Effect of Rhizobium Biofertilizer On The Growth of Selected Species of Tree Legumes

By

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A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE-641 043, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

MAY 1992

Acknowledgement

ACKNOWLEDGEMENT

The author wishes to express her profound sense of gratitude and heartfelt thanks to **Dr. (Mrs.) Nirmala K.Murthy**, B.Sc. (Hons.), M.S.(Iowa), Ph.D. (Madras), Dean Faculty of Science, Department of Biochemistry, Avinashilingam Institute of Home Science and Higher Education for women (Deemed University), Coimbatore for her keen interest, valuable guidance, timely encouragement, advice and suggestions rendered throughout the study.

The author records her immense sense of gratitude and sincere thanks to **Mrs. K.Usha**, Assistant Professor, Department of Biochemistry, Avinashilingam Institute of Home Science and Higher Education for women (Deemed University), Coimbatore for her kind help, constructive suggestions and able guidance throughout the study.

The author wishes to thank **Dr. (Mrs.) Rajammal P.Devadas**, M.A., M.Sc., Ph.D. (Ohio State), D.Sc. (Madras), Vice Chancellor, Avinashilingam Institute of Home Science and Higher Education for women (Deemed University), Coimbatore for the opportunity given.

The author wishes to place a special mention about the spontaneous and enthusiastic remarks and comments as well as the practical guidance rendered by **Dr.S.Kannaiyan**, M.Sc., (Ag.), Ph.D., Professor of Biotechnology, Tamil Nadu Agricultural University, Coimbatore.

The author wishes to record her deep sense of appreciation and heartfelt thanks, with esteemed reverence to retired Professor **N.S.Subba Rao**, Bangalore for his enthusiastic guidance and help that helped to improvise this study.

The author places her profound sense of gratitude and appreciation to **Miss.P. Vijayachamundeswari** and **Mrs.Sudha Srinivasan** for their timely help and valuable suggestion.

It is with pleasure the author wishes to thank all the staff members of the Department of Biochemistry, Avinashilingam Institute of Home Science and Higher Education for woman, Coimbatore for their encouraging words and guidance given whenever approached.

It is with a deep sense of gratitude that the author wishes to acknowledge **Mr.S. Duraisamy, Mr.N. Bhagyanathan** and **Ms.Radha** of THATHA clapce, Coimbatore - 3 for helping out with the printing.

The author wishes to thank all her fellow students who were all the time ready to sustain her with their cheerful disposition.

The author wishes to take this as an achievement in life solely motivated by her **parents, sisters** and all whom she loves in this world.

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Introduction

I. INTRODUCTION

The persistence of life on this planet depends on the recycling of biological elements. The biological cycles of carbon, oxygen, nitrogen, sulfur and other elements are fundamental to terrestrial and aquatic biology and the role of microbes in these cycles is crucial (Subba Rao, 1986).

Biological nitrogen fixation has emerged as an important component of ecosystems on this planet. In virgin forests, which are in ecological balance biological fixation provides an adequate input of nitrogen and other nutrients - phosphorus, sulfur, sometimes potassium or molybdenum - to the flora and fauna (Lawrie, 1986).

Perturbation of a balanced ecosystem whether by fire, vulcanism, inundation, drought or simple cultivation, renews or recycles all the biological elements.

In consequence fixed nitrogen frequently becomes limiting and hence biological nitrogen fixation becomes important in such a situation (Sprent and Raven, 1990).

Non-biological processes make a contribution; for example, nitrogen oxides can be formed in the atmosphere as a result of lightning flashes or combustion and become washed into the soil as nitrates. Man - made fertilizer also makes a significant contribution (Downie and Johnston, 1986).

The atmosphere contains 3.9 X 10^{15} tonnes of nitrogen. The biosphere receives annually 175 million tonnes of nitrogen by biological fixation. Thus the biological process is pre-eminent on a global scale (Sprent *et al.,* 1987).

Chemical fertilizer production consumes energy, now increasingly expensive and requires sophisticated industry. its use entrains run-off problems. Eutrophication of waters may then occur with attendant pollution problems, high nitrate levels in drinking water can actually be toxic. Biological nitrogen fixation is, thus, of great practical importance today, as well as for the future (Indrani *et al.*, 1988).

Escalating costs of production and transport of fertilizer, both in monetary and energy terms make exploitation of biological processes increasingly attractive and probably obligatory in the next century (Rai and Sinha, 1986).

While the world's population is increasing by about 80 million people per year the world's tropical forests are disappearing at an alarming rate. Deforestation is taking place at a rate of approximately 18-20 million hectares per year (Swaminathan, 1983).

All the loss of forest area is occuring in the tropics with very little change in closed forest area in the temperate zone. Loss of these forest resources in the developing world is a direct consequence of increasing population pressure and lack of economic alternatives for millions of the poor (Tewari, 1991).

The principal cause of tropical forest loss are the conversion and use of forest lands for agriculture, for fuel wood and for industrial logging (Kushalapa, 1991).

For a variety of reasons, wood is still the principal source of energy in rural areas and used for cooking and heating. However the demand for firewood has outstripped the natural regeneration and new planting so much so that in same areas though there is food to eat, not enough wood is available to cook the same (Chaturvedi, 1991).

The indiscrimate felling of valuable timber trees resulting in the denudation of forests is posing a serious threat not only to the environment but also to the very survival of humanity. The unrestricted removal of green cover also leads to the imbalance in soil ecosystems as floods and soil erosion may result in the loss of valuable top soil and siltation of rivers and dams which may further contribute to the change in the microclimate (Mukerji, 1991).

In a calculation made by a fuel wood committee of the Government of India made in 1982, it was concluded that a minimum of 3 million hectares of land will have to be planted under quick yielding fuel wood trees each year upto 2000 A.D. to meet the growing needs (Swaminathan, 1984).

Last decade there has been a rapid period of expansion of tree planting to meet industrial, rural community needs and support agriculture systems in agroforestry conditions, to revegatate degraded lands and protect areas from soil erosion and deforestation (Mitchell, 1985).

Legumes are the forefront of this new phase of tree planting, leguminous flora are being selected and advocated for meeting the stupendous demand of fodder, fuel and timber wood. Several international bodies, for example, Food and Agricultural Organisation (FAO), National Academy of Sciences (NAS) are actively promoting the use of woody legumes (Wilson *et al.*, 1986).

Nitrogen fixing plants can provide a useful and versatile tool for improving and maintaining forest productivity. Cropping systems under various afforestation programmes involving there plants will influence on the various productivity factors as well as enrich soil nitrogen status (Pokhriyal *et al.*, 1990).

To reaffirm the importance of trees and forests in everyday life, the Food and Agriculture Organisation of the United Nations (FAO) selected the caption "Trees for life" as the theme for World Food Day, 1991.

The only way by which effective reforestation can be achieved is by planting of all waste lands with fast growing nitrogen fixing trees which can provide the people with food, fodder, fertilizer, fuel and fibre. Forest genetic resources consisting leguminous and certain non-leguminous trees enrich soil fertility through biological nitrogen fixation (Abrol and Gill, 1985).

Biological nitrogen fixation is conducted principally by microbes usually in association with the roots of plants (Kondorosi and Kondorosi, 1986).

The recent return of interest and intensification of research into biological nitrogen fixation systems as alternatives to chemically produced fertilizer nitrogen

has focussed considerable attention on the legume-**Rhizobium** symbiosis (Young and Johnston, 1989).

In those countries where nitrogen fertilizer supplies and technological development are limited, a greater demand is being made on alternative and inexpensive sources of nitrogen and many laboratories have turned to nodulate legumes to meet this need (Sprent, 1986).

Nitrogen supply by the nodulated legume is dependent on nodules being formed by a strain of **Rhizobium** effective in nitrogen fixation in that host plant (Rao, 1992).

The nitrogen fixed by forage legumes is important not only for their own growth but also as a source of combined nitrogen for grasses and non-legume crops (Cable *et al.*, 1984).

Man has manipulated the environment to favour the growth and nitrogen fixation to forage legumes by inoculation of seed; mineral fertilization; irrigation; management of grazing and cutting; control of pests; diseases and weeds; and by the breeding and selection of superior genotypes (Henzell, 1982).

The ability of rhizobia to fix nitrogen in symbiosis with legume plants is of great economical interest (Streit *et al.*, 1991).

In tropical countries, rapid increases in population has put a tremendous strain on natural resources resulting in nitrogen depleted soil. Nitrogen fixing trees play an important role in land reclamation and soil enrichment. One such tree species that has attracted attention is **Leucaena leucocephala** (Lam.) de Wit. It is known to be the best forest tree for its quick growth and multiple uses (Punj and Gupta, 1988).

The rational use of nitrogen fixing symbiosis particularly in the context of energy conservation and increased demand for wood biomass on the forest ecosystem is an important aspect, deserves detailed studies on priority (Pokhriyal *et al.,* 1987).

It is especially attractive for legume crops from which sustained productivity is sought from the low input farming system in the tropics and subtropics by virtue of their ability to fix nitrogen through their symbiotic association with micro organisms such as rhizobia (Basu and Kabi, 1987).

Therefore the present investigation on the biological nitrogen fixation of certain leguminous trees is carried out with the following objectives :

1. Isolation, authentication and characterisation of rhizobial strains from root nodules of certain commonly occuring tree legumes.

2. Seed treatment with **Rhizobium** and its effect on nodulation.

3. Effect of **Rhizobium** inoculation on

    a. Plant root & shoot length.

    b. Plant biomass.

4. Effect of **Rhizobium** inoculation on

    a. Total Nitrogen content.

    b. Phosphorus content .

    c. Potassium content.

    d. Chlorophyll content.

    e. Amino nitrogen content.

5. Studies on the effect of fungicide (Bavistin) and **Rhizobium** inoculation on germination and seedling growth.

Review of Literature

II. REVIEW OF LITERATURE

The review of literature pertaining to the study "Effect of **Rhizobium** biofertilizer on the growth of selected species of tree legumes" is discussed under the following headings:

1. Introduction.
2. Biological Nitrogen fixation.
3. Enhancement of soil fertility by biofertilizers.
4. Disadvantages of chemical fertilizers.
5. **Rhizobium** Legume symbiosis.
6. **Rhizobium** biofertilizer and tree legumes.
7. Nodulation status of tree legumes.
8. Nitrogen fixing potential of tree legume rhizobia.
9. Role of tree legumes in ecodevelopment.
10. Fungicides and their effect on Rhizobial interaction.

INTRODUCTION

Millions of people around the world depend on trees and forests to help meet their basic needs for food and shelter.

Forests are useful to man in two distinct ways, as producers of a wide variety of goods commonly called "forest produce" and as custodians of favourable environmental conditions (Subba Rao, 1982).

The progressive disappearance of forests has of late become a matter of grave concern not only to environmentalists but also to the whole of humanity. The removal of tree cover from vast areas of land has resulted in an increasing frequency of floods all over the country, accelerated soil erosion and contributed to changes in the microclimate as well (Swaminathan, 1983).

The increasing deforestation is due to the steady population growth, increasing pressure on land and increasing demand for forest products especially for firewood. Trees are cut down only to be replaced by acres and acres of cement and concrete structures (Subba Rao, 1988).

Deforestation in the tropics has increased in recent years to become one of the most significant problems in world forestry (Hariharran, 1986).

Fast growing nitrogen fixing trees cover the opened up areas, establish reforestation and preserve the ecosystem. Agrotechnology based on quick yielding nitrogen fixing trees is highly remunerative since most legumes in the tropics fix 100-350 kg N/ha/year thus providing mineral nutrition to the flora and fauna (Jayaraman *et al.*, 1985).

In order to improve the nitrogen status of the soil and to support the required area of green cover, it is necessary to exploit the biological nitrogen fixation associated with tree legumes.

BIOLOGICAL NITROGEN FIXATION

The biological cycles of carbon, sulfur, nitrogen and other elements sustain the biosphere of this planet is a steady state with respect to the chemosphere (Postgate, 1982).

The transfer of nitrogen from the atmosphere to the biosphere takes place by nitrogen fixation. There are three major ways in which nitrogen is fixed:

1. Spontaneous fixation in the atmosphere from electrical discharge by lightning and combustion.
2. Industrial fixation by the Haber-Bosch process in nitrogen fertilizer production.
3. Biological fixation by microorganisms (Anon, 1981)

The global situation with reference to sources of nitrogen for crop production lies as 42 million metric tonnes and are supplied through biological fixation, signifying the potential for biological sources (Subba Rao, 1979).

Among the various sources of biologically fixed nitrogen, symbiotic legume-**Rhizobium** associates constitutes one of the major source.

ENHANCEMENT OF SOIL FERTILITY BY BIOFERTILIZERS :

Biofertilizers harness atmospheric nitrogen with the help of specialised soil microorganism. Such nitrogen fixing microorganism are either free living in soil or

have symbiotic associations with plants and directly or indirectly contribute toward nitrogen nutrition of plants (Biswas *et al.*, 1985).

Biofertilizers include **Rhizobia,** the symbiotic nitrogen fixing bacteria, *Azolla Anabaena* symbionts, the free lining organism like *Azospirillum, Azotobacter* and *Beijerinckia* (Tu and Miles, 1976).

Biofertilizers play a valuable role in utilizing the native sources of nutrients such as nitrogen, phosphorus, potassium for economical and better crop production (Rai and Sinha, 1986).

DISADVANTAGES OF CHEMICAL FERTILIZERS :

The present energy crisis has resulted in the escalating cost of chemical fertilizer (Dreyfus *et al.*, 1984).

Repeated and large scale use of chemical fertilizers lead to deterioration of soil quality. High sulfate ion concentration in soil by using ammonium sulfate gives more acidity and nitrates are left by potassium or sodium nitrate. This nitrate is washed off by rain into rivers and drinking water reserves thereby causing public health hazard (Satyaprakash Singh, 1987).

In the area of chemical fertilizers no major break through is yet visible to minimize the energy requirements of the conventional Haber-Bosch process for the production of ammonia. Installing fertilizer plants are not only expensive but also time-consuming (Rodgers *et al.*, 1979).

Also the rising cost of fossil fuels required or the Haber-Bosch process and environmental pollution problem caused by leaching of artificial fertilizer from soil have resulted in renewed interest in biological nitrogen fixation process (Rao and Venkateswaralu, 1987).

RHIZOBIUM-LEGUME SYMBIOSIS :

The activities of nitrogen fixing bacteria were harnessed for human benefit centuries before the discovery of either the bacteria or the nitrogen fixing process (Brill, 1977).

The **Rhizobium**-legume symbiosis is one of the best known symbiotic associations.

The **Rhizobium**-legume symbiosis was estimated to contribute annually 35 x 10^6 tonnes of nitrogen in different ecosystem (Vance and Johnson, 1981).

The contribution of the *Azolla-Anabaena* symbionts, free-living bacteria of the genera *Azotobacter, Beijerinckia*, the blue green algae and *Azospirillum* of nitrogen to soil through non-symbiotic process was found to be lesser than that of **Rhizobium**-legume symbiosis (Nambiar *et al.*, 1984).

Rhizobia are gram negative rods. Their classification is based on the cross-inoculation grouping which lies in ability of an isolate to form nodules on the roots of limited species of legummes which are related to one another (Vincent, 1982).

The symbiotic relationship between **Rhizobium** and legumes was found to be of great importance from agricultural productivity point of view (Danse *et al.*, 1987).

Symbiotic process is especially attractive for leguminous crops where sustained productivity is sought from the low input farming system in the tropics and sub-tropics because of their unusual ability to be self-sufficient for nitrogen supply (Halliday, 1982).

Often inoculation of legume seeds with **rhizobia** increases yield if the used inoculant strains are highly competitive in nodulation (Theis *et al.*, 1991).

The renowned Dutch Microbiologist Beijerinck (1888) was the first who recognized the bacterium causing root nodules in legumes and demonstrated that purified bacteria formed nodules in plants grown from sterilised seeds. Inoculation of legume seeds with the appropriate culture of **Rhizobium** was originally introduced as a means of ensuring the establishment of seedlings in nitrogen deficient soils, which lacked adequate population of nodulating bacteria (Bergerson, 1971).

It has been conceded that each of the partners - **Rhizobium** and legume is unable by itself to fix substantial amounts of nitrogen and the effective nitrogen

fixation is the phenotypic expression of the two associated genomes (Barnet *et al.*, 1985).

The capacity of certain legume-**Rhizobium** association to fix nitrogen under optimal conditions could be defined as 'potential nitrogen fixation' as opposed to 'actual nitrogen fixation' (Kremer & Peterson, 1982).

Symbiotic nitrogen fixation is profoundly influenced by the efficiency of rhizobial strain, appropriate host and environment. The delicate balance between the host plant and the micro-symbiont is easily disturbed by the environment stress (Philpolts, 1976).

Rhizobia inoculated on seeds of legumes were often exposed to adverse environment condition in the field which affected their survival and effectiveness. Different types of carriers were recommended for the survival of **rhizobia** (Somasegaram, 1985).

Currier and Strobel (1976) stated that **Rhizobium** species shared chemotaxis to plant-root exudates of both leguminous and non-leguminous plants.

The technology for establishing effective nitrogen fixing symbiosis between legume and rhizobia involved application of **Rhizobium** either to the legume seed coat prior to planting or directly into the soil in which the legume will be sown (Halliday, 1980).

RHIZOBIUM BIOFERTILIZER AND TREE LEGUMES:

Dreyfus and Dommergues (1981) studied 13 tree legume species belonging to Acacia which were classified into 3 groups. The first group nodulated effectively with slow growing cowpea type rhizobial strains, the second group nodulated with tropical fast growing strains and the third with both fast and slow growing strains.

Domingo (1983) and Quinones (1983) gave an account of the tree species which were found to contain **Rhizobium** in their roof nodules. The nodulated trees which were recorded in Philippines included *Instiga bituga* (Colab.) O. Ktze, **Leucaena leucocephala** (Lam.) de. Wit., *Cassia fistula* L., *Albizia procera*.

Pancholy (1991) studied the status of indigenous soil rhizobia associated with certain arid zone legume trees. In unsupplemented soil, seedlings of *Prosopis cineraria* and *Acacia tortilis* showed onset of nodulation at one month. Beyond three months nodulation was reduced.

Galliana (1990) established that plant nitrogen fixing ability depends on the **Rhizobium** strain.

Kucey and Hynes (1989) showed that once established in a soil the inoculated bacteria are subject to the same limitations as the rest of the soil microbial populations, increasing and decreasing in numbers in response to thier environment.

Factors affecting growth and survival of both host plants and rhizobia are involved. Some of the factors such as soil pH, water supply, mineral nutrition and temperature also apply to tree species (Stacey, 1990).

During the first hours after inoculation of legume seeds or seedlings, rhizobia are exposed to several stress factors including low pH, Manganese or Aluminium toxicity, drought and high temperature (Dowling *et al.*, 1986).

These factors affect growth and survival of **Rhizobium** and have been cited frequently for the failure of inoculation experiments (MacDermot *et al.*, 1990).

A single rhizobial strain may be able to infect tree saplings from different subfamilies. Host specificity varies widely (Young and Johnston, 1989).

Only little is known about competition for limiting nutrients either in the tree soil or in the rhizosphere. Inoculum strains have to compete for nutrients with other soil bacteria and indigenous rhizobia (Lowendorf, 1980).

Singleton and Tavares (1986) investigated the effects of rhizobial inoculation on the growth and nodulation of some important forest legumes. They concluded that the use of biofertilizers in forestry has a tremendous potentiality of nitrogen turnover from the atmosphere into the biosphere.

NODULATION STATUS OF TREE LEGUMES :

The legume-rhizobial symbiosis has been mostly studied with agricultural legumes of the temperate region. The leguminosae, one of the largest families in the plant kingdom, Comprises of over 550 genera and 13000 species. Of these 1200 (10%

of all the leguminous species) have been examined for nodulation and not all of them nodulate (Allen, 1981).

Norris (1973) found that rhizobia of **Leucaena** could be affected by acidity.

Diatloff (1973) showed that uninoculated Leucaena failed to nodulate and produce good growth. The simplest and cheapest way of ensuing maximum biomass producing ability was to inoculate the seed at planting with suitable rhizobia.

Basak and Goyal (1975) isolated and characterised the rhizobial strains from tree legumes such as *Acacia senegal* willd., *Acacia tortilis* lgm. etc. and reported that the rhizobia from these legumes were akin to those of cowpea group in their properties.

Lim & Ng (1977) found 33 species of tree legumes out of 35 to have nodules. The nodulated species largely belonging to Mimosoideae and Papilionoideae. Their report on nodulation of **Delonix regia** of the Caesalpinoideae constituted the first observation of nodulation in this species. However the nodules in **Delonix regia** were found only in young seedlings and not on matured tree roots.

Prasad and Dadwal (1984) studied the nodulation behaviour in different groups of **Leucaena leucocephala**. Inoculation could boost up the growth of nursery stock of **Leucaena leucocephala** by producing a healthy stock of high vigour for large scale plantation in silvipastoral system and social forestry programmes.

Sankpal and Konde (1985) studied the nodulation in **Leucaena leucocephala** and demonstrated that single strain inoculation which is recommended in order to a failure of a single strain.

Sufeng and Xiangyuan (1991) studied the possibility of inducing nodulation and nitrogen fixation in non-nodulated free legumes involving the use of 2,4-dichlor -phenoxy acetate or enzymic treatment.

Yanfu (1983) reported the induction of nodule like structures on wheat roots by treating wheat seedlings with 2,4-D together with rhizobium species.

The formation of nodular structures has been reported on rice plants and oil seed rape using enzymic treatment (Al Mallah *et al.*, 1989, 1990).

Sufeng and Xiangyuan (1991) examined nearly 20 species of tree legumes which belong to the enera *Cassia, Delonix, Gleditsa* for nodulation.

Sufeng and Xiangyuan (1984) reported that even given artificial inoculation with mixed rhizobial pure culture obtained from nine different species of nodulated tree legumes, all the Caesalpiniaceous species failed to nodulate.

Ducousso *et al* (1991) studied the occurence of nodulation among woody legume species they examined only one *Erythrophlem guinere* was found to bear nodules while 90% of Mimoisaceae were nodulated.

Lal and Khanna (1991) studied the survivability and renodulation potential of **Rhizobium** of *Acacia nilotica*. They showed that the plants inoculated with **Rhizobium** showed a 100% increase in dry matter over uninoculated control.

The studies of Li and Alexander (1986) indicate that successful nodulation by **Rhizobium** strains is affected by competition for nutrients in the rhizosphere and also at greater distances from the root.

The decomposition of root nodules of leguminous plants is believed to be an important potential source of mineral nitrogen release from nodulated plants (Whitney and Kanchiro 1976).

Nodules of all living legumes demonstrate high turnover and this appears to be accompanied by a rapid release of mineral nitrogen.

Pokhriyal *et al* (1990) carried out studies to screen 27 different leguminous fast growing tree species for nodule formation and nitrogen fixing activity.

NITROGEN FIXING POTENTIAL OF THE TREE LEGUME RHIZOBIA :

Hoeberg and Kvanstroem (1982) estimated the nitrogen fixing rate a four year old stand of the woody legume **Leucaeua leucocephala** to be in the order 110 + 30 kg/ha.

Barnet *et al* (1985) observed biological nitrogen fixation in two sand dune regions planted with Acacia species used in revegetation programmes. District seasonal variations were observed with higher activities of nitrogen fixation in the cooler months. The legume **Rhizobium** associations was the only sources of biologically fixed nitrogen.

Saginga *et al* (1986) reported that inoculation of **Leucaena leucocephala** with **Rhizobium** increased total nitrogen and dry matter of Leucaena that forest legumes and reported that inoculation with Rhizobium resulted in better nodulation over the uninoculated controls.

Sprent, J. R. & Sutherland (1991) studied nitrogen fixing woodly legumes in general.

ROLE OF TREE LEGUMES IN ECODEVELOPMENT :

Dobereiner and Campelo (1977) stressed the importance of legumes available in all 3 subfamilies of leguminosae for reforestation over other tree species such as Pinus, Eucalyptus etc in assisting the recuperation of eroded land and stressed the importance of inoculation of these tree species, especially in Mimioisoideae which represented a district cross inoculation group.

Tree species belonging to the subfamily Mimoisoideae-**Leucaena leucocephala,** *Prosopis Juliflora*, the caesal pinoideae - *Delonix regia, Cassia grandis*

were most beneficial in reforestation programmes and **Leucaena leucocephala** was assessed to be as a pioneer forest tree (Halliday & Somasegaran, 1982).

Basak and Goyal (1980) recommended the planting of tree legumes in erecting 'shelter belts' and 'wind breakers' in desert areas in containing the spreading deserts.

Sharifi *et al* (1982) studied the biomass and net primary production of *Prosopis grandulosa* a woody tree legume in desert conditions over other communities. The high above ground productivity of *Prosopis* in low rainfall conditions was found to be most suitable for its cultivation in marginal agricultural areas with low surface water resources.

FUNGICIDES AND THEIR EFFECT ON RHIZOBIAL INTERACTION :

Fungicides are used to protect the seeds from seed borne pathogens.

It is well known that crop production is adversely affected by plant diseases and pests and chemical treatment of both plant and soil controls diseases and pests to a great extent. Primary reliance in the control of plant diseases has been placed on fungicides since they play a major role in assuring production of crops (Vyas, 1983).

Bavistin, a broad spectum fungicide is a commercially used one, quite beneficially for legumes. This is chemically labelled as Methyl-2-benzimidazole carbamate.

When legume inoculants are used in conjuction with insecticides, pesticides, fungicides, weedicides, they exert a deleterious effect on the nitrogen-fixing organism.

Borges *et al.*, (1990) stated that the evermore intensive use of fungicides, herbicides and insecticides in soybean cultivation can affect **Bradyrhizobium japonicum** and thus the symbiosis.

Tu (1981) verified a decrease in nodulation and nitrogen fixation and in plant growth when pesticides Thiram Lindane or Diazinon were used for coating soybean seeds inoculated with **Bradyrhizobium japonicum.**

When seeds were treated with Penta chloro nitro benzene (PCNB) and inoculated with the bacterium, a reduction of 6% in cell viability was observed (Curley and Burton, 1975).

Several herbicides decrease the number, the size and dry weight of nodules in soybean plants (Gaur, 1980; Dunningan *et al.*, 1972).

Experimental Procedure

III. EXPERIMENTAL PROCEDURE

The present study evaluated the efficiency of nitrogen fixation in **Rhizobium** inoculated tree legume species - **Delonix regia** and **Leucaena leucocephala.**

COLLECTION OF NODULES AND ISOLATES OF *RHIZOBIUM* SPECIES FROM THE ROOTS OF *LEUCAENA LEUCOCEPHALA* :

Nodules of **Leucaena leucocephala** (Lam.) De wit., were colleted from the plants grown in Forestry Research Station, Mettupalayam and from various locations in and around Coimbatore. Large and pink coloured nodules were chosen for isolation since they were found to contain effective Rhizobial strains. The nodules were washed thoroughly in tap water to remove the adhering soil particles. The nodules were then exposed momentarily to 95 per cent ethonol and immersed in 0.1 per cent acidified mercurric chloride solution for 3 minutes (Mercurric chloride 0.1 g, concentrated hydrochloric acid, 0.5 ml and distilled water 100 ml).

These surface sterilized nodules were then washed in six changes of sterile distilled water. The nodules were transferred to sterile glass tubes containing 1-2ml of sterile distilled water and crushed with the blunt end of a sterilized glass rod. The milky fluid from the nodules was streaked on yeast extract mannitol agar incorporated with Congo red in sterile petri plates after which they were incubated at 28^{o}C for 4-8 days, for the development of colonies. The colonies which appeared transluscent, watery or whitishly opaque, without absorbing the Congo red were

picked out and transferred to yeast extract mannitol agar slants. Once they achieved good growth, the tubes were stored in a refrigerator until further use.

Composition of yeast extract mannitol agar (YEMA) medium (Allen, 1957)

Mannitol	10.0 g
Calcium carbonate	3.0 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Yeast extract	0.5 g
Agar	15.0 g
Distilled water	1000 ml
pH	6.8 - 7.0

Culturing of appropriate isolates of *Rhizobium* species.

Rhizobium isolates initially maintained in congored yeast extract mannitol agar slants were further multiplied in malt extract broth for inoculation purpose. The composition of the broth is given below :

Malt extract	10.0 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Yeast extract	1.0 g
Distilled water	1000 ml
pH	6.8

AUTHENTICATION AND CHARACTERISATION OF ROOT NODULE BACTERIA

Authentication of the rhizobial isolates by plant infection test in growth tubes (Somasegaran *et al.*, 1982).

The isolates were tested for their infectivity in growth tubes. For this large-sized glass tubes (300 x 40 mm) were made use of. Nitrogen free nutrient solution was taken in each tube to a height of 20 mm. Germination paper was inserted into each tube, the month plugged with cotton and sterilized. Cowpea (*Vigna unguiculata* (L.) *Walp*) var. Co.4 seeds were surface sterilized by treating them with 90% ethanol for 3 minutes, washed 5-6 times with sterile distilled water and then transfered to petri plates having 1% agar (W/V) and incubated overnight. The sproughted seeds were picked up using sterile forceps and placed in the holes made in the germination paper and the rhizobial suspension prepared out of the isolate was added (1ml containing 1 x 10^9 cells/ml per seed) and kept for observation. Those isolates which produced nodules were selected and used for characterisation and further studies.

CHARACTERISATION OF RHIZOBIAL ISOLATES.

The identity of the **Rhizobium** was established by performing the following tests :

i) Growth in Congo red yeast extract mannitol agar.

ii) Growth in Hofer's alkaline broth

iii) Growth in Lactose agar

iv) Growth in Peptone glucose agar

v) Reaction in litmus milk.

Growth in congored yeast extract mannitol agar

One ml of the appropriate dilution of rhizobial isolate was transferred to sterilised petri dishes and the Congo red yeast extract mannitol agar medium was added and mixed by rotating clockwise and anti-clockwise. The agar was allowed to solidify and the dishes were inverted and incubated at 26-28°C. Growth was observed upt 4-5 days for fast growing rhizobia and upto 10 days for slow growing rhizobia.

It was found that in congored incorporated agar medium, rhizobia developed as white transluscent, glistening elevated small colonies.

Congo red yeast extract mannitol agar medium

Mannitol	10.0 g
Calcium carbonate	3.0 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Yeast extract	0.4 g
Agar	15.0 g
Distilled water	1000 ml

pH	7.0
Congo red	0.025 g/1

Growth in Hofer's alkaline broth :

One ml of the appropriate cultures isolated from seven forage legumes, grown in malt extract broth was transferred into a 250 ml flask containing 100 ml of Hofer's alkaline broth. The flasks were then incubated at room temperature in a rotary shaker. Absence of growth in this medium indicated that the cultures were of rhizobia. The composition of the broth is given below :

Hofer's alkaline broth (Hofer, 1935)

Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Calcium carbonate	0.05 g
Yeast extract	1.0 g
Mannitol	10.0 g
Distilled water	1000 ml

(pH adjusted to 11.00 by adding approximately 28 ml of 1 N NaOH and 1 ml of 1.6 per cent thymol blue).

Growth in Lactose agar (Bernaerts and DeLey, 1963)

One ml of the appropriate cultures isolated from seven forage legumes was transferred into sterile petri dishes. Fifteen ml of liquified and cooled lactose agar medium which consisted of 1.0 per cent lactose in the place of mannitol in yeast extract mannitol agar medium was poured into the petri dish and mixed by rotation several times clock wise and anti-clock wise. Agar was allowed to solidify, the dishes were inverted and incubated at 26-28^{o}C for 4-5 days.

Benedict's reagent was prepared by taking 173 g sodium citrate and 100 g anhydrous sodium carbonate which were dissolved in 66 ml of distilled water. 17.3 g crystalline copper sulphate was dissolved in 100 ml distilled water. The latter solution was added to the former with constant stirring and made upto 100 ml with distilled water.

After a good growth of the culture was got, Benedict's reagent was poured over the agar medium containing lactose. The absence of yellow ring was considered to be positive reaction for **Rhizobium.**

Growth in Peptone glucose agar :

One ml each of the appropriate cultures of seven different forage legumes was transferred to sterile petri dishes. Fifteen ml of liquified and cooled peptone glucose agar was poured into the petri dishes and mixed by rotating several times clockwise and anti-clockwise. The agar was allowed to solidify, the dishes were

inverted and incubated at 28°C for 4-5 days. The positive reaction for rhizobia was indicated by the absence of growth. The composition of the medium is given below :

Peptone glucose agar

Peptone	10.0 g
Glucose	5.0 g
Bromocresol purple (1% alcoholic solution)	10.0 ml
Agar	10.0 g
Distilled water	1000 ml

Reaction in litmus milk (Rangaswami, 1966)

Litmus milk (80 mg of litmus granules taken in 300 ml of 40 per cent alcohol, boiled for a moment and 2 per cent of this litmus solution was added to the skim milk) was taken, autoclaved three times at 10 psi for ten minutes on three consecutive days. The tubes containing 5 ml of the litmus milk were inoculated with the isolates and incubated for one or two weeks at 27°C. Non-rhizobial forms of contaminants were revealed by their rapid decolourization of litmus.

PACKET CULTURE STUDIES

Effect of inoculation of *Rhizobium* on *Delonix regia* and *Leucaena leucocephala*.

The effect of inoculation with the rhizobial strains isolated from **Leucaena leucocephala** was studied under packet culture conditions on two tree legumes viz., **Delonix regia** and **Leucaena leucocephala**.

Seeds of **Delonix regia** and **Leucaena leucocephala** were surface sterilized with 95 per cent alcohol and subjected to heat treatment in boiling water at 80°C for 3 minutes. Then the seeds were treated with the Rhizobial strain cultured in malt extract broth. The seeds were sown in polythene packets containing unsterilized soil. The sampling was done on 30, 60, 90 and 120 days after sowing. During each sampling, 5 plants were removed from each treatment.

The plants were washed in running tap water to remove adhering soil particles and pressed between filter paper folds to remove water droplets.

Nodule number was counted and nodule fresh weight was determined. Root length, shoot length, and plant fresh weight were determined.

The fresh leaves were used for the determination of chlorophyll content. An extract was prepared out of the fresh leaves and was used for the estimation amino nitrogen content.

The saplings were dried at 80°C for 24 hours. The dried samples were powdered by using mortar and pestle. The powdered samples were subjected to a series of nutrient analysis (Nitrogen, Phosphorus and Pottasium).

The following parameters were observed to assess the effeciency of rhizobial isolates.

1. ***Nodule number*** : The plants were gently removed and the adhering soil particles was washed off under a stream of tap water and the nodules were counted.

2. ***Nodule fresh weight*** : The nodule were gently scraped off and their fresh weight for was recorded.
3. ***Root length*** : The roots were gently straightened and the length (cms) was measured.
4. ***Shoot length*** : The shoot length (cms) was measured from the ground level till the tip.
5. ***Plant fresh weight*** : The tree saplings were weighed and the weight was expressed as grams per plant.
6. ***Plant dry weight*** : The saplings were oven dried at 70^{o}C for 24 hours and the dry weights were recorded.
7. ***Total Nitrogen content*** : The total nitrogen content was estimated by the Microkjeldahl method (Humphries, 1956) as given in Appendix I.
8. ***Estimation of Phosphorus*** : The phosphorus content of the tree saplings was estimated on the 30th, 60th, 90th and 120 days after sowing. A detailed procedure of the estimation of phosphorus by the method of Jackson (1973), is given in Appendix II
9. ***Estimation of Potassium*** : Potassium content of all the plant samples was estimated by the method of Jackson (1973), a detailed procedure of which is given in Appendix III.
10. ***Estimation of chlorophyll*** : Chlorophyll content of leaves was measured by the method of Yoshida *et al.*, (1971). The procedure is given in detailed in Appendix IV.
11. ***Estimation of amino nitrogen*** : The amino nitrogen content in the leaves of the tree saplings was estimated by Ninhydrin method as given in Appendix V.

EFFECT OF BAVISTIN ON THE GERMINATION AND GROWTH OF BOTH *RHIZOBIUM* INOCULATED AND UNINOCULATED SEEDS OF *DELONIX REGIA* AND *LEUCAENA LEUCOCEPHALA* :

10 g of seeds of **Delonix regia** and **Leucaena leucocephala** were treated with four different concentrations i.e., 5 mg, 10 mg, 15 mg and 20 mg of Bavistin. This was done in duplicates. To one set of seeds of both of species, added the isolated Rhizobial strain, and then the moisture was completely dried out. The seeds were then stored for a week in polythene bags. After a week, the seeds were removed and allowed to germinate in sterile petri plates.

The per cent germination, root and shoot length and fresh weight was recorded on the 10th day (under lab conditions).

Statistical Analysis (Gupta, 1985)

The results obtained were subjected to statistical analysis.

The test of significance (t test) was calculated using the formula :

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S} \times \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

X_1 = Mean of first sample

X_2 = Mean of second sample

n_1 = number of observation in first sample

n_2 = number of observation in second sample

S = Combined Standard Deviation

$$\sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1 + n_2 - 2}}$$

S_1 = Standard Deviation in first sample

S_2 = Standard Deviation in second sample

Results and Discussion

IV. RESULTS AND DISCUSSION

The present study evaluated the effect of **Rhizobium** inoculation on the growth of two tree legumes namely **Delonix regia** and **Leucaena leucocephala**. Seeds of these two tree legumes were taken and treated with **Rhizobium** isolated from the root nodules of **Leucaena leucocephala.** They were then sown in polythene packets and allowed to germinate. On the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing, a few saplings were uprooted carefully and growth attributes (nodule number, nodule fresh weight, root and shoot length, plant fresh and dry weight) were measured. Biochemical constituents (Nitrogen, Phosphorus, Potassium, Chlorophyll and Amino Nitrogen) were also estimated in these tree saplings on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing.

The results of the above mentioned observations are discussed as follows :

1) Effect of *Rhizobium* on nodulation :

The effect of **Rhizobium** inoculation on the nodulation behaviour of **Delonix regia** and **Leucaena leucocephala** were studied. **Delonix regia** was found to contain no nodules. This result agreed with the report of Faria *et al.*, (1989) who stated that consistent negative reports regarding nodulation were obtained for genera such as *Delonix, Schizolobium, Peltophorum.*

Leucaena leucocephala was found to be nodulating.

Table I.

Effect of Rhizobium inoculation on the nodule number Leucaena leucocephala tree saplings

Age (days)	Nodule number Mean ± S.D.#		't' value$
	Uninoculated	Inoculated	
30	2.3 ± 0.7	3.5 ± 0.6	2.60*
60	4.7 ± 0.3	6.6 ± 0.3	5.37**
90	7.1 ± 0.2	10.8 ± 0.4	16.55**
120	11.0 ± 0.3	14.5 ± 0.2	19.41**

For 5 replications

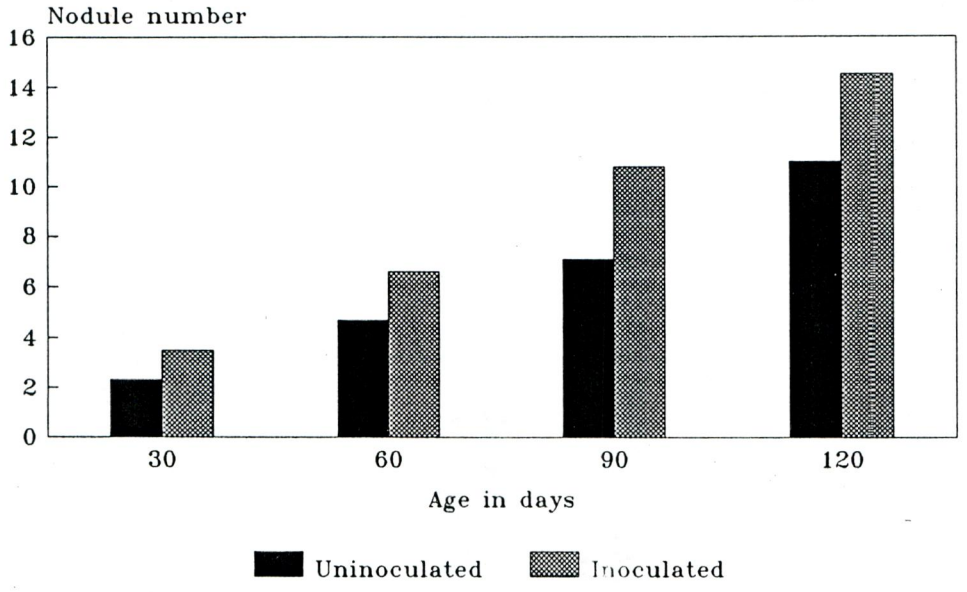
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not significant

FIG.1. EFFECT OF *RHIZOBIUM* INOCULATION ON THE NODULE NUMBER OF *L.LEUCOCEPHALA* TREE *SAPLINGS*



2) Nodule number :

Table I and Figure I shows the effect of **Rhizobium** inoculation on the nodule number of **Leucaena leucocephala** tree saplings taken on the 30th, 60th, 90th and 120th days after sowing.

The number of nodules was greater in the **Rhizobium** inoculated **Leucaena leucocephala** tree saplings than the uninoculated saplings.

The nodule number was 2.3, 4.7, 7.1 and 11.0 in uninoculated saplings on the 30th, 60th, 90th and 120th days respectively after sowing were as in inoculated saplings the number of nodules were 3.5, 6.6, 10.8 and 14.5 on 30, 60, 90 and 120 days respectively after sowing.

The difference in the nodule number between **Rhizobium** inoculated and uninoculated saplings was found to be significant.

3. Nodule fresh weight :

Table II and Figure II shows the fresh weight of nodules in **Leucaena leucocephala** saplings in both **Rhizobium** inoculated and uninoculated condition taken on the 30th, 60th, 90th and 120th days after sowing.

The fresh weight of nodules on the 30th, 60th, 90th and 120th days were found to be 0.035, 0.072, 0.109 and 0.169 g respectively in the uninoculated saplings and 0.054, 0.102, 0.166 and 0.223 g in the **Rhizobium** inoculated saplings of **Leucaena leucocephala**.

Table II.

Effect of Rhizobium inoculation on the nodule fresh weight of Leucaena leucocephala tree saplings

Age (days)	Nodule fresh weight Mean ± S.D.# (g)		't' value$
	Uninoculated	Inoculated	
30	0.035 ± 0.034	0.054 ± 0.023	0.93^{NS}
60	0.072 ± 0.036	0.102 ± 0.051	0.97^{NS}
90	0.109 ± 0.013	0.166 ± 0.022	4.49**
120	0.169 ± 0.021	0.223 ± 0.011	4.54**

For 5 replications

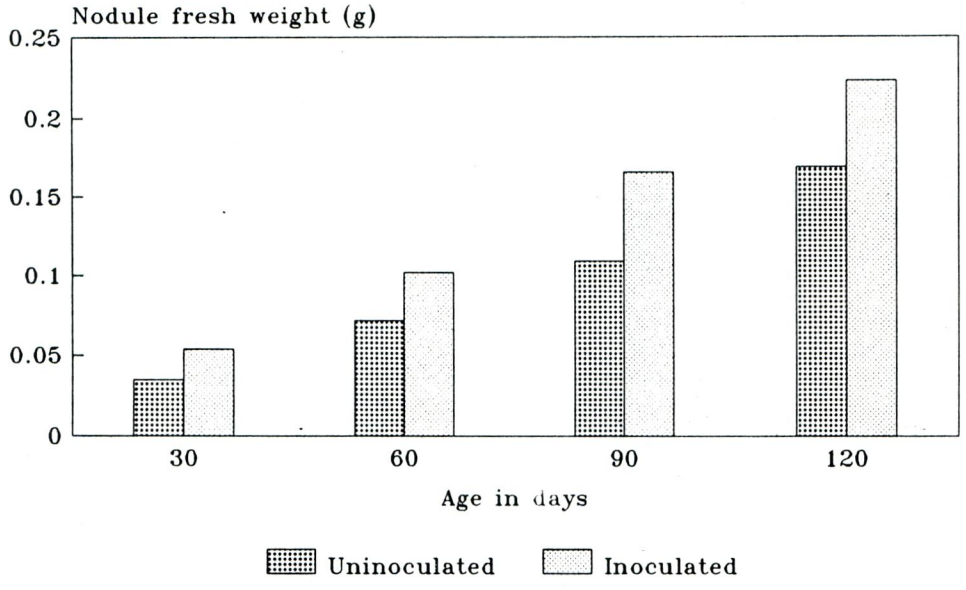
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.2. EFFECT OF *RHIZOBIUM* INOCULATION ON THE NODULAR FRESH WEIGHT OF *L. LEUCOCEPHALA* TREE SAPLINGS



The fresh weight of the nodules increased in both the **Rhizobium** inoculated and uninoculated **Leucaena leucocephala** saplings but the nodular fresh weight was greater in the **Rhizobium** inoculated saplings.

Pokhriyal *et al* ., (1987) reported that the nodule fresh weight was increased significantly in **Leucaena leucocephala** inoculated with **Rhizobium**.

The results of the present study showed that the difference in the nodule fresh weight between the **Rhizobium** inoculated and uninoculated **Leucaena leucocephala** saplings was increased significantly on the 90^{th} and 120^{th} days after sowing.

4) Root length :

Tabel III and Figure III represent the growth of **Delonix regia** and **Leucaena leucocephala** in terms of root length (cms) on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing.

Root length of the inoculated saplings of **Delonix regia** was found to be 9.9, 17.6, 32.8 and 40.8 cms and 7.4, 15.3, 30.1 and 37.4 cms for the uninoculated saplings on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days respectively after sowing.

The root length of the **Rhizobium** inoculated **Leucaena leucocephala** saplings was found to be 10.3, 20.5, 33.4 and 41.8 cms on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days respectively after sowing.

Table III.

Effect of Rhizobium inoculation on the root length of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Root length Mean ± S.D.# (cms)		't' value$
		Uninoculated	Inoculated	
Delonix	30	7.4 ± 0.46	9.9 ± 0.41	8.12**
regia	60	15.3 ± 0.75	17.6 ± 0.24	5.84**
	90	30.1 ± 0.31	32.8 ± 0.57	8.32**
	120	37.4 ± 0.42	40.8 ± 0.71	8.24**
Leucaena	30	9.7 ± 0.19	10.3 ± 0.10	5.59**
leucocephala	60	19.6 ± 0.24	20.5 ± 0.13	6.59**
	90	30.2 ± 0.11	33.4 ± 0.08	47.05**
	120	40.3 ± 0.05	41.8 ± 0.23	12.75**

For 5 replications

$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.3. EFFECT OF *RHIZOBIUM* INOCULATION ON THE ROOT LENGTH OF *D. REGIA* AND L. *LEUCOCEPHALA* TREE SAPLINGS

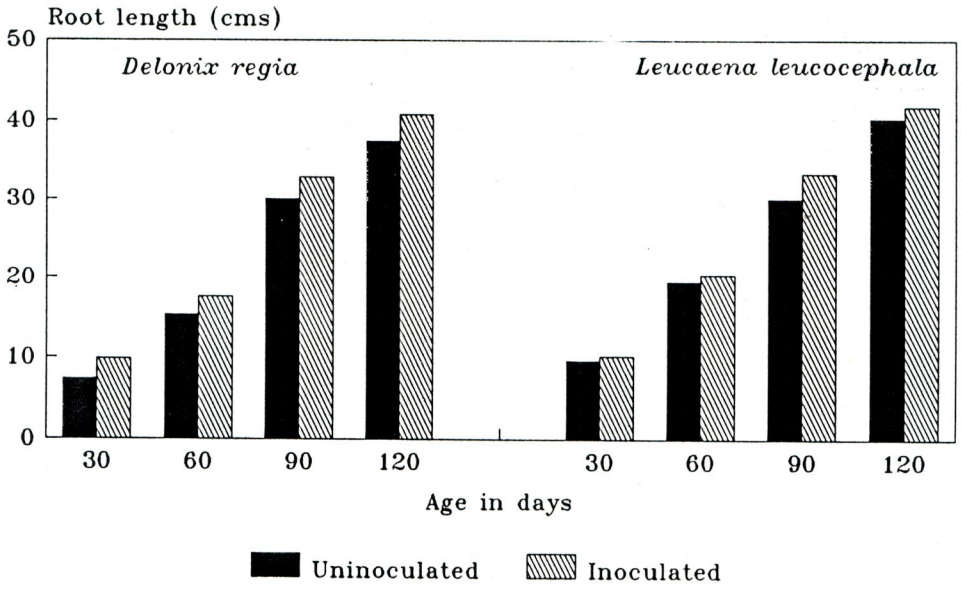


PLATE 1. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **DELONIX REGIA** TAKEN ON THE 30th DAY AFTER SOWING

PLATE 2. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **DELONIX REGIA** TAKEN ON THE 60th DAY AFTER SOWING

PLATE 1

Uninoculated Inoculated

PLATE 2

Uninoculated Inoculated

PLATE 3. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **DELONIX REGIA** TAKEN ON THE 90th DAY AFTER SOWING

PLATE 4. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **DELONIX REGIA** TAKEN ON THE 120th DAY AFTER SOWING

PLATE 3

Uninoculated Inoculated

PLATE 4

Uninoculated Inoculated

The roots of uninoculated **Leucaena leucocephala** saplings were 9.7, 19.6, 30.2 and 40.3 cms long on the 30th, 60th, 90th and 120th days respectively after sowing.

While both the groups of saplings grew, there was more rapid growth due to **Rhizobium** inoculation.

Basu and Kabi (1987) reported that due to **Rhizobium** biofertilizer application, root growth in **Leucaena leucocephala** was significantly enhanced, 6 months after sowing.

The results of the present study showed that due to **Rhizobium** inoculation highly significant increases in root length can be achieved.

5) Shoot length :

The growth of **Delonix regia** and **Leucaena leucocephala** in terms of shoot length (cms) taken on the 30th, 60th, 90th and 120th days after sowing is represented by Table IV and Figure IV.

The shoot length of the inoculated saplings of **Delonix regia** was found to be 14.1, 20.2, 26.6, and 31.7 cms on the 30th, 60th, 90th and 120th days respectively after sowing.

The shoot length of the uninoculated saplings of **Delonix regia** was found to be 12.4, 17.5, 24.3 and 29.8 cms on the 30th, 60th, 90th and 120th days respectively after sowing.

Table IV

Effect of Rhizobium inoculation on the shoot length of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Shoot length Mean ± S.D.# (cms)		't' value$
		Uninoculated	Inoculated	
Delonix regia	30	12.4 ± 0.05	14.1 ± 0.13	24.41**
	60	17.5 ± 0.62	20.2 ± 0.49	6.83**
	90	24.3 ± 0.24	26.6 ± 0.33	11.27**
	120	29.8 ± 0.41	31.7 ± 0.21	8.25**
Leucaena leucocephala	30	14.5 ± 0.09	16.3 ± 0.01	39.76**
	60	21.4 ± 0.21	23.3 ± 0.20	13.10**
	90	27.7 ± 0.14	32.8 ± 0.54	18.28**
	120	35.9 ± 0.36	38.1 ± 0.79	5.07**

For 5 replications

$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.4. EFFECT OF *RHIZOBIUM* INOCULATION ON THE SHOOT LENGTH OF *D. REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS

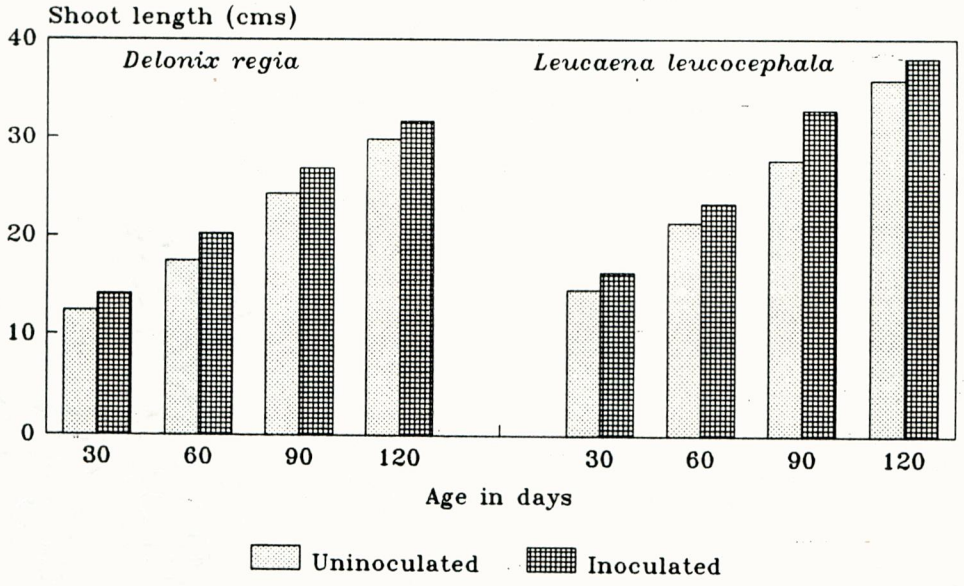


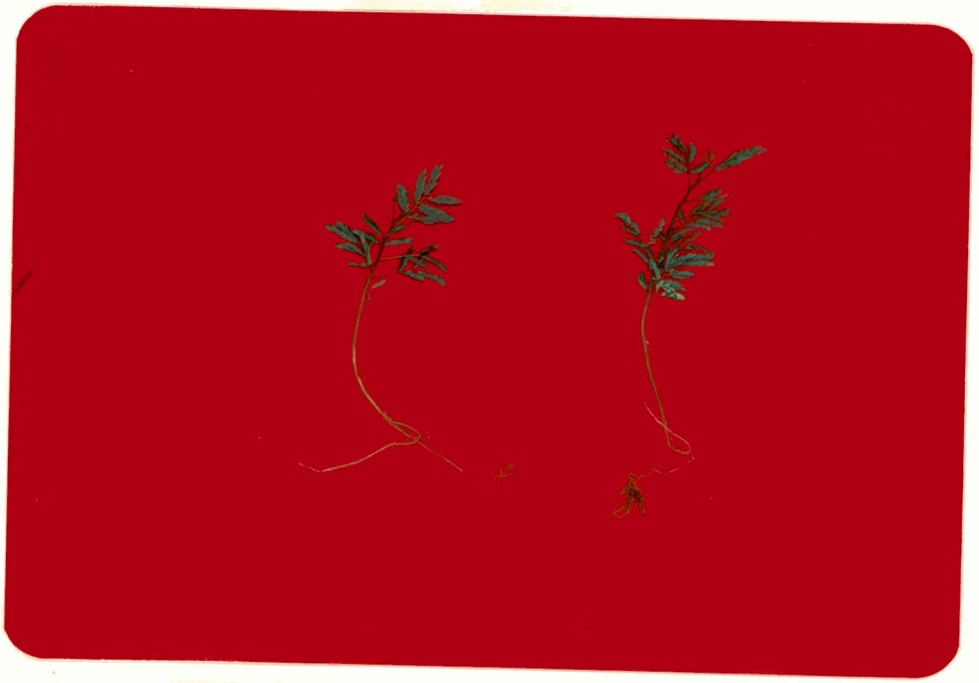
PLATE 5. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **LEUCAENA LEUCOCEPHALA** TAKEN ON THE 30th DAY AFTER SOWING

PLATE 6. EFFECT OF **RHIZOBIUM** INOCULATION ON THE GROWTH OF **LEUCAENA LEUCOCEPHALA** TAKEN ON THE 60th DAY AFTER SOWING

PLATE 5

Uninoculated Inoculated

PLATE 6



Uninoculated Inoculated

PLATE 7. EFFECT OF **RHIZOBIUM** INOCULATION ON THE **LEUCAENA LEUCOCEPHALA** TAKEN ON THE 90th DAY AFTER SOWING

PLATE 8. EFFECT OF **RHIZOBIUM** INOCULATION ON THE **LEUCAENA LEUCOCEPHALA** TAKEN ON THE 120th DAY AFTER SOWING

PLATE 7

Uninoculated Inoculated

PLATE 8

Uninoculated Inoculated

The shoot length of **Leucaena leucocephala** was found to be 16.3, 23.3, 32.8 and 38.1 cms in the **Rhizobium** inoculated saplings and 14.5, 21.4, 27.7 and 35.9 cms in the uninoculated saplings on the 30th, 60th, 90th and 120th days respectively after sowing.

The increase in the shoot length of the **Rhizobium** inoculated saplings over the uninoculated saplings was found to be statistically significant.

Plates 1-4 represent the growth of both **Rhizobium** inoculated and uninoculated **Delonix regia** tree saplings taken on the 30th, 60 th, 90th and 120th days after sowing.

Plates 5-8 represent the growth of both **Rhizobium** inoculated and uninoculated **Leucaena Leucocephala** tree saplings taken on the 30th, 60 th, 90th and 120th days after sowing.

6) Plant fresh weight :

The biomass of **Delonix regia** and **Leucaena leucocephala** in terms of fresh weight in grams on the 30th, 60th, 90th and 120th days after sowing is shown in Table V and Figure V.

The fresh weight of **Delonix regia** was 2.35, 3.49, 5.9 and 8.34 g for the **Rhizobium** inoculated tree saplings and 1.24, 2.2, 4.05 and 6.86 g for the

Table V

Effect of Rhizobium inoculation on the fresh weight of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Plant fresh weight Mean ± S.D.# (g)		't' value$
		Uninoculated	Inoculated	
Delonix	30	1.24 ± 0.66	2.35 ± 0.45	2.78*
regia	60	2.20 ± 0.78	3.49 ± 0.67	2.51*
	90	4.05 ± 0.73	5.90 ± 0.42	4.39**
	120	6.86 ± 0.59	8.34 ± 0.16	4.84**
Leucaena	30	1.07 ± 0.89	1.97 ± 0.22	1.96NS
leucocephala	60	1.97 ± 0.17	2.86 ± 0.06	9.87**
	90	2.35 ± 0.54	4.36 ± 0.36	8.19**
	120	3.29 ± 0.22	5.75 ± 0.61	9.59**

For 5 replications

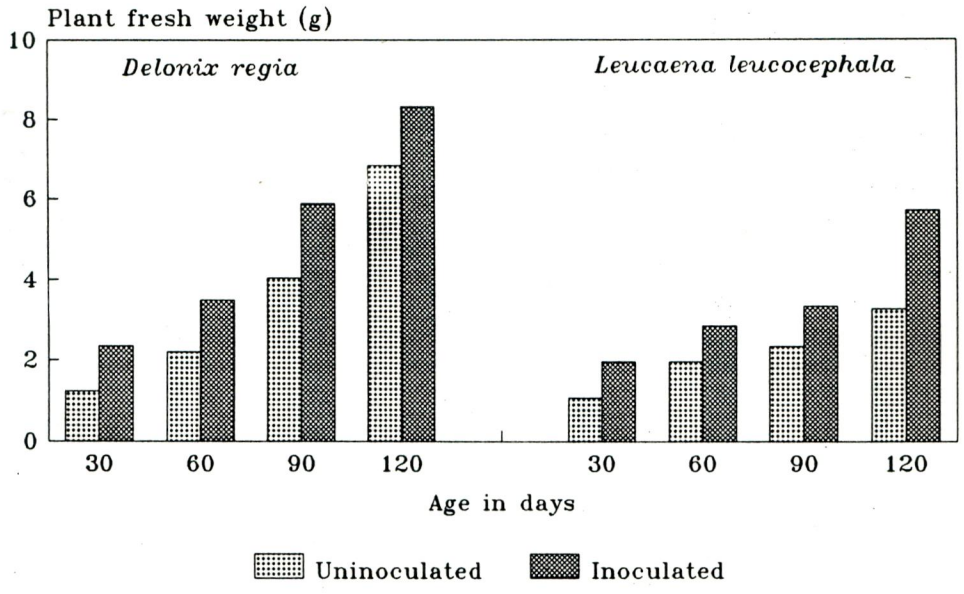
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.5. EFFECT OF *RHIZOBIUM* INOCULATION ON THE FRESH WEIGHT OF *D. REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS



uninoculated tree saplings on the 30th, 60th, 90th and 120th days respectively after sowing.

The fresh weights of **Leucaena leucocephala** on the 30th, 60th, 90th and 120th days after sowing was 1.97, 2.56, 4.36 and 5.75 g for the **Rhizobium** inoculated tree saplings and 1.07, 1.97, 2.35 and 3.29 g for the uninoculated saplings respectively.

Rhizobial inoculation significantly increased the plant fresh and dry weight of the inoculated saplings over the uninoculated ones (Punj and Gupta, 1988).

The present study showed that **Rhizobium** inoculation of **Delonix regia** resulted in significant increases in plant fresh weight whereas in **Leucaena leucocephala** significant increases in plant fresh weight were observed on the 60th, 90th and 120th days after sowing.

7) Plant Dry Weight :

The biomass of **Delonix regia** and **Leucaena leucocephala** in terms of dry weight in grams on the 30th, 60th, 90th and 120th days after sowing are shown in Table VI and Figure VI.

The plant dry weight of **Delonix regia** was 1.2, 1.83, 2.96 and 3.05 g for the **Rhizobium** inoculated tree saplings and 0.56, 0.99, 1.85 and 2.79 g for the uninoculated tree saplings on the 30th, 60th, 90th and 120th days respectively after sowing.

Table VI

Effect of Rhizobium inoculation on the dry weight of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Plant dry weight Mean ± S.D.# (g)		't' value$
		Uninoculated	Inoculated	
Delonix	30	0.56 ± 0.43	1.20 ± 0.47	2.01^{NS}
regia	60	0.99 ± 0.22	1.83 ± 0.10	6.18**
	90	1.85 ± 0.36	2.96 ± 0.15	5.69**
	120	2.79 ± 0.16	3.05 ± 0.28	1.61^{NS}
Leucaena	30	0.56 ± 0.15	0.70 ± 0.09	1.60^{NS}
leucocephala	60	0.99 ± 0.21	1.06 ± 0.18	0.51^{NS}
	90	1.24 ± 0.07	1.93 ± 0.31	4.34**
	120	1.96 ± 0.19	2.24 ± 0.23	5.88**

For 5 replications

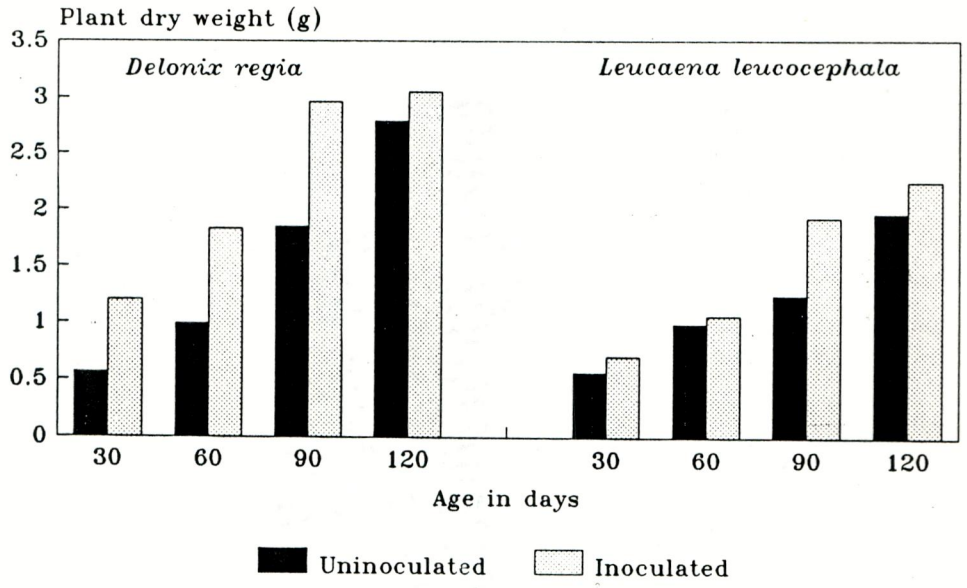
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.6. EFFECT OF *RHIZOBIUM* INOCULATION ON THE DRY WEIGHT OF D. *REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS



The dry weight of **Leucaena leucocephala** on the 30th, 60th, 90th and 120th days after sowing was 0.7, 1.06, 1.93 and 2.24 g for the **Rhizobium** uninoculated saplings and 0.56, 0.99, 1.24 and 1.96 g respectively for the uninocuated of tree saplings.

Punj *et al.*, (1987) reported a significant increase in the dry weight of trees pre – treated with **Rhizobium** over the uninoculated saplings.

The results of the present study showed that due to **Rhizobium** inoculation, the plant dry weight was significantly increased on the 90th and 120th days after sowing in **Leucaena leucocephala** saplings.

8) Nitrogen Content :

The total nitrogen content of **Delonix regia** and **Leucaena leucocephala** on the 30th, 60th, 90th and 120th days after sowing is represented by Table VII and Figure VII.

The total nitrogen content of **Delonix regia** was 1.26, 1.4, 1.57 and 1.69% on the 30th, 60th, 90th and 120th days respectively after sowing.

The total nitrogen content was found to be 1.32, 1.45, 1.63 and 1.74 % on the 30th, 60th, 90th and 120th days respectively after sowing in inoculated tree saplings of **Leucaena leucocephala.**

Table VII

Effect of Rhizobium inoculation on the total nitrogen content of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Total Nitrogen Mean ± S.D.# (%)		't' value$
		Uninoculated	Inoculated	
Delonix	30	1.08 ± 0.81	1.26 ± 0.59	0.36^{NS}
regia	60	1.12 ± 0.72	1.40 ± 0.85	0.50^{NS}
	90	1.23 ± 0.27	1.57 ± 0.19	2.06^{NS}
	120	1.37 ± 0.07	1.69 ± 0.62	1.03^{NS}
Leucaena	30	1.10 ± 0.41	1.32 ± 0.27	0.90^{NS}
leucocephala	60	1.19 ± 0.10	1.45 ± 0.63	0.82^{NS}
	90	1.32 ± 0.08	1.63 ± 0.31	1.94^{NS}
	120	1.46 ± 0.33	1.74 ± 1.72	0.71^{NS}

For 5 replications

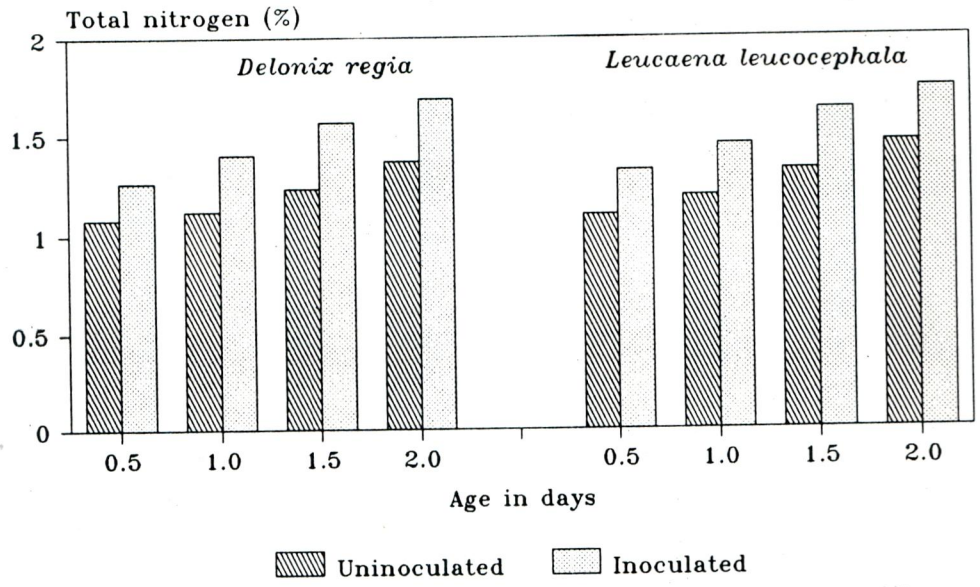
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.7.EFFECT OF RHIZOBIUM INOCULATION ON THE TOTAL NITROGEN CONTENT OF *D.REGIA* AND *L.LEUCOCEPHALA* TREE SAPLINGS



In uninoculated **Leucaena leucocephala** saplings, the total nitrogen content was found to be 1.10, 1.19, 1.32 and 1.46 % on the 30th, 60th, 90th and 120th days after sowing.

Rhizobium biofertilizer application had been reported to increase the total nitrogen, phosphorus and potassium uptake (Singh *et al.*, 1978; Prasad *et al.*, 1990).

However in the present study the difference in the nitrogen content between the **Rhizobium** inoculated and uninoculated tree saplings was found to be not significant.

9) Phosphorus content :

Table VIII and Figure VIII shows the phosphorus content of **Delonix regia** and **Leucaena leucocephala** as estimated on the 30th, 60th, 90th and 120th days after sowing.

The phosphorus content of **Delonix regia** was 0.298, 0.306, 0.322 and 0.367% on the 30th, 60th, 90th and 120th days respectively after sowing in inoculated tree saplings.

In uninoculated saplings the phosphorus content was 0.201, 0.286, 0.305, 0.321% for 30, 60, 90 and 120 days after sowing respectively.

The phosphorus content was found to be 0.288, 0.321, 0.345, 0.397% on the 30th, 60th, 90th and 120th days respectively after sowing in inoculated tree saplings

Table VIII

Effect of Rhizobium inoculation on the phosphrous content of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (days)	Phosphorus Mean ± S.D# (%)		't'value$
		Uninoculated	Inoculated	
Delonix	30	0.201 ± 0.086	0.278 ± 0.076	1.69NS
regia	60	0.286 ± 0.015	0.306 ± 0.035	1.05NS
	90	0.305 ± 0.036	0.322 ± 0.041	0.62NS
	120	0.321 ± 0.021	0.347 ± 0.024	1.64NS
Leucaena	30	0.221 ± 0.051	0.288 ± 0.076	1.46NS
leuocephala	60	0.301 ± 0.011	0.321 ± 0.034	1.12NS
	90	0.317 ± 0.014	0.345 ± 0.121	0.46NS
	120	0.360 ± 0.092	0.397 ± 0.070	0.64NS

For 5 replications

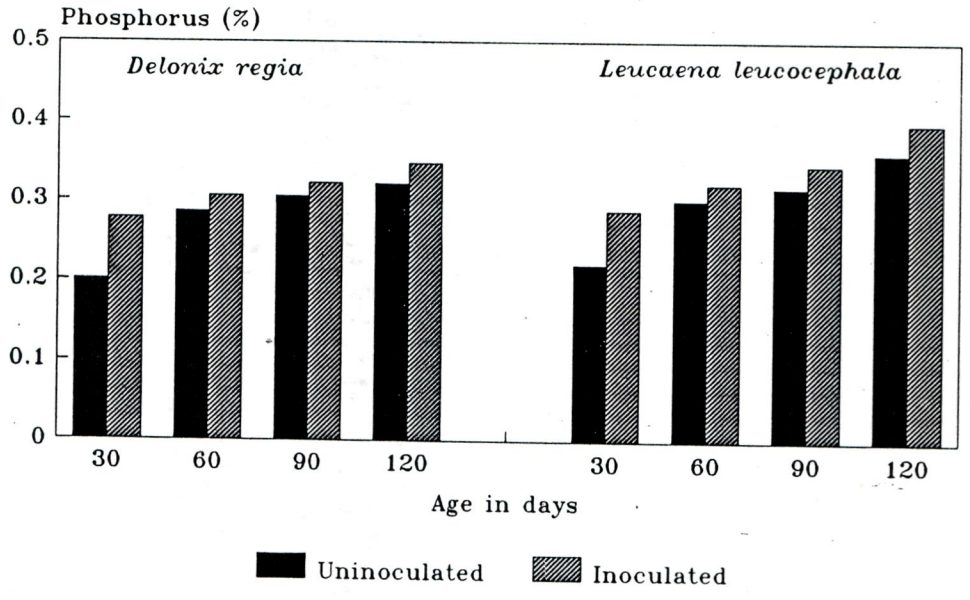
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.8. EFFECT OF RHIZOBIUM INOCULATION ON THE PHOSPHORUS CONTENT OF *D.REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS



of **Leucaena leucocephala** whereas in the uninoculated saplings, the phosphorus content was found to be 0.221, 0.301, 0.317 and 0.360% on the 30th, 60th, 90th and 120th days respectively after sowing.

The difference in the phosphorus content of **Rhizobium** inoculated and uninoculated tree saplings was found to be not significant.

10) Potassium content :

Potassium content in the tree saplings viz., **Delonix regia** and **Leucaena leucocephala** on the 30th, 60th, 90th and 120th days after sowing is shown in Table IX and Figure IX.

For the inoculated **Delonix regia** saplings, the potassium content was 1.63, 1.80, 1.85 and 1.92 % on the 30th, 60th, 90th and 120th days after sowing. For the uninoculated saplings, the potassium content was 1.56, 1.79, 1.82 and 1.90 % respectively.

The potassium content of **Leucaena leucocephala** saplings on the 30th, 60th, 90th and 120th days after sowing was 1.71, 1.83, 1.92 and 2.15 % respectively for the **Rhizobium** inoculated saplings and 1.62, 1.79, 1.85 and 2.02 % for the uninoculated saplings.

The difference in the potassium content of **Rhizobium** inoculated and uninoculated tree saplings was found to be not significant.

Table IX

Effect of Rhizobium inoculation on the potassium content of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (Days)	Potassium Mean ± S.D.# (%)		't' value$
		Uninoculated	Inoculated	
Delonix	30	1.56 ± 0.03	1.63 ± 0.11	1.23 NS
regia	60	1.79 ± 0.36	1.80 ± 0.19	0.05 NS
	90	1.82 ± 0.26	1.85 ± 0.22	0.18 NS
	120	1.90 ± 0.42	1.92 ± 0.17	0.09 NS
Leucaena	30	1.62 ± 0.24	1.71 ± 0.56	0.30 NS
leucacephla	60	1.79 ± 0.17	1.83 ± 0.37	0.20 NS
	90	1.85 ± 0.41	1.92 ± 0.16	0.32 NS
	120	2.02 ± 0.27	2.15 ± 0.05	0.95 NS

For 5 replications

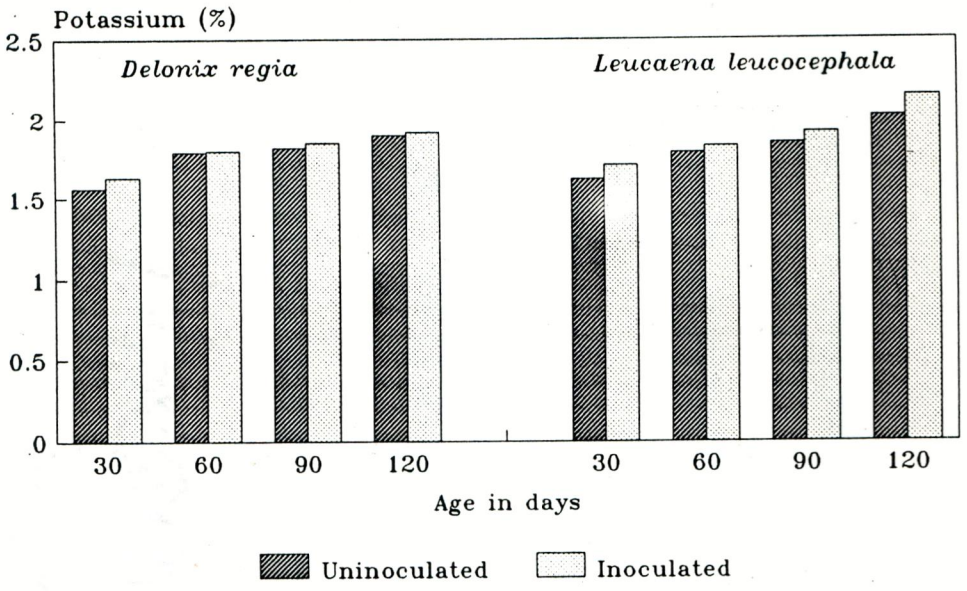
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.9. EFFECT OF *RHIZOBIUM* INOCULATION ON POTASSIUM CONTENT OF *D. REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS



11) Chlorophyll content :

The chlorophyll content of **Delonix regia** and **Leucaena leucocephala** on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing is represents in Table X and Figure X.

The chlorophyll content of **Delonix regia** was found to be 1.126, 1.310, 1.40 and 1.426 mg/g on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing in inoculated tree saplings.

In the uninoculated controls, the chlorophyll content was 1.067, 1.174, 1.203 and 1.245 mg/g on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days respectively after sowing.

The chlorphyll content was found to be 1.098, 1.226, 1.56 and 1.591 mg on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing in inoculated tree saplings of **Leucaena leucocephala.**

In uninoculated saplings, the chlorophyll content was found to be 1.012, 1.180, 1.302 and 1.379 mg on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing.

The difference between the chlorophyll contents in the leaves of **Rhizobium** inoculated and uninoculated **Leucaena leucocephala** saplings was found to be significant on the 30^{th}, 90^{th} and 120^{th} days after sowing.

Table X

Effect of Rhizobium inoculation on the chlorophyll content in the leaves of Delonix regia and Leucaena leucocephala tree saplings

Species	Age (Days)	Chlorophyll Mean ± S.D# (mg)		't' value$
		Uninoculated	Inoculated	
Delonix	30	1.067 ± 0.016	1.126 ± 0.101	1.15NS
regia	60	1.174 ± 0.126	1.310 ± 0.192	1.18NS
	90	1.203 ± 0.235	1.400 ± 0.005	1.68NS
	120	1.245 ± 0.096	1.1426 ± 0.136	2.17NS
Leucaena	30	1.012 ± 0.021	1.098 ± 0.045	3.46**
leucocephala	60	1.186 ± 0.058	1.226 ± 0.066	1.05NS
	90	1.302 ± 0.089	1.560 ± 0.095	3.96**
	120	1.379 ± 0.103	1.591 ± 0.056	3.62**

For 5 replications

$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.10 EFFECT OF *RHIZOBIUM* INOCULATION ON CHLOROPHYLL CONTENT OF *D. REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS

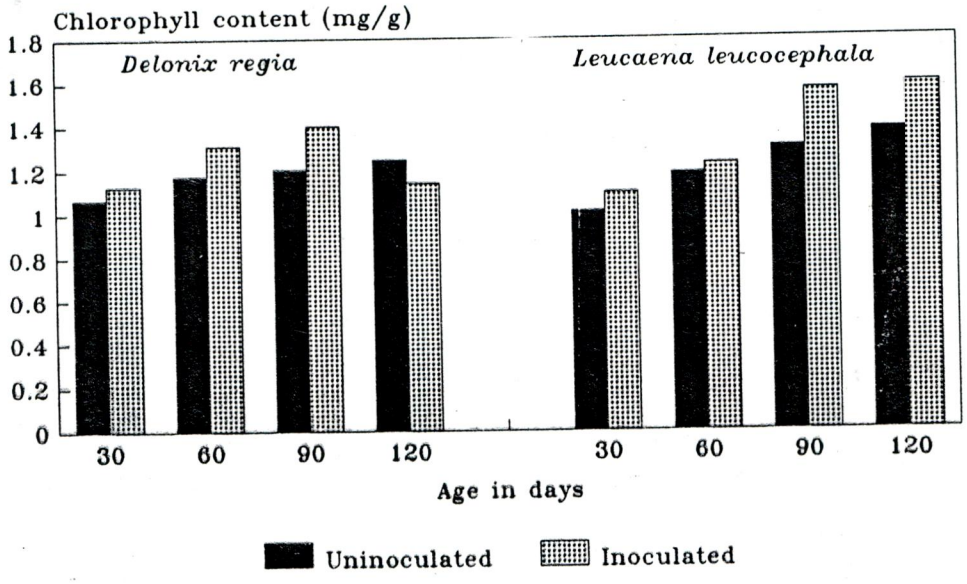


Table XI

Effect of Rhizobium Inoculation on the Amino nitrogen content in the leaves Delonix regia and Leucaena leucocephala tree saplings

Species	Age (Days)	Amino nitrogen Mean ± S.D.# mg/g		't'value$
		Uninoculated	Inoculated	
Delonix regia	30	1.21 ± 0.71	1.43 ± 0.84	0.40 NS
	60	1.35 ± 0.32	1.49 ± 0.36	0.58 NS
	90	1.40 ± 0.36	1.52 ± 0.22	0.57 NS
	120	1.46 ± 0.19	1.57 ± 0.17	0.85 NS
Leucaena leucacephala	30	1.39 ± 0.05	1.62 ± 0.24	1.92 NS
	60	1.43 ± 0.23	1.68 ± 0.35	1.19 NS
	90	1.52 ± 0.46	1.70 ± 0.25	0.69 NS
	120	1.56 ± 0.16	1.79 ± 0.19	1.92 NS

For 5 replications

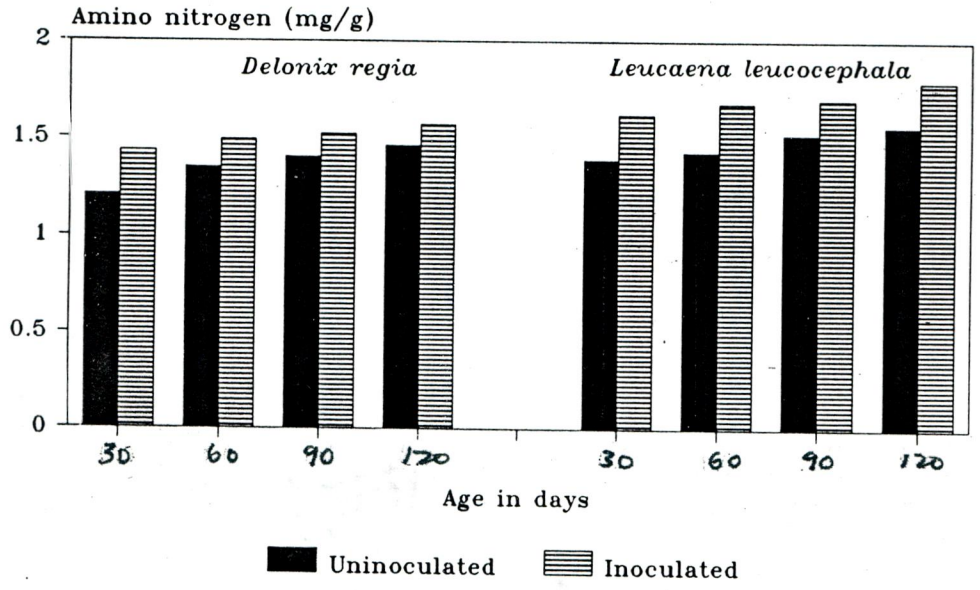
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.11. EFFECT OF RHIZOBIUM INOCULATION ON THE AMINO NITROGEN CONTENT *OF D.REGIA* AND *L.LEUCOCEPHALA* TREE SAPLINGS



12) Amino nitrogen content :

The amino nitrogen content in the leaves of **Rhizobium** inoculated and uninoculated saplings of **Delonix regia** and **Leucaena leucocephala** taken on the 30th, 60th, 90th and 120th days after sowing is shown in Table XI and Figure XI.

The amino nitrogen content of **Delonix regia** on the 30th, 60th, 90th and 120th days after sowing was found to be 1.43, 1.49, 1.52 and 1.59 mg respectively for the **Rhizobium** inoculated saplings and 1.21, 1.35, 1.40 and 1.46 mg respectively for the uninoculated saplings.

In the leaves of **Leucaena leucocephala** the amino nitrogen content on the 30th, 60th, 90th and 120th days after sowing was found to be 1.62, 1.68, 1.70 and 1.79 mg for **Rhizobium** inoculated saplings and 1.39, 1.43, 1.52 and 1.56 mg for the uninoculated saplings.

The difference in the amino nitrogen content in the leaves of inoculated and uninoculated saplings was found to be not significant.

13) Effect of different concentrations of Bavistin on both *Rhizobium* inoculated and uninoculated seeds of *Delonix regia* and *Leucaena leucocephala* on the 10th day :

a) *Seed germination*

The effect of Bavistin on the germintion of both the **Rhizobium** inoculated and uninoculated seeds of **Delonix regia** and **Leucaena leucocephala** is shown in Table XII and Figure XII.

Table XII

Effect of seed treatment with Bavistin at different concentrations and Rhizobium inoculation on the germination of seeds of Delonix regia and Leucaena leucocephala on the 10th day

Species	Bavistin Concentration mg/g	Seed germination Mean ± S.D.# (%)		't' value$
		Uninoculated	Inoculated	
Delonix	0.5	81.25 ± 6.25	83.50 ± 2.95	0.33^{NS}
regia	1.0	84.90 ± 3.95	91.71 ± 5.68	0.98^{NS}
	1.5	90.35 ± 2.45	92.60 ± 4.90	0.41^{NS}
	2.0	70.47 ± 3.66	73.31 ± 1.42	0.72^{NS}
Leucaena	0.5	85.05 ± 4.23	86.45 ± 6.05	0.19^{NS}
leucocephala	1.0	89.46 ± 2.74	90.06 ± 3.10	0.14^{NS}
	1.5	89.43 ± 1.15	93.95 ± 2.65	2.89*
	2.0	75.65 ± 1.00	75.93 ± 5.23	0.75^{NS}

\# For 5 replications

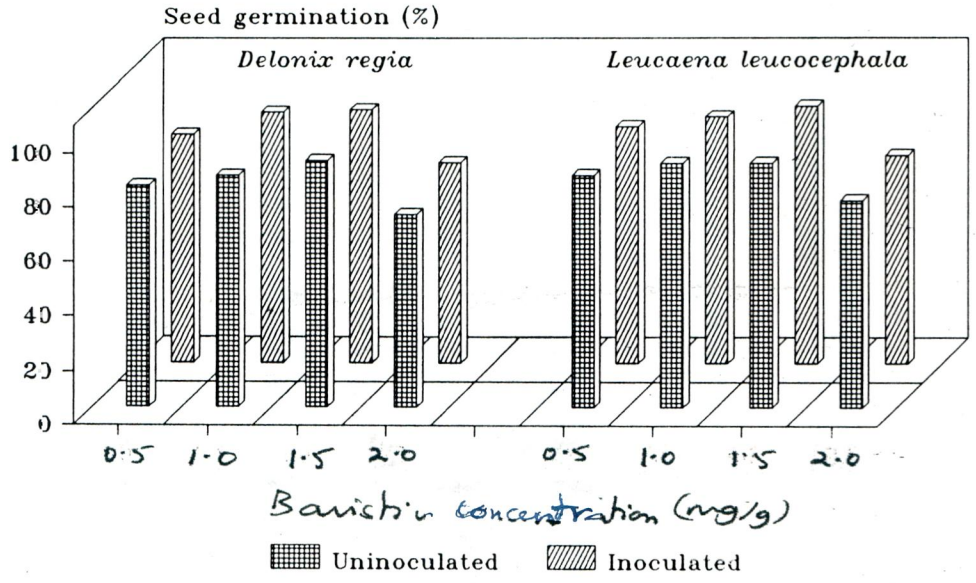
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.12.EFFECT OF *BAVISTIN* AND RHIZOBIUM ON SEED GERMINATION OF *D.REGIA* AND *L. LEUCOCEPHALA* TREE SAPLINGS



The difference in the percent germination between the **Rhizobium** inoculated and uninoculated seeds of **Delonix regia** and **Leucaena leucocephala** was found to be not significant for all the concentrations (0.5, 1.0, 1.5 and 2.0 mg/g) of Bavistin used.

There was a significant increase in the germination of **Rhizobium** inoculated **Leucaena leucocephala** when the concentration of Bavistin used was 1.5 mg/g.

b) *Root length* :

Table XIII and Figure XIII represent the effect of different concentrations of Bavistin on the root length of **Rhizobium** inoculated and uninoculated seedlings of **Delonix regia** and **Leucaena leucocephala** taken on the 10th day.

The results of the present study showed that there was no significant increase in the root length when the seeds of **Delonix regia** and **Leucaena leucocephala** were treated with Bavistin.

c) *Shoot length* :

Table XIV and Figure XIV represent the effect of different concentrations of Bavistin on the shoot length of **Rhizobium** inoculated and uninoculated seedlings of **Delonix regia** and **Leucaena leucocephala** taken on the 10th day.

The results of the present study showed that there was no significant increase in the shoot length when the seeds of **Delonix regia** and **Leucaena leucocephala** were treated with Bavistin.

Table XIII

Effect of Bavistin treatment at different concentrations on the root length of Rhizobium inoculated and uninoculated seedlings of Delonix regia and Leucaena leucocephala on the 10th day

Species	Bavistin Concentration mg/g	Root length Mean ± S.D.# (cms) Uninoculated	Inoculated	't' value$
Delonix	0.5	1.92 ± 0.31	2.11 ± 0.26	0.47 NS
regia	1.0	1.56 ± 0.63	2.31 ± 0.45	1.25 NS
	1.5	2.34 ± 0.71	2.56 ± 0.22	0.30 NS
	2.0	2.02 ± 0.23	2.50 ± 0.49	0.54 NS
Leucaena	0.5	1.98 ± 0.48	2.12 ± 0.53	0.19 NS
leucocephala	1.0	1.76 ± 0.51	2.24 ± 0.49	0.68 NS
	1.5	2.43 ± 0.36	2.77 ± 0.33	0.69 NS
	2.0	1.99 ± 0.11	2.55 ± 0.56	0.98 NS

For 5 replications

$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.13.EFFECT OF BAVISTIN TREATMENT AND RHIZOBIUM INOCULATION ON ROOT LENGTH OF *D. REGIA AND L. LEUCOCEPHALA*

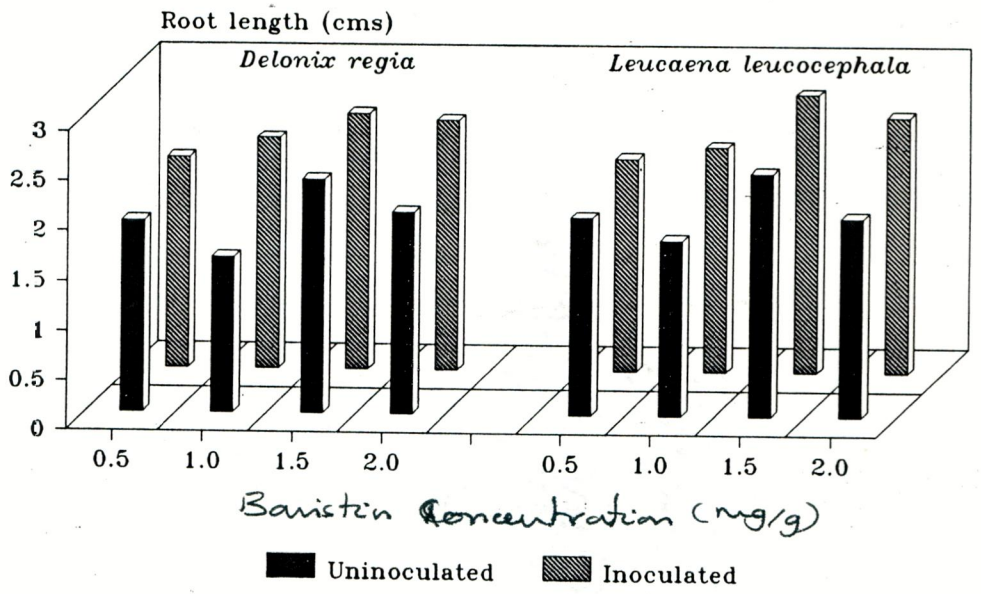


Table XIV

Effect of Bavistin treatment at different concentrations on the shoot length of Rhizobium inoculated and uninoculated seedlings of Delonix regia and Leucaena leucocephala on the 10th day

Species	Bavistin Concentration mg/g	Shoot length . Mean ± S.D.# (cms)		't' value $
		Uninoculated	Inoculated	
Delonix	0.5	3.99 ± 0.78	4.26 ± 0.31	0.32 NS
regia	1.0	3.36 ± 0.81	4.01 ± 0.57	0.66 NS
	1.5	4.05 ± 0.23	4.97 ± 0.42	0.84 NS
	2.0	3.76 ± 0.59	4.12 ± 0.16	0.59 NS
Leucaena	0.5	4.21 ± 0.36	4.37 ± 0.25	0.07 NS
leucocephala	1.0	4.30 ± 0.21	4.49 ± 0.73	0.25 NS
	1.5	4.56 ± 0.76	5.04 ± 0.55	0.51 NS
	2.0	4.28 ± 0.65	4.88 ± 0.44	0.77 NS

For 5 replications

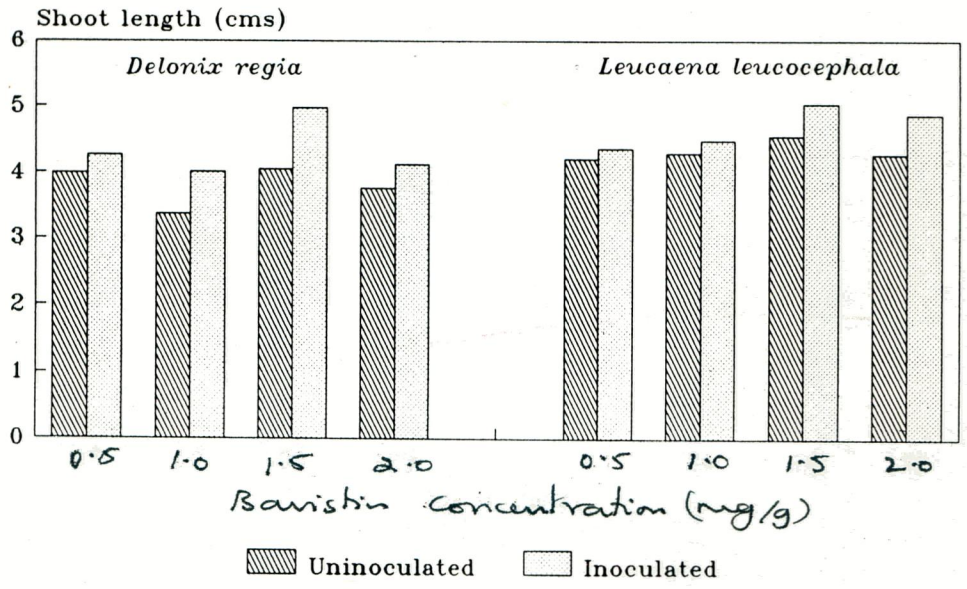
$ Groups compared: inoculated versus uninoculated

* Significant at 5% level

** Significant at 1% level

NS-Not Significant

FIG.14. EFFECT OF BAVISTIN TREATMENT AND RHIZOBIUM INOCULATION ON SHOOT LENGTH OF *D. REGIA* AND *L. LEUCOCEPHALA*



Summary and Conclusion

V. SUMMARY AND CONCLUSION

Nitrogen fixing trees can provide a useful and versatile tool for improving and maintaining forest productivity. The **Rhizobium**-legume symbiosis is one of the best known symbiotic associations which has contributed immensely to the biological fixation of atmospheric nitrogen.

The present study "Effect of **Rhizobium** biofertilizer on the growth of selected species of tree legumes" was undertaken to evaluate the efficiency of **Rhizobium** inoculation on the growth of two tree legume species viz., **Delonix regia** and **Leucaena leucocephala.**

Rhizobium strains were isolated and characterized from healthy nodules of **Leucaena leucocephala.** The seeds of **Delonix regia** and **Leucaena leucocephala** were treated with the isolated **Rhizobium** and allowed to germinate in polythene packets. Uninoculated controls were also maintained.

At the end of 30, 60, 90 and 120 days after sowing the tree saplings were carefully uprooted and subjected to a series of investigations (root and shoot lengths, plant fresh and dry weights, total nitrogen content, phosphorus content, potassium content, chlorophyll content and amino nitrogen contents of leaves). The T test of significance was carried out between the means of each parameter of the uninoculated and inoculated tree saplings. The results were discussed in chapter IV.

The present study showed that **Delonix regia** was found to contain no nodules.

However, **Leucaena leucocephala** saplings were found to be nodulating and the nodule number significantly increased due to **Rhizobium** inoculation.

On the 90^{th} and 120^{th} days the nodular fresh weight of **Rhizobium** inoculated **Leucaena leucocephala** saplings (0.166 and 0.223 grams respectively) was found to be significantly increased over that of the uninoculated saplings (0.109 and 0.169 grams respectively).

On 30, 60, 90 and 120 days, the root lengths of both **Delonix regia** and **Leucaena leucocephala** were found to increased significantly due to **Rhizobium** inoculation.

Significant increases in the shoot lengths were also obtained for the **Rhizobium** inoculated **Delonix regia** and **Leucaena leucocephala** tree sapling on the 30^{th}, 60^{th}, 90^{th} and 120^{th} days after sowing.

The data revealing these observations as shown in Tables III and IV. These increased rates of growth may be attributed to the nitrogen binding role of the Rhizobial symbionts that a lodged within the root issue.

From the results obtained it can be seen that **Leucaena leucocephala** grew faster **Delonix regia.** This enhancement in growth can be attributed to the specificity of the isolated strain towards **Leucaena leucocephala.**

The increase in biomass content of **Delonix regia** and **Leucaena leucocephala** in terms of plant fresh weight and dry weight was significantly due to **Rhizobium** inoculation. Highly significant increases were observed on the 90th and 120th days, the fresh weights of the **Rhizobium** inoculated saplings being 5.90 and 8.34 grams respectively for **Delonix regia** and 4.36 and 5.75 grams for **Leucaena leucocephala.** The fresh weights of the uninoculated saplings taken on the 90th and 120th days were 4.05 and 6.86 grams for **Delonix regia** and 2.35 and 3.29 grams for **Leucaena leucocephala.**

These observation showed that the **Rhizobium** inoculation was more effective as the age of the saplings increased.

Rhizobium inoculation did not significantly increase the total nitrogen content of **Delonix regia** and **Leucaena leucocephala** tree saplings.

The difference in the phosphorus and potassium content between the **Rhizobium** inoculated and uninoculated **Delonix regia** and **Leucaena leucocephala** tree saplings was found to be not statistically significant.

Rhizobium inoculation had little influence on the chlorophyll content of leaves of **Delonix regia** and **Leucaena leucocephala** on the 30th and 60th days after sowing. But on the 30th, 90th and 120th days chlorophyll content was significantly increased in the **Rhizobium** inoculated **Leucaena leucocephala** tree saplings.

The amino nitrogen content of the **Rhizobium** inoculated **Delonix regia** and **Leucaena leucocephala** was found to be not significantly enhanced when compared to the uninoculated saplings.

Hence it can be concluded from this study that the application of **Rhizobium** to tree legumes can help in achieving quick growth of trees and also bring about increased fertility. This study thus showed that an increase of certain biochemical constituents can be obtained by **Rhizobium** inoculation. Moreover, this study has proven to be agreeable with the fact that **Leucaena leucocephala** is a pioneer tree-due to its quick growth and nitrogen fixing capabilities.

Application of fungicides in potent plant protection scheme is largely undertaken. The effect of Bavistin treatment at different concentrations on the seed germination, root length and shoot length of both **Rhizobium** inoculated and uninoculated seedlings on the 10th day was also studied.

It was observed that there was a significant increase in the germination of **Rhizobium** inoculated **Leucaena leucocephala** when the concentration of Bavistin used was 1.5 mg/g.

No significant increase in the root and shoot lengths of **Delonix regia** and **Leucaena leucocephala** was observed by Bavistin treatment.

Forests - an investment for the future :

Of late, forest has become an issue of common concern in India mainly for three reasons; ecological consideration; commercial value and, exploitation of forest resources. The overall impact of forests with consequent widespread benefits to mankind is to soften the interaction of elements that comprise the ecological system. Large scale deforestation has brought the ecosystem to the brink of ecological collapse and drought and floods have become an annual feature.

Deforestation seems to have come to symbolise the situation of the over exploitation of natural resources. Unfortunately as an old Thai saying goes "experience is a comb which nature gives to a man after he is bald".

It is high time, people realise the consequences of continued uncontrolled forest exploitation and cooperate in the successful achievement of reforestation programmes.

Gaps of knowledge of biological nitrogen fixation in forest ecosystems are large. Therefore, there is a need to conduct nitrogen fixation studies on a long term basis and to screen and grade all nitrogen fixing tree species depending upon their nitrogen fixing performance under different agroclimatic conditions and then recommend them for different afforestation programmes.

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Appendix

APPENDIX I

ESTIMATION OF NITROGEN

Microkjeldahl method (Humphries, 1956)

Reagents

Diacid

1) 4:1 ratio of sulphuric acid and perchloric acid

2) **Mixed Indicator**

Dissolved 0.5 g bromocresol green and 1g of methyl red in 100 ml ethyl alcohol.

Procedure

1) Ground the plant sample and made it a fine powder.
2) Took 0.5 g of sample in a microkjeldahl flask.
3) Added 12 ml of diacid.
4) Digested the sample over a sand bath.
5) Made up the volume to 100 ml with distilled water.
6) Pipetted out 10 ml of the aliquot into a microkjeldahl distillation apparatus.
7) Kept at the delivery end 10 ml of 2% boric acid with mixed indicator in a 100 ml beaker.
8) Added 10 ml of 40% sodium hydroxide into the microkjeldahl distillation apparatus and steamed the distillate until a blue colour was reached.
9) After distillation, titrated against N/50 sulphuric acid until a wine red colour was got.

Calculation

$$\text{Nitrogen content (\%)} = \frac{0.00028 \times \text{Titre value} \times 100}{10} \times \frac{100}{0.5}$$

APPENDIX II

ESTIMATION OF PHOSPHORUS

Estimation of phosphorus was done by the method of Jackson (1973), in all the plant samples.

Reagent

Barteu Reagent

1) *Solution A* 25 g of Ammonium molybdate is dissolved in 400 ml of warm water.

2) *Solution B* 1.25 g of Ammonium metavanadate is dissolved in 300 ml of boiling water.

Solution A is aded to solution B and the volume is made upto 1000 ml.

Procedure

1) From the acid extract pipetted out 5 ml of the aliquot into a 25 ml volumetric flask.

2) Introduced a bit of red litmus paper.

3) Neutralised with Ammonia solution until litmus paper turned blue.

4) Again acidified it with concentrated nitric acid until litmus paper turned red.

5) Added 5 ml of Barteu reagent.

6) Made up the volume to 25 ml with distilled water.

7) After 30 minutes the intensity of yellow colour developed was read at 420 nm in a colorimeter.

8) Using the phosphorus standard curve, the phosphorus concentration in the sample was read in ppm.

Calculation

$$\text{Phosphorus content (\%)} = \frac{\text{P concentration in ppm}}{10^6} \times \frac{25}{5} \times \frac{100}{0.5} \times 100$$

Preparation of phosphorus standard curve

Analytical grade of potassium dihydrogen phosphate was dried at 40°C and 0.2195 g was dissolved in 400 ml of distilled water, 25 ml of 7N sulphuric acid and the volume was made upto 1000 ml. This is 50 ppm of phosphorus. Then 100 ml of this 50 ppm stock solution was diluted to 1000 ml to get 5 ppm of phosphorus solution. From this a series of phosphorus standards ranging from 0.01 ppm to 1.0 ppm were prepared. Finally 5.0 ml of this solution was pipetted out into a 25 ml volumetric flask. 5.0 ml of Barteu reagent was added and volume was made upto 25 ml. Intensity of the colour of each standard was measured on the colorimeter and a standard curve was constructed using meter readings and concentrations.

APPENDIX III

ESTIMAITON OF POTASSIUM

Potassium content of all the plant samples was estimated by the method of Jackson (1973).

Procedure

1) 5.0 ml of the acid extract was pipetted out into a vial and the transmission was read in flame photometer.
2) Using a potassium standard curve, the concentration of potassium was determined.

$$\text{Potassium content (\%)} = \frac{\text{K concentration in ppm}}{10^6} \times \frac{100}{5} \times \frac{100}{0.5} \times 100$$

Potassium standard curve

1.907 g of Analar grade Potassium chloride was dissolved in 100 ml distilled water to get 1000 ppm of potassium. 100 ml of 1000 ppm potassium was diluted to one litre to get 1000 ppm of potassium. From this a series of potassium standards ranging from 0-100 ppm were prepared and percentage transmission was read on a Flame photometer to construct a standard curve.

APPENDIX IV

ESTIMATION OF CHLOROPHYLL

Method of Yoshida *et al.*, (1971)

Reagent : 80% acetone.

Method :

Cut 1.0 g fresh leaves into small pieces and homogenised in a mortar with a pestle using excess acetone. Decanted and filtered the supernatant on a Buchner funnel through Whatman No.42 filter paper. Added sufficient quantity of 80% acetone and repeat the extraction. Transferred the contents from the mortar to the Buchner funnel and washed the brei with acetone until it became colourless. Pooled the filtrates and made up the volume to 100 ml in a volumetric flask. Transferred 5.0 ml of the extract into a 50 ml volumetric flask and diluted by making up the volume with 80% acetone. Measured the absorbance at 645 and 663 nm for the determination of total chlorophyll.

Calculation

The chlorophyll content was calculated on a fresh weight basis employing the following formulae :

$$\text{Total chlorophyll (mg/g)} = \frac{20.2 A_{645} + 8.02 A_{663}}{a \times 1000 \times W} \times V$$

$$\text{or} \qquad = \frac{27.8\ A_{652}}{a \times 1000 \times W} \times V$$

a - Length of path light in the cell (1 cm)

v - Volume of the extract in ml

w - Fresh weight of the sample.

APPENDIX V

ESTIMATION OF AMINO NITROGEN

Amino nitrogen content was estimated in leaf extract by Ninhydrin method.

Preparation of the leaf extract:

5 g of finely chopped leaf tissue was suspended in 25 ml. of 80% ethanol and extracted on a hot water for 5 mts. After cooling the material was homogenised by grinding with the help of Pestle and mortar and the extract was squeezed through cheese cloth. The residue was reextracted for 2 mts. in 10 ml. of hot 80% ethanol and filtered. The extracts were pooled and filtered through whatman No.42 filter paper. The filtrate was brought to 25 ml. by adding 80% ethanol. So that 5.0 ml. of the extract represented one g of the tissue.

Reagents :

Reagent A **:** - 10 ml of 1 N sodium hydroxide + 1.05 g citric acid. The solution was made up to 25 ml. Out of this take 20 ml. and add 32 mg. of stannous chloride.

Reagent B : 20 ml. of fresh methyl cellosolve solution + 800 mg. of ninhydrin.
Mix Reagent A and B.

Reagent 2 : Diluent solution : n. propanol 50 ml + distilled water 50 ml. (1:1).

Procedure :

To 1.0 ml. of the extract added 1.0 ml. of reagent 1. The tubes were placed for 20 minutes in boiling water bath. On removal from the bath added 5.0 ml. of reagent (2). The solution in the tubes were made upto 25 ml. and filtered through an ordinary filter paper. The colour developed was read at 570 nm in a colorimeter.