

**“EFFECT OF *RHIZOBIUM* INOCULATION AND  
NITROGEN FERTILIZER APPLICATION ON  
GROWTH PERFORMANCE OF *DALBERGIA SISSOO*  
TREE SEEDLINGS.”**

**M.Sc. (Ag.) THESIS**

**by**

**ANISH KUMAR**

**DEPARTMENT OF MICROBIOLOGY  
COLLEGE OF AGRICULTURE  
INDIRA GANDHI KRISHI VISHWAVIDYALAYA  
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TREE SEEDLINGS.”**

*Thesis*

Submitted to the

**Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)**

*by*

**ANISH KUMAR**

IN PARTIAL FULFILMENT OF THE  
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in

**AGRICULTURE  
(MICROBIOLOGY)**

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## CERTIFICATE - I

This is to certify that the thesis entitled "EFFECT OF *RHIZOBIUM* INOCULATION AND NITROGEN FERTILIZER APPLICATION ON GROWTH PERFORMANCE OF *DALBERGIA SISSOO* TREE SEEDLINGS", submitted in partial fulfillment of the requirements for the degree of "Master of Science in Agriculture" (Microbiology) of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by Mr. ANISH KUMAR under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate, award etc.) or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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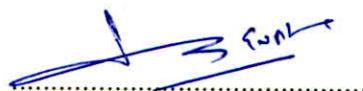
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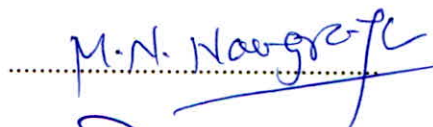
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Member Dr. S.B. Gupta



Member Dr.M.N. Naugraiya



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This is to certify that the thesis entitled “EFFECT OF *RHIZOBIUM* INOCULATION AND NITROGEN FERTILIZER APPLICATION ON GROWTH PERFORMANCE OF *DALBERGIA SISSOO* TREE SEEDLINGS.”, submitted by Mr. ANISH KUMAR to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in partial fulfillment of the requirements for the degree of M.Sc. (Ag.) in the Department of Microbiology has been approved by the external examiner and student’s advisory committee after an oral examination.

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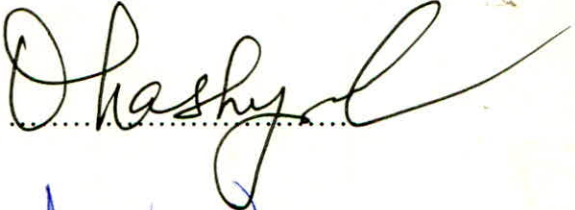
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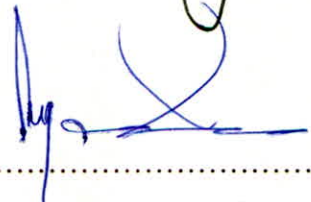
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*"Education plays fundamental role in personal and social development and teacher plays a fundamental role in imparting education. Teachers have crucial role in preparing young people not only to face the future with confidence but also to build up it with purpose and responsibility. There is no substitute for teacher pupil relationship". I start in the name of God-who has bestowed upon me all the physical and mental attributes that I possess and skills to cut through and heal a fellow human.*

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**College of agriculture, Raipur (C.G.)**

Date: 2/08/2010

  
**Anish Kumar**

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## LIST OF ABBREVIATIONS

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ABBREVIATIONS	FULL FORM
%	percent
/	Per
@	at the rate
°C	degree Celsius
BNF	Biological Nitrogen Fixation
CD	Critical Difference
cm	centimeter
DAT	Date after transplant
E.C.	Electrical Conductivity
<i>et al.</i>	and co-workers/ and others
Fig.	figure
g	gram
ha	hectare
hr	hours
i.e.	that is
kg	kilogram
mg	milligram
ml	milliliter
mm	millimeter
NFT	Nitrogen Fixing Tree
NPK	Nitrogen, Phosphorus and Potassium
pH	potentiality of hydrogen
SEm ±	Standard Error of mean
<i>Viz.</i>	for example

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## CHAPTER - I

### INTRODUCTION

Nitrogen is one of major plant nutrients and important for normal plant growth and development. A continuous tree growth depends upon the replenishment of rhizospheral N pool in forest soils. Excessive application of nitrogenous fertilizer is not only uneconomical but it also adversely affects the environment and crop growths (Kenney, 1982, Sehgal *et al* 1992). Thus the influence of Nitrogen Fixing Trees (NFTs) in soil N management should be given attention. *Dalbergia sissoo* is an important Nitrogen Fixing Tree (NFT) species belongs to family leguminasae, subfamily papilionoideae and widely used in agroforestry, afforestation programs and farm forestry. Being a fast growing NFTs, this species is highly valued for furniture and timber purpose.

Very often leguminous trees are established in the field by transplanting seedlings from nursery. To be successful after outplanting, sissoo seedlings must access sufficient nutrient from the soil and out compete others. In modern forestry, it is important to produce quality seedlings by inducing morpho - physiological changes in the plants for making them competent enough to bear the shock of field planting and enhancing their productivity. Successful establishment of nitrogen fixation tree (NFTs) in plantation programme is possible through the production of high quality tree seedling at nursery levels (Rensberg and Strijdom, 1985).

Role of inorganic fertilizer in boosting the growth of forest tree species either in nursery or plantation in terms of girth, height and also biomass increment has been

studied in several tree species (Totey *et al.*, 1986). However these fertilizers are costly and cumbersome to use over vast plantation areas besides causing soil pollution if not judiciously used (Troeh *et al.*, 1980, Prasad, 1988). This age of increasing prices along with the increasing demand of chemical fertilizers and depleting soil fertility necessitates the integrated nutrient management involving biofertilizers.

Bio-fertilizer has tremendous potential to provide plant nutrients as it helps to boost microbial population present in soil which in turn makes the insoluble nutrient available for growth of the plants. (Subba Rao, 1977). Biofertilizer hold a promise to improve the quality of tree seedlings which are better adapted to withstand the adverse condition. *Rhizobium* inoculation of NFTs at seedling stage helps in producing healthy stocks in nursery, capable of growing successfully when planted in field (Totey *et al.*, 2000). The role of *Rhizobium* bacteria trapping atmosphere N there by enhancing soil fertility is well establishment in agriculture. However its role in forestry needs to be explored. The effects of *Rhizobium* inoculation on growth and nodulation of some important forest legumes has been studied indicating tremendous potentiality of these biofertilizer in improving health of soil as well as plant stock (Kabi *et al* 1982). .

*Dalbergia sissoo* being an N-fixing tree can be utilized to its fullest extent only if it occurs successfully symbiotic relationship with *Rhizobium* strain. A study indicate that native *Rhizobium* in soil produce poor quality, low number of nodules, fixing insufficient atmosphere of nitrogen (Pancholy, 1992). This suggested that in *Dalbergia sissoo* the bio mass production and timber quality could be improved by inoculation

with specific *Rhizobium* culture. Therefore an attempt has been made to isolate *Rhizobium* associated with nodules of *Dalbergia sissoo* and applying *Dalbergia sissoo* specific *Rhizobium* inoculation to *sissoo* seedling to enrich the rhizobial population at rhizospher zone of *Dalbergia sissoo* seedling which could be effective for maintaining healthy vigorous nursery stocks for large scale plantation.

The efficiency of symbiosis depends upon most optimum soil and environmental condition. Major factors likely to influence response of legumes to inoculation are density of indigenous rhizobia in soil, availability of mineral N and effectiveness of indigenous rhizobia (Singleton *et al* 1992). Becker *et. al.* 1991 reported that legumes do not fix sufficient N to obtain maximum growth response and so addition of soil-N is required for enhancing the biomass at nursery stages of growth. The starter dose of N given to legumes also influenced the amount of N<sub>2</sub> fixed (Johanson *et al* 1975).

It was also observed that high level of soil nitrates could be a potent inhibitor for nitrogen fixation. Streeter (1988) observed that legumes could fix greater amount of N and contribute more N to soil in an optimum range of N level. At the same time the evaluation of optimum level of nitrogenous fertilizer in which the inoculant gets maximum activity is also necessary (Prasad *et al.*, 1998).

Therefore, the present investigation was undertaken to isolate, characterize the rhizobial bacteria from nodules of *Dalbergia sissoo* tree seedling and to evaluate the performance of isolated *Rhizobium* individually and in combination with different doses of nitrogen fertilizer on growth performance of *Dalbergia sissoo* seedlings.

Keeping these points in view, the experiment entitled “**Effect of *Rhizobium* inoculation and Nitrogen fertilizer application on growth performance of *Dalbergia sissoo* tree seedlings.**” was carried out with following objectives.

1. To see the response of legume tree seedling to *Rhizobium* inoculation.
2. Influence of Nitrogen application on biologically fixed amount of nitrogen by legume tree seedlings.
3. Population dynamics of inoculated *Dalbergia sissoo Rhizobium* under glass house conditions.

## CHAPTER – II

### REVIEW OF LITERATURE

The review of literature on work done past is essential to understand the problem in-depth and provides necessary guidelines as well as feedback for fulfillment of objectives of the study. So, this chapter deals with the review of study or research work relevant to the study.

1. To see the response of legume tree seedling to *Rhizobium* inoculation.
2. Influence of Nitrogen application on biologically fixed amount of nitrogen by legume tree seedlings.
3. Population dynamics of inoculated *Dalbergia sissoo Rhizobium* under glass house conditions.

#### 2.1 To see the response of legumes tree seedling to *Rhizobium*

##### **Inoculation:**

It has been an established fact that the bacterial inoculation of legumes is quite necessary for their proper establishment when grown in new place (Jenkins *et al.* 1954). Nodule bacteria (rhizobia), in association with leguminous hosts, fix at least  $90 \times 10^6$  metric tons of N annually in the world. This is more than twice the amount of N, used in chemical fertilizers and more than one-half the total amount of this element fixed biologically each year (Hardy and Holsten, 1972). Mahanta (1969) reported that rhizobia supply nitrogenous compounds to the host plant (leguminous plants). *Rhizobium* spp., have the ability to infect roots of leguminous plants, form nodules and work symbiotically with their host in fixing molecular N. The *Rhizobium* leguminous

plant association offers the greatest promise of all systems for providing the nutritious protein food which will be needed in the year's ahead (Pepler and Perlman, 1979).

Dreyfus and Dommergus (1981 a, b) reported that forest legume are nodulated by wide range of slow and fast growing strains of rhizobia, all the strain were not found to be equally effective in fixing nitrogen symbiotically. Even among the effective strains, deficiency of the strains may vary widely.

Kabi *et al.* (1982) investigated the effect of *Rhizobium* inoculation on the growth and nodulation of some important forest legume and came to a conclusion that use of biofertilizers in forestry has a tremendous potentiality of nitrogen turn over from the atmosphere.

Poi and Kabi (1983) found that inoculation significantly increased fresh weight and N<sub>2</sub> content of pot grown plants. Plants in adjacent fields had an average of 44 and 55 nodules/plant and recovery of introduced *Rhizobium* strains was poor due to the high competitive ability of native strains. Similarly, Wahhab and Bhuiya (1984) reported that oil and protein contents in groundnut were positively correlated with nodules/plant but not with nodule size.

Free - living N<sub>2</sub> - fixer develop slowly because of relatively limited habitat and energy sources. In contrast, symbiotically living micro organism provide relatively large amount of nitrogen, particularly those which from root nodules association between plant and *Rhizobium sp.* of bacteria (Tarrant 1983).

Successful establishment of nitrogen fixation tree (NFTs) in plantation programme is possible through the production of high quality tree seedling at nursery levels (Rensberg and Strijdom, 1985).

Prasad and Ram (1986) found that *Rhizobium* inoculation increased all the parameters over uninoculated control possibly owing to N<sub>2</sub> fixation and favorably affecting P solubilization in rhizosphere soil.

Basu and Kabi (1987) found that application of biofertilizer, *Rhizobium* or *Rhizobium* + *Azotobacter* combined, has enhanced nodulation and growth of seven forest legumes significantly. Activity of the inoculants was found to increase further, at least in some cases, due to plating of inoculated seed with lime. Biofertilizer application was found to augment dry matter production in different forest legumes of which *Leucaena leucocephala* registered the maximum response. Need for inoculating different tree legumes, as routine cultural practice, was emphasized.

Prasad (1988) studied the effect of *G. fasciculatum* and *Rhizobium* on biomass yield and nutrient uptake of *Dalbergia sissoo*. Maximum shoot length (116.2cm), root length (26.2cm), shoot dry weight (80.72g), number of leaves/plant (99.1), N (3.98 %), P (0.196 %) and K (1.96 %) were recorded in double inoculated plants.

Javid and Fisher (1989) reported that *D. sissoo* and *L. leucocephala* form effective symbiotic associations with native rhizobia strains from the arid plains of Pakistan. The *Rhizobium* associated with *L. leucocephala* was fast growing and acid producing, whereas that associated with *D. sissoo* was slow growing and alkali producing. *L. leucocephala* rhizobia had an acetylene-reducing potential of 0.38 micro mol/h C<sub>2</sub>H<sub>4</sub> per plant, whereas the activity of *D. sissoo* rhizobia was only 0.16 micro mol/h C<sub>2</sub>H<sub>4</sub> per plant. Specific nitrogenase activity (C<sub>2</sub>H<sub>2</sub> reduction) of *L. leucocephala* rhizobia nodules was 20.8 micro mol g<sup>-1</sup>h<sup>-1</sup> C<sub>2</sub>H<sub>4</sub>, whereas that of *D. sissoo* nodules was 27.9 micro mol g<sup>-1</sup>h<sup>-1</sup> C<sub>2</sub>H<sub>4</sub>. Strong correlations were observed

between number of nodules, nodule dry weight, shoot dry weight and acetylene reducing activity in both tree species.

Herrera *et al.*, 1993 ; Galiana *et al.* , 1994 reported that the inoculation on woody legume with selected rhizobial strains, which showed increased survival percentage in seedling and greater biomass production in all the inoculated trees.

Sharma *et al.* (1994) observed the effects of rhizobial inoculation on seed germination and seedling growth of *Dalbergia sissoo*, *Acacia auriculiformis* and *Acacia catechu*. No significant response to inoculation was observed in seed germination. However, seedlings, which were inoculated 1 and 2 weeks after transplanting in the nursery (at 2 months old), showed a positive growth response 3 months later. The best response was by *A. catechu*. Inoculated seedlings had more effective nodules than those not inoculated.

Toky *et al.* (1994) reported that nitrogen-fixing abilities of *Acacia nilotica* subsp. *indica*, a semi-arid Indian tree inoculated with *Rhizobium* AC-1 at 15, 30 and 60 p.p.m. of nitrogen fertilizer (urea) were assessed in sterilized sand in chillum jars. The increasing level of fertilizer did not significantly affect ( $p > 0.01$ ) the nodulation, it significantly ( $p < 0.01$ ) decreased nitrogenase activities, although there was a significant increase in the growth of plants and their nitrogen content. The effect of *Rhizobium* in increasing nitrogen concentration in plants at different levels of fertilizer was highly significant compared to uninoculated plants. The results are useful from the point of view of afforestation of wastelands with *Acacia nilotica* in semi-arid regions.

Jamaluddin *et al.* (1995) found that the effects of pure cultures of *Rhizobium* strains from the nodules of different forest tree species (*Dalbergia sissoo*, *Leucaena leucocephala*, *Pongamia pinnata* and *Albizia lebbek*) were tested on the growth of *A. lebbek* seedlings in the nursery. Seeds were scarified and inoculated before planting in polybags in soil/manure/sand (2:1:1). One seedling per bag was maintained, and plants were watered daily and grown for 3 months before uprooting and measuring. 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 controls, but inoculation with the *Rhizobium* strain from *Albizia lebbek* was more effective than with *Rhizobium* strains from the other legume species.

Varma *et al.* (1996) reported that application of *Rhizobium* broth (5ml/plant) and 100gm single superphosphate alone or in combination increased are plant growth (plant height, collar diameter, and root length). Height of seedling of *D. sissoo*, after a period an one year increased by 251.60%, collar diameter by 131.70% root lengths by 193.18% shoot fresh weight by 118.61%, shoot dry weight by 124.48%, root fresh weight by 113.57%, and root dry weight by 122.64% due to application of 5ml *Rhizobium* broth (bio-fertilizer) and 100g Single superphosphate per plant 113.57%

Purohit *et al.* (1997) observed seedling of NFT growing in Himalayan region and were assessed for their nodulation inoculation in term of nodule No. was highest in *D. sissoo* and *D. serica* but quantitatively the highest nodule weight per plant under the treatments. On the basis of nodule nitrogen, although *A. stioulata* had highest nitrogen fixed per plant, the rhizobial strain associated with *D. serica* seems to be highly efficient than other species.

Totey *et al.* (1997) found that a treatment involving dipping seeds in *Rhizobium* biofertilizer slurry for 24 h and then sowing increased germination of *Dalbergia sissoo* to 78% (compared with 50% in control untreated seeds). The treatment also increased shoot height and root length of 3-wk-old seedlings to 4.5 and 5.0 cm, respectively, as against 2.8 cm each in the control. The total length of seedlings from treated seeds was almost double that of controls.

Aryal *et al.* (1999) investigated effects of inoculation of *Rhizobium* suspension on nodulation and plant growth were examined with *Albizzia procera*, *Albizzia lebbeck* and *Leucaena leucocephala* seedlings grown on sterilized and non-sterilized soil media. Inoculation resulted in nodule number increases of 28.6, 29.02 and 23.9 times in sterilized soil and 3.4, 3.6 and 3.27 times in non-sterilized soil for *A. procera*, *A. lebbeck* and *L. leucocephala* seedlings respectively. Total dry mass increased by 127.6%, 66.7% and 60.7% in sterilized soil and 100%, 95.5% and 52.65% in non-sterilized soil for these three legume trees, respectively, after a period of two months. Significantly high inoculation responses of root length, root diameter, collar diameter, shoot length, and dry mass of root, shoot, leaves and nodules were also observed in both sterilized and non-sterilized soil media as compared to respective control treatments. The response to inoculation was strong in sterilized and modest in non-sterilized soils. The significantly higher response to *Rhizobium* inoculation over control in all the species tested suggested that application of *Rhizobium* greatly enhanced plant growth, nodulation, biomass production and nitrogen-fixing activity of the nodules.

Totey *et al.*(2000) observed application of 2gm coal based *Rhizobium* culture (biofertilizer) per plant has increased the relative height growth of one year old plantation of *D. sissoo* by 1.5 times as against control. Though interaction of coal based *Rhizobium* culture and single super phosphate was non-significant, the best combination was 2g coal based *Rhizobium* Bio-fertilizer and 75g ssp. per plant. Looking towards the easy availability and low investment, biofertilizer (particularly coal based *Rhizobium* culture) may be preferred over inorganic phosphatic fertilizers in order to boost up overall biomass of leguminous forest trees.

Khan *et al.* (2001) observed that a nursery experiment was conducted to study the impact of arbuscular mycorrhizas and *Rhizobium* inoculations alone and in combination with NPK fertilizers on the growth of *Dalbergia sissoo*. Maximum value for all the parameters (shoot height, collar diameter, root and shoot dry weight and total biomass) was recorded when full dose of fertilizers was applied and equally significant response was recorded with AM inoculations.

Morques *et al* (2002) reported that a higher biomass production was in inoculated seedlings of *Centrolobium tomentosum*, which was attributed to better growth, high nodulation and also relatively more nutrient uptake from control seedlings.

Chuohan and Pokhriyal (2002) in this study, *Albizia lebbek* seedlings were treated with and without inorganic nitrogen and inoculants with *Rhizobium*. It was observed that the plants treated with both nitrogen and *Rhizobium* performed better than those, which received either one of them or none (control). Growth parameter *i.e.* plant height, collar diameter and root length were observed to follow an increasing

pattern with growth irrespective of the nature of treatment. New leaf flushes appeared from April onwards reaching maximum in the month of August and September, followed a decreasing tend therefore.

Kaushik *et al.* (2003) observed the influence of *Glomus mosseae* on the nodulation and nutrient content of *Acaccia nilotica* and *Dalbergia sissoo*. Inoculation of VAM fungi significantly increase the number of nodules (34.30) in *A. nilotica* while in *D. sissoo* it was only 15.88. Highest concentration of N (1.82 %) and P (0.28 %) were recorded in *A. nilotica* compared to *D.sissoo* in which they were only 1.47 % and 0.22%, respectively . higher concentration (1.26%) of K was found in *D.sissoo*, while in *A. nilotica* it was 1.22%.

Mohanty *et al.* (2004) found that a nursery experiment in polypots was conducted during July to October 2000 at the silviculture nursery of OUAT, Bhubaneswar, Orissa, India to determine the effect of *Rhizobium* inoculations on growth and biomass production of *Dalbergia sissoo* in acid lateritic soil amended with lime and farmyard manure (FYM). The result showed that application of *Rhizobium* culture with FYM to the lime amended soil influenced the seedling vigour, viz., shoot length, root length shoot and root biomass, root volume and root nodules.

Mahmood, and Javaid (2005) reported that the rhizobia from the root nodules of leguminous plants *Albizia lebbeck*, *Dalbergia sissoo*, *Leucaena leucocephala*, *Pithecellobium dulce*, *Prosopis glandulosa*, *P. juliflora* and *Vigna unguiculata*, which were growing in and around Karachi, Pakistan, were tested for their ability to produce root nodules on *V. unguiculata*. The effects of the symbiosis on dry matter production and total nitrogen content of the host species were also recorded. Isolates from all the

leguminous plants produced nodules on *V. unguiculata*. Isolates from *D. sissoo*, *L. leucocephala*, *Pithecellobium dulce* and *Prosopis glandulosa* were the most effective in nitrogen fixation and significantly increased the dry weight and nitrogen content of the host plants.

Okunomo *et al.* (2007) investigated the effect of *Rhizobium* inoculation on the seedling growth and development of *Albizia niopoides* in a glass house at Nigeria. There were four treatment in all namely 1 ml. *Rhizobium* inoculation 5 ml., 10ml. and control the parameter considered were height collar diameter, leaf number and leaf area. 10 ml. *Rhizobium* inoculations gave maximum height of 93.6 cm. diameter increments of 0.77 mm. (14WAP), leaf number 14.7 and leaf area 193.4. the inoculated treatments produced nodule number ranging between 336.7 and 496.0 while uninoculated treatment gave 247.6 nodules dry matter production was directly proportional to the quantity of *Rhizobium* inoculation applied. 10ml. *Rhizobium* broth produced the highest nodule dry matter of 24.7 g. which was significantly different from the control (13.97 g.)

Mahmood and Athar (2008) studied Cross-inoculation experiments were conducted in the greenhouse to test the rhizobia isolated from nodules of seven tree legumes for their effectiveness in *Vigna mungo* plants. The tree legumes included *Albizia lebbeck*, *Dalbergia sissoo*, *Leucaena leucocephala*, *Pithecellobium dulce*, *Prosopis cineraria*, *Prosopis glandulosa* and *Prosopis juliflora*, all growing under arid environment. Rhizobia from these legumes formed nodules on the roots of *Vigna mungo* except isolates from *Albizia lebbeck*. Dry weight and nitrogen contents of *Vigna mungo* plants increased significantly ( $P < 0.05$ ) in response to cross inoculation

as compared to uninoculated control. Rhizobia from *Leucaena leucocephala* and *Prosopis glandulosa* showed significant increase in dry weight ( $P < 0.05$ ) and nitrogen contents ( $P < 0.05$ ) than other inoculated treatments. The natural rhizobia of wild tree legumes growing under arid environment show higher tolerance to prevailing adverse conditions like salt stress, elevated temperatures and drought. These rhizobia may be used to inoculate wild as well as crop legumes cultivated in reclaimed desert lands. These rhizobia may have specific traits that can be transferred to other rhizobia through genetic engineering tools. The cross infection of agriculturally important legumes with isolates from wild legumes may prove a useful means of increasing nitrogen contents within these plants.

Merbach *et al.* (2008) reported that the effect of *Rhizobium* inoculation increased the symbiotic  $N^2$  fixation of lupins in absence of mineral nitrogen. In presence of mineral N the effect of *Rhizobium* inoculation was not significant. For getting high yield of lupins, a *Rhizobium* inoculation is essential.

## **2. 2 Influence of Nitrogen application on biologically fixed amount of nitrogen by legume tree seedlings:**

Seedling quality specification has traditionally been based on certain morphological character such as sturdiness (height/Diameter ratio), root/shoot ratio and some other root features (Lavender and Cleary, 1974, Schmidt-Vogt, 1974).

Hussain *et al.* (1986) reported that the adverse effect on nitrogen fertilization on nodulation of *A. Procera*. Summerfield (1977) and Dazzo and Brill (1978), also found that excessive nitrogen application reduce root hair infection, nodule number. Similarly observation reported by (Kenney, 1982; Sehgal *et al.*, 1992). Excessive

application of nitrogenous fertilizers is not only uneconomical but it also adversely affects the environment and crop quality

Beneficial effect of low nitrogen doses on the growth and development of the plant have been reported by many investigators (Prasad *et al* 1998; Eaglesham, *et al.* 1983 and Katoch *et al.* 1983)

Pokhriyal *et al.* (1987) observed that polypotted plants of *Albizia lebbeck*, *Acacia nilotica*, *D. sissoo* and *L. leucocephala* were transferred to 12-inch diam. earthen pots filled with well-sieved soil and grown for 3 months in a glasshouse. Four plants of each species were then uprooted and measurements made of ht, fresh wt. and numbers of nodules, and nitrogenase activity (using the acetylene reduction assay). Max. values were observed for *L. leucocephala* for all parameters, followed by *A. lebbeck*, *A. nilotica* and *D. sissoo*.

Prasad (1988) inorganic fertilizers indicates that their increased use result not only in soil pollution but also soil deterioration. Similar observation also earlier reported by (Troch *et al.*, 1980; Kinhal, 1985).

Streeter (1988) observed that legume could fix greater amount of N and contribute more N to soil in an optimum range of level. Therefore, it is necessary to evaluate the optimum dose of N fertilizer in which efficient strains on inoculation can successfully fix nitrogen.

Pancholy (1992) suggested that in *P. juliflora*, the biomass production and fodder quality could be improved seed inoculation with efficient rhizobial cultures.

Aryal *et al.* (2000) observed that the nodulation status and measure nitrogenase activity of some important legume tree species in a nursery. Nitrogenase activity was

highest in *L. leucocephala* (4913.59 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup>) followed by *Albizia procera* (2080 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup>). Seedling height, nodule fresh weight, root fresh weight and nitrogenase activity (per nodule, per gram nodule fresh weight, per gram root fresh weight and per gram root dry weight) were also highest in *L. leucocephala*.

The value was significantly reduced in case with MLU (Moderate level of urea) and HLU (High level of urea) amended soil. This may be, due to the fact that the higher rates of N fertilizer may have produced nutrient imbalance thus rendering unavailable the other elements in soil. (Jha *et al.*, 2000).

Singh *et al.* (2000) seeds of 3 nitrogen fixing tree species (*Dalbergia sissoo*, *Acacia nilotica* and *Prosopis cineraria*) were sown in polythene bags in sand/FYM/soil media at different ratios. In all treatments, the germination percentage was highest in *D. sissoo*, lower in *P. cineraria* and lowest in *A. nilotica*. Among the growing medium treatments, sand/FYM/soil at a ratio of 1:1:1 gave the maximum germination in *D. sissoo* (91%), while for *P. cineraria* maximum germination (90%) was recorded at a ratio of 2:1:1 and for *A. nilotica* maximum germination (84.0%) was recorded at a medium ratio of 1:1:2. For *P. cineraria* and *A. nilotica* the same media also gave maximum shoot and root length, while for *D. sissoo* maximum shoot and root length was recorded in a medium ratio of 1:1:2 (which gave the third best germination of 89%). The number of nodules was more in *Prosopis cineraria* than in the other 2 species.

Ahmed *et al.* (2006) investigated the effect of *Rhizobium* inoculation and nitrogen on performance of mungbean (*Vigna radiata* L.), The research material consisted of mungbean variety (NM- 98) with treatments of seed and soil inoculation

and nitrogen levels at 15, 30 and 45 kg ha<sup>-1</sup>. Data were recorded on, number of nodules per plant, root length, plant height at maturity and biological yield per plant. Soil and seed inoculation in combination with N fertilizer positively affected the growth and nodule formation of green gram. Among all the treatments, seed inoculation + 15 kg N ha<sup>-1</sup> was found effective.

Anjum *et al.*, (2006) investigated that the mungbean (*Vigna radiata* L.) is capable of fixing atmospheric nitrogen through *Rhizobium* species living in its root nodules. To evaluate the effect of inoculations and nitrogen levels on performance of mungbean, a pot experiment was conducted during spring 2004. Mungbean variety NM-98 was sown at 20 kg ha<sup>-1</sup> in pots. Seed and soil inoculation, and nitrogen levels at 15, 30 and 45 kg ha<sup>-1</sup> were applied. Data on recorded on number of pods per plant, number of seeds per plant, 100- seed weight and seed yield were recorded. Yield and yield components of mungbean crop were significantly affected by both inoculation and fertilizer application. Seed inoculation was more affective and gave better results than soil inoculation.

Bora *et al.* (2006) a pot experiment was conducted by using isolates inoculates of *Rhizobium* and *Rhizobium* + recommended doges of fertilizer at different levels of N to evaluate their individual and combined effect on symbiotic traits and growth and biomass production of *Albizia procera*. Seven strain of *Rhizobium* were isolates from *Albizia procera* seedlings. Among them Ab (1) 100, Ab (m) 98 and Ab (1) 32 were found to be efficient, having positive effect on growth, nodulation and biomass production .Nodulation was recorded maximum in Ab (1) 32 inoculated seedling, being 69.82% higher than control (uninoculated ones). The combined effect of

*Rhizobium* inoculation along with application different levels of N dosages of fertilizer showed significant increase in growth, nodulation and biomass production at low level urea-amended soil (20kg/h). Nodulation was found to be suppressed in medium level urea (40kg/h) application although there was a significant increase of growth and biomass production of plant. However, an exception was noticed in *Ab* (m) 98 inoculated seedlings where in significantly high value of nodulation was recorded when medium level urea was applied to soil. Stunted growth and poor nodulation was observed when high level urea (60kg/h) was too applied.

Khan *et al.* (2006) microbial inoculants as effective microorganism was used to find out their influence on seedlings growth and development of *Albizia procera* (Roxb) Benth. The seedlings were grown in a mixture of forest top soil and cow dung (3:1) kept in polybags. The E M solution at different concentrations, (0.1, 0.5, 1, 2.5 and 10%) was incorporated before and after a week of sowing the seeds. Germination as well as physical growth parameter like shoot and root length, vigor index collar diameter leaf no., fresh and dry weight of shoot and root and total biomass increment were measured. The nodulation status influenced by EM was also observed along with the measurement of chemical parameters viz. chlorophyll a, b and carotnoid. Both germination and the measured physical growth parameters were found significantly ( $P < 0.05$ ) higher in seedlings treated with different concentrations of EM solution in comparison to control. Max growth was observed at 2% concentrations followed by 5% and 1%. Nodulation was higher at 0.1% conce.of EM. But in normally decreased with the increased of concentrations. Leaf pigments were also Significantly ( $P < 0.05$ ) higher in most of the treatments with respect to control. The result of the present study

indicate that the EM technology might be useful to improve the growth of seedlings in the nursery. These also indicate that the associated beneficial organisms along with the polybags soil might be of value in improving the degraded soil or poor field soil for better nutrient and water uptake during the initial growth of transplanted seedlings.

Huda *et al.* (2007) studied the influence of different inorganic fertilizers (phosphorous and potassium) on the nodulation and growth of *Dalbergia sissoo* grown in the nursery. Before seeds sowing, different combinations of P, K fertilizers were incorporated with the nutrient-deficient natural forest soils, and then amended with cowdung (soil: cowdung = 3:1). Nodulation status (nodule number, shape, fresh weight, dry weight and color) in the roots and the plant growth parameters (length of shoot and root, collar diameter, fresh and dry weight of shoot and root) were recorded 60 days after seeds sowing. Nodulation status and growth of the plants varied significantly ( $P < 0.05$ ) in the soils amended with fertilizers in comparison to the control. The highest nodule number (62), fresh (0.50 g) and dry (0.07 g) weights were recorded with the dose of PK at the rate of 160 kg/hm<sup>2</sup>. Nodule shape and color also varied widely in different treatments. In case of plant growth parameters, shoot and root length, collar diameter, fresh and dry weight of the plants took on a significant difference ( $P < 0.05$ ) among various combination of fertilizers. From the study, it is revealed that PK at the rate of 160 kg/hm<sup>2</sup> fertilizer with soil and cowdung mixture (soil: cowdung = 3:1) is recommended for optimum growth and nodule formation of *D. sissoo* in degraded soils at a nursery level.

Uddin *et al.* (2008) the effects of different inorganic fertilizers (Urea and Triple Super Phosphate (TSP)) on seedling growth and nodulation capabilities of four agroforestry tree species (*Albizia chinensis*, *A. saman*, *Acacia nilotica* and *Sesbania sesban*) were compared. The nodulation of these seedlings were treated with different fertilizer treatments (at the rate of urea 40 kg(hm-2), urea 80 kg(hm-2), TSP 40 kg(hm-2), TSP 80 kg(hm-2), (Urea+TSP) 40 kg(hm-2) and (Urea+TSP) 80 kg(hm-2) after one month of seed germination. The results revealed that the seedling growth was enhanced significantly with moderate fertilizer treatment. In some cases, the higher levels of fertilizers reduced the seedling growth. The study also revealed that the nodulation in nodule number and size was significantly inhibited by the application of N fertilizer (Urea), while it was increased significantly with the application of P fertilizer (TSP). This study improved our understanding and provided insights that would be useful to the farmers in their efforts to amend the soil with inorganic fertilizers in order to enhance plant growth and biological nitrogen fixation.

### **2.3 Population dynamics of inoculated *Dalbergia sissoo Rhizobium* under glass house conditions:**

Louw and Webley (1959) reported that count of bacteria in the root region increased during the growing period of the plant. According to Nutman (1975) stimulation of the rhizobia is greatest at places where lateral roots emerge and generally extends to 10-20 mm from the root surface into the soil. Increased growth of *Rhizobium* in the rhizosphere is a response to excretion of energy source, amino acids, growth factors, especially B group vitamins and enzymes by plant roots. The rhizosphere stimulation is a response to a complex mixture of substances. It was

demonstrated by Rovira (1956), who was unable to replace fully the stimulating effect of root exudation by mixture of glucose, soil extract, amino acids and all growth factors known to be excreted by roots.

*Rhizobium* is pre-eminently a rhizosphere organism multiplying on the root surface and in the root surroundings, and within the mucilage layer of both legumes and non-legumes, but especially legumes (Rovira, 1961).

Nutman (1963, 1965) suggested that "A given legume tends to promote the multiplication of bacteria able to infect it more than others", and, "Individual strains of nodule bacteria are more strongly stimulated by those hosts they are able to infect than by other legumes", citing only a reference by Wilson (1930) in support of these statements. Dart and Mercer (1964) present the opposite viewpoint. They state that there is no evidence that legume roots selectively stimulate the growth of *Rhizobium* rather than other organisms. Further, they state that the *Rhizobium* strains which nodulate a particular legume are not preferentially stimulated in that hosts rhizosphere over other *Rhizobium* strains, citing Krasil'nikov (1958) and Purchase (private communication).

The growth of two strains of *R. japonicum* (strains USDA 110 and CB 1809), and two strains of *R. leguminosarum* (strains Hawaii 5-0 and Nitragin 92A3) was followed in the rhizospheres of soybean, pea, and corn growing in non-sterile soil. Rhizosphere soil was sampled at 35 days over four successive growth cycles. The numbers of each strain were determined by membrane filter immunofluorescence, using strain-specific fluorescent antibodies. Nodule occupancy of the strains on their appropriate host was determined by immunofluorescence. No specific stimulation of

rhizobia in the rhizospheres of their homologous host plants was observed. Counts of all strains, as well as total bacteria were generally in the following order: soybean rhizosphere > pea > corn > fallow soil. Strain CB 1809 occupied a slightly higher percentage of soybean nodules than USDA 110, whereas Nitragin 92A3 dominated Hawaii 5-0 in pea nodules. Fifteen percent of soybean nodules were doubly infected, as were 3.5% of pea nodules. The four strains together comprised 1.5 to 2.6% of the total rhizosphere bacteria of the legumes and 2 to 9% of the total bacteria in the corn rhizosphere. Rhizobia comprised 3 to 5% of the total bacteria in fallow soil. These data are not suggestive of an overwhelming increase of homologous rhizobia in the rhizospheres of their respective host legumes. Specific stimulation of growth of *Rhizobium* in the legume rhizosphere does not appear to be a contributor to specificity in the *Rhizobium*-legume symbiosis under the conditions of this study. (Robert Baines Woolfenden II 1982).

Postma *et al.*(1991) the influence of cell surface properties on attachment to soil particles and on population dynamics of introduced bacteria was studied in sterilized and nonsterilized loamy sand and silt loam. *Rhizobium leguminosarum* RBL5523 and three TnS mutants (RBL5762, RBL5810, and RBL5811) with altered cell surface properties were used. Cellulose fibrils were not produced by RBL5762. Both RBL5810 and RBL5811 produced 80 to 90% less soluble exopolysaccharides and RBL5811 had, in addition, an altered lipopolysaccharide composition. In sterilized soil the total numbers of cells as well as the number of particle-associated cells of RBL5523 and RBL5810 were, in general, higher as compared with cell numbers of RBL5762 and RBL5811. Differences between strains in percentage of particle-associated cells in sterilized soil were only found at high inoculum densities, when

populations increased little. In the nonsterilized silt loam, final population sizes, as well as numbers of particle-associated cells, of the parental strain (RBL5523) were higher than those of strains with altered cell surface properties after 56 and 112 days of incubation. But in general, differences in survival among the strains were not very marked. The importance of association with soil particles or aggregates for the survival of introduced cells was affirmed by the pronounced increase of the percentage of particle-associated cells during incubation in nonsterilized as well as sterilized soil. However, no clear relation among altered cell surface properties, particle association, and survival was found.

Dan Turk *et al.* 1993 investigated to determine the relationship between leguminous tree yield response to inoculation and indigenous rhizobial population density, an inoculation experiment was conducted in pots using four soils and six tree species: *Acacia auriculiformis* A. Cunn. Ex. Benth., *A. mangium* Wild., *A. mearnsii* De Wild., *Leucaena diversifolia* (Schlecht.) Benth., *Robinia pseudoacacia* L. and *Sesbania grandiflora* Poir. Densities of indigenous rhizobia were determined by most probable number (MPN) plant-infection assays. Statistically significant increases in shoot N due to inoculation were observed most frequently in soils with  $< 50$  rhizobia  $g^{-1}$  soil. Inoculation resulted in significant increases in shoot N ( $P < 0.05$ ) for *R. pseudoacacia* and *A. mearnsii* in three and one soils, respectively, despite the presence of  $> 1000$  rhizobia  $g^{-1}$  soil. A hyperbolic model best described the relationship between response to inoculation and the density of indigenous rhizobia. Incorporating an index of available soil N into the hyperbolic model reduced residual mean square values, indicating that available mineral N attenuates the response to inoculation.

## CHAPTER - III

### MATERIALS AND METHODS

This chapter deals with the description of the materials used and the methods or techniques adopted during the course of present study entitled “Effect of *Rhizobium* inoculation and nitrogen fertilizer application on growth performance of *Dalbergia sissoo* tree seedlings” The details of materials used along with geographical situation, climate and the experimental technique adopted under the experiment are briefly described below.

#### 3.1 Experimental site and Geographical situation

The experiment was conducted in the glass house of Dept. of Microbiology, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during 2009-2010 with *Dlbergia sissoo* (Shisham). Two weeks old *D. sissoo* seedlings grown in nursery were collected from Central Forest Nursery, Jora (Raipur). Raipur is situated in plains of Chhatishgarh at 21°16' N latitude and 81°36' E longitude with an altitude of 289.60 meter above mean sea level (MSL).

#### 3.2 Climatic condition:

Raipur, comes under sub humid region, receiving an average rainfall of 1200-1400 mm out of which about 85 per cent is received during the rainy season ( June to September) and the rest 12 per cent during winter season( October – February).The place experiences a short mild winter, January being the coolest and dry hot summer, May being the hottest month. Soil surface temperature of this region crosses 60 °C, air temperature touches to 48 °C and humidity drops up to 3 to 4 per cent during summer

season and mercury level drops to as low as 60 °C during December and January.

Appendix –I.

### **3.3 Experimental details:**

The experiment was conducted in polythene bags in the glass house under controlled conditions. The details of the experiment are given below.

#### **3.3.1 Particulars of Experiment:**

**Location:** - Glass house of Dept. of Microbiology, CoA, Raipur.

**Tree seedling:-** *Dalbergia sissoo*

**Duration of study:** - August to January (2009-2010)

**No. Of Treatments:** - 6

**No. Of Replication-** 4

**Design** – Factorial Completely Randomised Design (CRD) with one factor –

age (at 4 different ages /DAT of seedlings , at which observations are recorded)

**Total No. Of seedling maintained** – 96 seedlings (4 Repli. X 6 Treat. X 4 ages = 96)

#### **3.3.2 Design and plan of taking observation:**

The experiment was laid out in factorial CRD with 6 treatments, replicated 4 times with one factor as age of seedlings (4) at which observations are recorded. A total no. of 96 poly bags (4 Repli. X 6 Treat. X 4 ages = 96) were maintained for experiment.

Date of seedling transplantation and treatment application 15 August 2009 (30days old seedling).

1<sup>st</sup> observation at 30DAT (Days after transplant) - (at 2 month old seedling).

2<sup>nd</sup> observation at 60DAT - (at 3month old seedling).

3<sup>rd</sup> observation at 90 DAT - (at 4 month old seedling).

4<sup>th</sup> observation at 120DAT (at 5month old seedling).

Hence total times of observation as per different ages of seedling/ as per DAT = 4

### 3.3.3 Details of treatments

T1	Control
T2	Inoculated ( <i>Rhizobium</i> )(@5 ml inoculated broth/seedling)
T3	Un-inoculated + N <sub>1</sub> (2g. N/seedling)
T4	Un-inoculated + N <sub>2</sub> (4g. N/seedling)
T5	Inoculated + N <sub>1</sub> (2g N/seedling)
T6	Inoculated + N <sub>2</sub> (4g N/seedling)

### 3.3.4 Filling of polythene bags:

Soils were randomly collected from a depth of 6 inches (15cm) from soil surface and thoroughly mixed to form a composite sample. The soils were processed and sieved through 2mm sieve. Soil, Sand and FYM were uniformly mixed in 2:1:1 ratio and filled in each polythene bag (size 12"x9" of 5 kg capacity) upto 4.5 kg capacity. The moisture content in FYM used was 83 %.

### 3.3.5 Isolation of *Rhizobium* and preparation of inoculum:

Species- isolate of *Rhizobium* was isolated from fresh nodule of *D. sissoo* seedling and culture broth was prepared using YEMA media (Appendix II). The isolated *Rhizobium* was multiplied in the departmental laboratory and thereafter inoculated as per treatment.

For isolation of *Rhizobium*, preferably pink nodule was removed carefully from seedling of one collected nodulated *D. sissoo*. Detached fresh nodule was surface sterilized by immersing in 1% HgCl<sub>2</sub> for 2-3 minutes and then rinsed with sterile distilled water for at least 6-7 times. This followed by soaking in 98% ethanol for 1 minute and again rinsed for 8-9 times with distilled water. The sterilized nodule was then crushed together in a small aliquot of sterile water with the help of a glass rod in a test tube. Loopful of fluid obtained from crushed nodule was spread on the surface of YEMA media plate, (Yeast Extract Mannitol Agar Media, Appendix II) as described by Vincent 1970 and incubated at 28 °C. Loopful of growth of bacterial colony was again restreaked in YEMA slant until a pure culture was obtained. To prepare the culture suspension, the isolate was grown in sterilized YEM broth for 7 days on a rotary shaker. *D. sissoo* -*Rhizobium* isolate was inoculated to 50 ml nutrient broth in 250ml conical flask and incubated at 28±2 °C for 48 hours. This broth culture was then used for seedling inoculation treatment.

### 3.3.6 Transplantation and caring:

Seedlings of *Dalbergia sissoo* (Two weeks old) grown in nursery were collected from Central Forest nursery, Jora, Raipur for experimental study. Uniform size

seedlings were shifted in polythene bags ( of 5Kg capacity 9x12” sized previously filled with 4.5 Kg mixture of soil-sand-FYM in 2:1:1 proportion) so that a single seedling in each polythene bag was maintained. . Phosphorus and Potassium are to be applied @ 2.5g Phosphorus and 2.0g Potassium per seedling through SSP and MOP respectively at 4-5cm deep in polybags at the time of transplantation of seedlings. A total number of 96 polybags were maintained for this experiment consisting of 6 treatments, 4 replications and 4 plants per each treatment and replication for taking 4 times of observations at different seedling ages (6x4x4) and the seedlings were allowed to grow up to 5 months (Aug-Jan). The seedlings were regularly watered and hand weeded in the polybag as per need.

### **3.3.7 Methods of treatment given:**

After transplantation, treatments were given. Nitrogen was given through urea at the time of transplantation of seedlings as per treatment and *Rhizobium* through seedling root inoculation. For root inoculation of seedlings matured broth of *Dalbergia sissoo-Rhizobium* was diluted with sterilized water. For this purpose 250ml of matured broth was taken and 1000 ml of sterilized water was added to it so that 1:4 dilution was attained. In this diluted broth sugar was added @ 0.5gm/100ml broth as a sticking agent which also served as ready food material for *Rhizobium*. After dipping of 1month old seedling in this broth they were transplanted in poly bags as per treatment.

### **3.4 Observations recorded:**

- ❖ Characterization of *D. sissoo-Rhizobium* isolates (Gram's staining)
- ❖ Physico-chemical and microbial properties of collected soil used in filling polythene bags

- ❖ Morphological growth parameter of *Dalbergia sissoo* seedlings at 30 Days interval after transplant/ giving treatment/ (30DAT (2 m old.), 60DAT (3 m old.), 90DAT (4 m old.) & 120DAT (5 m old.). (Shoot length, Root length, No. of leaves/seedling, No. of short branches/seedling). Collar Diameter of 5m old seedling at 120DAT.
- ❖ Biomass study in different component of *Dalbergia sissoo* seedlings at one month interval after giving treatment (30DAT (2 m old.), 60DAT (3 m old.), 90DAT (4 m old.) & 120DAT (5 m old.). Shoot, leaf and root dry weights were recorded month wise.
- ❖ Nodulation study in *D. sissoo* seedlings.( Nodule number, nodule fresh and dry weights/seedling)
- ❖ Nitrogen content in different components of *D. sissoo* seedlings at 60DAT 3month old and at 120DAT, 5month old seedlings
- ❖ Nutrient(P & K) content in different components of *D. sissoo* seedlings at 5 month, old (120DAT) seedlings.
- ❖ Population dynamics of inoculated *Dalbergia sissoo Rhizobium* under glass house conditions.

#### **3.4.1 Gram staining of *Dalbergia sissoo*- bacteria isolates:**

Gram staining was conducted as per the procedure. Firstly thin and uniform smears of bacteria were made on glass slides. Then they were air dried followed by fixing the smears by heat. Then each smear was covered with crystal violet for 30 seconds followed by washing each slide with distilled water for few seconds. Then each

slide was covered with Gram's iodine solution for 60 seconds. These slides were then washed with 95 per cent ethyl alcohol to wash off the iodine solution. Ethyl alcohol was added drop by drop until no more colour flowed from the smear. Again the slides were washed with distilled water and drained. Safranin was then applied to the slides for 30 seconds. Then they were washed with distilled water and blot dried with absorbent paper and left for air drying. The bacteria that appeared purple were referred to as Gram-positive and those which appeared pink were described as Gram-negative (Aneja, 2003)

### **3.4.2 Physico – chemical analysis of soil samples:**

#### **Sampling and processing:**

Collected Soil sample for filling polythene bags were analysed for physico-chemical and microbial properties. The fresh soil sample was kept for microbial analysis. Then the soil samples were air dried, ground in a wooden mortar with wooden pestle and sieved through 2mm sieve and stored in sampling polythene bags with proper labelling for analysis purpose. The analysis for different parameters was done following standard methods as given below.

- **Moisture content of soil:**

Sample was weighed, oven dried at 105 °C and weighed again for moisture content determination by gravimetric method (Singer and Munns, 1992).

$$\text{Gravimetric water content} = \frac{\text{Weight loss}}{\text{Dry weight}}$$

- **Soil pH:**

The pH of the soil was determined in 1:2.5 soil: water suspension, by using pH meter.(Jackson , 1967)

- **Electrical conductivity:**

Electrical conductivity (E.C.) was determined in soil water suspension (1:2.5) by conductivity bridge as described by Jackson (1973)

- **Organic Carbon:**

Organic carbon in soil was determined by wet oxidation method of by Walkley and Black as described by page *et.al.* (1982)

- **Available N:**

Available N was determined by alkaline  $\text{KMnO}_4$  method as outlined by Subbiah and Asija (1965). Twenty gram soil sample was taken in one litre boiling flask and 200 ml distilled water, 100 ml of 0.32 per cent  $\text{KMnO}_4$  and 100 ml of 2.5 per cent NaOH were then added in sequence. The flask was connected to the condenser immediately after adding NaOH and the content was boiled on heater to collect about 150 ml distillate in 10 ml boric acid solution containing mixed indicator Ammonium - N in distillate was determined by titrating against 0.02 N  $\text{H}_2\text{SO}_4$

- **Available P:**

Soil phosphorus was extracted by 0.5 M  $\text{NaHCO}_3$  as described by Olsen et al. (1954) and phosphorus in the extract was determined spectrophotometrically by ascorbic acid method of Watanabe and Olsen (1965).

- **Available K:**

It was determined by extracting the soil with Ammonium acetate solution and was estimated by Flame Photometer (Hanway and Heidel, 1952).

### **3.4.3 Morphological growth parameters:**

The following morphological growth parameters were recorded in *D. sissoo* subjected to different treatments. The observations were recorded on 4 randomly selected plants in each treatment at monthly interval and data were systematised for statistical analysis.

#### **Plant sampling and processing:**

Four plants replicates from each treatment were randomly selected (for recording the growth data through sampling process at monthly interval after giving treatment.) At each observation the seedling were uprooted carefully without damaging the root system. The root were washed in running tap water and parameters such as root length, shoot length, no. of short branch per seedling, no. of leaves per seedling were recorded in fresh samples. Month wise observations were recorded after given treatment.

- **No. of leaves:**

No. of leaves per seedling was counted separately in each treatment

- **No. of short branches:**

No. of short branches per seedling was counted separately in each treatment

- **Shoot and Root length:**

Shoot and Root length of seedlings were measured by graduated scale divided in cm and mm.

- **Collar diameter:**

Collar diameter of seedlings was measured at the collar portion (2cm from soil surface on the stem) using Digital Vernier calliper.

### 3.4.4 Biomass Accumulation

- **Shoot, Root and leaf biomass:**

The plant components *Viz* leaves, shoot, root were collected from seedling at monthly interval and oven dried at 70 °C for 3-4 days up to the attainment of constant weight. Then final dry weight of root, shoot and leaves were recorded.

The seedling quality parameters *Viz* sturdiness (the ratio of height to diameter), root/shoot ratio (on biomass basis) were computed. To quantify the morphological quality of seedlings, Dickson quality- Index (Q.I) was calculated as per Dickson *et al* 1960, and also following Chauhan and Sharma (1997)

$$QI = \frac{Tw}{H/D + Rw / Sw}$$

<b>QI</b>	=	Quality Index
<b>Tw</b>	=	Total dry weight of seedlings (g/seedling)
<b>H</b>	=	Seedling height (mm)
<b>D</b>	=	Collar diameter (mm)
<b>Sw</b>	=	Shoot dry weight (g/seedling)
<b>Rw</b>	=	Root dry weight (g/seedling)

### 3.4.5 Nodulation study:

Uprooted plants were washed carefully so that nodules were not disturbed. Nodules were washed, wrapped in blotting paper. Fresh and dry weights were taken; number of nodules per seedling was recorded.

### 3.4.6 Nutrient content in Plant components:

The oven dried different plant components *Viz* leaves, shoot, root samples were ground into powder through Wiley mill and used for N, P and K analysis. The methods employed are given below.

- **Total N :** Total N in sample was determined by Kjeldahal method (Jackson-1958) by digesting 0.1g sample in 10ml conc.  $H_2SO_4$  followed by distillation and titration.
- **Total P and K:** The samples were digested in diacid ( $HNO_3$  and  $HClO_4$  9:4 ratio). Estimation of Phosphorus in the plant sample was done by vanado- molybdate yellow colour method using spectrophotometer (Jackson, 1973). The yellow colour developed was read at 490 nm using a spectrophotometer. Estimation of Potassium in the plant sample was estimated by Flame Photometer (Hanway and Heidel, 1952).

The nutrient concentrations of each component were multiplied with their respective biomass to obtain nutrient contents.

### 3.4.7 Microbial analysis:

- **Sampling:**

About 25g of soil sample was kept as such in sealed polythene bag to prevent the moisture loses and properly stored in refrigerator for quantitative analysis of microbes

Microbial analysis with respect to *Rhizobium* count in soil was done by dilution plating method( Subba Rao, 1988).For bacterial counting serial dilution of soil samples were done by taking 1g of soil in 9ml sterilized water in a dilution tube and it was shaken in a shaker for 30 minutes. After shaking the dilution tube (No.1) was kept for 30 minutes to allow the soil particles to settle down, in this way  $10^1$  dilution of soil sample was obtained. Now 1.0 ml of the bacterial suspension from dilution tube No. 1 was drawn out with the help of autopipette and transferred to another dilution tube No.2 containing 9 ml sterilized water resulting  $10^2$  dilution. It was again shaken in a rotary shaker for 5 minutes. Again 1.0ml suspension was drawn from dilution tube No.2 for  $10^3$  dilution and in this way serial dilution of a soil sample was carried out upto desired dilution and finally a complete set of desirable dilutions of each soil sample was obtained.

About 20 ml of the appropriate sterilized and partially cooled agar media was poured in to the sterilized Petri plates containing 1 ml aliquot of appropriate dilution at the bottom which was drawn out from the respective dilution with the help of a sterilized tip of an auto pipette and the plates were incubated at  $28^{\circ}\text{C}$  in the incubator. Counting of rhizobial colonies was started after 24 hours of incubation. Counted colonies were marked with the instant marker to avoid the repeated counting and the process of counting was continued up to 7 days of incubation. Colony counting was done on colony counter.

Plating of each samples was done in duplicate and mean values were worked out for each samples. One control was also incorporated with each set of plating. After counting of colonies, rhizobial population was calculated on the basis of per gm of dry

soil using following formula (Schmidt and Caldwell, 1967.). Rhizobial population density in the YEM broth was also estimated by using the same formula.

**Number of *Rhizobium* per gm of oven dry soil:**

$$= \frac{\text{No of colony forming units (cfu) x dilution}}{\text{Dry weight of one gm moist sand sample x aliquot taken}}$$

The operation of making serial dilutions, setting of plates, inoculation with appropriate media was done in sterilized atmosphere of Laminar flow.

**3.5 Statistical analysis:**

All observations recorded from this experimental study were tabulated in a systemic manner. The final observations of morphological growth parameters, biomass and nodulation were statistically analysed using ANOVA for factorial completely randomised design and others by ANOVA for CRD. The significant difference were tested through F-test at 5% level of significance .The standard error of means  $SEM_{\pm}$  and CD were calculated where F-test was significant for comparing treatment means. (Panse and Shukhatme (1978)).

## CHAPTER IV

### RESULT AND DISCUSSION

The investigation entitled “**Effect of *Rhizobium* inoculation and Nitrogen fertilizer application on growth performance of *Dalbergia sissoo* tree seedlings.**” was conducted at the Department of Microbiology, College of Agriculture Raipur, Chhattisgarh during the year 2009-2010 to see the response of legume tree seedling (*Dalbergia sissoo*) to *Rhizobium* inoculation and the influence of Nitrogen on biologically Nitrogen fixation.

The study comprised of (i) isolation of *Rhizobium* isolates from nodules of *Dalbergia sissoo* and preparation of *Dalbergia sissoo Rhizobium* inoculated broth. (ii) Evaluation of treatment effects comprised of inoculation of *Dalbergia sissoo-Rhizobium*, N fertilization alone and along with each other given to *Dalbergia sissoo* seedlings raised in Polythene bags to assess the response of legumes tree seedling to Biofertilizer alone and along with N fertilizer.

The findings obtained from these studies are depicted and attempts were made to establish cause and affect relationship for important parameters which are discussed in this chapter. The findings are divided into the following parts. First part of deals with the characterization of *Dalbergia sissoo Rhizobium* isolates by Gram staining technique. Second part involves about properties of soil used in filling the polythene bags for the purpose of raising *Dalbergia sissoo*, the test plant. Third part deals with influence of *Rhizobium* inoculation alone and along with different dose of Nitrogen fertilizer on (a) morphological growth parameters, (b) biomass accumulation (c) nodulation study and

(d) nutrient content in *Dalbergia sissoo* seedlings. While fourth part concerned with the effect of legume tree seedling and also different treatments on *Rhizobium* population in rhizosphere soil of *D. sissoo* after completion of experimental study.

#### **4.1: Isolation and characterization of *Dalbergia sissoo*- *Rhizobium*:**

Isolation of *Rhizobium* was made from nodulated *Dalbergia sissoo* seedlings collected from nursery. For the purpose of isolation of *Rhizobium*, YEMA (Appendix II) media was used and the isolated colonies were maintained on nutrient agar slope. *Rhizobium* isolates from tree legume (*Dalbergia sissoo*) produced translucent, round and gummy colonies which varied in size between 1.5 to 2.00 mm. The isolate was motile. (Plate-2). The *Dalbergia sissoo* -*Rhizobium* isolates were treated for Gram staining reaction. A loopful of the culture was used to prepare a smear for gram staining. After the gram- staining the bacteria assumed a red colour which indicated that it was a Gram -ve stain. Gram staining of the cultured isolates was done to provide information as presumptive tests of the isolates. The authentication of the isolates was performed using sub culturing method. The broth culture of this authenticated *Rhizobium* was used for *D. sissoo* inoculants for the experiment. For this a YEM broth was prepared and loopful of pure isolate of *Dalbergia sissoo Rhizobium* was transferred to sterilized YEM broth as inoculant. The broth was grown in a shaker for 3-6 days for the purpose of giving *Rhizobium* inoculation treatment to *Dalbergia sissoo* seedling as per experimental study. The isolation method of *Rhizobium* from *D. sissoo* nodule, preparation of *D. sissoo Rhizobium* inoculated broth and characterization of this isolate were clearly depicted in Plate 1 and 2 respectively. Similar type of *Rhizobium* isolation was also carried out in YEMA media in *Albizzia procrera* and various morphological



Plate 2 : Characterization of selected *Dalbergia sissoo* -*Rhizobium* isolate



Plate 1 : Isolation of *Rhizobium* from nodulated plant of *Dalbergia sissoo*

and biochemical tests like colony characteristics, gram reaction are also carried by Bora *et al* (2006).

#### **4.2 Properties of collected soils used in filling polythene bags for raising sissoo seedling:**

Collected soils used in filling polythene bags to raise *Dalbergia sissoo* seedling for experimental purpose were vertisol and were analyzed for their Physico-chemical and biological properties. The data presented in Table-1, clearly showed that, soils were neutral in reaction (pH 7.34), having medium organic carbon status (6.8g/Kg soil). The soil was low in mineralizable Nitrogen status (235.8 Kg/ ha.), low in available (Olsen's) Phosphorus content are (8.4 Kg/ha.) but higher in status with respect to available Potassium content (385Kg/ha.). The population of *Rhizobium* enumerated in YEMA media (APPENDIX II) showed no. of *Rhizobium* colonies of  $5.4 \times 10^2$  cfu /g of soil. (Table -1).

These soils were mixed with sand and FYM (the moisture content of FYM was 83%) in 2:1:1 proportion and polybags were filled with these mixtures. This has been shown in plate 3(a). After root inoculation of *Dalbergia sissoo* seedlings of 30 days old as per treatment description were transferred and raised in polythenebags, so that one seedling was maintained in each polybag. *Dalbergia sissoo*-*Rhizobium* inoculated broth used as treatment alone and along with two levels of Nitrogen to assess the response of legume tree seedling, *Dalbergia sissoo*. This type of study was also carried out by Totey *et.al* (2000) in *D. sissoo* and *Albizzia procera*,. Chauhan and Pokhriyal (2002) in *Albizzia lebbek*, Bora *et. al.* in year 2006 in *A. procera* who studied the combined effect of *Rhizobium* inoculation along with application of different levels of N dosages

**Table-1: Properties of soil used in filling polythene bags  
for experimental purpose**

<b>S. No.</b>	<b>Properties</b>	<b>Vertisol</b>
1	pH (1:2.5)	7.34
2	E. C. (m. mhos cm <sup>-1</sup> )	0.18
3	Organic carbon (g/kg soil)	6.8
4	Mineralizable N (kg/ha.)	235.8
5	Available P (Kg/ha.)	8.4
6	Available K (Kg/ha.)	385
7	<i>Rhizobium</i> population Per g. of soil	5.4 x10 <sup>2</sup>

of fertilizer. A general view of *Dalbergia sissoo* seedlings as affected by treatments was shown in Plate 3 (b).

### **4.3: Influence of *Rhizobium* inoculation and Nitrogen fertilizer application either alone or along with each other on growth performances of *Dalbergia sissoo*.**

#### **4.3.1 Morphological growth parameters of *Dalbergia sissoo*:**

Results on morphological growth parameters of legume tree species Viz. *Dalbergia sissoo* seedlings treated with different treatments are presented in Table 2 and Fig. 1 & 2.

- **Shoot length**

The shoot length of *Dalbergia sissoo* significantly varied with age of seedlings and different treatments (Table-2 and Fig. 1). As per the passage of time, the shoot length increased in all treatments. But the increasing rate with time varied with different treatments. Highest seedling height (64.8cm) was found in T<sub>5</sub>, when *Rhizobium* inoculation was given along with lower nitrogen level, followed by *Rhizobium* inoculation only (62.4) at 120DAT (plate-5 b). But initially at 30 and 60 DAT, T<sub>2</sub>, i.e., when only *Rhizobium* inoculation was given showed Maximum shoot length in *Dalbergia sissoo* seedlings(49.67cm) followed by T<sub>5</sub> (47.84cm) (plate 5 a). Among inorganic N application, T<sub>3</sub> i.e., application of less dose of N influence the shoot length much over T<sub>4</sub> higher dose of N fertilization and control. When *Rhizobium* inoculation was given with higher N level, the effect was less (52.7 cm height at 120DAT as compared to T<sub>2</sub> and T<sub>5</sub>. The seedling heights as affected by different treatments at two



a) General view of experiment at the time of transplanting



b) Growth performance of *Dalbergia sissoo* at 60 DAT

Plate 3 : General view of experiment at the time of transplanting and at 60 DAT

different stages (at 3 month old at 60DAT and 5 month old at 120DAT) are shown in plate 5 (a) and (b) and also depicted in Fig. 1.

However, both the treatments T<sub>2</sub> and T<sub>5</sub> showed consistently highest shoot length in *Dalbergia sissoo* seedlings followed by T<sub>6</sub> and T<sub>3</sub>. At 5 month old seedling height was 64.8cm at T<sub>5</sub> and 62.4cm at T<sub>2</sub> which were 2.13 and 2.05 times higher over control respectively.

Higher N (Nitrogen) level does not influence much with respect to shoot length when applied alone and even when applied along with *Rhizobium* inoculation as compared to T<sub>5</sub> and T<sub>2</sub>. However, T<sub>6</sub> showed better performance over T<sub>4</sub> and T<sub>1</sub>. At 120DAT the height of *Dalbergia sissoo* seedlings ranged from 30.4 to 64.8 cm under different treatments. Interaction of treatment and age of seedlings showed significant variation in shoot length (Table-2). Totey *et al* 2000 observed application of 5ml *Rhizobium* broth per plant to *A. procera* increased the relative height of 1 year old plantation by 1.2 times over control. Inoculation of *Rhizobium* broth @ 10ml /plant gave 93.6cm height in *Albizia niopoides* over uninoculated ones 77.2cm at 120DAT by Okunomo, *et. al.* (2007)

#### • Number of leaves

Number of leaves in *Dalbergia sissoo* seedlings was significantly influenced by different treatments and age (Table-2). *Rhizobium* inoculation alone (T<sub>2</sub>) and *Rhizobium* inoculation along with N application at lower dose (N<sub>1</sub>) were most effective in increasing the no. of leaves compared to other treatments at different age of seedlings.

At 30 and 60 DAT, (2 and 3 month old stage of *D. sissoo*) *Rhizobium* inoculation only (T<sub>2</sub>) promoted maximum no. of leaves in *Dalbergia sissoo* followed by

**Table- 2: Effect of *Rhizobium* inoculation and N application on Morphological growth parameters of *D. sissoo* seedlings at different stages.**  
Morphological growth parameters

Treatment	Seedling Height (cm.)				No. of leaves/seedling				Root length (cm.)				No of branches /seedling							
	Days after transplant				Days after transplant				Days after transplant				Days after transplant							
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
<b>T1</b>	18.50	26.70	28.20	30.40	25.95	20.00	32.00	46.00	56.00	38.50	15.30	23.40	25.80	27.20	22.925	0.00	2.00	3.00	3.00	2.00
<b>T2</b>	38.45	49.67	54.80	62.40	51.33	46.00	84.00	92.00	114.00	84.0	28.30	38.60	42.50	48.20	39.400	3.00	5.00	6.00	7.00	5.25
<b>T3</b>	28.74	38.80	47.50	55.80	42.71	32.00	47.00	68.00	96.00	60.75	21.60	32.40	34.10	36.20	31.075	2.00	2.00	4.00	4.00	3.00
<b>T4</b>	23.20	30.00	38.20	43.40	33.70	28.00	38.00	64.00	77.00	51.75	18.20	28.50	31.20	33.00	27.725	1.00	2.00	3.00	3.00	2.25
<b>T5</b>	36.30	47.84	55.60	64.80	51.135	44.00	72.00	85.00	128.00	82.25	29.20	40.10	42.20	46.00	39.375	3.00	3.00	5.00	7.00	4.50
<b>T6</b>	35.20	43.30	51.60	52.70	45.70	36.00	60.00	77.00	85.00	64.50	23.10	34.50	38.20	39.20	33.75	2.00	3.00	5.00	5.00	3.75
<b>Factor</b>	<b>SEm(±)</b>				<b>CD (5%)</b>	<b>SEm(±)</b>				<b>CD (5%)</b>	<b>SEm(±)</b>				<b>CD (5%)</b>	<b>SEm(±)</b>				<b>CD (5%)</b>
<b>Treatment (T)</b>	0.39				1.10	0.85				2.40	0.08				0.23	0.20				0.56
<b>Days (D)</b>	0.32				0.90	0.69				1.96	0.07				0.19	0.16				0.46
<b>Interaction (T x D)</b>	0.78				2.20	1.70				4.79	0.16				0.45	0.40				1.13

T<sub>1</sub>- Control T<sub>2</sub>- Inoculated (*Rhizobium*) T<sub>3</sub>- Un-inoculated + N<sub>1</sub> T<sub>4</sub> - Un-inoculated + N<sub>2</sub> T<sub>5</sub>- Inoculated + N<sub>1</sub> T<sub>6</sub>- Inoculated + N<sub>2</sub>

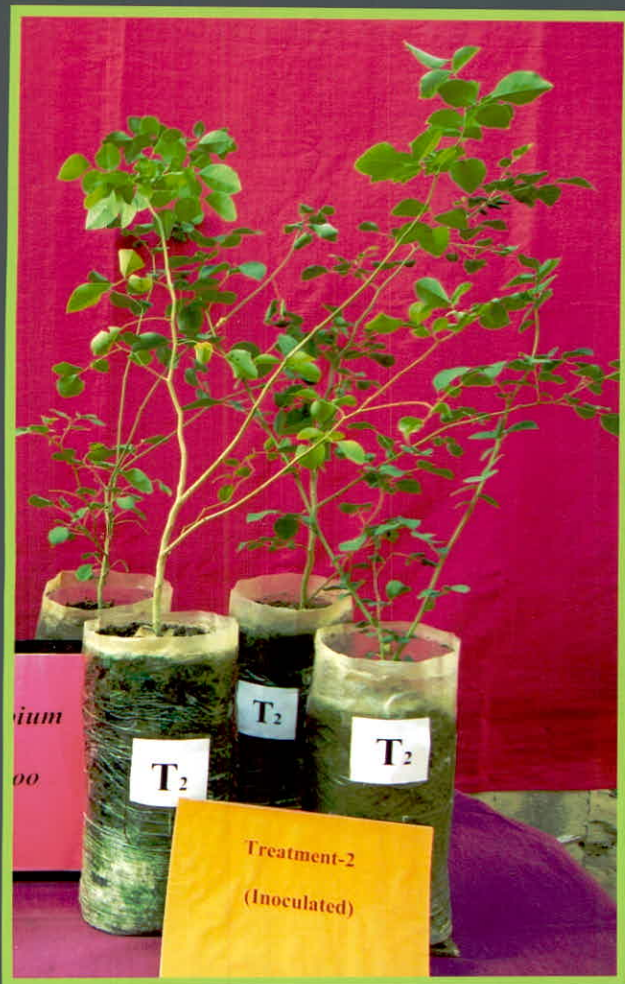


Plate 4 : Better growth performance of *Rhizobium* inoculated *Dalbergia sissoo* at 120 DAT

*Rhizobium*+ N<sub>1</sub> (T<sub>5</sub>) but at 120DAT, (5 month old *D. sissoo*) *Rhizobium* + N<sub>1</sub> showed significantly highest no. of leaves over *Rhizobium* inoculation only. However, both the treatments T<sub>2</sub> and T<sub>5</sub> significantly differ from T<sub>6</sub> (*Rhizobium*+ N<sub>2</sub>) as for as no. of leaves concerned. Among inorganic N fertilization, higher dose of N (N<sub>2</sub>) did not influence much when applied alone or along with *Rhizobium*. However, least no. of leaves/seedlings was found at control. The no. of leaves per *Dalbergia sissoo* seedlings were 46, 84, 92 and 114 under *Rhizobium* inoculation at 30,60,90 and 120 DAT which were 2.3, 2.62, 2 and 2.03 times greater than untreated seedlings (control).

At 5 month old, the no. of leaves per seedlings ranged between 56 to 128 in different treatments, being significantly highest (128) at T<sub>5</sub> which was 1.33, 1.66 and 2.28 times greater over T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> (uninoculated ones) followed by (114) in T<sub>2</sub> while it reduced to 85 at T<sub>6</sub>. Again at T<sub>3</sub> the leaf no./seedling was 96 at 120DAT and at T<sub>4</sub> it reduced to 77.

*Rhizobium* inoculation to *Dalbergia sissoo* seedlings increased the leaf number, and further increased when applied along with less level of N. Lower dose of N influenced *Rhizobium* inoculation (T<sub>5</sub>) significantly over higher dose of N (T<sub>6</sub>). Interaction effects also showed significant. Almost similar observations were recorded earlier by Chauhan *et.al.* (2002) in *A. lebbeck*.

#### • Root length

It is evident from data given in Table (2) that root length of *Dalbergia sissoo* was significantly affected by treatments and age of seedlings. With increase age, the root length of seedlings increased but the increasing rate was more in 30 and 60 DAT after that at 90 and 120 DAT root length increased at slower rate. T<sub>2</sub> and T<sub>5</sub> consistently

gave maximum root length compared to other treatments while lowest was found in control. Initially at 30 and 60 DAT, significantly maximum root length of (29.2cm and 40.1cm) respectively was observed at T<sub>5</sub>, but at 90 and 120 DAT, only *Rhizobium* inoculation exhibited significantly highest root length(42.5 cm and 48.2cm) followed by T<sub>5</sub>(42.2 cm and 46.0cm) respectively. However, both the treatments significantly differ from T<sub>6</sub> (39.2cm). As per only inorganic N application concerned, lower level of N affected significantly giving root length of 36.2cm while it was 33cm at T<sub>4</sub> and 27.2cm at T<sub>1</sub> at 120 DAT. Even higher level of N when applied with *Rhizobium* inoculation dose not affect so significantly as per T<sub>5</sub> and T<sub>2</sub>. However, it (T<sub>6</sub>) has significant effect over control, and T<sub>4</sub>. At 3, 4, and 5 month( 60,90 and 120 DAT, *Dalbergia sissoo* attained 38.6, 42.5 and 48.2cm of root length at T<sub>2</sub> respectively which were 1.64, 1.64 and 1.77 times greater than control.

Among different treatments, T<sub>5</sub> and T<sub>2</sub> were best for promoting the root growth in seedlings followed by T<sub>6</sub>, T<sub>3</sub> and T<sub>4</sub> whereas T<sub>1</sub> was least effective in influencing root length. At 120DAT, 5 month *Dalbergia sissoo* showed maximum root length of 48.2cm at T<sub>2</sub> followed by 46 cm at T<sub>5</sub>. As per inorganic nitrogenous fertilizer concerned, root length of 36.2cm and 33cm were found at T<sub>3</sub> and T<sub>4</sub> respectively. Interaction of treatment x age of seedling was found significant for root length.

Totey *et al.*, (1996) found that with application of *Rhizobium* biofertilizer which is specific to *D. sissoo* only on growth of 3 weeks old seedling of *D. sissoo* remarkable increase in shoot length, *i.e.* almost double height of seedling (9.5cm) over control (5.6cm). Also root length and marked increased in biomass was found. Biofertilizer helps boost microbial population present in soil which in turn makes the insoluble

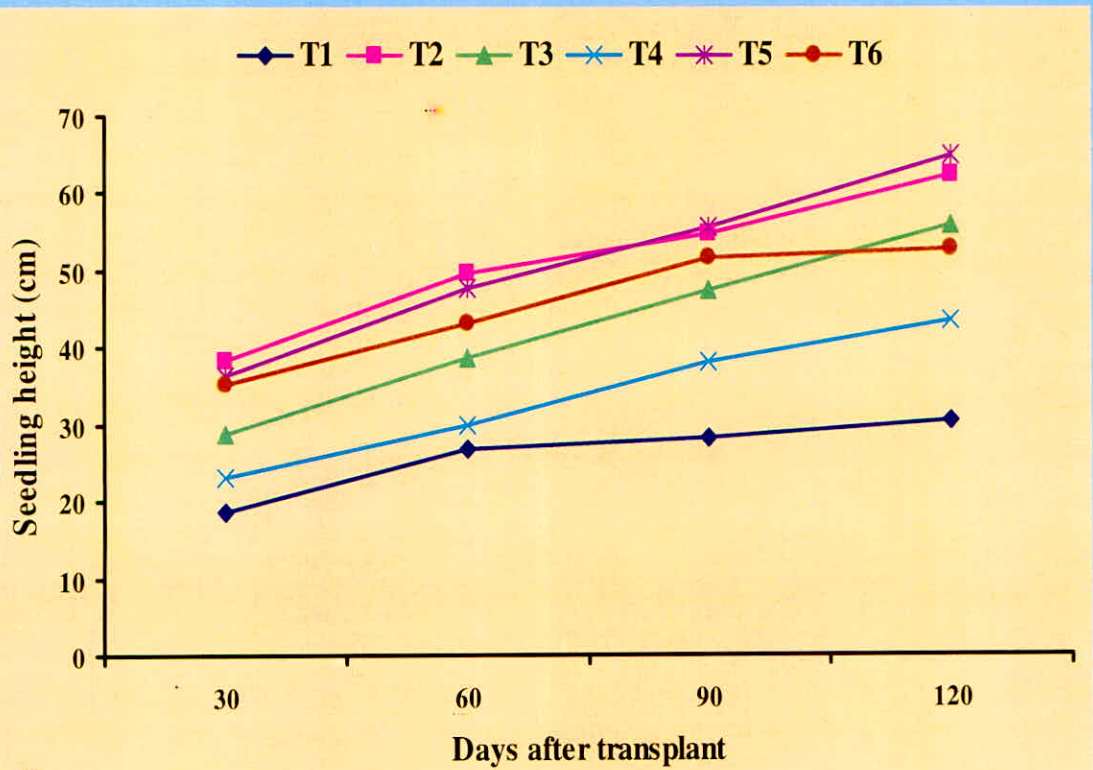


Fig. 1: Effect of *Rhizobium* inoculation and N fertilizer application on seedling height of *Dalbergia sissoo*

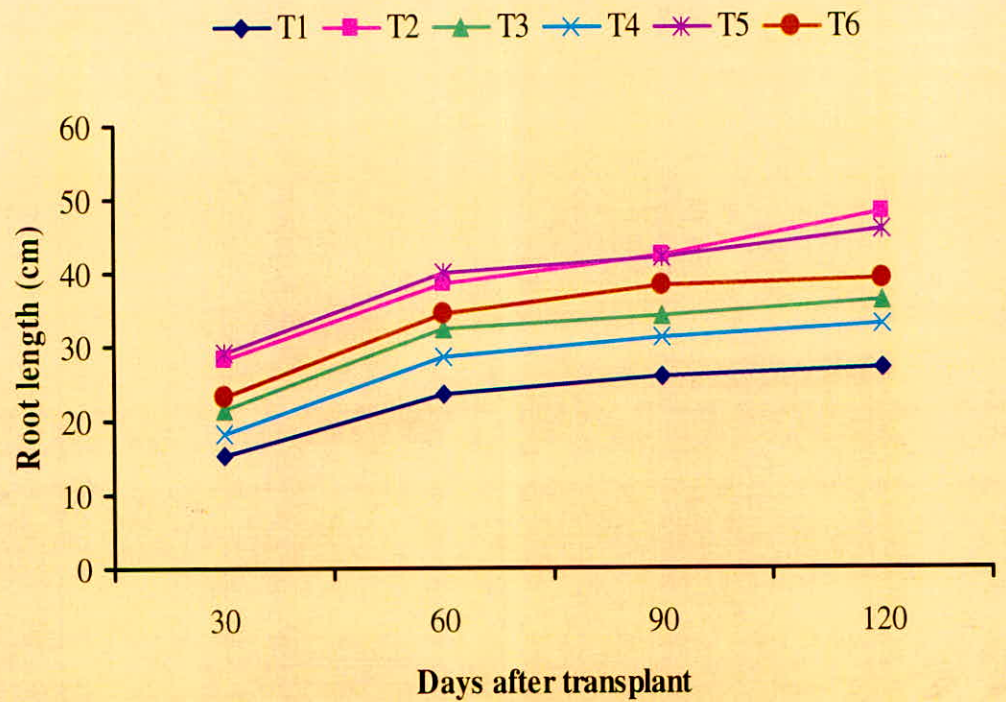


Fig. 2: Effect of *Rhizobium* inoculation and N fertilizer application on root length of *Dalbergia sissoo*

nutrients available for growth of plant. Chauhan (1999) had also reported that *Rhizobium* inoculation enhanced the growth in *Albizzia* seedlings as compared to control.

- **Number of small branches:**

In inoculated seedlings significantly bear more no. of small branches/seedling as compared to uninoculated ones (inorganic N fertilization and control). Among inoculated treatments, *Rhizobium* inoculation only affected much which was at par with *Rhizobium* +N<sub>1</sub> seedlings showed maximum 7 no. of branches /seedling at T<sub>5</sub> and T<sub>2</sub> treatments at 5 month old stage while 5 no. of branches/seedling were seen at T<sub>6</sub> treatment. Number of branches ranged from 0 to 3 in all treatments at 2month, 2 to 5 at 3month, 3 to 6 at 4month and 3 to 7 at 5month age *D. sissoo*. Interaction effect was also significantly affected for no. of branches. (Table-2). Thus it can be inferred from above that plant height, root length as well as growth is significantly influenced by application of *Rhizobium* biofertilizer. The effect of root inoculation of *Rhizobium* was highly significant over control in *D. sissoo* which was shown in plate 4. Similar results were obtained by Basu and Kabi (1987) in *Albizzia lebbeck* and *D. sissoo*. Kinhal (1985) also obtained better performance in bamboo plantation. Due to application of biofertilizer containing *Azotobacter*. At initial growth stage 60 DAT T<sub>2</sub> was significantly better over T<sub>5</sub> which can be seen in plate5 (a). But at latter stage at 120DAT ,T<sub>5</sub> showed better growth which can be seen in plate 5(b) Less N application along with *Rhizobium* influence in increasing *Rhizobium* symbiosis for a longer period. However as per mean value of morphological growth parameters, the effect of T<sub>2</sub> and T<sub>5</sub> was at par. But both the treatments significantly differ from T<sub>6</sub>. Further when only N effect was concerned,

T<sub>3</sub> was more significant over T<sub>4</sub> while minimum growth was found in control T<sub>1</sub>. As mentioned above the effect of 2 levels of N on sissoo seedlings alone and along with *Rhizobium* has been shown in Plate 6.

Similar observations were also found by Chauhan and Pokhriyal in *Albizzia*(2002).

- **Collar diameter:**

Collar diameter (in mm) of seedlings was measured at 120DAT in *Dalbergia sissoo* seedlings of 5 month old at 120 DAT as affected by different treatments. (Table-3). Collar diameter observed was significantly ( $P \leq 0.05$ ) influenced by *Rhizobium* inoculation being 8.01, 8.27 and 7.74 at T<sub>2</sub>, T<sub>5</sub> and T<sub>6</sub> respectively while 7.51, 7.14 and 6.02 at T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> respectively. Among different treatments *Rhizobium* inoculation +N<sub>1</sub> (T<sub>5</sub>) alone was most significantly effective and enhanced maximum collar diameter of 8.27mm followed by T<sub>2</sub> (*Rhizobium* +N<sub>1</sub>) 8.01mm whereas it was lowest in untreated seedling (control)6.02mm. Collar diameter of *Dalbergia sissoo* seedlings varied between 6.02 to 8.27mm under different treatments. (Table 6) Bora *et al* (2006) also found out the inoculation of *Rhizobium* in seedlings of *A. procera* significantly influenced the root length, shoot length, collar diameter and nodulation.

Application of inorganic Nitrogen fertilizer alone also significantly influenced collar diameter as compared to control, however lower dose of N (N<sub>1</sub>), T<sub>3</sub> significantly increased collar diameter(7.51mm) followed by N<sub>2</sub> (T<sub>4</sub>) 7.15mm and control (T<sub>1</sub>)6.02mm. Among all treatments Collar diameter was comparatively low T<sub>4</sub>, while minimum was found at T<sub>1</sub> and maximum at T<sub>2</sub> and T<sub>5</sub> followed by T<sub>6</sub>. (Table 3)

**Table – 3: Collar Diameter of *Dalbergia sissoo* at 120DAT as affected by *Rhizobium* inoculation and N fertilizer application**

S. No.	Treatment	Collar Diameter (C.D.) in mm
1	Control	6.02
2	Inoculated ( <i>Rhizobium</i> )	8.01
3	Un-inoculated + N <sub>1</sub>	7.51
4	Un-inoculated + N <sub>2</sub>	7.15
5	Inoculated + N <sub>1</sub>	8.27
6	Inoculated + N <sub>2</sub>	7.74
	<b>SEm(±)</b>	<b>0.03</b>
	<b>CD (5%)</b>	<b>0.10</b>



a) Performance at 60 DAT



B) Performance at 120 DAT

Plate 5 : Effect of treatments on growth performance of *Dalbergia sissoo*



Plate 6 : Influence of N levels on *Rhizobium* inoculated *Dalbergia sissoo*

#### 4.3.2 Biomass accumulation in different components of *Dalbergia sissoo* seedlings as affected by different treatments:

The data on stem biomass, root biomass and leaf biomass as well as total biomass per seedling was given in table 4 and 5 and figure 3 to 7.

- **Leaf biomass**

Leaf biomass of *Dalbergia sissoo* seedlings at different age varied significantly among different treatments. Giving *Rhizobium* inoculation showed significantly maximum leaf biomass over uninoculated seedlings. (Table 4)

At initial stage *Rhizobium* inoculation only gave maximum leaf biomass of 1.103g/seedling which was 5 times higher over control, while at 5month old at 120 DAT maximum leaf biomass 7.21 g/seedling found in T<sub>5</sub> which was 3.27 higher over control. Among inorganic fertilization, T<sub>3</sub> gave biomass of 5.01g/ seedling which was 1.4 and 2.2 times greater than T<sub>4</sub> and T<sub>1</sub> at 120DAT of *Dalbergia sissoo*. Leaf biomass ranged from 0.219 to 7.21g/ seedling under different treatments and age of seedling. However *Dalbergia sissoo* exhibited maximum leaf biomass in T<sub>2</sub> and T<sub>5</sub> followed by T<sub>6</sub> and then T<sub>3</sub> while minimum was found at T<sub>1</sub>. Interaction of treatment and age was found to be significant for leaf biomass. (Table 4)

- **Stem biomass**

Stem biomass was significantly affected by different age of seedling and under different treatments. Seedlings inoculated with *Rhizobium* inoculation showed significantly higher stem biomass accumulation over control and uninoculated ones. Among inoculated treatments, at 60 and 90 DAT (3month and 4month stage) by application of only *Rhizobium* inoculation (T<sub>2</sub>), the maximum stem biomass was seen

while at latter stage at 120 DAT (4 and 5month old) application of lower level of N with *Rhizobium* inoculation gave significantly maximum stem biomass of 13.15g/ seedling. Under T<sub>5</sub>, the stem biomass was 1.68, 5.53, 8.473 and 13.15g/seedling and under T<sub>2</sub>, the shoot biomass was 1.44, 6.095, 9.267 and 12.58g/seedling at 2, 3, 4 and 5 month stage respectively. But application of higher dose of N along with *Rhizobium* inoculation showed stem biomass of 11.35g/ seedling, which was significantly decreased compared to T<sub>2</sub> and T<sub>5</sub>. Further application of only inorganic N, there was also significant increase in stem biomass over control. Among dose of N fertilizer, shoot biomass was observed / seedling higher at N<sub>1</sub> over N<sub>2</sub>. At 5month old age, T<sub>5</sub> gave maximum shoot biomass of 13.15g followed by T<sub>2</sub> 12.58g which were 1.9 and 1.82 times greater over T<sub>1</sub>, control respectively.

The stem biomass significantly increased from 1.68g/ seedling to 13.15g/ seedling throughout the study period. Interaction of treatment x age was found significant for stem biomass (Table.4) and this can also be depicted in Fig3. As per mean value over ages treatment effect of T<sub>2</sub> and T<sub>5</sub> were at par and both were significantly high over T<sub>6</sub> w.r.to stem biomass content.

#### • **Root biomass**

Root biomass of *Dalbergia sissoo* seedlings varied significant by *Rhizobium* inoculation and N fertilization either singly or in combination. (Table-4). *Rhizobium* inoculation either alone or in combination with N significantly increased the root biomass over uninoculated. However, at all ages, highest root biomass was seen (0.833 , 4.86, 8.48 and 17.24g/ seedling at T<sub>2</sub>, and at 30,60,90 and 120 DAT respectively, Overall *Rhizobium* inoculation only and *Rhizobium* inoculation along with lower dose



of N fertilizer showed maximum accumulation of root biomass *i.e.* 17.24 and 16.95 g/seedling which were 1.2 and 2.4 times greater than T<sub>6</sub> and control. Application of inorganic N fertilization influenced root biomass as compared to control. In lower level of N (N<sub>1</sub>), the accumulation of root biomass (11.81g/ seedling) was significantly higher over high level of N (N<sub>2</sub>) at 120DAT which was 1.2 and 1.6 times more than T<sub>4</sub> and T<sub>1</sub> respectively.

Root biomass ranged from 7.01g to 17.24g under different treatments at 120DAT, being highest at T<sub>2</sub> followed by T<sub>5</sub> then T<sub>6</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub>. Interaction effect of treatment and age of seedlings was found to be significant. (Table 4). This can also be seen in Fig.-3

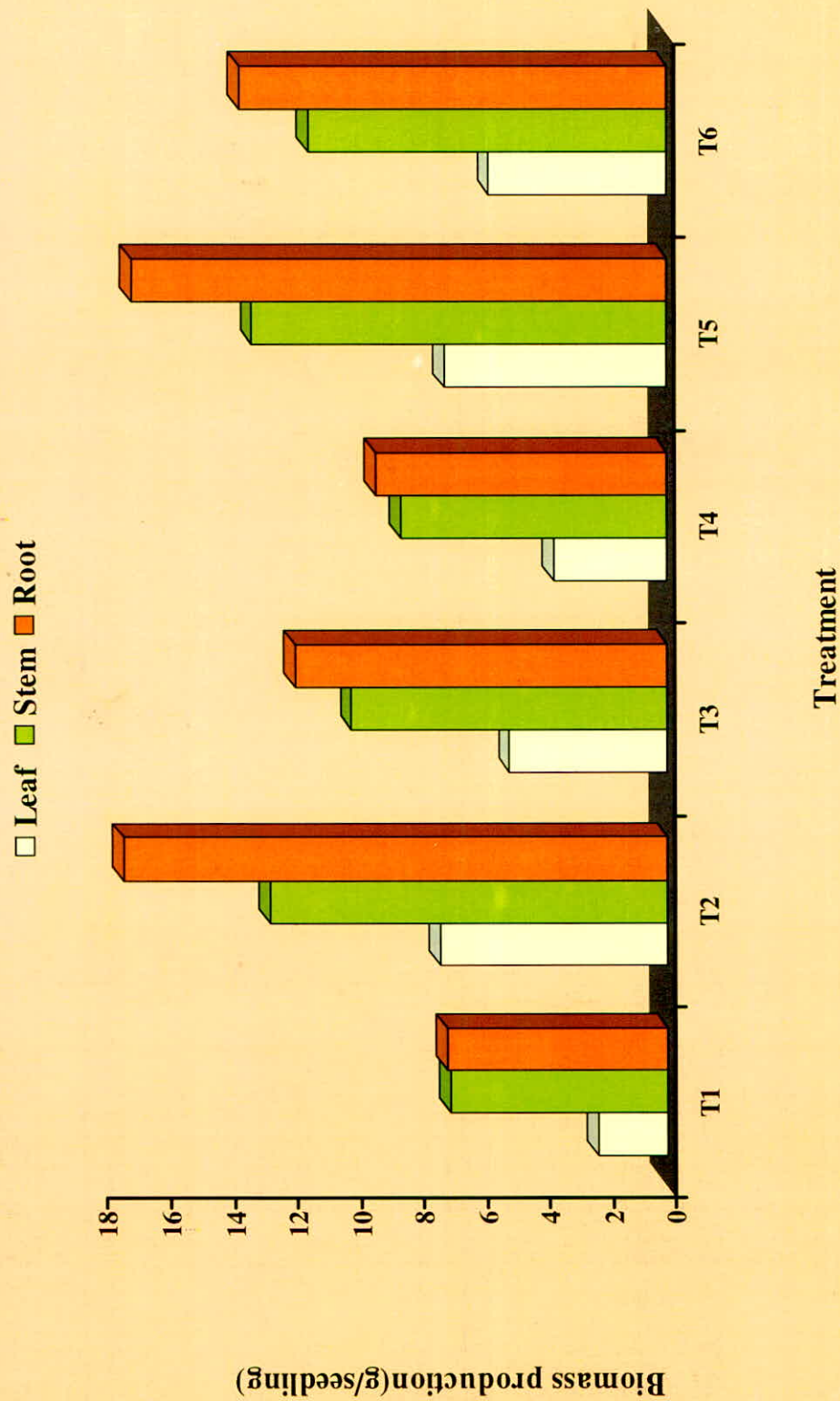
- **Total biomass**

Data on above ground and below ground as well as total biomass as affected by different treatments are presented in Table-5 and also depicted in Fig. 4, 5, 6 and 7.

As per described above, the above ground, below ground as well as total biomasses of seedling were significantly influenced by *Rhizobium* inoculation alone (T<sub>2</sub>) and along with N<sub>1</sub> (T<sub>5</sub>). Maximum total biomass was obtained 37.4 g/ seedling at 120DAT under treatment T<sub>5</sub> followed by 36.9g/ seedling at T<sub>2</sub> which were significantly higher over T<sub>6</sub> (30.6g/ seedling ). At 120 DAT T<sub>2</sub> and T<sub>5</sub> were at par and had significant influence in total biomass accumulation in *Dalbergia sissoo* seedling over others.

Total biomass of *Dalbergia sissoo* ranged from 16.14 to 37.4 g/ seedling under different treatments at 120DAT. 131 % increase in total biomass at 120DAT was found due to *Rhizobium* inoculation over control. These observations are in agreement with





**Fig. 3: Effect of *Rhizobium* inoculation and N fertilizer application on biomass accumulation in different components of *Dalbergia sissoo* at 120 DAT.**

**Table- 5: Effect of *Rhizobium* inoculation and N fertilizer application on Total Biomass production by *D. sissoo* seedlings at different stages**

Treatment	Above ground biomass (g/seedling)					Below ground biomass (g/seedling)					Total biomass (g/seedling)				
	Days after transplant					Days after transplant					Days after transplant				
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
<b>T1</b>	0.642	1.865	4.649	9.120	4.07	0.219	2.206	4.355	7.02	3.45	0.861	4.071	9.004	16.140	7.52
<b>T2</b>	2.543	9.335	14.253	19.640	11.44	0.833	4.92	8.602	17.324	7.92	3.376	14.255	22.855	36.96	19.36
<b>T3</b>	1.517	5.544	9.616	15.020	7.92	0.479	2.975	6.518	11.840	5.45	1.996	8.519	16.134	26.860	13.37
<b>T4</b>	1.29	2.866	7.351	12.030	5.88	0.444	2.586	6.107	9.2680	4.60	1.734	5.452	13.458	21.290	10.48
<b>T5</b>	2.646	8.37	13.089	20.360	11.12	0.663	4.438	8.152	17.040	7.57	3.309	12.808	21.241	37.40	18.69
<b>T6</b>	1.605	7.172	9.618	17.00	8.85	0.525	3.11	7.481	13.60	6.18	2.13	10.282	17.099	30.60	15.03
<b>Factor</b>	<b>SEm(±)</b>					<b>SEm(±)</b>					<b>SEm(±)</b>				
	<b>CD (5%)</b>					<b>CD (5%)</b>					<b>CD (5%)</b>				
<b>Treatment (T)</b>	0.14					0.02					0.13				
<b>Days (D)</b>	0.11					0.02					0.11				
<b>Interaction(Tx D)</b>	0.27					0.05					0.27				

T<sub>1</sub>- Control T<sub>2</sub>- Inoculated (*Rhizobium*) T<sub>3</sub>- Un-inoculated + N<sub>1</sub> T<sub>4</sub> - Un-inoculated + N<sub>2</sub> T<sub>5</sub>- Inoculated + N<sub>1</sub> T<sub>6</sub>- Inoculated + N<sub>2</sub>

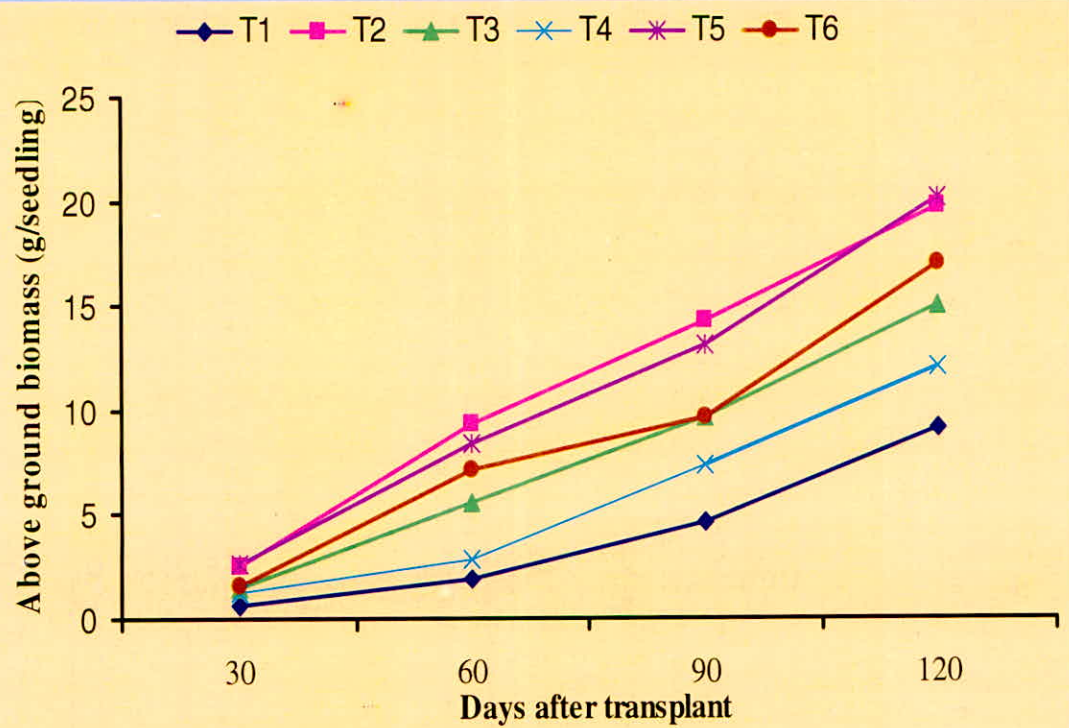


Fig. 4: Effect of *Rhizobium* inoculation and N fertilizer application on above ground biomass accumulation in *Dalbergia sissoo*

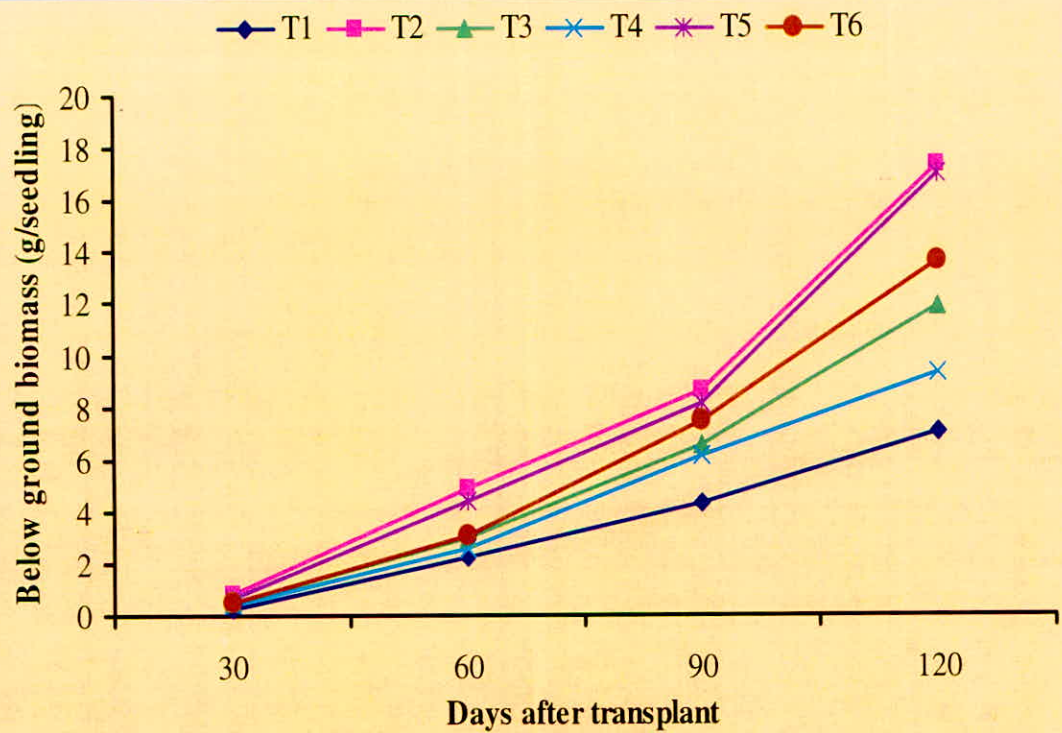


Fig. 5: Effect of *Rhizobium* inoculation and N fertilizer application on below ground biomass accumulation in *Dalbergia sissoo*

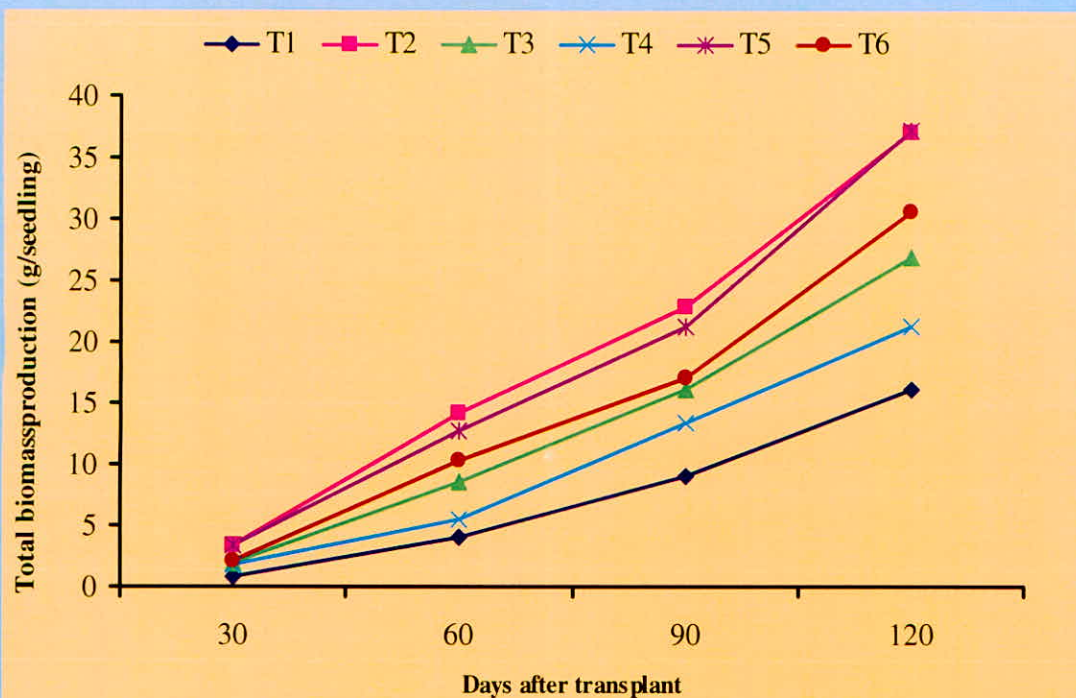


Fig. 6: Effect of *Rhizobium* inoculation and N fertilizer application on total biomass production by *Dalbergia sissoo* at different stages

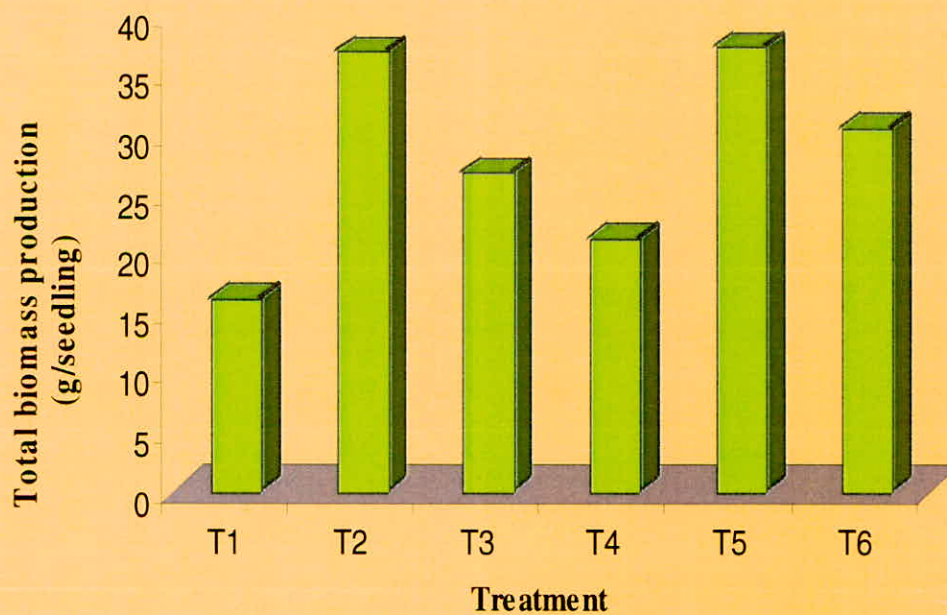


Fig. 7: Effect of *Rhizobium* inoculation and N fertilizer application on total biomass accumulation in *Dalbergia sissoo* at 120 DAT

the earlier reports on inoculation on woody legumes with selected rhizobial strains which showed increased survival percentage in seedling and greater biomass production in inoculated trees by Herrera *et al* 1993, Galiana *et al* 1994.

As per Verma *et al* (1996) application of *Rhizobium* broth 5ml /plant significantly increased plant height. Collar diameter, root length of *Dalbergia sissoo*. Also increase in shoot fresh weight by 118.61%, shoot dry weight by 124.48%, root dry weight 122.04 % due to application of 5ml *Rhizobium* broth biofertilizer and 100g SSP per plant as against control. .

Result indicated that the survival and growth of legume sp. *i.e.* *D. sissoo* were significantly higher in *Rhizobium* inoculation over uninoculated treatment. Similar to growth, biomass of legume seedling *D. sissoo* was significantly influenced by inoculation with *Rhizobium*. The biomass of seedling is mainly depends on diameter, height and root growth. The higher biomass under inoculation treatments as attributed to higher Collar diameter, plant height, under this treatment. This is in line with the findings of Morqueset *al* (2002), where a higher biomass was reported in inoculated seedlings of *Centrolobium tomentosum*, which was attributed to better growth, high nodulation and also relatively more nutrient uptake from control seedlings. This clearly indicates the better response of *D. sissoo* for *Rhizobium* as compared to inorganic fertilization. In contrary Singh *et al* (2000) demonstrated a better response of inoculation for increasing growth and development in *A. procera*.

### **Seedling quality parameters**

Seedling can better be compared on the basis of certain quality parameters (Table-6) which are calculated from various seedling traits. Seedling quality

**Table-6: Quality parameters of *D. sissoo* seedling at 120 DAT as affected by *Rhizobium* inoculation and N fertilizer application**

Treatment	Seedling height (cm)	Collar diameter (mm)	Sturdiness	Shoot biomass g/seedling (A.G.)	Root biomass g/seedling (B.G.)	Root: Shoot ratio	Total dry weight g/seedling	Quality Index (Q.I.)
T <sub>1</sub>	30.4	6.02	50.5	9.12	7.02	0.76	16.14	0.314
T <sub>2</sub>	62.4	8.01	77.9	19.64	17.32	0.88	36.96	0.469
T <sub>3</sub>	55.9	7.51	74.4	15.02	11.84	0.79	26.86	0.357
T <sub>4</sub>	43.4	7.15	60.7	12.03	9.27	0.77	21.30	0.346
T <sub>5</sub>	64.8	8.27	77.9	20.36	17.04	0.84	37.40	0.475
T <sub>6</sub>	52.7	7.74	68.1	17.00	13.60	0.80	30.60	0.444

**T<sub>1</sub>-Control**                      **T<sub>2</sub>-Inoculated (*Rhizobium*)**                      **T<sub>3</sub>-Un-inoculated + N<sub>1</sub>,**  
**T<sub>4</sub> - Un-inoculated + N<sub>2</sub>**                      **T<sub>5</sub>- Inoculated + N<sub>1</sub>,**                      **T<sub>6</sub>- Inoculated + N<sub>2</sub>**

specification have been based on certain morphological characters such as sturdiness (height/diameter ratio), root/shoot ratio (Lavender and Cleary, 1974, Schmidt-vogot, 1974).

The seedling quality specifications are also fairly better in inoculated treatments. The treatments when compared on the basis of seedling quality parameter (Table-6) the T<sub>5</sub> and T<sub>2</sub> treatments gave good values for sturdiness, root/shoot ratio and Dickson quality index. Higher root/shoot ratio helps in survival and growth after planting (Chauhan and Sharma, 1997).

The Dickson quality index (QI) reflects the overall quality of the seedling. Higher the value of this index, the better will be the seedling. Highest QI (0.475) was found in T<sub>5</sub> followed by 0.469 in T<sub>2</sub>. Inoculation of *Rhizobium* would be helpful for production of quality seedling in nursery, which the experimental results also, confirms (Table-6). Inoculation of bio-fertilizer increased the seedling quality and seedling growth in *Dalbergia sissoo* at nursery.

#### **4.3.3 Nodulation study:**

The nodular properties like nodule no., nodule fresh weight and nodule dry weight mg/seedling as affected by different treatments and different age of seedlings are presented in Table-7

The no. of nodules per seedling ranged from 2 to 56 under different treatments and at different ages of seedlings. It was lowest when seedlings were not inoculated with *Rhizobium* and highest nodules no. was obtained in seedlings where *Rhizobium* inoculation along with low level of N was given. At 60DAT (3months age) nodulation began in inoculated seedlings but delay nodulation has been observed in uninoculated

seedlings. At 60DAT nodule number/seedling was 21 under T<sub>2</sub> which was significantly higher, but at 90 and 120 DAT, T<sub>5</sub> showed significantly higher nodulation. It was seen that nodulation decreased considerably in the control, but could not be completely eliminated as is evident from sparse nodulation in the control plants. With *Rhizobium* inoculation nodules number increase to 52/seedling where as in control (02) and further increased to 56 due to starter dose of N<sub>1</sub> (lower level). But with N<sub>2</sub> (higher dose) nodulation decreased considerably (26/seedling). However, highest was obtained in T<sub>5</sub> (56) followed by T<sub>2</sub> (52) at 90 DAT. While in only N fertilization maximum nodule no. was found (22) at T<sub>3</sub>, (09) at T<sub>4</sub> and minimum (04) at T<sub>1</sub>. *Dalbergia sissoo* nodules were round ball like off pink coloured and small nodules.

Similar observation was also found by Pokhriyal *et al* (1987) who found same nodulation in *Dalbergia sissoo* and studied nodule behaviour in *Leuceana*, *Acacia*, *Albizia* and *Dalbergia sissoo* tree species.

The fresh weight of nodules varied from 18 to 184mg/seedling. Those were higher in inoculated seedling as compared to uninoculated ones. The size of nodules was considerably larger in *Rhizobium* and N treated plants as compared to untreated ones. Significant increase in the weight of nodules of plants receiving *Rhizobium* + N<sub>1</sub> treatment over control was observed. Less N dose is equally parallel to ensure the efficiency of N<sub>2</sub> fixation. Hence the lower dose of N as starter dose is important to ensure in increasing the efficiency of N<sub>2</sub> fixation. Whereas significant decrease in nodulation as compared to T<sub>2</sub> was observed in T<sub>6</sub> i.e., giving N at higher dose along with *Rhizobium* inoculation. (Table-7)

**Table- 7: Effect of *Rhizobium* inoculation and N fertilizer application on nodulation behaviour of *D. sissoo* seedlings at different stages.**

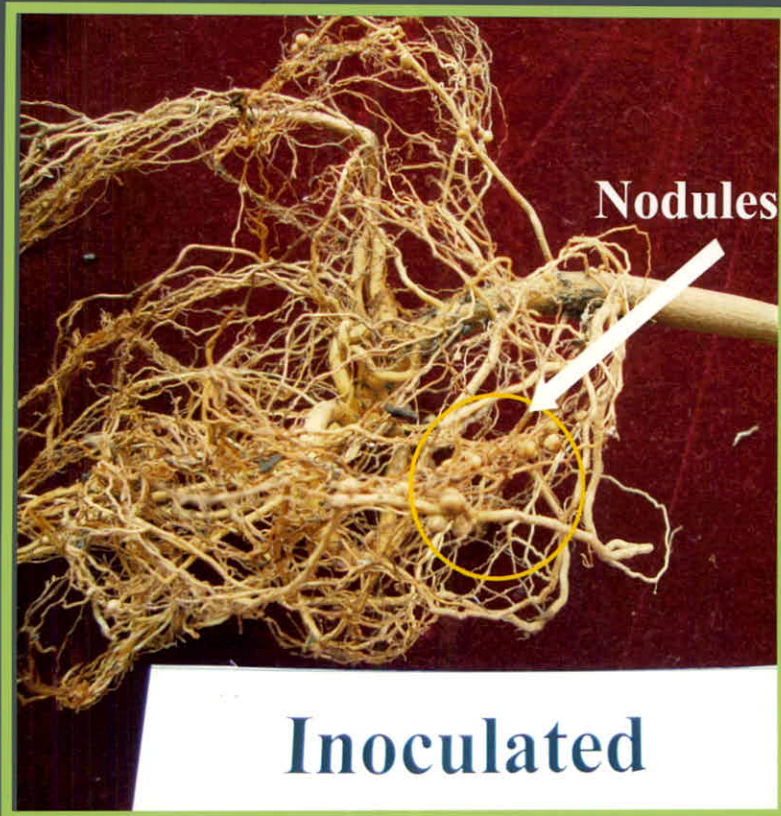
Treatment	No. of nodules/seedling				Fresh weight of nodules (mg/seedling)				Dry weight of nodules (mg/seedling)						
	Days after transplant				Days after transplant				Days after transplant						
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
T <sub>1</sub>	0.00	0.00	2.00	4.00	1.50	0.00	0.00	18.12	18.00	10.50	0.00	0.00	4.75	4.58	2.33
T <sub>2</sub>	0.00	21.00	52.00	42.00	28.75	0.00	98.10	195.30	128.10	105.25	0.00	56.75	114.00	84.25	63.75
T <sub>3</sub>	0.00	0.00	22.00	12.00	8.50	0.00	0.00	90.00	52.50	35.50	0.00	0.00	45.00	28.00	18.25
T <sub>4</sub>	0.00	0.00	9.00	7.00	4.00	0.00	0.00	50.00	48.00	24.50	0.00	0.00	20.00	18.00	9.50
T <sub>5</sub>	0.00	18.00	56.00	46.00	30.00	0.00	96.20	184.50	132.30	103.00	0.00	48.00	122.00	87.75	64.44
T <sub>6</sub>	0.00	18.00	26.00	26.00	17.50	0.00	93.40	168.20	104.20	91.25	0.00	42.00	101.00	62.25	51.31
<b>Factor</b>	<b>SEm(±)</b>	<b>CD (5%)</b>		<b>SEm(±)</b>	<b>CD (5%)</b>		<b>SEm(±)</b>	<b>CD (5%)</b>							
Treatment(T)	0.53	1.50		0.79	2.24		0.81	2.28							
Days (D)	0.44	1.23		0.65	1.83		0.66	1.86							
Interaction (TxD)	1.07	3.01		1.59	4.48		1.62	4.55							

T<sub>1</sub>- Control      T<sub>2</sub>- Inoculated (*Rhizobium*)      T<sub>3</sub>- Un-inoculated + N<sub>1</sub>  
T<sub>4</sub>- Un-inoculated + N<sub>2</sub>      T<sub>5</sub>- Inoculated + N<sub>1</sub>      T<sub>6</sub>- Inoculated + N<sub>2</sub>

The dry weight of nodules ranged between 4mg to 88mg at 120DAT under different treatments. At 90DAT highest dry weight of nodules observed which becomes slightly senescent at latter stage (at 120DAT) of *Dalbergia sissoo*. Significantly maximum dry weight of nodules (122mg/seedling) observed at 90DAT under treatment T<sub>5</sub>. (Table-7) *Rhizobium* inoculated seedling showed significantly higher nodule biomass over uninoculated ones, lower level of N dose when applied with *Rhizobium* inoculation influenced the nodulation and ensure to increase BNF, but reverse happened in case of high level of N, when applied with *Rhizobium* inoculation. Inoculation of *L. leucocephala* with its specific *Rhizobium* could bring out an amazing effect so far nodulation, growth and dry matter production of these legumes are concerned. This is an agreement with the report made earlier by Kabi *et al.*, (1982). Nodule biomass plays an important role in the N fixation activity of plant. This type of study was also carried out by Pokhriyal in 1987 in *Leuceana*, *Acacia*, *Albizzia* and *Dalbergia sissoo*. Summerfield *et al.*, 1977 and Dazzo and Bell (1978) also found that excessive N fertilizer application reduce root hair infection, nodulation no. and weight.

Biofertilizer application of *Rhizobium* or application of *Rhizobium* + *Azotobacter* combined has enhanced nodulation and growth of Seven forestry legume significantly and also found to augment dry matter production by Basu and Kabi (1987).

The magnitude of these data showed that inoculation with *Rhizobium* alone significantly influenced the nodular properties at different stages of the seedling and further increased with application of lower dose of N as starter dose. (Table 7)



**Plate 7 : Nodulation study of *Rhizobium* inoculated *Dalbergia sissoo* at 90 DAT**

#### 4.3.4 Nutrient status in leguminous plants

Inoculation with *Dalbergia sissoo Rhizobium* and Nitrogen fertilizer application influenced the nutrient concentration in different seedling components Viz., leaves, root and shoots. At 120DAT, seedlings where application of *Rhizobium* alone and along with lower level of N fertilizer showed higher nutrient concentration than only inorganic N fertilization and control. The N and K concentration were higher in leaves followed by roots and shoots where as P concentration was higher in root followed by leaves and then stem. (Table 8-11)

- **Nitrogen concentration in different plant components**

Nitrogen concentration as well as N content in different components of *D. sissoo* seedlings at 60 DAT was given in Table 8 and at 120DAT in Table -9

Nitrogen concentrations in leaves were affected by different treatments, highest N concentrations (1.88 %) was found in T<sub>5</sub> followed by T<sub>2</sub> (1.85 %) Which were 1.14 and 1.12 times greater over control at 60DAT.

Among inorganic N (Nitrogen) fertilization, T<sub>3</sub> (lower dose of N) showed higher N concentration in leaves, shoot and root of *Dalbergia sissoo* as compared to T<sub>4</sub>. Also at T<sub>5</sub> maximum N concentration in leaves, shoot and root of *Dalbergia sissoo* being 1.88, 0.70 and 0.95 % respectively while minimum was found at control.

In leaf, the N concentration ranged from 1.65% to 1.88%, in stem, it ranged from 0.58% to 0.70% while in root it ranged from 0.82% to 0.95 % respectively under different treatments at 60 DAT. (Table -8) Comparatively less concentration of N in leaf, stem and root of *D. sissoo* seedlings at 120DAT was found over N concentration at 60DAT. (Table 9). In leaf, the N concentration ranged from 1.58% to 1.81%, in stem, it ranged

from 0.52% to 0.65% while in root it ranged from 0.74% to 0.82 % respectively under different treatments at 120DAT.(Table -9)

*Rhizobium* inoculation showed higher N concentration over uninoculated ones. Further due to application of lower dose of N along with *Rhizobium* the concentration increased in leaf and almost remained equal as T<sub>2</sub> in stem and root but reverse happened at higher level of N when higher N was applied along with *Rhizobium* N concentration decreased than T<sub>2</sub>. Application of inorganic N fertilizer only also influenced in concentration of N over control. . (Table -8 and Table -9)

- **Nitrogen content in different plant components:**

At 60DAT in inoculated seedlings maximum N content was found in **leaves** followed by roots, while uninoculated seedling Nitrogen content was highest in root followed by leaves. *Rhizobium* inoculation singly showed significantly highest N content in leaf, stem and root i.e.,59.94mg/seedling ,42.66 mg/seedling and 46.17 mg/seedling respectively followed by T<sub>5</sub> containing 53.4,38.71and41.7mg/seedling while at T<sub>6</sub> it was significantly reduced. Among two levels of N, lower level of N influence significantly over higher N level. Inoculated seedlings showed significantly higher N content over only N fertilization and control. (Table 8). N uptake by shoot of *D. sissoo* was 102mg/seedling while at control it was 18mg/seedling at 60DAT.( Table 8)., N content varied from 10.56 to59.94mg/seedling in leaf, 7.05-42.66mg/seedling in stem and 18.1 to 46.2mg/seedling as affected by different treatments at 60DAT(Table 8).

*Rhizobium* inoculated seedling showed maximum N content ranging 98.87 to 130.50mg/seedling, while uninoculated ones contain leaf N ranging 34.76 to 85.2mg/seedling in leaf at 120DAT. Among two levels of N, lower level of N influence

**Table- 8: Effect of *Rhizobium* inoculation and N fertilizer application on N concentration % and N accumulation (mg/seedling) in different components of *D. sissoo* seedlings at 60 DAT.**

Treatment	Concentration Nitrogen %			Accumulation of Nitrogen (mg)/seedling			N uptake by shoot (mg)	
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf+ Stem	
Control	1.65	0.58	0.82	10.56	7.052	18.09	17.612	
Inoculated ( <i>Rhizobium</i> )	1.85	0.70	0.95	59.94	42.66	46.17	102.6	
Un-inoculated + N <sub>1</sub>	1.76	0.62	0.87	21.86	26.67	25.88	48.53	
Un-inoculated + N <sub>2</sub>	1.74	0.60	0.84	14.96	12.036	21.72	26.996	
Inoculated + N <sub>1</sub>	1.88	0.70	0.95	53.39	38.71	41.705	92.10	
Inoculated + N <sub>2</sub>	1.80	0.66	0.89	36.75	33.86	27.30	70.61	
SEm(±)				0.05	0.07	0.05	0.10	
CD (5%)				0.16	0.19	0.16	0.31	

**Table-9: Effect of *Rhizobium* inoculation and N fertilizer application on N concentration % and N accumulation (mg/seedling) in different components of *D. sissoo* seedlings at 120DAT**

Treatment	Concentration of Nitrogen (%)			Accumulation of Nitrogen (mg/seedling)			Nitrogen uptake by shoot(mg)
	Leaf	Stem	Root	Leaf	Stem	Root	
Control	1.58	0.52	0.74	34.76	35.93	51.87	70.69
Inoculated ( <i>Rhizobium</i> )	1.77	0.64	0.82	124.96	80.51	141.36	205.47
Un-inoculated + N <sub>1</sub>	1.70	0.54	0.79	85.17	54.04	93.3	139.21
Un-inoculated + N <sub>2</sub>	1.67	0.52	0.76	60.12	43.83	70.3	103.95
Inoculated + N <sub>1</sub>	1.81	0.65	0.82	130.50	85.47	138.9	215.97
Inoculated + N <sub>2</sub>	1.75	0.62	0.80	98.87	70.37	108.32	169.24
SEM (±)				0.04	0.17	0.17	0.18
CD (5%)				0.13	0.51	0.49	0.52

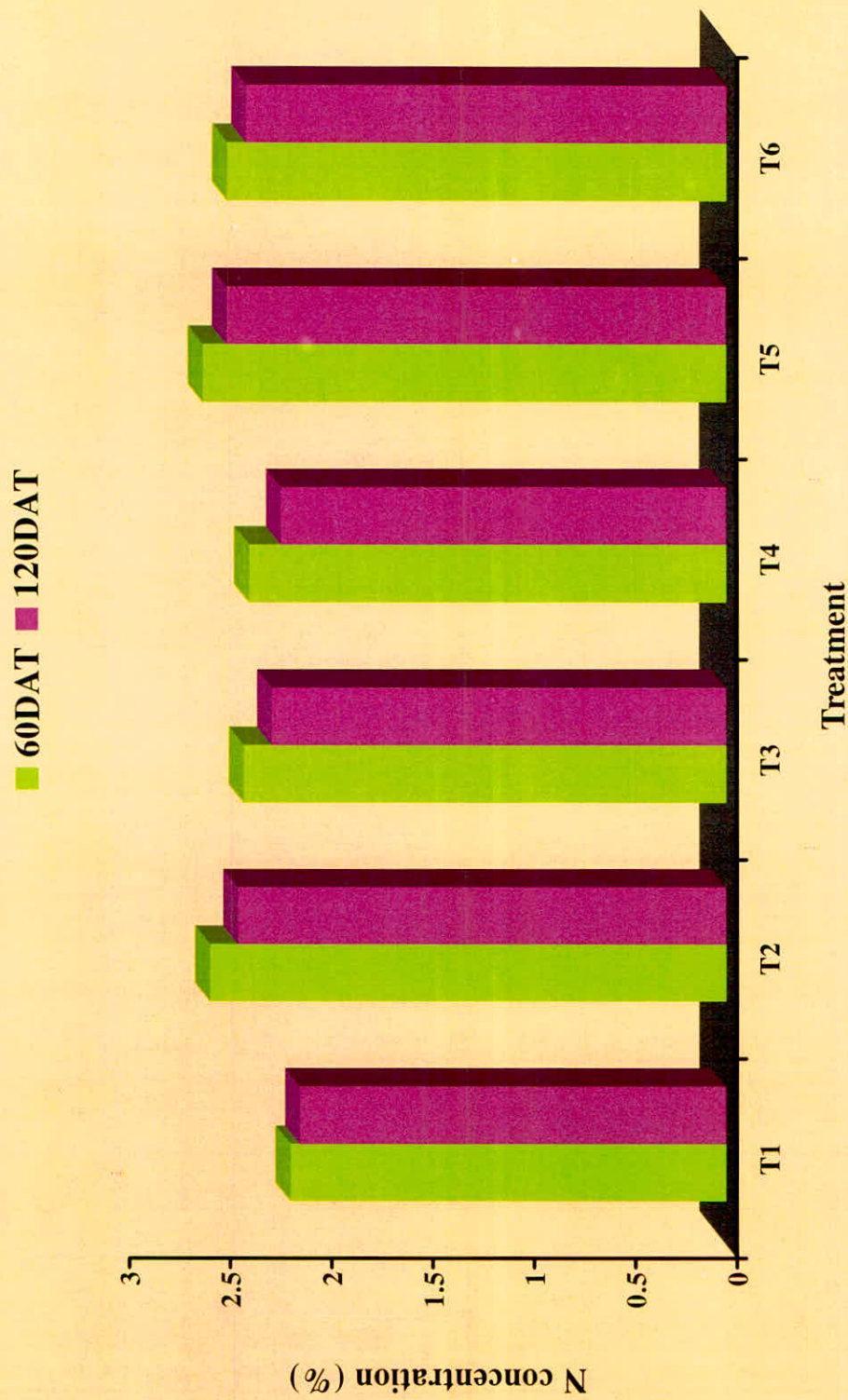


Fig. 8 : Effect of *Rhizobium* inoculation and N fertilizer application on N concentration in shoot of *Dalbergia sissoo* at 60 and 120 DAT.

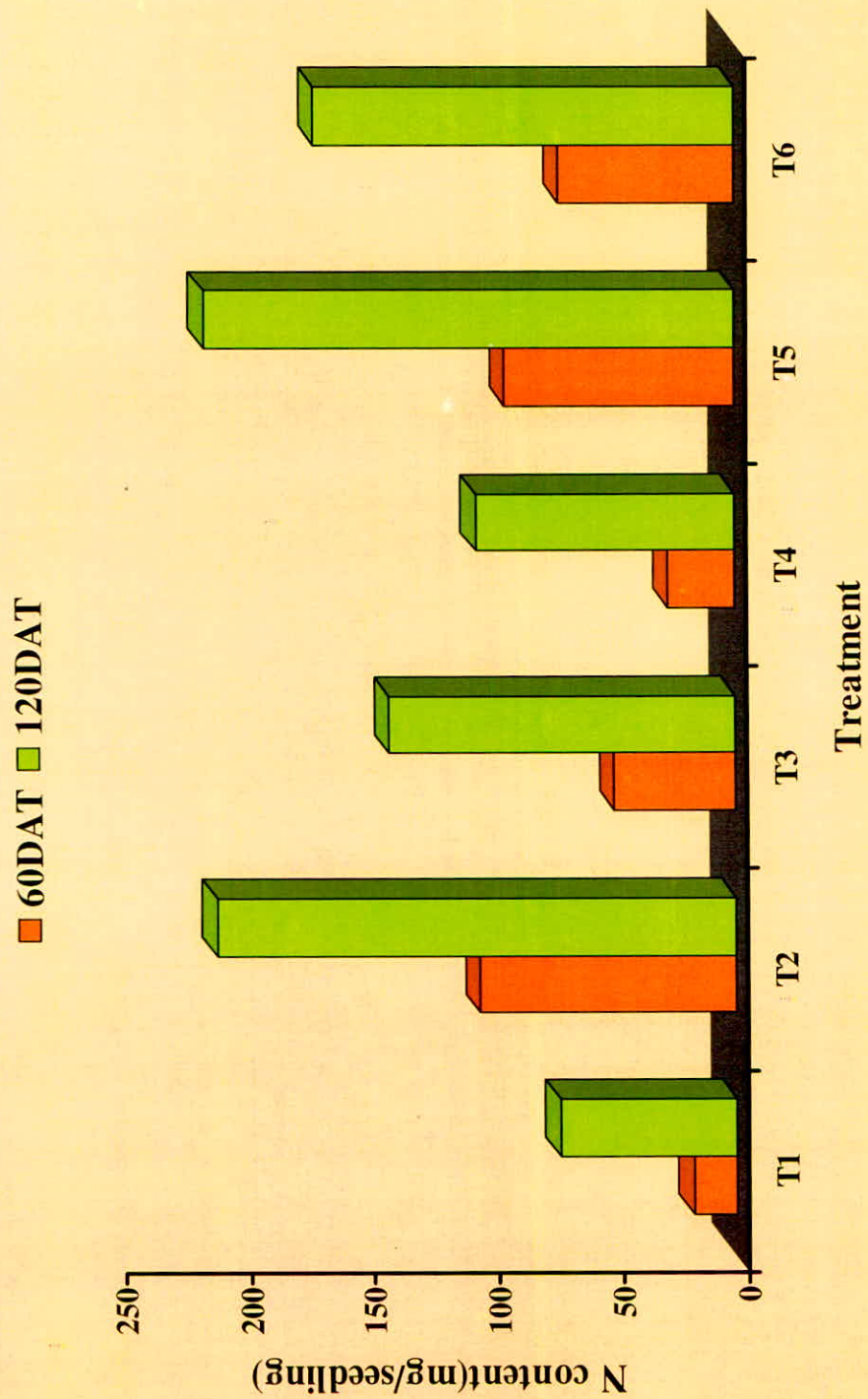
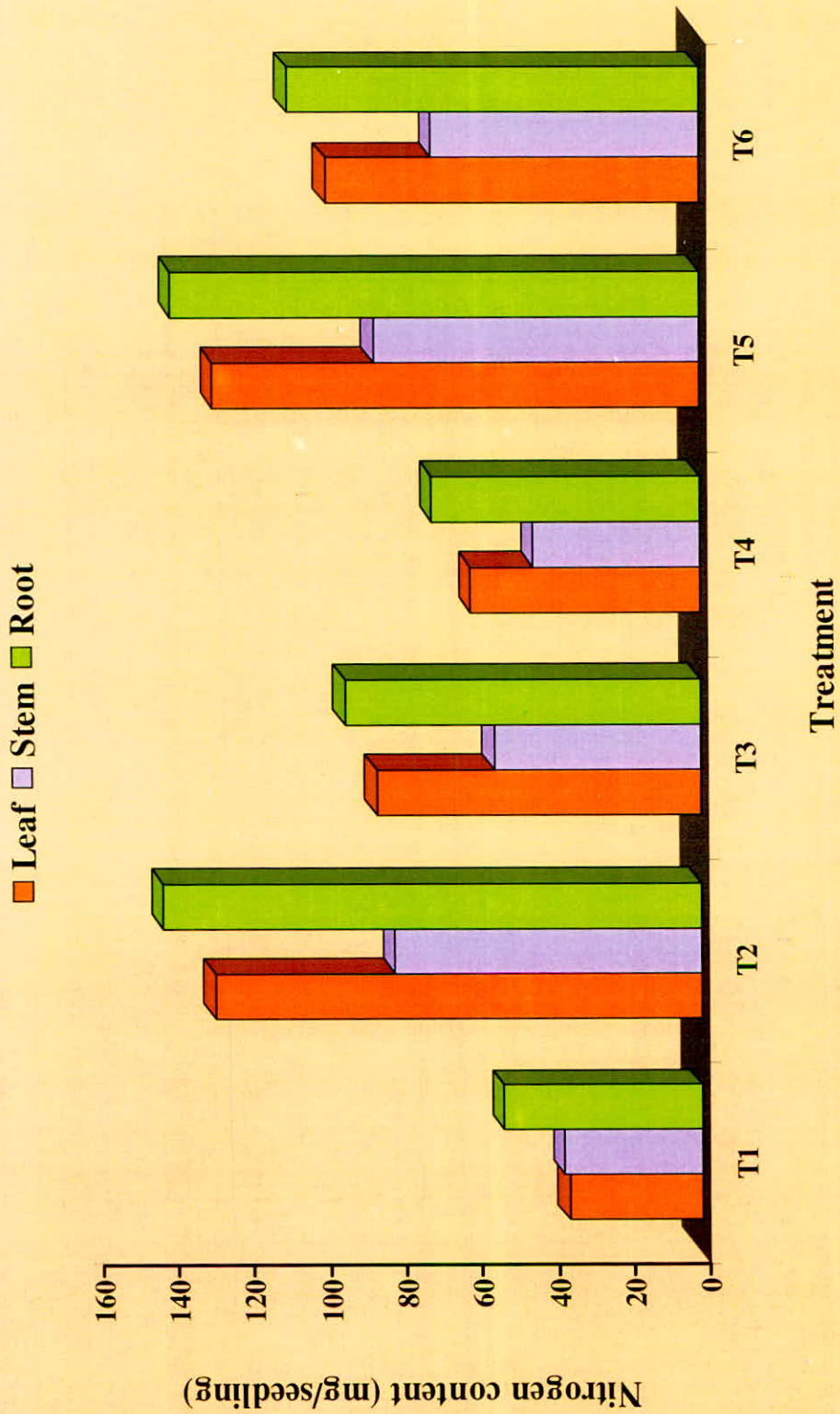


Fig. 9: Effect of *Rhizobium* inoculation and N fertilizer application on N content in shoot of *Dalbergia sissoo* at 60 and 120 DAT.



**Fig. 10 : Effect of *Rhizobium* inoculation and N fertilizer application on N content in different components of *Dalbergia sissoo* at 120 DAT.**

significantly over higher N level. In  $R + N_1$ , *Dalbergia sissoo* showed significantly maximum Nitrogen content 216mg/seedling in shoot followed by 205 mg/seedling at  $T_2$  while minimum 70.69mg/seedling at control.( Table-9) N content varied from 34.76 to 130.5mg/seedling in leaf, 35.93 to 85.47 mg/seedling in stem and 51.87 to 141.36mg/seedling in root as affected by different treatments at 120DAT( Table 9). Concentration of N as well as N content in shoot of *D. sissoo* at 60 and 120DAT was given in Fig. 8 and 9. Nitrogen content in different components at 120 DAT as affected by different treatments was clearly depicted in Fig.-10.

- **Phosphorus concentration in different seedling components:**

Concentration of P as well as accumulation of P in different components of *D. sissoo* at 120DAT was given in Table 10.

Phosphorus concentration in leaves of *Dalbergia sissoo* was affected due to different treatments, being highest in inoculated seedlings, where was minimum in uninoculated seedlings and was lowest in untreated seedlings *i.e.*, control. Among inoculated, when *Rhizobium* inoculation when given along with lower N level showed maximum P concentration in leaves (0.08%) while at *Rhizobium* only it was (0.07%). However, phosphorus concentration in leaves ranged from 0.05 to 0.08% with various treatments.

In shoot, phosphorus concentration ranged from 0.04 to 0.06% being highest under  $T_5$  and  $T_2$ . *Rhizobium* inoculated seedlings showed 0.06% of P concentration at  $T_2$  which was 1.5 times greater than control. While among N fertilization,  $T_3$  showed 0.05% P in shoot which was 1.2 times more over  $T_4$  and  $T_1$ .



**a) Measurement of collar diameter**



**b) Estimation of biologically fixed amount of nitrogen**

In root, P concentration ranged from 0.05 to 0.09%. Maximum (0.09%) P concentration was found in root of *Dalbergia sissoo* treated with T<sub>5</sub> at 120DAT. Which was 1.8 times more over T<sub>1</sub>. (Table 10)

- **Phosphorus content in different plant components at 120DAT**

Among different seedling components roots showed higher p content than stem and leaves. In roots, the Phosphorus content ranged from 3.5 to 15.25mg/seedling whereas in shoot, it ranged from 3.86 to 13.62mg/seedling under different treatments.

Leaves of *Dalbergia sissoo* (at 120DAT) contained 5.00 and 5.77mg P/seedling when inoculated with *Dalbergia sissoo Rhizobium* isolate alone and along with lower level N (N<sub>1</sub>) respectively.

In shoot, *Rhizobium* inoculation seedling showed P content of 13.62, 12.78 and 7.93mg/seedling under T<sub>5</sub>, T<sub>2</sub> and T<sub>6</sub> treatments. These were significantly higher over uninoculated treatments. In uninoculated treatment P content in shoot ranged from 3.86 to 8.0mg/seedling being highest under T<sub>3</sub> followed by T<sub>4</sub> and T<sub>1</sub>. Table 10

- **Potassium concentration in different components of *D. sissoo* seedling at 120DAT:**

Concentration of K as well as accumulation of K in different components of *D. sissoo* at 120DAT was given in Table 11.

Application of *Rhizobium* alone and along with N fertilizer application significantly affected Potassium concentration in leaves, stem and root of *Dalbergia sissoo* (120 DAT). Maximum concentration was found in *Rhizobium* inoculation along with lower dose of N followed by *Rhizobium* inoculation only. The concentration of potassium in leaves ranged from 0.78 to 0.95% (Table-11).

**Table-10: Effect of *Rhizobium* inoculation and N fertilizer application on P concentration (%) and P accumulation (mg/seedling) in different components of *D. sissoo* seedlings at 120DAT**

Treatment	Concentration of Phosphorous %				Accumulation of Phosphorous (mg/seedling)			Above Ground P content (mg/seedling)
	Leaf		Root		Leaf	Stem	Root	
	Leaf	Stem	Leaf	Root				
Control	0.05	0.04	0.05	0.05	1.1	2.76	3.5	3.86
Inoculated ( <i>Rhizobium</i> )	0.07	0.06	0.08	0.08	4.94	7.84	13.79	12.78
Un-inoculated + N <sub>1</sub>	0.06	0.05	0.07	0.07	3.00	5.0	8.26	8.00
Un-inoculated + N <sub>2</sub>	0.06	0.04	0.06	0.06	2.16	3.37	5.55	5.53
Inoculated + N <sub>1</sub>	0.08	0.06	0.09	0.09	5.77	7.85	15.25	13.62
Inoculated + N <sub>2</sub>	0.06	0.04	0.06	0.06	3.39	4.54	8.12	7.93
SEm(±)					0.12	0.05	0.07	0.13
CD(5%)					0.36	0.16	0.19	0.39

In stem potassium concentration ranged from 0.55 to 0.69% and in root it ranged from 0.55 to 0.71% under different treatments. *Rhizobium* inoculation along with lower N level exhibited maximum (0.95%) K concentration in leaf, whereas it was minimum (0.55%) in stem and root at control. Among N levels, lower one influenced significantly over the higher level. (Table 11)

• **Potassium content in different seedling components:**

Potassium content was higher in roots followed by stem and leaf of *sissoo* seedlings at 120DAT. In shoot potassium content ranged from 55.16 to 159.23mg/seedling and in root it ranged from 38.56 to 120.34mg/seedling under various treatments. *Rhizobium* inoculated seedlings along with lower N levels (T<sub>5</sub>) showed significantly maximum K content followed by *Rhizobium* inoculated only (T<sub>2</sub>).

Among uninoculated treatments, potassium content in leaves of *Dalbergia sissoo* was found as 41.58 mg/seedling at T<sub>3</sub>, 28.8 mg/seedling at T<sub>4</sub> and 17.16 mg/seedling at T<sub>1</sub>. Inoculated seedling showed significantly higher Potassium content in leaf over uninoculated ones being 66.36, 68.5 and 45.2 mg/seedling at T<sub>2</sub>, T<sub>5</sub> and T<sub>6</sub> respectively.

Significantly maximum potassium content in stem of *Dalbergia sissoo* was seen under R+N<sub>1</sub> treatment (90.73 mg/seedling) followed by *Rhizobium* only (85.54 mg/seedling). Moreover, these were 1.28 and 1.2 times greater over T<sub>6</sub>. Inorganic N application also showed higher K content in stem of 60.06 mg/seedling at T<sub>3</sub> followed by 50.58mg at T<sub>4</sub> while minimum was at control (38.0 mg/seedling).

Potassium content was significantly highest (120.34) in the roots of *Rhizobium* inoculated along with less N application (T<sub>5</sub>) seedlings, followed by T<sub>2</sub>(117.23

mg/seedling) which were 1.34 and 1.31 times greater than T<sub>6</sub>. Minimum was found at T<sub>1</sub> (38.56 mg/seedling). (Table-11). N, P and K content in *D. sissoo* at 120DAT as affected by *Rhizobium* inoculation alone and along with N application was shown in Fig.-11.

Similar trend in nutrient concentration was reported by Kaushik *et al.*, (2003). The application of *Rhizobium* also increased the uptake of nutrients, which enhanced the growth and development of seedling. The *Rhizobium* alone and along with N<sub>1</sub>, showed higher N, P and K concentration than uninoculated ones. P uptake by seedlings is more in inoculated ones over uninoculated. The adequate supply of moisture, mineral, nutrients, ensure the better growth and development of seedlings.

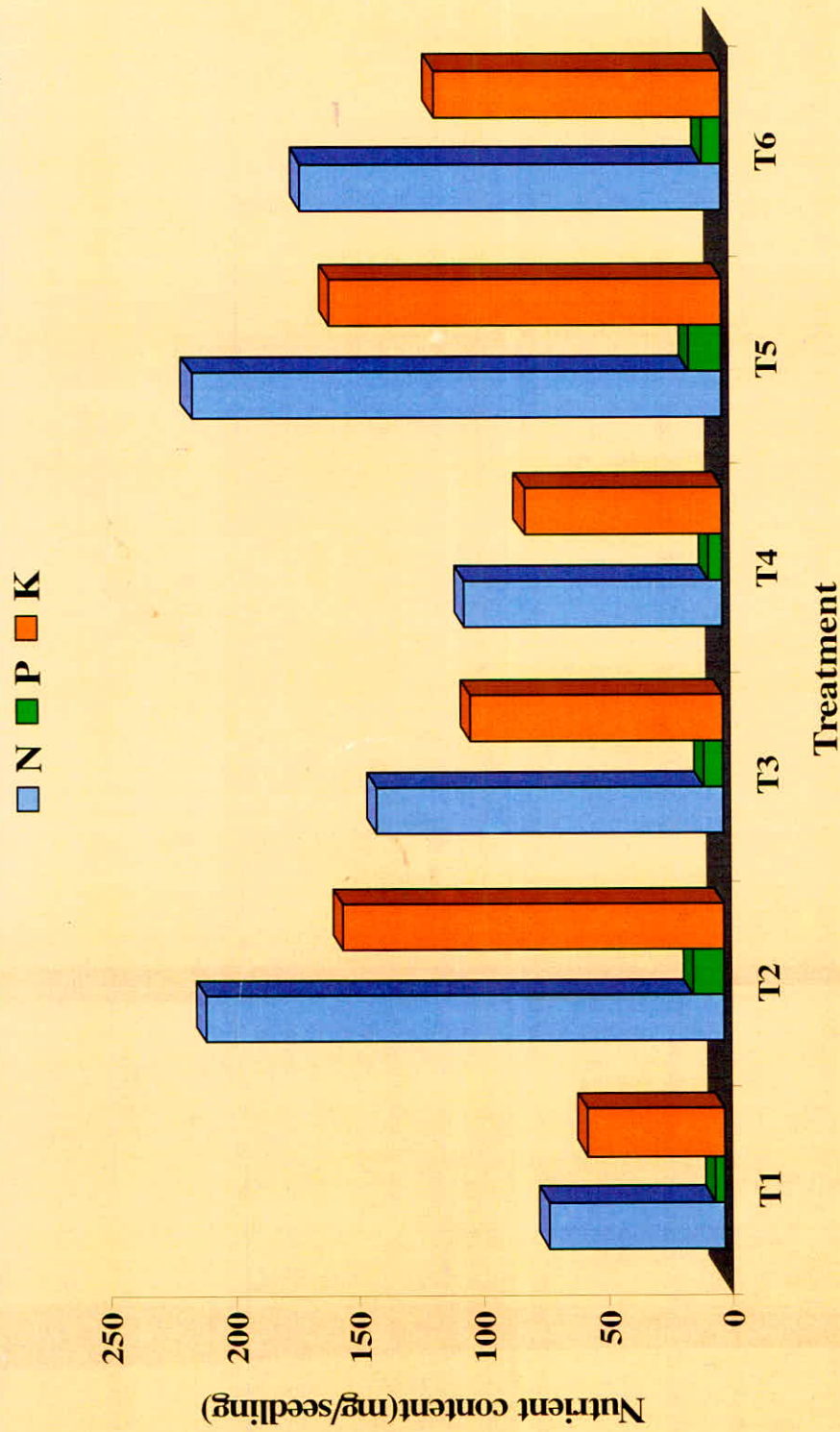
It is well established fact that *Rhizobium* and N fertilization enhanced nodulation, dry weight and nutrients (N, P and K) contents in legume sp. Prasad (1998) reported the highest N, P and K concentration in dual inoculation seedling of *D. sissoo* compared single inoculation of *Rhizobium*/Vam and uninoculated treatments. Increased

High N status in seedling was found in inoculated seedling due to increased BNF and increased P status in inoculated along with N<sub>1</sub> plants suggests more efficient absorption of P from the soil. The higher status of K in R + N<sub>1</sub> treated seedling might be due to synergistic effect of N and P which mediated the efficient uptake of K in seedlings. Such synergistic effect of increased uptake of one nutrient facilitated by other nutrients in leguminous species was also reported by Prasad (1998).

The better uptake of nutrient might be facilitated through microbial inoculants. Further application of less N dose (N<sub>1</sub>) along with *Rhizobium* inoculation showed a +ve

**Table-11: Effect of *Rhizobium* inoculation and N fertilizer application on K concentration (%) and K accumulation (mg/seedling) in different components of *D. sissoo* seedlings at 120DAT**

Treatment	Concentration of Potassium %			Accumulation of Potassium (mg/seedling)			Above Ground K content (mg/seedling)
	Leaf	Stem	Root	Leaf	Stem	Root	
Control	0.78	0.55	0.55	17.16	38.00	38.56	55.16
Inoculated	0.94	0.68	0.68	66.36	85.54	117.23	151.90
Un-inoculated + N <sub>1</sub>	0.83	0.6	0.64	41.58	60.06	75.58	101.64
Un-inoculated + N <sub>2</sub>	0.80	0.6	0.62	28.80	50.58	57.35	79.38
Inoculated + N <sub>1</sub>	0.95	0.69	0.71	68.50	90.73	120.34	159.23
Inoculated + N <sub>2</sub>	0.80	0.62	0.66	45.2	70.37	89.36	115.57
SEm(±)				0.03	0.06	0.05	0.06
CD(5%)				0.09	0.17	0.14	0.18



**Fig. 11: Nutrient content in *Dalbergia sissoo* at 120 DATs affected by *Rhizobium* inoculation and N application**

influence in nutrient uptake by sissoo seedlings. The result revealed that the nutrient N, P and K contents were higher in *Rhizobium* +N1 treated plants and also in only *Rhizobium* inoculated plants as compared to other treatments. The nutrient content mainly depends on the nutrient concentration of components and its biomass in seedlings. The *Rhizobium* inoculation only and *Rhizobium* + N1 treatment not only increased the nutrient status but also resulted higher biomass. Due to higher biomass and more nutrient concentration, the nutrient content in different components of seedling was higher in inoculated treatments.

#### **4.4 *Rhizobium* population in rhizosphere soil of *Dalbergia sissoo***

##### **legume seedlings:**

Data on *Rhizobium* population per g of soil in rhizosphere soil of *Dalbergia sissoo* as affected by different treatment effect at 120DAT was given in Table 12.

In initial soil *Rhizobium* population per g of soil was  $5.4 \times 10^2$  which increased to  $2.4 \times 10^3$  in control due to influence of leguminous tree. Further it increased significantly to  $3.64 \times 10^3$ ,  $3.83 \times 10^3$  and  $3.65 \times 10^3$  in inoculated treatments at T<sub>2</sub>, T<sub>5</sub> and T<sub>6</sub> respectively at 120DAT. In T<sub>5</sub> highest population was found. So N at less dose has a positive correlation in increasing *Rhizobium* population at rhizosphere zone of *Dalbergia sissoo* when inoculation was given along with N<sub>1</sub>, but reverses in case of N<sub>2</sub>.

Application of inorganic N fertilization only, *Rhizobium* population was found higher ( $2.67 \times 10^3$ ) at T<sub>3</sub> and  $2.52 \times 10^3$  at T<sub>4</sub> while at control it was  $2.4 \times 10^3$  at 120DAT. Microbes decompose organic matter and release nutrients. More microbes are an advantage for nutrient transformation and plant growth. Rhizosphere effect of

leguminous plant and also by adding inoculation was evident from soil biological properties under *Dalbergia sissoo*. (Table –12).

The effect of NFT, was pronounced in *Rhizobium* inoculated than control. This is ascribed due to efficient recycling of nutrients under this treatment. The more microbial population in rhizosphere soil was established by planting of legume tree, which also reported by Singh *et al.*, 2000. The application of N1 to *Rhizobium* inoculated seedling of *D. sissoo* further enhanced the *Rhizobium* population.

Both *Rhizobium* alone and along with lower dose of N improve the growth of *D. sissoo* seedling through fixation of atmospheric N in root nodules and the increased nodulation helps in N status in plants, which is essential for growth and development of seedlings. The result revealed that higher growth was observed in lower doses of N than the higher level of N, (N<sub>2</sub>). Thus difference in growth and development of seedlings are mainly attributed to nutrient status of *i.e.* at higher N dose, the effect of *Rhizobium* in biological Nitrogen fixation is reduced as compared to *Rhizobium* inoculation only. Higher dose of N fertilizer also reduced the seedling growth by the initiation of toxic fertilizer effects. The earlier worker also showed the poor effect of inoculation of *Rhizobium* at higher N dose. Similar observation was also reported by Uddin *et al.* 2007 for *A. lebbeck* in response to application of phosphorus fertilizer -ve effect of commercial fertilizers on seedling growth at higher dose was also observed by Kadeba (1978) who reported that the addition of excess fertilizer on *Pinus caribacea* depressed growths and increased mortality of seedlings. The finding of Vander Driessche (1980) also supported those who reviewed both the +ve and -ve effects of N fertilizer application on seedling growth.

**Table 12: *Rhizobium* population in rhizosphere soil of *Dalbergia sissoo* as affected by *Rhizobium* inoculation and N application at 120 DAT.**

Treatment	Rhizobium population per g of soil
T1 (Control)	2.4 x 10 <sup>3</sup>
T2(Inoculated ( <i>Rhizobium</i> ))	3.64 x 10 <sup>3</sup>
T3(Un-inoculated + N <sub>1</sub> )	2.67 x 10 <sup>3</sup>
T4(Un-inoculated + N <sub>2</sub> )	2.52 x 10 <sup>3</sup>
T5 (Inoculated + N <sub>1</sub> )	3.83 x 10 <sup>3</sup>
T6 (Inoculated + N <sub>2</sub> )	3.65 x 10 <sup>3</sup>
SEm (±)	104.8
CD at 5%	311.4

Inorganic N fertilizer can have an effect being it +ve and -ve, on plant nodulation, N<sub>2</sub> fixation and seedling growth of legume plants (Huda *et al.*, 2007). High concentration of inorganic N in soil normally inhibit symbiotic- N- fixation (Hungria and Vargas2000), but not always (Davidson and Robson, 1986).

The effect of inorganic nitrogen fertilizer on seedling growth and nodulation capability of some agroforestry tree speices *Acacia chinensis*, *A. nilotica* ,and *Sesbania sesban* was carried out by Uddin, Khan *et al.*, (2008 ), revealed that the seedlings growth was enhanced significantly with moderate fertilizer treatment . While at higher level of fertilizer reduce the seedling growth. The present study also reveled that the nodulation, was significantly reduced by application of higher level nitrogen fertilizer .This beneficial effect of low N dosages on the growth and development of the plant have been reported by many investigators (Prasad *et al.*, 1998) Eaglesham *et al.*, 1983 and Katoch *et al.*, 1983.

The present findings showed that *Rhizobium* application alone and along with N is comparatively better for improving growth and development of legume seedling *i.e.* *D. sissoo* as it influences other process such as photosynthesis, uptake of trace element and plant hormones along with nodulation and N fixation. Inoculation with *Rhizobium* alone and in combination with different N levels (15, 30, 45, Kg/ha.) on yield and yield component of mungbean crop was studied by Anjum *et al.*, (2006) observed inoculation and 15Kg N/ha. significantly increased no. of pods per plants as compared to other higher N doses. Chetti *et al.*, (1995) studied the effect of N and *Rhizobium* inoculation on the productivity of ground nut and repotted that both N application and inoculation had significant +ve effects. Bora *et al.*, 2006 observed both root and shoot biomass was

considerably high in *A. procera* inoculation seedling growth and in LLU (low level urea). The value was significantly reduced in case with MLU (Moderate level of urea) and HLU (High level of urea) amended soil. This may be, due to the fact that the higher rates of N fertilizer may have produced nutrient imbalance thus rendering unavailable the other elements in soil. (Jha *et. al.*, 2000).

*SUMMARY, CONCLUSION AND SUGGESTIONS  
FOR FUTURE RESEARCH WORK*

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## CHAPTER V

### SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

The present investigation entitled “Effect of *Rhizobium* inoculation and Nitrogen fertilizer application on growth performance of *Dalbergia sissoo* tree seedlings” was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture Raipur, Chhattisgarh during August-January, 2009-10 with the objective to see the response of legume tree seedlings (*Dalbergia sissoo*) to *Rhizobium* inoculation and the influence of Nitrogen on biologically Nitrogen fixation.

The above experiment was carried out in polythene bags with 6 treatments consisting of *Dalbergia sissoo* -*Rhizobium* inoculation @ 5 ml inoculated broth/seedling, 2 levels of N fertilization (2g and 4g N/seedling) alone and along with each other including one control. These treatments were treated with 4 replications on *Dalbergia sissoo* seedlings as factorial CRD to study the root inoculation effect alone and along with N on growth performances of sissoo seedlings at 4 different stages (DAT). The results have been furnished and discussed in the preceding chapter.

The salient findings of the investigation have been summarized in this chapter in following points:

1. The *Dalbergia sissoo Rhizobium* isolate from nodulated *Dalbergia sissoo* seedling was tested for Gram staining reaction and results showed the isolate was gram negative.
2. The soil sample used for raising *Dalbergia sissoo* seedlings in polythene

bags contain *Rhizobium* population of  $5.4 \times 10^2$  per g soil and mineralizable Nitrogen was low in status (235.8Kg/ha). After completion of experiment at 120 DAT, *Rhizobium* population(/g soil) in rhizosphere soil of *D. sissoo* was  $2.4 \times 10^3$  at control due to influence of legume tree seedling, further due to *Dalbergia sissoo Rhizobium* inoculation treatment it increased significantly to  $3.64 \times 10^3$  and further increased to  $3.83 \times 10^3$  when *Rhizobium* inoculation was given along with less N dose ( $N_1$ ). But when inoculation was given along with higher N ( $N_2$ ), the *Rhizobium* population significantly decreased to ( $3.65 \times 10^3$ ).

3. The isolate of *Dalbergia sissoo - Rhizobium* were tested for their impact on morphological growth parameters of *Dalbergia sissoo* seedlings. Results revealed that *Rhizobium* inoculation alone and along with less N dose significantly influenced the growth of *Dalbergia sissoo*. Seedling height increased by 2.05 and 2.13 times over control with *Rhizobium* inoculation only ( $T_2$ ) and *Rhizobium* + less N ( $T_5$ ) treatments respectively. Significantly highest shoot length (64.8cm) was found in  $T_5$  over  $T_2$  (62.4cm) at 120 DAT. Root length was significantly high and maximum (48.2cm) at  $T_2$  followed  $T_5$  (46cm) at 120 DAT. Initially under  $T_2$  the number of leaves/seedlings showed significantly maximum (84) at 60 DAT, but latter at 120 DAT it was maximum up to 128 at  $T_5$ . Similarly no of small branches/seedlings was highest (07) in both  $T_2$  and  $T_5$  which significantly increased over uninoculated ones (03).
4. These growth parameters followed an increasing trend with age of seedlings from August to January irrespective of different treatments applied to seedlings. The increasing rate was significantly higher in inoculated seedlings.

5. Collar diameter of sissoo seedlings showed significant effect at T<sub>2</sub> and T<sub>5</sub> over uninoculated seedlings being maximum (8.27mm) at T<sub>5</sub> followed by (8.01mm) at T<sub>2</sub>, while minimum (6.02mm) at control. Application of N<sub>1</sub> with inoculation was significant over only inoculation.
6. With *Rhizobium* inoculation, maximum nodule no. /seedlings was 52 and further increased to 56 due to applying N<sub>1</sub> along with *Rhizobium* at 90 DAT, while with N<sub>2</sub> along with *Rhizobium* it decreased to 26. Again nodule no./seedlings were 22 and 9 at T<sub>3</sub> and T<sub>4</sub> respectively. Very sparse nodulation (04) was seen at control. Similar trend was observed in case nodule fresh weight and nodule dry weight. At 120DAT the effect of less dose of N when applied with inoculation was significant over T<sub>2</sub> w.r.to nodulation. Earlier initiation of nodulation was observed in inoculated seedlings as compare to uninoculated ones.
7. Biomass production was significantly affected by *Rhizobium* inoculation singly and in combination with N<sub>1</sub> in *D. sissoo* at different age. Root biomass was higher over shoot biomass followed by leaf biomass. Biomass accumulation ranged from 2.20 to 7.21g/seedling for leaf, 6.91to 13.15g/seedling for stem and 7.01to 17.24g/seedling for roots at 120 DAT under different treatments. Total biomass accumulation by *D. sissoo* seedling at 120 DAT was increase by 130 % and 132 % at T<sub>2</sub> and T<sub>5</sub> respectively over control.
8. Quality Index (as per Dickson) showed higher values for inoculated seedlings over uninoculated. 50% increase in QI of sissoo seedlings at 120 DAT was observed in inoculated seedlings over control. Maximum Quality Index of 0.475

was found for seedlings receiving *Rhizobium* inoculation along with less N (N<sub>1</sub>) followed by 0.469 when only *Rhizobium* inoculation was given. While it was reduced (0.444) at higher N along with inoculation.

9. N concentrations were highest in leaves followed by roots then stem component of *Dalbergia sissoo*. Maximum N concentration in leaf, stem and root were 1.88 %, 0.70%, 0.95% was found at 60DAT which decrease to 1.81%, 0.65% and 0.82% at 120DAT under T<sub>5</sub> respectively. P concentration was highest in roots followed by leaf then stem of *D. sissoo*. The maximum P concentration (0.08%, 0.06% and 0.09% in leaf, stem and root respectively at 120DAT) was at T<sub>5</sub> followed by T<sub>2</sub>.

Like Nitrogen, K concentration was also highest in leaves followed by roots then stem of *Dalbergia sissoo*. Maximum K concentration of 0.95%, 0.69%, 0.71% were found in leaf, stem and root respectively at T<sub>5</sub> followed by T<sub>2</sub>.

10. Nitrogen uptake in shoot gained extra atmospheric N of 85mg/seedling and 74.5mg/seedling at *Rhizobium* inoculation only and *Rhizobium* along with N<sub>1</sub> respectively at 60DAT. Significantly highest N uptake by shoot of *D. sissoo* was (102.6 mg/seedling) found at T<sub>2</sub> followed by T<sub>5</sub> ( 92.1 mg/seedling ). N uptake was reduced by 1.45 times at T<sub>6</sub> giving N<sub>2</sub> with *Rhizobium* from T<sub>2</sub>. At 120DAT N uptake by shoot of *D. sissoo* was significantly high 216 mg/seedling at T<sub>5</sub> followed by T<sub>2</sub> 205 mg/seedling. Nitrogen uptake in shoot gained extra atmospheric N of 135mg/seedling and 145 mg/seedling at *Rhizobium* inoculation only and *Rhizobium* along with N<sub>1</sub> respectively at 120DAT.
11. Utilization of Phosphorus and Potassium by seedling was also significantly

influenced by *Rhizobium* only and *Rhizobium* along with less N application. Significantly maximum P uptake by shoot of *D. sissoo* (13.62 mg/seedling) was observed at T<sub>5</sub> followed by 12.78mg/seedling at T<sub>2</sub>. Similar trend was observed in case of K uptake by seedling.

12. Inoculation treatments were highly significant as compared to N fertilization only and control. Less N dose (T<sub>3</sub>) influenced more in increasing growth of *D. sissoo* over higher N dose (T<sub>4</sub>) and both treatments showed significant effect over control. Application of less N along with *Rhizobium* inoculation (T<sub>5</sub>) showed significant effect w.r. to growth parameters and biomass accumulation, nodulation and nutrient content in *sissoo* seedlings. Higher N fertilization dose did not favour the growth of *sissoo* seedlings when given alone and along with *Rhizobium* inoculation resulting less shoot length, root length no. of leaves and no. of branches as compared to T<sub>2</sub> and T<sub>5</sub>.

## CONCLUSIONS:

Keeping in view of above mentioned findings, broad conclusions drawn from the experiment are stated here as below.

1. Root inoculation of *Dalbergia sissoo* seedlings with effective *D. sissoo* -*Rhizobium* (@ 5 ml inoculum/seedling) was found significantly effective and has tremendous potential in improving growth, biomass production, nodulation and nutrient content over only inorganic N application treatments and control. This is attributed by providing more rhizobia in rhizospheral zone of tree seedling.
2. N application at sub optimal level (2g/seedling) along with *Rhizobium* inoculation enhanced BNF through enhancing the efficiency of *Rhizobium* in symbiotic relationship. In terms of growth performances and nutrient uptake by *D. sissoo*, a positive response was found when N fertilization in less level was given along with *Rhizobium* inoculation in *D. sissoo* seedling.
3. Based on results it can also be inferred that application of N fertilizer at the rate of 2g/seedling along with *Rhizobium* inoculation has the potential to improve the growth of *Dalbergia sissoo* seedlings in nursery. This finding may be helpful in improving initial growth response of *Dalbergia sissoo* plants in afforestation programmes.

## SUGGESTIONS FOR FUTURE WORK:

1. As NFT species are of great importance in traditional agro-forestry system, the detailed field investigation was recommended to ensure the long term growth performance of selected NFTs species in response of inoculation and N fertilizer application in natural stands.

2. A screening and grading of different NFTs depending upon their N-fixing performance on location wise is needed.
3. Screening of efficient *Rhizobium* strain of NFTs may be need-full to increase effect of N fixation and boost growth of tree.
4. Tree Rhizobia may have specific traits and the cross inoculation of agriculturally important legumes with Rhizobial isolates from tree legumes may prove a useful mean of increasing nutrient content within these plants. Field trials are needed to prove the effectiveness of the isolate form tree legumes in increasing nutrient content of cultivated pants.

**“Effect of *Rhizobium* inoculation and Nitrogen fertilizer application on growth performance of *Dalbergia sissoo* tree seedlings.”**

**BY**

**Anish kumar**

***ABSTRACT***

The present experiment was conducted in glass house of Department of Microbiology, College of Agriculture, Raipur, Chhattisgarh in the year 2009-10 with six treatments comprised of *Dalbergia sissoo* -*Rhizobium* inoculation @ 5 ml inoculum/seedling, 2 levels of N fertilization (N<sub>1</sub> and N<sub>2</sub> as 2g and 4g N/seedling) alone and along with each other including one control to evaluate the response of legume tree seedling (*Dalbergia sissoo*) to *Rhizobium* inoculation and the influence of Nitrogen on biological Nitrogen fixation. This involved isolation of *Rhizobium* isolate from nodule of *D. sissoo*.

Results revealed that root inoculation of *D. sissoo* seedlings with *D. sissoo* -*Rhizobium* inoculum was found significantly effective in improving growth, biomass production, nodulation and nutrient content over only inorganic N application and control. A positive response was found when N in less level was given along with *Rhizobium* inoculation. Significantly maximum root length (48.2cm), root biomass (17.24g/seedling) and root/ shoot ratio (0.88) were obtained in sissoo seedlings receiving only inoculation at 120 days after transplant (DAT) while combined effect of inoculation along with lower level of N showed maximum seedling height (64.8cm), shoot biomass (20.4g/seedling) and collar diameter (8.27mm). 129% and 132 % increase in total biomass over control was found in inoculation only and along with N<sub>1</sub>. Significantly maximum nodulation (no.56/seedling and dry weight 122mg / seedling) was found in *Rhizobium* + N<sub>1</sub> treatment followed by *Rhizobium* inoculation only. Significantly higher N, P, K content in shoot of *D. sissoo* (216.0, 13.62 and 159.23 mg /seedling respectively) was found when N<sub>1</sub> was applied along with inoculation. Inoculation with N<sub>1</sub> treatment resulted maximum *Rhizobium* population per g of rhizosphere soil of *D. sissoo* at 120DAT ( $3.83 \times 10^3$ ).

Root inoculation of *D. sissoo* seedlings with species- *Rhizobium* showed significant effect and further application of N at lower level along with *Rhizobium* inoculation was found most effective in increasing symbiotic traits, growth performances and nutrient uptake by *D. sissoo* seedlings.

**Department of Microbiology  
College of Agriculture,  
Raipur.**

Date: 2.08.2010



**Smt. D. Dash  
Chairman  
Advisory committee**

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## APPENDIX I

### Weekly Meteorological data (30 July 2009 to 21 January 2010)

Week Number	Temperature (°C)		Rainfall (mm)	Relative Humidity (%)		Wind Velocity (Kmph)	Sun shine (hours)
	Max.	Min.		I	II		
31	32.05	26.6	2	81	57	9.2	6.4
32	31.8	25.4	99.6	87	74	8.5	3.8
33	30.7	25.4	50.2	93	77	6.1	3.2
34	30.2	24.4	99.2	93	78	6.3	3.1
35	30.8	25.2	17.2	95	83	4.8	3.9
36	31.2	25.3	17.2	95	83	4.8	3.9
37	32.2	24.7	8.6	92	62	5	6.6
38	33	25.8	0.4	92	62	3.3	7.7
39	32.9	25.1	6.4	90	65	4.3	4.3
40	30.6	24.8	50.8	92	79	6.5	4.4
41	31.9	22.1	0.8	93	53	1.9	6.9
42	32.7	20	0	92	41	1.7	9.2
43	31.3	15.3	0	94	25	2	9.3
44	30.7	16.7	0	92	34	1.9	7.6
45	30.4	20.9	0	90	56	3.4	4.6
46	29.8	21.6	49.5	91	68	4	3.6
47	26.4	13	0	93	40	4.8	7.8
48	26.9	10.8	0	93	32	1.6	8.9
49	28.6	13.1	0	93	36	1.5	7.7
50	29.5	14.9	0	93	39	1.7	7.1
51	26.1	13.5	19.2	94	52	2	6.4
52	26.1	11.6	0.1	91	36	2	7.1
1	25.6	8.6	0	88	29	2.2	8.4
2	26.6	14.7	15.4	87	49	2	5.1
3	25.7	9.3	0	94	32	1.3	7.6

## APPENDIX II

### Chemical composition of media

**Yeast Extract Mannitol Agar Media (YEMA) for rhizobia (Subba Rao, 1988).**

#### Composition of the medium

▪ Mannitol	10.0 g
▪ $K_2 HPO_4$	0.5 g
▪ $MgSO_4 \cdot 7H_2O$	0.2 g
▪ NaCl	0.1 g
▪ Yeast Extract	1.0 g
▪ Agar	15.0 g (Broth culture not add)
▪ Distilled water	1000.0 ml
▪ Congo red solution (1%)	2.5 ml
▪ pH =	7.0