EFFECT OF *RHIZOBIUM* INOCULATION WITH DIFFERENT LEVELS OF NITROGEN ON PERFORMANCE OF KARANJ (*Pongamia pinnata*) SEEDLINGS

M.Sc. (Ag.) THESIS

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CERTIFICATE - I

This is to certify that the thesis entitled "**EFFECT OF *RHIZOBIUM* INOCULATION WITH DIFFERENT LEVELS OF NITROGEN ON PERFORMANCE OF KARANJ (*Pongamia pinnata*) SEEDLINGS"**, submitted in partial fulfilment of the requirements for the degree of **"Master of Science in Agriculture" (Agricultural Microbiology)** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Ku. SUJATA DARPAN** 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 her.

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Member Dr. S.B. Gupta _____

Member Shri S. S. Porte _____

Member Dr. R.R. Saxena _____

Member Dr. (Smt.) J. Ganguli _____

CERTIFICATE – II

This is to certify that the thesis entitled "**EFFECT OF *RHIZOBIUM* INOCULATION WITH DIFFERENT LEVELS OF NITROGEN ON PERFORMANCE KARANJ (*Pongamia pinnata*) SEEDLINGS",** submitted by **Ku. SUJATA DARPAN** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in partial fulfilment of the requirements for the degree of **M.Sc. (Ag.)** in the **Department of Agricultural Microbiology** has been approved by the external examiner and student's advisory committee after an oral examination.

Date: **External Examiner**

Major Advisor

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Dean Faculty

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Director of Instructions

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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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Date: _____

Sujata darpan

C O N T E N TS

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
%	Per cent
/	Per
@	at the rate
^{0}C	degree Celsius
BNF	Biological Nitrogen Fixation
CD	Critical Difference
cm	centimeter
DAT	Days after transplant
E.C.	Electrical Conductivity
et al.	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 $\pm$	Standard Error of means
Viz.	for example
w.r.t.	with respect to

CHAPTER - I

INTRODUCTION

Pongamia pinnata, commonly known as 'Karanj' is one of the important Nitrogen Fixing Tree (NFT) species belongs to family Leguminosae, sub-family Paplionaceae. A medium –sized tree,with a short bole and spreading crown, it grows up to 18 m height or some times even more and 1.5 m in girth. Karanj is an indigenous remarkable tree species found throughout India along road sides, canals and railway tracks. The wood is used for furniture, small turnery article and also as veneer for ply work. It is one of important species, which can play a role in reducing the energy crisis as the oil is directly used for supply of raw material for biodiesel production (Shrinivasa, 2001). The oil extracted from the seeds is used for soap making, lubrication, leather tanning and for medicinal purposes (Anon, 1969). Among the oil bearing trees, *Pongamia pinnata* (Karanj) has large industrial and economic potential and again being a Nitrogen Fixing Tree (NFT) it is important for soil N management.

Considering the emerging importance of this species as a potential tree born oil seed and an important NFT species, the ultimate objective is to develop fast growing, high yielding, superior quality in order to replace the wild natural forest stands so as to obtain higher forest productivity per unit area and time.

Very often trees are established in the field by transplanting seedlings from nursery. To be successful after out planting, Karanj 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 seedlings 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 necessitates the integrated nutrient management involving biological sources of nutrient for sustainable crop production.

Biofertilizer improve the quality of tree seedlings which are better adopted to withstand the adverse conditions and has tremendous potential to provide plant nutrients by boosting the microbial population present in soil which in turn makes the insoluble nutrients available for growth of the plants. (Subba Rao, 1977). *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 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).

It was therefore felt necessary to take up research programme, by applying *Rhizobium* biofertilizer at nursery to Karanj which could be effective for maintaining quality nursery stocks for large scale plantation. Karanj being an N-fixing tree can be

utilized to its fullest extent only if it occurs successfully symbiotic relationship with *Rhizobium*. A study indicate that native *Rhizobium* in soil produce uneffective and low number of nodules with low BNF. (Pancholy, 1992). This suggested that in Karanj the seed yield, bio mass production and timber quality can be improved by inoculation with specific *Rhizobium* inoculant. Therefore, an attempt was made to isolate *Rhizobium* from nodules of Karanj and applying the effective isolates to Karanj seedling for maintaining healthy vigorous nursery stocks for large scale plantation.

The efficiency of symbiosis depends upon most optimum soil and environmental conditions. 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 rhiozobia (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, sturdiness at nursery stages of growth. 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 *Rhizobium* from nodules of Karanj tree seedling, their characterization and to evaluate their effect individually and in combination with different doses of nitrogen fertilizer on growth performance of Karanj seedlings.

Keeping these points in view, the experiment entitled **"Effect of *Rhizobium* inoculation with different levels of nitrogen on performance of Karanj (*Pongamia pinnata)* seedlings"** was carried out with following objectives:

- To see the response of Karanj seedling to *Rhizobium* inoculation.
- Influence of nitrogen application on biologically fixed amount of nitrogen by Karanj seedling.
- Population dynamics of inoculated Karanj-*Rhizobium.*

CHAPTER – II
REVIEW OF LITERATURE

To review the work carried out in past is essential to understand the problem in-depth and provides necessary guidelines as well as feedback for fulfilment of objectives of the study. So, this chapter deals with the review of study or research work relevant to the study.

1. Response of legume tree seedling to *Rhizobium* inoculation.
2. Influence of nitrogen application on BNF by legume tree seedlings.
3. Population dynamics of inoculated Karanj-*Rhizobium* under glass house conditions.

2. 1 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 $90x10^6$ 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 (Peppler 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 very widely.

Sinha and Basu (1981) studied the nodules from the plants members of Papilionaceae, there was a large variation in the amount of IAA in the nodules, those of *Pongamia pinnata* (L.) Pierre contained quite a large amount. More than 50% of the IAA became oxidized during extraction by the endogenous oxidizing enzymes. The IAA was metabolized in the nodules, The micro-symbiont (*Rhizobium sp.)* produced an appreciable amount of IAA in the culture when supplied with L-tryptophan.

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_2 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_2 – fixer develop slowly because of relatively limited habitat an 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).

Dayama (1985) found that in pot experiments, foliar sprays of sucrose (0-5%) were applied to seedlings weekly for 6 wk. Nodulation was greatest with 2% sucrose, but seedling dry wt., ht. and N content were linearly related to sucrose concentration, being greatest with 5% sucrose.

Prasad and Ram (1986) found that *Rhizobium* inoculation increased all the parameters over uninoculated control possibly owing to N_2 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 come eases, 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 bimass 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 C2H4 per plant, whereas the activity of *D. sissoo* rhizobia was only 0.16 micro mol/h C2H4 per plant. Specific nitrogenase activity (C2H2 reduction) of *L. leucocephala* rhizobia nodules was 20.8 micro mol g 1h-1 C2H4, whereas that of *D. sissoo* nodules was 27.9 micro mol g-1h-1 C2H4. Strong correlations were observed between number of nodules, nodule dry weight, shoot dry weight and acetylene reducing activity in both tree species.

Thamizhchelvan *et al.* (1991) reported that the *Rhizobium* strains Ent 01, Alb 01 and Aca 01 were isolated from root nodules of the tree legumes *Enterolobium saman, Albizzia lebbeck* and *Acacia arabica*], resp. Cultures of the strains were used to inoculate seedlings. After 30 days the plants were harvested and observed for nodulation. Aca 01 was fast growing and tolerant to alkaline pH (10.5) and sensitive to acid pH (3.0). Alb 01 and Ent 01 were slow growing, tolerant of acid pH (3.0) and sensitive to alkaline pH (10.5). The fast-grower (Aca 01) was gelatinase positive. All three were urease and nitrate reductase positive. Fast and slow growing strains nodulated *Albizia saman, Albizia lebbek, Acacia nilotica, Leucaena leucocephala* and *Pongamia pinnata*. Ent 01 was the most efficient strain in terms of dry matter accumulation and total nitrogen content in host plants.

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 and Dutta (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. lebbeck* 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.

Verma *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.

Venkatesh *et al*., (1998) studied the efficacy of biofertilizer with inoculations with *Rhizobium, phosphobacterin* and *vesicular-arbuscular mycorrhiza* individually and in combination on the growth, biomass, biochemical parameters and nutrient yield of pungam (*Pongamia pinnata*) seedlings under nursery conditions. The results showed enhanced shoot and root length, total dry matter and nutrient uptake due to triple inoculation with *Rhizobium, phosphobacterin* and *VAM*. Inoculation with a combination of *Rhizobium, phosphobacterin* and *VAM* would improve the nursery performance of pungam.

Rahangdale and Gupta (1998) reported that the efficiency of 12 *VAM* inoculants was evaluated through pot experiments with 6 forest tree species - *Albizia lebbeck, A. procera, Acacia nilotica, Dalbergia sissoo, Gmelina arborea* and *Pongamia pinnata.* Observations were made of percentage root colonization, shoot height, dry biomass and P content in root tissue, after 150 days. A significant increase in plant height, dry biomass and tissue P was observed in the *VAM* inoculated plants. Data analysis using Duncan's multiple range test enabled selection of the most appropriate *VAM* inoculants for each host.

Aryal and Mridha (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.

Srivastava *et al.* (1999) a survey was made of the nodulation of 1 year old polybag seedlings of 6 leguminous species in 3 forest nurseries. The highest number of nodules per plant were found in *Albizia lebbeck* (17.78) followed by *Pongamia pinnata* (8.67), *Prosopis cineraria* (8.0), *Dalbergia sissoo* (2.89) and *Acacia nilotica* (2.11). No nodules were observed on *Delonix regia.* Biomass production was highest in *Albizia lebbeck*, followed by *Pongamia pinnata, Prosopis cineraria, Dalbergia sissoo, Delonix regia* and *Acacia nilotica*. In *Albizia lebbeck*, the number of nodules was positively correlated with biomass. There were differences between the 3 nurseries in average seedling growth parameters and average numbers of nodules per seedling, which may be related to nursery practices.

Totey *et al*.(2000) observed application of 2gm coal based *Rhizibium* 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.

Srivastava *et al.* (2001) reported that the use of biofertilizer, particularly *vesicular arbuscular mycorrhizas* fungi, has a great importance in forestry as it provides minerals, wide absorption area of root zone, water uptake and tolerance to water stress conditions, etc. In these harsh conditions, *VAM* fungi can play a significant role in survival of plants. In the present study, some economically important tree species namely *Cordia myxa, Artocarpus integer, Dalbergia sissoo, Pongamia pinnata, Mangifera indica* and *Alestronia sp*. were selected for the study. *VAM* inoculated seedlings of above tree species performed better in term of shoot height, root height, biomass and percentage of colonization. The shoot height increase was maximum (32.2%) in A. integer and minimum (11.2%) in *Mangifera indica.*

Manjula *et al.* (2001) reported that the antibacterial activity of karanj and neem seed oil in vitro against *Pseudomonas aeruginosa, Bacillus circulans, Vibrio cholerae, Escherichia coli, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shigella boydii, Staphylococcus spp., Salmonella typhimurium, Salmonella typhi* and *Salmonella paratyphi* was assessed. Using the tube dilution technique, it was observed that 57.14 and 21.42% of the pathogens were inhibited at 500 micro l/ml, 14.28 and 71.42% at 125 micro l/ml and 28.57 and 7.14% at 250 micro l/ml of karanj and neem

oils, respectively. The activity was mainly due to the inhibition of cell-membrane synthesis in the bacteria.

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 batter than those, which received either one of them or none (control). Growth parameter *i.e.* plant heath, 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.

Kumudha, P. (2006) a study was conducted with the seeds of *Pongamia pinnata* to elucidate the effects of *Rhizobium* (18 g/pot), *phosphobacteria* (18 g/pot) and *VAM* fungi (45 g/pot) individually and in conjunction on germination, seedling growth, physiological and biochemical parameters such as chlorophyll 'a', chlorophyll 'b', total chlorophyll, total soluble carbohydrates, reducing sugars, total free amino acids, total proteins, total free phenolics N, P, K, Ca and Mg under pot culture condition. The results indicated that in *P. pinnata* seeds inoculated with all the employed biofertilizers showed better performance compared to control. Combined

inoculation with *Rhizobium + phosphobacteria* and single inoculation with *Rhizobium* improved most of the investigated biometric as well as biochemical parameters.

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 went 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 umber ranging between 336.7 and 496.0 while uninculated 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.

Waddar, and Lakshman, (2008) found that indigenous arbuscular mycorrhizal (AM) fungus was isolated from rhizosphere soils of *Pongamia pinnata* to examine the role of indigenous *VAM* fungi on growth of *Pongamia pinnata* and evaluating their interaction with introduced *Glomus mosseae, Glomus fasciculatum* and *Rhizobiurn phaseoli*. Young seedlings of *Pongamia pinnata* inoculated with indigenous *AMF Gloous fasciculatum* showed significant growth and the plants introduced with Glomus mosseae did not show significant effect on growth. The dual inoculation with indigenous *Glomus fasciculatum* and introduced *Glomus mosseae* depicted growth response was much greater than the single inoculation. The tripartite system of indigenous and introduced *VAM* with *Rhizobium phaseoli* improved significant

increase plant height, plant dry matter, nodulation number, N and P content of *Pongamia pinnata* over non-inoculated plants with either *AMF or Rhizobium* alone.

Verma *et al*. (2008) response of *Arbuscula mycorhiza* (AM) fungi, *Azospirillum, phosphate-solubilizing bacteria* (PSB) and a companion fungus (CF) (*Aspergillus fumigatus*) was stuied on growth of Aonla (*Emlica officinalis*) in nursery. Application of *AM* fungi and *PSB* in combination produced maximum plant height followed by combination of all tree the four treatments. Maximun diameter of seedling was obtained in *Azospirillum* treatments followed by *Azospirillum* along with the companion fungus, followed by combination of *AM* fungi and companion fungus and companion fungus and *PSB.* Maximum colonization was in *AM* fungi fungus combination and combination of *AM* fungi, companion fungus and *Azospirillum*. On the basis of above application of *AM* fungi along with companion fungus or *Azospirillum* and companion fungus is recomded to boost the growth of Aonla in nursery.

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).

Kamire and Sonar (1979). in experiments at Rahuri, Maharashtra, *Pongamia glabra* Vent. and *Azadirachta indica* Juss. (oil seed) cakes of both species reduced the rate of mineralization of urea in a medium black, calcareous clay soil (pH 8.1). The effect was more marked with the karanj cakes

Singh (1984) observed that potted seedlings of ht. 9-12 cm were fertilized with deoiled seed cakes of karanj (*Pongamia pinnata*), castor (*Ricinus communis*), salmeal (*Shorea robusta*) and neem (*Azadirachta indica*), by mixing cake with soil from around the seedlings at a rate per pot equivalent to 100 kg/ha N. Pots were kept in a greenhouse and ht. recorded every month until the end of Dec., when seedlings were measured and weighed. All fertilized seedlings grew better than untreated controls. The most effective fertilizer cake (on the basis of shoot ht. and total dry wt.) was karanj.

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) inorga nic fertilizers indicate 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.

Gupta, Adarsh and Bhandari (1988) found that deoiled seed cakes of *Shorea robusta* meal, *Pongamia pinnata* and *Ricinus communis* were applied at doses equiv. to 100 kg/ha nitrogen to seedlings (about 1 yr old) in forest soil in polypots. Seedling ht. was recorded monthly for 1 yr; shoot ht. and shoot and root dry wt. were measured at 2 yr old. All treatments increased growth, with the best ht. growth occurring with sal meal and the best dry wt. increases (shoots 96.66% and roots 47.2%) occurring with karanj cake.

Lewis (1988) recommended nomenclature and common synonyms are given for 7 species, in response to queries from Nitrogen Fixing Tree Association (NFTA). They are *Pongamia pinnata* (syn. *Derris indica) Albizia lebbeck* (syn. *A. lebbek*), *Albizia saman* (syn. *Samanea saman* and *Desmodium saman*), *Desmodium gyroides* (syn. *Codariocalyx gyroides*), *Faidherbia albida* (syn. *Acacia albida*), *Flemingia macrophylla* (syn. *F. congesta*), and *Paraserianthes falcataria* (syn. *Albizia falcataria*).

Natanam *et al.* (1989) reported that the Karanja kernel contained crude protein (CP) 20.5, ether extract (EE) 33.2, crude fiber (CF) 3.8, nitrogen free extract (NFE) 39.7, available carbohydrate (ACHO) 33.3, total ash 2.8, calcium 0.51 and phosphorus 0.38% DM. Expeller Karanja cake contained CP 24.3, EE 14.2, CF 3.9, NFE 52.0, ACHO 26.2, total ash 5.6, Ca 0.76 and P 0.48%. Values for solvent extracted cake were 26.9, 1.7, 5.5, 60.2, 19.0, 5.7, 0.87 and 0.55%, respectively. Concentration (g/16% nitrogen) of leucine was the highest (7.87) and methionine was the lowest (1.45) amongst amino acids. In Karanja oil, oleic acid had the highest concentration (41.9%) followed by linoleic (18%) and palmitic acids (11.4%). The kernel, expeller and solvent extracted cakes contained 1.65, 31.6 and 3.41% tannins, respectively, and trypsin inhibitor values of 5.3, 8.7 and 8.2% of protein, respectively.

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

Arjunam, Antony and Ponnamma (1994) observed the germination percentage of seeds of *Pongamia pinnata* collected from a 12-yr-old tree at Coimbatore, Tamil Nadu. Seeds were graded into large, medium and small sizes and their germination percentage were recorded. Large sized seeds germinated better (98%) than medium sized (80%) and small sized seeds (70%) and biomass production was higher in seedlings produced from larger seeds. The germination rate was decreased to 50% after 5 months of storage in large seeds, 4 months in medium sized seeds and 2 months in small sized seeds.

Naidu and Swamy (1994) reported that the deoiled seed cakes of *Brassica latifolia,Pongamia glabra [P. pinnata], Azadirachta indica* and *Ricinus communis*

were applied as fertilizer to potted saplings of *Terminalia bellerica* . All four deoiled seed cakes promoted better sapling growth than that in control unfertilized trees, with best growth and biomass production promoted by *M. longifolia* deoiled seed cake.

Chaukiyal and Pokhriyal (1996) nitrate assimilation, leaf area and biomass accumulation studies were carried out in the first to twelfth compound leaves of *Pongamia pinnata*. On the basis of standardization, maximum in-vivo nitrate reductase activity was observed at pH 7.5 pH in (0.1M) phosphate buffer with 0.5 M potassium nitrate. In individual leaves, an increase in the nitrate assimilation activity, leaf area and leaf biomass was observed up to the sixth leaf, while there was no definite pattern observed among individual leaflets in each compound leaf with the growth and leaflet position. The growth behavior of individual leaflets is discussed in detail.

Prakash, Ponnambalam,and Sushamani (1998) suggested that in one month-old uniform seedlings of *Pongamia pinnata, Tamarindus indica, Ceiba pentandra, Acacia ferruginea, Delonix regia, Albizia lebbeck, Cassia siamea* and *Acacia mellifera* were inoculated with 109 bacteria/g of peat of *phosphobacterium* (*Pseudomonas striata*), *Azospirillum* or *Rhizobium* spp. before transplanting in the nursery. Seedling growth was recorded at 30-day intervals over 7 months. The effects of bacterial inoculation varied with tree species, bacterial species and growth parameter (height, collar diameter or volume). *Phosphobacterium* was generally effective in increasing height over control (non-inoculated) values (except in *Acacia mellifera* and *C. pentandra*). The only significant improvement in collar diameter was in *D. regia* and *S. siamea*. The best volume response was in *A. mellifera* and *A. ferruginea* with *Azospirillum.*

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 C2H4 h-1) followed by *Albizia procera* (2080 nmol C2H4 h-1). 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.

Chaukiyal, Sheel and Pokhriyal (2000) study that the nodulation behavior and nitrogenase activity in relation to nitrogen treatment (0, 40, 80, 100 kg N ha-1, applied as a split dose in July and September) and seasonal variations in potted *Pongamia pinnata* seedlings, kept in the open at the Forest Research Institute, Dehra Dun, India. However, no definite trend was observed due to the different nitrogen treatments in the nodule biomass and nitrogenase activity, although the 40 kg N ha-1 treatment was more effective than the others.

Jakowska (2003) this paper provides a description of *Pongamia pinnata*, which is a nitrogen fixing and fodder tree of the Fabaceae family. The medicinal and culinary uses of *P. pinnata* are discussed, as well as the use of the brown oil, extracted from the seeds of *P. pinnata*, as biofuel.

The nutritive value of stems leaves and fruits of *Pongamia pinnata, Albizia lebbeck, Moringa oleifera, Pithecellobium dulce* and was investigated. Crude protein (29.07%), nitrogen free extract (71.78%) and total carbohydrate (85.53%) were found maximum in *P. pinnata*. Crude fibre (29.30%) was higher in *A. lebbeck*. Crude fat (16.84%) and total ash (14.57%) were higher in M. oleifera, while organic matter (95.38%) was found higher in Pithecellobium dulce. Kapoor *et al.* (2004)

According to Chaukiyal and Pokhriyal (2005) four different nitrogen doses (i.e., 40 kg N/ha, 80 kg/ha, 100 kg N/ha and 0 kg N/ha as control) were applied on the potted leguminous *Pongamia pinnata* seedlings in two equal split doses. N-40 treatment was able to enhance NR activity during winter and summer, whereas, N-80 in rainy season. However, among different fertilizer treatments, lowest activity was recorded in control.

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-1. 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-1 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-1 in pots. Seed and soil inoculation, and nitrogen levels at 15, 30 and 45 kg ha-1 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) 1oo, 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, (o.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 collor 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. cholorophyll a, b and carotnoid. Both geremination and the measured physical growth perameters 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 usefull 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 batter 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/hm2. 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/hm2 fertilizer with soil and cow dung mixture

(soil: cow dung = 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.

Rao and Sharma (2010) found that the *Pongamia pinnata* is a medium –sized glabrous tree this plant has long been used in India and neighboring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel. Extract of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory and analgesic activities. Its oil is a source of biodiesel. It has also alternative source of energy. This is an attempt to

compile and document information on different aspect of *Pongamia pinnata* and its potential use as a source of biodiesel

2.3 Population dynamics of inoculated Karanj *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-' 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-' *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 with different levels of Nitrogen on performance of Karanj (*Pongamia pinnata)* 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 Agricultural Microbiology, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during 2010-2011 with *Pongamia pinnata* (Karanj). Seeds of Karanj 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 15 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 ^{0}C, air temperature touches to 48 ^{0}C and humidity drops up to 3 to 4 per cent during summer

season and mercury level drops to as low as 60 ^{0}C during December and January. (Appendix –I)

3.3 Experimental details:

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

3.3.1 Particulars of Experiment:

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

Tree species: - *Pongamia pinnata* (Karanj)

Duration of study: - September to March (2010-2011)

No. of treatments: - 08

No. of replications: - 03

Growth stages: - 05

Design –Completely Randomised Design (CRD)

3.3.2 Design and plan of taking observations:

The experiment was laid out in CRD with 8 treatments, replicated 3 times. A total no. of 96 seedlings (3 Repli. X 8 Treat. X 4 times uprooting) were maintained for the experiment (Plant sampling observations were carried out at 4 different growth stages of Karanj plant i.e., 60, 90,120 and 150 DAT of seedlings)

Date of seedling transplantation and treatment application: 1st October 2010. Observations were taken at 30 days interval up to 150DAT of Karanj plant.

1st observation at 30 DAT (Day after transplant): 1/11/2010- (at 1 month old seedling).

2nd observation at 60 DAT: 1/12/2010 - (at 2 months old seedling).

3rd observation at 90 DAT: 1/01/2011- (at 3 months old seedling).

4th observation at 120 DAT: 1/02/2011(at 4 months old seedling).

5th observation at 150 DAT: 1/03/2011 (at 5months old seedling).

1st observation was based on morphological parameters only while from 2nd onwards biomass and nodulation were also included.

3.3.3 Details of treatments

T_1 Control (Un-inoculated)

T_2 Inoculated

T_3 Inoculated + N_1

T_4 Inoculated + N_2

T_5 Inoculated + N_3

T_6 Un-inoculated + N_1

T_7 Un-inoculated + N_2

T_8 Un-inoculated + N_3

- *Rhizobium* sp. was isolated from the nodules of Karanj & culture broth was prepared for root inoculation of germinated Karanj seedlings.
- Nitrogen was given through Urea (50mg, 150mg, 400mg/seedling as N_1, N_2 and N_3 respectively.

3.3.4 Isolation of *Rhizobium* sp. and preparation of inoculum:

Rhizobium was isolated from fresh nodule of Karanj *(Pongamia pinnata)* seedling and culture broth was prepared using YEM media (Appendix II). The isolated *Rhizobium* was multiplied in the departmental laboratory and thereafter inoculated as per treatment.

For isolation of *Rhizobium,* preferably pink nodules were removed carefully from one collected nodulated Karanj seedling. Detached fresh nodules were surface sterilized by immersing in 1% $HgCl_2$ for 2-3 minutes and then ringed with sterile water for at least 6-7 times. This followed by dipping in 98% ethanol for 1 minute and again ringed for 8-9 times with sterilized water. The surface sterilized nodules were then crushed together in a small aliquot of sterile water with the help of a glass rod in a test tube. Loopful of crushate was spread on the surface of YEMA media plate with the help of spreader as described by Vincent 1970 and were incubated at 28 ^{0}C for 4-7 days. A single discrete colony was transferred to YEMA slant to maintain the isolate. To prepare the culture suspension for experimentation the isolate was inoculated in sterilized YEM broth in conical flasks which were kept on a rotary shaker for 7 days.

3.3.5 Filling of polythene bags:

Surface soils (15cm deep) was collected and thoroughly mixed to form a composite sample. The soils were processed and sieved through 2mm sieve. A well mixed 5kg mixture of Soil, Sand and FYM (3:1:1) was filled in high density polythene bags (12”x 10” size). The moisture content in FYM used was 84 %. A total no. of 96 poly bags were prepared for experimentation and in each poly bag one seedling was maintained.

3.3.6 Transplantation and aftercare:

Seeds of Karanj *(Pongamia pinnata)* were collected from Forest nursery, Raipur and were aloud to germinate by pre treating with bavistin and soaked in water for half an hour and then sown in trays filled with equal proportion of field soil, sand and FYM on 1st September, 2010. Light irrigation was given by sprinkling the water as and when required. 8-12 days were required to acquire complete germination. Uniform size germinated seedlings were selected for root inoculation treatment and after inoculation seedlings were transplanted in filled polythene bags 12x10" and a single healthy seedling was transplanted to each polybag. Phosphorus and Potassium were applied commonly @ 2.0g Phosphorus and 1.5g Potassium per seedling through SSP and MOP respectively and were placed at 4-5cm depth in polybags before transplantation of seedlings. A total number of 96 polybags were maintained for this experiment and the seedlings were allowed to grow up to 5 months /150 Days (Oct-March). The seedlings were regularly watered and hand weeded in the polybags as and when considered necessary to keep the seedling free from weeds. The growth performance of Karanj plants was recorded at regular interval.

3.3.7 Seedling treatment Method:

Rhizobium was inoculated through seedlings root dipping mehtod. For root inoculation of germinated Karanj seedlings, matured broth of Karanj- *Rhizobium* was diluted with sterilized aqueous so that 1:4 dilution was attained. In this diluted broth sugar was added @ 0.5g/100ml broth as a sticking agent which also served as ready food material for *Rhizobium.* After seedlings root treatment they were transplanted in

poly bags as per treatment. In uninoculated pots seedlings were dipped in same amount of nutrient broth but not inoculated with *Rhizobium*. Nitrogen through urea was given in water soluble form after seven days of transplantation of seedlings as per treatment description.

3.4 Observations recorded:

- Characterization of Karanj-*Rhizobium* isolate.
- Physico-chemical and microbial properties of collected soil used for filling the polythene bags.
- Morphological growth parameters *viz* Shoot length, No. of leaves/seedling, Collar Diameter of Karanj seedlings at 30 days intervals after transplant *i.e.*30, 60, 90, 120 and 150 DAT.
- Fresh and oven dried biomass of stem, leaf and root at different stages of transplanting.
- Nodulation study in Karanj plants (nodule number, nodule fresh and oven dried weights/seedling) at 60, 90,120 and 150 DAT.
- Nitrogen content in plants at 90 and 150 DAT.
- Incidence of insect/ pest if any.
- Population dynamics of *Rhizobium* and total bacteria

3.4.1 Characterization of Karanj-*Rhizobium* isolate

The Karanj-*Rhizobium* isolate was characterized by Gram staining and colony morphological characters.

3.4.1.1 Gram reaction of Karanj isolates:

The Gram's reaction was observed and bacteria were classified as gram positive and / or gram negative. The bacterial shape was observed after simple staining with crystal violet.

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 iodine solution for 60 seconds (Appendix-III).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)

3.4.1.2 Colony morphological characters

The colony characters *viz,* margin, elevation, size and colour were observed on agar medium and recorded. 1 ml of appropriate dilution of Karanj-*Rhizobium* was transferred to Petri plates containing YEMA with congored medium.The plates were incubated at room temperature for 2-3 days. The phenotype and growth pattern were observed.

3.4.2 Physico – chemical analysis of soil samples:

Sampling and processing:

Soil used for filling the polybags was analysed for physico-chemical and microbial properties and fresh soil sample was kept in refrigerator for microbiological analysis. The soil sample used for physical and chemical analysis was 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 ^{0}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 $KMnO_4$ method as outlined by Subbiah and Asija (1965).

- **Available P:**

Soil phosphorus was extracted by 0.5 M $NaHCO_3$ 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 Karanj (*Pongamia pinnata*) subjected to different treatments .The observations were recorded on 3 randomly selected plants in each treatment at monthly interval.

Plant sampling and processing:

Three plants as 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 seedlings were uprooted carefully without damaging the root system. The roots were washed in running tap water and parameters

such as shoot length, no. of leaves per seedling and collar diameter were recorded in fresh samples. Month wise observations were recorded after giving treatments.

- **No. of leaves:**

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

- **Shoot length:**

  Shoot length of seedlings was 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. Fresh biomasses of these were taken by weighing different components and then these were oven dried at 70 ^{0}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 quantity 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)

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

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

3.4.5 Nodulation study:

Roots of uprooted plants were washed carefully so that nodules are not damaged, then no. of nodules and their fresh weight was recorded. After recording the fresh weight, the nodules were kept in small papers begs was dried in hot air oven at 60^0c till their constant oven dry weight is obtained.

3.4.6 Nitrogen content in Plant:

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

- **Total N:** Total N in sample was determined by Kjeldahal method (Jackson-1958) by digesting sample in conc. H_2SO_4 followed by distillation and titration.

The nitrogen content of each component were multiplied with their respective biomass to obtain nitrogen uptake.

3.4.7 Microbial analysis:

- **Sampling:**

About 25g of soil sample was kept from polybag under different treatments in small polythene bags to prevent the moisture loses and properly stored in refrigerator for quantitative analysis of microbes.

Enumeration of microbial population in rhizosphere soil

Rhizosphere soil samples from different treatments were collected after completion of experiment for enumeration of total bacteria and *Rhizobium*. Enumeration was done by serial dilution and plating technique (Subba Rao, 1988). Ten fold serial dilutions were prepared for each soil sample and 1 ml aliquots from the appropriate dilutions (10^{-3} for *Rhizobium* and 10^{-6} for total bacteria) were transferred to sterile Petri dishes. Plating was done using Congo red yeast extrtact manitol agar medium for *Rhizobium*, and nutrient agar for bacteria. The plates were incubated at 28 ^{0}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, the population were expressed as cfu g^{-1} of dry soil using following formula.

Number of *Rhizobium*/ Total bacteria per gram of oven dry soil:

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

The operation of making serial dilutions, setting of plates and 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, nodulation and nitrogen uptake in different components of Karanj plants were statistically analysed using ANOVA for completely randomised design (CRD). The significant difference were tested through F-test at 5% level of significance .The standard error of means SEm± 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 with different levels of nitrogen on performance of Karanj (*Pongamia pinnata)* seedlings"** was conducted under glass house condition at the Department of Agricultural Microbiology, College of Agriculture Raipur, Chhattisgarh during the year 2010-2011 to see the response of legume tree seedling (*Pongamia pinnata*) to *Rhizobium* inoculation and the influence of inorganic nitrogen on BNF.

The study comprised of (i) isolation and characterization of *Rhizobium* isolate from nodules of Karanj (ii) Inoculation effects alone and along with different levels of N fertilization.

The findings obtained from these studies are discussed in this chapter.

4.1: Isolation and colony characteristics of Karanj - *Rhizobium*:

Rhizobium isolate from tree legume, Karanj produced translucent, round and gummy colonies with entire edge which varied in size between 1.5to 2.00 mm. (Table 1). Karanj- *Rhizobium* colony morphological characteristics can also be observed in Plate-2. After the gram- staining the bacteria assumed a red colour which indicated that it was a Gram -ve stain. Gram staining of the cultured isolate 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 was used as root inoculants for the experiment. For this YEM broth was prepared and loopful of pure

isolate of Karanj-*Rhizobium* was transferred to sterilized YEM broth as inoculant of seedlings as per experimental study. The isolation method of *Rhizobium* from Karanj nodule, preparation of Karanj -*Rhizobium* inoculated broth and characterization of this isolate were clearly depicted in Plate 1 and 2 respectively.

4.2 Properties of collected soils used in filling polythene bags for raising Karanj seedling:

Collected soil used in filling polythene bags to raise Karanj plants for experimental purpose was vertisol and was analyzed for their Physico-chemical and biological properties. The data presented in Table–2, clearly showed that, soil was slightly alkaline in reaction (pH 7.6), having medium organic carbon status (6.3g/kg soil). The soil was low in mineralizable Nitrogen status (240.4 kg/ ha.), low in available (Olsen's) Phosphorus content (11.6 kg/ha.) but higher in status with respect to available Potassium content (416 kg/ha.). The population of *Rhizobium* enumerated in YEMA media showed no. of *Rhizobium* of $4.6x10^2$ cfu/g of dry soil (Table –2).

The soil was mixed with sand and FYM (the moisture content of FYM was 84%) in 3:1:1 proportion and polybags were filled with these mixtures. This has been shown in plate 3. Seedling root inoculation and transplantation of seedlings in polybags has shown in Plate 3. 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 lebbeck*, 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 of fertilizer. A general view of Karanj (*Pongamia pinnata*) seedlings as affected by treatments was shown in Plat-4.

4.3: Inoculation effects alone and along with different nitrogen levels on growth parameters of Karanj (*Pongamia pinnata*).

4.3.1 Morphological growth parameters of Karanj:

Results on morphological growth parameters of Karanj plants treated with different treatments are presented in Table 3 and Fig. 1 &2.

- **Plant height**

The plant height of Karanj significantly varied with age of seedlings and different treatments (Table- 3 and Fig. 1) at various stages of growth. According to age of the plant, the plant height increased in all treatments. But the increasing rate with time varied with different treatments being higher in inoculated treatments. Inoculated Karanj plants showed significantly higher seedling height ranging from 17.8 to 22.3cm, 24.26 to 27.8cm, 28.3 to 34.5cm, 36.2 to 42.4cm and 42.8 to 51.85cm at 30, 60, 90,120 and 150 DAT respectively as compared to control and uninoculated ones in which height ranged from 13.14 to 19.84cm, 18.21 to 23.63cm, 19.13 to 27.43cm, 22.5 to 32.16cm and 25.37 to 37.86 cm at 30, 60, 90,120 and 150DAT respectively.

Significantly highest plant height (51.85cm) was found in T_4, when *Rhizobium* inoculation was given along with N_2 nitrogen level, followed by T_3 i.e., *Rhizobium* inoculation with N_1 nitrogen level (48.36cm) at 150DAT (plate-6 & 7). Higher nitrogen level N_3 does not influence much with respect to plant height of Karanj plant when applied along with *Rhizobium* inoculation as compared to N_2 and N_1. The treatment T_4 showed significantly highest plant height followed by T_3 and then T_5 and

T_2. However T_2 and T_5 were at par with respect to plant height of Karanj. The effect of inoculation on plant height can be visualized from Plate 5 & 6.

In case of inorganic N fertilizer alone (T_6, T_7 and T_8) the plant height was significantly influenced as compared to control (T_1). Among inorganic N fertilizer, the treatment T_8 i.e., at higher dose of N (N_3) increased the shoot length (37.86cm) significantly followed by T_7 (34.3cm) and T_6 (30.2cm) while it was minimum (25.37 cm) at control, T_1 at 150 DAT. The seedling heights as affected by different treatments at various stages of growth (Fig. 1).

However, both the treatments T_4 and T_3 showed consistently highest plant height in Karanj seedlings followed by T_5 and T_2. At 5 month old seedling height was 51.85cm at T_4 which was 1.21 times higher over T_2 and 2.04 times higher over control. (Table-3). 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 *Albizzia niopoides* over uninoculated ones 77.2cm at 120DAT by Okunomo, *et al.*, (2007)

- **Number of leaves**

Number of leaves in Karanj seedlings was significantly influenced by different treatments and age (Table-3). *Rhizobium* inoculation alone (T_2) and *Rhizobium* inoculation along with different levels of N applications were most effective in increasing the no. of leaves compared to uninoculated treatments and control at different growth stages.

The number of leaves per seedling ranged from 13.32 to 50.67 in inoculated seedlings in 30 to 150 DAT, (1 to 5 month old age of Karanj) while it ranged from 11.37 to 30.8 in inorganic nitrogen fertilization and 8.62 to 18.5 in control in 30 to 150 DAT.

Rhizobium inoculation along with lower nitrogen levels N_2 and N_1 i.e., (T_4 and T_3) promoted maximum no. of leaves in Karanj plant followed by *Rhizobium* inoculation only and *Rhizobium* + N_3 (T_5). At 5 month old Karanj plant i.e., at 150 DAT highest no. of leaves 50.67 per seedling were observed at *Rhizobium* + N_2 showing significant increase over other treatments. Maximum no. of leaves were found in T_4 followed by T_3, T_2 and then T_5. Higher dose of N (N_3) along with *Rhizobium* inoculation did not influence much as compared to N_2 and N_1. However, both the treatments T_2 and T_5 were at par as per no. of leaves concerned. Among inorganic N fertilization, higher dose of N (N_3) i.e., T8 influenced much when applied alone over T_7 and T_6. However, least no. of leaves/seedling were found with control. The no. of leaves per Karanj seedlings were 15, 24,32,42,and 51 under *Rhizobium* inoculation + N_2 at 30,60,90 120 and 150 DAT which were 1.6, 1.8, 2.46 3.0 and 2.75 times greater than untreated seedlings (control). (Table 3)

At 5 month old, the no. of leaves per seedlings ranged between 18 to 51 in different treatments, being significantly highest (51) at T_4 which was 2.37, 1.92 and 1.65 times greater over T_6, T_7 and T_8 (uninoculated ones) followed by (47) in T_3 while it reduced to 35 at T_5. *Rhizobium* inoculation to Karanj seedlings increased the leaf number, and further increased when applied along with less levels of N. Lower doses of N influenced *Rhizobium* inoculation (T_4 and T_3) significantly over higher dose of N (T_5). Almost similar observations were recorded earlier by Chauhan *et.al.* (2002) in *A. lebbeck.*

- **Collar diameter:**

Collar diameter (in mm) of seedlings was measured at different stages of growth in Karanj plants up to 5 month. The data as affected by different treatments at 30, 60, 90, 120 and 150 DAT are given in table-3 and (Figure 2).

It is evident from data given in table 3 that collar diameter of Karanj was significantly affected by various treatments and increased with advancement in age of Karanj plant. Collar diameter (mm) observed was significantly influenced by *Rhizobium* inoculation being 5.94, 6.28, 6.44 and 6.01 at T_2, T_3, T_4 and T_5 respectively while 5.67, 5.30, 4.78, and 4.00 at T_8, T_7, T_6 and T_1 respectively. T_4 and T_3 consistently gave maximum collar diameter compared to other treatments while lowest was found in control. Among different treatments *Rhizobium* inoculation $+N_2$ (T_4) was most significantly effective and enhanced maximum collar diameter of 6.44mm followed by T_3 (*Rhizobium* $+N_1$) 6.28mm, T_5 (6.01mm) and T_2 (5.94mm). Whereas it was lowest in untreated seedling (control) 4.00mm. However, these inoculated treatments significantly differ from other treatments consisting only nitrogen

applications and control. As per only inorganic N application concerned, higher level of N affected significantly giving collar diameter of 5.67mm at T_8 while it was 5.30mm at T_7 and 4.78mm at T_6 at 150 DAT. However, application of higher levels of N (T_7 and T_8) has significant effect over lowest N dose (T_6) and control (T_1). At 3, 4, and 5 month (90 , 120 and 150 DAT), *P. pinnata* attained 5.38, 6.00 and 6.44mm of collar diameter at T_4 respectively which were 1.67, 1.60 and 1.61 times greater than control. Collar diameter of Karanj seedlings varied between 1.71 to 6.44mm under different treatments at different growth stages of plant ages. (Table 3)

Among different treatments, T_4 and T_3 were best for promoting the growth in Karanj plants w.r.t height, no. of leaves and collar diameter followed by T_5, T_2 and T_8 whereas T_1 was least effective in influencing the growth of Karanj plant. However inoculation of seedlings alone and along with N was found significant for collar diameter in Karanj plants. The growth of Karanj plants as affected by different treatments has shown in Plate 5, 6 and 7 describing effect of inoculation, effect N along with inoculation and effect of N application only respectively.

Totey *et al.,* (1997) 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 nutrients available for growth of plant. Chauhan (2002) had also reported that *Rhizobium* inoculation enhanced the growth in *Albizzia* seedlings as compared to control.

Thus it can be inferred that plant height, collar diameter as well as growth is significantly influenced by application of *Rhizobium* biofertilizer. The effect of root inoculation *Rhizobium* was highly significant over control in Karanj which was shown in plate 5. Similar results were obtained by Basu and Kabi (1987) in *Albizzia lebbeck* and *D. sissoo*. Kinhal (1985) also obtaianed better performance in bomboo plantation due to application of biofertilizer containing *Azotobacter*. 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_2 and T_5 was at par. But both the treatments significantly differ from T_1, T_6 T_7 and T_8. Further when only N effect was concerned, T_8 was more significant over T_7 while minimum growth was found in control T_1. As mentioned above the effect of 3 levels of N on Karanj seedlings alone and along with *Rhizobium* has been shown in Plate 6 and 7. Similar observations were also found by Chauhan and Pokhriyal (2002) in *Albizzia.*

Inoculated seedlings significantly showed more no. of leaves/seedling, shoot length and collar diameter as compared to uninoculated ones (inorganic N fertilization and control). Among inoculated treatments, *Rhizobium* inoculation along with N_2 affected much which was at par with *Rhizobium* +N_1 seedlings (Table-3). 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.

4.3.2 Biomass accumulation in different components of Karanj *(Pongamia pinnata)* plants as affected by different treatments:

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

- **Leaf biomass**

Leaf biomass of Karanj plants at different ages varied significantly among different treatments. Inoculation of *Rhizobium* showed significantly maximum leaf fresh and dry biomass over uninoculated seedlings. (Table 4 and 5)

Fresh weight of leaves (g/ seedling)varied from 2.43 to 5.74, 3.69 to 8.94, 4.32 to 11.69, 4.14 to 14.73 at 60,90,120 and 150 DAT respectively under different treatments, while dry weight of leaves (g/ seedling) varied from 0.84 to 2.46, 1.20 to 2.87, 1.69 to 4.24, 1.67 to 5.85 at 60,90,120 and 150 DAT. (Table 4 and 5)

Rhizobium inoculation along with N_2 (T_4) gave significantly maximum leaf fresh biomass of 14.73g/seedling followed by T_3 (13.38 g/ seedling), T_2 (10.37g/seedling) and T_5 (9.86g/seedling) at 5 month old at 150 DAT. However there was no significant difference was observed between T_2 andT_3 i.e., only *Rhizobium* inoculation and *Rhizobium* inoculation along with higher N dose, N_3. Further inoculated treatments significantly increased leaf fresh biomass over uninoculated and control. Among uninoculated treatments, T_8 i.e., application of higher N dose only showed significantly increased fresh biomass of 9.06 (g/seedling) followed by 7.93(g/seedling) at T_7 and 5.86 (g/seedling) at T_6 while minimum was found at control 4.14(g/seedling) (Table 4)

Rhizobium inoculation along with N_2 (T_4) and N_1 (T_3) significantly increased leaf dry weight up to 5.85 and 5.64 g/seedling which were 3.5 and 3.37 times higher over control respectively, at 5 month old at 150 DAT. Maximum leaf biomass ranging 2.46 to 5.85 g/seedling was found in T_4 followed by T_3 (2.18 to 5.64g/seedling), T_5 (1.87 to 4.66g/seedling) and then T_2 (1.76 to 4.26g/seedling) under different ages of Karanj plants. However treatments T_4 and T_3 were at par. Among inorganic fertilization, application of N alone increased leaf dry biomass significantly as compared to control. Further higher nitrogen doses N_3 and N_2 i.e., T_8 and T_7 gave biomass of 3.62 and 3.49 g/ seedling which were 1.6 and 2.1 times greater than T_6 and T_1 respectively at 150DAT of Karanj plant. However treatments T_7 and T_8 were at par and significantly differ from T_6. Leaf biomass (dry) ranged from 0.84 to 5.85g/ seedling under different treatments and age of seedling. However Karanj plants exhibited maximum leaf dry biomass in T_4 and T_3 followed by T_5 and then T_2 while minimum was found at T_1 (Table 5).

- **Stem biomass**

Stem biomass of Karanj was significantly affected by different treatments under different age. (Table 4 & 5) Seedlings inoculated with *Rhizobium* inoculation showed significantly higher stem biomass accumulation over control and uninoculated ones.

Stem fresh weight g/seedling was observed maximum at T_4 (11.96) followed by T_3 (11.21) and T_5 (10.01). However there was no significant difference in between T_3 and T_4 and between T_5 and T_2. Among only N applications, T_8 and T_7 were at par showing 8.59 (g/seedling) and 8.49(g/seedling) fresh stem biomass which were significantly differ from lower N dose treatment T_6 (7.12g/seedling) while minimum

was found at control 3.49 (g/seedling) at 150 DAT. Fresh stem weight varied from 2.03 to 11.96 (g/seedling) under different treatments at various ages of Karanj plant (Table 4).

Among inoculated treatments, application of *Rhizobium* with N_2 (T_4) produced significantly maximum stem dry biomass ranging 1.46 to 4.86 g/seedling at 60 to150 DAT followed by T_3 application of lower level of N (N_1) with *Rhizobium* inoculation (1.45 to 4.56 g/ seedling). But application of higher dose of N along with *Rhizobium* inoculation showed stem biomass of 1.14 to 4.00 g/ seedling which was significantly decreased compared to T_4 and T_3. Whereas under T_2, only *Rhizobium* application, it was 1.37 to 3.88 g/seedling (Table 5).

Further application of only inorganic N, there was also significant increase in stem biomass over control. Among doses of N fertilizer, shoot biomass was observed higher at N_3 over $N_2$and N_1. At 5 month old age, T_8 gave shoot biomass of 3.41g followed by T_7, 2.81g and T_6, 2.0 g/seedling where at control it was 1.44 g/ seedling.

The stem biomass significantly increased from 0.59g/ seedling to 4.86g/ seedling throughout the study period. Treatment effect was found significant for stem biomass (Table.5) and this can also be depicted in Fig3. As per mean value over ages, treatment effect of T_4 3.16 (g/seedling) and T_3 2.90 (g/seedling) were observed maximum w.r.to stem biomass content which were 2.72 and 2.5 times greater over T_1, control respectively.

- **Root biomass**

Root biomass of Karanj seedlings varied significant by *Rhizobium* inoculation and N fertilization either singly or in combination. (Table-4 and 5). *Rhizobium* inoculation either alone or in combination with N significantly increased the root biomass over

uninoculated. Nitrogen fertilizer application only also showed significant increase in root biomass over control.

Fresh weight of root varied from 2.66 to 13.87 (g/seedling) at different ages and treatments. Inoculation of *Rhizobium* along with lower N dose N_1 (T_3) produced significantly maximum root fresh weight of 13.87 (g/seedling) at 150DAT followed by T_4 13.28 (g/seedling) and T_5 11.87 (g/seedling) and T_2 11.54 (g/seedling. However, treatments T_2 and T_5 were at par w.r.t. fresh root biomass accumulation. The mean data over ages also showed that T_3 exhibited maximum root fresh biomass of 9.30 (g/seedling) followed by 9.04 (g/seedling) at T_4, Among N applications, T_8 showed significantly maximum value of 10.76 (g/seedling) over T_7 (9.47g/seedling), while minimum were 9.47(g/seedling) at T_6 and 5.14(g/seedling) at T_1 at 150 DAT (Table 4).

However, at 2,3,4 month ages, highest root dry biomass was seen 1.57 , 3.56 and 4.16 g/ seedling at T_3, but at 5 month age at 150 DAT T_4 gave significantly maximum root dry biomass accumulation of 5.89 (g/seedling) in Karanj plant . Overall *Rhizobium* inoculation along with lower doses of N fertilizer (N_1 and N_2) showed maximum accumulation of dry root biomass *i.e.*5.89 and 5.43 g/seedling which were 2. 38 and 2.61 times greater than control. There was no significant difference between T_2 and T_5 however T_5 showed higher biomass (4.92g/seedling) than T_2 (4.81g/seedling) at 150 DAT. Application of inorganic N fertilization significantly influenced root biomass (dry) as compared to control. In highest level of N (N_3), the accumulation of root biomass (dry) (3.92g/ seedling) was significantly higher over low levels of N (N_2 and N_3) at 150 DAT which was 1.38 and 1.98 times more than lower N dose treatments and control respectively.

Root biomass ranged from 1.71g to 5.89g/seedling under different treatments at 150 DAT, being highest at T_4 followed by T_3 then T_5, T_2, T_8, T7, T_6 and T_1. Effect of treatments was found to be significant (Table 5 & in Fig.-3)

- **Total dry biomass accumulation**

Data on above ground and below ground as well as total dry biomass as affected by different treatments at various ages of Karanj plants are presented in Table-6 and also depicted in Fig. 5, 6 7 and 8.

As per described above, the above ground, below ground as well as total biomasses of seedlings were significantly influenced by *Rhizobium* inoculation alone (T_2) and along with N (T_3, T_4 and T_5). Mean value of above ground dry biomass over all the stages of growth showed that maximum value of 7.01 (g/seedling) was obtained in treatment T_4 followed by T_3 (6.54g/seedling). The AG biomass ranged from 3.92 to 10.71(g/seedling) in T_4, 3.63 to 10.2(g/seedling) in T3, 3.01 to 8.66 (g/seedling) in T_5 and 3.13 to 8.14(g/seedling) in T_2 from 60 to 150 DAT. Among N applications, highest AGB was in T_8 (2.77 to 7.03g/seedling), followed by T_7 (2.52-6.3g/seedling) and T_6 (2.48- 4.24g/seedling) while minimum was seen at control (1.43-3.11g/seedling). Similar trend was observed in case of below ground biomass accumulation in Karanj plant being highest under T_3 (1.57-4.52g/seedling) followed by T_4 (1.53- 4.37g/seedling), T_5 and T_2.

Dry biomass at different ages under treatments was depicted in Fig.5 showing significantly maximum total biomass was obtained 16.78 g/ seedling at 150 DAT under treatment T_4 followed by 15.78g/ seedling at T_3 which were significantly higher over T_5 (13.66g/ seedling) and T_2 (13.036g/seedling). Among N applications only, T_8

exhibit higher biomass of 10.97(g/seedling) followed by T_7 9.817(g/seedling) and T_6 6.554(g/seedling) while minimum was found in T_1 4.82 at 150DAT .At 150 DAT the effect of treatments were significant in total biomass accumulation in Karanj seedlings over control. The graph of above ground and below ground biomass as affected by treatments at different ages was given in Fig. 6 & 7 respectively.

Total biomass of Karanj ranged from 2.23-5.4(g/seedling), 4.34-9.25 (g/seedling), 4.84-12.17(g/seedling), 4.82-16.78g/ seedling under different treatments at 60, 90,120 and 150 DAT respectively. 158 % increase in total biomass was found due to *Rhizobium* inoculation with N_2 over control when mean value over ages was concerned. 29 % increase in total biomass was observed when N_2 dose of N fertilizer was given along with *Rhizobium* inoculation as compared to *Rhizobium* inoculation only at 150 DAT. And inoculation of *Rhizobium* only significantly increase the biomass by 151% over control. (Table 6). Total fresh and dry biomass accumulation in Karanj plants at 150DAT was shown in fig.8.

These observations are in agreement with 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. Karanj *(P.pinnata)* were significantly higher in *Rhizobium* inoculation over uninoculated treatments. Similar to growth, biomass of Karanj plants was significantly influenced by inoculation with *Rhizobium*. The biomass 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 Morques*et 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 Karanj *(P. pinnata)* plants to *Rhizobium* inoculation 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-7) which are calculated from various seedling traits. Seedling quality 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 fairy better in inoculated treatments. The treatments when compared on the basis of seedling quality parameter (Table-7) the T_4 and T_3 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 (0.207) was found in T_4 followed by o.203 in T_3. Inoculation of *Rhizobium* would be helpful for production of quality seedling in nursery, which the experimental results also, confirms (Table-7) being QI of 0.179,0.203.,0.207 and 0.185 at T_2,T3,T_4 and T_5 respectively while application of N only showed QI of 0.103, 0.150 , 0.163 at T_6,T_7,T_8 while minimum was at control 0.075. Inoculation of bio-fertilizer increased the seedling quality and seedling growth in *Pongamia pinnata* at nursery.

4.3.3 Nodulation study:

The nodular properties like nodule no., nodule fresh weight and nodule dry weight as affected by different treatments and different age of seedlings are presented in table-8 and Fig. 4.

The no. of nodules per seedling ranged from 2 to 47 under different treatments and at different ages of seedlings. It was lowest in uninoculated seedlings and highest nodule no. was obtained in seedlings where *Rhizobium* inoculation along with N_2 levels of N was given. At 60DAT nodulation began in inoculated seedlings but delayed nodulation was observed in uninoculated seedlings. At 120 DAT, significantly maximum nodulation was observed in Karanj plants in all treatments. Inoculated seedlings showed higher nodulation (being 31.66, 36. 66, 46.66, 32.33/seedling at T_2, T_3, T_4, T_5 respectively over uninoculated ones where nodule no. ranged from 2.66 to 18.33/seedling at 120 DAT. Among inoculated treatments T_4 showed significantly maximum no. of nodules/seedling 47 followed by T_3, 37/seedling, then T_5 and T_2. It was seen that nodulation decreased consider in the control, but could not be

completely eliminated as is evident from sparse nodulation in the control plants (03/seedling). With *Rhizobium* inoculation nodules number increase to 32/seedling and further increased to 47 and 37 due to starter dose of N_2 and N_1 respectively. But with N_3 (higher dose) nodulation did not increased considerably (33/seedling). While in only N fertilization, among uninoculated treatments, maximum nodule no. was found (18.33/seedling) at T_8, followed by (12/seedling) at T_7, and (06/seedling) at T_6 whereas minimum was seen in control 2.66/seedling. Karanj nodules were round ball like off pink / dull white colored and somewhat medium size 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 0.017 to 1.75 g/seedling under different treatments at various growth stages of Karanj plant. Those were higher in inoculated seedlings as compared to uninoculated ones. The size of nodules was considerably larger in *Rhizobium* and less levels N treated plants as compared to untreated ones. Significant increase in the weight of nodules of plants receiving *Rhizobium* + N_2 (1.75g/seedling) treatment over other treatments was observed. Less N dose is equally parallel to ensure the efficiency of N_2 fixation. Hence the lower dose of N as starter dose is important to ensure in increasing the efficiency of N_2 fixation showing nodule fresh weight of 1.75 and 1.56 (g/seedling) at T_4 and T_3 respectively while it was 1.32(g/seedling) at T_2 at 120DAT. The fresh nodule weight (g/seedling)

was 1.06 at T_8, 0.757 at T_7 and 0.31 at T_6 while minimum was seen in control T_1 (0.024) at 120 DAT. (Table- 8)

The dry weight of nodules per seedling ranged between 0.002 to 0.006g at 60DAT, 0.002 to 0.20g at 90DAT, 0.003 to 0.374g at 120DAT and 0.003 to 0.186g at 150DAT under different treatments. At 120DAT highest dry weight of nodules observed which becomes slightly senescent at latter stage (at 150DAT) of Karanj. Significantly maximum dry weight of nodules (0.374g/seedling) observed at 120DAT under treatment T_4. (Table-8) *Rhizobium* inoculated seedling showed significantly higher nodule biomass g/seedling over uninoculated ones, being in the range of 0.004 – 0.287 in T_2, 0.004 – 0.36l in T_3, 0.006-0.374 in T_4 and 0.002- 0.256 in T_5 at different stage of growth of Karanj plant. Lower levels of N dose N_1 and N_2 when applied with *Rhizobium* inoculation influenced the nodulation and ensure to increase BNF, but reverse happened in case of high level of N, N_3 when applied with *Rhizobium* inoculation. However inoculated seedlings showed significantly better nodulation as compared to only N fertilization. The nodule dry weight g/seedling ranged from 0-0.127 in T_8, 0- 0.096 in T_7 and 0- 0.06 in T_6 whereas minimum was found in control T_1 0-0.003. The effect of treatments on nodule no. and dry weight at 120DAT has shown in Fig.-4.Also better nodulation in inoculated seedlings over control has clearly shown in Plate 8. Effect of N levels on nodulation in Karanj shown in Plate 9.

`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 N_1 and N_2 doses of N as starter dose. (Table 8)

4.3.4 Nitrogen status in Karanj plants

Inoculation with Karanj (*P. pinnata*) *Rhizobium* and nitrogen fertilizer application influenced the nitrogen concentration in different seedling components *viz*., leaves, root and shoots. At 90 and 150DAT, application of *Rhizobium* alone and along with N fertilizer showed higher nutrient concentration in seedlings than only inorganic N fertilization and control. The N concentration was higher in leaves followed by roots and shoots. (Table 9 and 10) Fig. 9 and 10.

- **Nitrogen concentration in different plant components**

Concentration of nitrogen as well as accumulation of N in different components of Karanj plants at 90 DAT was given in Table 9 and at 150DAT in Table -10.

Nitrogen concentrations in leaves were affected by different treatments, highest N concentrations (1.52 %) was found in T_4 followed by T_3 (1.46 %) which were 1.71 and 1.66 times greater over control at 90DAT. Among inorganic N (Nitrogen) fertilization, T_8 showed higher N concentration (%) in leaves (1.32), as compared to T_7 (1.26) and T6 (1.13) while minimum was found in control (0.85%). Similar trend were also observed in stem and root N conc. of Karanj plant.

Also at T_4 maximum N concentration in shoot and root of Karanj plant being 0.51 and 0.67 % respectively followed by T_3 while minimum was found at control. At T_8, T_7 and T_6 the N concentration in shoot were 0.38, 0.34 and 0.30% and in root were 0.51, 0.47, and 0.42 % respectively.

In leaf, the N concentration ranged from 0.85 % to 1.52% in stem, it ranged from 0.28% to 0.51% while in root it ranged from 0.38% to 0.67 % respectively under

different treatments at 90 DAT. (Table -9) Comparatively less concentration of N in leaf, stem and root of Karanj seedlings at 150DAT was found over N concentration at 90DAT. (Table 10). In leaf, the N concentration ranged from 0.78 % to 1.26%, in stem, it ranged from 0.16% to 0.35% while in root it ranged from 0.27% to 0.53 % respectively under different treatments at 150DAT.(Table -10).Highest N conc. (%) of 1.26,0.35,0.53 were found in leaf, stem and root respectively at T_4 followed by T_3, T_5 and T_2.

Rhizobium inoculation showed higher N concentration over uninoculated ones. Further due to application of N_2 doses of N along with *Rhizobium* the concentration increased in leaf, in stem and root. Application of inorganic N fertilizer only also significantly influenced in concentration of N over control. . (Table -9 and Table -10)

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

Among different seedling components leaves showed higher N content than stem and root. In leaves, the N content ranged from 10.2 – 43.35 mg/seedling whereas in stem, it ranged from 3.56 to 14.09mg/seedling and in root it was 7.1 – 22.85(mg/seedling) under different treatments at 90 DAT (Table 9), Fig.-10.

Significantly highest N content in leaf, stem and root i.e., 43.35 mg/seedling, 14.09 mg/seedling and 22.85 mg/seedling respectively were observed when inoculated with Karanj- *Rhizobium* isolate with N_2 level of N followed by inoculation with Karanj- *Rhizobium* isolate with N_1 level of N being 38.77, 11.81 and 22.442 mg/seedling in leaf, stem and root respectively.

Rhizobium inoculation singly showed N content of 31.57,9.21and 17.057 mg / seedling in leaf, stem and root, while at T_5 it was 30.58,8.52,17.454 mg /seedling in leaf,

stem and root respectively. Among two levels of N, lower levels of N influence significantly over higher N level when applied with *Rhizobium*. Inoculated seedlings showed significantly higher N content over only N fertilization and control. (Table 9). N uptake by shoot of Karanj was 57.44mg/seedling at T_4 while at control it was 13.7mg/seedling at 90DAT.(Table 9).

N content was higher in leaves followed by root and stem of Karanj seedlings at 150DAT. In leaf N content ranged from 13.03- to 73.7 mg/seedling, in stem it ranged from 2.31 to 17.06mg/seedling and in root 4.61 -31.201 mg/seedling under various treatments at 150 DAT. *Rhizobium* inoculated seedlings along with lower N levels (T_4 and T_3) showed significantly maximum N content followed by *Rhizobium* inoculated seedlings along with higher N level T_5 and *Rhizobium* inoculated only (T_2). Table 10

Among inoculated treatments, N content in stem of Karanj was found as 10.88 mg/seedling at T_2, 14.58 mg/seedling at T_3 , 17.06 mg/seedling at T_4 and 12.36 mg/seedling at T_5. Inoculated seedling showed significantly higher N content in stem over uninoculated ones being 4.0, 6.19 and 8.514mg/seedling at T_6, T_7 andT_8 respectively whereas at control it was minimum *i.e*., 2.31mg/seedling at 150 DAT.

Significantly maximum N content in leaf of Karanj was seen under *Rhizobium* +N_2 treatment (73.7 mg/seedling) followed by *Rhizobium* + N_1 (67.73 mg/seedling). Moreover, these were 1.5 and 1.3 times greater over T_2 and T_5 respectively. Inorganic N application also showed higher N content in leaf of 39.07 mg/seedling at T_8 followed by 35.59mg at T_7 while minimum was at control (13.03 mg/seedling).

N content was significantly highest (31.2 mg/seedling) in the roots of *Rhizobium* inoculated along with N_2 application (T_4) seedlings, followed by T_3 (27.78 mg/seedling) and T_5 23.65 mg/seedling when *Rhizobium* inoculated along with N_3 application which were 1.5,1.34 and1.14 times greater than T_2 when *Rhizobium* inoculated alone. Minimum was found at T_1 (4.61 mg/seedling) (Table-10). Inorganic N application also showed higher N content in root of 14.50 mg/seedling at T_8 followed by 12.25 mg at T_7 while 6.70 mg/seedling at T_6. Concentration of N as well as N uptake in shoot of Karanj at 90 and 150DAT was given in Fig. 11.

Among three levels of N, less level N_1 and N_2 influenced significantly over higher N level, N_3. In *R* + N_2 , Karanj plant showed significantly maximum Nitrogen content 90.75mg/seedling in shoot followed by 82.31 mg/seedling at *Rhizobium* + N_1 while 66.41mg/seedling at *Rhizobium* + N_3.(Table-10). Among only N applications higher N level N_3 at T_8 showed significantly higher N content 47.58 over lower N level, N_1 (24.00) while minimum was 15.34 mg/seedling. N content 47.58 mg/seedling varied from 13.03 to73.69mg/seedling in leaf , 2.31 to 17.06 mg/seedling in stem and 4.61 to 31.2mg/seedling in root as affected by different treatments at 150DAT (Table 10).

Similar trend in nutrient concentration was reported by Kaushik *at 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, showed higher N concentration than uninoculated ones. N uptake by seedlings is also 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 N 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.

High N status in seedling was found in inoculated seedling due to increased BNF. The higher growth in *Rhizobium* + N_2 treated seedling might be due to synergistic effect of lower N levels as starter dose with *Rhizobium* inoculation which mediated the efficient uptake of N 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_1 and N_2) along with *Rhizobium* inoculation showed a +ve influence in nutrient uptake by Karanj seedlings. The result revealed that the nutrient contents were higher in *Rhizobium* along with N 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* + N treatments 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 Observation of any insect/pest incidence in Karanj plants under glass house condition as affected by different treatments

Observations of insects/pests in Karanj plants under glass house condition was taken at 30 days intervals to see whether there was any occurrence of insect/pests under different treatments at various growth stages i.e., 30, 60, 90, 120 and 150 DAT. No pests were recorded in Karanj plants at different stages of growth in all the treatments except some plants at control at 60 DAT. An average of 7 mites was recorded in some Karanj plants at control at 60 days after transplanting. The mites were red in coloured identified as *Tetranychus* spp. a shown below. The damaged leaves at control due to mites appeared as thick and crinkled shown below. The infected leaves were picked destroyed. Except control in all other inoculated and N treatments, no mites/any other damaged leaves were observed. Thus, from the above studies it can be concluded that mites were observed only on those plants in which no treatment was given, in other case inoculated and N treated Karanj plants were observed as healthy plants with healthy leaves and no insect/pest attack. So, inoculation might have offered some resistance against mites, which needs further confirmation by detail studies.

***Tetranychus* spp.**

Infected leaves of Karanj plant at Control

4.5 *Rhizobium* and total bacterial population in rhizosphere soil of Karanj (*Pongamia pinnata*) legume plants:

Data on *Rhizobium* population per g of soil in rhizosphere soil of *P. pinnata* (Karanj) as affected by different treatment effects at 150DAT was given in Table 11

In initial soil *Rhizobium* population per g of soil was $4.8x10^2$ which increased to 2.32 $x10^3$ in control due to influence of leguminous tree. Further it increased significantly to $3.26x10^3$, $3.84x10^3$, $4.02x10^3$ and 3.31 x 10^3 in inoculated treatments at T_2, T_3 T_4 and T_5 respectively. In T_4 highest population was found followed by T_3. So, N at less level N_1 and N_2 has a positive correlation in increasing *Rhizobium* population at rhizosphere zone of Karanj when inoculation was given along with N, but in case of higher N dose *i.e.,* N_3 was not so effective when applied along with *Rhizobium* inoculation. Application of inorganic N fertilization only, *Rhizobium* population was found higher ($2.96x10^3$) at T_8 and $2.78x10^3$ at T_7 while at T_6 it was $2.72x10^3$ at 150DAT.

Similar trend of total bacterial population was also found in rhizosphere zone of Karanj plant at 150 DAT being maximum 5.65 x 10^6 at T_4 followed by 5.24 x 10^6 at

T_3 while at control the population was 1.65 x 10^6/g of soil. 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 Karanj. (Table – 11).

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 N_1 and N_2 to *Rhizobium* inoculated seedling of *P. pinnata* further enhanced the *Rhizobium* population.

Rhizobium alone and along with lower doses of N improve the growth of Karanj 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 when lower doses of N were applied as starter dose along with *Rhizobium* inoculation than the higher level of N, (N_3). 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 with less N doses. 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. Inorganic N fertilizer can have an effect being it +ve and –ve, on plant nodulation, N_2 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 Vargas 2000), 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 *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 when given along with *Rhizobium* inoculation .This beneficial effect of low N doges 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.* Karanj 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 plant 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 reported 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).

CHAPTER V

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

The present investigation entitled "Effect of *Rhizobium* inoculation with different levels of nitrogen on performance of Karanj (*Pongamia pinnata)* seedlings" was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture Raipur, Chhattisgarh during September-March, 2010-11 with the objective to see the response of legume tree seedlings (*Pongamia pinnata*) to *Rhizobium* inoculation and the influence of nitrogen on biologically fixed Nitrogen.

The above experiment was carried out in polythene bags with 8 treatments (T_1 to T_8) consisting of one control (T_1), seedling root inoculation with Karanj–*Rhizobium* (T_2), 3 levels of N fertilization (N_1-50mg, N_2-150mg and N_3-400mg N/seedling) along with *Rhizobium* inoculation (T_3,T_4,T_5) and 3 levels of N fertilization alone (T_6,T_7,T_8). These treatments were replicated thrice Karanj (*Pongamia pinnata*) seedlings to study the root inoculation effect alone and along with different doses of N on growth performances of Karanj plants at 4 different stages. 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. Karanj- *Rhizobium* isolate from nodulated Karanj plant was tested for Gram staining reaction and results showed the isolate was gram negative. The isolate

on YEMA media produced white translucent colonies of circular shape and raised, smooth surface with entire edge and creamy white in colour.

2. The soil sample used for raising Karanj plants in polythene bags contain *Rhizobium* population of 4.8 x 10^2 per g soil and mineralizable nitrogen was low in status (240.4kg/ha). After completion of experiment at 150 DAT, *Rhizobium* population(/g soil) in rhizosphere soil of Karanj was 2.32 x10^3 at control due to influence of legume tree, further due to root inoculation treatment it increased significantly to 3.26 x10^3 and further increased to 3.84 x10^3 and 4.02 x$10^3$when *Rhizobium* inoculation was given along with N_1 and N_2 doses respectively.

3. The isolate, Karanj - *Rhizobium* was tested for their impact on morphological growth parameters of Karanj plants. *Rhizobium* inoculation alone and along with less N doses significantly influenced the growth of Karanj. Seedling height increased by 1.68 and 2.04 times over control with *Rhizobium* inoculation only (T_2) and *Rhizobium* + N_2 (T_4) treatments respectively. Significantly highest shoot length (51.85cm) was found in T_4 followed by 48.36cm in T_3 and 43.87cm in T_5over T_2 (42.8cm) at 150 DAT. Among inorganic N application, seedling height was significantly higher at T_8 (37.86cm) over T_7 (34.3cm) and T_6 (30.2cm) whereas at T_1 it was 25.37cm.

4. *Rhizobium* inoculation with less N doses, N_1 and N_2 promoted maximum number of leaves in Karanj plants. Number of leaves/seedlings was highest (51) in T_4 and 46 in T_3 which significantly increased over control (18) at 150 DAT.

5. Collar diameter of Karanj plants showed significant effect in inoculated treatments over uninoculated seedlings being maximum (6.44mm) at T_4 followed by (6.28mm) at T_3, 6.01mm at T_5 and 5.94mm at T_2 while minimum (4.00mm) at control. While among application of N only, T_8 showed collar diameter of 5.67mm which was significantly more over T_6.

6. These growth parameters followed an increasing trend with age of seedlings from the month of October to March irrespective of different treatments applied to seedlings. The increasing rate was significantly higher in inoculated seedlings.

7. Earlier initiation of nodules was observed in inoculated seedlings as compare to uninoculated ones. At the age of 3 – 4 months of Karanj plant, nodulation was higher, however significantly maximum nodulation was observed at 4 months age/ 120 DAT.

8. With *Rhizobium* inoculation, maximum average nodule no./seedlings was 32 and further increased to 37 due to applying N_1 along with *Rhizobium* at 120 DAT, while with N_2 along with *Rhizobium* it again increased to 47. Application of higher Nitrogen dose N_3 with *Rhizobium* inoculation, the no. of nodule did not increase significantly (33). Again nodule no./seedlings were 18 and 12 at T_8 and T_7 respectively. Very sparse nodulation (03) was seen at control.

9. Similar trend was observed in case nodule fresh weight and nodule dry weight. The effect of less doses of N (N_1 and N_2) when applied with inoculation was significant over only *Rhizobium* inoculation alone and along

with higher N dose, N_3 w.r.to nodulation. At 120 DAT significantly maximum nodule dry weight (g/ seedling) (0.374) was found at T_4 followed by 0.361 at T_3, 0.287 at T_2 and 0.256 at T_5 while at control it was minimum (0.003).

10. Biomass production was significantly affected by *Rhizobium* inoculation singly and in combination with N_1 in Karanj at different age. Leaf biomass was higher than stem biomass from 60 to 150 DAT of Karanj plants. Biomass accumulation ranged from 1.67 to 5.85g/seedling for leaf, 1.44to 4.86g/seedling for stem and 1.71to 5.89g/seedling for roots at 150 DAT under different treatments. Total biomass accumulation by Karanj seedling at 150 DAT was increased by 2.7, 3.4 and 2.2 times in T_2 (only inoculation), T_4 (inoculation with N_2) and T_8 (at higher dose N_3 only) respectively over control.

11. *Rhizobium* inoculation with N_2 treatment produced significantly maximum shoot biomass and total biomass of 10.7 and 16.8 g/seedling respectively against 3.11 and 4.82 g/seedling at control.

12. Quality Index (as per Dickson) was higher for inoculated seedlings over uninoculated. Two times increase in QI of Karanj seedlings at 150 DAT was observed in inoculated seedlings over control. Maximum Quality Index of 0.207 was found for seedlings receiving *Rhizobium* inoculation along with N_2 dose of N while in only *Rhizobium* inoculated Karanj plants showed QI of 0.179. Among only N fertilization, T_8 i.e., application of higher N dose showed better quality seedlings having QI of 0.163 whereas at T_7 and T_6 it were 0.15 and 0.103 respectively. While QI was minimum (0.075) at control.

13. No incidence of pests were recorded in Karanj plants in all treatments at stages except not all at control at 60 DAT. Mites (*Tetranycus* spp.) were seen only on those Karanj plants by which leaves of some Karanj plants appeared as thick and crinkled in control pots. In inoculated and N treated Karanj plants no pests or not any other damaged leaves were observed, these were healthy plants with healthy leaves and no pest attack.

14. N concentrations were highest in leaves followed by roots then stem component of Karanj plant. Maximum N concentration in leaf, stem and root were 1.52 %, 0.51%, 0.67% was found respectively at 90DAT under T_4 followed by T_3 while at control these were 0.85%, 0.28% and 0.38% in leaf, stem and root respectively. At 150DAT N concentration was decreased compared to 90DAT being 1.26%, 0.35% and 0.53% in leaf, stem and root respectively.

15. In root inoculated Karanj seedlings, N uptake by shoot increased significantly being 40.78 mg/seedling against control (13.76 mg/seedling) at 90 DAT. Further addition of N_1 and N_2 doses of Nitrogen indicated that N uptake by Karanj plant increased. Significantly highest N uptake by shoot of Karanj plant was (57.44 mg/seedling) found at T_4 followed by T_3 (50.58 mg/seedling) at 90DAT while at T_5 it was 39.1 mg/seedling. At 150DAT N uptake by shoot of Karanj was significantly high 90.75 mg/seedling at T_4 followed by T_3 82.31 mg/seedling. Nitrogen uptake in shoot gained extra atmospheric N of 44mg/seedling and 75 mg/seedling at *Rhizobium* inoculation only and *Rhizobium* along with N_2 respectively at 150DAT.

16. Inoculation treatments were highly significant w.r. to growth parameters, biomass accumulation, nodulation and nitrogen content in Karanj seedlings as compared to N fertilization only and control. Application of less N doses along with *Rhizobium* inoculation (T_3 and T_4) showed significant effect over only *Rhizobium*. As compared to T_3 and T_4 higher N along with *Rhizobium* inoculation did not favour the growth of Karanj plants (T_5). However T_5 showed significant effect as compared to T_6, T_7 and T_8.Again among only N fertilization, the effects were in order of $T_8 > T_7 > T_6$. All treatments showed significant effect over control.

CONCLUSIONS:

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

1. Seedling root inoculation of Karanj plants with Karanj-*Rhizobium* was found significantly effective in increasing the morphological growth, biomass production, nodulation and nitrogen uptake over only inorganic N application treatments and control.
2. Among different levels of nitrogen doses, significantly positive response was found when N fertilization in less level (50 and 150mg/seedling) was given along with *Rhizobium* inoculation as compared to higher N level (400mg/seedling).
3. Biologically fixed amount of nitrogen by Karanj seedlings was enhanced significantly when nitrogen at sub optimal level (150mg/seedling) was applied along with *Rhizobium* inoculation.

Based on results it can be inferred that application of N fertilizer at the rate of 150mg/seedling along with *Rhizobium* inoculation has tremendous potential in improving initial growth response of Karanj plants in nursery. This finding may be helpful in producing quality planting stock of Karanj for afforestation programmes.

SUGGESTIONS FOR FUTURE WORK:

1. As NFT species are of great importance in traditional agro-forestry system, the detailed field investigation is 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 with different levels of Nitrogen on performance of Karanj (*Pongamia pinnata)* seedlings.

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ABSTRACT

A polybag experiment was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture, Raipur, Chhattisgarh during the year 2010-11 with eight treatments replicated thrice to assess the impact of *Rhizobium* inoculation on performance of legume tree seedling, Karanj and influence of Nitrogen on biological Nitrogen fixation. The treatments comprised of seedling root inoculation with Karanj -*Rhizobium* alone and along with 3 levels of N fertilization(N_1, N_2 and N_3 as 50mg, 150mg and 400mg N/seedling) and application of 3 levels of N alone including one control. This involved isolation of *Rhizobium* from nodule of Karanj. The isolate produced white translucent round colonies, raised and smooth surface on YEMA media.

Results revealed that seedling root inoculation of Karanj significantly effective in increasing growth, biomass production, nodulation and nitrogen content over only inorganic N application and control. Further application of N_2 dose along with *Rhizobium* inoculation showed significant effect over only inoculation**.** Significantly maximum seedling height (51.9cm), shoot biomass (10.7g/seedling), total biomass 16.8 g/seedling and collar diameter (6.44mm) were obtained in Karanj plants receiving inoculation with N_2 at 150 days after transplant. Nodulation was found significantly maximum(no.47 and dry weight 0.374g per seedling) in *Rhizobium* + N_2 treatment at 120 DAT against only *Rhizobium* (32, 0.287g/seedling) while very sparse nodulation (03) at control. Significantly higher N uptake mg/seedling (90.75) by shoot of Karanj was at R + N_2 against 15.34 mg/seedling at control at 150DAT. Seedling root inoculation with *species- Rhizobium* along with N_2 level of nitrogen application was found most effective in increasing symbiotic traits, growth performances and N uptake by Karanj plants.

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APPENDIX I

Weekly Meteorological data (1 September 2010 to 1 March 2011)

Week Number	Temperature (^{0}C)		Rainfall (mm)	Relative Humidity (%)		Wind Velocity (Kmph)	Sun shine (hours)
	Max.	Min.		I	II		
35	30.3	25.2	71.4	94	82	5.3	3.6
36	31.0	25.3	129.6	93	77	3.5	5.2
37	31.7	25.0	51.0	94	72	2.2	5.8
38	30.2	24.7	141.0	95	73	2.7	3.9
39	32.2	24.5	5.8	90	54	1.4	8.3
40	32.0	24.3	0.0	88	56	2.5	8.8
41	31.8	22.7	10.2	88	50	2.5	7.9
42	30.3	23.9	8.4	92	69	3.6	13.5
43	31.2	20.5	46.0	94	52	1.7	8.5
44	26.5	19.2	0.7	92	56	2.9	2.8
45	29.5	20.6	0.0	90	55	3.2	7.3
56	31.5	20.6	6.5	93	34	2.1	7.9
47	31.6	18.1	0.0	88	37	1.7	9.3
48	31.7	18.7	0.6	89	43	1.1	7.5
49	25.7	17.6	55.0	83	52	4.3	4.0
50	26.2	15.0	2.2	92	39	2.0	6.8
51	25.8	8.2	0.0	92	26	1.8	9.4
52	27.6	11.8	0.0	92	33	1.6	8.8
1	24.3	9.0	0.0	83	31	2.4	7.9
2	26.1	7.3	0.0	87	24	1.6	9.5
3	28.3	10.4	0.0	79	31	2.5	9.9
4	29.8	12.9	0.0	85	31	1.3	9.1
5	29.9	14.0	0.0	82	35	2.7	8.5
6	31.4	13.1	0.0	83	27	2.1	9.4
7	32.1	16.8	0.0	74	31	2.9	8.9
8	27.1	15.7	12.2	84	51	3.0	5.6
9	32.0	16.5	0.0	81	30	2.3	9.1

APPENDIX II

Chemical composition of media

Yeast Extract Mannitol broth media (YEM broth) for Rhizobia (Subba Rao, 1988)

Composition of the medium

- Mannitol 10.0 g
- $K_2 HPO_4$ 0.5 g
- $MgSO_4, 7H_2O$ 0.2 g
- NaCl 0.1 g
- Yeast Extract 1.0 g
- Distilled water 1000.0 ml
- Congo red solution (1%) 2.5 ml
- pH 7.0

APPENDIX III

1. **Gram staining solution (Vincent 1970)**

Solution-1 Crystal violet solution

Crystal violet	10g
Ammonium oxalate	4g
Ethanol	100g
Distilled water	400ml

Solution-2 Iodine solution

Iodine	1g
Potassium iodide	2gm
Ethanol	25g
Distilled water	100ml

Solution-3 Alcohol

Distilled water	5ml
Ethanol	95ml

Solution -4 Counter stain

2.5%Safranin in ethanol	10ml
Distilled water	100ml

APPENDIX IV

Nutrient Agar media for total bacteria

Composition of the medium

- K_2HPO_4 1.0g
- KNO_3 0.5g
- $MgSO_4$ 0.2g
- $CaCl_2$ 0.1g
- NaCl 0.1g
- $FeCl_3$ Trace
- Asperagine 0.5g
- Mannitol 1.0g
- Agar-Agar 15.0g
- Distilled water 1000ml

Table – 1: Characteristics of Karanj- *Rhizobium* isolate

	Properties	**Karanj- *Rhizobium* isolate**
1.	Gram staining	Gram –ve bacteria
2.	Shape	Short rod
Colony characters		
3.	Growth on YEMA	White translucent
4.	Shape/form of colony	Circular
5.	Size	1.5-2.00 mm
6.	Edge/margin	Entire
7.	Elevation	Convex (raised)
8.	Surface	Smooth
9.	Colour	Creamy white

Table-2: Properties of soil used in filling polythene bags for experimental purpose

S. No.	**Parameters**	**Values**
	Soil	**Vertisol**
1	**pH (1:2.5)**	**7.6**
2	**E. C. (m. mhos cm^{-1})**	**0.21**
3	**Organic carbon (g/kg soil)**	**6.3**
4	**Mineralizable N (kg/ha.)**	**240.4**
5	**Available P (kg/ha.)**	**11.6**
6	**Available K (kg/ha.)**	**416**
7	Rhizobium *population* **Per g. of soil**	**4.8×10^2**

Table- 3: Effect of *Rhizobium* inoculation with different levels of N on morphological growth parameters of Karanj plants at different stages.

Treatmen	Morphological growth parameters																	
	Seedling Height (cm)						No. of leaves/seedling						Collar Diameter (mm)					
	Days after transplant						Days after transplant						Days after transplant					
	30	60	90	120	150	Mean	30	60	90	120	150	Mean	30	60	90	120	150	Mean
T_1	13.14	18.21	19.13	22.5	25.37	19.68	8.62	13.5	13.5	14.21	18.5	13.67	1.71	2.5	3.22	3.75	4.00	3.03
T_2	17.8	24.26	30.16	37.5	42.8	30.50	13.32	20.34	22.33	28.8	37.75	24.50	2.78	4.04	4.77	5.48	5.94	4.60
T_3	22.3	26.0	33.13	40.76	48.36	34.11	14.12	21.46	30.5	37.12	46.45	29.93	3.10	4.45	5.25	5.84	6.28	4.99
T_4	21.6	27.8	34.5	42.4	51.85	35.63	14.64	23.52	32.33	41.86	50.67	32.60	3.48	4.56	5.38	6.00	6.44	5.18
T_5	18.5	25.14	28.3	36.2	43.87	30.40	13.85	19.42	24.5	29.4	35.25	24.49	2.93	4.24	5.06	5.51	6.01	4.76
T_6	13.87	19.23	22.6	28.6	30.2	22.9	11.37	16.71	18.00	18.66	21.5	17.24	2.17	3.12	3.85	4.07	4.78	3.60
T_7	16.2	21.24	24.36	30.2	34.3	25.27	11.54	17.71	18.66	20.42	26.5	18.97	2.35	3.49	4.30	4.87	5.30	4.07
T_8	19.84	23.63	27.43	32.16	37.86	28.18	12.5	17.98	19.67	23.12	30.8	20.81	2.65	3.84	4.68	4.97	5.67	4.37
SEm(±)	1.102	1.207	1.36	1.04	0.723		0.38	0.547	0.535	0.506	1.201		0.107	0.277	0.185	0.203	0.23	
CD (5%)	3.305	3.62	4.09	3.11	2.17		1.14	1.64	1.603	1.52	3.602		0.32	0.832	0.555	0.608	0.689	

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table-4: **Effect of *Rhizobium* inoculation with different levels of N on fresh biomass accumulation in different components of Karanj plants at different stages.**

Treatment	Fresh weight of leaves (g/seedling)					Fresh weight of stem (g/seedling)					Fresh weight of root (g/seedling)				
	Days after transplant					Days after transplant					Days after transplant				
	60	90	120	150	Mean	60	90	120	150	Mean	60	90	120	150	Mean
T_1	2.43	3.69	4.32	4.14	3.64	2.03	3.23	3.31	3.49	3.01	2.66	3.75	5.06	5.14	4.16
T_2	5.00	6.40	9.97	10.37	7.93	4.12	5.31	7.31	9.29	6.50	4.06	8.31	9.66	11.54	8.50
T_3	5.54	7.19	10.82	13.38	9.23	4.56	5.39	8.06	11.21	7.30	4.22	9.07	10.07	13.87	9.30
T_4	5.74	8.94	11.69	14.73	10.28	4.74	6.24	8.65	11.96	7.90	4.18	8.88	9.85	13.28	9.04
T_5	4.86	6.12	8.83	9.86	7.41	3.62	3.99	7.13	10.01	6.19	3.69	7.88	9.04	11.87	8.12
T_6	4.05	4.42	5.38	5.86	4.92	3.02	3.50	4.38	7.12	4.50	2.71	5.81	7.52	7.82	5.97
T_7	4.45	4.68	6.52	7.93	5.90	3.29	3.79	5.86	8.49	5.36	3.18	6.44	7.95	9.47	6.77
T_8	4.50	5.00	8.48	9.06	6.77	3.41	3.92	6.15	8.59	5.51	3.31	6.58	8.11	10.76	7.19
SEm(±)	0.234	0.34	0.31	0.25	-	0.18	0.27	0.23	0.39	-	0.26	0.29	0.28	0.31	-
CD (5%)	0.701	1.03	0.94	0.77	-	0.51	0.79	0.69	1.16	-	0.77	0.84	0.82	0.93	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table-5: **Effect of *Rhizobium* inoculation with different levels of N on dry biomass accumulation in different components of Karanj plants at different stages.**

Treatment	Dry weight of leaves (g/seedling)					Dry weight of stem (g/seedling)					Dry weight of root (g/seedling)				
	Days after transplant					Days after transplant					Days after transplant				
	60	90	120	150	Mean	60	90	120	150	Mean	60	90	120	150	Mean
T_1	0.84	1.20	1.69	1.67	1.35	0.59	1.27	1.34	1.44	1.16	0.8	1.87	1.81	1.71	1.55
T_2	1.76	2.25	3.54	4.26	2.96	1.37	2.14	3.07	3.88	2.61	1.43	3.10	3.76	4.81	3.28
T_3	2.18	2.65	4.08	5.64	3.63	1.45	2.46	3.15	4.56	2.90	1.57	3.56	4.16	5.43	3.69
T_4	2.46	2.87	4.24	5.85	3.86	1.46	2.76	3.56	4.86	3.16	1.53	3.41	4.00	5.89	3.70
T_5	1.87	2.14	3.78	4.66	3.11	1.14	1.86	2.44	4.00	2.38	1.36	2.90	3.88	4.92	3.27
T_6	1.46	1.70	2.00	2.24	1.85	1.02	1.41	1.71	2.00	1.53	1.02	2.14	2.40	2.31	2.00
T_7	1.50	1.82	3.28	3.49	2.52	1.02	1.48	2.00	2.81	1.82	1.22	2.55	2.87	3.50	2.54
T_8	1.72	1.96	3.47	3.62	2.70	1.05	1.65	2.23	3.41	2.09	1.24	2.80	3.22	3.92	2.80
SEm(±)	0.09	0.055	0.175	0.09	-	0.034	0.07	0.04	0.09	-	0.07	0.09	0.08	0.14	-
CD (5%)	0.29	0.17	0.524	0.27	-	0.102	0.19	0.13	0.28	-	0.19	0.29	0.21	0.41	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table- 6: **Effect of *Rhizobium* inoculation with different levels of N on Total dry biomass production by Karanj plants 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				
	60	90	120	150	Mean	60	90	120	150	Mean	60	90	120	150	Mean
T_1	1.43	2.47	3.03	3.11	2.51	0.80	1.88	1.81	1.71	1.55	2.23	4.34	4.84	4.82	4.06
T_2	3.13	4.39	6.61	8.14	5.57	1.43	3.22	4.04	4.89	3.39	4.57	7.61	10.65	13.03	8.96
T_3	3.63	5.11	7.23	10.2	6.54	1.58	3.73	4.52	5.59	3.85	5.20	8.84	11.76	15.79	10.39
T_4	3.92	5.63	7.8	10.71	7.01	1.54	3.61	4.38	6.08	3.90	5.46	9.24	12.18	16.79	10.91
T_5	3.01	4.00	6.22	8.66	5.48	1.36	3.01	4.14	4.99	3.38	4.38	7.01	10.36	13.66	8.84
T_6	2.48	3.11	3.71	4.24	3.39	1.02	2.15	2.46	2.31	2.00	3.50	5.26	6.17	6.56	5.37
T_7	2.52	3.30	5.28	6.30	4.36	1.22	2.58	2.967	3.51	2.56	3.74	5.88	8.24	9.81	6.92
T_8	2.77	3.61	5.7	7.03	4.78	1.24	2.84	3.34	3.94	2.84	4.01	6.46	9.04	10.98	7.62
SEm(±)	0.118	0.071	0.191	0.113	-	0.065	0.09	0.08	0.138	-	0.148	0.112	0.19	0.187	-
CD (5%)	0.355	0.214	0.574	0.340	-	0.195	0.29	0.21	0.416	-	0.446	0.337	0.59	0.561	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table-7: Quality parameters of *P.pinnata* (Karanj) plants at 150 DAT as affected by *Rhizobium* inoculation with different levels of N fertilizer

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_1	25.37	4.00	63.42	3.11	1.71	0.54	4.82	0.075
T_2	42.80	5.94	72.05	8.14	4.89	0.60	13.04	0.179
T_3	48.36	6.28	77.00	10.20	5.58	0.55	15.78	0.203
T_4	51.85	6.44	80.51	10.71	6.07	0.57	16.78	0.207
T_5	43.87	6.01	72.99	08.66	4.99	0.58	13.66	0.185
T_6	30.20	4.78	63.18	4.24	2.31	0.54	6.55	0.103
T_7	34.30	5.30	64.71	6.30	3.52	0.56	9.82	0.150
T_8	37.86	5.67	66.77	7.03	3.94	0.56	10.97	0.163

T_1-Control, T_2-Inoculated (*Rhizobium*), T_{3-} inoculated + $N_{1,}$ T_{4-} inoculated + $N_{2,}$ T_5- inoculated + $N_{3,}$ T_6- uninoculated + $N_{1,}$ T_7- uninoculated+$N_{2,}$ T_8 – uninoculated+N_3

Table- 8: Effect of *Rhizobium* inoculation with different levels of N on nodulation behaviour of Karanj plants at different stages..

Treatment	No. of nodules/seedling					Fresh weight of nodules (g/seedling)					Dry weight of nodules (g/seedling)				
	Days after transplant					Days after transplant					Days after transplant				
	60	90	120	150	Mean	60	90	120	150	Mean	60	90	120	150	Mean
T_1	-	1.66	2.66	-	2.16	-	0.021	0.024	-	0.02	-	0.002	0.003	-	0.0025
T_2	2.33	18.33	31.66	15.00	16.83	0.028	1.02	1.32	0.76	0.79	0.004	0.121	0.287	0.086	0.12
T_3	2.66	20.33	36.66	17.33	19.24	0.028	1.04	1.56	0.82	0.87	0.004	0.178	0.361	0.154	0.17
T_4	3.00	24.33	46.66	20.00	23.50	0.031	1.12	1.75	0.88	0.94	0.006	0.208	0.374	0.186	0.20
T_5	1.00	17.66	32.33	9.66	15.17	0.017	0.87	1.06	0.68	0.66	0.002	0.104	0.256	0.078	0.11
T_6	-	3.00	6.66	2.66	4.10	-	0.21	0.31	0.03	0.19	-	0.010	0.060	0.004	0.02
T_7	-	7.00	12.33	5.33	8.22	-	0.52	0.757	0.36	0.55	-	0.024	0.096	0.017	0.04
T_8	-	9.00	18.33	6.66	11.33	-	0.62	1.06	0.48	0.72	-	0.042	0.127	0.024	0.07
SEm(±)	0.333	0.928	1.44	0.726	-	0.0015	0.019	0.033	0.025	-	0.0005	0.0033	0.0071	0.0033	-
CD (5%)	0.999	2.78	4.31	2.18	-	0.0044	0.057	0.1	0.076	-	0.0015	0.01	0.021	0.01	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table- 9: Effect of *Rhizobium* inoculation with different levels of N fertilization on N concentration % and N uptake (mg/seedling) in different components of Karanj plants at 90 DAT.

Treatment	Concentration of Nitrogen %			Concentration of Nitrogen % in shoot	Nitrogen uptake (mg/seedling)			N uptake by shoot (mg/seedling)	Extra N gain through BNF (mg/seedling)
	Leaf	Stem	Root	Leaf + Stem	Leaf	Stem	Root	Leaf+ Stem	Leaf+ Stem
T_1	0.85	0.28	0.38	1.13	10.20	3.56	7.109	13.76	-
T_2	1.40	0.43	0.55	1.83	31.57	9.21	17.075	40.78	27.02
T_3	1.46	0.48	0.63	1.94	38.77	11.81	22.442	50.58	36.82
T_4	1.52	0.51	0.67	2.03	43.35	14.09	22.852	57.44	43.68
T_5	1.43	0.46	0.60	1.89	30.58	8.52	17.454	39.1	25.34
T_6	1.13	0.30	0.42	1.43	19.21	4.23	8.970	23.44	9.68
T_7	1.26	0.34	0.47	1.6	22.92	5.027	11.990	27.94	14.18
T_8	1.32	0.38	0.51	1.70	25.85	6.27	14.294	32.12	18.36
SEm (±)	-	-	-	-	1.59	0.486	1.077	1.44	-
CD (5%)	-	-	-	-	4.77	1.458	3.231	4.34	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table-10: Effect of *Rhizobium* inoculation with different levels of N fertilization on N concentration % and N uptake (mg/seedling) in different components of Karanj plants at 150 DAT

Treatment	Concentration of Nitrogen (%)			Concentration of Nitrogen (%) in shoot	Nitrogen uptake (mg/seedling)			N uptake by shoot (mg/seedling)	Extra N gain through BNF (mg/seedling)
	Leaf	Stem	Root	Leaf+ Stem	Leaf	Stem	Root	Leaf+ Stem	Leaf+ Stem
T_1	0.78	0.16	0.27	0.94	13.03	2.31	4.61	15.34	-
T_2	1.14	0.28	0.43	1.42	48.58	10.88	20.69	59.46	44.12
T_3	1.20	0.32	0.51	1.52	67.73	14.58	27.79	82.31	66.97
T_4	1.26	0.35	0.53	1.61	73.69	17.07	31.20	90.75	75.41
T_5	1.16	0.31	0.48	1.47	54.05	12.37	23.64	66.42	51.08
T_6	0.91	0.20	0.29	1.11	20.40	4.00	6.70	24.00	8.66
T_7	1.02	0.22	0.35	1.24	35.59	6.19	12.25	41.78	26.44
T_8	1.08	0.25	0.37	1.33	39.07	8.51	14.50	47.58	32.24
SEm(±)	-	-	-	-	1.725	0.706	1.139	2.03	-
CD (5%)	-	-	-	-	5.173	2.117	3.416	6.11	-

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3- inoculated + N_1, T_4- inoculated + N_2, T_5- inoculated + N_3, T_6- uninoculated + N_1, T_7- uninoculated+N_2, T_8 – uninoculated+N_3

Table 11: *Rhizobial* and total bacterial population in rhizosphere soil of Karanj plant as affected by different treatments at 150 DAT.

Treatment	***Rhizobium* population per g of soil**	**Bacterial population per g of soil**
T_1**(Control)**	2.32×10^3	1.65×10^6
T_2**(Inoculated (*Rhizobium*)**	3.26×10^3	4.46×10^6
T_3**(Inoculated +** N_1**)**	3.84×10^3	5.24×10^6
T_4**(Inoculated +** N_2**)**	4.02×10^3	5.65×10^6
T_5**(Inoculated +** N_3**)**	3.31×10^3	4.55×10^6
T_6**(Un-inoculated +** N_1**)**	2.72×10^3	2.27×10^6
T_7**(Un-inoculated +** N_2**)**	2.78×10^3	2.58×10^6
T_8**(Un-inoculated +** N_3	2.96×10^3	3.12×10^6
SEm (±)	0.078×10^3	0.055×10^6
CD at 5%	0.235×10^3	0.167×10^6

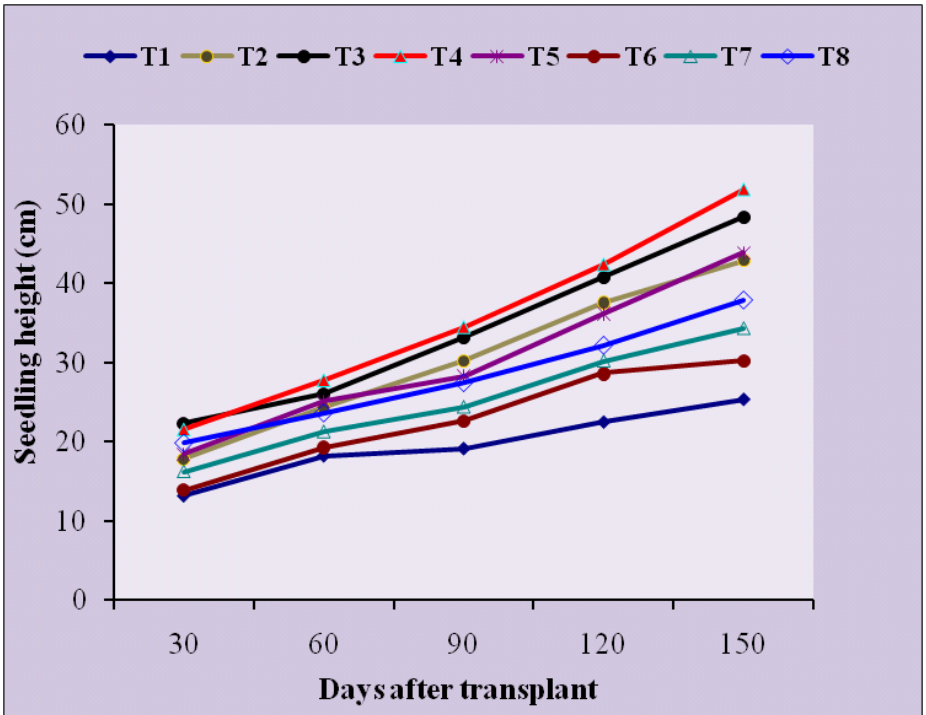


Fig. 1: Effect of *Rhizobium* inoculation with different levels of Nitrogen on seedling height of Karanj

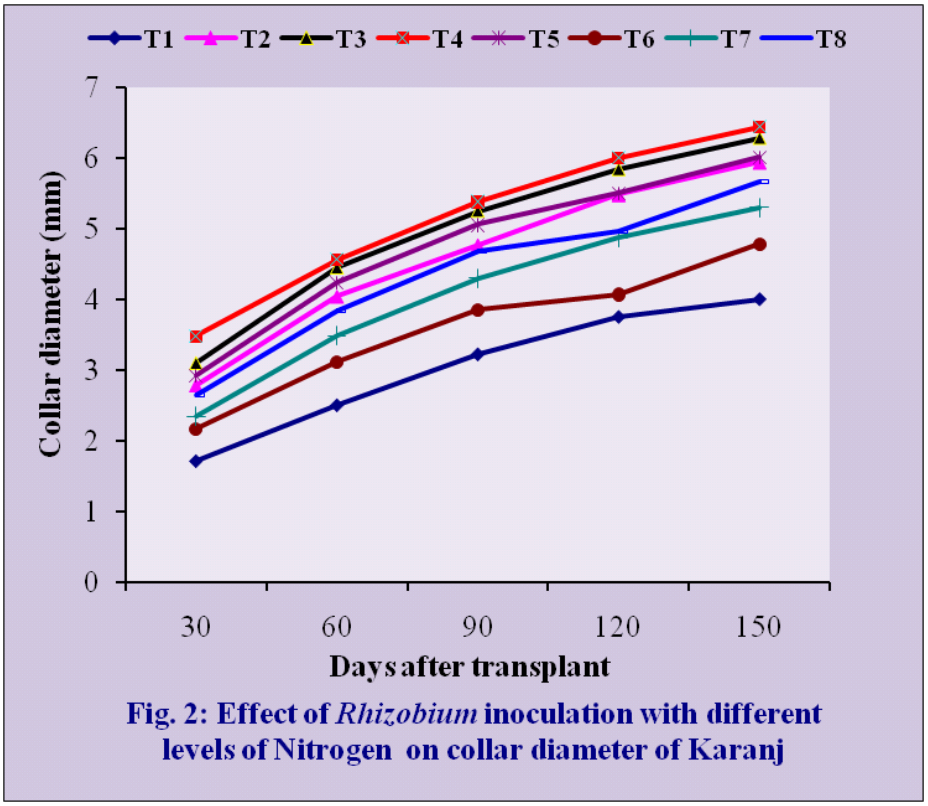


Fig. 2: Effect of *Rhizobium* inoculation with different levels of Nitrogen on collar diameter of Karanj

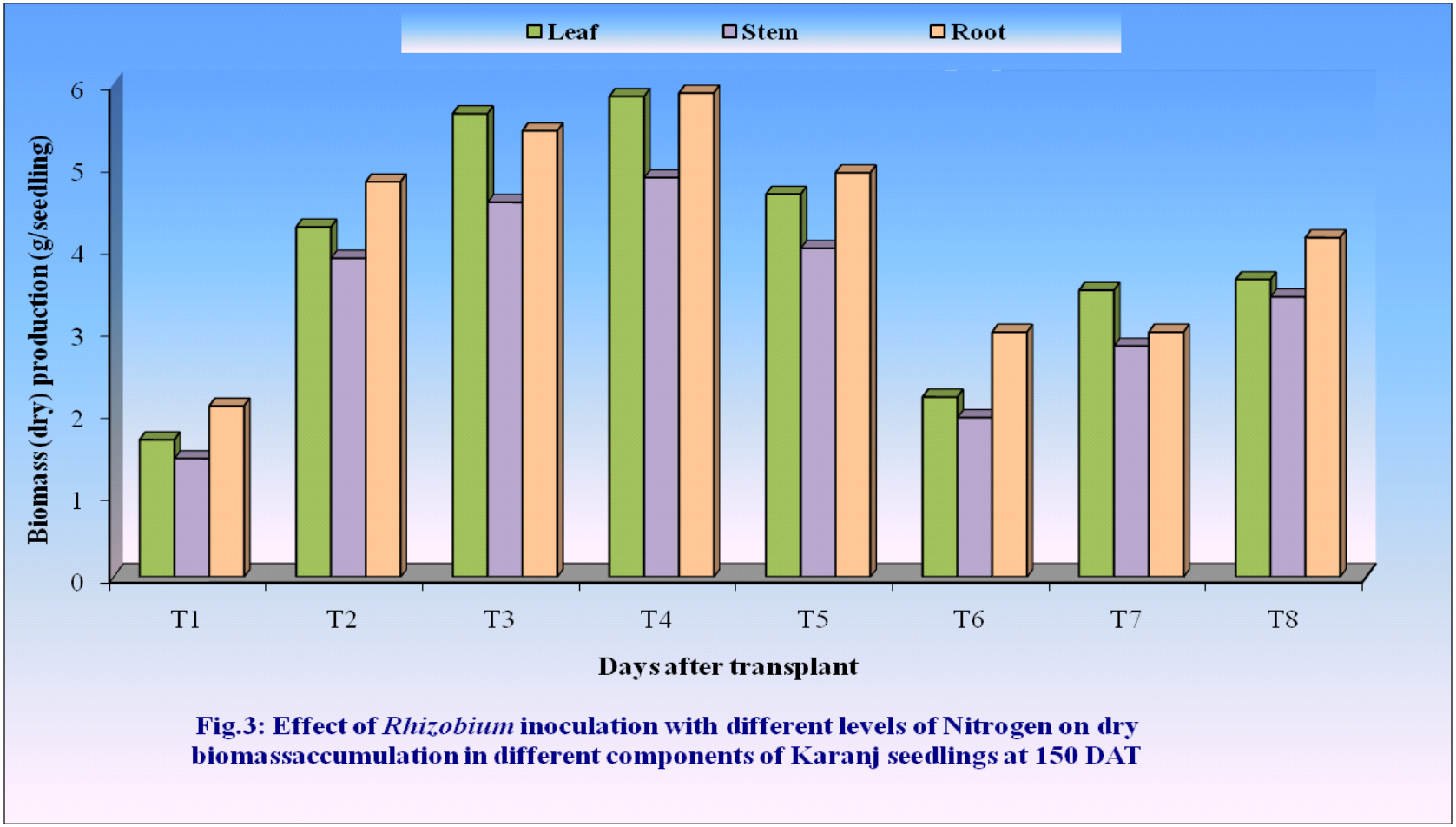


Fig.3: Effect of *Rhizobium* inoculation with different levels of Nitrogen on dry biomassaccumulation in different components of Karanj seedlings at 150 DAT

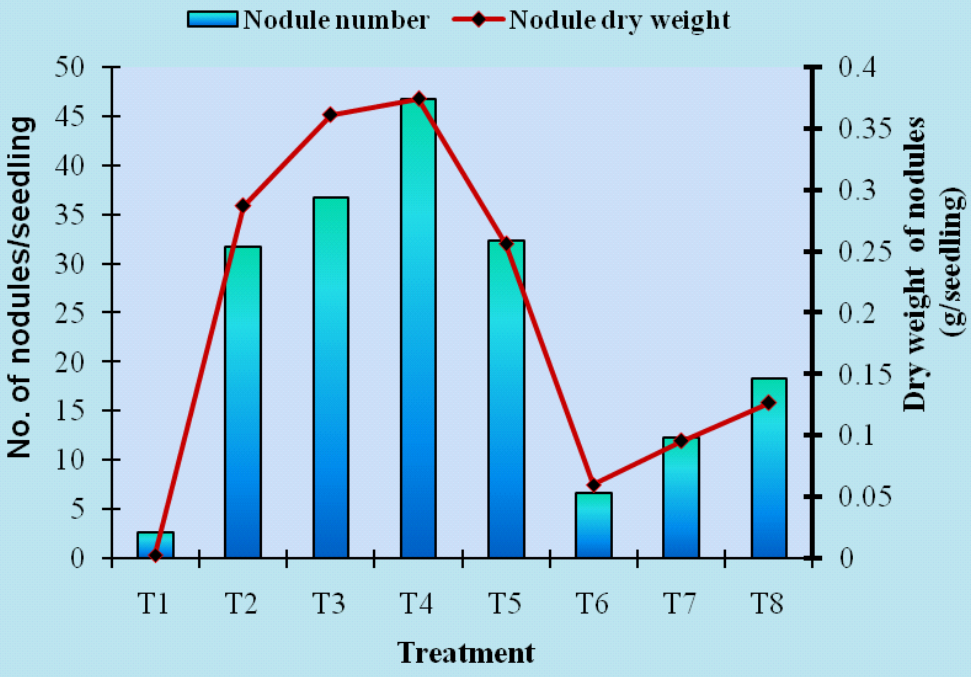


Fig. 4: Effect of *Rhizobium* inoculation with different levels of Nitrogen on nodule number and nodule dry weight of Karanj at 120 DAT.

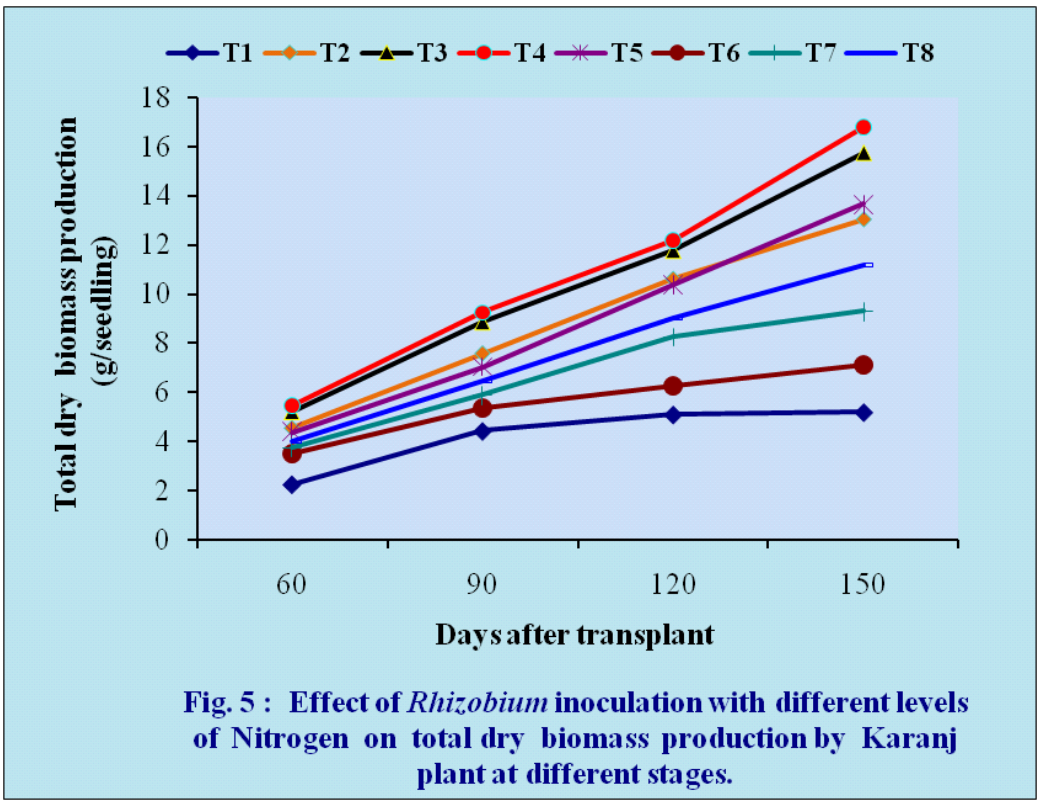


Fig. 5 : Effect of *Rhizobium* inoculation with different levels of Nitrogen on total dry biomass production by Karanj plant at different stages.

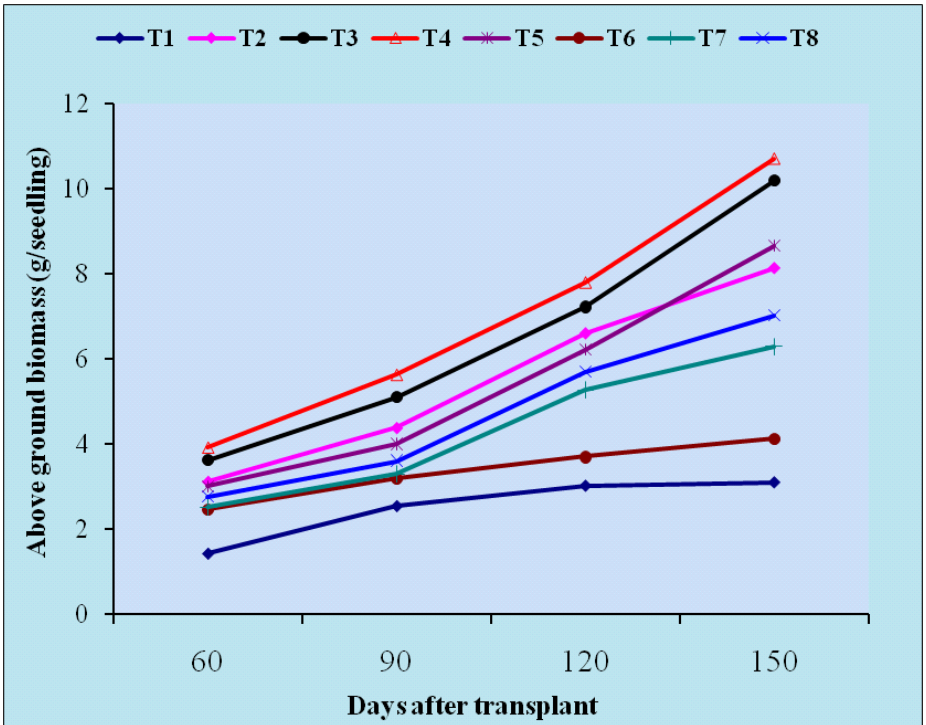


Fig. 6: Effect of *Rhizobium* inoculation with different levels of Nitrogen on above ground biomass(dry) accumulation in Karanj.

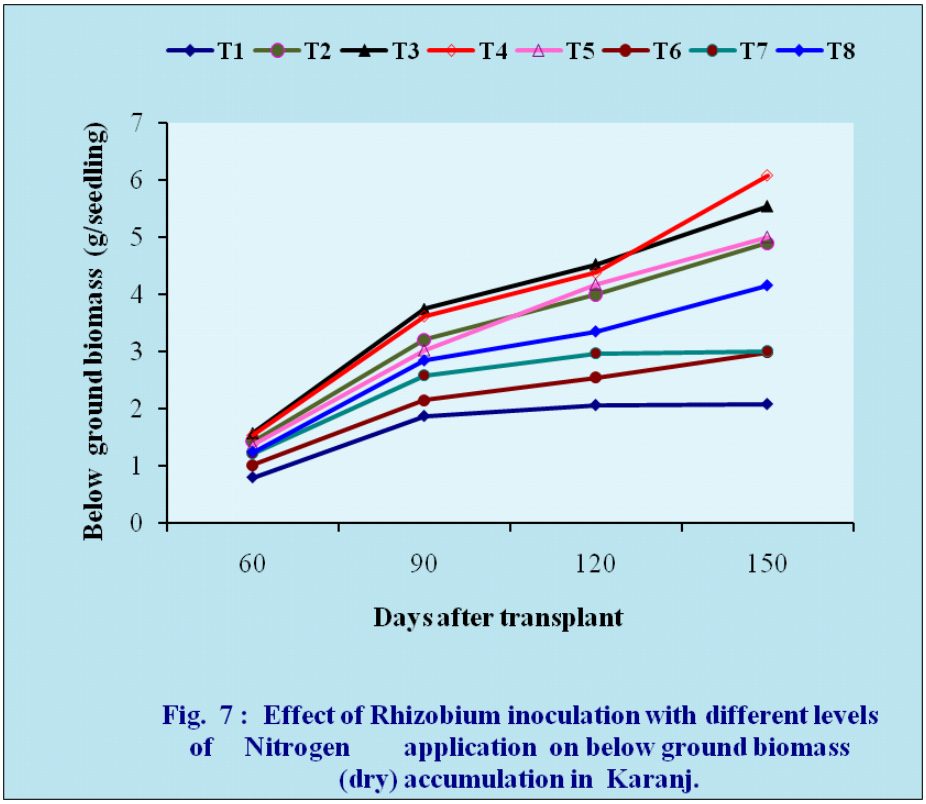


Fig. 7 : Effect of Rhizobium inoculation with different levels of Nitrogen application on below ground biomass (dry) accumulation in Karanj.

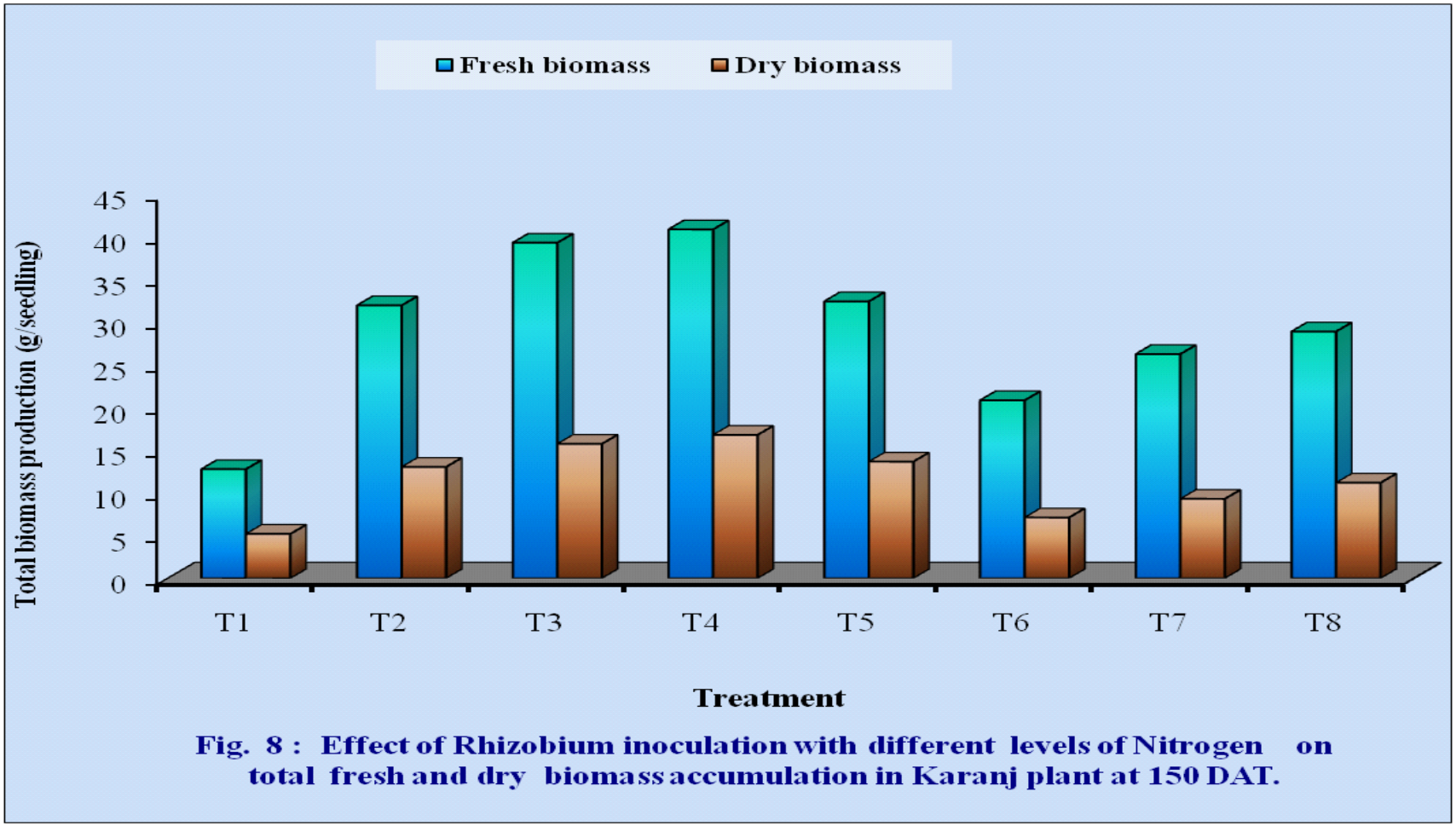


Fig. 8 : Effect of Rhizobium inoculation with different levels of Nitrogen on total fresh and dry biomass accumulation in Karanj plant at 150 DAT.

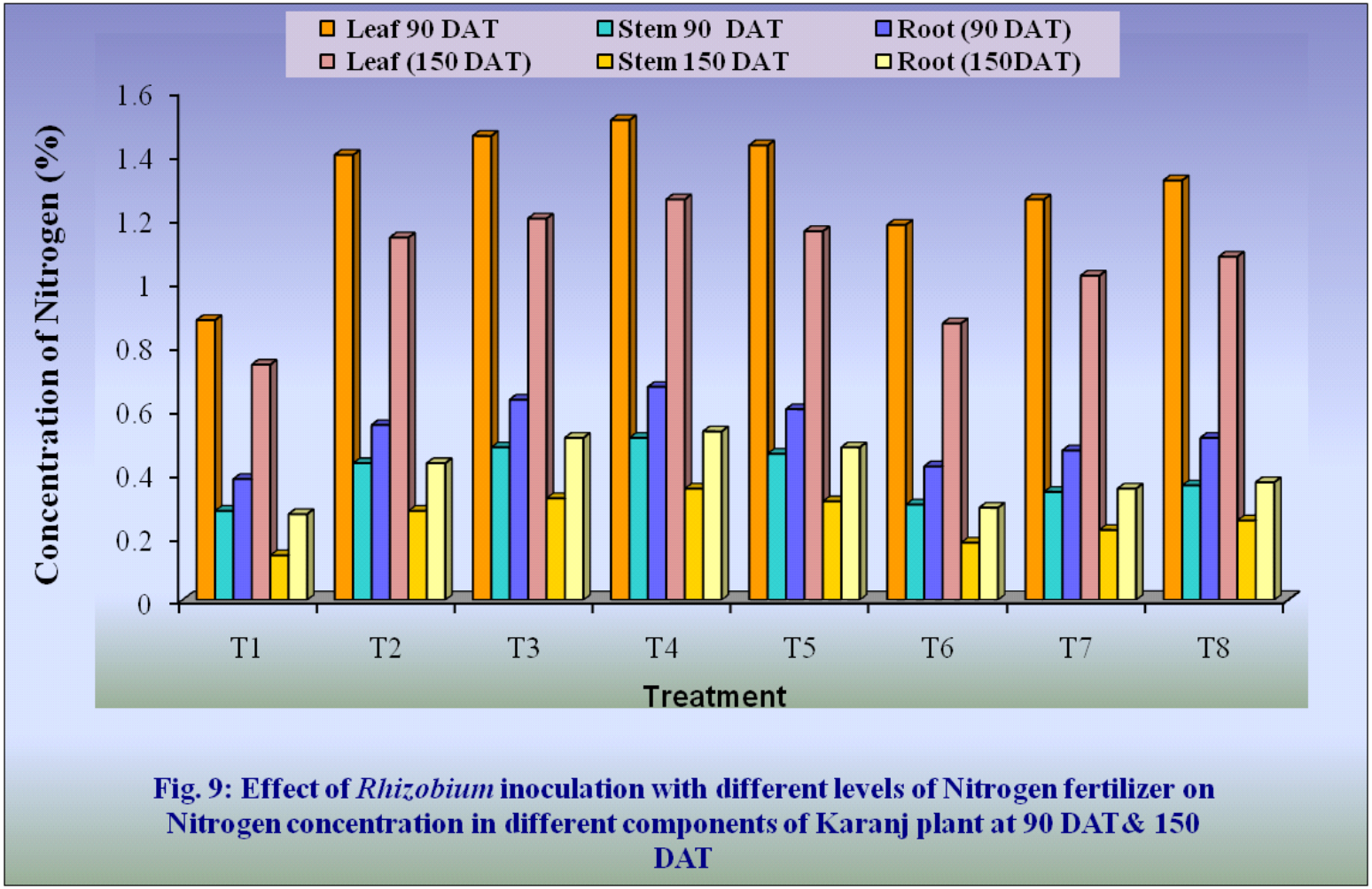


Fig. 9: Effect of *Rhizobium* inoculation with different levels of Nitrogen fertilizer on Nitrogen concentration in different components of Karanj plant at 90 DAT & 150 DAT

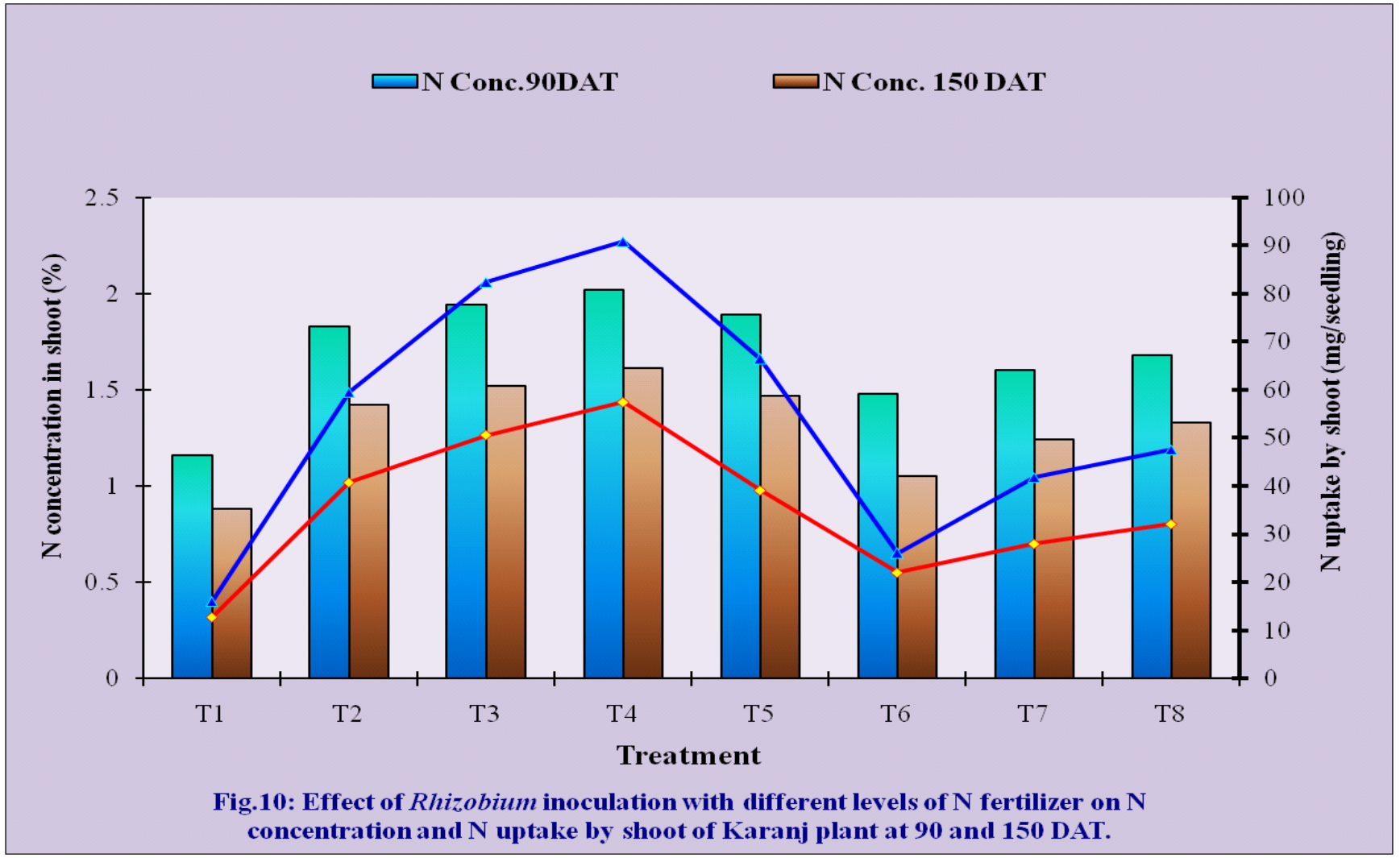


Fig.10: Effect of *Rhizobium* inoculation with different levels of N fertilizer on N concentration and N uptake by shoot of Karanj plant at 90 and 150 DAT.

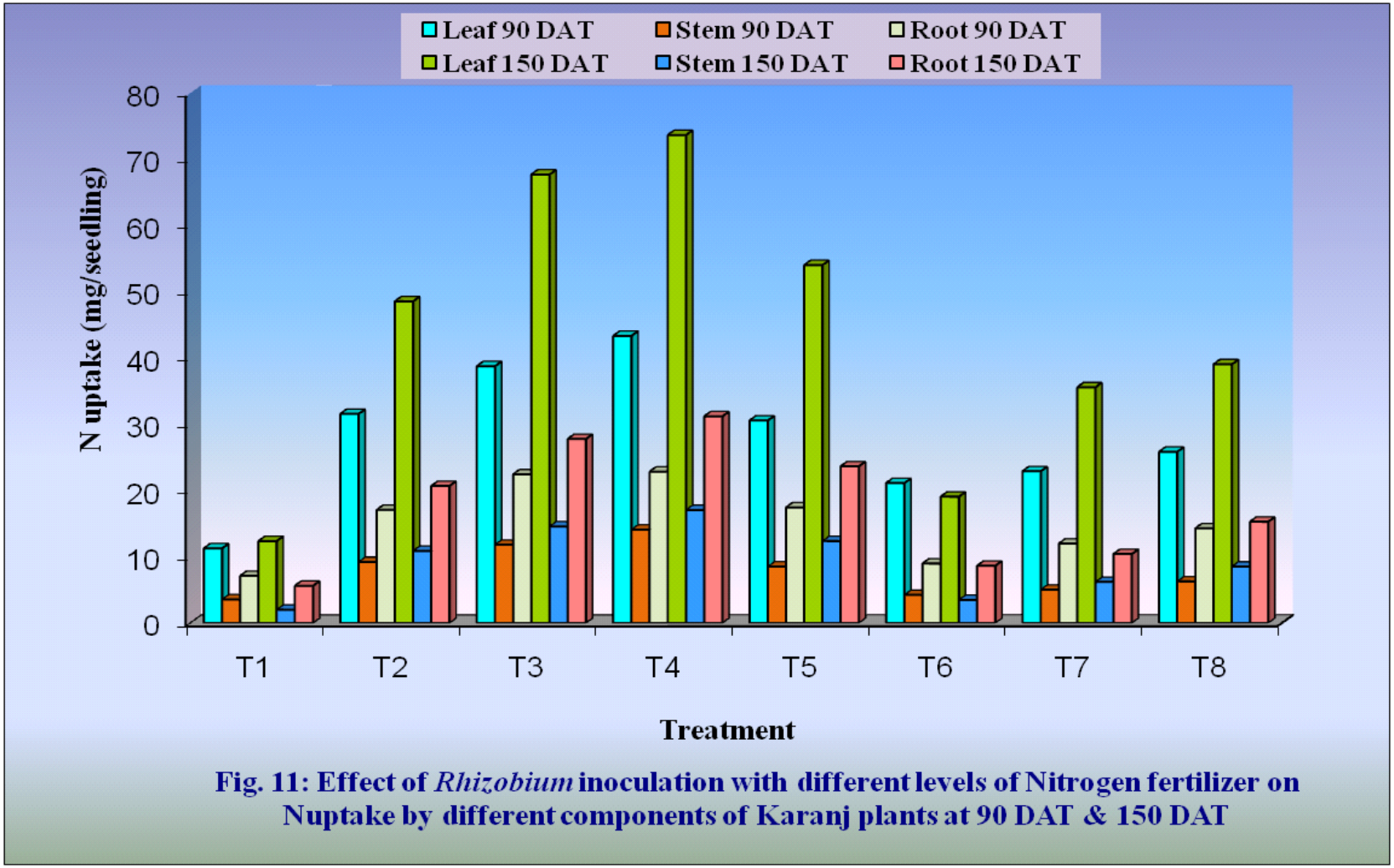
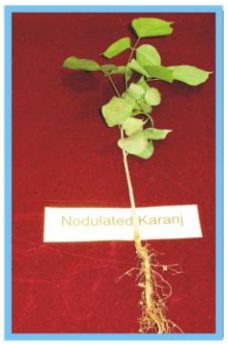


Fig. 11: Effect of *Rhizobium* inoculation with different levels of Nitrogen fertilizer on Nuptake by different components of Karanj plants at 90 DAT & 150 DAT



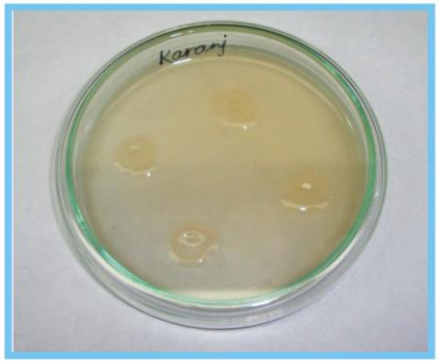


Plate 1 : Isolation of *Rhizobium* from nodulated plant of Karanj (*Pongamia pinnata*)

Plate 2 : Characterization of selected Karanj - *Rhizobium* isolate

A) Root inoculation of germinated Karanj seedlings

B) Experiment at the time of transplanting

Plate 3 : General view of root inoculation and transplantation of Karanj plants

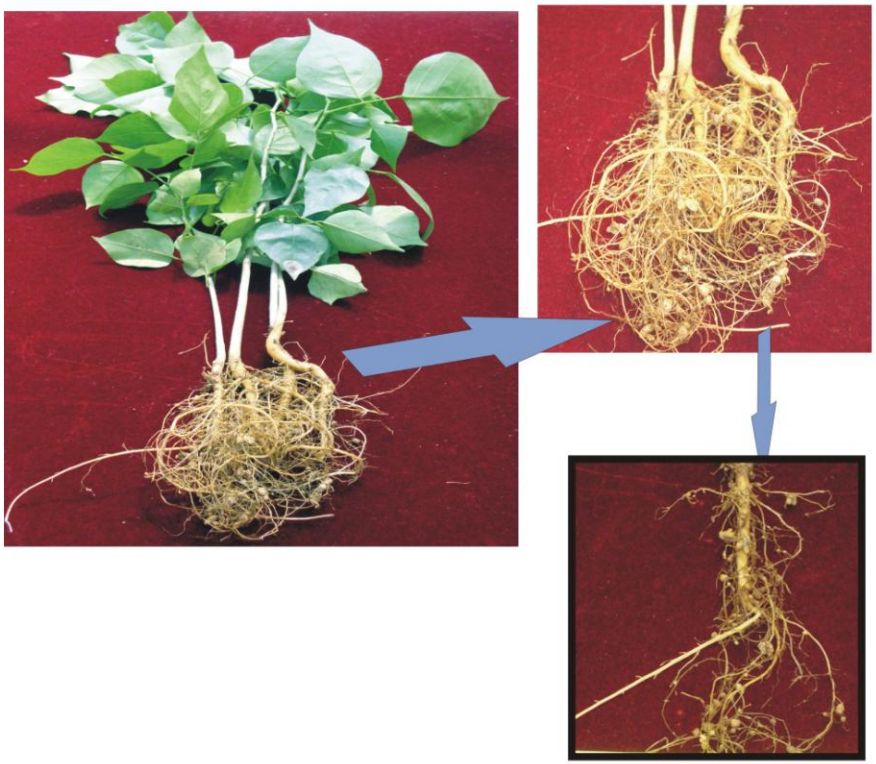
A) At 30 DAT

B) At 120 DAT

Plate 5 : Better growth performance of *Rhizobium* inoculated Karanj

Plate 6 : Influence of N levels on inoculated Karanj

Plate 7 : Influence of different levels of Nitrogen on Karanj



Inoculated

Control

Plate 8 : Nodulation study of *Rhizobium* inoculated Karanj at 120 DAT

N_1 N_2 N_3

A) Inoculated

N_1 N_2 N_3

B) Uninoculated

Plate 9 : Treatment effects on nodulation in Karanj

Plate 10 : Recording of different observations related to the experiment