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Effect of Bio-Fertilizer on Growth and Nutrient Uptake of *Acacia nilotica* L. Seedlings under Nursery Conditions

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ABSTRACT

An experiment was carried out under nursery management conditions with the inoculation of *Azospirillum brasilense,* Phosphorus Solubilizing Bacteria (*Bacillus polyxyma*) and Vesicular Arbuscular Mycorrhiza (VAM) in single, dual and triple combination on *Acacia nilotica* seedlings to assess their effect on seedling growth, dry weight, nutrient uptake and chlorophyll content. The treatments comprised of T_1- *Azospirillum brasilense*, T_2- PSB (*Bacillus polyxyma*), T_3- *Vesicular Arbuscular Mycorrhiza* (VAM), T_4- *Azospirillum brasilense* + PSB (*Bacillus polyxyma*), T_5- *Azospirillum brasilense* + VAM, T_6- PSB (*Bacillus polyxyma*) + VAM*,* T_7- *Azospirillum brasilense* + PSB (*Bacillus polyxyma*) + VAM*,* T_8- Control. The objective of the study was to determine the best combination of bio-fertilizer which may be useful for better production and establishment of nursery stock of *A. nilotica* resulting in less failure of seedlings thus being economically more advantageous to agro forestry farmers, nursery growers and foresters. The results divulged that the treatment T_7 (*Azospirillum brasilense + Bacillus polyxyma + VAM*) consisting of three bio-fertilizers proved to be most efficacious in production of quality seedlings of *Acacia nilotica.*

Key words: Biofertilizer*, Acacia nilotica, Azospirillum brasilense,* PSB *(Bacillus polyxyma), Vesicular Arbuscular Mycorrhiza*

Acacia nilotica, also called as a Gum Arabic Tree is a common multipurpose species found in Indian subcontinent and widely recognized as 'Babul'. It is considered as one of the principal Nitrogen Fixing Tree (NFT) species belonging to family Fabaceae, sub-family *Mimosoideae*. Babul is one of the most common species which is found in subtropical and humid areas of the earth (Kaur *et al.* 2005, Ahmad *et al.* 2017) and is locally confined to Indian and African continent, particularly in Indo-Pak areas. It is well grown in arid and semi-arid regions and extensively used in the farmlands as linear plantation both public and private plantations (Singh *et al.* 2009). *Acacia nilotica* (Babul) being the major agroforestry species especially for semi-arid and dry areas, is widely planted on farm lands, wasteland, roadsides and generally recommended for afforestation in the entire nation. It provide many useful materials such as gums, silage, tannins, furniture wood, timber wood and is also exercised as medicinal plant (Cooperband *et al.* 2002). It yields nutritious fodder for livestock as well as firewood for daily requirement and wood for multiple purposes (Sarra *et al.* 2005). *Acacia nilotica* (Babul) is a tree of height about 5 to 20 m with a spherical crown which is thick; branches and stems are generally menacing to black collared, greyish pink laceration, bark is fissured giving off reddish inferior gum (Ali *et al*. 2012). The tree has instantly straight, light, thin, grey spines in axillary pairs, usually in 4 to 10 pairs; 4 to 8.5 cm long in immature plants, adult plants generally lack spikes. The leaves are bi-pinnate, with 3 to 6 pairs of pinnulae and 10 to 30 duo leaflets, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2 to 1.5 cm in thickness of an intense golden-yellow

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colour set up moreover axillary on peduncles 2 to 3 cm long situated at last part of the branches while pods are sturdily tensed, white-grey, thick and hairy (Baravker *et al.* 2008).

Nitrogen, phosphorous and potassium are essential nutrients necessary for augmentation of a tree, which are lacking in majority of soils. The extensive use of chemical fertilizers poses serious collateral problems such as environmental pollution, pest resistance development and food safety decline (Lin *et al.* 2020). Usage of chemical fertilizers has led to severe ecological problems as well as deterioration of human health. However, bio-fertilizers provide substitute to chemical inputs and having an ability of mobilizing the nutritionally imperative components from non-useable to useable form (Rani 1999). Bio-fertilizers enhance the seedling characteristics which boost seedlings capacity to survive in unfavorable circumstances as they have tremendous potential to supply plant nutrients by multiplying the number of microbes residing in soil that in turn makes the insoluble nutrients available for the growth of a tree (Kelel *et al*. 2014). In tropical countries, especially the ones like India, where soils are mainly deficit in basic nutrients like Phosphorus and Nitrogen, restitution and sustainable management of fertility status of soil, is an essential and serious environmental dilemma.

The production of good quality seedlings and their establishment in nursery is imperative for getting good survival and better establishment. The above restrictions may be considered by usage of bio-fertilizers like *Azospirillum spp.*, Phosphorus Solubilizing Bacteria (PSB), and *Vesicular Arbuscular Mycorrhizae* (VAM). Bio fertilizers like *Azospirillum*, Phosphorus Solubilizing Bacteria and Mycorrhizae are accomplished of increasing the mineral nutrition of trees and richness of available soil. Microorganisms like *Azospirillum* fix atmospheric nitrogen, Mycorrhizae form symbiotic connotation with plant and make unavailable form of phosphorus to plants (Gurumurthy *et al.* 2014). Phosphorus Solubilizing Bacteria are competent of solubilizing missing shape of phosphorus into contemporary form and make it obtainable to trees (Veena *et al.* 2009). Therefore, the effect of bio-fertilizers on the growth and nutrient uptake of *Acacia nilotica* was assessed in an experiment under nursery conditions.

MATERIALS AND METHODS

A nursery-based trial was experimented at Institute of Agricultural Sciences, Bundelkhand University, Jhansi (Uttar Pradesh). The experiment was conducted in randomized block design (RBD) with 8 treatments replicated three times thus having total 24 replicates. Treatments in the research experiment included T_1- *Azospirillum brasilense*, T_2- Phosphorus Solubilizing Bacteria (*Bacillus polyxyma*), T_3- VAM, T_4- *Azospirillum brasilense* + Phosphorus solubilizing bacteria (*Bacillus polyxyma*), T_5- *Azospirillum brasilense* + VAM, T_6- Phosphorus solubilizing bacteria (*Bacillus polyxyma*) + VAM, T_7- *Azospirillum brasilense* + Phosphorus Solubilizing Bacteria (*Bacillus polyxyma*) + VAM, T_8- Control.

Collection and transplantation

Seeds of *Acacia nilotica* were collected from Jhansi Forest Department and were allowed to germinate by pre-treating with hot water and then sown in trays containing the soil, sand and FYM in the ratio of 2:1:1. *Azospirillum brasilense* and *Bacillus polyxyma* were procured from Biofertilizer Centre, J.N.K.V.V. (Jabalpur) whereas VAM (G.I and A.S.) culture was collected from CAFRI, Jhansi. After ten days, seedlings were transplanted in nursery in Agriculture field of Institute of Agricultural Sciences in polybags containing potting mixture with amendment of 10-10 grams of *Azospirillum brasilense* and *Bacillus polyxyma* and 20 grams VAM in the respective treatment.

Observation and statistical analysis

The observation for growth parameters like height and diameter, at a time gap of 30 days for 180 days consequently, were undertaken whereas total biomass, chlorophyll, nitrogen and phosphorous content at the end of 180 days of sowing was estimated. Nitrogen and phosphorous of the oven dried seedling samples were predicted by Microkjeldahl, using Barton's reagent and Tri acid Mixture, correspondingly (Jackson 1967) respectively, while potassium was estimated using a flame photometer. The chlorophyll content was determined using method of Hiscox and Israelstem (1979). To find out the various effects, the data has been statistically analyzed using randomized block design following the procedure outlined by Panse and Sukhamte (1967).

RESULTS AND DISCUSSION

Diameter

The present research was conducted for the purpose of measuring the impact of bio-fertilizers on growth and establishment of *Acacia nilotica* (Babul) under nursery conditions. It is inferred from the analysis of data that after 180 days of inoculation of bio-fertilizer, combination of *Azospirillum brasilense* + PSB + VAM (T_7) was found to be most efficacious in increasing the diameter of the seedlings. There was a significant increase in shoot diameter over control which was 49.59% in case of T_7, followed by combination of *Azospirillum brasilense* + PSB with 38.39% increase over control (T_8).

Shoot length

In case of shoot length, significant difference was found in *Acacia nilotica* seedlings inoculated with bio- fertilizer in comparison to control. The maximum shoot length was recorded (70.41cm) with T_7 (*Azospirillum brasilense* + PSB + VAM) which is 33.91% more than control whereas the minimum value was recorded in case of uninoculated control i.e. T_8.

Root length

The maximum root length was found 28.33cm with treatment T_7 *Azospirillum brasilense* + PSB (*Bacillus polyxyma*) + VAM which was statistically on par with other treatments followed by T_6 i.e. dual combination of PSB + VAM

(27.04c.m.) and T_5 i.e. *Azospirillum brasilense* + *VAM* (26.53). However, the minimum value (18.65 cm) of root length was found with uninoculated seedlings (T_8).

Root dry weight

From the (Table 1), it is inferred that maximum root dry weight was observed in treatment T_7 (*Azospirillum brasilense* + PSB + VAM) whereas minimum was found with T_0 (control) where no biofertilizer inoculation was done. The percentage increase in root dry weight was about 51.90% in comparison to seedlings where no treatment was given.

Shoot dry weight

As far as shoot dry weight is concerned, the highest value (3.090 gm) was obtained with application of T_7 (*Azospirillum brasilense* + PSB + VAM) which was significantly higher than control (2.48 gm) followed by T_6 (*Azospirillum brasilense* + PSB) (3.41 gm) respectively.

Number of nodules

The maximum number of nodules (39.7) was found with the application of *Azospirillum brasilense* + PSB + VAM (T_7). However, control showed the minimum number of nodules (23.8).

Table 1 Effect of application of bio-fertilizer application on growth, biomass and nodulation of *Acacia nilotica* L. seedlings under nursery condition after 180 days of sowing

Treatments	Collar diameter (mm)	Shoot length (cm)	Root length (cm)	Root dry weight g/plant	Shoot dry weight g/plant	No. of nodules/ seedling
T_1	7.88	52.54	20.48	0.910	2.660	26.1
T_2	7.35	48.85	20.85	0.920	2.510	28.6
T_3	8.45	55.64	21.48	0.990	2.780	31.6
T_4	9.30	58.29	24.52	1.060	2.900	36.6
T_5	9.65	59.39	26.53	1.100	2.980	38.1
T_6	9.52	59.17	27.04	1.170	2.810	38.4
T_7	10.35	62.31	28.33	1.220	3.090	39.7
T_8	6.72	46.53	18.65	0.910	2.480	23.8
SE(m)	0.178	1.389	0.20	0.011	0.026	0.289
C.D.	0.456	4.254	0.61	0.033	0.081	0.886
C.V.	3.568	4.347	1.4	1.812	1.641	1.525

Nitrogen, Phosphorus and Potassium uptake

The amount of nitrogen uptake in the plants inoculated with different bio-fertilizers (alone and in combinations) were observed. The uptake of nitrogen was found highest (67.580 mg/plant) in the seedlings inoculated with *Azospirillum brasilense* + PSB + VAM (T_7) followed by (T_5) *Azospirillum brasilense* + VAM (63.349 mg/plant) in comparison to uninoculated control. While the lowest nitrogen uptake (46.442 mg/plant) was found in T_2 (PSB) inoculated plants when compared to other combination of bio-fertilizers whereas uninoculated control seedlings set yielded minimum value amongst all treatments (Table 2). Interestingly, the combination (*Azospirillum brasilense* + PSB + VAM) showed highest synthesis of nitrogen concentration in the seedlings. All the bio-fertilizers in single and combinly inoculated, revealed almost maximum amount of nitrogen as compared to uninoculated control as depicted in (Table 2).

The amount of phosphorus uptake in the plants inoculated with different bio-fertilizers (alone and in combinations) was observed. The phosphorus uptake was found highest in the *A. nilotica* seedlings inoculated with *Azospirillum brasilense* + PSB + VAM (T_7) (0.0739 mg/plant). While the lowest phosphorus uptake was found with T_1 *Azospirillum brasilense* (0.0527 mg/plant) when compared to other combination of bio-fertilizers (Table 2). Hence, the combination (*Azospirillum brasilense* + PSB + VAM) presents highest phosphorus uptake value in *A. nilotica* seedlings. In case of potassium uptake, the greatest value (37.928 mg/plant) was observed with T_7 (*A. brasilense* + PSB + VAM) which was found to be 36.4% more than (T_0) control whose value was observed 27.798 mg/plant.

The (Table 2) elucidates the content of *chlorophyll* in seedlings of *Acacia nilotica*. The present study provides an insight on the effect of following bio-fertilizers viz. *Azospirillum brasilense*, Phosphate Solubilizing Bacteria (PSB) and Vesicular Arbuscular Mycorrhiza (VAM) separately and collectively in seedlings of *A. nilotica*. The table describes all the bio-fertilizers inoculated plants, either single or in combination, possessed greater amount of chlorophyll as compared to uninoculated control. The content of *chlorophyll a* was observed significantly higher (1.264 mg/g) in seedlings inoculated with T_7 (*Azospirillum brasilense* + PSB + VAM) followed by T_6 (PSB + VAM) (1.258 mg/g), T_4 (*Azospirillum brasilense* + PSB) (1.253 mg/g) in comparison to uninoculated control T_0 (1.120 mg/g). While the greatest chlorophyll b content (0.698) was found with T_7 (*Azospirillum brasilense* + PSB + VAM) inoculated seedlings when compared to other combination of bio-fertilizers and uninoculated control (Table 2). However, in case of total chlorophyll content, the treatment T_7 (*Azospirillum brasilense* + PSB + VAM) yielded the highest value of 1.962 mg/g which was significantly higher as compared to control T_0 (1.799 mg/g).

This experimental research, conducted on effect of bio-fertilizers on growth and nutrient uptake of A. *nilotica* seedlings showed that the inoculation of consortium of three biofertilizers viz. *Azospirillum brasilense*, Phosphorus Solubilizing Bacteria (PSB) and Vesicular Arbuscular Mycorrhiza (VAM) in single, double and triple combination yielded positive response to *A. nilotica* as revealed by results of the study. However, the growth, establishment and nutrient uptake with triple combination of bio-fertilizers produced best result which was significantly superior over un-inoculated control. Bio-fertilizer application was efficient in getting better growth and biomass at the end of 180 days in nursery. Also, the inoculation of consortia of these three bio-fertilizers showed better nutrient uptake and chlorophyll content of babul seedlings. The results obtained are in strong conformity with those of Rajendran and Jayashree (2007), Mohan and Rajendran (2014), Verma *et al.* (2008), Sharma and Chaubey (2015), Mohan and Rajendran (2017).

Table 2 Effect of application of bio-fertilizers on N, P and K uptake and Chlorophyll (a and b) of *Acacia nilotica* seedlings under nursery conditions

Treatments	N mg/plant	P mg/plant	K mg/plant	Chlorophyll a mg/g of fresh plant	Chlorophyll b mg/g of fresh plant	Total Chlorophyll mg/g of fresh plant
T_1	51.646	0.0527	29.631	1.248	0.694	1.942
T_2	46.442	0.0572	29.498	1.224	0.682	1.906
T_3	55.695	0.0575	32.045	1.240	0.690	1.930
T_4	58.766	0.0641	34.452	1.253	0.692	1.945
T_5	63.349	0.0655	35.496	1.250	0.695	1.945
T_6	56.834	0.0701	35.422	1.258	0.692	1.950
T_7	67.580	0.0739	37.928	1.264	0.698	1.962
T_8	43.663	0.0452	27.798	1.120	0.679	1.799

The significant increase in growth parameters like shoot length, collar diameter, root length and biomass are due to increased uptake of phosphorus, potassium and nitrogen, which is evident from the results of the laboratory analysis. The enhanced height, collar diameter and biomass of *A. nilotica* as well as better nutrient uptake, observed due to inoculation of single or multiple bio-fertilizers is attributed to the production of various growths promoting hormones by the beneficial microorganisms such as IAA, Gibberellic acid, and cytokinins (Veena *et al.* 2009) as well as the availability of fixed nitrogen by *Azospirillum*, phosphate solubilization (Akshitha *et al.* 2014), phosphate mobilization by the mycorrhizal fungi (Gurumurthy *et al.* 2014) and the synergistic growth promotion by the consortia of bio-fertilizers used (Bora 2006).

Seedlings treated with *Azospirillum* gave better growth in comparison to control. The results are in conformity with earlier work of Rodriguez-Barrueco *et al.* (1991), who found an increase in growth of *C. cunninghamiana* seedlings *with A. brasilense* as compared to uninoculated control. It can be due to accumulation of nitrogen and increased root biomass (Wong and Stenberg 1979), along with manufacture of chemicals like gibberellin and cytokinin (Tien *et al.* 1979), which altogether aids in better growth of the seedlings. Superior growth and better nutrient uptake over control was observed with application of PSB in the present investigation. Such results have been given by Mohammad and Prasad (1988) in *Eucalyptus camaldulensis* and Young (1990) in *Leucaena leucocephala.* The underlying cause might be transformation of insoluble form of phosphorus into soluble form, which plants uptake due to their readily availability. In present trial, seedlings which were inoculated with VAM, resulted in greater growth and nutrient uptake over uninoculated ones i.e. control. It has been already revealed by Abbott and Robson (1984) that VAM enhances the mineral nutrition of the host seedling, which leads to better growth. It may be ascribed to expanded captivating surface area because of expanded extraneous mycelial network arisen from the interrelationship of Vesicular Arbuscular Mycorrhiza accompanying the root complex of host plant (Howeler *et al.* 1981). In case of combined application of two and three bio-fertilizers, the improved results in collar diameter, shoot height, root length and dry matter of *A. nilotica* seedlings may be on account of enhanced nitrogen concentration owing to Azospirillum (Gunjal and Patil 1992), greater phosphorus solubilisation by PSB (Kuccy 1987) and AM fungi (Young *et al*. 1988).

It is inferred from the evidences of the investigation that the bio-fertilizer application has a profound effect on height, diameter, root growth, nodulation and biomass as well as nutrient uptake of *A. nilotica* seedlings. Although, all bio-fertilizers influenced the growth and nutrient uptake of seedlings but combined application especially combination of three i.e. *Azospirillum brasilense* + PSB (*Bacillus polyxyma*) + VAM was found as the most effective treatment which can save time and expenses to a larger extent for the farmers engaged in tree cultivation, nursery owners as well as foresters.

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