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Influence of Biofertilizers (A-*Mycorrhiza* and *Rhizobium)* and Inorganic Fertilizers (NPK) on Growth and Development of *Dalbergia sissoo*

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ABSTRACT

A factorial randomized design experiment was conducted to assess the interaction between various doses of NPK X *Rhizobium,* NPK X AM, AM X *Rhizobium* in *Dalbergia sissoo* at SFRI, Jabalpur. The maximum interaction (in terms of total biomass) was observed between *Rhizobium* $_3$ (R_3 -20 g culture/pl of 10^7 population per gm) with NPK $_3$ (250 kg N, 30 kg P20S and 150 kg K/ha). Similarly, the response of mycorrhiza was maximum with NPK $_1$ (500 kg N, 75 kg P20S and 350 kg K/ha) with AM $_3$ (V_3 -200 chlamydospores with 5 gm AM infected roots). There was maximum response between *Rhizobium* $_3$ and AM $_2$ in producing total biomass. The three way interaction produced excellent results in growth enhancement and biomass production in *D. sissoo.* The light doses of NPK, AM and *Rhizobium* (NPK $_3$ AM $_3$ and *Rhizobium* $_3$) rendered maximum growth and maximum biomass in comparison to other treatments.

Key words : A-Mycorrhiza, Biofertilizers, *Growth, Dalbergia sissoo* and *Rhizobium,*

Soil fertility is diminishing gradually due to soil erosion, loss of nutrients, accumulation of salts, water logging, unbalanced nutrient compensation, pollution and contamination of soil. Fertility problems are greatly solved by addition of mineral fertilizers. The high cost of fertilizers, the gap between supply and demand and their adverse effect on environment have led agricultural scientists to look for alternate strategies. Further, knowing the deleterious effect of using only the chemical fertilizers, use of soil microorganisms which can fix atmospheric nitrogen, solubilize phosphorus or stimulate plant growth through synthesis of growth promoting substances will be environmentally a benign approach for nutrient management of ecosystem (Babu *et al.,* 1998).

A promising trend for increasing the efficiency of biofertilizers is the use of different mixtures of biopreparations as nitrogen fixing bacteria and arbuscular mycorrhizal symbiosis was studied (Das *et al.,* 2001, Hauka *et al.,*1996, Sansamna *et al.,* 1998). This study was designed to explore the combined effect of rhizobium and AM in the presence of different levels of NPK on *Dalbergia sissoo* one of the important multipurpose tree species in agroforestry systems widely distributed in drier tracts of Indian peninsula.

MATERIAL AND METHODS

Pot culture

Seeds of *Dalbergia sissoo* Roxb. (Sheesham) were obtained from State Forest Research Institute, Jabalpur, Madhya Pradesh. Seeds of uniform size and colour were selected and soaked overnight in distilled water. Fine imbibed seeds were sown in polythene bags filledup with 5 kg of sterilized soil and sand mixture (1:1 V/V). After germination and seedling emergence, single healthy seedling was retained per bag and bags were arranged in pot culture and the design was completely factorial randomized design.

Isolation, production and inoculation of AM

AM fungi were isolated from rhizospheric soil, collected from the natural stands of *D.sissoo,* as per the procedure adopted by Daniel and Skipper (1982). *Glomus interadices, Glomus mosseae* and *Acaulospora scrobiculata,* predominant AM species isolated and were multiplied as per Sieverding (1991) method. Each AM fungi was grown separately and equal number of spores of each fungus were mixed together and the mixed inoculum was used for inoculating tree seedlings which contained about 100 spores per 5g of substrate.

Treatments

Different doses of nitrogen, phosphorus, potash and AM were given to the 30 days old seedlings. Nitrogen was applied in the form of urea, phosphorus as single superphosphate and potash as muriate of potash and different levels i.e. 300 kg N 75 kg P_2O_5 and 250 kg k/ha. [NPK_1], 200 kg N. 5O kg P_2O_5 and 150 kg k/ha [NPK_2] and 120 kg N. 30 Kg P_2O_5 and 75 kg k/ha [NPK_3] The dose of NPK was calculated by including present status of available nutrients of the soil.

Different doses of AM spores i.e. 0, (AM_0) 200 (AM_1) 300 (AM_2), and 500 spores $plant^{-1}$ (AM_3) were added to different pots. Along with Rhizobium Ro Control R_1 *Rhizobium* 60 gm/Pl, R_2 *Rhizobium* 40gm/pl and R_3 *Rhizobium* 20 gm/pl were maintained. Rhizobium strain isolated from *Dalbergia sissoo* procured from Regional Biofertilizer Development Centre, Jabalpur, Madhya Pradesh.

Plants were raised in nursery and watered to maintain moisture content of soil near field capacity. The inoculum was added to different pots at 0.5 cm. depth in and around root zone followed by appropriate watering

The shoot length was recorded from the ground level to the base of the last full opened leaf. Stem diameter (collar diameter) was measured by Vernier callipers at the lowest internode. One year old plants were harvested in October and roots of the seedlings cleared by washing the soil with water and number of nodules per seedling were recorded. Fresh weights of shoots and roots were taken and the dry weight was obtained by drying the material in an oven at 70°C for 72 hr.

RESULTS AND DISCUSSION

Effect of chemical fertilizers and *Rhizobium*

The chemically fertilized seedling of *D.sissoo* responded well in terms of shoot length, root length, total biomass along with nodule number and gave significantly different results from uninoculated seedlings. While *Rhizobium* inoculated seedlings exhibited significant collar diameter shoot root biomass and thereby total biomass. Interaction of fertilizer and *Rhizobium* was significant for plant shoot height and biomass production. Although application of chemical fertilizer and *Rhizobium* did not affect the collar diameter and root length of seedlings but implicitly the response was beneficial in comparison to control seedlings. The data (Table1) showed great variation in plant growth parameters and various treatments. For instance maximum shoot height and collar diameter were obtained in seedlings by application of NPK_2 along with *Rhizobium* $_3$ while root length was the maximum (68.33 cm/pl.) in *Rhizobium*$_1$ inoculated seedlings in comparison to other treatments

The plants require enough quantity of nutrients for their luxurious growth and biomass production. It was interesting to note that fertilization by NPK_3 along with *Rhizobium*$_3$ rendered maximum biomass rather than higher dose of NPK and other biofertilizer treatments. The application of NPK and *Rhizobium* alone could not affect seedling shoot biomass but combined effect of these substances enhanced shoot biomass of *D. sissoo.* Root biomass was influenced by *Rhizobium* application. However NPK_3 and *Rhizobium*$_3$ again proved their suitability for maximum root biomass production of *D. sissoo*.

The total biomass was also affected by application of NPK and *Rhizobium.* The *Rhizobium* inoculated seedlings responded higher than chemically fertilized seedlings. However, interaction of chemical and biofertilizer (*Rhizobium*$_3$) exponentially increased total biomass in comparison to other treatments. The *Rhizobium*$_3$ (20g inoculum) might be appropriate amount of bacteria suitable for plant growth rather than other higher dose treatment which might have competed with themselves for their food which resulted in slow growth of plants or no effect.

Effect of chemical fertilizers and *AM*

Mycorrhiza is also one of the most profound symbionts associated in growth of *D.sissoo*. The inoculation of AM influenced shoot development , root elongation, shoot biomass and total biomass of seedlings significantly at P 0.05. Chemical fertilizer also played the same role. The most illustrative result was observed on seedlings inoculated by AM along with NPK fertilizer in combination (Table 2).This resulted in the maximum shoot length and diameter in combination of NPK_2 and AM_3 treatment. Exceptionally, root length was high in NPK_1 treated seedlings in comparison to other treatments. The low dose of NPK could not influence root elongation. Shoot biomass was the maximum in NPK_2 in combination with AM_3 while root biomass was maximum in high dose of NPK_1 with high dose of AM_3.The total biomass was also maximum in NPK_1 with AM_3. However, inoculation of AM and NPK alone also exhibited considerable high biomass in comparison to control but extent of response was maximum in seedlings treated with AM and NPK in combination.

Table1. Interaction of NPK and *Rhizobium* on growth and biomass of *Dalbergia sissoo*

	NPK_0	NPK_1	NPK_2	NKP_3	Mean	CD at P<0.05
Shoot length(cm)						
R0	64.67	68.00	62.00	72.33	68.92	NPK-7.10
R1	74.67	59.00	48.67	68.33	60.17	R-NS
R2	63.33	59.33	77.67	79.00	64.75	NPK x R-11.7
R3	52.67	96.67	98.33	89.33	71.25	
Mean	63.83	65.42	68.83	67.00		
Diameter(cm)						
R0	0.326	0.360	0.613	0.526	0.45	NPK-NS
R1	0.493	0.466	0.423	0.356	0.43	R-0.08
R2	0.436	0.393	0.500	0.450	0.44	NPK x R-0.16
R3	0.410	0.390	0.650	0.610	0.51	
Mean						
Root length(cm)						
R0	40.00	56.67	43.33	43.67	45.92	NPK-12.2
R1	68.33	54.00	49.33	66.00	59.42	R-NS
R2	46.33	43.67	40.00	34.33	41.08	NPK x R-NS
R3	44.33	52.33	46.33	47.00	47.50	
Mean	49.75	51.67	44.75	47.75		
No. of nodule $plant^{-1}$						
R0	09.00	20.67	34.00	25.67	22.33	NPK-12.47
R1	44.33	34.00	24.67	20.00	30.75	R-NS
R2	80.67	39.67	42.00	30.00	35.58	NPK x R-NS
R3	06.33	25.00	22.00	22.33	18.92	
Mean	22.58	29.83	30.67	24.50		
Dry Shoot biomass(g/pl)						
R0	1.88	2.60	3.79	2.96	2.81	NPK-NS
R1	2.24	2.78	1.74	1.17	1.98	R-7.57
R2	2.38	1.79	2.63	3.01	2.45	NPK x R-1.01
R3	1.72	2.47	5.01	5.88	3.77	
Mean	2.05	2.41	3.29	3.25		
Root biomass(g/pl)						
R0	1.88	2.55	3.23	1.94	2.34	NPK- NS
R1	2.64	2.67	1.63	1.69	2.16	R-0.75
R2	2.28	1.30	2.45	1.71	1.94	NPK x R-1.01
R3	1.68	3.57	3.64	4.75	3.41	
Mean	2.06	2.53	2.74	2.52		
Total biomass (g/pl)						
R0	3.51	5.16	7.03	4.90	5.15	NPK-1.43
R1	4.55	5.46	3.38	2.87	4.06	R-1.43
R2	4.67	3.10	5.08	4.73	4.39	NPK x R-2.86
R3	3.40	5.71	8.65	10.63	7.10	
Mean	4.03	4.86	6.03	5.78		

Table 2. Interaction of NPK and *AM fungi* on growth and biomass of *Dalbergia sissoo*

	NPK_0	NPK_1	NPK_2	NKP_3	Mean	CD at P<0.05
Shoot length(cm)						
V0	64.67	68.00	62.00	72.33	66.75	NPK-5.87
V1	74.67	59.00	48.67	68.33	62.67	AM-5.87
V2	63.33	59.33	77.67	79.00	69.33	NPKxAM-11.7
V3	52.67	96.67	98.33	89.33	84.25	
Mean	63.83	70.75	71.67	77.25		
Diameter(cm)						
V0	0.32	0.42	0.43	0.49	0.42	NPK-NS
V1	0.49	0.48	0.38	0.43	0.45	AM-NS
V2	0.43	0.42	0.58	0.42	0.48	NPKxAM-0.148
V3	0.39	0.59	0.59	0.34	0.48	
Mean	0.41	0.48	0.49	0.42		
Root length(cm)						
V0	40.00	77.00	52.67	36.67	51.58	NPK-9.30
V1	68.33	39.67	40.67	51.67	50.08	AM-9.30
V2	46.33	50.67	28.67	34.00	39.92	NPKxAM-18.5
V3	44.32	48.33	35.00	32.67	40.08	
Mean	49.75	53.39	39.25	38.75		
No. of nodule $plant^{-1}$						
V0	9.00	19.33	45.00	42.00	28.83	NPK-9.89
V1	42.67	25.00	46.67	31.00	36.33	AM-9.89
V2	30.67	7.66	22.33	24.33	21.25	NPKxAM-19.7
V3	6.66	19.33	16.00	15.67	14.42	
Mean	22.25	17.83	32.50	28.25		
Dry Shoot biomass(g/pl)						
V0	1.63	2.31	3.88	2.81	2.66	NPK-NS
V1	2.64	2.01	2.19	2.86	2.43	AM-NS
V2	2.28	2.04	1.54	2.95	2.20	NPKxAM-1.42
V3	1.68	4.41	3.37	2.49	2.99	
Mean	2.06	2.69	2.74	2.78		
Root biomass(g/pl)						
V0	1.88	2.08	3.03	3.46	2.61	NPK-0.54
V1	2.34	2.35	1.70	2.28	2.17	AM-0.54
V2	2.20	1.83	3.21	3.57	2.70	NPKxAM-1.09
V3	1.72	7.18	4.98	4.83	4.68	
Mean	2.03	3.36	3.23	3.54		
Total biomass (g/pl)						
V0	3.51	4.40	5.91	6.67	5.02	NPK-0.90
V1	4.98	4.36	3.90	5.15	4.60	AM-.90
V2	4.48	3.87	4.75	6.52	4.90	NPKxAM-1.79
V3	3.40	11.60	8.35	7.33	7.67	
Mean	4.09	6.06	5.73	6.32		

Table 3. Interaction of *AM* and *Rhizobium* on growth and biomass of *Dalbergia sissoo*

	AM_0	AM_1	AM_2	AM_3	Mean	CD at P<0.05
Shoot length(cm)						
R0	64.67	66.67	73.00	71.00	68.83	AM-4.73
R1	72.33	65.33	64.33	50.67	63.17	R-4.73
R2	62.00	63.33	57.67	64.00	61.75	AMxR-8.58
R3	72.33	52.67	82.67	62.00	67.42	
Mean	67.83	62.00	69.42	61.92		
Diameter(cm)						
R0	0.326	0.36	0.61	0.53	0.459	AM-NS
R1	0.490	0.56	0.47	0.42	0.469	R-NS
R2	0.430	0.50	0.40	0.64	0.495	AMxR-0.165
R3	0.490	0.41	0.48	0.45	0.458	
Mean	0.434	0.44	0.49	0.51		
Root length(cm)						
R0	40.00	56.67	43.33	47.00	46.75	AM-NS
R1	36.67	29.67	38.33	56.00	40.17	R-NS
R2	52.67	36.67	54.67	41.33	46.33	AMxR-16.42
R3	36.67	65.67	35.33	41.67	44.83	
Mean	41.50	47.17	42.92	46.50		
No. of nodule $plant^{-1}$						
R0	9.00	20.33	34.00	25.00	22.08	AM-NS
R1	42.00	68.67	36.00	12.33	39.75	R-NS
R2	45.00	8.00	13.33	65.67	33.00	AMxR-27.5
R3	42.00	16.00	10.33	43.33	27.92	
Mean	34.50	28.25	23.42	36.58		
Dry Shoot biomass(g/pl)						
R0	1.88	2.00	3.79	2.96	2.66	AM-0.554
R1	3.14	3.67	2.06	1.34	2.55	R-0.554
R2	3.03	2.60	2.10	3.70	2.86	AMxR-1.108
R3	3.46	1.67	5.57	3.04	3.43	
Mean	2.88	2.48	3.38	2.76		
Root biomass(g/pl)						
R0	1.63	1.89	3.24	1.93	2.17	AM-0.419
R1	2.73	2.95	2.14	1.54	2.34	R-0.419
R2	3.88	2.22	2.14	3.90	3.04	AMxR-0.84
R3	2.81	2.46	3.77	4.40	3.36	
Mean	2.76	2.38	2.94	2.82		
Total biomass (g/pl)						
R0	3.52	3.86	7.03	4.89	4.83	AM-0.96
R1	5.87	6.63	4.20	2.88	4.87	R-0.96
R2	6.92	4.82	4.24	8.28	6.06	AMxR-1.91
R3	6.27	4.13	9.35	7.44	6.80	
Mean	5.64	4.87	6.20	5.87		

Table 4. Interaction of NPK, *AM* and *Rhizobium* on growth and biomass of *Dalbergia sissoo*

Treatments	Shoot length (cm)	Diameter (cm)	Root length (cm)	No. of nodule plant^{-1}	Shoot weight (g/pl)	Root weight (g/pl)	Total biomass (g/pl)
NoVoRo	64.67	0.32	40.00	9.00	1.88	1.63	3.52
$N_1V_1R_1$	72.67	0.65	33.30	19.33	4.30	3.48	7.78
$N_2V_2R_2$	83.00	0.53	45.00	17.66	3.85	2.85	6.67
$N_3V_3R_3$	85.33	0.56	53.00	14.67	5.89	4.70	10.60
CD at P<0.05	8.19	0.20	10.50	NS	2.46	NS	4.99
SE	3.34	0.08	4.32	NS	1.00	NS	2.03

Effect of dual inoculation of symbiotic microorganism AM *and Rhizobium*

Rhizobium and AM both are very important symbiotic microorganisms which invade roots of sissoo simultaneously. Dual inoculation increased the biomass of forestry species (Paroha *et al.,* 2000). They perform dually rather than competing with each other. This was evident from this study (Table 3). Inoculation with AM and *Rhizobium* noticed significant increment in shoot height and biomass of *D. sissoo* when inoculated alone or with any combination. However, the effect of treatments on diameter, root length and nodulation of seedling did not exhibit apparent result. Interaction of these organism furnished best performance, however, it was varied from dose and parameter but net effect was positive in comparison to control seedlings of *D. sissoo.* And the r*hizobium* was found dominant over AM fungi. Therefore, the low dose of *rhizobium* was enough for better growth and biomass production of plant .The shoot length was maximum in seedlings inoculated by *Rhizobium* $_3$ along with AM_2 while root length was maximum in *Rhizobium* along with AM_1. The nodulation, an indicative of *rhizobium* population in rhizosphere was boosted by high dose of $Rhizobium_1$ starter inoculums rather than low dose of AM and *rhizobium*, The shoot biomass was maximum in seedlings treated with *rhizobium*$_3$ and AM_2 while root biomass was maximum in *rhizobium*$_3$ and AM_3 treatment . But eventually the treatment of *Rhizobium*$_3$ and AM_2 found best in terms of total biomass production of *D. sissoo* seedlings. The other treatment such as Rhizobium $_2$ along with low population of AM also enhanced the total biomass in a notable extent.

Effect of mixed inoculation (AM, *Rhizobium* and chemical fertilzier)

The triple inoculation of AM, *Rhizobium* and chemical fertilizer (NPK) was also made in varying quantities .Table 3 indicates the response of these treatments. It is found that shoot height of D. *sissoo* seedling responded maximum through N_3, V_3 and R_3 while corresponding diameter in these seedling was not achieved, Similarly root length of seedling was maximum in N_3 V_3 and R_3 while nodulation status in root was encouraged by N_1V_1 and R_1 treatment. However, other treatments also increased root nodulation in comparison to uninoculated control. The seedling rendered maximum shoot root and total biomass merely in the presence of low amount of nutrient, AM and *Rhizobium* (*ie.,* N_3 V_3 R_3). Significantly higher response was noticed by Reddy and Ahlawat (2001) when *Rhizobium leguminosarum* and VAM was used together in lentil plants. A significant increase in yield, nutrient uptake and nitrogen fixation have been observed in coffee (Swarupa, 1996), coconut (Sansamma *et al.,* 1998) grown in soil fertilized with combined inoculants of different strains. Present results are in agreement with these reports.

Based on the results it can be concluded that light dose of nutrient @ 250 kg N, 30 kg P and 130 kg P ha^{-1} along with 20 g of *Rhizobium* and 10 g of AM (contained 200 chlamydospore and 5gm roots) produced maximum growth and biomass in case of *Dalbergia sissoo* seedlings. While higher dose of NPK caused detrimental effect due to toxic effect on *D. sissoo.* Similarly inoculation of AM and *Rhizobium* alone also influenced the growth if inoculated in relatively large quantity.

LITERATURE CITED

Babu R S, Sankaranarayanan C and Joshi G, **1998.** Indian Journal of Nematology 281 77-80.

Daniels B A and Skipper HD, 1982. *Mycorrhizal Research*, 29-35.

Das AK Bera MK and Mohiuddein M, **2001.** *Environment and Ecology* 19 145-148.

Hauka FIA, El-Sawah MMA and Selim AEI, **1996.** *Proc 7TH Conf. Agronomy Univ. Mansoura*, 239-252.

Paroha Seema Chandra K K and Tiwari K P 2000. Synergistic role of VAM and Azotobacter inoculation on Growth and biomass production in forestry spicies Journal of Tropical Forestry 16: (1), 13-21.

Reddy S V and Ahlawat I P 2001. Dry matter accumulation and nutrient uptake in lentil in relation to cultivars, phosphorus, zinc and biofertilizers. Research on Crops 2(1): 21-24.

Sansamma G Pillai G and George S, 1998. Forage Research 24 101-106.

Sieverding E 1991. Vasicular-arbuscular Mycorrhiza Management in Tropical Agrosystem.GTZ Publication Germany P-229-245.

Swarupa S G 1996. Study on the effect of biofertilizers on growth of CXR Coffee seedlings. Journal of Coffee Research 45: 217-223.

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