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Effect of microbial inoculation on biomass production and nitrogen fixation of *Acacia nilotica*

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ABSTRACT

A green house study was conducted at the Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur during 2015-16 to assess the response of legume tree seedlings (*Acacia nilotica*) to *Rhizobium* and PSB inoculation and to assess the influence of inoculation on biologically fixed nitrogen. The treatments were inorganic fertilization only, inoculation with *Rhizobium*, PSB alone and along with 25 per cent N including control. Dual inoculation of *A. nilotica* with *Rhizobium* and PSB along with 25 % nitrogen recorded significantly higher dry biomass accumulation (7.66 g per plant and 4.41 g per plant in shoot and root respectively at 90 days after planting (DAP). Nitrogen uptake by *A. nilotica* significantly increased from 40.19 mg per plant at control to 104.74 mg per plant at *Rhizobium* +PSB + 25 % N. However, maximum extra N gain was 64.55 mg per seedling over control and 37 per cent increase in shoot dry biomass accumulation at 90 DAP were obtained due to dual inoculation + 25 % N applied in *A. nilotica*. The more microbial population in rhizosphere soil was established by inoculation effect of legume tree. Dual inoculation along with addition of 25 % nitrogen was significantly effective in enhancing growth performances and N fixation in *Acacia nilotica*.

Key words : *Acacia nilotica*, biomass and N fixation, PSB, *Rhizobium*

INTRODUCTION

Acacia nilotica, commonly known as Indian Gum-Arabic Tree and locally 'Babul' is one of the important nitrogen fixing tree (NFT) species that belongs to family Fabaceae, sub-family Mimo soideae. This species provides nutritious fodder for livestock, firewood for local people and wood for a variety of purposes (Sarr et al., 2005). Due to nitrogen fixing ability and fast rate of litter decomposition, they also help in ameliorating the soil, so improving soil health (Basu et al., 1987). Successful establishment of NFTs in plantation programme is possible through the production of high quality tree seedlings at nursery levels. For successful planting, seedlings must access sufficient nutrient from the soil and compete with others. Therefore, 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.

Excessive application of nitrogenous fertilizer is not only uneconomical, but also adversely affects the environment and crop quality (Tandan, 2000; Arun et al., 2007). This necessitates use of biological sources of nutrient for sustainable production and better soil health. Thus, the influence of NFTs in soil N management should be given attention. Being leguminous tree, it enhances soil fertility by maintaining the N balance in soil through its association with effective rhizobia and also sufficient fall of senescing leaves. The effects of rhizobial and PSB inoculation on growth and nodulation of some important forest legumes have been studied indicating tremendous potential of these biofertilizers in improving plant system (Totey et al., 2000). Consequently, systematic inoculation of nursery seedlings with local strains of micro organism capable of forming an effective symbiosis with the plant is recommended. There were several previous reports dealing isolation and application of beneficial microbes, either individually or combined, to assess their effects on the growth and biomass production of several trees (Kumar et al., 2013; Bhagat et al., 2014).

Therefore, the present investigation was carried out to isolate and evaluate the *Rhizobium* sp. and PSB from *A. nilotica* nodules and rhizosphere soils of *A. nilotica* and applying these inoculants in nursery alone and in combination with nitrogen for improving growth performances and N uptake above all for the production of healthy seedling stocks and productive plantation.

MATERIALS AND METHODS

A green house experiment was conducted at Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during *kharif* 2015-2016 with *Acacia nilotica*. The experiment was laid out in complete randomized design (CRD) with six treatments replicated four times. Treatments in the experiment included T_1-Control, T_2-Inorganic Fertilization (N, P, K as 1.5:1:1 g urea, SSP, MOP per seedling), T_3-*Rhizobium* inoculation, T_4-PSB inoculation, T_5-*Rhizobium*+ 25% N, T_6-*Rhizobium* + PSB + 25% N.

Isolation and inoculum preparation

Rhizobium was isolated from fresh nodule from nodulated *A. nilotica* plant grown locally and pure culture was identified by streaking selected colonies on the YEMA (Yeast Extract Mannitol Agar) media. The isolated *Rhizobium* was multiplied and a single discrete colony was transferred to YEMA slant to maintain the isolate (Vincent, 1970). Culture broth was prepared by inoculating in sterilized YEM (Yeast Extract Mannitol) media and shaken for seven days.

Soil samples were collected to a depth of 30 cm from rhizosphere soil from *A. nilotica* grown locally for the purpose of isolation of PSB. The appearance of clearing zone around bacterial colonies after 96h of growth at 30^{o}C was used as indicator for positive phosphorus solubilization. To prepare the culture suspension PSB were inoculated in sterilized Pikovaskya's broth in conical flask incubated respectively at pH (7.0) and temperature at 28 ± 2^o C for 48 hours and then were kept on a rotary shaker for seven days. These cultures were then used for inoculation treatment (Subba Rao, 2000).

Transplantation, observation and statistical analysis

A well mixed 5 kg mixture of soil, sand and compost in 3:1:1 ratio was filled in each polythene bag (12" × 10" size). The soil of the experimental site was vertisol having pH 7.6, organic carbon 6.3 g kg^{-1} soil, mineralizable nitrogen status (204.4 kg ha^{-1}), low in available (Olsen's) phosphorus content (11.6 kg ha^{-1}), but higher in status with respect to available potassium content (416 kg ha^{-1}). The analysis was done as per Page et al. (1982). In initial soil *Rhizobium* population per g of soil was found 1.4×10^5 and PSB population in initial soil sample was 1.1×10^3.

Seeds of *Acacia nilotica* collected from naturally grown area of *A. nilotica* were allowed to germinate in trays containing field soil and sand (2:1) and then uniform seedlings of 15 days old were selected for experiment purpose. Experimental site Raipur is situated in plains of Chhattisgarh at 20^o 16' N latitude and 81^o 36' E longitude with an altitude of 289.6 meter above mean sea level. After inoculation with matured broth of *A. nilotica* – *Rhizobium* and PSB as per treatments, seedlings were transplanted so that a single healthy seedling was maintained in each polybag. In uninoculated pots, seedlings were dipped in same amount of nutrient broth but not inoculated with *Rhizobium* and PSB. Nitrogen through urea was given in water soluble form after seven days of transplantation of seedlings as per treatment description. Phosphorus and potassium were applied commonly to all polybags through SSP and MOP respectively.

For inorganic fertilization, nitrogen, phosphorus and potassium were applied to polybags through urea, SSP and MOP respectively in water soluble form to the soil in polybag as per treatment. The seedlings were allowed to grow up to 90 days. Other package of practices were uniformly adopted in all the treatments for growing healthy crop. Seedling biomass accumulation and N uptake by plants after harvest on four randomly selected plants were recorded in each treatment. The dry weight was recorded after drying in the hot air oven at 70°C to constant weight. N concentration in shoot and root vis-a-vis their uptake by seedlings were analyzed following the standard procedures outlined by Page et al. (1982).

Population dynamics of *Rhizobium* and PSB in rhizosphere soil of pot grown *A. nilotica* were recorded after harvest of plant. The operation of making serial dilutions, setting of plates and inoculation with appropriate media was done in sterilized atmosphere of laminar flow.

Number of Rhizobium or PSB per gram of oven dry soil

$$= \frac{\text{No of colony forming units (cfu)} \times \text{dilution}}{\text{Dry weight of 1 g moist soil sample} \times \text{aliquot taken}}$$

All observations were statistically analyzed using analysis of variance for completely randomised design. The significant difference was tested through F-test at 5% level of significance (Panse and Shukhatme, 1978).

RESULTS AND DISCUSSION

Biomass accumulation

The data on oven dried shoot biomass and root biomass are given in Table 1 and the total biomass per seedling in Fig. 1. Significantly higher biomass yield was recorded with the application of *Rhizobium* + PSB inoculation along with 25% N (7.66 per seedling respectively as shoot dry biomass) over rest of the treatments. Significantly lower biomass accumulation of 5.58 g per seedling were recorded by the control in shoot dry biomass than all other treatments. There was an increase of 37% dry biomass in shoot with *Rhizobium* + PSB + 25 % N over control. Similar results were reported by Chauhan and Pokhriyal (2002) in *Albizzia* and Kumar et al. (2013) in *D. sissoo*. The lower yields were mainly due to low biological nitrogen fixation (BNF) during development stages. This resulted in poor uptake of N, P and K and might have not met the plant requirement of these nutrients for growth and development as evidenced by lower growth parameters resulting in lower biomass yield (Table 1).

Dual inoculation along with less N dose significantly influenced the growth of *A. nilotica* followed by *Rhizobium* inoculation along with less N dose. Biomass accumulation ranged from 5.58 to 7.66 per seedling for shoot, 0.92 to 2.25 per seedling for root at 90 DAP under different treatments. *Rhizobium* + PSB inoculation with 25% N treatment produced significantly maximum shoot biomass and total biomass per seedling, respectively against control.

Total biomass accumulation by *A. nilotica* seedling at 90 DAP was increased by 1.52 times in over T_1 control (Fig. 1). Treatment effect was found significant for total biomass production and this is also depicted in Fig. 1. However, dual inoculation in T_6 showed significantly higher biomass. 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).

Result indicated that the survival and growth of legume sp. i.e. *A. nilotica* were significantly higher in inoculation over uninoculated treatments. This is in line with the findings of Morques et al. (2002) and Komy (2005), where a higher biomass was reported in inoculated seedlings of *Centrolobium tomentosum*, which was attributed to relatively more nutrient uptake from control seedlings. This clearly indicates the better response of *A. nilotica* plants to both PSB and *Rhizobium* inoculation as compared to single application, inorganic fertilization. Similarly, Singh et al. (2000) demonstrated a better response of inoculation for increasing growth and development in *A. procera*.

Table 1. Effect of *Rhizobium* and PSB inoculation on biomass accumulation in *Acacia nilotica* at 90 DAP.

Treatments	Dry weight of shoot (per seedling)	Dry weight of root (per seedling)
T_1 Control	5.58	0.92
T_2 Inorganic Fertilization	6.56	1.30
T_3 Rhizobium Inoculation	7.24	1.84
T_4 PSB Inoculation	7.06	2.05
T_5 Rhizobium+ 25% N	7.36	2.02
T_6 Rhizobium + PSB + 25% N	7.66	2.25
SEm (±)	0.13	0.06
CD(5%)	0.40	0.17

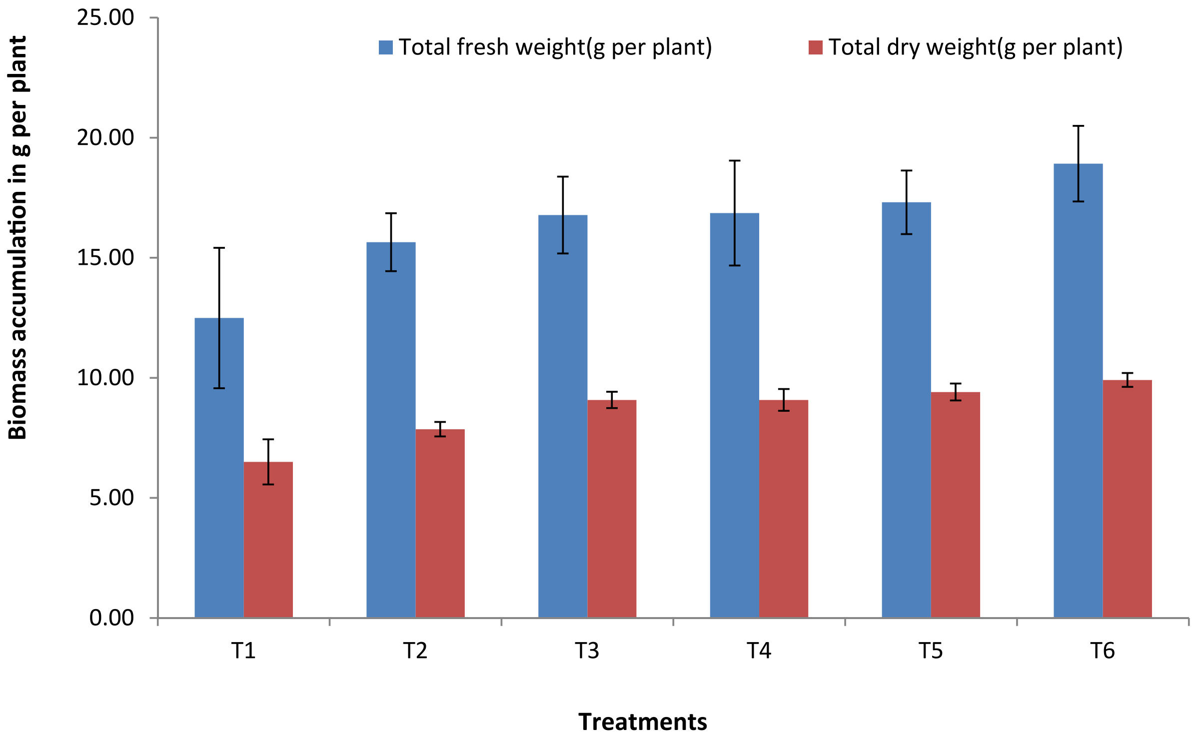

Fig. 1. Effect of inoculation on total biomass accumulation in *A. nilotica* at 90 DAP

Nitrogen status in *Acacia nilotica*

Concentration of nitrogen as well as accumulation of N in shoot and root of *A. nilotica* plants at 90 DAP was given in Table 2. The N concentration of shoots of *A. nilotica* was significantly influenced by the different treatments and significantly higher concentration of N in plant (1.37%) and 0.46% was recorded in shoot and root respectively at 90 DAP with *Rhizobium* + PSB + 25% N than rest of the treatments. Significantly lower N concentration was recorded 0.72% and 0.27% in shoot and root respectively of *A. nilotica* plant by the control over rest of the treatments (Table 2). Significantly higher N uptake in shoot and root i.e., 104.74 mg per seedling and 10.23 mg per seedling were observed respectively when inoculated with *A. nilotica- Rhizobium+PSB* isolate with 25% N (T6) followed by inoculation with *A. nilotica- Rhizobium* + 25% N (T_5) being 92.44 and 8.37 mg per seedling in shoot and root respectively. N uptake in shoot of *A. nilotica* plant varied from 40.19 to 104.74 mg per seedling being significantly maximum in T_6 which gains 64.55 extra N through BNF at 90 DAP (Table 2). Extra N gain through BNF in shoot was maximum at T_6 (64.55 mg per seedling) followed by T_5 and T_3, 52.25 and 36.81 mg per seedling respectively at 90 DAP. Control recorded significantly lowest N uptake by shoot and root of plant (40.19 and 2.47 mg per seedling. respectively) than rest of treatments. Similar trend in nutrient concentration was reported by Bora et al. (2006). Dual inoculation of *Rhizobium* and PSB along with N fertilization enhances nodulation, dry weight and N contents in legume sp.

The higher growth in dual inoculation along with 25% N treated seedling might be due to synergistic effect of lower N levels as starter dose with *Rhizobium* inoculation which mediated the efficient uptake of N in seedlings. High N status in seedling was found in inoculated seedling due to increased BNF. Further P intake by PSB enhanced BNF, which mediated the efficient uptake of N in seedlings. The better uptake of nutrient might be facilitated through microbial inoculants. Similar observations were recorded by Chauhan and Pokhriyal (2005) for nitrogen fixation in *Pongamia pinnata*.

Table 2. N concentration (%) and N uptake (m per seedling) in shoot and root of *Acacia nilotica* plants at 90 DAP as affected by *Rhizobium* and PSB inoculation

Treatment	Concentration of nitrogen %		Nitrogen uptake (m per seedling)		Extra N gain in shoot through BNF (m per seedling)
	Shoot	Root	Shoot	Root	
T_1	0.72	0.27	40.19	2.47	-
T_2	0.84	0.30	55.16	3.87	14.97
T_3	1.06	0.38	77.00	6.89	36.81
T_4	0.88	0.32	62.08	6.43	21.89
T_5	1.26	0.41	92.44	8.37	52.25
T_6	1.37	0.46	104.74	10.23	64.55
SEm (±)	-	4.78		0.29	-
CD (5%)	-	-	14.19	0.86	-

T_1-Control, T_2-Inorganic fertilization (Urea, SSP, MOP: @1.5:1:1 g per seedling), T_3-Rhizobium Inoculation, T_4-PSB Inoculation, T_5-Rhizobium+ 25% N, T_6-Rhizobium + PSB + 25% N

***Rhizobium* and PSB population in rhizosphere soil of *Acacia nilotica* (Babul) legume plants:**

In initial soil *Rhizobium* population per g of soil was found 1.4 × 10^5 which increased to 2.01 × 10^5 in control at 90 DAP due to influence of leguminous tree. At 90 DAP highest population was seen (7.00 × 10^5) in T_6 followed by T_5 and T_3 (Fig. 2). The more microbial population in rhizosphere soil was established by planting of legume tree, which also reported by Singh et al. (2000) and Revathi et al. (2013). PSB population in initial soil sample was 1.1 × 10^3 which increased to 1.63 × 10^3 in control at 90 DAP. Highest population was seen in T_6 (3.02 × 10^3) followed by T_4 and T_5. The effect of inoculation in NFT was pronounced in PSB population density over control. This is ascribed due to efficient recycling of nutrients under this treatment. The application of *Rhizobium* and PSB inoculated seedling of *A. nilotica* further enhanced the *Rhizobium and PSB* population (Fig. 2).

Rhizobium and PSB inoculation with lower doses of N improve the growth of *A. nilotica* seedling through fixation of atmospheric N. This beneficial effect of low N doses on the growth and development of the plant have been reported by many investigators (Prasad et al., 1998; Eaglesham et al., 1983). This work is strongly supported by Krishnaveni et al. (2010). Similar study was also carried out in *Albizzia lebbeck* (Chauhan and Pokhriyal, 2002) and in *D. sissoo* and *A. procera* (Totey et al., 2000).

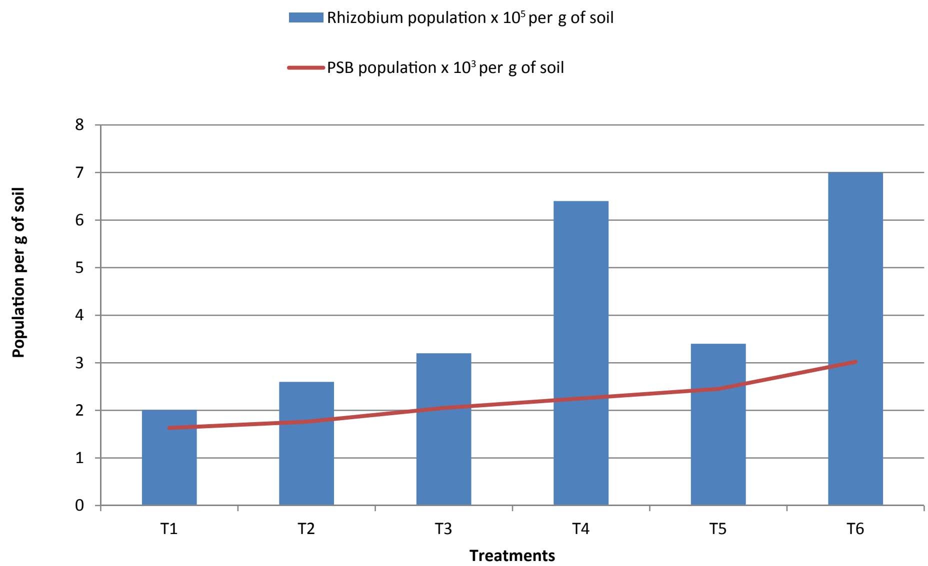

Fig. 2. Effect of inoculation on *Rhizobium* and PSB population in rhizosphere soil of *A. nilotica* at 90 DAP

CONCLUSION

It can be inferred that dual inoculation with *Rhizobium* and PSB along with application of 25% N was significant in improving initial growth response of *Acacia nilotica* plants in nursery enhancing BNF spread. This finding may be helpful in producing quality planting stock of *A. nilotica*. In the seedling production of *A. nilotica*, due consideration should be given to application of microbial organisms such as *Rhizobium* and P solubulizing bacteria as improvement in seedling quality is essential for the survival of seedlings in degraded low soil fertility area.

The study further suggests that as NFT species are of great importance in traditional agro-forestry system, the detailed field investigation is recommended to ensure the long term growth performance of selected NFTs species in response of inoculation in natural stands.

REFERENCES

Arun, K.S. 2007. Bio-fertilizers for sustainable agriculture. Mechanism of P-solubilization. 6th ed., Agribios Publishers, Jodhpur, India, pp. 196-197.

Basu, P.K. and Kabi, M.C. 1987. Effect of application of biofertilizers on the growth and nodulation of seven forest legumes. *Indian Forester* **113** (4): 249-257.

Bhagat, P.K., Dash, D., Raj, A. and Jhariya, M. K. 2014. Effect of *Rhizobium* inoculation on growth and biomass accumulation in *Leucaena leucocephala*. *Ecoscan*. **5**: 65-74.

Chauhan, S.P. and Pokhriyal, T.C. 2005. Nitrogen fertilization vis-a-vis nitrate assimilation in different plant parts of *Pongamia pinnata* Pierre seedlings. *Indian J. For.* **13** (1): 31-43.

Chauhan, Y.S. and Pokhriyal, T.C. 2002. Effect of nitrogen and Rhizobium inoculation treatments on some growth parameters in *Albizia lebbek* (L). benth seedling. *Indian Forester* **110**: 316-321

Eaglesham, A.R., Hassouna, J. and Seegers, R. 1983. Fertilizer N-affect on N fixation by cowpea and soybean. *Agron. J.* **75**: 61-66.

Herrera, M.A., Salmanca, C.P. and Burer, J.M. 1993. Inoculation of woody legumes with selected arbusculae mycorrhizal fungi and rhizobia to recover decertified mediterranran ecosystem. *Appl. Environ. Microbiol.* **59**: 129-133.

Komy, M.A.H. 2005. Co immobilization of *Azospirillum lipoferum, Bacillus megaterium* for successful phosphorus, nitrogen nutrition of wheat plants. *Food Technol. Biotechnol*. **43** (1): 19–27.

Krishnaveni, M.S. 2010**.** Studies on phosphate solubilizing bacteria (PSB) in rhizosphere and non-rhizosphere soils in different varieties of Foxtail Millet (*Setaria italica) Int. J. Agric. Food Sci. Technol*. **1**(1): 23-39.

Kumar, A., Dash, D. and Jhariya, M.K. 2013. Impact of Rhizobium on growth, biomass accumulation and nodulation in *Dalbergia sissoo*. *BioScan* **8**(2): 553-560.

Morques, M.S., Pafano, M. and Scotti, M.R.M.M.L. 2002. Dule inoculation of wood legume (*Centrolobium tomentosum*) with Rhizobia and Mycorrhizal fungi in south-eastern Brazil. *Agrofor. Syst.* **52**: 107-117.

Page, A.L., Millar, R.H. and Keeny, D.R. 1982. Methods of soil analysis, part-2. Chemical and microbiological properties of Phosphorus. ASA Monograph, 9, pp. 403-430.

Panse, V.G. and Shukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research. New Delhi, pp.145-156.

Prasad, P. and Nautiyala, A.R. 1998. Response of two multipurpose tree legumes to different fertilizer treatments at nursery level. *Indian J. For*. **21**: 237-279.

Revathi, R., Mohan, V. and Jha, M.N. 2013. Integrated nutrient management on the growth enhancement of *Dalbergia sissoo* Roxb. seedlings. *J. Acad. Indus. Res.* **1**(9): 550-557.

Sarr, A., Diop, B., Peltier, R., Neyra, M and Lesuer, D. 2005. Effect of rhizobial inoculation methods and host plant provenances on nodulation and growth of *Acacia senegal* and *Acacia nilotica*. *New Forests* **29**(1): 75–87.

Singh, R.R., Kaushik, J.C. and Singh, J. 2000. Effect of soil media on germination, shoot and root length and number of nodules of three nitrogen fixing tree species seedlings. *Indian J. For.* **23**(2): 157-159.

Subba Rao, N.S. 2000. *Soil Microbiology*. 4th ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Calcutta, p. 407.

Tandan, H.L.S. 2000. Fertilizer, organic manures, recyclable waste and biofertilizers. Fertilizer Development and Consultation Organization, New Delhi, p. 98.

Totey, N.G., Verma, R.K., Verghees, M., Shadangi, D.K., Khatri, P.K. and Pathak, H. D. 2000. Effect of (*Rhizobium*) Biofertilizers and varying levels of phosphorus on the growth of forest legumes (*D. sissoo & Albizia procera*). *Indian J. For.* **23**(2): 205-207.

Vincent, J.M. 1970. *A manual for the practical study of root nodule bacteria*. IBP Handbook No. 15, Blackwell Scientific Publications, Oxford, pp. 14-15.