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RESEARCH ARTICLE

Interaction Effects of Different Nutrient Levels, Rhizobia and Arbuscular Mycorrhizal Fungi on Growth and Biomass of *Dalbergia sissoo* Roxb. seedling under Nursery Condition

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Abstract

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A factorial randomized block experiment was conducted to determine the influence of different levels of Rhizobia and AMF under three levels of macronutrient (NPK) combinations on growth and biomass of Dalbergia sissoo seedlings. The treatment consist Nutrients (N1, N2, N3). Rhizobia (R1, R2, R3) and AMF (AMF1, AMF2, AMF3) alone and in its different combinations. The effect of different treatments and their combinations showed significant difference in all the attributes of plants compared to uninoculated control. In three way interaction, the maximum significant records for plant height, root length, shoot and root dry biomass were obtained in plants received with tripartite inoculation of N3xR3xAMF3 in which the magnitude of increase were 31.94, 32.5, 229.05 and 188.34% respectively compared to control, while N x AMF was found best among dual inoculation categories and nutrient added plants exhibited maximum growth and biomass among single treatment component. As for as the level of dose was concerned, the lowest level of nutrient, Rhizobia and AMF (N3xR3xAMF3) was scored Ist most effective treatment in biomass production (10.59 g pl⁻¹) while the highest level of nutrient and lowest level of AMF (N1 x AMF3) was IInd best treatment (9.59 g pl⁻¹ biomass). The moderate level of nutrient with lowest level of Rhizobia (N2x R3) scored III^{rd} position (8.65 g pl⁻¹ total dry biomass) compared to control (3.58 g pl⁻¹ total dry biomass). Among treatments without any combination, the nutrient was found most effective as it increased the mean total biomass up to 62.01% while AMF inoculation registered 28.49% and Rhizobia 25.13% increase compared to control. It was also observed that there was synergistic interaction between Rhizobia and AMF, however this combination performed low than the treatment added with nutrient.

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INTRODUCTION

Excessive industrial exploitation, clearing of forest land for agricultural and firewood purposes have led to deforestation during recent decades (Bandyopadhyay and Shyamsundar, 2004). Planting of trees along roadsides, on degraded lands and as part of agro-forestry is being practiced for environmental protection and economic gain. The increase in forest cover is urgently required in India due to rapidly growing population and industries. This is only possible through massive plantations in all the possible sites but success of any plantation depends on the quality of planting stock, nutrient, water management and protection. In general, significant percentage of plantation is failed every year in the country as poor seedlings is deployed for planting with no use of fertilizer in forest species. *Dalbergia sissoo* is one such species of tropical regions has great potential in improving soil nutrient status and even

withstand under harsh condition in the site and also domesticated for fodder, fuel and timber production. It is planted extensively for waste land development, nutrient improvement in agroforestry and farm forestry system with agriculture due to its fast growing nature and high income to farmers. In addition, it is a favourable species for plantations in canal bank, side of railway tracks and road sides. The demand of quality nursery stock of multipurpose trees like *D. sissoo* is being increased day by day due to higher survival of healthy seedling compared to normal once. Being a leguminous tree species *D. sissoo* forms association with Rhizobia and saves nitrogen requirements through nodule formation and atmospheric N fixation. AMF contain widespread symbiotic interactions that are commonly described as the result of co-evolution between fungi and plants, where both partners benefit from the reciprocal nutrient exchange (Bonfante and Genre, 2008). Now a days a special attention is paid to the potential role of these fungi in agricultural and production of quality nursery stock for afforestation and rejuvenation of wastelands due to their ability to increase water and nutrient uptake. Such increase may be due to the increase in the absorption of the roots as a result of the wide extension of fungus mycelium in the soil around the root system that allows the plant to have access to higher volume of soil (Hayman, 1983). Symbiotic relationship between different plants and AM fungi on the exterior part of the root improve plant performance under drought and nutrient stress soil.

Dual inoculation of biofertilizers results in to greater increase in plant growth than inoculation with only one (Chalk et al., 2006, Chandra, 2013). Inoculation alone or a combination of beneficial microorganisms including AMF, Rhizobia, PGPR and PSB have been observed to increase production in many agriculture crops (Jain et al., 2008, Hussain et al., 2013) But the report of interaction of AMF, Rhizobia in different levels and doses have been limited and scarce particularly in forest species. However, in forestry few research reports are available to demonstrate, that biofertilizers stimulate the growth and biomass and enhance the uptake of nutrient (Rangarajan et al., 1987, Huang et al., 1985).

In many circumstances, merely inoculation of bio-inoculant does not give apparent results but they requires suitable partner and neighbors with appropriate combinations for efficient performance. In addition, soil plays a major role in the efficacy of biofertilizers besides host species, therefore, suitable inoculums doses need to determine for different bio-inoculant and nutrients for different forest species. The objective of the present investigation was therefore to investigate the effect of different nutrient levels, Rhizobia and AMF and their interactions on growth and biomass of *D. sissoo* under nursery condition.

Material and Methods

The study was conducted at State Forest Research Institute, Jabalpur, India during rainy season in the month of June-July. The mean maximum temperature in summer is over 44°C. Three phenotypically superior seeds of *Dalbergia sissoo* were sown in polythene bag of 5 kg capacity filled with nursery soil and sand mixture by1:1 ratio by volume. After 30 days of seed sowing one healthy seedling was retained in each bag and the experiment was arranged as per factorial randomized block design. The various combinations of fertilizer and microbial inoculants were applied as per experiment design. The treatment given to seedling is as below:-

Control - No fertilizer and microbial cultures

- N1 High dose (560 kg N, 75 kg P_2O_5 and 350 kg K_2O per ha)
- N2 Medium dose (400 kg N, 50 kg P_2O_5 and 250 kg K_2O per ha)
- N3 Low dose (250 kg N, 30 kg P_2O_5 and 150 kg K_2O per ha)
- R1 Rhizobia (60 g inoculum 2.17 X 10^7 bacteria per g culture)
- R2 Rhizobia (40 g inoculum 2.17 X 10^7 bacteria per g culture)

R3 - Rhizobia (20 g inoculum 2.17 X 10^7 bacteria per g culture)

AMF 1- (500 Acaulospora scrobiculata and Funneliformis mosseae +25g infected roots)

AMF 2 - (300 Acaulospora scrobiculata and Funneliformis mosseae +15g infected roots)

AMF 3 - (200 Acaulospora scrobiculata and Funneliformis mosseae +10 g infected roots)

(To get the required level of nutrients in N1 2.092g Urea + 0.703g SSP + 0.980g MOP were mixed in polythene bag mixture, similarly in N2 1.222g Urea + 0.312g SSP + 0.550g MOP and in N3 0.407g Urea + 0g SSP + 0.034g MOP per polythene bag were mixed) The initial test value of soil and sand mixture was 175kg N, 30kg P_2O_5 and 122kg K_2O).

Preparation of Rhizobia and AMF cultures:- The fresh root nodules were collected from local *D. sissoo* plantations and surface sterilized with 0.05% Hgcl₂ for 5 minutes followed washing with sterilized water. The nodules were crushed and inoculated in YEMA media for 7 days at 26°C then grown colonies of Rhizobia were

placed in broth culture. Mass multiplication was done in biofermenter at 300 rpm stirrer speed for 6 days. The culture contained 2.17×10^7 Rhizobia populations per gram of substrate.

Arbuscular Mycorrhizal Fungi inoculum was prepared by isolating AMF spores from 15 year old *Dalbergia sissoo* plantation of Jabalpur provenance. Soil dominated with Acaulospora scrobiculata and Funneliformis mosseae was wet sieved by using sieving and decanting technique (Gerdemann and Nicolson, 1964) and sucrose centrifugation techniques. The spores were inoculated in sterilized substrate for multiplication with Panicum grass and maize using potting method. The colonization status of AMF in root was regularly checked using Philips and Hayman, 1970).

Inoculation of Rhizobia and AMF: After 30 days of seedling age 60g (R1), 40g (R2) and 20g (R3) Rhizobia culture were inoculated in each seedling as per experiment by making 3-4 small holes around root zone and culture was placed in to hole and watered. Similar method was also followed for the inoculation of AMF and 50g (AMF1), 30g (AMF2) and 20g (AMF3) AMF culture were inoculated. AMF inoculum consist 200 spores per 10g substrate and infected roots of Panicum trap plant.

After 6 months of the treatment six seedlings per treatment represented each replicate were selected and harvested to record plant height (cm) and root length (cm). Shoot dry weight (g pl⁻¹) and root dry weight of the seedling were recorded after keeping the harvested plant materials in hot air oven at 90°C for 3 days and total biomass (g pl⁻¹) was determined by summing shoot and root biomass of the seedling. The experiment included 39 treatments which were the combination between three nutrient levels, Rhizobia and AMF arranged in a factorial randomized blocks design with 3 replicates. The collected data were subjected to statistical analysis of variance by using the Least Significant Difference (LSD) at 5% level according to Snedecor and Cochran (1980).

Results and Discussion

Effects of Nutrient, Rhizobia and AMF

The results revealed significant differences among the treatments for all morphological parameters considered in the present investigation at harvesting age at P< 0.05. It is evident from the data that with the use of nutrient, Rhizobia and AMF alone enhanced plant height compared to the control (Table 1). Mean plant height, root length and shoot dry weight increase became 23.0, 36.37 and 82.93% respectively due to nutrient addition compared to control which were highest among single treatments. Similarly, the total biomass of plant also increased maximum reached to 62.01% with the addition of nutrients as compared to control. The higher growth and development of plant under nutrient application was due to readily availability of N, P, K the most essential nutrients needed to plant for cell division, elongation and for other physiological activities as also reported by Lalitha et al. (2011). In addition, Moreira et al. (2010) illustrated that phosphorus and nitrogen are the most limiting factor for plant growth and also required for AMF and Rhizobia symbiosis. It was deduced from the data that AMF fungi was second most effective treatment in D. sissoo next to nutrient as mean total biomass of the plant was improved 28.49% than control. However, lengthwise the root and shoot development observed poor than the Rhizobia inoculated plants. The medium level of nutrient consisted 400, 50 and 250 kg of N, P2O5 K respectively was found most effective treatment as it is likely that the mineral dosage utilized in this treatment was sufficient to meet the demand of the D. sissoo at seedling age, followed by moderate level of AMF (300 spores + 15g infected) and high level of Rhizobia as compared to all other treatment with different levels without combinations. Similar findings have been reported by Mortimer et al. (2007) Nazir et al. (2011), they all reported significant effects of AMF and Rhizobia in plant growth and development. The highest significant increase in root dry weight obtained in plant grown under AMF (44.11%) was due to extended absorptive area through their mycelium network of AMF and changing unavailable phosphorus to available form and translate to root system cause increase in plant growth parameters (Ghobanian et al., 2011).

Interaction Effect of Nutrient and Rhizobia

The combinations of different levels of nutrient with Rhizobia levels were found to have significant influence on all morphological characters (Table 2). It was observed that moderate level of nutrient with lowest level of Rhizobia (N2xR3) was most effective in promoting the plant parameters significantly compared to control and other combinations of nutrient with Rhizobia except in root length. As for plant height, shoot dry weight, root dry weight and total biomass, the highest significant results were observed of 61.57, 166.48, 114.11 and 141.62% respectively in plants with N2xR3 treatment compared to control. The positive interaction of nutrient with Rhizobia also enhanced mean plant height (30.74%), Root length (32.20%) and total dry biomass (59.21%) than control. In conformity with the findings of the present study, Leigh et al. (2009) and Moreira et al. (2010) who worked on leguminous trees observed that Rhizobia enhances growth and biomass. This was due to the association between the plants and the Rhizobia to fix N for readily availability of N which enhanced growth and plant vigor and improved the plant physiological activities. It was also noticed that nutrient addition improve the efficacy of Rhizobia in *D. sissoo*, results in to higher growth and biomass than single inoculations.

Interaction Effect of Nutrient and AMF

Evaluating of the results showed in table 3 revealed that high level of nutrient with lowest level of AM fungi (N1xAMF3) treatment significantly highest effect on all parameters of plant considered in the present investigation except plant height which was highest in N2xAMF3 however the difference between two treatment was insignificant at P<0.05%. The root length, shoot dry weight and root dry weight of N1xAMF3 treated plants was enhanced 30.82, 175.53 and 159.41% compared with the control. Among other interactions N2xAMF3 found to have second best treatment as most of the plant parameters improved. This was in line with the findings of other workers (Leigh, 2009, Moreira, 2010) who have reported that P and N addition boosts AMF development in plants. These plants rendered higher response with inoculation of mycorrhiza because of more strong relationship with mycorrhiza to fulfill their phosphorus and other nutrient requirement and shared some photosynthetes with fungus to maintain the symbiosis (Hobbie, 2006). However, as compared to plants under highest nutrient level (N1) the interaction effect was higher, this indicating the positive effect of AMF with nutrients. This supports the results of Zandavalli et al. (2004) who found that the increase in yield due to synergistic interaction between nutrient and AMF. Our results were also in line with Kuntal et al. (2007) who studied the effect of chemical fertilizers N, P and K alone or in combinations with AMF results showed significant increase in biomass due to bio fertilizer applications. This result in accordance with Linderman and Davis (2004), Abdel-Rahman et al. (2011) and Aster et al. (2012). Mycorrhizal inoculated plants improved their biomass by enhanced translocation of nutrients and water from rhizosphere due to higher root colonization (Rejali et al. 2008).

Interaction Effect of Rhizobia and AMF

It is evident from the data in Table 4 that combined inoculation of Rhizobia with AMF also had a significant effect on plant development and biomass than un-inoculated control. The interaction effect of lowest level of Rhizobia combined with the Moderate level of mycorrhiza (R3xAMF2) could significantly increase plant height, root length, shoot dry weight, root dry weight and total biomass at highest rate reached to 51.21, 53.82, 116.48, 121.76 and 118.99% respectively compared with their controls. Our result correlate with the findings of Mortimer et al. (2007) and Bhuiyan, (2004) that both Rhizobia and mycorrhizal symbiosis acts synergistically on promoting plant growth as similar in case of present study. In addition, Jia et al. (2004) reported that inoculation of AMF promotes biomass and photosynthetic rate in plant because of the enhanced P and N supply due to AMF and Rhizobia. These findings are in agreement with that of Aysan and Demir (2009), Askar and Rashad (2010) and Xiurong et al. (2011). Nutrient in these combinations (Rhizobia and AMF) was lacking resulted in a comparative low biomass than nutrient added treatments (NxR and NxAMF), this proved that supplementary addition of nutrient is important for making strong relationship with the host.

Tripartite Effect of Nutrient, Rhizobia and AMF

The interaction effects of tripartite application of nutrient+ Rhizobia+AMF was impressive in improving all the growth attributes of D. sissoo in comparison to uninoculated control (Table 5). The treatment of N3xR3xAMF3 had a pronounced effect in enhancing the growth parameters like plant height, root length, shoot and dry biomass. The magnitude of increase was 31.94, 32.5, 229.05 and 188.34% respectively over control seedlings. With reference to other treatments these tri-combination expressed best result and boosted growth and biomass in D. sissoo. The high response of low levels of NxRxAMF might be due to efficient N fixation and the uptake by the root through fungal mycelium from substrate (Leigh et al. 2009). Moreover, it has been widely documented that legume plants negatively regulate nodulation when supplied with high level of N (Vargas et al. 2000, Zang et al. 2000) also as in case of present study where the addition of high nutrient did not result as equal to the lowest nutrient treatment combined with Rhizobia and AMF. It is well known that AM fungi can improve the nutrient status of their host plants (Smith and Read, 2008; Kim et al., 2010), this not only benefitted Rhizobia, but also lead to increase photosynthesis, making a greater proportion of photosynthates available to the Rhizobia nodules (Mortimer et al., 2008). It is also thought that the plant-Rhizobia system benefits from the presence of AM fungi because the mycorrhiza ameliorate not only P deficiency but also any other nutrient deficiencies that might be limiting to Rhizobia (Smith, 2002). Also, Nautiyal et al. (2010) found that the dual inoculation of Cicer arietinum L. with Rhizobia and AMF significantly enhanced the number of nodules and the dry weight per plant.

The mean total biomass with respect to different treatments are given in Fig. 1 revealed that plants with N x R x AMF exhibited maximum total biomass (8.35 g pl-1) followed with N x AMF (6.27 g pl-1) compared to control seedling (3.58 g pl-1). This indicates that nutrient plays an essential role in improving the efficacy of symbiosis. This was conformity with the finding of Moreira et al. (2010) who reported the positive role of N for the establishment of Rhizobia- plant relationship and P for AMF-plant symbiosis. Control plants which were deprived from any microsymbionts and nutrients showed poor growth compared to treated seedlings. Mean total biomass illustrated in figure -1 depicts that the combined inoculation of two or three treatment components found better than

single treatment in *D. sissoo* because of the better exploitation of multiple input by plant through the help Rhizobia and AMF under supplemented nutrient condition.

Conclusion

Nutrient was the main limiting factor for the growth of *D. sissoo* under nursery condition. The dual inoculation with AMF and Rhizobia, despite alleviating the effects of nutrient omission, was not sufficient to ensure satisfactory plant growth. It was observed that the addition of NPK not only required for plant growth and biomass production but equally needed for symbiosis with Rhizobia and AMF. Among different treatments, tripartite application of lowest level of Nutrients, Rhizobia and AMF sufficiently enhanced the plant height, root length, shoot weight, root weight and total dry biomass at maximum extent. Interestingly, the interaction of nutrient of highest level and lowest level of AMF3 registered maximum growth and biomass compared to other dually inoculated plants in the present investigation. In general, dual inoculation with AMF and Rhizobia increase the growth of *D. sissoo*, indicating the addition of inorganic fertilizers particularly NPK. Comparing the growth in single inoculation, nutrient application was found best than Rhizobia and AMF fungi.

Table -1: Effects of individual treatment (without any combination) of different levels of nutrient, Rhizobia and AM fungi on plant growth and dry biomass of *Dalbergia sissoo* Roxb.

Treatments	Plant Height	Root Length	Shoot Dry	Root Dry	Total Dry
	(cm)	(cm)	Weight (g	Weight (g pl ⁻¹)	Biomass (g pl
			pl ⁻¹)		1)
Control	54.67	40.00	1.88	1.70	3.58
N1	68.00	63.67	3.58	2.60	6.18
N2	68.50	56.33	3.79	2.53	6.32
N3	65.30	43.67	2.96	1.94	4.90
Mean	67.26	54.55	3.44	2.35	5.80
LSD 0.05	4.35	5.32	1.01	NS	1.43
R1	74.67	60.33	2.34	2.64	4.98
R2	63.33	46.33	2.38	2.28	4.66
R3	52.60	44.30	1.92	1.88	3.80
Mean	63.53	50.32	2.21	2.26	4.48
LSD 0.05	4.05	NS	0.57	0.75	1.43
AMF1	65.80	46.00	2.34	2.19	4.53
AMF2	69.00	56.67	2.30	3.24	5.54
AMF3	65.33	43.33	1.82	1.93	3.75
Mean	66.71	48.66	2.15	2.45	4.60
LSD 0.05	3.87	2.60	0.54	0.41	0.90

Table -2: Interaction effects of different nutrient levels with Rhizobia on morphological characteristics of *Dalbergia sissoo* Roxb.

Treatments	Plant Height	Root Length	Shoot Dry	Root Dry	Total Dry
	(cm)	(cm)	Weight (g	Weight (g pl ⁻¹)	Biomass (g pl ⁻
			pl ⁻¹)		1)
N1 XR1	59.00	54.00	2.70	2.67	5.37
N1 XR2	59.33	43.67	2.79	1.89	4.68
N1 XR3	76.67	52.33	2.79	3.57	6.36
N2 XR1	48.67	49.33	1.99	1.83	3.82
N2 XR2	77.67	40.00	2.63	2.45	5.08
N2 XR3	88.33	66.33	5.01	3.64	8.65
N3 X R1	68.33	60.00	1.89	1.99	3.88
N3 X R2	79.00	43.33	3.01	1.91	4.92
N3 XR3	86.33	67.00	4.80	3.75	8.55
Treatments Mean	71.48	52.88	3.06	2.63	5.70

LSD 0.05	11.72	3.05	1.01	1.00	2.86

Table -3: Influence	of different	levels	of nutrient	addition	in soi	and	Arbuscular	Mycorrhizal	Fungi on
growth and biomass	of Dalbergia	a sissoa	o Roxb.						

Treatments	Plant Height	Root Length	Shoot Dry	Root Dry	Total Dry
	(cm)	(cm)	Weight (g	Weight (g pl ⁻¹)	Biomass (g pl ⁻
			pl ⁻¹)		1)
N1 XAMF1	59.00	39.67	2.35	2.01	4.36
N1 XAMF2	59.33	50.67	1.89	2.04	3.93
N1 XAMF3	96.67	52.33	5.18	4.41	9.59
N2 XAMF1	48.67	40.67	2.00	2.19	4.19
N2 XAMF2	77.67	28.67	3.21	2.84	6.05
N2 XAMF3	98.33	35.00	4.98	3.37	8.35
N3 X AMF1	68.33	39.67	2.28	2.86	5.14
N3 X AMF2	79.00	34.00	3.57	2.95	6.52
N3 XAMF3	89.33	47.67	4.83	3.29	8.32
Treatments Mean	75.15	40.93	3.65	2.88	6.27
LSD 0.05	7.00	8.56	0.99	1.02	1.79

Table -4:Interaction effect of different inoculums load of Rhizobia and Arbuscular Mycorrhizal Fungi on morphological characteristics of *Dalbergia sissoo* Roxb.

Treatments	Plant Height	Root Length	Shoot Dry	Root Dry	Total Dry
	(cm)	(cm)	Weight (g	Weight (g pl ⁻¹)	Biomass (g pl
			pl ⁻¹)		1)
R1 XAMF1	65.33	49.67	3.07	2.90	5.97
R1 XAMF2	65.03	38.33	2.06	2.14	4.20
R1 XAMF3	64.87	36.00	1.94	1.74	3.68
R2 XAMF1	65.33	36.67	2.60	2.22	4.82
R2 XAMF2	64.67	54.67	2.10	2.14	4.24
R2 XAMF3	78.70	61.53	3.40	3.90	7.30
R3 X AMF1	62.67	45.67	1.97	2.46	4.43
R3 X AMF2	82.67	61.33	4.07	3.77	7.84
R3 XAMF3	73.00	61.07	3.04	3.40	6.44
Treatments Mean	69.14	49.43	2.69	2.74	5.43
LSD 0.05	4.58	6.42	1.10	0.84	1.11

Table - 5: Interaction effect of different inoculums load of Rhizobia and Arbuscular Mycorrhizal Fungi on morphological characteristics of *Dalbergia sissoo* Roxb.

Treatments	Plant Height	Root Length	Shoot Dry	Root Dry	Total Dry
	(cm)	(cm)	Weight (g	Weight (g pl ⁻¹)	Biomass (g pl⁻
			pl ⁻¹)		1)
N0R0AMF0	64.67	40.00	1.79	1.63	3.52
N1R1AMF1	72.67	33.33	4.30	3.48	7.78
N2R2AMF2	83.00	45.00	3.85	2.85	6.67
N3R3AMF3	85.33	53.00	5.89	4.70	10.60
Treatments Mean	80.33	43.76	4.68	3.67	8.35
LSD 0.05	8.19	6.58	1.46	0.56	1.99



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