial culture - Agroforester group C	2.73	3.83	3.53	2.63	3.18
Uninoculated	2.37	3.17	2.27	3.07	2.72
Mean	3.48	3.37	3.12	3.68	

C.D (0.05) for isolates = 0.063 SEM (±) = 0.022
C.D (0.05) for Lime = 0.040 SEM (±) = 0.014
C.D (0.05) for isolates × Lime = 0.126 SEM (±) = 0.045

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 27: Influence of liming and inoculation of *Rhizobium* on N uptake of *A. mangium* seedlings

Rhizobium isolates	N uptake (g plant^{-1})				
	L_0	L_1	L_2	L_3	Mean
MRh-2	0.016	0.017	0.028	0.024	0.021
MRh-13	0.019	0.020	0.021	0.029	0.022
MRh-6	0.013	0.013	0.023	0.028	0.019
MRh-3	0.019	0.021	0.026	0.026	0.023
MRh-10	0.029	0.035	0.043	0.036	0.036
ARh-1	0.006	0.009	0.010	0.011	0.009
ARh-2	0.005	0.005	0.009	0.009	0.007
Commercial Culture-Agroforester - Group A	0.023	0.027	0.037	0.052	0.035
Commercial Culture-Agroforester - Group C	0.006	0.013	0.017	0.008	0.011
Uninoculated	0.004	0.009	0.007	0.006	0.006
Mean	0.014	0.017	0.022	0.023	

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003

C.D (0.05) for Lime = 0.005 SEM (±) = 0.002

C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 =0 kg ha^{-1}

L_1 =500 kg ha^{-1}

L_2 =1000 kg ha^{-1}

L_3 =1500 kg ha^{-1}

seedlings. The interactions between lime levels and inoculation with rhizobial isolates varied significantly. Maximum uptake was observed in seedlings limed at L_3 level and inoculated with "Agroforester - Group A" and in the seedlings inoculated with MRh-10 and applied with lime at the level of L_2.

Increasing levels of lime increased the N - uptake by the seedlings.

4.3.12. Soil nitrogen

The influences of rhizobial inoculation and application of lime were significant on soil N (Table28). The inoculation with all the MRh-isolates and the culture, "Agroforester - Group A" resulted in increase in soil N over control. ARh isolates were inferior and showed no significant difference from uninoculated - control.

The interactions between rhizobial inoculation and lime application were significant. The isolates MRh-6, MRh-2, MRh-3, and MRh-13 with lime level L_2 were not significantly different from MRh-2 and MRh-13 with L_1 level of lime. Uninoculated control without lime performed poorly.

4.4. Efficiency of rhizobial isolates under different soil fertility

4.4.1. Number of nodules and effective nodules

Inoculation with rhizobial isolates showed significant influence on number of nodules and effective nodules (Table 29 and 31). All the inoculated seedlings were significantly superior to uninoculated seedlings. Seedlings inoculated with MRh-6 showed significantly higher number of nodules and effective nodules followed by seedlings inoculated with MRh-13. Uninoculated seedlings produced lowest number of nodules and effective nodules.

Interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on number of nodules and effective nodules. Increase in N and P levels significantly increased the number of nodules. However, at P_2 level significant reduction was observed in the number of nodules. Inoculated seedlings produced significantly higher number of nodules and effective nodules than uninoculated seedlings at all levels of applied N and P. The seedlings inoculated with MRh-6 and applied with highest dose of N (N_2) recorded maximum number of nodules and effective nodules. Uninoculated seedlings did not show any appreciable differences due to fertilizer N and recorded lowest number of nodules. Generally addition of N-fertilizer contributed significantly higher number of nodules and effective nodules than addition of P fertilizer in inoculated plants. Inoculated seedlings without P-fertilizer showed higher number of nodules and effective nodules than seedlings without N-fertilizer.

The interactions between N and P were also significant. All the isolates responded favourably to fertilizer application by increasing the number of nodules and effective nodules(Table 30 and 32). Fertilizer levels N_1P_1 and N_2P_1 resulted in higher number of nodules and effective nodules followed by N_1P_2 and N_2P_2 levels. Seedlings without fertilizer N and P showed lowest numbers of nodules and effective nodules.

Interactions between rhizobial inoculation and application of N and P fertilizer were highly significant. Seedlings inoculated with MRh-6 and fertilized with N_2P_1, N_1P_1 and N_1P_2 levels showed no significant difference from seedlings inoculated with MRh-10 and fertilized with N_1P_1 and recorded highest number of nodules and effective nodules. Lowest numbers of nodules and effective nodules

Table 28: Influence of liming and inoculation of *Rhizobium* on soil nitrogen

Rhizobium isolate	Soil nitrogen (%)				
	L_0	L_1	L_2	L_3	Mean
MRh - 2	0.084	0.098	0.098	0.084	0.091
MRh - 13	0.084	0.089	0.098	0.084	0.089
MRh - 6	0.084	0.098	0.103	0.084	0.092
MRh - 3	0.093	0.084	0.098	0.089	0.091
MRh - 10	0.089	0.098	0.093	0.084	0.091
ARh -1	0.079	0.084	0.089	0.098	0.087
ARh - 2	0.075	0.084	0.089	0.093	0.085
Commercial culture - Agroforester group A	0.084	0.084	0.093	0.084	0.086
Commercial culture - Agroforester group C	0.079	0.079	0.075	0.075	0.077
Uninoculated	0.075	0.075	0.084	0.089	0.080
Mean	0.082	0.087	0.092	0.087	
Control (Before experiment)	0.079	0.084	0.089	0.089	0.085

C.D (0.05) for isolates = 0.008 SEM (±) = 0.003
C.D (0.05) for Lime = 0.005 SEM (±) = 0.002
C.D (0.05) for isolates × Lime = 0.016 SEM (±) = 0.006

L_0 = 0 kg ha^{-1}
L_1 = 500 kg ha^{-1}
L_2 = 1000 kg ha^{-1}
L_3 = 1500 kg ha^{-1}

Table 29: Influence of inoculation of *Rhizobium* isolates and application of N and P on number of nodules in *A. mangium* seedlings

Rhizobium isolate	Number of nodules						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	31.1	72.3	80.4	60.8	67.9	55.2	61.3
MRh - 13	72.0	78.9	85.5	58.0	89.6	88.9	78.8
MRh - 6	56.2	102.3	120.2	58.0	112.1	108.7	92.9
MRh - 3	32.4	67.8	98.8	57.6	72.4	69.0	66.3
MRh - 10	39.7	83.2	81.2	43.3	84.1	76.7	68.0
Uninoculated	06.0	08.8	07.6	06.2	07.2	08.9	7.4
Mean	39.6	68.9	79.0	47.3	72.2	67.9	

C.D (0.05) for Isolates = 3.1 SEM (±) = 1.1
C.D (0.05) for N = 2.2 SEM (±) = 0.77
C.D (0.05) for P = 2.2 SEM (±) = 0.77
C.D (0.05) for Isolates × N = 5.3 SEM (±) = 1.9
C.D (0.05) for Isolates × P = 5.3 SEM (±) = 1.9

Table 30: Influence of N and P on number of nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Number of nodules								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	23.3	36.3	33.6	70.3	68.3	78.3	88.7	99.0	53.7
MRh - 13	41.0	70.7	104.3	69.0	88.3	79.3	64.0	109.7	83.0
MRh - 6	12.0	61.7	95.0	38.7	136.7	131.7	123.3	138.0	99.3
MRh - 3	20.0	48.3	29.0	35.7	85.0	82.7	117.0	84.0	95.3
MRh - 10	31.7	40.3	47.0	46.7	130.3	72.7	51.7	81.7	110.3
Uninoculated	04.3	03.3	10.3	09.0	09.3	08.0	05.3	09.0	8.3
Mean	22.1	43.4	53.2	44.9	86.3	75.4	75.0	86.9	75.0

C.D (0.05) for N × P = 3.7 SEM (±) = 1.3
C.D (0.05) for Isolates × N × P = 9.1 SEM (±) = 3.3

Table 31: Influence of inoculation of *Rhizobium* isolates and application of N and P on number of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Number of effective nodules						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	23.5	58.1	57.3	45.4	53.0	40.6	46.3
MRh - 13	54.3	56.6	53.8	35.1	68.1	61.4	54.9
MRh - 6	48.6	85.8	86.7	45.3	89.8	85.9	73.7
MRh - 3	23.2	44.6	71.0	41.3	53.4	44.0	46.3
MRh - 10	29.4	58.1	45.3	27.2	62.7	43.0	44.3
Uninoculated	04.1	03.3	41.7	02.8	03.8	5.6	04.0
Mean	30.5	51.0	53.1	32.9	55.1	46.7	

C.D (0.05) for Isolates = 2.21 SEM (±) = 0.79
C.D (0.05) for **N** = 1.6 SEM (±) = 0.56
C.D (0.05) for P = 1.6 SEM (±) = 0.56
C.D (0.05) for Isolates × N = 3.8 SEM (±) = 1.36
C.D (0.05) for Isolates × P = 3.8 SEM (±) = 1.36

Table 32: Influence of N and P on number of effective nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Number of effective nodules								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	16.3	28.0	26.3	52.7	56.0	65.7	67.3	75.0	29.7
MRh – 13	17.0	58.3	87.7	54.0	66.7	49.0	34.3	79.3	47.7
MRh – 6	12.0	47.0	86.7	34.0	118.	105.0	90.0	104.0	66.0
MRh - 3	12.0	37.7	20.0	23.3	60.3	50.0	88.7	62.3	62.0
MRh - 10	20.0	31.0	37.3	33.0	101.3	40.0	28.7	55.7	51.7
Uninoculated	03.0	03.3	06.0	02.7	03.7	03.7	02.7	04.3	07.0
Mean	13.4	34.2	44.0	33.3	67.7	52.2	51.9	63.4	44.0

C.D (0.05) for N × P = 2.7 SEM (±) = 0.96
C.D (0.05) for isolates × N × P = 6.6 SEM (±) = 2.4

were recorded by uninoculated seedlings and seedlings inoculated with MRh-6 without fertilizer application (N_0P_0).

4.4.2. Weight of nodules and effective nodules

Fresh weights and dry weights of nodule and effective nodules were influenced significantly by rhizobial inoculation (Table 33,35,37 and 39). All the five rhizobial isolates tested were effective in increasing the fresh and dry weights of nodules and effective nodules. The most effective isolate was MRh-6 followed by MRh-10.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on fresh weights and dry weights of nodules and effective nodules. Increase in doses of N and P resulted in significant increase in the weights of nodules and effective nodules. Inoculated seedlings showed higher weights of nodules and effective nodules at all levels of applied N and P. Seedlings inoculated with the isolate MRh-6 with lower and higher dose of N (N_1 and N_2) did not differ significantly from seedlings inoculated with MRh-10 and applied with N_2 level of N. Seedlings inoculated with MRh-6 and applied with higher dose of P (P_2) showed higher fresh and dry weights of nodules. Uninoculated unfertilized seedlings (N_0P_0) recorded lowest fresh and dry weights of nodules. Seedlings inoculated with MRh-2 and MRh-6 recorded higher nodule and effective nodule weights, at N_1 level than at P_1 level. But seedlings inoculated with MRh-13, MRh-3 and MRh-10 recorded higher weights of nodule at P_1 level than at N_1 level. Higher dose of N (N_2) contributed higher nodule weights in seedlings inoculated with MRh-2, MRh-10 and MRh-3.

Interaction effects of N and P were also significant. Seedlings with fertilizer levels N_2P_1 and N_1P_2 recorded maximum weights of nodules among the interactions, followed by N_1P_1. Seedlings without fertilizer (N_0P_0) recorded lowest weight of nodule (Table 34,36,38 and 40).

Interactions between rhizobial inoculation and N and P applications were highly significant on fresh weights and dry weights of nodule and effective nodules. Highest values for both nodule characters were shown by seedlings inoculated with MRh-6 and applied with N_1P_2 level of fertilizer followed by seedlings inoculated with MRh-6 or MRh-3 with a fertilizer level of N_2P_1. Lowest value of nodule-weight was observed in uninoculated seedlings, followed by seedlings inoculated with MRh-2 and MRh-6 without fertilizers (N_0P_0).

4.4.3. Collar diameter

There was significant difference in collar diameter of plants due to the influence of *Rhizobium* inoculation (Table 41). All the five rhizobial isolates were effective in increasing the collar diameter of the seedlings. The most effective isolate was MRh – 6, other four isolates were on par.

The collar diameter increased with increasing levels of N and P. The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on collar diameter. Seedlings inoculated with MRh-6 recorded the highest collar diameter with N_2P_2 level of fertilizer. At higher dose of N (N_2), uninoculated seedlings also recorded higher collar diameter than some inoculated seedlings. The isolates MRh-2, MRh-13, MRh–3 and MRh–10 reduced the collar diameter of seedlings at high N level. All inoculated seedlings recorded a

Table 33: Influence of inoculation of *Rhizobium* isolates and application of N and P on fresh weight of nodules in *A. mangium* seedlings

Rhizobium isolates	Fresh weight of nodules (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.36	1.02	1.13	0.71	0.83	0.98	0.840
MRh - 13	0.62	1.24	0.92	0.49	1.22	1.08	0.930
MRh - 6	0.77	1.54	1.48	0.75	1.17	1.86	1.260
MRh - 3	0.56	0.98	1.11	0.51	1.19	0.96	0.890
MRh - 10	0.55	1.27	1.47	0.77	1.29	1.24	1.100
Uninoculated	0.09	0.20	0.23	0.14	0.18	0.20	0.170
Mean	0.49	1.04	1.06	0.56	0.98	1.05	

C.D (0.05) for Isolates = 0.054 SEM (±) = 0.019
C.D (0.05) for N = 0.038 SEM (±) = 0.014
C.D (0.05) for P = 0.038 SEM (±) = 0.014
C.D (0.05) for Isolates × N = 0.093 SEM (±) = 0.033
C.D (0.05) for Isolates × P = 0.093 SEM (±) = 0.033

Table 34: Influence of N and P on fresh weight of nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Fresh weight of nodules (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	0.13	0.50	0.46	1.22	0.66	1.20	0.77	1.34	1.28
MRh – 13	0.31	0.72	0.83	0.25	1.97	1.51	0.90	0.98	0.89
MRh – 6	0.15	0.79	1.39	0.73	0.89	3.00	1.38	1.84	1.21
MRh – 3	0.23	1.06	0.40	0.79	0.88	1.28	0.50	1.63	1.21
MRh – 10	0.32	0.71	0.61	0.75	1.35	1.72	1.25	1.79	1.38
Uninoculated	0.04	0.09	0.14	0.15	0.22	0.22	0.23	0.24	0.22
Mean	0.20	0.65	0.64	0.65	0.99	1.49	0.84	1.30	1.03

C.D (0.05) for **N** × P = 0.066 SEM (±) = 0.024
C.D (0.05) for Isolate × **N** × P = 0.162 SEM (±) = 0.058

Table 35. Influence of inoculation of *Rhizobium* isolates and application of N and P on dry weight of nodules in *A. mangium* seedlings

Rhizobium isolate	Dry weight of nodules (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.092	0.262	0.289	0.180	0.213	0.251	0.215
MRh - 13	0.159	0.319	0.236	0.125	0.313	0.275	0.238
MRh - 6	0.198	0.394	0.379	0.193	0.300	0.477	0.324
MRh - 3	0.144	0.251	0.285	0.130	0.304	0.246	0.227
MRh - 10	0.140	0.326	0.377	0.198	0.329	0.316	0.281
Uninoculated	0.023	0.050	0.060	0.036	0.047	0.050	0.044
Mean	0.126	0.267	0.271	0.144	0.251	0.269	

C.D (0.05) for Isolates = 0.017 SEM (±) = 0.006
C.D (0.05) for N = 0.012 SEM (±) = 0.004
C.D (0.05) for P = 0.012 SEM (±) = 0.004
C.D (0.05) for Isolates × N = 0.030 SEM (±) = 0.011
C.D (0.05) for Isolates × P = 0.030 SEM (±) = 0.011

Table 36: Influence of N and P on dry weight of nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Dry weight of nodules (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	0.033	0.127	0.117	0.311	0.168	0.307	0.197	0.343	0.328
MRh – 13	0.080	0.184	0.212	0.065	0.504	0.388	0.231	0.250	0.226
MRh – 6	0.037	0.202	0.355	0.188	0.227	0.767	0.354	0.472	0.310
MRh - 3	0.058	0.271	0.103	0.202	0.225	0.327	0.128	0.416	0.309
MRh - 10	0.082	0.183	0.155	0.192	0.346	0.441	0.320	0.458	0.553
Uninoculated	0.010	0.023	0.037	0.038	0.056	0.057	0.060	0.062	0.056
Mean	0.050	0.165	0.163	0.166	0.254	0.381	0.215	0.334	0.264

C.D (0.05) for N × P = 0.021 SEM (±) = 0.007

C.D (0.05) for Isolates × N × P = 0.051 SEM (±) = 0.018

Table 37: Influence of inoculation of *Rhizobium* isolates and application of N and P on fresh weight of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of effective nodules (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.19	0.64	0.62	0.47	0.51	0.49	0.49
MRh - 13	0.55	0.49	0.55	0.29	0.68	0.61	0.53
MRh - 6	0.51	1.02	0.88	0.51	0.85	1.05	0.80
MRh - 3	0.44	0.64	0.71	0.37	0.84	0.58	0.60
MRh - 10	0.44	0.67	0.74	0.54	0.69	0.62	0.61
Uninoculated	0.44	0.06	0.05	0.04	0.05	0.06	0.05
Mean	0.36	0.59	0.59	0.37	0.60	0.57	

C.D (0.05) for Isolates = 0.017 SEM (±) = 0.006
C.D (0.05) for N = 0.012 SEM (±) = 0.004
C.D (0.05) for P = 0.012 SEM (±) = 0.004
C.D (0.05) for Isolates × N = 0.030 SEM (±) = 0.011
C.D (0.05) for Isolates × P = 0.030 SEM (±) = 0.011

Table 38: Influence of N and P on fresh weight of effective nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizboum isolate	Fresh weight of effective nodules (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	0.10	0.23	0.27	0.61	0.61	0.71	0.70	0.70	0.48
MRh – 13	0.30	0.60	0.76	0.20	0.69	0.59	0.40	0.74	0.49
MRh – 6	0.12	0.49	0.92	0.54	0.96	1.55	0.86	1.10	0.69
MRh – 3	0.20	0.83	0.28	0.52	0.62	0.78	0.38	1.08	0.68
MRh – 10	0.28	0.56	0.50	0.52	0.77	0.70	0.82	0.74	0.65
Uninoculated	0.04	0.04	0.04	0.05	0.06	0.07	0.04	0.06	0.06
Mean	0.17	0.46	0.46	0.41	0.62	0.73	0.53	0.74	0.51

C.D (0.05) for N × P = 0.021 SEM (±) = 0.007

C.D (0.05) for isolates × N × P = 0.051 SEM (±) = 0.018

Table 39: Influence of inoculation of *Rhizobium* isolates and application of N and P on dry weight of effective nodules in *A. mangium* seedlings

Rhizobium isolate	Dry weight of effective nodules (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.048	0.154	0.150	0.113	0.123	0.116	0.117
MRh - 13	0.133	0.117	0.131	0.072	0.162	0.147	0.127
MRh - 6	0.122	0.244	0.211	0.121	0.204	0.253	0.193
MRh - 3	0.105	0.153	0.171	0.088	0.202	0.139	0.143
MRh - 10	0.107	0.160	0.177	0.130	0.165	0.148	0.148
Uninoculated	0.009	0.015	0.013	0.010	0.013	0.014	0.012
Mean	0.087	0.141	0.142	0.089	0.145	0.136	

C.D (0.05) for Isolates = 0.002 SEM (±) = 0.0006
C.D (0.05) for N = 0.004 SEM (±) = 0.001
C.D (0.05) for P = 0.004 SEM (±) = 0.001
C.D (0.05) for Isolates × N = 0.009 SEM (±) = 0.003
C.D (0.05) for Isolates × P = 0.009 SEM (±) = 0.003

Table 40: Influence of N and P on dry weight of effective nodules in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Dry weight of effective nodules (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.024	0.055	0.064	0.146	0.146	0.171	0.167	0.168	0.115
MRh - 13	0.071	0.144	0.183	0.047	0.165	0.140	0.097	0.178	0.118
MRh - 6	0.029	0.117	0.221	0.129	0.231	0.372	0.206	0.263	0.165
MRh - 3	0.048	0.199	0.067	0.124	0.149	0.187	0.091	0.258	0.164
MRh - 10	0.066	0.134	0.120	0.126	0.186	0.169	0.197	0.176	0.157
Uninoculated	0.008	0.009	0.010	0.013	0.014	0.017	0.010	0.015	0.015
Mean	0.041	0.110	0.111	0.097	0.148	0.176	0.128	0.176	0.122

C.D (0.05) for **N** × **P** = 0.002 SEM (±) = 0.0007
C.D (0.05) for Isolate × **N** × P = 0.016 SEM (±) = 0.006

Table 41: Influence of inoculation of *Rhizobium* isolates and application of N and P on collar diameter of *A. mangium* seedlings

Rhizobium isolates	Collar diameter (mm)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	2.26	4.29	4.10	3.40	3.50	3.75	3.55
MRh – 13	2.33	3.80	4.35	3.12	3.67	3.69	3.49
MRh – 6	2.70	4.78	5.11	3.83	4.17	4.59	4.20
MRh – 3	2.28	3.99	4.21	2.86	3.90	3.70	3.49
MRh – 10	2.13	4.01	4.34	2.93	3.58	3.96	3.49
Uninoculated	2.12	3.44	4.50	2.44	3.58	4.05	3.36
Mean	2.30	4.05	4.43	3.10	3.74	3.96	

C.D (0.05) for Isolates = 0.072 SEM (±) = 0.026
C.D (0.05) for N = 0.051 SEM (±) = 0.018
C.D (0.05) for P = 0.051 SEM (±) = 0.018
C.D (0.05) for Isolates × N = 0.125 SEM (±) = 0.045
C.D (0.05) for Isolates × P = 0.125 SEM (±) = 0.045

higher collar diameter than uninoculated ones, without application. Inoculation of MRh-2, MRh-13, MRh-6 and MRh–10 resulted in higher collar diameter as compared to MRh-3, when P fertilization were given. At P_1 level, MRh-6 and MRh –3 showed superiority over others. However, at P_2 level, MRh-6 alone showed superiority over the control. At lower level of P also this inoculant performed well. The proportionate increase in collar diameter due to N application was more than that due to P application.

Interactions between N and P were also significant. N_2P_2 level was significantly superior to all other levels. This was followed by N_2P_1 and N_1P_2levels. Unfertilized seedlings (N_0P_0) showed lowest collar diameter (Table 42).

Interactions between rhizobial inoculation and N and P application were highly significant (Table 42). Highest collar diameter was recorded in plants inoculated with the isolate MRh-6 with fertilizer level of N_2P_2. It was also observed that with N_2P_2 level uninoculated seedlings recorded diameter which was on par with inoculated seedlings except seedlings inoculated with MRh-6, which was superior to all others.

4.4.4. Height of seedlings

Effects of inoculation of different rhizobial isolates on height of fertilized seedlings were significant (Table 43). All the five rhizobial isolates tested were effective in increasing the height of the seedlings. Inoculation of seedlings with the isolate MRh-13 and MRh-6 showed maximum increase in height over others, followed by MRh-10 and MRh-2 which were comparable with each other. Uninoculated seedlings showed minimum height.

Table 42: Influence of N and P on collar diameter of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Collar diameter (mm)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	1.71	2.73	2.35	4.65	3.67	4.56	3.85	4.12	4.33
MRh – 13	1.80	2.48	2.71	3.43	3.74	4.23	4.14	4.78	4.13
MRh – 6	1.53	3.26	3.29	4.93	4.32	5.09	5.01	4.94	5.38
MRh – 3	1.60	2.75	2.47	3.25	4.18	4.53	3.72	4.78	4.12
MRh – 10	1.74	2.35	2.29	3.53	3.76	4.73	3.52	4.64	4.85
Uninoculated	1.34	2.37	2.66	1.92	4.21	4.21	4.06	4.16	5.28
Mean	1.62	2.66	2.63	3.62	3.98	4.56	4.05	4.57	4.68

C.D (0.05) for N × P = 0.089 SEM (±) = 0.032
C.D (0.05) for Isolates × N × P = 0.217 SEM (±) = 0.077

Table 43: Influence of inoculation of *Rhizobium* isolates and application of N and P on shoot height of *A. mangium* seedlings

Rhizobium isolates	Shoot height (cm)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	17.19	30.21	31.18	23.84	25.40	29.34	26.19
MRh – 13	20.18	31.76	33.57	24.15	30.83	30.52	28.50
MRh – 6	20.84	30.77	33.30	23.99	27.59	33.32	28.30
MRh – 3	18.24	26.72	32.10	21.90	29.18	25.99	25.69
MRh – 10	19.71	30.63	28.45	24.76	25.99	28.31	26.22
Uninoculated	11.92	16.70	23.14	14.11	18.29	19.37	17.25
Mean	18.01	27.80	30.27	22.130	26.12	27.83	

C.D (0.05) for Isolates = 0.231 SEM (±) = 0.083
C.D (0.05) for **N** = 0.164 SEM (±) = 0.058
C.D (0.05) for P = 0.164 SEM (±) = 0.058
C.D (0.05) for Isolates × N = 0.401 SEM (±) = 0.143
C.D (0.05) for Isolates × P = 0.401 SEM (±) = 0.143

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence. Increase in doses of N and P resulted significant increase in shoot height. Inoculated seedlings were taller than uninoculated ones at all levels of applied N and P. The seedlings inoculated with isolates MRh-13 and MRh-6 with higher dose of N (N_2) were taller followed by MRh-13 with N_1, level and MRh-3 with N_2 level. Seedlings inoculated with MRh-6 with higher dose of P were taller followed by MRh-13 with lower and higher dose of P. Uninoculated seedlings without fertilizers were the shortest among all. Generally N-fertilized plants showed significantly superior height than P-fertilized plants. But in seedlings inoculated with MRh-6, N_2 and P_2 levels recorded maximum height, even though between N_2 and P_2 levels, there was no significant difference. Seedlings without N-fertilizer (N_0) were inferior to seedlings without P (P_0).

Interactions between N and P had significant influence on height of inoculated plants (Table 44). Without applied N (N_0), the addition of P at 50 kg ha^{-1} (P_1) significantly increased the height. While 100 kg ha^{-1} of P (P_2) significantly reduced the height below P_1. Without P application, addition of N-fertilizer significantly increased the height. All the fertilized seedlings were significantly superior to unfertilized seedlings. Seedlings with a fertilizer dose of N_2P_2 were the tallest. This was followed by the seedlings with N_2P_1 level of fertilizer.

Interactions between rhizobial inoculation and N and P application were highly significant on seedling height. Tallest seedlings were observed when the seedlings inoculated with MRh-13 were fertilized with N_2P_1 level. This was followed by inoculation of MRh-3 with fertilizer level of N_2P_1 and inoculation of MRh-6 with

Table 44: Influence of N and P on shoot height of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Shoot height (cm)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	15.29	18.56	17.74	28.63	26.06	35.93	27.60	39.59	34.35
MRh – 13	16.99	21.30	22.27	29.30	30.13	35.83	26.17	41.07	33.47
MRh – 6	11.76	23.10	27.67	30.71	26.25	35.33	29.50	33.43	36.97
MRh – 3	15.53	23.60	15.60	24.25	26.57	29.36	25.92	37.37	33.00
MRh – 10	15.10	22.30	21.73	29.59	29.22	33.09	29.60	24.82	30.52
Uninoculated	09.76	12.30	13.70	12.07	18.97	19.07	20.49	23.60	25.33
Mean	14.07	20.19	19.78	25.76	26.20	31.44	26.55	31.98	32.27

C.D (0.05) for N × P = 0.283 SEM (±) = 0.101
C.D (0.05) for Isolate × N × P = 0.694 SEM (±) = 0.248

fertilizer level of N_2P_2. Uninoculated seedlings with no fertilizer (N_0P_0) resulted in shortest seedlings.

4.4.5. Number of phyllodes

Different rhizobial isolates and fertilizer levels had significant effects on number of phyllodes per seedling (Table 45). Inoculation with rhizobial isolate MRh-6 resulted in highest number of phyllodes per seedling. This was followed by isolate MRh-10 and MRh-2. The isolates MRh-13 and MRh-3 were on par with the uninoculated control.

Increasing dose of P from 0 to 100 kg ha^{-1} (P_2) significantly increased the number of phyllodes. All the inoculated seedlings produced higher number of phyllodes than uninoculated seedlings at all levels of applied N and P. The seedlings inoculated with MRh-6 and given a N dose of 200 kg ha^{-1} showed maximum number of phyllodes. This treatment was followed by inoculation with MRh-6 and application of 100 kg of N ha^{-1}. The seedlings inoculated with MRh-10 combined with an N dose of 100 kg ha^{-1} also showed superiority.

Application of P alone significantly increased the number of phyllodes. Without P application, increasing levels of N also significantly increased the number of phyllodes. The fertilizer levels N_2P_2 and N_1P_2 did not differ significantly and were significantly superior to other levels. The seedlings without fertilizer N and P (N_0P_0) showed the lower number of phyllodes (Table 46).

Highly significant interactions were observed between rhizobial inoculation and N and P application for number of phyllodes. The highest number of phyllodes was observed in seedlings inoculated with the isolate MRh-6 and applied with higher

Table 45: Influence of inoculation of *Rhizobium* isolates and application of N and P on number of phyllods of *A. mangium* seedlings

Rhizobium isolates	Number of phyllods						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	7.0	13.4	11.2	10.2	9.3	12.1	10.5
MRh – 13	7.0	10.6	11.3	07.7	10.5	10.4	09.6
MRh – 6	7.5	16.1	20.0	11.5	14.6	17.4	14.5
MRh – 3	6.6	09.0	11.8	07.0	11.0	09.5	09.1
MRh – 10	6.6	15.6	12.5	09.7	11.1	14.0	11.6
Uninoculated	5.3	11.6	11.2	05.8	10.5	11.7	09.4
Mean	6.7	12.7	13.0	08.7	11.2	12.5	

C.D (0.05) for Isolates = 0.42 SEM (±) = 0.15
C.D (0.05) for N = 0.29 SEM (±) = 0.11
C.D (0.05) for P = 0.29 SEM (±) = 0.11
C.D (0.05) for Isolates × N = 0.72 SEM (±) = 0.26
C.D (0.05) for Isolates × P = 0.72 SEM (±) = 0.26

Table 46: Influence of N and P on number of phyllodes of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Number of phyllodes								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	5.6	7.3	8.0	14.6	11.3	14.3	10.3	09.3	14.0
MRh – 13	5.0	7.6	8.3	08.3	11.6	11.6	10.0	12.6	11.3
MRh – 6	5.0	8.0	9.6	12.3	16.3	19.6	17.3	19.6	23.0
MRh – 3	3.3	8.6	8.0	07.0	10.0	10.0	10.6	14.3	10.6
MRh – 10	5.6	7.3	7.0	13.3	14.3	19.3	10.3	11.6	15.6
Uninoculated	3.0	6.3	6.6	07.6	13.3	14.0	07.0	12.0	14.6
Mean	4.6	7.5	7.9	10.5	12.8	14.8	10.9	13.2	14.8

C.D (0.05) for N × P = 0.51 SEM (±) = 0.18
C.D (0.05) for Isolates × N × P = 1.3 SEM (±) = 0.45

dose of N and P (N_2P_2) followed by same inoculant with a fertilizer level of N_1P_2 and N_2P_1. The lowest number of phyllodes was observed in the seedlings without inoculation and fertilizer application (N_0P_0).

4.4.6. Depth of tap root and number of lateral roots

Inoculation with rhizobial isolates and application of N and P fertilizers and their interactions had significant effect on depth of tap root and number of lateral roots (Table 47 and 49). All the inoculated seedlings showed higher root depth and more number of lateral roots than uninoculated seedlings. The seedling inoculated with MRh-6 showed maximum depth and number of roots. The seedlings inoculated with other isolates though superior to uninoculated control did not show significant difference among themselves. Increasing doses of N and P increased the depth and number of roots significantly. Inoculated seedlings produced significantly higher number and depth of roots than uninoculated seedlings at all levels of N and P. Inoculation with the isolate MRh-6 at N_1 level resulted in higher number of lateral roots. The root depth was superior in seedlings inoculated with MRh-6 and applied with N_1 and N_2 levels of N. The isolates MRh-2 and MRh-3 with N_2 levels of N also performed well. Inoculated seedlings with higher dose of P (P_2) showed higher number and depth of roots followed by inoculated seedlings with lower dose of P. The proportion of increase in number of roots and its depth due to N fertilization was more than that due to P fertilization. Seedlings applied with a fertilizer level of N_2P_2 showed root depth followed by N_2P_1, N_1P_2 and N_2P_0. The number of lateral roots was superior with the fertilizer levels N_1P_2 followed by N_2P_2 and N_2P_1 which showed no significant difference among themselves. Unfertilized seedlings (N_0P_0) showed lowest number of roots and they were shorter too (Table 48 and 50).

Table 47: Influence of inoculation of *Rhizobium* isolates and application of N and P on root depth of *A. mangium* seedlings

Rhizobium isolate	Root depth (cm)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	27.20	36.77	42.07	34.80	36.20	35.03	35.34
MRh - 13	30.09	36.68	39.26	34.83	34.59	36.60	35.34
MRh - 6	29.53	43.56	43.16	35.03	38.69	41.92	38.75
MRh - 3	27.73	37.81	42.01	33.68	37.51	36.37	35.85
MRh - 10	27.17	37.49	39.91	33.03	34.16	37.38	34.86
Uninoculated	25.26	29.10	37.89	25.32	32.53	34.39	30.75
Mean	27.83	36.90	40.72	32.88	35.61	36.95	

C.D (0.05) for Isolates = 1.190 SEM (±) = 0.425
C.D (0.05) for N = 0.842 SEM (±) = 0.300
C.D (0.05) for P = 0.842 SEM (±) = 0.300
C.D (0.05) for Isolates × N = 2.061 SEM (±) = 0.735
C.D (0.05) for Isolates × P = 2.061 SEM (±) = 0.735

Table 48: Influence of N and P on root depth of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Root depth (cm)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	27.50	29.20	24.90	36.83	35.13	38.33	40.07	44.27	41.87
MRh - 13	30.33	28.77	31.17	33.23	34.50	42.30	40.93	40.50	36.33
MRh - 6	27.13	32.23	29.23	39.43	43.90	47.33	40.33	39.93	49.20
MRh - 3	25.63	30.83	26.73	34.27	36.73	42.43	41.13	44.97	39.43
MRh - 10	27.50	26.70	27.30	32.57	38.40	41.50	39.03	37.37	43.33
Uninoculated	22.67	26.80	26.30	21.13	36.57	29.60	32.17	34.23	47.27
Mean	26.79	29.09	27.61	32.91	37.54	40.25	38.94	40.21	42.99

C.D (0.05) for N × P = 1.458 SEM (±) = 0.520
C.D (0.05) for Isolates × N × P = 3.570 SEM (±) = 1.274

Table 49: Influence of inoculation of *Rhizobium* isolates and application of N and P on number of lateral roots of *A. mangium* seedlings

Rhizobium isolate	Number of lateral roots						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	09.9	16.5	21.0	12.3	16.4	18.6	15.8
MRh - 13	12.2	19.4	23.8	15.5	19.1	20.8	18.5
MRh - 6	11.5	27.0	24.7	19.2	20.6	23.4	21.1
MRh - 3	12.6	23.1	22.3	16.4	19.7	21.8	19.3
MRh - 10	11.0	21.5	19.8	14.2	19.0	19.2	17.4
Uninoculated	06.3	15.7	21.1	11.4	14.1	17.6	14.4
Mean	10.6	20.6	22.2	14.8	18.2	20.3	

C.D (0.05) for Isolates = 0.70 SEM (±) = 0.25
C.D (0.05) for N = 0.50 SEM (±) = 0.18
C.D (0.05) for P = 0.50 SEM (±) = 0.18
C.D (0.05) for Isolate × N = 1.2 SEM (±) = 0.43
C.D (0.05) for Isolate × P = 1.2 SEM (±) = 0.43

Table 50: Influence of N and P on number of lateral roots of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Number of lateral roots								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	3.6	11.0	15.0	14.6	17.0	18.0	18.6	21.3	23.0
MRh – 13	9.3	13.0	14.3	13.6	21.0	23.6	23.6	23.3	24.6
MRh – 6	7.3	11.3	16.0	23.3	26.3	31.3	27.0	24.3	23.0
MRh – 3	8.0	15.3	14.6	21.3	19.6	28.3	20.0	24.3	22.6
MRh – 10	9.0	12.0	12.0	17.6	23.0	24.0	16.0	22.0	21.6
Uninoculated	4.3	05.0	09.6	11.6	16.0	19.6	18.3	21.3	23.6
Mean	6.9	11.2	13.6	17.0	20.5	24.1	20.6	22.7	23.1

C.D (0.05) for N × P = 0.86 SEM (±) = 0.31

C.D (0.05) for isolates × N × P = 2.1 SEM (±) = 0.75

Highly significant interactions were observed between inoculation, N and P application, for both parameters. Both MRh-6 and MRh-3 recorded highest number of lateral roots with the fertilizer level N_1P_2, the former isolate showing superiority over the later. The lowest number of roots were recorded by seedlings inoculated with MRh-2 and uninoculated seedlings at N_0P_0 level of fertilizer. In general, without N, when P was applied, the number of lateral roots produced was more in all the isolates. The deepest root was observed in seedling inoculated with the isolate MRh-6 and applied with fertilizer level N_2P_2. This was followed by the same isolate with N_1P_2 level of fertilizer. Uninoculated seedlings produced shorter roots at lower levels of fertilizer. However, at N_2P_2 level, the uninoculated seedlings produced deeper roots than many of the rhizobial inoculated seedlings.

4.4.7. Leaf area

There were significant differences in leaf area of seedlings due to rhizobial inoculation (Table 51). All the five rhizobial isolates were effective in increasing the leaf area of the seedlings. The most effective isolate was MRh-6 followed by MRh-13.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on leaf area. Increase in doses of N and P fertilizers significantly increased the leaf area of the seedlings. The inoculated seedlings showed more leaf area than uninoculated ones at all levels of applied N and P. seedlings inoculated with MRh-6 and fertilized with low dose of N (N_1) recorded higher leaf area. This was followed by seedlings inoculated with MRh-13 and applied with the fertilizer level of N_1. Seedlings inoculated with MRh-6 and fertilized with higher dose of P (P_2) also showed high leaf area followed by MRh-13

Table 51: Influence of inoculation of *Rhizobium* isolates and application of N and P on leaf area of *A. mangium* seedlings

Rhizobium isolates	Leaf area (cm^2)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	44.07	186.92	254.05	142.16	172.85	170.03	161.68
MRh - 13	107.47	428.35	347.00	184.16	419.25	279.42	294.27
MRh - 6	131.92	445.10	371.20	203.57	310.34	434.31	316.07
MRh - 3	83.61	176.62	235.11	125.46	194.94	174.94	165.11
MRh - 10	101.54	26.7	308.16	138.98	240.82	276.64	218.81
Uninoculated	29.12	91.96	181.42	52.86	94.72	154.92	100.84
Mean	82.96	262.61	282.82	141.20	238.82	248.38	

C.D (0.05) for Isolates = 2.633 SEM (±) = 0.939
C.D (0.05) for N = 1.862 SEM (±) = 0.664
C.D (0.05) for P = 1.862 SEM (±) = 0.664
C.D (0.05) for Isolates × N = 4.561 SEM (±) = 1.627
C.D (0.05) for Isolates × P = 4.561 SEM (±) = 1.627

with lower dose of P (P_1). Uninoculated -unfertilized seedlings showed very low leaf area. In seedlings inoculated with MRh-6 application of P_2 contributed more leaf area than N_2.

Interactions between N and P significantly influenced leaf area of the inoculated seedlings. Seedlings with fertilizer level N_2P_2 showed maximum leaf area followed by N_2P_1 (Table 52).

Interactions between rhizobial inoculation, N and P application showed highly significant influence on leaf area of the seedlings. Highest leaf area was recorded in plants inoculated with the isolate MRh-13 and applied with fertilizer level N_1P_1 followed by the isolate MRh-6 with N_1P_2. Uninoculated, unfertilized seedlings recorded lowest leaf area.

4.4.8. Weights of leaf, stem and root

There were significant differences in fresh and dry weights of leaves, stems and roots of the seedlings due to rhizobial inoculation (Table 53,55,57,59,61and 63). All the five rhizobial isolates tested were effective in increasing the fresh and dry weights of the leaf, stem and roots. The most effective isolate was MRh-6 followed by MRh-13. The weight of roots in seedlings inoculated with MRh-13 and uninoculated seedlings were on par and showed very low values.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on fresh and dry weights of leaf,stem and root. Increase in dose of N from N_1 to N_2 and P from P_1 to P_2 significantly increased the leaf, stem and root weights. Inoculated seedlings produced significantly higher leaf and stem weights than uninoculated seedlings at all levels of applied N and P.

The seedlings inoculated with MRh-6 and MRh-10 recorded higher values of stem and leaf weights when higher dose of N (N_2) was applied, followed by MRh-13 with same dose of N. Inoculated seedlings produced significantly higher root weights than uninoculated seedlings with low dose of N (N_1) as well as without N-fertilizer. At higher dose of N (N_2), all the inoculated seedlings except MRh-6 showed low root weights than uninoculated ones. Seedlings inoculated with MRh-6 and fertilized with high dose of N (N_2) or P (P_2) recorded maximum root weight.

Interactions between N and P showed significant and influence on fresh and dry weights of leaf, stem and root (Table 54,56,58,60,62 and 64). The fertilizer levels N_2P_1 and N_2P_2 recorded maximum stem and leaf weights followed by N_1P_2. Unfertilized seedlings (N_0P_0) recorded minimum leaf, stem and root weights.

Interactions between rhizobial inoculation, N and P-fertilizers showed highly significant influence on leaf, stem and root weights. Highest values of leaf, stem and root weights were shown by seedlings inoculated with MRh-6 and applied with a fertilizer level N_1P_2, followed by seedlings inoculated with MRh-10 and MRh-13 with a fertilizer level N_2P_1 and MRh-13 with a fertilizer level of N_1P_1. Uninoculated seedlings when applied with higher dose of N and P (N_2P_2) also showed higher root weight. Lowest weight of leaf, stem and root were observed in uninoculated unfertilized seedlings.

4.4.9. Above ground biomass and total biomass

There were significant differences in biomass of the seedlings due to rhizobial inoculation (Table 65 and 67). The most effective isolate in increasing the biomass was MRh-6 followed by MRh-13. Most ineffective isolate was MRh-2.

Table 52: Influence of N and P on leaf area of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Leaf area (cm^2 $plant^{-1}$)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	22.08	45.09	65.04	151.22	197.08	212.45	253.17	276.37	232.61
MRh – 13	28.06	137.21	157.15	282.30	739.72	263.03	242.11	380.82	418.08
MRh – 6	15.87	136.56	243.34	282.20	348.77	704.33	312.65	445.68	355.27
MRh – 3	30.12	149.98	70.72	144.17	179.87	205.82	202.10	254.96	248.27
MRh – 10	59.39	97.49	147.74	177.17	242.72	320.32	180.38	382.25	361.87
Uninoculated	13.13	28.61	45.62	54.40	101.68	119.81	91.05	153.88	299.33
Mean	28.11	99.16	121.60	181.91	301.64	304.29	213.58	315.66	319.24

C.D (0.05) for **N** × P = 3.225 SEM (±) = 1.150

C.D (0.05) for Isolates × **N** × P = 7.90 SEM (±) = 2.820

Table 53: Influence of inoculation of *Rhizobium* isolates and application of N and P on fresh weight of leaves in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of leaves (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	2.02	3.35	8.34	3.32	5.07	5.32	4.57
MRh - 13	2.82	9.39	10.58	5.00	9.49	8.30	7.60
MRh - 6	3.45	8.48	9.87	4.21	7.37	10.22	7.27
MRh - 3	2.78	5.59	7.01	3.88	5.83	5.67	5.13
MRh - 10	2.60	7.34	11.13	5.66	6.70	8.72	7.02
Uninoculated	1.44	2.98	4.91	1.64	3.49	4.21	3.11
Mean	2.52	6.19	8.64	3.95	6.32	7.07	

C.D (0.05) for Isolates = 0.218 SEM (±) = 0.078
C.D (0.05) for N = 0.154 SEM (±) = 0.055
C.D (0.05) for P = 0.154 SEM (±) = 0.055
C.D (0.05) for Isolates × N = 0.377 SEM (±) = 0.135
C.D (0.05) for Isolates × P = 0.377 SEM (±) = 0.135

Table 54: Influence of N and P on fresh weight of leaves in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Fresh weight of leaves (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.70	2.51	2.84	3.17	3.98	2.90	6.08	8.72	10.22
MRh - 13	0.76	3.33	4.36	5.86	13.33	8.98	8.38	11.82	11.56
MRh - 6	0.51	4.78	5.05	4.22	6.27	14.96	7.89	11.06	10.66
MRh - 3	0.78	4.19	3.37	4.52	5.78	6.46	6.35	7.51	7.17
MRh - 10	1.53	2.37	3.91	5.16	6.27	10.58	10.27	11.47	11.65
Uninoculated	0.27	1.67	2.39	2.24	2.99	3.71	2.40	5.80	6.53
Mean	0.76	3.14	3.65	4.20	6.43	7.93	6.89	9.40	9.63

C.D (0.05) for N × P = 0.267 SEM (±) = 0.095

C.D (0.05) for Isolates × N × P = 0.653 SEM (±) = 0.233

Table 55: Influence of inoculation of *Rhizobium* isolates and application of N and P on dry weight of leaves in *A. mangium* seedlings

Rhizobium isolate	Dry weight of leaves (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	0.45	0.78	2.20	0.85	1.18	1.38	1.14
MRh – 13	0.62	2.69	3.33	1.54	2.59	2.51	2.21
MRh – 6	1.07	3.04	3.42	1.49	2.52	3.52	2.51
MRh – 3	0.75	1.50	2.71	0.94	2.20	1.82	1.65
MRh – 10	0.63	2.19	3.54	1.45	2.29	2.62	2.12
Uninoculated	0.39	0.59	1.26	0.44	0.81	0.99	0.75
Mean	0.65	1.80	2.74	1.12	1.93	2.14	

C.D (0.05) for Isolates = 0.076 SEM (±) = 0.027
C.D (0.05) for N = 0.054 SEM (±) = 0.019
C.D (0.05) for P = 0.054 SEM (±) = 0.019
C.D (0.05) for Isolates × N = 0.132 SEM (±) = 0.047
C.D (0.05) for Isolates × P = 0.132 SEM (±) = 0.047

Table 56: Influence of N and P on dry weight of leaves in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Dry weight of leaves (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.15	0.63	0.56	0.75	0.93	0.65	1.66	1.99	2.94
MRh - 13	0.18	0.75	0.93	2.00	3.15	2.93	2.44	3.88	3.66
MRh - 6	0.11	1.34	1.76	1.39	2.58	5.13	2.96	3.64	3.66
MRh - 3	0.15	1.15	0.96	1.46	1.76	1.28	1.21	3.71	3.23
MRh - 10	0.32	0.57	1.01	1.18	2.34	3.06	2.87	3.94	3.79
Uninoculated	0.06	0.51	0.61	0.49	0.64	0.63	0.77	1.28	1.73
Mean	0.16	0.82	0.97	1.21	1.90	2.28	1.98	3.08	3.17

C.D (0.05) for N × P = 0.093 SEM (±) = 0.033
C.D (0.05) for isolates × N × P = 0.229 SEM (±) = 0.082

Table 57: Influence of inoculation of *Rhizobium* isolates and application of N and P on fresh weight of stem in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of stem (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.93	3.31	3.33	2.22	2.38	2.98	2.52
MRh - 13	1.27	3.83	3.87	2.15	3.72	3.10	2.99
MRh - 6	1.63	3.49	3.92	1.70	2.91	4.42	3.01
MRh - 3	1.10	2.46	3.61	1.57	2.85	2.76	2.39
MRh - 10	0.94	2.73	3.92	1.91	2.75	2.92	2.53
Uninoculated	0.62	2.04	3.12	1.33	2.12	2.32	1.93
Mean	1.08	2.98	3.63	1.81	2.79	3.08	

C.D (0.05) for Isolates = 0.135 SEM (±) = 0.048
C.D (0.05) for N = 0.096 SEM (±) = 0.034
C.D (0.05) for P = 0.096 SEM (±) = 0.034
C.D (0.05) for Isolates × N = 0.235 SEM (±) = 0.084
C.D (0.05) for Isolates × P = 0.235 SEM (±) = 0.084

Table 58: Influence of N and P on fresh weight of stem in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Fresh weight of stem (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.54	1.28	0.98	3.98	1.88	4.07	2.14	3.97	3.88
MRh - 13	0.57	1.13	2.10	3.08	5.09	3.32	2.79	4.94	3.87
MRh - 6	0.38	1.69	2.82	1.90	3.15	5.41	2.83	3.90	5.03
MRh - 3	0.47	1.64	1.20	1.80	2.15	3.44	2.44	4.75	3.64
MRh - 10	0.46	1.16	1.20	2.18	2.98	3.02	3.10	4.12	4.55
Uninoculated	0.19	0.70	0.97	1.40	2.24	2.46	2.39	3.43	3.54
Mean	0.43	1.27	1.54	2.39	2.92	3.62	2.61	4.18	4.09

C.D (0.05) for N × P = 0.166 SEM (±) = 0.059
C.D (0.05) for Isolates × N × P = 0.406 SEM (±) = 0.145

Table 59: Influence of inoculation of *Rhizobium* isolates and application of N and P on dry weight of stem in *A. mangium* seedlings

Rhizobium isolate	Dry weight of stem (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	0.38	1.57	1.56	1.03	1.18	1.30	1.17
MRh – 13	0.45	1.85	1.97	1.15	1.75	1.37	1.42
MRh – 6	0.67	1.91	2.06	1.05	1.42	2.17	1.55
MRh – 3	0.44	1.09	1.32	0.72	1.01	1.12	0.95
MRh – 10	0.31	1.18	1.85	0.83	1.08	1.43	1.11
Uninoculated	0.28	0.81	1.08	0.51	0.75	0.90	0.72
Mean	0.42	1.40	1.64	0.88	1.20	1.38	

C.D (0.05) for Isolates = 0.078 SEM (±) = 0.028
C.D (0.05) for N = 0.055 SEM (±) = 0.020
C.D (0.05) for P = 0.055 SEM (±) = 0.020
C.D (0.05) for Isolates × N = 0.135 SEM (±) = 0.048
C.D (0.05) for Isolates × P = 0.135 SEM (±) = 0.048

Table 60: Influence of N and P on dry weight of stem in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Dry weight of stem (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.10	0.63	0.42	2.00	0.78	1.95	0.99	2.14	1.55
MRh - 13	0.15	0.40	0.79	1.56	2.46	1.53	1.74	2.41	1.78
MRh - 6	0.097	0.83	1.08	1.27	1.31	3.14	1.78	2.12	2.29
MRh - 3	0.139	0.64	0.52	0.89	1.06	1.32	1.13	1.33	1.51
MRh - 10	0.15	0.29	0.49	0.77	1.14	1.63	1.57	1.91	2.17
Uninoculated	0.04	0.34	0.47	0.55	0.89	0.98	0.94	1.02	1.27
Mean	0.11	0.52	0.63	1.18	1.27	1.75	1.36	1.80	1.76

C.D (0.05) for N × P = 0.096 SEM (±) = 0.034
C.D (0.05) for Isolates × N × P = 0.235 SEM (±) = 0.084

Table 61: Influence of inoculation of *Rhizobium* isolates and application of N and P on fresh weight of roots in *A. mangium* seedlings

Rhizobium isolate	Fresh weight of roots (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	1.22	4.54	5.14	2.74	4.18	3.99	3.63
MRh - 13	1.86	4.75	5.36	2.45	4.61	4.91	3.99
MRh - 6	1.81	6.56	8.24	4.46	5.13	7.02	5.54
MRh - 3	1.32	5.25	6.14	3.21	5.29	4.21	4.24
MRh - 10	1.10	5.16	6.14	2.71	5.40	4.30	4.13
Uninoculated	0.92	4.24	6.80	2.61	4.13	5.22	3.99
Mean	1.37	5.08	6.30	3.03	4.79	4.94	

C.D (0.05) for Isolates = 0.045 SEM (±) = 0.016
C.D (0.05) for N = 0.032 SEM (±) = 0.011
C.D (0.05) for P = 0.032 SEM (±) = 0.011
C.D (0.05) for Isolates × N = 0.078 SEM (±) = 0.028
C.D (0.05) for Isolates × P = 0.078 SEM (±) = 0.028

Table 62: Influence of N and P on fresh weight of roots in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolate	Fresh weight of roots (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh – 2	0.42	2.11	1.10	4.51	3.34	5.78	3.28	7.07	5.08
MRh – 13	0.57	2.07	2.94	2.30	5.67	6.26	4.47	6.08	5.54
MRh – 6	0.37	1.56	3.51	4.30	5.23	10.14	8.70	8.60	7.41
MRh – 3	0.55	2.29	1.12	3.67	5.20	6.88	5.40	8.39	4.64
MRh – 10	0.57	1.05	1.69	4.87	5.16	5.45	2.68	9.98	5.75
Uninoculated	0.28	0.73	1.76	3.58	4.31	4.81	3.98	7.33	9.09
Mean	0.46	1.64	2.02	3.87	4.82	6.55	4.75	7.91	6.25

C.D (0.05) for N × P = 0.055 SEM (±) = 0.020
C.D (0.05) for isolates × N × P = 0.135 SEM (±) = 0.048

Table 63: Influence of inoculation of *Rhizobium* isolates and application of N and P on dry weight of roots in *A. mangium* seedlings

Rhizobium isolate	Dry weight of roots (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	0.35	1.30	1.47	0.78	1.19	1.14	1.04
MRh – 13	0.53	1.35	1.53	0.70	1.31	1.40	1.14
MRh – 6	0.52	1.87	2.35	1.27	1.46	2.00	1.58
MRh – 3	0.38	1.50	1.75	0.91	1.51	1.20	1.21
MRh – 10	0.31	1.47	1.75	0.77	1.54	1.22	1.18
Uninoculated	0.26	1.21	1.94	0.74	1.18	1.49	1.14
Mean	0.39	1.45	1.80	0.86	1.36	1.41	

C.D (0.05) for Isolates = 0.017 SEM (±) = 0.006
C.D (0.05) for N = 0.012 SEM (±) = 0.004
C.D (0.05) for P = 0.012 SEM (±) = 0.004
C.D (0.05) for Isolates × N = 0.030 SEM (±) = 0.011
C.D (0.05) for Isolates × P = 0.030 SEM (±) = 0.011

Table 64: Influence of nitrogen and phosphorus interaction on dry weight of roots in *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Dry weight of roots (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.12	0.60	0.31	1.29	0.95	1.65	0.93	2.02	1.45
MRh - 13	0.16	0.59	0.84	0.66	1.62	1.79	1.28	1.73	1.58
MRh - 6	0.11	0.44	1.00	1.23	1.49	2.89	2.48	2.45	2.11
MRh - 3	0.16	0.65	0.32	1.05	1.48	1.96	1.54	2.39	1.32
MRh - 10	0.16	0.30	0.48	1.39	1.47	1.55	0.76	2.84	1.64
Uninoculated	0.08	0.21	0.50	1.02	1.23	1.37	1.14	2.09	2.59
Mean	0.13	0.47	0.58	1.10	1.37	1.87	1.35	2.25	1.78

C.D (0.05) for **N** × P = 0.021 SEM (±) = 0.007
C.D (0.05) for Isolate × N × **P** = 0.051 SEM (±) = 0.018

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on plant biomass. Increase in doses of N and P resulted in significant increase in above ground biomass and total biomass of the seedlings. The inoculated seedlings showed significantly higher above ground biomass and total biomass than uninoculated ones at all levels of applied N and P. Seedlings inoculated with isolates MRh-6, MRh-13 and MRh-10 and applied with higher dose of N (N_2) did not differ significantly among themselves and recorded greater above ground biomass followed by MRh-6 with lower dose of N (N_1). Seedlings inoculated with MRh-6 and applied with higher dose of N (N_2) showed maximum total biomass followed by MRh-10 with same dose of N. Uninoculated unfertilized seedlings were inferior in biomass production, followed by seedlings inoculated with MRh-2, MRh-3 and MRh-10 without fertilizer application.

Among P applied treatments, seedlings inoculated with isolate MRh-6 and applied with higher dose of P (P_2) recorded maximum biomass followed by MRh-13 and MRh-6 with lower dose of P (P_1). Generally N-fertilized plants had higher biomass than P-fertilized plants. N_1 dose N contribute higher biomass in all the inoculated seedlings than P_1 dose of P except MRh-3 and MRh-10. In all the inoculated seedlings, higher dose of N (N_2) contributed more biomass than higher dose of P (P_2).

Interactions between N and P had significant influence on above ground biomass and total biomass of inoculated plants (Table66 and 68). Addition of N fertilizer at lower and higher dose without application of P fertilizer and addition of P fertilizer at lower and higher dose without application of N fertilizer had significantly increased the biomass of the seedlings. Seedlings with fertilizer level N_2P_2 and N_2P_1

Table 65: Influence of inoculation of *Rhizobium* isolates and application of N and P on above ground biomass of *A. mangium* seedlings

Rhizobium isolates	Above ground biomass (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh – 2	0.83	2.35	3.76	1.88	2.37	2.68	2.31
MRh – 13	1.07	4.54	5.30	2.69	4.34	3.87	3.63
MRh – 6	1.74	4.95	5.48	2.54	3.94	5.69	4.05
MRh – 3	1.19	2.59	4.04	1.66	3.21	2.94	2.60
MRh – 10	0.95	3.37	5.39	2.29	3.37	4.05	3.23
Uninoculated	0.68	1.39	2.33	0.95	1.56	1.89	1.46
Mean	1.07	3.19	4.38	2.00	3.13	3.52	

C.D (0.05) for Isolates = 0.130 SEM (±) = 0.046
C.D (0.05) for N = 0.092 SEM (±) = 0.033
C.D (0.05) for P = 0.092 SEM (±) = 0.033
C.D (0.05) for Isolates × N = 0.225 SEM (±) = 0.080
C.D (0.05) for Isolates × P = 0.225 SEM (±) = 0.080

Table 66: Influence of N and P on above ground biomass of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Above ground biomass (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.25	1.26	0.98	2.75	1.71	2.58	2.68	4.13	4.49
MRh - 13	0.33	1.14	1.73	3.56	5.61	4.45	4.18	6.28	5.43
MRh - 6	0.21	2.17	2.83	2.67	3.90	8.27	4.78	5.76	5.95
MRh - 3	0.29	1.79	1.48	2.36	2.82	2.59	2.33	5.04	4.74
MRh - 10	0.47	0.87	1.50	1.95	3.48	4.69	4.44	5.76	5.96
Uninoculated	0.10	0.85	1.08	1.04	1.53	1.61	1.71	2.30	2.99
Mean	0.27	1.34	1.59	2.38	3.17	4.03	3.34	4.87	4.92

C.D (0.05) for N × P = 0.159 SEM (±) = 0.057

C.D (0.05) for isolates × N × P = 0.390 SEM (±) = 0.139

Table 67: Influence of inoculation of *Rhizobium* isolates and application of N and P on total biomass of *A. mangium* seedlings

Rhizobium isolates	Total biomass (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	1.22	3.79	5.37	2.78	3.68	3.94	3.46
MRh - 13	1.73	6.01	6.96	3.46	5.82	5.42	4.90
MRh - 6	2.38	7.06	8.04	3.93	5.61	7.94	5.83
MRh - 3	1.67	4.24	5.96	2.66	4.92	4.28	3.95
MRh - 10	1.37	5.00	7.31	3.19	5.07	5.42	4.56
Uninoculated	0.95	2.61	4.29	1.70	2.75	3.39	2.62
Mean	1.55	4.79	6.32	2.95	4.64	5.06	

C.D (0.05) for Isolates = 0.132 SEM (±) = 0.047
C.D (0.05) for **N** = 0.093 SEM (±) = 0.033
C.D (0.05) for P = 0.093 SEM (±) = 0.033
C.D (0.05) for Isolates × N = 0.229 SEM (±) = 0.082
C.D (0.05) for Isolates × P = 0.229 SEM (±) = 0.082

Table 68: Influence of N and P on total biomass of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Total biomass (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.40	1.92	1.36	4.18	2.81	4.40	3.75	6.32	6.05
MRh - 13	0.56	1.88	2.75	4.27	7.39	6.38	5.55	8.19	7.13
MRh - 6	0.34	2.73	4.05	4.02	5.62	11.54	7.42	8.47	8.23
MRh - 3	0.49	2.64	1.87	3.53	4.45	4.74	3.97	7.69	6.22
MRh - 10	0.70	1.30	2.10	3.46	5.13	6.42	5.40	8.78	7.75
Uninoculated	0.18	1.07	1.59	2.07	2.78	2.99	2.85	4.41	5.60
Mean	0.45	1.92	2.29	3.59	4.69	6.08	4.82	7.31	6.83

C.D (0.05) for **N** × P = 0.162 SEM (±) = 0.058
C.D (0.05) for Isolates × N × P = 0.396 SEM (±) = 0.141

were not significantly different and recorded higher values among this interactions followed by N_1P_2. Seedlings fertilized with N_2P_1 recorded maximum total biomass followed by N_2P_2 level. Lower biomass was recorded by seedlings with lower dose of P (P_1), which did not differ significantly from unfertilized seedlings.

Interactions between rhizobial inoculation and N and P application were highly significant. Highest above ground biomass was recorded by seedlings inoculated with MRh-6 and fertilized with N_1P_2 level of fertilizers followed by MRh-13 with N_2P_1, MRh-10 with N_2P_2 and MRh-6 with N_2P_2 level of fertilizers. Highest total biomass was recorded seedlings inoculated with MRh-6 with N_1P_2 level. Lowest biomass was recorded by inoculated and uninoculated seedlings without fertilizers.

4.4.10. Plant nitrogen content

There were significant differences in plant nitrogen content due to influence of rhizobial inoculation (Table 69). All the five isolates were effective in increasing nitrogen content of the seedlings. The most effective isolate was MRh-3.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on the plant nitrogen. All the inoculated seedlings except MRh-6 showed higher N than uninoculated ones at all levels of applied N. Among fertilizer levels, higher dose of N (N_2) contributed higher content of plant N than the lower dose. Seedlings inoculated with MRh-6 and applied with lower dose of N showed lower N than uninoculated seedlings with same dose of N. Unfertilized (N_0) seedlings inoculated with MRh-10 showed higher content of N followed by seedlings inoculated with MRh-2 and MRh-3 and applied with lower dose of N. All the rhizobial inoculated plants produced significantly higher

Table 69: Influence of inoculation of *Rhizobium* isolates and application of N and P on plant nitrogen of *A. mangium* seedlings

Rhizobium isolates	Plant nitrogen (%)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	2.388	2.202	2.128	2.166	2.351	2.201	2.239
MRh - 13	2.353	2./370	2.016	2.029	2.209	2.501	2.246
MRh - 6	2.264	1.650	2.182	1.929	2.090	2.078	2.032
MRh - 3	2.333	2.408	2.280	2.192	2.532	2.297	2.340
MRh - 10	2.508	1.942	2.209	2.114	2.310	2.234	2.220
Uninoculated	1.542	1.829	1.912	1.748	1.780	1.756	1.761
Mean	2.231	2.067	2.121	2.030	2.212	2.178	

C.D (0.05) for Isolates = 0.0296 SEM (±) = 0.0105
C.D (0.05) for N = 0.0209 SEM (±) = 0.0075
C.D (0.05) for P = 0.0209 SEM (±) = 0.0075
C.D (0.05) for Isolates × N = 0.0512 SEM (±) = 0.0183
C.D (0.05) for Isolates × P = 0.0512 SEM (±) = 0.0183

percentage of N than uninoculated seedlings at all levels of applied P. Seedlings with fertilizer level N_0P_1 showed high N followed by N_0P_2. The level N_2P_0 did not show significant differences from control (N_0P_0). The levels N_1P_1, N_2P_1, N_1P_2 and N_2P_2 were on par (Table 70).

The interactions between rhizobial inoculation and application of N and P fertilizers were highly significant. Seedlings inoculated with the isolate MRh-13 and fertilized with higher dose of P (N_0P_2) recorded highest percent of N followed by MRh-10 with lower dose of P (N_0P_1). The lowest percentage of N was shown by uninoculated seedlings without fertilizers (N_0P_0) followed by MRh-6 and MRh-10 with lower dose of N (N_1P_0) and MRh-6 with lower dose of N and P.

4.4.11. Plant nitrogen uptake

There were significant differences in N uptake of seedlings due to rhizobial inoculation (Table71). All the five rhizobial isolates were effective in increasing the N uptake of seedlings. The most effective isolate was MRh-6 followed by MRh-13.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on N uptake. Increases in doses of N and P fertilizers significantly increased the N uptake. The inoculated seedlings showed significantly higher N uptake than uninoculated seedlings at all levels of applied N and P. Seedlings inoculated with the isolate MRh-6 and applied with higher dose of N (N_2) showed maximum N uptake followed by seedlings inoculated with MRh-10 with same dose of N. With the higher dose of P also seedlings inoculated with the isolate MRh-6 was superior in N uptake. The isolate MRh-13 showed comparable N uptake, both with higher and lower dose of P. In all the inoculated seedlings, higher

Table 70: Influence of N and P on plant nitrogen of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Plant nitrogen (%)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	2.350	2.630	2.183	2.130	2.407	2.070	2.017	2.017	2.350
MRh - 13	2.000	2.147	2.913	2.183	2.577	2.350	1.903	1.903	2.240
MRh - 6	2.163	2.297	2.333	1.440	1.530	1.980	2.183	2.443	1.920
MRh - 3	2.277	2.613	2.110	2.280	2.443	2.500	2.020	2.540	2.280
MRh - 10	2.387	2.820	2.317	1.513	1.780	2.333	2.443	2.130	2.053
Uninoculated	1.323	1.603	1.700	1.920	1.980	1.587	2.000	1.757	1.980
Mean	2.083	2.352	2.259	1.911	2.153	2.137	2.094	2.132	2.137

C.D (0.05) for N × P = 0.0362 SEM (±) = 0.0129

C.D (0.05) for Isolates × N × P = 0.0887 SEM (±) = 0.0316

Table 71: Influence of inoculation of *Rhizobium* isolates and application of N and P on N uptake of *A. mangium* seedlings

Rhizobium isolates	N uptake (g plant^{-1})						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh-2	0.030	0.082	0.115	0.058	0.082	0.088	0.076
MRh-13	0.044	0.144	0.141	0.070	0.129	0.130	0.110
MRh-6	0.055	0.124	0.176	0.076	0.119	0.160	0.118
MRh-3	0.040	0.102	0.139	0.057	0.124	0.100	0.094
MRh-10	0.034	0.101	0.159	0.067	0.108	0.119	0.098
Uninoculated	0.015	0.047	0.082	0.033	0.050	0.062	0.048
Mean	0.036	0.100	0.135	0.060	0.102	0.110	

C.D (0.05) for isolates = 0.005 SEM (±) = 0.002
C.D (0.05) for **N** = 0.004 SEM (±) = 0.001
C.D (0.05) for P = 0.004 SEM (±) = 0.001
C.D (0.05) for isolates × N = 0.009 SEM (±) = 0.003
C.D (0.05) for isolates × P = 0.009 SEM (±) = 0.003

dose of N (N_2) resulted in higher N uptake than with higher dose of P (P_2).

Interactions between N and P had significant influence on N uptake of seedlings. Seedlings fertilized with N_2P_1 recorded maximum N uptake. The N uptake observed in unfertilized seedlings was significantly lower.

Interactions between rhizobial inoculation, N and P applications were highly significant (Table 72). Highest N uptake was recorded in seedlings inoculated with MRh-6 and fertilized at N_1P_2 level. The seedlings inoculated with MRh-6 and MRh-3 fertilized at N_2P_1 level. Lowest uptake was recorded in uninoculated unfertilized seedlings.

4.4.12. Soil nitrogen content

Effects of inoculation of different rhizobial isolates on soil nitrogen were significant (Table 73). All the five rhizobial isolates tested were effective in increasing the soil nitrogen percentage. Inoculation with the isolate MRh-3 showed higher soil N percentage.

The interactions between rhizobial inoculation and application of N and P fertilizers showed significant influence on soil nitrogen. Addition of lower dose (P_1) and higher dose (P_2) of P contributed significant increase in soil N above unfertilized treatment (P_0) but did not differ significantly among themselves. Unfertilized seedlings inoculated with the isolate MRh-3 and MRh-6, seedlings inoculated with MRh-13, MRh-3 and MRh-10 and fertilized with N_1 level of N, all the inoculated and uninoculated seedlings with higher dose of N (N_2) recorded higher percentage of soil N. Fertilizer P increased in soil N, when inoculated with rhizobial isolates MRh-6. In

Table 72: Influence of N and P on N uptake of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	N uptake (g plant^{-1})								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh-2	0.009	0.050	0.029	0.089	0.068	0.091	0.075	0.127	0.142
MRh-13	0.011	0.040	0.080	0.093	0.190	0.150	0.106	0.156	0.160
MRh-6	0.007	0.063	0.094	0.058	0.086	0.228	0.162	0.207	0.158
MRh-3	0.011	0.069	0.039	0.080	0.109	0.118	0.080	0.195	0.142
MRh-10	0.016	0.036	0.049	0.052	0.102	0.150	0.132	0.187	0.159
Uninoculated	0.002	0.017	0.027	0.040	0.055	0.047	0.057	0.077	0.111
Mean	0.010	0.046	0.053	0.069	0.102	0.131	0.102	0.158	0.145

C.D (0.05) for N × P = 0.007 SEM (±) = 0.002
C.D (0.05) for isolates × N × P = 0.016 SEM (±) = 0.006

Table 73: Influence of inoculation of *Rhizobium* isolates and application of N and P on soil nitrogen of *A. mangium* seedlings

Rhizobium isolates	Soil nitrogen (%)						
	N_0	N_1	N_2	P_0	P_1	P_2	Mean
MRh - 2	0.093	0.092	0.095	0.089	0.100	0.092	0.093
MRh - 13	0.092	0.101	0.095	0.900	0.096	0.101	0.096
MRh - 6	0.101	0.092	0.096	0.087	0.098	0.104	0.096
MRh - 3	0.106	0.100	0.104	0.096	0.112	0.101	0.103
MRh - 10	0.090	0.101	0.090	0.095	0.093	0.093	0.094
Uninoculated	0.076	0.089	0.098	0.086	0.092	0.086	0.088
Mean	0.093	0.096	0.096	0.090	0.099	0.096	

C.D (0.05) for Isolates = 0.0054 SEM (±) = 0.0019
C.D (0.05) for N = 0.0038 SEM (±) = 0.0014
C.D (0.05) for P = 0.0038 SEM (±) = 0.0014
C.D (0.05) for Isolates × N = 0.0093 SEM (±) = 0.0033
C.D (0.05) for Isolates × P = 0.0093 SEM (±) = 0.0033

seedlings inoculated with the isolate MRh-3, lower dose of P (P_1) contributed significant increase in soil N.

Interactions between N and P showed significant differences. Lower and higher dose of N did not differ significantly in contributing to soil N without the application of P, but significantly increased the nitrogen above unfertilized (N_0) treatments. The soil N content was significantly higher in pots applied with N_1P_1, N_0P_2, N_1P_2, N_2P_0, N_0P_1 and N_2P_2 levels. Soil N in pots without fertilizer (N_0P_0) was the lowest (Table 74).

Interactions between rhizobial inoculation, N and P application were highly significant. Inoculation with the isolate MRh-3 with a fertilizer level of N_0P_1 and inoculation with MRh-6 with a fertilizer level N_0P_2 recorded highest values of soil N and showed no significant difference from MRh-2 and MRh-6 with a fertilizer level of N_0P_1; MRh-2, MRh-13 and MRh-3 with N_0P_2; MRh-13, MRh-3 and MRh-10 with N_1P_0. All these inoculated pots combined with fertilizer application showed significantly superior soil N than uninoculated seedlings without fertilizer (N_0P_0).

Table 74: Influence of N and P on soil nitrogen of *A. mangium* seedlings inoculated with *Rhizobium* isolates

Rhizobium isolates	Soil nitrogen (%)								
	N_0P_0	N_0P_1	N_0P_2	N_1P_0	N_1P_1	N_1P_2	N_2P_0	N_2P_1	N_2P_2
MRh - 2	0.084	0.098	0.098	0.084	0.103	0.089	0.098	0.098	0.089
MRh - 13	0.084	0.093	0.098	0.098	0.098	0.107	0.089	0.098	0.098
MRh - 6	0.084	0.103	0.117	0.089	0.089	0.098	0.089	0.103	0.098
MRh - 3	0.093	0.117	0.107	0.098	0.107	0.093	0.098	0.112	0.103
MRh - 10	0.089	0.093	0.089	0.098	0.098	0.107	0.098	0.089	0.084
Uninoculated	0.075	0.079	0.075	0.084	0.098	0.084	0.098	0.098	0.098
Mean	0.085	0.097	0.097	0.092	0.099	0.096	0.095	0.100	0.095

C.D (0.05) for N × P = 0.0066 SEM (±) = 0.0024

C.D (0.05) for isolates × N × P = 0.0162 SEM (±) = 0.0058

Discussion

DISCUSSION

The nodulation characteristics of *Acacia mangium* and the response of the rhizobial isolates from *A. mangium* to N, P and lime levels are discussed in this chapter.

5.1. Nodulation characteristics of *A. mangium* plantations

5.1.1. Nodule characteristics

The mangium plantations in the state showed good nodulation potential. The plantations varied in age, girth of the trees (GBH) and soil pH, The surveyed plantations were of age 2 to 9 years, indicating that *Rhizobium* was active not only in seedlings but also in grown up trees. The lowest GBH was in plantations of Adimali (Idukki) and largest from Thiruvazhamkunnu (Palakkad).The soil of the plantations were acidic with the pH values ranging from 4.9 to 6.2.

The number of nodules per unit volume of soil varied from 6.3 to 24.3. The highest number of nodules were recorded from the plantation in Muvatupuzha (Ernakulam district) followed by that from Thiruvazhamkunnu (Palakkad district) and Vellanikkara (Trichur district). The nodules were round or fingershaped or collaroid or astragalloid. Thirteen isolates of rhizobia were obtained from mangium plantations, representing different districts of Kerala except Kasargod. These isolates are designated as MRh-1 to MRh-13. These thirteen isolates showed variation in colony characteristics, growth rate and the viable count of bacteria. The isolates from Mananthavadi in Wynad district (MRh-3) and Muvatupuzha in Ernakulam district (MRh-7) were fast growers whereas the other eleven isolates were slow growers (Table 1). Maximum count of viable bacteria was observed for

the isolate from Mananthavadi (MRh-3) followed by the isolate from Perambra (MRh-2) and Muvatupuzha (MRh-7).

These data indicate that, the fast growers (*Rhizobium* spp) as well as the slow growers (*Bradyrhizobium* spp) are involved in nodulation of *A. mangium* in Kerala. This is contrary to earlier reports. So far it has been reported that, the nodulation in *A. mangium* is by *Bradyrhizobium* spp. The survey in Australia (Galiana, 1991), China and French Guyana (Souvannavong and Galiana, 1991), could isolate only *Bradyrhizobium* from *A. mangium*. Kerala Forest Research Institute conducted a survey throughout the state and isolated *Rhizobium* as well as (*Bradyrhizobium*) from *A. auriculiformis* (Sharma *et al.*, 1995), but they have not surveyed any mangium plantations.

The number of nodules per unit volume of soil observed in field survey or the GBH of the tree were not having any deducible relationship with the growth rate of rhizobia. Similarly, there was no perceptible relationship between the rate of growth and viable count of bacteria per millilitre. Since the soil pH of all the plantations were acidic, it is presumed that, all these isolates of rhizobia are tolerant to acidity. Plant infection test conducted had confirmed the nodulation ability of all the 13 rhizobial isolates.

5.2. Competition from native ineffective rhizobia

The five selected isolates from *A. mangium* and *A. auriculiformis* and two commercial cultures from Agroforester™ seeds, Hawaii showed different responses in sterilized and unsterilized soils.

5.2.1. Nodulation characteristics

The number of nodules and weight of nodules showed wide variation in sterilized and unsterilized soils. In general, the nodulation characteristics and the general growth of the seedlings were superior in unsterilized soils. However, a clear comparison of the rhizobial isolates and cultures were possible only from the results from sterilized soil. The isolates MRh-2, MRh-13 and MRh-10 produced more number of nodules and effective nodules in sterilized soil as compared to unsterilized soil. The five isolates from *A. auriculiformis* (ARh-isolates) were relatively ineffective and produced few number of nodules and effective nodules in sterilized soil. The ARh-isolates gave relatively high number of nodules and effective nodules under unsterilized soil condition. The number of nodules observed in seedlings inoculated with ARh-isolates and uninoculated seedlings was high. This points towards the possibility of native rhizobia forming nodules on roots of mangium, thus competing for nodulation sites. The number of effective nodules in these cases were not in proportion to the total number of nodules, implying nodulation by ineffective local strains of rhizobia also. The prominent variations in nodulation pattern observed in sterilized and unsterilized soils indicate that, some soil factors in unsterilized soil are complementing the performance of the isolates from *A. auriculiformis*. Probably the native population of the rhizobia and/or mycorrhizae or other micro-organisms present in the unsterilized soil are having complementary influence on the performance of rhizobial isolates from *A. auriculiformis*. The complementary influences between rhizobia and VAM has been reported in *A. mangium, A. auriculiformis* and *Paraserianthes falcataria* (Dela Cruz *et al.*, 1988). Dual inoculation between *Rhizobium* and mycorrhiza was found to be capable of fulfilling the requirements of N and P in plants to a great extent (Punj and

Gupta, 1988 b). In the present study the VAM and other beneficial soil micro-organisms may be killed in the process of sterilization, resulting in a general loss of vigour of seedlings grown in sterilized soil. The lower number of nodules and effective nodules recorded in unsterilized soil in the case of isolates MRh-2, MRh-13, MRh-10 may be because of the competition from native rhizobia. Similar instance of competition from native rhizobia has been reported in *L. leucocephala* (Prinsley *et al.*, 1987. South, 1982). The culture "Agroforester - Group A", though did not show much variation in the number of nodules per seedlings in sterilized and unsterilized soil, number of effective nodules produced in sterilized soil was almost double than that in the unsterilized soil. Almost similar patterns of results were observed when the fresh and dry weight of the nodules and effective nodules in sterilized and unsterilized soils were compared.

5.2.2. Growth characteristics

When the seedling growth parameters like fresh and dry weights of leaves, stem and roots were compared, the seedlings in unsterilized soil were superior to those grown in sterilized soil. Even in uninoculated seedlings, the seedling growth was much superior in unsterilized soil as compared to sterilized soil. Evidently there are some factors in the unsterilized soil which are encouraging the growth of the seedlings. It may be due to the presence of other beneficial micro-organisms like mycorrhiza or organic matter decomposing and mineralising organisms. There are a number of reports on better performance of legumes due to the inoculation with VAM or due to double inoculation with VAM and rhizobia (Chang *et al.*, 1986 and Dela Cruz *et al.*, 1988). There were no perceptible pattern of growth differences between uninoculated and inoculated seedlings in unsterilized soil.

In sterilized soil most of the inoculated seedlings showed superior performance compared to uninoculated seedlings. The fresh and dry weight of leaves, stem and roots of seedlings inoculated with rhizobial isolates MRh-3, MRh-2, MRh-13, MRh-6, MRh-10 and "Agroforester - Group A" showed superior performance. Two cultures from A-auriculiformis *viz.*, ARh-1 and ARh-2 were superior to uninoculated control where as ARh-3, ARh-4, ARh-5 and "Agroforester - Group C" were on par with uninoculated control. In sterilized soil, the leaf area, above ground biomass, total biomass and soil N were more in inoculated seedlings (Fig.2,3,4 and 5). The plant N content showed superiority only in seedlings inoculated with the isolate MRh-3; all other isolates being on par with uninoculated seedlings. The superiority of the mangium isolates from Kerala (MRh-2, MRh-13, MRh-3, MRh-6) were very much evident from the data on leaf area of the seedlings, above ground biomass, total biomass and soil-N content. As the Agroforester - Group C and the isolates from *A. auriculiformis* (ARh-1, ARh-2, ARh-3, ARh-4 and ARh-5) were on par with uninoculated seedlings, with respect to these characters, rhizobia identified for *A. auriculifirmis* cannot be recommended for mangium. So it is evident that mangium is specific in its rhizobial inoculation requirement. Specificity of mangium for nodulation has been reported earlier also (Galiana *et al.*, 1991, Turk and Keyser, 1992). Miller *et al.* (1991) found that some Australian soil contained a varied populations of strains of rhizobia and *A. mangium* nodulated and fixed N poorly with two of the four strains tested, only three out of 33 isolates from different locations were effective.

In unsterilized soil, the effects of inoculation were not consistent. With many isolates the inoculated and uninoculated seedlings showed no significant difference. The soil N content were more with isolates MRh-2, MRh-13, MRh-10 and other

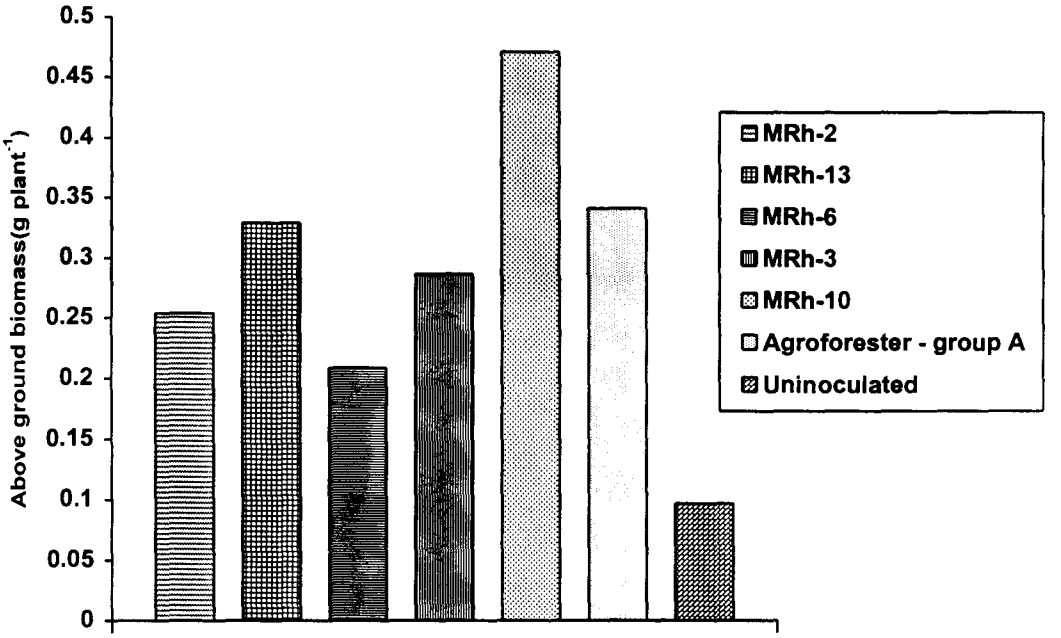

Fig. 2: Influence of rhizobial inoculation of *A. mangium* seedlings on above ground biomass (sterilized soil)

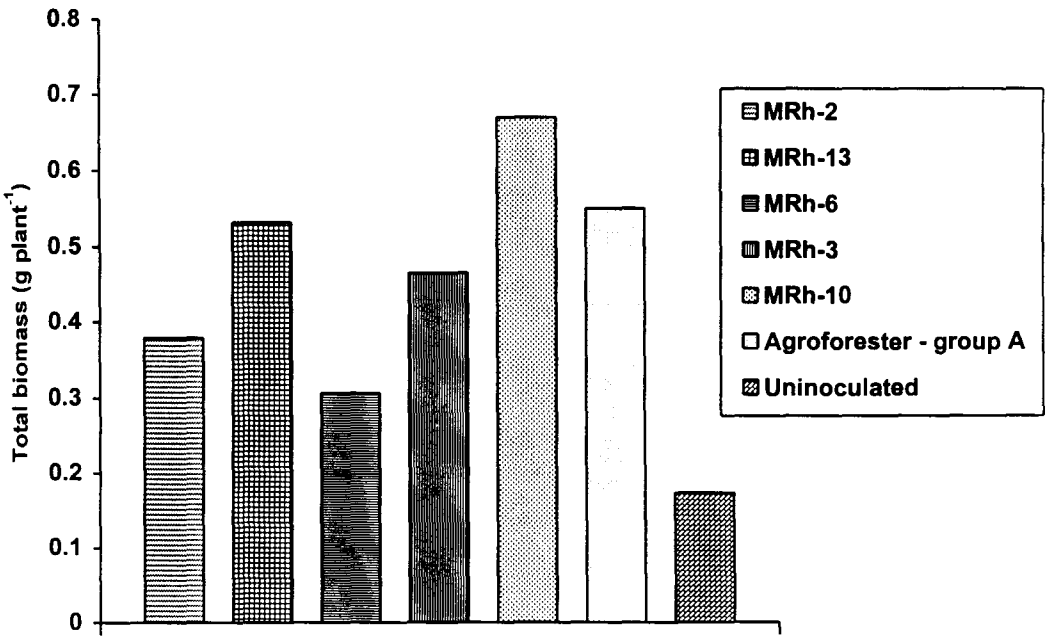

Fig. 3: Influence of rhizobial inoculation of *A. mangium* seedlings on Total biomass (sterilized soil)

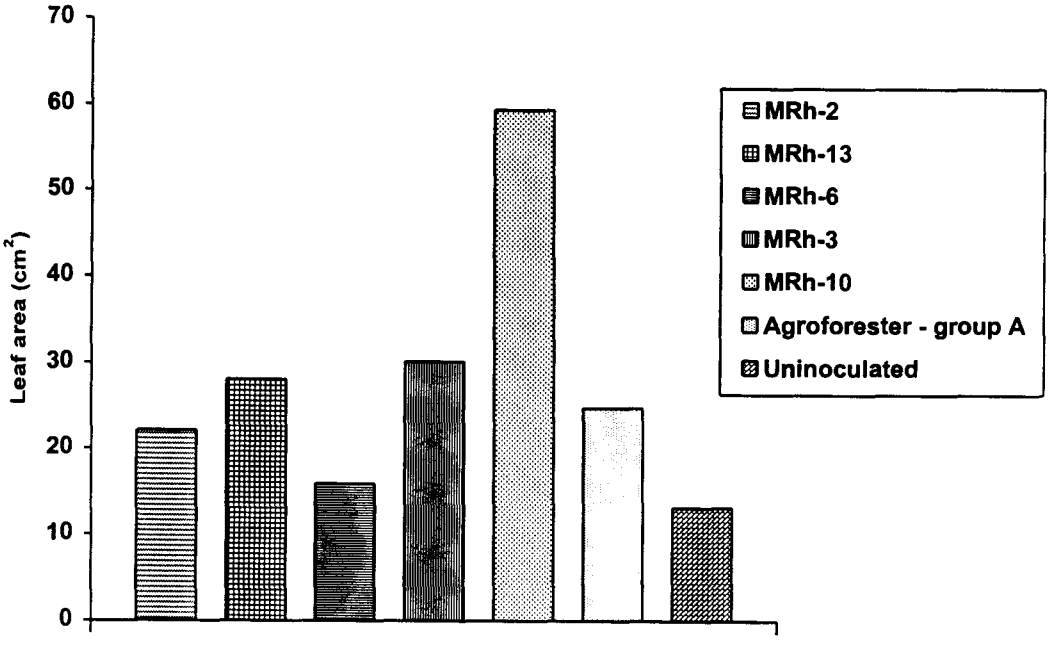

Fig. 4: Influence of rhizobial inoculation on leaf area of *A. mangium* seedlings (sterilized soil)

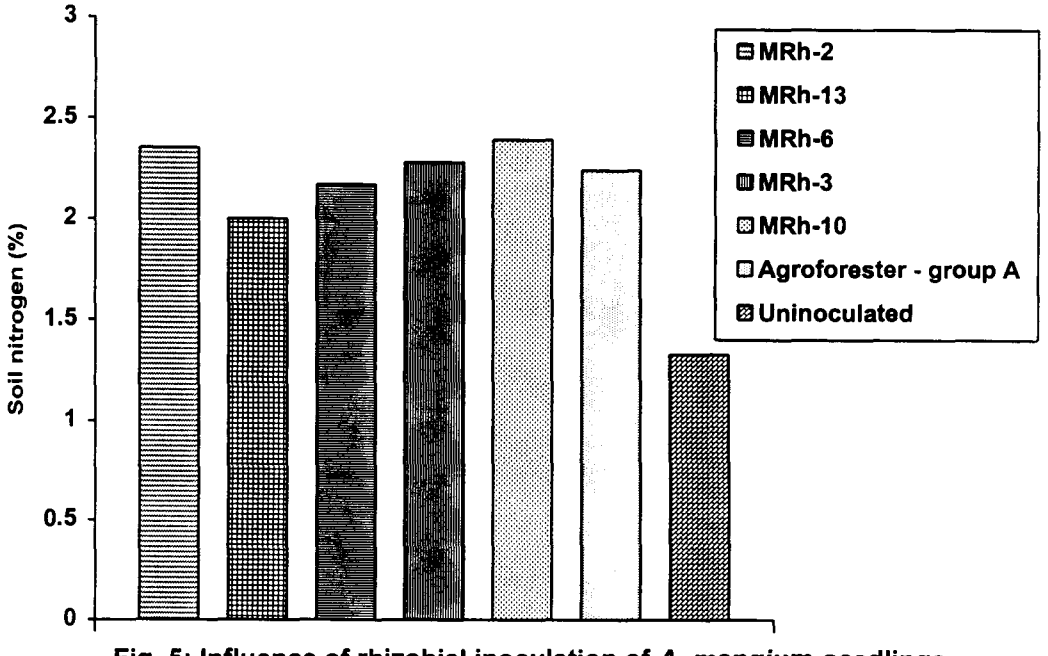

Fig .5: Influence of rhizobial inoculation of *A. mangium* seedlings on soil nitrogen (sterilized soil)

isolates were on par or inferior to uninoculated control. The plant N content was superior only in seedlings inoculated with the isolate MRh-6. The more number of effective nodules and the high plant N content of the seedlings inoculated with MRh-6 in unsterilized soil corroborates the evidence for the ability of this isolate to compete with local ineffective strains of rhizobia and fix N efficiently. In sterilized soil also this isolate produced high number of effective nodules and the number of ineffective nodules were negligible. The leaf area, above ground biomass, total biomass and N uptake showed two to eight fold increase in unsterilized soil. However, these data do not indicate any definite pattern so as to enable the evaluation of different rhizobial isolates. A meaningful evaluation of the isolates may be possible only with sterilized soil, eventhough the general vigour of the seedlings is poor as compared to unsterilized soil. Contrary to these results, Dommergues (1987) observed that tree seedlings grown in unsterilized soil exhibited a survival rate which was less than half of that in sterilized soils.

5.3. Efficiency of the cultures under different soil acidity

The influences of graded levels of lime applied to moderate the soil acidity, on the performance of the selected rhizobial isolates are discussed below.

5.3.1. Nodulation characteristics

Number of nodules and effective nodules in mangium seedlings showed significant variation due to the application of lime. The total number of nodules per seedlings were decreased with L_1 and L_3 levels of lime. However, L_2 level of lime resulted in significantly more number of nodules per seedlings. The total number of nodules observed in seedlings inoculated with MRh-2, MRh-13 and MRh-10 and

"Agroforester - Group A" were considerably more than the seedlings inoculated with other isolates and uninoculated seedlings showing the potential of these isolates in N_2 - fixation. The isolates from *A. auriculiformis*, though produced more number of nodules than the uninoculated seedlings, were much inferior to the isolates from *A. mangium*. The "Agroforester - Group A" produced more number of nodules with increasing levels of lime up to L_2 level, whereas MRh-13 showed lesser number of nodules with increasing levels of lime. The number of effective nodules also were relatively high in MRh-2, MRh-13, MRh-6 and MRh-10 and the maximum number was observed in MRh-10 (Fig. 6). The number of effective nodules also were less in seedlings inoculated with isolates of *A. auriculiformis* but still superior to uninoculated seedlings. The overall effect of liming was reduction in number of effective nodules. In "Agroforester - Group A", unlike the total number of nodules, the number of effective nodules decreased due to lime application. Lime application also decreased the number of effective nodules produced by MRh-2, MRh-13, MRh-3 indicating the preference of these isolates to a pH of 5.5. In ARh-1, ARh-2 and "Agroforester - Group C", lime application increased the number of effective nodules indicating the preference of these isolates for soil pH from 6 to 6.5. The fresh and dry weight of nodules also showed similar pattern of results.

5.3.2. Growth characteristics

Rhizobial isolates MRh-10 and "Agroforester - Group A" induced better seedling growth when lime was applied at the rate of L_2 and L_3 . This was evident from the seedling characters like collar diameter, height, number of phyllodes, depth and number of roots, leaf area and biomass. On the otherhand, the isolate MRh-13 showed its prominent influence with out lime application. At all levels of lime, the

fresh and dry weights of leaves, stem and roots were very low in uninoculated seedlings as compared to seedlings inoculated with mangium isolates. Leaf area, above ground biomass and total biomass of the seedlings also showed same trend (Fig. 7,8 and 9).

5.3.3. Nitrogen content of plant and soil

The plant N content decreased due to the application of lime in seedlings inoculated with the isolate MRh-2 MRh-10 or "Agroforester - Group A" (except at L_3 level). In the case of MRh-13, MRh-6 and MRh-3, lime application up to L_2 level decreased the plant N content where as L_3 level increased the plant N content significantly indicating the preference of these isolates for acidic soils. The high plant N content may be due to improvement in uptake by the plant due to reasons other than inoculation. In uninoculated seedlings also, plant N content increased with increasing levels of lime except at L_2 level which was on par with L_0. In general, the seedlings inoculated with mangium isolates showed higher plant N content as compared to auriculiformis isolates and uninoculated seedlings. Nitrogen uptake of the seedlings increased due to liming. The uptake of N in uninoculated seedling was considerably less at all levels of lime. Maximum N uptake was observed in limed seedlings inoculated with MRh-10 and "Agroforester – Group-A". Soil N content did not show much variation either with inoculation or with lime application.

The data on plant nitrogen show the superiority of the mangium - isolates from the state. Overall results indicate that nodulation characteristics like total number of nodules, effective nodules, fresh and dry weight of nodules and effective nodules were adversely affected by lime application, in seedlings inoculated with mangium isolates from the State, except MRh-10. On the otherhand, in rhizobial

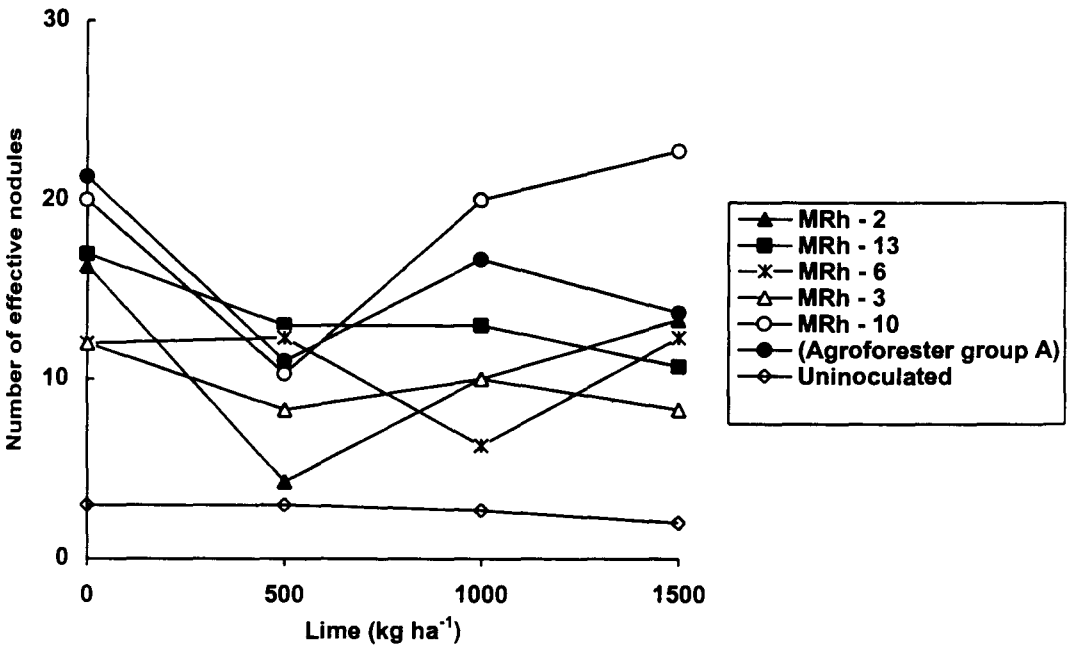

Fig.6: Influence of liming and inoculation of *Rhizobium* on number of effective nodules in *A. mangium* seedlings

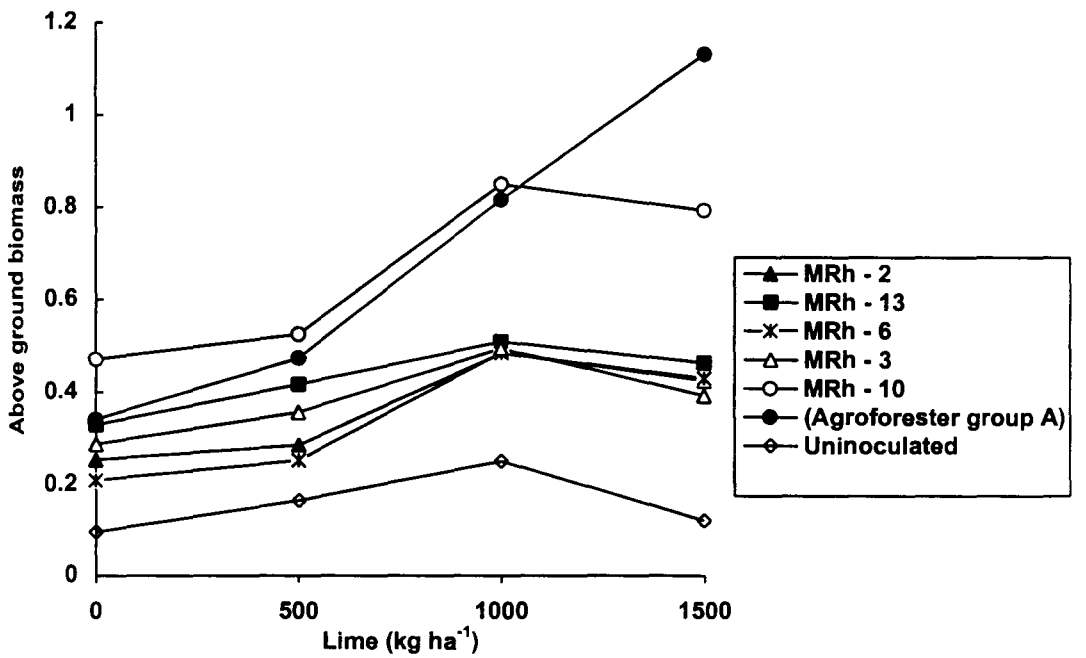

Fig.7 : Influence of liming and inoculation of *Rhizobium* on above ground biomass of *A.mangium* seedlings

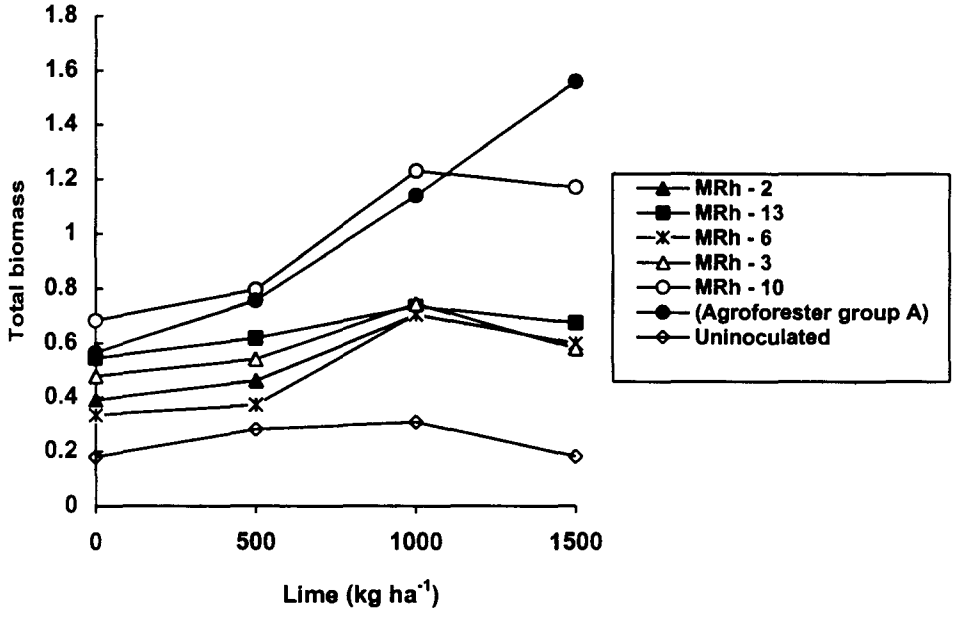

Fig.8: Influence of liming and inoculation of *Rhizobium* on total biomass of *A. mangium* seedlings

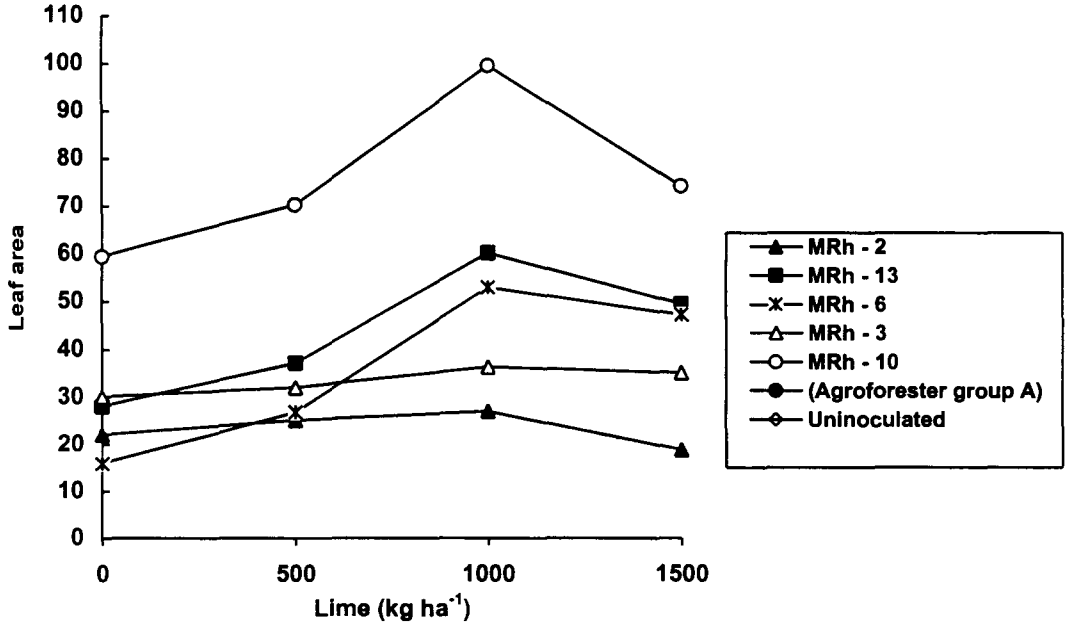

Fig.9: Influence of liming and inoculation of *Rhizobium* on leaf area of *A. mangium* seedlings

culture for mangium from Hawaii, "Agroforester - Group A", lime application up to 1000 kg ha^{-1} increased the of number of nodules. As the mangium isolates were collected from plantations with acidic soils (Table 1), these cultures may be adapted to low soil pH and amending the pH by liming may be of disadvantage. "Agroforester - Group A", mangium culture from Hawaii may be adapted to higher pH. Rhizobial isolates adapted to different soil pH were isolated by several workers. The critical soil pH reported for establishment of rhizobial association of *Leucaena* was between 4.45 and 4.70 below which species cannot be established satisfactorly (Ahmad and Ng. 1981). In a study conducted in Kerala (Balasundaran and Ali, 1987) the *Leacaena* seedlings survived at pH levels 4.1 and 5.1 and the growth was poor at pH 5.7. Rhizobial strains isolated from root nodules of *Sesbania formosa, Acacia farnesiana* and *Dalbergia sisoo* were well adapted to grow on soil pH 12.0 while bacterial strain from *Albizia lebbek* and *Acacia nilotica* grew well at pH 9.0 (Surange *et al.*, 1997). Umali - Garcia (1988) reported that, liming of soil to pH 6.5, improved the performance of *Rhizobium - A. mangium* symbiosis. Liming of acid soils improves greatly the survival of rhizobia (Basu *et al.*, 1987), but may also induce nutritional disorders like deficiencies of P, Zn, Cu and Fe (Franco and Munns, 1982). Manguiat *et al.* (1984) reported that in *Albizzia procera*, the overall effect of lime pelleting was detrimental, possibly because of fixation of available soil P by Ca ions.

The mangium isolates MRh-10 and "Agroforester – Group A" showed higher number and weight of nodules and effective nodules with higher levels of lime. The seedling growth characteristics also indicated the requirement of liming for the efficient performance of these rhizobial isolates. However, the isolate MRh-10 produced relatively higher values for most of the nodulation and plant growth

characteristics even without application of lime. This points to the versatility of this isolate; may be this isolate is adapted to wide range of soil pH. The nodulation and growth characteristics of the seedlings inoculated with the isolate MRh-13 were superior without lime application indicating the suitability of this isolate for acidic soils and not at all suitable for near – neutral or alkaline soils.

5.4. Nodulation efficiency of the rhizobial isolates under different soil fertility

The influences of application of graded levels of nitrogen and phosphorus fertilizers on nodulation and N_2 -fixation efficiency of the selected rhizobial isolates are discussed in this section.

5.4.1. Nodulation characteristics

Application of N and P fertilizers increased the total number of nodules, number of effective nodules and fresh and dry weight of nodules. Total number of nodules and number of effective nodules increased with increasing levels of N, whereas, with P fertilizers, the increase was observed only up to 50 kg ha^{-1} . The response to application of N, was much more conspicuous in the isolate MRh-6 as compared to other isolates (Fig. 10). The isolate MRh-6 also showed good response to application of P (Fig. 11). In all the isolates, maximum number of nodules were observed when P was applied at the rate of 50 kg ha^{-1}. Over all, MRh-6 was superior to all others followed by MRh-13, MRh-10, MRh-3 and MRh-2. Uninoculated seedlings recorded very low number of nodules.

The rhizobial isolates showed significant interactions between combined application of N and P fertilizers. Highest number of nodules were observed in MRh-6 with 200 kg ha^{-1} of N and 50 kg ha^{-1} of P (N_2P_1). However, this level of fertilizers

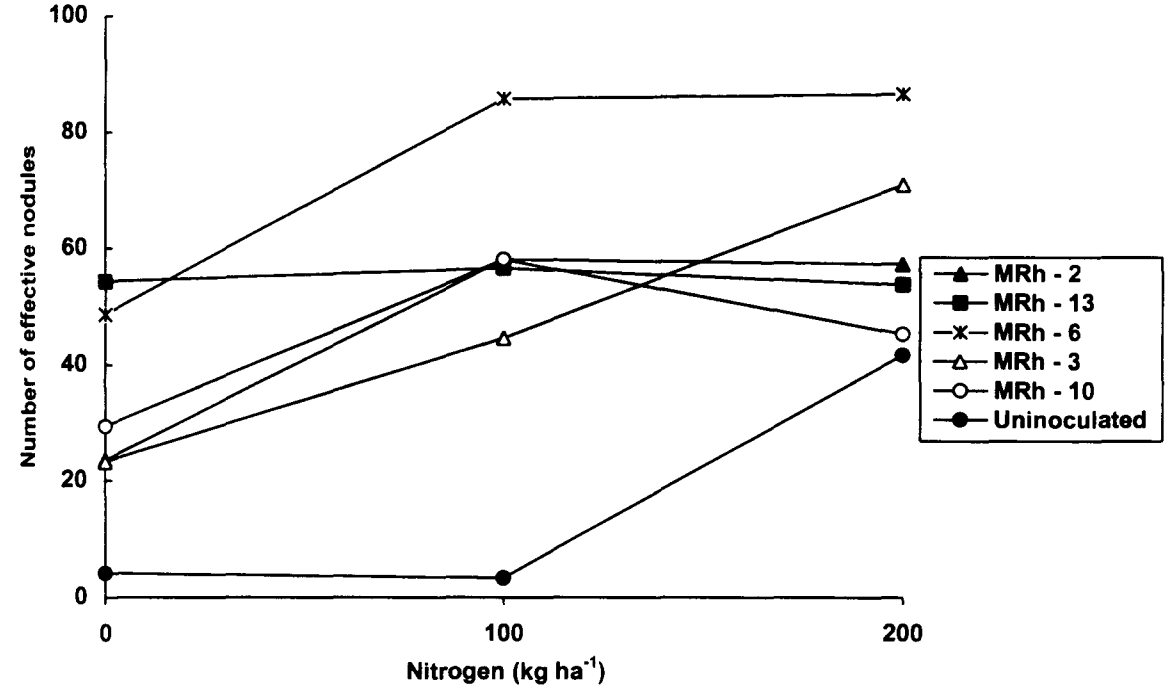

Fig.10: Influence of inoculation of *Rhizobium* Isolates and application of N on number of effective nodules in A.mangium seedlings

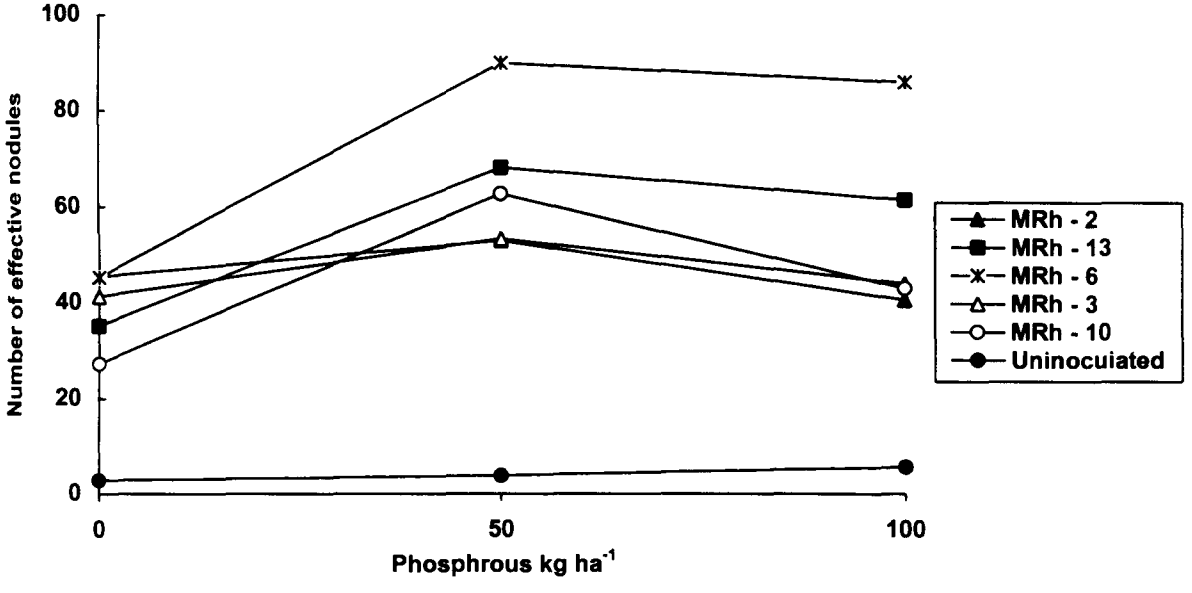

Fig.11: Influence of inoculation of *Rhizobium* isolates and application of P on number of effective nodules in *A. mangium* seedlings

was on par with 100 kg ha^{-1} of N and 100 kg ha^{-1} of P (N_1P_2) and 100 kg ha^{-1} of N and 50 kg ha^{-1} of P (N_1P_1). Application of N and P showed complementary effects. In MRh-2, MRh-13 and MRh-10, maximum number of nodules were recorded with 200 kg ha^{-1} of N and 50 kg ha^{-1} of P . Whereas, in MRh-3, it was with 200 kg ha^{-1} of N (N_2P_0). Over all, the number of nodules were best with 100 kg N and 50 kg P ha^{-1}. The number of effective nodules also showed almost similar trend, the highest number being in MRh-6 with a fertilizer level of 100 kg ha^{-1} N and 50 kg ha^{-1} P which was superior to all other isolates at different levels of fertilizers. This indicates the necessity for a starter dose of N and P for these isolates to perform well. The requirements of N and P for nodulation by rhizobia were reported in *A. mangium* (Cabahug, 1991) *Leucaena* (Sanginga *et al.*, 1988a and b). Though these are N_2-fixing species, starter dose of N is required for effective symbiosis between rhizobia and the plant (Harper, 1974; Eaglesham *et al.*, 1977; Kang, 1975 and Sanginga *et al.* 1986). Jones (1985) reported favourable growth responses of nodulated *Leucaena* seedlings following initial application of N. Phosphorus is reported to have energy transfer functions in *Rhizobium* and plant (Sprent, 1979). In *Leucaena*, P stimulated nodulation and growth (Benge, 1989, Hu and Chang, 1981). Contrary to the above results Vadez *et al.* (1995) reported that, *A. mangium* does not have high requirements of P for growth and N_2-fixation.

The fresh and dry weight of nodules also increased with increasing levels of N and P (Table 33 and 35). However, fresh and dry weight of nodules showed significant increase only up to N_1 and P_1 levels.

The interaction effects of N and P were observed on the fresh weight of nodules also. With P_0 levels, increasing N levels did not show much influence on the

weight of nodules. Similarly higher levels of P without N did not show much response. The highest values of fresh weight of nodules were obtained with N_1P_2 followed by N_2P_1. Inoculation with the isolate MRh-13 with a fertilizer dose of 100 kg ha^{-1} N and 50 kg ha^{-1} of P (N_1P_1) resulted in maximum nodule fresh weight. In the isolate MRh-6, highest nodule fresh weight was observed with 200 kg N and 50 kg P per hectare. The fresh weight and dry weight of nodules and effective nodules increased with increasing N and P levels. However, the increase in nodule weight due to increasing the fertilizer dose from N_1 to N_2 and P_1 to P_2 were only marginal. The interactions between the isolates, N and P showed almost the same trend as that of fresh weight of nodules. Maximum fresh and dry weight of nodules were observed with N_2P_1 level in the isolate MRh-6 followed by N_1P_1 in MRh-13. From the nodule characteristics, it is evident that, MRh-6 is a superior isolate followed by MRh-13 at a fertilizer level of N_1P_1 and MRh-3 at a fertilizer level of N_2P_1 . Increase in number and weight of nodules in *A. mangium* due to application of N and P fertilizers were reported by Mao *et al.* (1989), Cali (1991) and Cabahug (1991). For most other legumes, N application depressed nodule initiation rather than nodule development, resulting in decreased number and mass of nodules (Kang, 1975; Hussain *et al.*, 1986 and Becana and Sprent, 1987).

5.4.2. Growth characteristics

The collar diameter, height of seedlings, number of phyllodes, number of lateral roots and depth of tap root increased due to inoculation with rhizobial isolates and application of N and P fertilizers. The superiority of the seedlings in these characters by increasing the fertilizer dose from N_0 to N_1 and P_0 to P_1 were evident in inoculated seedlings, whereas, the increase in growth characters by increase in

fertilizer dose from N_1 to N_2 and P_1 to P_2 were only marginal in uninoculated seedlings. Inoculations with isolate MRh-6 combined with 200 kg N and 100 kg P ha^{-1} (N_2 P_2) resulted in maximum collar diameter, height and number of phyllodes. However, maximum depth of tap root and number of lateral roots were observed in seedlings inoculated with MRh-6 combined with fertilizer application at the rate of 100 kg N and 100 kg P (N_1P_2) per hectare. In MRh-10 and MRh-13, maximum diameters and heights were observed with fertilizer levels of N_2P_2 and N_2P_1 respectively. The maximum number of phyllodes was observed in MRh-13 with fertilizer level of N_2P_1 while in MRh-10, maximum number was observed with N_1P_2. In uninoculated seedlings, the diameter, height, number of phyllodes, number of lateral roots, and root depth were relatively very low at all levels of fertilizer application. Better performance of the inoculated seedlings observed at N_1P_1 or N_2P_1 level indicate the complementary influences of rhizobial inoculation and N and P application.

The fresh and dry weight of leaves, stem and root also increased due to inoculation with rhizobial isolates and application of N and P fertilizers. Inoculation with isolate MRh-6 combined with N_1P_2 level of fertilizer resulted in maximum fresh weight of leaves. In the case of isolate MRh-13, the maximum weight of leaves was observed with 100 kg N and 50 kg P ha^{-1} (N_1P_1). However, maximum leaf and root dry weight was observed in seedlings inoculated with MRh-6 combined with fertilizer application at the ratio of 100 kg N at 100 kg P (N_1P_2). In MRh-10 and MRh-13, the peak values on dry weight of leaves were observed with a fertilizer dose of 200 kg N and 50 kg P (N_2P_1). Similarly the highest value of dry weight of stem was observed in seedlings inoculated with MRh-6 combined with a fertilizer dose of 100 kg N and 100 kg P (N_1P_2). Seedlings inoculated with isolates MRh-10 MRh-3, MRh-13 and

uninoculated seedlings showed highest dry weight when the fertilizer level was increased to 200 kg N and 100 kg P. The isolates MRh-2, MRh-13, MRh-3 and MRh-10 showed its best influence when a fertilizer level of N_2P_1 was given. The superiority of the isolate MRh-6 was evident from the leaf area of the seedlings also (Fig. 16 and 17). The isolate MRh-6 recorded maximum leaf area when the fertilizer levels were increased to N_1P_2. The increase in leaf area by increasing the fertilizer dose from 0 to 100 kg N and 0 to 50 kg P ha^{-1} were 215 and 69 per cent respectively, increase in leaf area by further increase in N and P were only marginal. The increase in leaf area of the seedlings inoculated with MRh-6 and MRh-13, at the fertilizer dose of 100kg N and 50 kg P ha^{-1} were 140 per cent and it was substantially higher than that recorded by other isolates at different levels of fertilizers. The isolates MRh-2, MRh-3 and MRh-10 showed significant influence on the leaf area of the seedlings when the fertilizer levels were increased to N_2P_1 level. In the uninoculated seedlings, the leaf area was very low at all the levels of fertilizer application.

5.4.3. Plant biomass

The above ground biomass of the seedlings was also maximum due to the inoculation with the isolate MRh-6 followed by inoculation with the isolate MRh-13. The above ground biomass in uninoculated seedlings were 115 per cent less than the inoculated seedlings. This was much more evident at higher levels of fertilizers (Fig. 12 and 13). The maximum biomass production was in seedlings inoculated with the isolate MRh-6 combined with fertilizer application at the rate of 100 kg N and 100 kg P per hectare (N_1P_2). Other isolates showed its best influence at fertilizer levels of N_2P_1 or N_2P_2. In uninoculated seedlings the above ground biomass

was low. However, the biomass increased with increasing levels of fertilizers. The total biomass of the seedlings also showed similar pattern as that of above ground biomass (Table 68, Fig. 14 and 15).

The increase in plant height, collar diameter above ground biomass and total biomass in inoculated seedlings may be because of the higher photosynthetic fixation of carbon from the increased leaf area of the seedlings consequent to rhizobial inoculation and feritlizer application (Table 41, 43, 65 and 67). There were 200 to 600 per cent increase in leaf area of the seedlings due to inoculation with rhizobial isolates and application of fertilizers (Table 52). The increase in leaf area observed due to inoculation of rhizobial isolates may be because of the symbiotic association between *Rhizobium* and the plant. The symbiotic activity might have increased the N content of the plant (Table 69) which might have induced the production of more leaf area. Cali (1991) also observed that, the rhizobial inoculation independently improved the height, biomass nodule weight and N content and uptake in *A. mangium.* Inoculated plants had significantly greater N contents in the above ground parts of *A. senegal,* than uninoculated plants (Badji *et al.*, 1987). Nitrogen being an important element for vegetative growth especially for leaf development (Michin *et al.*, 1981), the rhizobial inoculation may have helped the plants to derive more N from atmosphere and to improve its vegetative growth. Positive effects of *Bradyrhizobium* on the growth of *A. mangium* is also demonstrated by Brunck *et al.* (1990). The influence of fertilizer application on nodulation and growth of tree legumes were also reported by several other workers. An increase in dry matter production and growth parameters were obtained when N_2-application and inoculation were combined in seedlings of *Enterolobium contortisiliqum* (Ribeiro *et al.*, 1986). Garza *et al.* (1987) reported that, in *Leucaena*

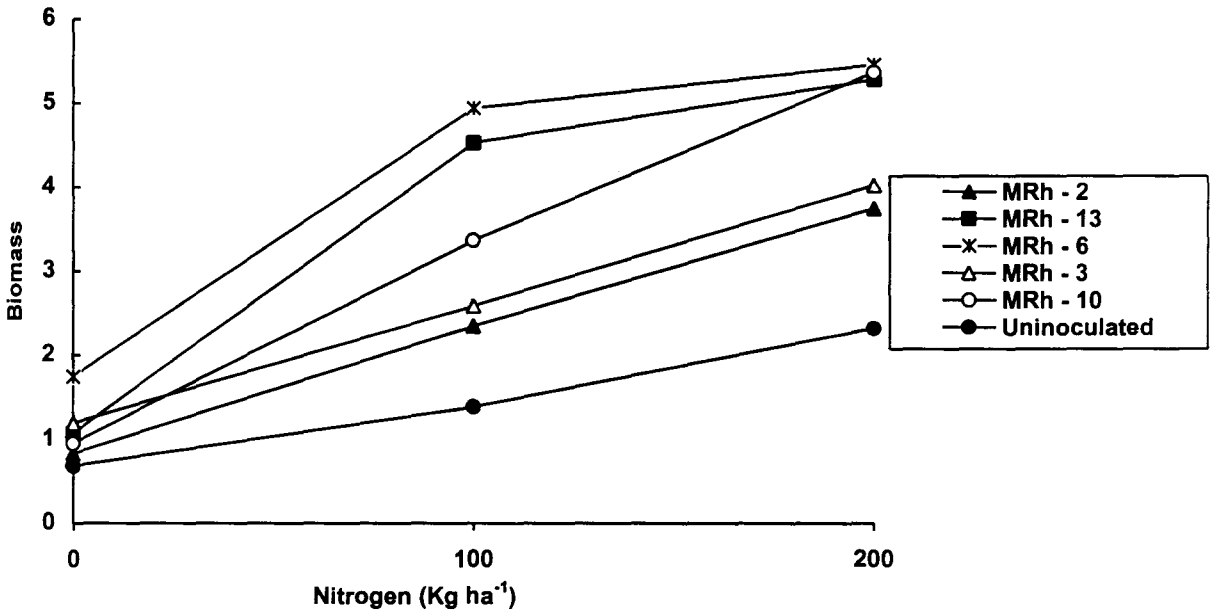

Fig.12: Influence of inoculation of *Rhizobium* isolates and application of N on above ground biomass of *A. mangium* seedlings

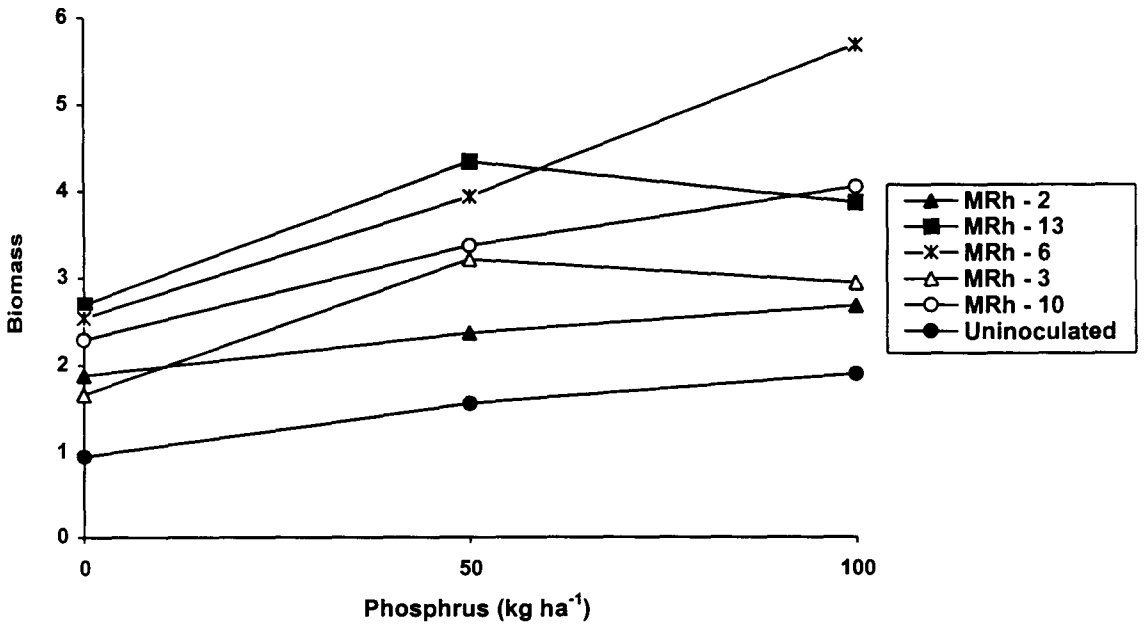

Fig.13: Influence of inoculation of *Rhizobium* isolates and application of P on above ground biomass of *A. mangium* seedlings

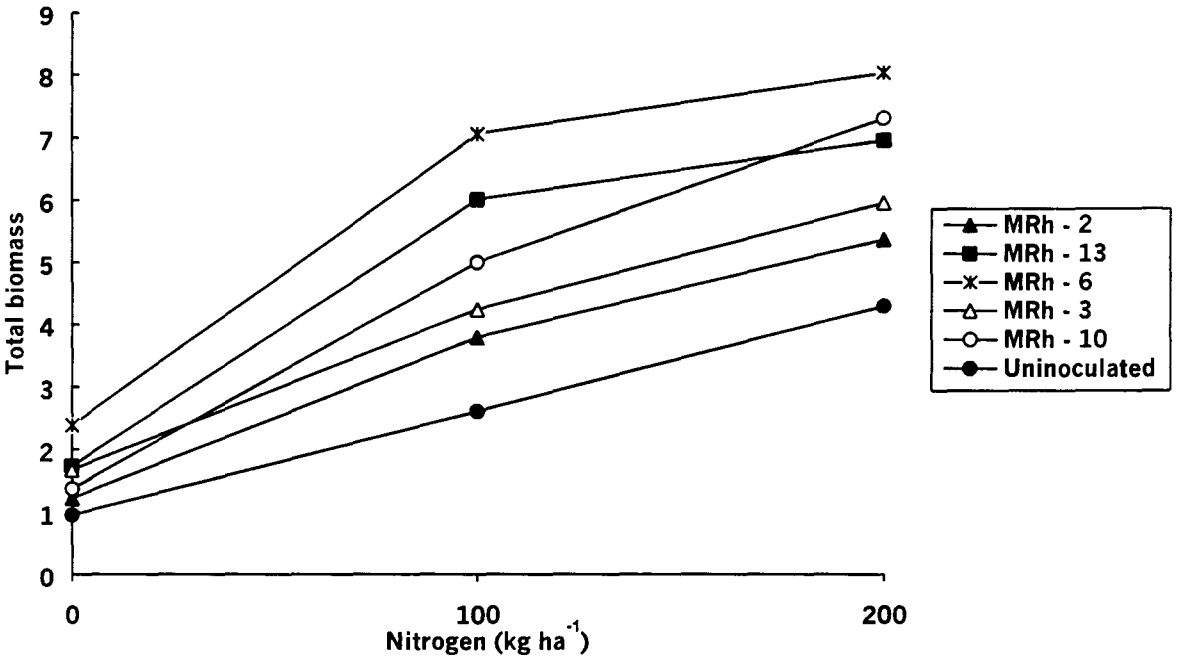

Fig.14: Influence of inoculation of *Rhizobium* isolates and application of N on total biomass of *A. mangium* seedlings

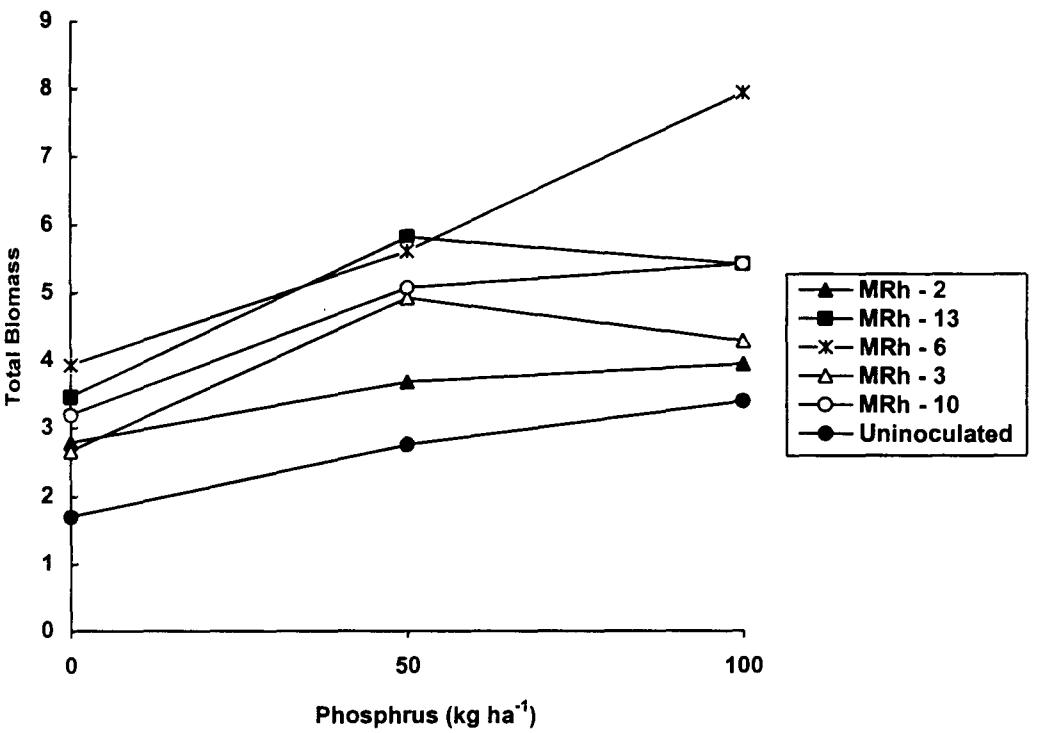

Fig.15: Influence of inoculation of *Rhizobium* isolates and application of P on total biomass in *A. mangium* seedlings

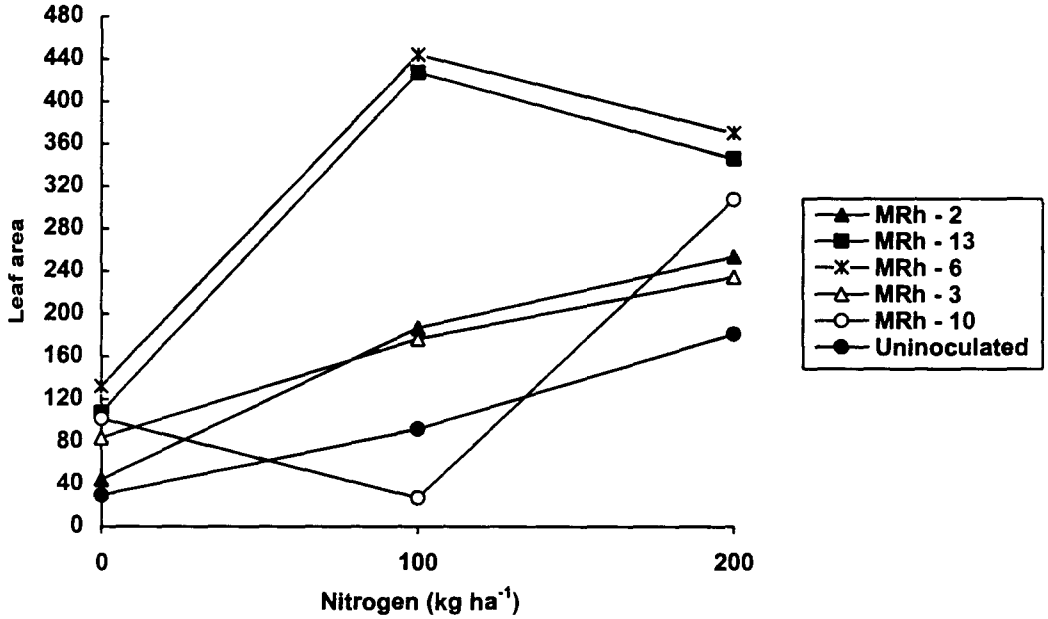

Fig.16: Influence of inoculation of *Rhizobium* isolates and application of N on leaf area of *A. mangium* seedlings

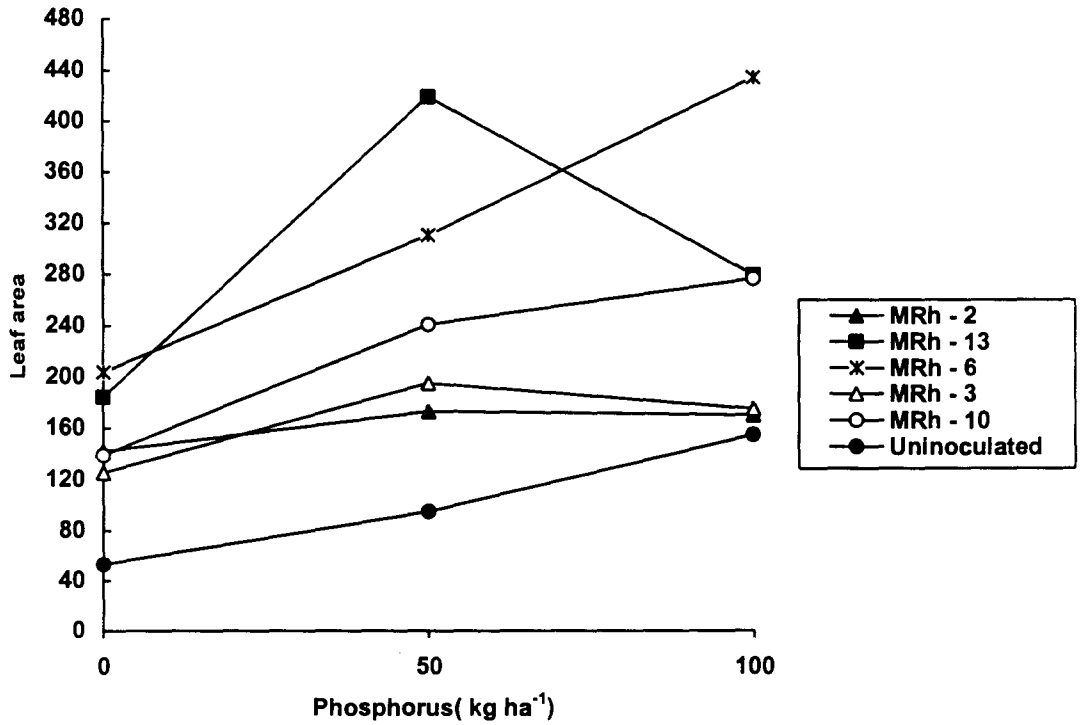

Fig.17: Influence of inoculation of *Rhizobium* isolates and application of P on leaf area of *A. mangium* seedlings

leucocephala best dry matter and total N were observed in two soil types due to rhizobial inoculation combined with P application. Application of P was found to increase the number of nodules per plant, nodule dry weight, shoot height, dry matter of stem and leaves in *Robinia pseudeoacacia* (Reinsvold and Pope, 1987). They recommended that, fertilizer P and N are necessary to increase nodulation and enhance N_2-fixation by *Robinia* on severely disturbed sites.

5.4.4. Plant nitrogen content

The N content of the seedling was maximum when it was inoculated with the isolate MRh-3 and this isolate was superior to all others. The plant N content in the seedlings inoculated with the isolate MRh-2, MRh-13 and MRh-10 were on par but inferior to MRh-6. All these isolates increased the plant N (30 per cent) as compared to uninoculated seedlings. The highest plant N with most of the isolates were observed with relatively low doses of N. When the interactions were considered, the highest plant N content was observed in seedlings inoculated with MRh-13 with a fertilizer level of N_0P_2. The isolates MRh-2, MRh-3 and MRh-10 showed its best influence on plant N content at a fertilizer of level N_0P_1. The isolate MRh-6 showed its best performance with respect to plant N content with a high fertilizer level of N_2P_1. Obviously MRh-2, MRh-13, MRh-3 and MRh-10 may help in best establishment and development of mangium seedlings in low fertility soils whereas the isolate MRh-6 may be successful only in fairly fertile soils or with a supplementary dose of N and P.

5.4.5. Plant nitrogen uptake

The N uptake was maximum in seedlings inoculated with the isolate MRh-6

followed by inoculation of the isolate MRh-13. The N uptake observed in uninoculated seedling were 35 per cent less than the inoculated seedlings. This was much more evident at higher levels of fertilizers. The maximum uptake was in seedlings inoculated with the isolate MRh-6 combined with fertilizer application at the rate of 100 kg N and 100 kg P per hectare. Other isolates showed its best influence at fertilizer levels of 200 kg N and 50 kg P or 200 Kg N and 100 kg P (Table 72). In uninoculated seedlings, the N uptake increased with increasing levels of fertilizers and the highest value was recorded with 200 kg N and 100 kg P per hectare. The influence of rhizobial inoculation in increasing the N uptake of mangium was reported by Cali (1991).

Throughout the investigation it was observed that, the isolate MRh-6 gave promising performance only with higher levels of fertilizers. With lower level of fertilizers, this isolate did not show much influence on the seedling growth. At higher levels of fertilizers MRh-6 recorded maximum collar diameter, height, biomass, leaf area and N uptake. Though the isolate MRh-6 induced maximum plant N content at a fertilizer level of N_2P_1, the plant N level observed with fertilizer level of N_0P_2 were comparable with N_2P_1 level.

Considering the plant N content alone, the N_2-fixing efficiency of the cultures under poor fertility conditions of the soil are rated in the order MRh-13> MRh-10> MRh-2>MRh-3> MRh-6. The high plant N content recorded at higher levels of fertilizers may also be due to the effect of fertilizers rather than due to the effect of inoculation of rhizobial isolates alone. However, at lower levels of fertilizers especially with N_0 level, the increase in plant N content observed must be due to the efficiency of the *Rhizobium* isolates in symbiotic N_2-fixation. Majority of the isolates

of rhizobia were capable of increasing the plant nitrogen content. Since N content of plant was also considered as a criterian for the efficiency of the inoculated strains, these attributes were also given weightage in identifying the best strain.

5.4.6. Soil nitrogen content

The soil N content showed less variation as compared to plant N content. The soil N was maximum in pots inoculated with the isolate MRh-3. All other isolates were on par in this respect. However, in many cases, the inoculated pots showed higher value of soil N as compared to uninoculated pots. The soil N content was highest in pots inoculated with MRh-3 combined with fertilizer application at the level of N_0P_1 and the isolate MRh-6 with the fertilizer level of N_0P_2. The isolate MRh-2 showed highest soil N at a fertilizer level of N_1P_1, MRh-13 at a fertilizer level of N_1P_2 and MRh-10 at a fertilizer level of N_1P_0. In uninoculated pots, maximum soil N was with N_1P_1 level. The soil N in pots inoculated with MRh-10 was on par with uninoculated pots. The other four isolates showed more soil N than uninoculated control at N_0P_1, N_0P_2, N_1P_1 and N_1P_2 levels of fertilizers respectively. Potential nitrogen contributions through N_2-fixation and the death and decay of nodules is high. Sprent (1983) reported that the nitrogen rich roots and nodules of NFTs are expected to add substantial amounts of N when they decay.

Summary

SUMMARY

Investigations were carried out at the College of Forestry, Vellanikkara, Trichur during the period April 1996 to December 1997 to study the nodulation behaviour of *Acacia mangium* Willd. (mangium) and efficiency of the rhizobial isolates from plantations of different agroclimatic regions of Kerala under varying soil fertility and soil acidity conditions. The salient results of the study are summarised here under.

1. Root nodules were observed in mangium plantations throughout the state. The soil of these plantations were acidic with pH values ranging from 4.9 to 6.2.

2. Laboratory studies indicated that all the rhizobial isolates showed variation in growth rate and viable count of bacteria per millilitre in Yeast mannitol broth. Maximum count was observed for the isolates from Mananthavadi (MRh-3) followed by the isolate from Perambra (MRh-2). The isolates from Mananthavadi (MRh-3) and Muvattupuzha (MRh-7) were fast growing (*Rhizobium*) species whereas other isolates were slow growing (*Bradyrhizobium*) spp.

3. Pot culture experiments showed that, inoculated seedlings in unsterilized soil performed well in terms of nodulation and growth characteristics as compared to seedlings grown in sterilized soil.

4. *Rhizobium* isolates from mangium plantations of the state and commercial culture for mangium from Hawaii performed better while the isolates from *A. auriculiformis* were not effective on mangium.

5. Liming and rhizobial inoculation influenced the nodulation and growth

characteristics, and N uptake in mangium. All the inoculated seedlings were superior to uninoculated ones. The isolates performed differently under varying soil pH.

6. Lime adversely affected the number and weight of nodules and effective nodules but improved the seedlings growth characters like collar diameter, shoot height, biomass and leaf area of mangium seedlings inoculated with rhizobial isolates. The commercial culture of mangium, "Agroforester - Group A" responded differently and resulted in significant increase in nodulation and growth characters due to lime application.

7. Application of fertilizer N and P improved the efficiency of inoculation. All the inoculation seedlings were superior to uninoculated ones at all levels of Nitrogen and Phosphorus.

8. Rhizobial inoculation and application of N and P fertilizers significantly improved the number and weight of nodules and effective nodules, growth characters of the seedlings like collar diameter, plant height, biomass, leaf area and N uptake.

9. Among rhizobial isolates, MRh-6 (Vellanikkara) which was a poor performer in unfertilized soil proved to be the best in fertilized soil.

10. MRh-13 (Trivandrum) and MRh-10 (Mavelikkara) performed well in varying levels of soil pH and also showed its superiority in fertilized and unfertilized soils.

Conclusion

In Kerala soils, mangium is nodulated by fast growing (*Rhizobium* spp.) as well as slow growing (*Bradyrhizobium* spp.) rhizobia. The effectiveness of the

rhizobial isolates collected from different agroclimatic regions varied. The 13 isolates from Kerala were adapted to acidic soil conditions, whereas the culture from Hawaii (commercial culture "Agroforester - Group A") was adapted to higher soil pH. Addition of N and P fertilizers may help to improve the nodulation characteristics and seedling growth characteristics of mangium seedlings. Among rhizobial isolates, MRh-6 (Vellanikkara) which was a poor performer in unfertilized soil, proved to be best in fertilized soil. The isolates MRh-10 (Mavelikkara), MRh-13 (Trivandrum) and MRh-3 (Mananthavadi) performed well even when N and P were not applied. So these isolates may be suited to poor fertility soil also. The requirement of a starter dose of N for early establishment and N_2-fixation of mangium seedlings is indicated.

Since inoculation requirement of leguminous trees may vary with site, the strains of symbionts for the mangium need to be selected to suit specific edaphic and climatic factors. Otherwise their impact may not be positive. As effective strains have been selected, it is proposed that, further field trials may be conducted to study the N_2-fixing efficiency of these isolates under varying environmental stress conditions.

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*** Originals not seen**

Appendix

Appendix I

Weather parameters during the study period (April 1996 to December 1997)

Year	Month	Temperature (°C)		Relative humidity (%)	Rainfall (mm)
		Maximum	Minimum		
1996	April	34.6	25.0	73	152.0
1996	May	32.8	25.2	77	95.6
1996	June	30.5	23.8	85	400.3
1996	July	28.8	23.1	90	588.7
1996	August	29.1	23.6	87	310.0
1996	September	29.2	23.7	84	391.6
1996	October	30.1	22.9	82	219.3
1996	November	31.5	23.6	72	23.1
1996	December	30.5	21.8	68	60.8
1997	January	32.0	22.9	62	0
1997	February	33.9	21.8	61	0
1997	March	35.7	24.0	60	0
1997	April	35.2	24.5	67	8.2
1997	May	34.4	24.5	72	63
1997	June	31.2	23.0	82	720.5
1997	July	28.6	21.8	90	979.2
1997	August	29.0	22.8	87	636.8
1997	September	30.6	23.4	82	164.0
1997	October	32.2	23.6	77	194.7
1997	November	31.6	23.2	78	211.3
1997	December	31.7	23.8	72	66.7

NODULATION BEHAVIOUR OF *Acacia mangium* Willd. IN RESPONSE TO *Rhizobium* INOCULATION

By
DHANESHKUMAR, P.

ABSTRACT OF A THESIS

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Kerala Agricultural University

Department of Tree Physiology and Breeding
COLLEGE OF FORESTRY
VELLANIKKARA, THRISSUR
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ABSTRACT

An investigation was carried out during April 1996 to December 1997 at the College of Forestry, Kerala Agricultural University, Vellanikkara to study the nodulation characteristics of *Acacia mangium* Willd. plantation and to isolate and compare the efficiency of rhizobia from different agroclimatic regions of Kerala. The efficiency of these rhizobial isolates under varying conditions of soil fertility and acidity were also evaluated.

Survey conducted throughout the state showed rhizobial association and nodulation in all mangium plantations. The soil of these plantations were acidic with pH values ranging from 4.9 to 6.2.

Experiments conducted to study the rhizobial characteristics indicated that, the fast growers (*Rhizobium* spp.) as well as the slow growers (*Bradyrhizobium* spp.) are involved in nodulation of *A. mangium* in Kerala.

The efficiencies of different rhizobial isolates were studied in polypots using sterilized and unsterilized soil - sand mixture. Two other polypot experiments were conducted to study the response of the rhizboial isolates to liming and N and P application. The experiments were laid out in C.R.D with three replications. Seedlings raised in unsterilized soil performed better as compared to seedlings in sterilized soil. However, the response to inoculation was more prominent in sterilized soil. In sterilized soil, seedlings inoculated with *Rhizobium* isolates from mangium ;MRh-3 (Mananthavadi), MRh-2(Perambra), MRh-13 (Trivandrum), MRh-6 (Vellanikkara) and MRh-10 (Mavelikkara) and commercial mangium culture ("Agroforester - Group A") showed superiority in nodulation and growth characteristics while the isolates from *A. auriculiformis* and commercial culture of *A. auriculiformis*, ("Agroforester - Group C") were not much effective.

Seedling - response to inoculation varied with rhizobial isolates, and mangium showed its specificity in *Rhiozbium* requirement for nitrogen fixation.

The application of lime adversely affected nodulation capacity of the rhizobial isolates of mangium from Kerala, indicating the adaptations of these isolates to acidic soils. The nodulation efficiency of commercial culture "Agroforester - Group A" was improved by lime application, indicating that this strain is adapted to soils of higher pH.

The nodulation and nitrogen fixing efficiency of the isolates were improved by supplementating fertilized N and P. The need for a starter dose of N and P for effective nodulation and efficient N_2-fixation in mangium seedlings. MRh-6 (Vellanikkara) performed poorly in unfertilized soil, but showed superiority when applied with N and P fertilizers, indicating the sensitivity of this isolate to site conditions. As the rhizobial isolates from different agroclimatic regions of Kerala responded differently under varying soil pH and doses of N and P, inoculation of selected strains of rhizobia depending on site conditions may be needed for successful establishment of mangium seedlings.