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Inoculation Effects of *Rhizobium* and Phosphorous Solubulizing Bacteria on Growth and Nodulation of *Acacia nilotica*

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A B S T R A C T

A study was conducted in green house of Department of Agricultural Microbiology, College of Agriculture, Chhattisgarh during 2015-16 to study the effect of *Rhizobium* and Phosphorous Solubulizing Bacteria inoculation on growth performance of *Acacia nilotica*. The treatments were inorganic fertilization only, inoculation with *Rhizobium*, PSB alone and along with 25 per cent N including control. The *Rhizobium* isolate from nodule of *A. nilotica* was found good in its growth at 30^0C and 6.5-7.5 pH but showed tolerance up to 50^0C and pH 5.5 to 8. *PSB* isolate, isolated from rhizosphere zone of *A. nilotica* plant was found to survive at pH 5, however pH 6 to 7 was found most suitable for its growth. *A. nilotica - PSB* showed well growth at temperature 30^0C and decreases with increasing temperature from 35^0C to 50^0C. Dual inoculation of *A. nilotica* with *Rhizobium* and PSB along with addition of 25 % nitrogen recorded significantly higher shoot length (78.75 cm), root length (53.75cm), collar diameter (6.15mm) and shoot dry biomass accumulation (7.66 g plant^{-1}) over other treatments at 90 DAT. Significantly higher nodulation (38no. and dry weight of nodules 0.283g plant^{-1}) also recorded with *Rhizobium* + *PSB* +25 % N at 60 DAT.

Introduction

Restoration and maintenance of soil fertility is a basic but critical ecological problem especially in the tropics where the soils are nutrient deficient particularly P and N. The degraded soils are reclaimed through afforestation programmes with multifarious forest legume tree species along with beneficial microbes. *Acacia nilotica*, commonly known as Indian Gum-Arabic Tree and locally 'Babul' is one of the important Nitrogen Fixing Tree (NFT) species belongs to family Fabaceae, sub-family Mimosoideae. This species provides nutritious fodder for livestock, firewood for local people for daily needs and wood for a variety of purposes (Sarra *et al.*, 2005). It is extensively planted in farm lands, wasteland, roadside avenues and is important for afforestation programme. Nitrogen and phosphorous are essential nutrient for plant growth and development. Use of inorganic fertilizers has led to worldwide ecological problems as well as affects the human health. However, biofertilizers offer an alternative to chemical inputs, which have an ability of mobilizing the nutrionally important elements from non-useable to useable form and known to increase yield. Biofertilizer improve the quality of tree seedlings which are better adopted to withstand the adverse condition as bio-fertilizer has tremendous potential to provide plant nutrients by boosting in microbial population present in soil which in turn makes the insoluble nutrients available for growth of the plants (Kelel, 2014)**.**

Hence for successful plantation in waste and degraded land, quality seedling stocks should be raised in nursery which necessitates the use of microbial inoculants further for N management in soil and to enhance biological N fixation, biofertilizer application contribute significantly. This type of study was also carried out by Totey *et al.,* (2000), Krishnaveni (2010). Besides N fixing organisms, it is now established that the enhanced growth of plant is also due to absorption of ions especially P from the soil and subsequent transfer to plants (Rani *et al.,* 1999). Inoculation of tree legume with PSB can stimulate the growth. The purpose of present investigation was to screen and identify the stress tolerant potential isolate for improvement of *A. nilotica*.

So considering these facts, to increase biomass productivity, seedling quality and to improve BNF of *A. nilotica* and to improve fertility of degraded land, there is need to adopt the eco-friendly management strategies which will be economically feasible. There were several previous reports dealing isolation, screening and application of beneficial microbes, either individually or combined, to assess their effects on the growth and biomass production of several trees (Bora *et al.,* 2006; Kumar *et al.,* 2013; Bhagat *et al.,* 2014). Therefore, the present investigation was carried out to isolate and characterize the *Rhizobium* sp. from *A. nilotica* nodules and PSB from rhizosphere soils of *A. nilotica* and to assess the effect of these inoculants alone and in combination with nitrogen for improving growth and nodulation above all for the production of healthy seedling stocks.

Materials and Methods

A greenhouse pot experiment was conducted at Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2015-2016 with *Acacia nilotica*. The experiment was laid out in complete randomized design (CRD) with 6 treatments replicated four times. Treatments in the experiment included T_1-Control, T_2-Inorganic Fertilization (N,P,K as 1.5:1:1g Urea, SSP, MOP per seedling), T_3-*Rhizobium* Inoculation, T_4-PSB Inoculation, T_5-*Rhizobium*+ 25% N, T_6-*Rhizobium* + PSB + 25% N. The *Rhizobium* strains isolated from *A. nilotica* nodules and PSB strain isolated from rhizosphere soils of *A. nilotica* were used for inoculation. 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 before transplantation in as per treatment.

Isolation, screening and inoculum preparation

Rhizobium was isolated from fresh nodule of collected *Acacia nilotica* (Babul) seedling naturally grown in the nearby Agriculture College campus 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 using YEM Yeast Extract Mannitol).

Soil samples were collected to a depth of 30cm from rhizosphere soil from *Acacia nilotica* grown in different locations of District, Chhattisgarh during January, 2015 for the purpose of isolation of PSB from collected soil samples (Table 1). The appearance of clearing zone around bacterial colonies after 96h of growth at 30^0C was used as indicator for positive Phosphorus solubilization.

Preparation of inoculums

To prepare the culture suspension for experimentation, the isolate *Acacia nilotica* -*Rhizobium* and PSB were inoculated in sterilized YEM broth and Pikovaskya's broth in conical flask incubated respectively at pH (7.0) and temperature at 28 ± 2^0C for 48 hours and then were kept on a rotary shaker for 7 days. These cultures were then used for inoculation treatment.

Characterization of isolates

Gram staining and colony morphology

Acacia nilotica –Rhizobium and PSB isolates were activated by inoculating in YEM and Pikovskaya's media respectively of pH (7.0) and temperature (28°C) and characterized for Gram staining, colony morphology (Aneja, 2003).

Assessment of acidity and temperature tolerance potency of *isolates*

For acidity tolerance, the pH level of YEM broth was adjusted by HCl / NaOH and then inoculated with species *Rhizobium* isolate. After completion of 3 days incubation period survival of *Rhizobium* were recorded. The effect of pH on PSB isolates was determined by inoculating isolates on Pikovskaya's agar plates, the pH level of broth was adjusted by HCl/NaOH. After completion of 3 days incubation period survival of PSB were observed for the halo zone formation.

YEM broth inoculated with *Acacia nilotica* –*Rhizobium* isolate were subjected to different temperatures 30-55^0C for 30 minutes in water bath. After thermal shocks, broth cultures were cooled down to room temperature and isolate was inoculated into petriplates containing specific medium with 4 replications. All the inoculated petriplates were incubated in incubator at 28± 2^0C for 2-6 days (Benson, 1990). Observations were recorded for survival and/or growth of inoculums. The effect of temperature on phosphate solubilizing ability of selected phosphate solubilizing isolates was determined by placing PVK broth tubes containing 3-4 days old inoculated bacterial culture in water bath and exposed to 30^0C-55^0C. These bacterial cultures were then spot inoculated on plates containing PVK medium and further observed for clear zone formation by them.

Sample collection, experimental site, transplantation, observation and statistical analysis

Soils were randomly collected from a depth of (15cm) from soil surface from agricultural fields and thoroughly mixed to form a composite sample. A well mixed 5 Kg mixture of soil, sand and compost in 3:1:1 ratio was filled in each polythene bag (12"x 10" size). The soil of the experimental site was vertisol having pH 7.6, organic carbon 6.3 g/kg soil, mineralizable Nitrogen status (204.4 kg/ ha.), low in available (Olsen's) Phosphorus content (11.6 kg/ha.) but higher in status with respect to available Potassium content (416 kg/ha.). The analysis was done as per Page *et al.,* (1982).

Seeds of *Acacia nilotica* were collected from naturally grown area of *A. nilotica* and were allowed to germinate by soaking in water for half an hour and then sown in trays containing field soil and sand (2:1). Uniform size seedlings of 15 days old were selected for experiment purpose. Experimental site Raipur is situated in plains of Chhattisgarh at $21^0$16'

N latitude and $81^0$36’ E longitude with an altitude of 289.60 meter above mean sea level (MSL). Seedlings were inoculated with matured broth of *Rhizobium* and PSB prior to transplantation as per treatments and were allowed to grow up to 3 months /90 Days. In uninoculated pots, seedlings were dipped in same amount of nutrient broth but not inoculated with *Rhizobium* and PSB. Observations were taken at 30 days interval up to 90 DAT of A. nilotica plant. Morphological growth parameters *viz.,* Shoot length, Root length, Collar Diameter of *Acacia nilotica* seedlings were recorded at 30 days intervals after transplant. Also nodulation behaviour in *Acacia nilotica* plants at different stages of transplanting was recorded. The dry weight was recorded after drying in the hot air oven at 70°C to constant weight. All observations were statistically analyzed using ANOVA for CRD. The significant differences were tested through F-test at 5% level of significance (Panse and Sukhatme, 1978).

Results and Discussion

Isolation and colony morphology of *Acacia nilotica* - *Rhizobium* and phosphate solubilizing bacteria

A. nilotica- Rhizobium (isolated from nodule of locally grown nodulated *A. nilotica* plant. On YEMA media *Rhizobium* isolate produced translucent, nearly round and gummy colonies which were Convex in elevation having nearly entire margin varied in size between 1.5 to 2.00 mm (Table 1). After the gram-staining the bacteria assumed a red colour which indicated that it was Gram -ve. Native PSB isolate required for the study was screened from 11 isolates, isolated from rhizospheric soils of *A. nilotica* tree commonly grown in different locations of two districts, Raipur(22.35°N 82.68°E) and Mungeli (22.40°N 81.40^0E) of Chhattishgarh on the basis of their qualitative phosphate soubilization and stress tolerant character. (Tables 1 and 2) Soil sample of known amount were allowed to grow in PVK broth containing 0.05 g tri calcium phosphate in each test tubes in incubator cum shaker at 30℃ temp for 7 days. After efficient growth of microbes in tubes they were streaked on a plate containing PVK agar. The selection of PSB was made after screening the bacterial colonies developed on plates for their morphological similarities to *Bacillus* and *Pseudomonas* genera, as it was reported by Komy (2005). This work is in line with Krishnaveni (2010). Native PSB isolates from 11soil samples were further grow on the Pikovskaya's agar (PKV) plates, out of which only 4 isolates were found capable of forming a clear zone on solid media Pikovskaya. The isolation studies of PSB are in co-ordination with the work done by Krishnaveni (2010) and Kelel (2014). Out of 11 PSB isolates, isolates namely PSB-1, PSB-2, PSB-5 and PSB-10 were found forming clearing zone. Colonies of all the isolates were found to be round and yellowish in colour. All the isolates were gram negative (Table 1).

Selected isolates were identified based on staining, morphological, cultural and biochemical tests according to Bergey's Manual of Systematic Bacteriology and were found belonged to *Pseudomonas* genera. The isolate PSB-2 was observed better growth and clearing zone as compared to other three PSB isolates. The efficacy of P solubilization by individual isolate on agar medium revealed that PSB formed clear zones by solubilising suspended tri-calcium phosphate.

Traditionally, the clearing/halo zone around the colony is used as an indicator for P solubilization. Malboobi *et al.,* (2009) found that the active growth of bacteria correlates well with P solubilization; hence the colony growth diameter of the PSB should be viewed merely as a first indication of the P solubilization potential only.

Acidity and temperature tolerance behavior

Rhizobium isolate of *Acacia nilotica* was observed as potent acidity tolerant isolates and survive as low as pH 5. But pH 6 to 7 was found most suitable pH for its growth and shows survivality upto pH 8 (Table 2). Segura (1995) observed 5 strains of *Rhizobium* out of 50 screened to be tolerant to acidity up to pH 4.5 and 5 in liquid and solid culture. Mpepereki *et al.*, (1997), however observed growth of lathyrus-*Rhizobium* upto even upto pH 4. *Acacia nilotica* - *Rhizobium* can grow well at temperature 30^0C and 35^0C while with increase in temperature growth reduces, but it can also tolerate upto 50^0C temperature. It shows minimum growth at 50 ^{0}C (Table 2).

A total of 04 bacterial isolates (PSB-1, PSB-2, PSB-5 and PSB-10) capable of forming a clear zone on solid media Pikovskaya were studied for phosphate solubilization ability under stress conditions. The effect of pH on phosphate solubilisation was tested by growing strains on PVK medium of which pH was adjusted (from 5 to 8.0) using HCl and NaOH, the isolate PSB-2 has shown solubilization zone in plate assay and good colony growth. *PSB* isolate of *Acacia nilotica* was the most potent acidity tolerant isolates and survive as low as pH 5. pH 6 to 7 was found most suitable pH for its growth and shows survivelity upto pH 8 (Table 2).

Similarly to assess the effect of high temperature, these isolates were kept at 30 to 60 ^{0}C temperature for 30 minutes and then after allowed to grow on PVK plates. *Acacia nilotica* - *PSB* shows full growth at temperature 30 ^{0}C. Growth decreases while increasing temperature from 35^0C to 55^0C. It shows minimum growth at 55^0C. PSB-2 isolates out of 04 isolates was efficient to form solubilization zone. PSB-2 isolates was selected for inoculation purpose as it was found to be potential phosphate solubilizers and stress tolerant (Table 2).

Kelel (2014) studied that PSB colonies isolated from the Acacia plant rhizophere soil demonstrated high phosphate liberating activity and survived at temperatures to 47^oC over a pH range of 4.5 to 8.5. These results provide a strong baseline source for application in agriculture when a bio-fertilizer is required. Surange *et al.*, (1997) also characterized Rhizobium from root nodules of leguminous trees. Identification of stress tolerant crop beneficial microbes is certainly useful in order to formulate those cultures which are able to survive / persist for longer period and work more efficiently under climatic conditions o Chhattishgarh Plains.

Fig.1 Effect of *Rhizobium* and PSB inoculation on nodule no and nodule dry weight in *A. nilotica*

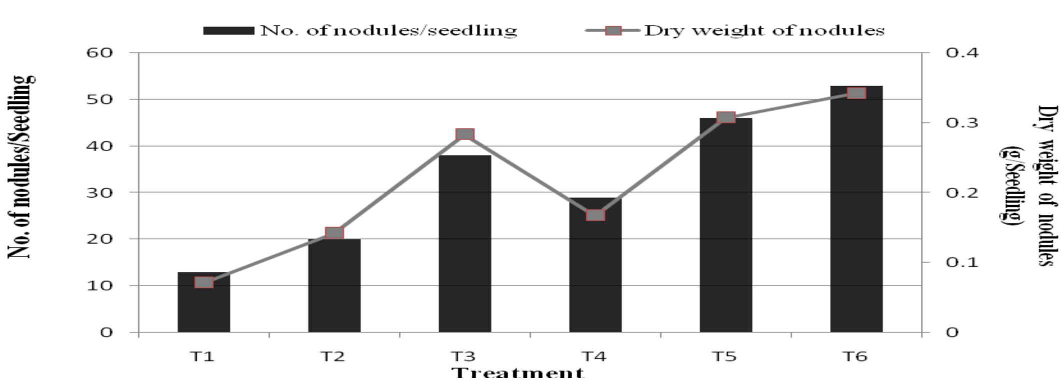

Table.1 Isolation and colony characterization of native *Rhizobium* and PSB isolates for *A. nilotica*

Locations			Isolates	Colony growth	Gram staining	Colony morphology
District	**Block**	**Village**				
Isolation of *Rhizobium*						
Raipur	Dharsiva	Jora	*A. nilotica-Rhizobium*	+++	-ve	Forming Round, White translucent, Nearly Entire margin, Convex (slightly flat) Smooth colony on YEMA medium
PSB isolates forming colony growth on PKV medium						
Mungeli	Mungeli	Sangwakapa	PSB-1	++	-ve	Smooth, round and Yellowish, Showing clearing zone
		Devrikhurd	PSB-2	+++	-ve	Smooth, round and Yellowish, Showing clearing zone
		Kodvabani	PSB-3	+	-ve	Not forming clearing zone
	Lormi	Dumraha	PSB-4	+	-ve	Not forming clearing zone
		Chilfi	PSB-5	++	-ve	Smooth, round and Yellowish, Showing clearing zone
		Sahaspur	PSB-6	+	-ve	Not forming clearing zone
	Pathriya	Pathriya	PSB-7	+	-ve	Not forming clearing zone
		Dharmpura	PSB-8	+	-ve	Not forming clearing zone
		Bhaisatrai	PSB-9	+	-ve	Not forming clearing zone
Raipur	Rajim	Rajim	PSB-10	++	-ve	Smooth, round and Yellowish, Showing clearing zone
		Navapara	PSB-11	+	-ve	Not forming clearing zone

"–'' indicates negative result and "+" indicates positive result (+++ Good growth, ++ Medium growth, + Poor growth)

Table.2 Acidity and thermal tolerance behavior

	Growth in different pH							
	5.0pH	**5.5pH**	**6.0 pH**	**6.5pH**	**7.0pH**	**7.5 pH**	**8.0 pH**	**Control**
selected PSB isolates								
PSB-1	+	+	++	++	++	++	+	++
PSB-2	+	+++	+++	+++	+++	+++	+	+++
PSB-5	+	+	++	++	++	++	+	++
PSB-10	+	+	++	++	++	++	+	++
Growth behavior of *Acacia nilotica - Rhizobium* isolate **in YEMA of different pH**								
	++	++	+++	+++	+++	++	+	+++
	Growth in PVK medium after subjected to thermal shock at different temperature for 30 minutes							
	30 ^{0}C	**35 ^{0}C**	**40 ^{0}C**	**45 ^{0}C**	**50 ^{0}C**	**55 ^{0}C**	**60^{0}C**	**Control**
PSB-1	++	++	++	++	+	-	-	++
PSB-2	+++	+++	+++	+++	+	+	-	+++
PSB-5	++	++	++	++	+	-	-	++
PSB-10	++	++	++	++	+	+		++
Growth in YEMA medium after subjected to thermal shock at different temperature for 30 minutes Growth behavior of isolate								
A.nilotica - Rhizobium	+++	+++	++	++	+	-	-	+++

"–'' indicates negative result and "+" indicates positive result (+++ Good growth, ++ Medium growth, + Poor growth)

Table.3 Inoculation effects on growth parameters of *Acacia nilotica* at 90 Days after transplant

Treatments	Morphological growth parameters		
	Shoot length (cm)	Root length (cm)	Collar Diameter (mm)
T1	58.50	36.50	5.05
T2	63.00	41.75	5.25
T3	76.00	44.50	5.73
T4	69.25	48.75	5.45
T5	77.25	46.50	5.85
T6	78.75	53.75	6.15
SEm (±)	1.39	0.78	0.10
CD (5%)	4.13	2.30	0.30

T_1-Control T_2-Inorganic Fertilization T_3-Rhizobium Inoculation, T_4-PSB Inoculation, T_5-Rhizobium+ 25% N, T_6-Rhizobium + PSB + 25%

Table.4 Inoculation effects on nodulation behavior of *Acacia nilotica* plants

Treatment	No. of nodules/seedling		Fresh weight of nodules (g/seedling)		Dry weight of nodules (g/seedling)	
	Days after transplant		Days after transplant		Days after transplant	
	60	90	60	90	60	90
T1	13.00	6.00	0.41	0.11	0.071	0.002
T2	20.00	8.00	1.18	0.21	0.142	0.004
T3	38.00	16.00	1.32	0.69	0.283	0.074
T4	29.00	12.00	1.22	0.32	0.167	0.052
T5	46.00	20.00	1.41	0.92	0.307	0.076
T6	53.00	22.00	1.52	1.14	0.342	0.088
SEm (±)	1.21	0.88	0.05	0.03	0.01	0.01
CD (5%)	3.61	2.62	0.15	0.08	0.03	0.02

T_1-Control T_2-Inorganic Fertilization T_3-Rhizobium Inoculation, T_4-PSB Inoculation, T_5-Rhizobium+ 25% N, T_6-Rhizobium + PSB + 25%

Rhizobium and PSB inoculation effects on growth parameters of *Acacia nilotica*

Results on morphological growth parameters of *Acacia nilotica* plants treated with different treatments are presented in (Table 3) Dual inoculation along with less N dose significantly influenced the growth of *A. nilotica* followed by *Rhizobium* inoculation along with less N dose.

Shoot length increased by 1.35 and 1.32 times over control with *Rhizobium* + PSB + 25% N (T6) and *Rhizobium* inoculation along with 25% N (T5) treatments respectively at 90DAT. Significantly highest shoot length (78.75cm) was found in T6 followed by 77.25cm in T5 and 76.00cm in T3 at 90 DAT. In Inorganic Fertilization, shoot length was found (63.00cm) whereas at T1 it was 58.50cm.

However effect of *Rhizobium* inoculation was significant with respect to shoot length as compared to only PSB inoculation, inorganic fertilizer treatment and control, but T3, T5 and T6 were at par. Totey *et al.,* 2000 observed application of 5ml *Rhizobium* broth per plant to *A. procera* increased the relative height of 1 year old plantation by 1.2 times over control.

Dual inoculation of *Rhizobium and PSB* along with 25% N dose significantly influenced the root growth of *Acacia nilotica* increasing root length by 1.47 times over control. Significantly highest root length (53.75cm) was found in T6 followed by 48.75cm in T4, 46.50cm in T5 and 44.50cm in T3 whereas at T1 it was 36.50cm at 90 DAT. Inoculated *Acacia nilotica* plants showed significantly higher root length ranging from 44.50 to 53.75cm, at 90 DAT as compared to control and uninoculated ones in which root length were 36.5 to 41.75cm at 90 DAT respectively under T_1 and T_2. It is evident from data that collar diameter of *Acacia nilotica* plants also showed significant effect in dual inoculated treatments over singly inoculation and uninoculated seedlings being maximum (6.15mm) at T6 followed by (5.85mm) at T5, 5.73mm at T3 and 5.45mm at T4 while minimum (5.05mm) at control at 90 DAT. However inoculation of seedlings alone and along with N was found significant for collar diameter in *Acacia nilotica* plants. But the treatments T_1 and T_2 were at par (Table 3).

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 growth. Biofertilizer helps boost microbial population present in soil which in turn makes the insoluble nutrients available for growth of plant. Similar observations were also found by Chauhan and Pokhriyal (2002) in Albizzia. Similar observations were also found by Chauhan and Pokhriyal (2002) in *Albizzia* and Kumar *et al.*, (2013) in *D. sissoo*. Inoculated seedlings significantly showed higher shoot length, root length and collar diameter as compared to uninoculated ones (inorganic N fertilization and control). Among inoculated treatments, *Rhizobium* and PSB inoculation along with N affected much followed by *Rhizobium* + N treated seedlings.

Rhizobium and PSB Inoculation effects on nodulation behaviour of *Acacia nilotica*

The nodular properties like nodule no., nodule fresh weight and nodule dry weight as affected by different treatments and different age of seedlings are presented in (Table 4 and Fig. 1). The no. of nodules per seedling ranged from 6 to 53 under different treatments and at different ages of seedlings. With *Rhizobium* inoculation +PSB with 25% N significantly maximum average nodule no./seedlings was 53.00 followed by only with *Rhizobium* with 25% *N (*38 nodule no./seedlings) whereas very sparse nodulation (13) was seen in control at 60 DAT. With *Rhizobium* inoculation nodules number increase to 38/seedling and further increased to 46 and 53 due to starter dose of N and PSB inoculation along with Rhizobium. *Acacia nilotica* nodules were irregular in shape, elongated and somewhat medium to big size nodules (Table 4) (Fig. 1).

Similar trend was observed in case of nodule fresh weight and nodule dry weight. The effect of dual inoculation along 25% of N was significant over only *Rhizobium* inoculation alone. The fresh weight of nodules varied from 0.11 to 1.52 g/seedling under different treatments at various growth stages of *Acacia nilotica* plant. At 60 DAT significantly maximum nodule dry weight (g/ seedling), 0.342 was found at T6 followed by 0.307 at T5, 0.283 at T3 and 0.167 at T4 while at control it was minimum (0.071).

Inoculation of *A. nilotica with* its specific *Rhizobium* and PSB could bring out an amazing effect so far nodulation, growth and dry matter production of these legumes are concerned. This is an agreement with the report made earlier by Bora *et al.*, (2006). The magnitude of these data showed that inoculation with *Rhizobium* alone and along with PSB significantly influenced the nodular properties at different stages of the seedling.

The present findings showed that both *Rhizobium* and PSB application alone and along with N is comparatively better for improving growth and development of legume seedling *i.e. A. nilotica* as it influences other process such as photosynthesis, uptake of trace element and plant hormones along with nodulation and N fixation. Dual inoculation with *Rhizobium* and PSB treatments were highly significant w.r.t. growth parameters and nodulation in *Acacia nilotica* seedlings as compared to other treatments. Due to inoculation in *Acacia nilotica* shoot length increased from 58.50 to 78.75cm, collar diameter from 5.05to 6.15mm and Nodulation increased 0.071to 0.342 g/seedling. As compared to T4, PSB inoculation, significant effect was obtained at T6 (R+ PSB +25%N) showing maximum nodule no. and dry weight i.e. 53/seedling and 0.342/seedling respectively. Co-inoculation of *Acacia nilotica* with *Rhizobium*+PSB along with application of less N dose (25% of RD) was found significantly effective in increasing the morphological growth and nodulation. This work is strongly supported by Moroque *et al.,* (2002), Mahanty *et al.*, (2004), Krishnaveni (2010), Kelel (2014). This type of study was also carried out by Bora *et al.*, in year 2006 in *A. procera* who studied the combined effect of *Rhizobium* inoculation along with application of different levels of N dosages of fertilizer, observed both root and shoot biomass was considerably high in *A. procera* inoculation seedling growth and in LLU (low level urea).

Based on the results obtained in the present investigation, it can be concluded 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. This finding may be helpful in producing quality planting stock of *Acacia nilotica* for afforestation programmes for the purpose of wasteland management.

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 and N fertilizer application in natural stands. Improvement in seedling quality is essential for the survival of seedlings in degraded areas of low soil fertility and biological activity.

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