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IMPACT OF *RHIZOBIUM* ON GROWTH, BIOMASS ACCUMULATION AND NODULATION IN *DALBERGIA SISSOO* SEEDLINGS

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ABSTRACT

The present experiment was conducted with six treatments comprised of *Dalbergia sissoo-Rhizobium* inoculation, 2 levels of N fertilization alone and along with each other including control. Results revealed that root inoculation of *D. sissoo* seedlings with *D. sissoo-Rhizobium* inoculum was found significantly effective in improving growth, biomass production and nodulation over only inorganic N application and control. A positive response was found when N in less level was given along with *Rhizobium* inoculation**.** Significantly maximum root length (48.2cm), root biomass (17.24 g/seedling) and root/shoot ratio (0.88) were obtained in sissoo seedlings receiving only inoculation at 120 days after transplant (DAT) while combined effect of inoculation along with lower level of N showed maximum seedling height (64.8cm), shoot biomass (20.4 g/seedling) and collar diameter (8.27mm). Significantly maximum nodulation (56/seedling and dry weight 122 mg/seedling) was found in *Rhizobium* + N_1 treatment followed by *Rhizobium* inoculation only. Root inoculation of *D. sissoo* seedlings with *Rhizobium* showed significant effect and further application of N at lower level along with *Rhizobium* inoculation was found most effective in increasing symbiotic traits and growth performances by *D. sissoo* seedlings.

INTRODUCTION

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*., 1997). However these fertilizers are costly and cumbersome to use over vast plantation areas besides causing soil pollution if not judiciously used (Troeh *et al.,* 1980; Prasad, 1988). This age of increasing prices along with the increasing demand of chemical fertilizers and deleting soil fertility necessitates the integrated nutrient management involving biofertilizers. 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).

The process of inoculating microbes to the soil in a forest nursery could be an effective method to achieve higher growth and establishment of tree species on afforestation sites. Since application of fertilizer in forestry operation is minimal or nonexistent, development of a proper poly-mix (combination of microbial isolates and organic fertilizers) for use in nursery could be a cost effective process. Microbial application at the nursery stage has been reported to enhance the productivity of forest trees like *Albizzia*, *Acacia* and *Dalbergia* (Rahangdale and Gupta, 1998; Sahgal *et al*., 2004; Thatoi *et al*., 1993; Verma *et al*., 1996). Becker *et al.* (1991) reported that legumes do not fix sufficient N to obtain maximum growth response and so addition of soil-N is required for enhancing the biomass at nursery stages of growth. The starter dose of N given to legumes also influenced the amount of N_2 fixed (Johanson *et al*., 1975). It was also observed that high level of soil nitrates could be a potent inhibitor for nitrogen fixation. Streeter (1988) stated 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).

Inorganic N fertilizer can have an effect being it positive or negative on plant nodulation, N_2 fixation and seedling growth of leguminous plants (Huda *et al*., 2007). Phosphorous has been shown to increase plant growth and stimulate nodulation in legumes (Rasanen and Lindstrom, 2003). Potassium stimulates transport of molecules through the membrane, enzyme activity and cell growth (Ashley *et al*., 2006). Nitrogen is involved in cell division and biosynthesis of molecules in plant growth (Buchanan *et al*., 2000). High concentration of inorganic N in the soil normally inhibits symbiotic nitrogen fixation (Hungria and Vargas, 2000). Diouf *et al.* (2003) have done the optimization of inoculation of *L. leucocephala* and *Acacia mangium* with *Rhizobium* under greenhouse conditions. The physiological stage of the bacterial culture had no effect on nodulation and growth of the seedlings of *A. mangium* inoculated and cultivated *in vitro* for four months. For *L. leucocephala*, the number of nodules was significantly higher when the seedlings were inoculated with a bacterial culture in stationary phase. *Dalbergia sissoo* being an N-fixing tree can be utilized to its fullest extent only if it occurs successfully symbiotic relationship with *Rhizobium* strain. A study indicate that native *Rhizobium* in soil produce poor quality, low number of nodules and fixing insufficient atmosphere nitrogen (Pancholy, 1992).

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

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 1200-1400mm 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 60ºC, air temperature touches to 48ºC and humidity drops up to 3 to 4 per cent during summer season and mercury level drops to as low as 60ºC during December and January.

Species- isolate of *Rhizobium* was isolated from fresh nodule of *D. sissoo* seedling and culture broth was prepared using YEMA media. Isolation of *Rhizobium* from nodules was performed using the methods of Vincent (1970). To prepare the culture suspension, the isolate was grown in sterilized YEMA broth for 7 days on a rotary shaker. *D. sissoo -Rhizobium* isolate was inoculated to 50mL nutrient broth and incubated at 28+/-2ºC for 48h. This broth culture was then used for seedling inoculation treatment.

Uniform size seedlings were shifted in polythene bags (of 5Kg capacity 9x12" sized previously filled with 4.5Kg mixture of soil-sand-FYM in 2:1:1 proportion). After transplantation, nitrogen was given through urea at the time of transplantation of seedlings as per treatment and *Rhizobium* through seedling root inoculation. A total number of 96 polybags were maintained for this experiment consisting of 6 treatments, 4 replications and 4 plants per each treatment and replication for taking 4 times of observations at different seedling ages. For the estimation of biomass accumulation the plant components viz., leaves, shoot and root were collected from seedling at monthly interval and oven dried at 70ºC for 3-4 days up to the attainment of constant weight. Then final dry weight of root, shoot and leaves were recorded. The seedling quality parameters viz., sturdiness (the ratio of height to diameter), root/shoot ratio (on biomass basis) were computed. To quantify the morphological quality of seedlings, Dickson quality- Index (Q.I) was calculated as per Dickson *et al*. (1960) and also following Chauhan and Sharma (1997).

For the nodulation study uprooted plants were washed carefully so that nodules were not disturbed. Fresh and dry weights were taken and number of nodules per seedling was recorded. All observations recorded from this experimental study were tabulated in a systemic 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$\pm$ and CD were calculated where F-test was significant for comparing treatment means (Panse and Shukhatme, 1978).

RESULTS AND DISCUSSION

Shoot length

The shoot length or seedling height of *Dalbergia sissoo* presented in the Table 1 which showed significant variation with age of seedlings and different treatments. It is evident that there are prominent variation in the seedlings height of control and those of treated seedlings. As per the passage of time, the shoot length increased in all treatments. But the increasing rate with time varied with different treatments. Highest seedling height (64.80cm) was found in T_5, when *Rhizobium* inoculation was given along with lower nitrogen level, followed by *Rhizobium* inoculation only. Among inorganic N application T_3 *i.e.,* application of less dose of N influence the shoot length much over T_4 higher dose of N fertilization and control. However, both the treatments T_2 and T_5 showed consistently highest shoot length in *Dalbergia sissoo* seedlings followed by T_6 and T_3. Higher N (Nitrogen) level does not influence much with respect to shoot length when applied alone and even when applied along with *Rhizobium* inoculation as compared to T_5 and T_2. Interaction of treatment and age of seedlings showed significant variation in shoot length (Table 1). Totey *et al*. (2000) observed application of 5 ml *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 120 DAT reported by Okunomo *et al*. (2007). Similar estimated were also presented by Revathi *et al*. (2013) and Dash *et al*. (2013) in *Dalbergia sissoo* seedlings that inoculation of different fungal and bacterial strains resulted in enhancement of plant height as compared to uninoculated control. Bisht *et al*. (2009) have also found the significant impact of inoculant on growth of *sissoo* over the control.

Number of leaves

Number of leaves in *Dalbergia sissoo* seedlings was significantly influenced by different treatments and age (Table 1). *Rhizobium* inoculation alone (T_2) and *Rhizobium* inoculation along with N application at lower dose (N_1) were most effective in increasing the no. of leaves compared to other treatments at different age of seedlings. Among inorganic N fertilization, higher dose of N (N_2) did not influence much when applied alone or along with *Rhizobium*. However, least no. of leaves/seedlings was found at control. The no. of leaves per seedlings ranged between 56 to 128 in different treatments. *Rhizobium* inoculation to *Dalbergia sissoo* seedlings increased the leaf number and further increased when applied along with less level of N. Interaction effects also showed significant. Almost similar observations were recorded earlier by Chauhan and Pokhriyal (2002) in *A. lebbeck*.

Table 1: Effect of *Rhizobium* inoculation and N application on Morphological growth parameters of *D. sissoo* seedlings at different stages

Treat.	Morphological growth parameters Seedling Height (cm.) Days after transplant					No. of leaves/seedling Days after transplant					Root length (cm.) Days after transplant					No of branches /seedling Days after transplant				
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
T1	18.50	26.70	28.20	30.40	25.95	20.00	32.00	46.00	56.00	38.50	15.30	23.40	25.80	27.20	22.925	0.00	2.00	3.00	3.00	2.00
T2	38.45	49.67	54.80	62.40	51.33	46.00	84.00	92.00	114.00	84.00	28.30	38.60	42.50	48.20	39.40	3.00	5.00	6.00	7.00	5.25
T3	28.74	38.80	47.50	55.80	42.71	32.00	47.00	68.00	96.00	60.75	21.60	32.40	34.10	36.20	31.075	2.00	2.00	4.00	4.00	3.00
T4	23.20	30.00	38.20	43.40	33.70	28.00	38.00	64.00	77.00	51.75	18.20	28.50	31.20	33.00	27.725	1.00	2.00	3.00	3.00	2.25
T5	36.30	47.84	55.60	64.80	51.135	44.00	72.00	85.00	128.00	82.25	29.20	40.10	42.20	46.00	39.375	3.00	3.00	5.00	7.00	4.50
T6	35.20	43.30	51.60	52.70	45.70	36.00	60.00	77.00	85.00	64.50	23.10	34.50	38.20	39.20	33.75	2.00	3.00	5.00	5.00	3.75
Factor		SEm(±)		CD(5%)		SEm(±)		CD(5%)		SEm(±)		CD(5%)		SEm(±)		CD(5%)				
Treatment (T)		0.39		1.10		0.85		2.40		0.08		0.23		0.20		0.56				
Days (D)		0.32		0.90		0.69		1.96		0.07		0.19		0.16		0.46				
Interaction (TxD)		0.78		2.20		1.70		4.79		0.16		0.45		0.40		1.13				

T_1-Control T_2-Inoculated (*Rhizobium*) T_3-Un-inoculated + N_1 T_4-Un-inoculated + N_2 T_5-Inoculated + N_1 T_6-Inoculated + N_2

Root length

It is found that the treatments and age of the seedlings both had significant impact on root length of sissoo (Table 1). With the increase in age the root length of seedlings increased but the increasing rate was more in 30 and 60 DAT after that at 90 and 120 DAT root length increased at slower rate. T_2 and T_5 consistently gave maximum root length compared to other treatments while lowest was found in control. Among different treatments T_5 and T_2 were best for promoting the root growth in seedlings whereas T_1 was least effective in influencing root length. Interaction of treatment and age of seedling was found significant for root length. 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 over control. 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 and Pokhriyal (2002) had also reported that *Rhizobium* inoculation enhanced the growth in *Albizzia* seedlings as compared to control.

Number of small branches

In inoculated seedlings, significantly bear more no. of small branches/seedling as compared to uninoculated ones. Among inoculated treatments *Rhizobium* inoculation only affected much which was at par with *Rhizobium* + N_1 seedlings showed maximum 7 no. of branches/seedling at T_5 and T_2 treatments at 5 month old stage while 5 no. of branches/seedling were seen at T_6 treatment (Table 1). Interaction effect was also significantly affected for no. of branches. Thus, it can be inferred from above that plant height, root length as well as growth is significantly influenced by application of *Rhizobium* biofertilizer. The effect of root inoculation of *Rhizobium* was highly significant over control. Similar results were obtained by Basu and Kabi (1987) in *Albizzia lebbeck* and *D. sissoo*. Kinhal (1985) also obtained better performance in bamboo plantation due to application of biofertilizer containing *Azotobacter*. At initial growth stage 60 DAT T_2 was significantly better over T_5. But at latter stage at 120 DAT T_5 showed better growth. 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. Similar observations were also found by Chauhan and Pokhriyal (2002) in *Albizzia*.

Collar diameter

Table 2: Collar Diameter of *Dalbergia sissoo* at 120DAT as affected by *Rhizobium* inoculation and N fertilizer application

Treatment	Collar Diameter (C.D.) in mm
Control	6.02
Inoculated (*Rhizobium*)	8.01
Un-inoculated + N_1	7.51
Un-inoculated + N_2	7.15
Inoculated + N_1	8.27
Inoculated + N_2	7.74
SEm (±)	0.03
CD (5%)	0.10

Table 3: Effect of *Rhizobium* inoculation and N fertilizer application on biomass accumulation in different components of *D. sissoo* seedlings at different stage

Treatment	Dry weight of leaves (g/seedling) Days after transplant					Dry weight of stem (g/seedling) Days after transplant					Dry weight of root (g/seedling) Days after transplant				
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
T1	0.219	0.640	1.442	2.200	1.12	0.423	1.216	3.207	6.910	2.94	0.219	2.206	4.350	7.010	3.45
T2	1.103	3.240	4.986	7.060	4.10	1.440	6.095	9.267	12.580	7.34	0.833	4.860	8.488	17.240	7.85
T3	0.652	1.242	3.258	5.010	2.54	0.865	4.302	6.358	10.010	5.38	0.479	2.975	6.473	11.810	5.43
T4	0.532	0.860	1.701	3.600	1.67	0.755	2.006	5.650	8.430	4.21	0.444	2.586	6.087	9.250	4.59
T5	0.966	2.840	4.616	7.210	3.91	1.680	5.530	8.473	13.150	7.20	0.663	4.390	8.030	16.950	7.51
T6	0.680	2.042	3.433	5.650	2.95	0.925	5.130	6.185	11.350	5.89	0.525	3.068	7.380	13.540	6.13
Factor	SEm (±)			CD (5%)		SEm (±)			CD (5%)		SEm (±)			CD (5%)	
Treatment (T)	0.04			0.11		0.12			0.35		0.02			0.06	
Days (D)	0.03			0.09		0.10			0.29		0.02			0.06	
Interaction (TxD)	0.08			0.22		0.25			0.70		0.05			0.14	

T_1-Control, T_2-Inoculated (*Rhizobium*), T_3-Un-inoculated + N_1, T_4- Un-inoculated + N_2, T_5- Inoculated + N_1, T_6- Inoculated + N_2

Table 4: Effect of *Rhizobium* inoculation and N fertilizer application on Total Biomass production by *D. sissoo* seedlings at different stages

Treatment	Above ground biomass (g/seedling) Days after transplant					Below ground biomass (g/seedling) Days after transplant					Total biomass (g/seedling) Days after transplant				
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
T1	0.642	1.865	4.649	9.120	4.07	0.219	2.206	4.355	7.02	3.45	0.861	4.071	9.004	16.140	7.52
T2	2.543	9.335	14.253	19.640	11.44	0.833	4.92	8.602	17.324	7.92	3.376	14.255	22.855	36.96	19.36
T3	1.517	5.544	9.616	15.020	7.92	0.479	2.975	6.518	11.840	5.45	1.996	8.519	16.134	26.860	13.37
T4	1.29	2.866	7.351	12.030	5.88	0.444	2.586	6.107	9.2680	4.60	1.734	5.452	13.458	21.290	10.48
T5	2.646	8.37	13.089	20.360	11.12	0.663	4.438	8.152	17.040	7.57	3.309	12.808	21.241	37.40	18.69
T6	1.605	7.172	9.618	17.00	8.85	0.525	3.11	7.481	13.60	6.18	2.13	10.282	17.099	30.60	15.03
Factor			SEm (±)		CD (5%)	SEm (±)			CD (5%)		SEm (±)			CD (5%)	
Treatment (T)			0.14		0.38	0.02			0.06		0.13			0.38	
Days (D)			0.11		0.31	0.02			0.06		0.11			0.31	
Interaction (TxD)			0.27		0.76	0.05			0.14		0.27			0.76	

T_1- Control T_2- Inoculated (*Rhizobium*) T_3- Un-inoculated + N_1 T_4- Un-inoculated + N_2 T_5- Inoculated + N_1 T_6- Inoculated + N_2

Table 5: Quality parameters of *D. sissoo* seedling at 120 DAT as affected by *Rhizobium* inoculation and N fertilizer application

Treatment	Seedling height (cm)	Collar diameter (mm)	Sturdiness	Shoot biomass g/seedling (A.G.)	Root biomass g/seedling (B.G.)	Root: Shoot ratio	Total dw g/seedling	Quality Index (Q.I.)
T_1	30.4	6.02	50.5	9.12	7.02	0.76	16.14	0.314
T_2	62.4	8.01	77.9	19.64	17.32	0.88	36.96	0.469
T_3	55.9	7.51	74.4	15.02	11.84	0.79	26.86	0.357
T_4	43.4	7.15	60.7	12.03	9.27	0.77	21.30	0.346
T_5	64.8	8.27	77.9	20.36	17.04	0.84	37.40	0.475
T_6	52.7	7.74	68.1	17.00	13.60	0.80	30.60	0.444

Collar diameter was measured at 120 DAT in *Dalbergia sissoo* seedlings as affected by different treatments (Table 2). Collar diameter observed was significantly (Pd″0.05) influenced by *Rhizobium* inoculation. Among different treatments *Rhizobium* inoculation + N_1 alone was found most significantly effective and enhanced maximum collar diameter whereas it was lowest in untreated seedling. Collar diameter of *Dalbergia sissoo* seedlings varied between 6.02 to 8.27mm under different treatments. Bora *et al*. (2006) also reported the inoculation of *Rhizobium* in seedlings of *A. procera* significantly influenced the root length, shoot length, collar diameter and nodulation. Application of inorganic N fertilizer alone also significantly influenced collar diameter as compared to control. Revathi *et al*. (2013) stated that inoculation with rhizobium was impressive in improving the growth attributes like shoot length, root length, collar diameter and shoot and root biomass in Shisham.

Biomass accumulation in different components of *Dalbergia sissoo*

The findings on stem biomass, root biomass and leaf biomass as well as total biomass per seedling were given in Table 3 and 4. The leaf biomass of *Dalbergia sissoo* seedlings at different age varied significantly among different treatments. Giving *Rhizobium* inoculation showed significantly maximum leaf biomass over uninoculated seedlings (Table 3). At initial stage *Rhizobium* inoculation only gave maximum leaf biomass of 1.103 g/seedling which was 5 times higher over control, while at 5 month old at 120 DAT maximum leaf biomass 7.21 g/seedling found in T_5 which was 3.27 higher over control. Leaf biomass ranged from 0.219 to 7.21 g/seedling under different treatments and age of seedling. Interaction of treatment and age of seedlings was also significant for leaf biomass.

In case of stem biomass (Table 3), seedlings inoculated with *Rhizobium* showed significantly higher stem biomass accumulation over control and uninoculated ones. Among inoculated treatments at 60 and 90 DAT by application of only *Rhizobium* inoculation (T_2), the maximum stem biomass was seen while at latter stage at 120 DAT application of lower level of N with *Rhizobium* inoculation gave significantly maximum stem biomass of 13.15 g/seedling. The stem biomass significantly increased from 1.68 g/seedling to 13.15 g/seedling throughout the study period. Interaction of treatment and age was found significant for stem biomass.

Root biomass of *sissoo* varied significant by *Rhizobium* inoculation and N fertilization either singly or in combination. *Rhizobium* inoculation either alone or in combination with N significantly increased the root biomass over uninoculated. Application of inorganic N fertilization influenced root biomass as compared to control. Root biomass ranged from 7.01-17.24g under different treatments. Significant interaction effect of treatment and age of seedlings were found for root biomass.

Data on above ground and below ground as well as total biomass are presented in Table 4. As per described above, the above ground, below ground as well as total biomasses of seedling were significantly influenced by *Rhizobium* inoculation alone and along with N_1. Maximum total biomass was obtained 37.4g/seedling at 120 DAT under treatment T_5 followed by 36.9 g/seedling at T_2 which were significantly higher over T_6. 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) and Galiana *et al*. (1994). As per Verma *et al*. (1996) application of *Rhizobium* broth 5mL/plant significantly increased plant height, collar diameter and root length of *sissoo*. Also increase in shoot fresh weight by 118.61%, shoot dry weight by 124.48% and root dry weight 122.04% due to application of 5mL *Rhizobium* broth biofertilizer and 100g SSP/plant as against control.

Result indicated that the survival and growth of legume species *i.e.*, *D. sissoo* were significantly higher in *Rhizobium* inoculation over uninoculated treatment. Similar to growth, biomass of *sissoo* seedling was significantly influenced by inoculation with *Rhizobium*. The biomass of seedling is mainly depends on diameter, height and root growth. The higher biomass under inoculation treatments as attributed to higher collar diameter and plant height. This is in line with the findings of Mohan (2000); Srivastava *et al*. (2001); Manoharan *et al*. (2010). Marques *et al*. (2001) find a higher biomass 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 *sissoo* for *Rhizobium* as compared to inorganic fertilization. Singh *et al*. (2000) demonstrated a better response of inoculation for increasing growth and development in *A. procera*. Kannan *et al*. (2013) reported the bioinoculants efficiency for growth and biomass of *Dalbergia* seedlings was significantly increased at 60, 120 and 180 DAT. In general, combined treatments are significantly better in performance compared to others including uninoculated control.

Seedling quality parameters

Seedling can better be compared on the basis of certain quality parameters (Table 5) which are calculated from various seedling traits. Seedling quality specifications have been based on certain morphological characters such as sturdiness (height/ diameter ratio) and root/shoot ratio (Lavender and Cleary, 1974,

Table 6: Effect of *Rhizobium* inoculation and N fertilizer application on nodulation behavior of *D. sissoo* seedlings at different stages.

Treatment	No. of nodules/seedling Days after transplant					Fresh weight of nodules (mg/seedling) Days after transplant					Dry weight of nodules (mg/seedling) Days after transplant				
	30	60	90	120	Mean	30	60	90	120	Mean	30	60	90	120	Mean
T_1	0.00	0.00	2.00	4.00	1.50	0.00	0.00	18.12	18.00	10.50	0.00	0.00	4.75	4.58	2.33
T_2	0.00	21.00	52.00	42.00	28.75	0.00	98.10	195.30	128.10	105.25	0.00	56.75	114.0	84.25	63.75
T_3	0.00	0.00	22.00	12.00	8.50	0.00	0.00	90.00	52.50	35.50	0.00	0.00	45.00	28.00	18.25
T_4	0.00	0.00	9.00	7.00	4.00	0.00	0.00	50.00	48.00	24.50	0.00	0.00	20.00	18.00	9.50
T_5	0.00	18.00	56.00	46.00	30.00	0.00	96.20	184.50	132.30	103.00	0.00	48.00	122.0	87.75	64.44
T_6	0.00	18.00	26.00	26.00	17.50	0.00	93.40	168.20	104.20	91.25	0.00	42.00	101.0	62.25	51.31
Factor	SEm (±)		CD (5%)			SEm (±)		CD (5%)			SEm (±)		CD (5%)		
Treatment(T)	0.53		1.50			0.79		2.24			0.81		2.28		
Days (D)	0.44		1.23			0.65		1.83			0.66		1.86		
Interaction (TxD)	1.07		3.01			1.59		4.48			1.62		4.55		

T_1-Control T_2-Inoculated (*Rhizobium*) T_3-Un-inoculated + N_1 T_4-Un-inoculated + N_2 T_5-Inoculated + N_1 T_6-Inoculated + N_2

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 the T_5 and T_2 treatments gave good values for sturdiness, root/shoot ratio and Dickson quality index. Higher root/shoot ratio helps in survival and growth after planting (Chauhan and Sharma, 1997). The Dickson quality index (QI) reflects the overall quality of the seedling. Higher the value of this index the better will be the seedling. Highest QI (0.475) was found in T_5 followed by 0.469 in T_2. Inoculation of *Rhizobium* would be helpful for production of quality seedling in nursery which the experimental result also confirms. Inoculation of biofertilizer increased the seedling quality and growth in *Dalbergia sissoo* at nursery (Revathi *et al*. 2013).

The nodular properties like nodule number, nodule fresh weight and nodule dry weight mg/seedling are presented in Table 6. The no. of nodules per seedling ranged from 2-56 under different treatments and at different ages of seedlings. It was lowest when seedlings were not inoculated with *Rhizobium* and highest nodules no. was obtained in seedlings when *Rhizobium* inoculation along with low level of N was given. At 60 DAT nodulation began in inoculated seedlings but delay nodulation has been observed in uninoculated seedlings. It was seen that nodulation decreased considerably in the control but could not be completely eliminated as in evident from sparse nodulation in the control plants. *Dalbergia sissoo* nodules were round ball like of pink coloured and small nodules. Similar observation was also found by Pokhriyal *et al*. (1987) who found same nodulation in *Dalbergia sissoo* and studied nodule behaviour in *Leuceana, Acacia, Albizia* and *Dalbergia sissoo* tree species.

The fresh weight of nodules varied from 18-184 mg/seedling (Table 6). Those were higher in inoculated seedling as compared to uninoculated ones. The size of nodules was considerably larger in *Rhizobium* and N treated plants as compared to untreated ones. Significant increase in the weight of nodules of plants receiving *Rhizobium* + N_1 treatment over control 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.

The dry weight of nodules ranged between 4-88 mg at 120 DAT under different treatments (Table 6). At 90 DAT highest dry weight of nodules observed which becomes slightly senescent at latter stage. Significantly maximum dry weight of nodules observed at 90 DAT. *Rhizobium* inoculated seedling showed significantly higher nodule biomass over uninoculated ones, lower level of N dose when applied with *Rhizobium* inoculation influenced the nodulation and ensure to increase BNF, but reverse happened in case of high level of N when applied with *Rhizobium* inoculation. This is an agreement with the report made earlier by Kabi *et al*. (1982) that inoculation of *L. leucocephala* with its specific *Rhizobium* could bring out an amazing effect so far on nodulation, growth and dry matter production of legumes concerned. This type of study was also carried out by Pokhriyal *et al*. (1987) in *Leuceana, Acacia, Albizzia* and *Dalbergia sissoo*. Summerfield *et al*. (1977) and Dazzo and Brill (1978) also found that excessive N fertilizer application reduce root hair infection, nodulation no. and weight. Biofertilizer application of *Rhizobium* or application of *Rhizobium* with *Azotobacter* combined has enhanced nodulation and growth of seven forestry legume significantly and also found to augment dry matter production (Basu and Kabi, 1987). The magnitude of these data showed that inoculation with *Rhizobium* alone significantly influenced the nodular properties at different stages of the seedling and further increased with application of lower dose of N as starter dose. Similar trend were also reported by Kannan *et al*. (2013).

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