Eco. Env. & Cons. 20 (3) : 2014; pp. (1059-1065)
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ISSN 0971–765X

Influence of *Rhizobium* inoculation on N, P and K content in *Dalbergia sissoo* Roxb.

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(Received : 1 February, 2014; accepted 5 March, 2014)

ABSTRACT

The present experiment was conducted in glass house of Department of Microbiology, College of Agriculture, Raipur, Chhattisgarh in the year 2009-10 with six treatments comprised of *Dalbergia sissoo-Rhizobium* inoculation @ 5 mL inoculum/seedling, 2 levels of N fertilization (N_1 and N_2 as 2g and 4g N/seedling) alone and along with each other including one control to evaluate the response of *Dalbergia sissoo* to *Rhizobium* inoculation and the influence of nitrogen on biological nitrogen fixation. This involved isolation of *Rhizobium* isolate from nodule of *D. sissoo*. Results revealed that inoculation with *Dalbergia sissoo-Rhizobium* and nitrogen fertilizer application influenced the nutrient concentration in different seedling components. *Rhizobium* inoculation showed higher N concentration over uninoculated ones. Significantly higher N, P and K content in shoot of *D. sissoo* (216.0, 13.62 and 159.23 mg/seedling, respectively) was found when N_1 was applied along with inoculation. Root inoculation of *D. sissoo* seedlings with species *Rhizobium* showed significant effect and further application of N at lower level along with *Rhizobium* inoculation, was found most effective in terms of increasing symbiotic traits and nutrient uptake by *D. sissoo* seedlings.

Key words : *Dalbergia sissoo, Inoculation, Nutrient uptake, Rhizobium*

Introduction

Soil microorganisms play significance role in biogeochemical processes impacting plant nutrition and leading to plant productivity and soil fertility improvement (Kumar *et al.*, 2013). Introduced microorganisms can affect plant growth, health and also soil quality. Thus, any change in microbial communities might affect plant-microorganism interactions. Microbial populations in the rhizosphere may benefit the plant in a variety of ways. Organisms in the rhizosphere produce organic compounds that affect the proliferation of the plant root system. The combination of bioinoculants is a major cause for success of both the plant establishment and the sustainability of bioinoculants and confirms the beneficial effects of microbial consortium over conventional single inoculant application method (Raja *et al.*, 2006). Fertilizer application enhanced the efficiencies of N, P and K uptake, whereas reduced their usage efficiencies. Though soil type did not affect microbial inoculation response, fertilizer application significantly affected plant response to microbial inoculation (Muthukumar and Udaiyan, 2006).

Dash and Gupta (2011) reported that the roots of tree legumes grown in different pots inoculated with mineral solubilizing bacteria and fungi to evaluate AM infection and colonization. As compared to fungal inoculation, bacterial inoculation encouraged increase in percent AM colonization in the roots.The microbial inoculants were used in single form or in combinations (Amutha and

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Kannaiyan, 2004; Aseri and Rao, 2005; Zaidi and Khan, 2006; Anil *et al.*, 2007; Gaikwad *et al.*, 2008). The effects of the inoculation of *Rhizobium* and phosphate solubilizing bacterium (*Bacillus megaterium* var. *phosphaticum*), singly or in combination gave better result than the uninoculated control (Sengupta *et al.*, 2002; Marimuthu*et al.*, 2002; Jat *et al.*, 2003; Mathew and Hameed, 2003; Kashyap *et al.*, 2004; Purbey and Sen, 2005). Singh and Tilak (2001) stated that the synergistic effect of combined phosphorus fertilizers and inert sources of natural P along with bioinoculants. Sumana and Bagyaraj (2002) studied the interaction between VAM fungus and nitrogen fixing bacteria and their influence on growth and nutrition on different tree species. Soil compaction induced a limitation in root and shoot growth that was reflected by a decrease in the microbial population and activity. Enhancement of growth, nodulation and N_2 fixation in *D. sissoo* by *Rhizobium* and *Glomus fasciculatum* in the form of dual inoculation was studied by Rao *et al.* (2003).

Dalbergia sissoo is an important Nitrogen Fixing Tree (NFT) species belongs to family leguminasae, subfamily papilionoideae and widely used in agroforestry, afforestration programs and farm forestry in the Indian subcontinent (Huda *et al.*, 2007). Being a fast growing NFTs, this species is highly valued for furniture and timber purpose. Very often leguminous trees are established in the field by transplanting seedlings from nursery. To be successful after outplanting, sissoo seedlings must access sufficient nutrient from the soil and out compete others. In modern forestry, it is important to produce quality seedlings by inducing morpho-physiological changes in the plants for making them competent enough to bear the shock of field planting and enhancing their productivity. Successful establishment of nitrogen fixation tree (NFTs) in plantation programme is possible through the production of high quality tree seedling at nursery levels. Observations have also been made on its usefulness as afforestation species for rehabilitation of degraded forest land under the inoculated conditions (Singh and Bhati, 2005).

Materials and Methods

The experiment was conducted in the glass house in Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during 2009-2010 with *Dalbergia sissoo* (Shisham). Two weeks old *D. sissoo* seedlings grown in nursery were collected from Central Forest Nursery, Jora (Raipur). Raipur is situated in plains of Chhattisgarh at 21°16′ N latitude and 81°36′ E longitude with an altitude of 289.60 meter above mean sea level (MSL). 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-September) and the rest 12 per cent during winter season (October-February). The place experiences a short mild winter, January being the coolest and dry hot summer, May being the hottest month. Soil surface temperature of this region crosses 60^{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.

Particulars of Experiment

Location	Glass house of Dept. of Microbiology, CoA, Raipur
Tree seedling	*Dalbergia sissoo*
Duration of study	2009-2010
No. of Treatments	6
No. of Replication	4
Design	Factorial CRD with one factor- age (at 4 different ages / DAT of seedlings, at which observations are recorded)
Total No. of seedling maintained	96 seedlings (4 Repli. X 6 Treat. X 4 ages = 96)
Plan of observations	30 DAT (Days after transplant), 60 DAT, 90 DAT, 120 DAT

Details of treatments

T1	Control
T2	Inoculated (*Rhizobium*)(@5 ml inoculated broth/ seedling)
T3	Un-inoculated + N_1 (2g. N/seedling)
T4	Un-inoculated + N_2 (4g. N/seedling)
T5	Inoculated + N_1 (2g N/seedling)
T6	Inoculated + N_2 (4g N/seedling)

Nutrient content in Plant components

The oven dried different plant components *viz.*, leaves, shoot, root samples were ground into powder through Wiley mill and used for N, P and K analysis.Total N in sample was determined by Kjeldahal method (Jackson,1958) by digesting 0.1g sample in 10ml conc. H_2SO_4 followed by distillation and titration. The samples were digested in diacid (HNO_3 and $HclO_4$ 9:4 ratio). Estimation of Phosphorus in the plant sample was done by Vanado- molybdate yellow colour method using spectrophotometer (Jackson, 1973). The yellow colour developed was read at 490 nm using a spectrophotometer. Estimation of Potassium in the plant sample was estimated by Flame Photometer (Hanway and Heidel, 1952). The nutrient concentrations of each component were multiplied with their respective biomass to obtain nutrient contents.

All observations recorded from this experimental study were tabulated in a systematic manner. These observations were statistically analysed using ANOVA for factorial completely randomised design. The significant differences 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).

Results and Discussion

Nutrient status in leguminous plants

Inoculation with *Dalbergia sissoo-Rhizobium* and Nitrogen fertilizer application influenced the nutrient concentration in different seedling components *viz.*, leaves, root and shoots. At 120 DAT, seedlings where application of *Rhizobium* alone and along with lower level of N fertilizer showed higher nutrient concentration than only inorganic N fertilization and control. The N and K concentration were higher in leaves followed by roots and shoots where as P concentration was higher in root followed by leaves and then stem (Table 1-4).

Nitrogen concentration in different plant components

Nitrogen concentration as well as N content in different components of *D. sissoo* seedlings at 60 DAT was given in Table 1 and at 120 DAT in Table 2. Nitrogen concentrations in leaves were affected by different treatments, highest N concentrations (1.88%) was found in T_5 followed by T_2 (1.85%) which was 1.14 and 1.12 times greater over control at 60 DAT. Among inorganic N (Nitrogen) fertilization, T_3 (lower dose of N) showed higher N concentration in leaves, shoot and root of *sissoo* as compared to T_4.

Table 1. Effect of *Rhizobium* inoculation and N fertilizer application on N concentration % and N accumulation (mg/seedling) in different components of *D. sissoo* seedling at 60 DAT

Treatment	Concentration N %			Accumulation of N(mg)/ seedling			N uptake by shoot (mg)
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf+ Stem
Control	1.65	0.58	0.82	10.56	7.052	18.09	17.612
Inoculated (*Rhizobium*)	1.85	0.70	0.95	59.94	42.66	46.17	102.6
Un-inoculated + N_1	1.76	0.62	0.87	21.86	26.67	25.88	48.53
Un-inoculated + N_2	1.74	0.60	0.84	14.96	12.036	21.72	26.996
Inoculated + N_1	1.88	0.70	0.95	53.39	38.71	41.705	92.10
Inoculated + N_2	1.80	0.66	0.89	36.75	33.86	27.30	70.61
SEm (±)				0.05	0.07	0.05	0.10
CD (5%)				0.16	0.19	0.16	0.31

Table 2. Effect of *Rhizobium* inoculation and N fertilizer application on N concentration % and N accumulation (mg/seedling) in different components of *D. sissoo* seedling at 120 DAT

Treatment	Concentration N %			Accumulation of N(mg)/ seedling			N uptake by shoot (mg)
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf+ Stem
Control	1.58	0.52	0.74	34.76	35.93	51.87	70.69
Inoculated (*Rhizobium*)	1.77	0.64	0.82	124. 96	80.51	141.36	205.47
Un-inoculated + N_1	1.70	0.54	0.79	85.17	54.04	93.3	139.21
Un-inoculated + N_2	1.67	0.52	0.76	60.12	43.83	70.3	103.95
Inoculated + N_1	1.81	0.65	0.82	130.50	85.47	138.9	215.97
Inoculated + N_2	1.75	0.62	0.80	98.87	70.37	108.32	169.24
SEm (±)				0.04	0.17	0.17	0.18
CD (5%)				0.13	0.51	0.49	0.52

Also at T_5 maximum N concentration in leaves, shoot and root of *sissoo* being 1.88, 0.70 and 0.95%, respectively while minimum was found at control.

In leaf, the N concentration ranged from 1.65% to 1.88%, in stem it ranged from 0.58% to 0.70% while in root it ranged from 0.82% to 0.95 %, respectively under different treatments at 60 DAT. Comparatively less concentration of N in leaf, stem and root of *D. sissoo* seedlings at 120 DAT was found over N concentration at 60 DAT. In leaf, the N concentration ranged from 1.58% to 1.81%, in stem it ranged from 0.52% to 0.65% while in root it ranged from 0.74% to 0.82%, respectively under different treatments at 120 DAT. *Rhizobium* inoculation showed higher N concentration over uninoculated ones. Further, due to application of lower dose of N along with *Rhizobium* the concentration increased in leaf and almost remained equal as T_2 in stem and root but reverse trend recorded at higher level of N when higher N was applied along with *Rhizobium* N concentration decreased than T_2. Application of inorganic N fertilizer only also influenced in concentration of N over control.

Nitrogen content in different plant components

At 60 DAT in inoculated seedlings maximum N content was found in leaves followed by roots, while uninoculated seedling nitrogen content was highest in root followed by leaves. *Rhizobium* inoculation singly showed significantly highest N content in leaf, stem and root *i.e.*, 59.94 mg/seedling, 42.66 mg/seedling and 46.17 mg/seedling, respectively followed by T_5 containing 53.4,38.71 and 41.7mg/seedling while at T_6 it was significantly reduced. Among two levels of N, lower level of N influence significantly over higher N level. Inoculated seedlings showed significantly higher N content over only N fertilization and control. N uptake by shoot of *D. sissoo* was 102 mg/seedling while at control it was 18 mg/seedling at 60 DAT. N content varied from 10.56 to 59.94 mg/seedling in leaf, 7.05-42.66 mg/seedling in stem and 18.1 to 46.2 mg/seedling as affected by different treatments at 60 DAT. *Rhizobium* inoculated seedling showed maximum N content ranging 98.87 to 130.50 mg/seedling, while uninoculated ones contain leaf N ranging 34.76 to 85.2 mg/seedling in leaf at 120 DAT. Among two levels of N, lower level of N influence significantly over higher N level. In *Rhizobium*+N_1, *Dalbergia sissoo* showed significantly maximum nitrogen content *i.e.*, 216 mg/seedling in shoot followed by 205 mg/seedling at T_2 while minimum (70.69 mg/seedling) at control. N content varied from 34.76 to 130.5 mg/seedling in leaf, 35.93 to 85.47 mg/seedling in stem and 51.87 to 141.36 mg/seedling in root as affected by different treatments at 120 DAT.

Phosphorus concentration in different seedling components

Concentration of P as well as accumulation of P in different components of *D.sissoo* at 120 DAT was given in Table 3. Phosphorus concentration is leaves of *Dalbergia sissoo* was affected due to different treatments, being highest in inoculated seedlings, whereas minimum in uninoculated seedlings and lowest in untreated seedlings *i.e.*, control. Among inoculated, when *Rhizobium* inoculation when given along with lower N level showed maximum P concentration in leaves (0.08%) while at *Rhizobium* only it was 0.07%. However, phosphorus concentration in leaves ranged from 0.05 to 0.08% with various treatments.

In shoot phosphorus concentration ranged from 0.04 to 0.06% being highest under T_5 and T_2. *Rhizobium* inoculated seedlings showed 0.06% of P concentration at T_2 which was 1.5 times greater than control. While among N fertilization, T_3 showed 0.05% P in shoot which was 1.2 times more over T_4 and T_1. In root, P concentration ranged from 0.05 to 0.09%. Maximum (0.09%) P concentration was found in root of *Dalbergia sissoo* treated with T_5 at 120 DAT which was 1.8 times more over T_1.

Phosphorus content in different plant components at 120 DAT

Among different seedling components roots showed higher P content than stem and leaves. In roots the phosphorus content ranged from 3.5 to 15.25 mg/seedling whereas in shoot it ranged from 3.86 to 13.62mg/seedling under different treatments. Leaves of *Dalbergia sissoo* (at 120 DAT) contained 5.00 and 5.77 mg P/seedling when inoculated with *Dalbergia sissoo-Rhizobium* isolate alone and along with lower level N (N_1), respectively. In shoot *Rhizobium* inoculation seedling showed P content of 13.62, 12.78 and 7.93 mg/seedling under T_5, T_2 and T_6 treatments. These were significantly higher over uninoculated treatments. In uninoculated treatment P content in shoot ranged from 3.86 to 8.0 mg/seedling being highest under T_3 followed by T_4 and T_1.

Potassium concentration in different components

of *D. sissoo* seedling at 120 DAT

Concentration of K as well as accumulation of K in different components of *D. sissoo* at 120 DAT was given in Table 4. Application of *Rhizobium* alone and along with N fertilizer application significantly affected potassium concentration in leaves, stem and root of *Dalbergia sissoo*. Maximum concentration was found in *Rhizobium* inoculation along with lower dose of N followed by *Rhizobium* inoculation only. The concentration of potassium in leaves ranged from 0.78 to 0.95%. In stem potassium concentration ranged from 0.55 to 0.69% and in root it ranged from 0.55 to 0.71% under different treatments. *Rhizobium* inoculation along with lower N level exhibited maximum (0.95%) K concentration in leaf, whereas it was minimum (0.55%) in stem and root at control. Among N levels lower one influenced significantly over the higher level.

Potassium content in different seedling components

Potassium content was higher in roots followed by stem and leaf of *sissoo* seedlings at 120 DAT. In shoot potassium content ranged from 55.16 to 159.23 mg/seedling and in root it ranged from 38.56 to 120.34 mg/seedling under various treatments. *Rhizobium* inoculated seedlings along with lower N levels (T_5) showed significantly maximum K content followed by *Rhizobium* inoculated only (T_2). Among uninoculated treatments, potassium content in leaves of *Dalbergia sissoo* was found as 41.58 mg/seedling at T_3, 28.8 mg/seedling at T_4 and 17.16 mg/seedling at T_1. Inoculated seedling showed significantly higher potassium content in leaf over uninoculated ones being 66.36, 68.5 and 45.2 mg/seedling at T_2, T_5 and T_6, respectively. Significantly maximum potassium content in stem of *Dalbergia sissoo* was seen under *Rhizobium*+N_1 treatment (90.73 mg/seedling) followed by *Rhizobium* only (85.54

Table 3. Effect of *Rhizobium* inoculation and N fertilizer application on P concentration % and P accumulation (mg/seedling) in different components of *D. sissoo* seedling at 120 DAT

Treatment	Concentration P %			Accumulation of P(mg)/ seedling			Above Ground P content (mg/ seedling)
	Leaf	Stem	Root	Leaf	Stem	Root	
Control	0.05	0.04	0.05	1.1	2.76	3.5	3.86
Inoculated (*Rhizobium*)	0.07	0.06	0.08	4.94	7.84	13.79	12.78
Un-inoculated + N_1	0.06	0.05	0.07	3.00	5.0	8.26	8.00
Un-inoculated + N_2	0.06	0.04	0.06	2.16	3.37	5.55	5.53
Inoculated + N_1	0.08	0.06	0.09	5.77	7.85	15.25	13.62
Inoculated + N_2	0.06	0.04	0.06	3.39	4.54	8.12	7.93
SEm (±)				0.12	0.05	0.07	0.13
CD (5%)				0.36	0.16	0.19	0.39

Table 4. Effect of *Rhizobium* inoculation and N fertilizer application on K concentration % and K accumulation (mg/seedling) in different components of *D. sissoo* seedling at 120 DAT

Treatment	Concentration K %			Accumulation of K (mg)/ seedling			Above Ground K content (mg/ seedling)
	Leaf	Stem	Root	Leaf	Stem	Root	
Control	0.78	0.55	0.55	17.16	38.00	38.56	55.16
Inoculated (*Rhizobium*)	0.94	0.68	0.68	66.36	85.54	117.23	151.90
Un-inoculated + N_1	0.83	0.6	0.64	41.58	60.06	75.58	101.64
Un-inoculated + N_2	0.80	0.6	0.62	28.80	50.58	57.35	79.38
Inoculated + N_1	0.95	0.69	0.71	68.50	90.73	120.34	159.23
Inoculated + N_2	0.80	0.62	0.66	45.2	70.37	89.36	115.57
SEm (±)				0.03	0.06	0.05	0.06
CD (5%)				0.09	0.17	0.14	0.18

mg/seedling). Moreover, these were 1.28 and 1.2 times greater over T_6. Inorganic N application also showed higher K content in stem of 60.06 mg/seedling at T_3 followed by 50.58 mg/seedling at T_4 while minimum was at control (38.0 mg/seedling). Potassium content was significantly highest (120.34) in the roots of *Rhizobium* inoculated along with less N application (T_5) seedlings followed by T_2 (117.23 mg/seedling) which were 1.34 and 1.31 times greater than T_6. Minimum was found at T_1 (38.56 mg/seedling).

Similar trend in nutrient concentration was reported by Kaushik *et al.* (2003). The application of *Rhizobium* also increased the uptake of nutrients, which enhanced the growth and development of seedling. The *Rhizobium* alone and along with N_1, showed higher N, P and K concentration than uninoculated ones. P uptake by seedlings is more in inoculated ones over uninoculated. The adequate supply of moisture, mineral, nutrients, ensure the better growth and development of seedlings. It is well established fact that *Rhizobium* and N fertilization enhanced nodulation, dry weight and nutrients (N, P and K) contents in legume sp. The effects of the inoculation of *Rhizobium* singly or in combination with others gave better outcomes over the uninoculated control (Sengupta *et al.*, 2002; Marimuthu *et al.*, 2002; Jat *et al.*, 2003; Mathew and Hameed, 2003; Kashyap *et al.*, 2004; Purbey and Sen, 2005). Singh and Tilak (2001) stated that the synergistic effect of combined phosphorus fertilizers and inert sources of natural P along with bioinoculants. This is in line with the findings of Marques *et al.* (2001), 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. Prasad *et al.* (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 and increased P status in inoculated along with N_1 plants suggests more efficient absorption of P from the soil. The higher status of K in *Rhizobium*+N_1 treated seedling might be due to synergistic effect of N and P which mediated the efficient uptake of K in seedlings. Such synergistic effect of increased uptake of one nutrient facilitated by other nutrients in leguminous species was also reported by Prasad *et al.* (1998). Mahmood and Javaid (2005) studied on leguminous plants *Albizia lebbeck, Dalbergia sissoo, Leuceana leucocephala, Pithecellobium dulce, Prosopis glandulosa, P. juliflora* and *Vigna unguiculata* and reported significantly increased the dry weight and nitrogen content of the host plants. Similar findings were also made by Mahmood and Athar (2008) in the study of seven tree legumes for their effectiveness in *Vigna mungo* plants. They reported nitrogen contents of *Vigna mungo* plants increased significantly ($P<0.05$) in response to cross inoculation as compared to uninoculated control. Rhizobia from *Leuceana leucocephala* and *Prosopis glandulosa* showed significant increase in dry weight ($P<0.05$) and nitrogen contents ($P<0.05$) than other inoculated treatments. The better uptake of nutrient might be facilitated through microbial inoculants. Further, application of less N dose (N_1) along with *Rhizobium* inoculation showed a +ve influence in nutrient uptake by *sissoo* seedlings. The result revealed that the nutrient N, P and K contents were higher in *Rhizobium* + N_1 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_1 treatment not only increased the nutrient status but also increase the biomass. Due to higher biomass and more nutrient concentration, the nutrient content in different components of seedling was higher in inoculated treatments.

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