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EFFECT OF BIOINOCULANTS ON QUALITY SEEDLING PRODUCTION OF *AZADIRACHTA INDICA* (A.) JUSS.

C. ALAGESABOOPATHI and K. RAJENDRAN*
Department of Botany, Government Arts College (Autonomous), Salem - 636 007. Tamil Nadu, India.
Email : alagesaboopathi@rediffmail.com
*P.G. and Research Department of Botany, Thiagarajar College, Madurai - 625 009. Tamil Nadu, India.

The present investigation deals with the effect of bioinoculants on quality seedling production of *Azadirachta indica* (A.) Juss. Nursery experiments were conducted to select the suitable bioinoculant and their combinations to improve the quality production of *A. indica*. *A. indica* seeds were treated with biofertilizers and germinated in nursery mother bed with a potting mixture of unsterilized substrate (Sand : Red Soil : Farm Yard Manure) and 7 days after transplanting 10 gm of carrier based bioinoculants applied individually and in combinations with *Azospirillum* + *Azotobacter* + Arbuscular Mycorrhizae. Control seedlings were also maintained for comparing growth performance. Root length, shoot length, basal diameter, biomass, nutrient content, concentration of chlorophyll and protein in plant tissue were estimated after three months of inoculation. The highest growth and biomass in the shoot was recorded in seedlings inoculated with *Azospirillum* + *Azotobacter* + Arbuscular Mycorrhizae followed by inoculation of *Azospirillum*. Enhanced nutrient concentration was found in seedlings inoculated with combination of all treatments. Among all the treatments *Azospirillum* and combination with other biofertilizers was found to be the most effective in increasing the growth, biomass and quality of seedlings.

Keywords : Arbuscular Mycorrhizae; *Azospirillum; Azotobactor;* Bioinoculants; Seedling.

Introduction

Increasing pressure on the demands for timber, fuel, fodder, medicine and insecticide have led to an emphasis on research on *Azadirachta indica* (A.) Juss. *A. indica* commonly called Neem is a fascinating multipurpose tree species belongs to the Meliaceae and is native of India and Burma. From time immemorial its derivatives have found use in agriculture, medicine and live stock production[1]. *A. indica* has a wide adaptability and establishes well in arid and semi arid regions. Although, it is assumed that *A. indica* is capable of sustaining itself even on nutrient depleted soils, some recent studies indicate the importance of soil nutrient in the growth of *A. indica*[2,3]. A major limiting factor in propagation of *A. indica* in nurseries is the slow growth of the seedlings.

Bioinoculants are cost effective, eco-friendly and natural inputs providing alternate source of plant nutrients, thus increasing farm income by providing extra yields and reducing input cost also. Bioinoculants increase crop yield by 20-30 percent, replace chemical N and P by 25 percent, stimulate plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne diseases.

Bioinoculants widely used in agriculture crops. *Azospirillum* is an important non-symbiotic associative, nitrogen fixing rhizosphere bacteria and fixes atmospheric nitrogen in soil[4]. It augments nitrogen fixation[5]. Rice responds well to *Azospirillum* inoculation[6]. Phosphobacterium also produces auxin and gibberellin, which may have favourable effect on plant growth[7]. The simulative effect of phosphobacteria inoculation on plant growth in phosphorus deficient soil has been reported[8]. Inoculation of unsterilized soil with phosphobacteria enhanced collar diameter, fresh weight and dry weight of *Eucalyptus camaldulensis* when compared to uninoculated control[9]. In *Leucaena leucocephala* an increase of 33.2 percent in plant height was observed with phosphobacteria[10].

The soil used for the production of planting stock in nurseries of forest department and local nurseries in Tamil Nadu, India are very low in nutrient content and beneficial microbial population. Though the soil is mixed with farm yard manure (FYM), the quality of seedling is very poor due to insufficiency of desired microorganisms and the rate of mineralization and nitrogen fixation is very low.

Hence, the present study was undertaken to find out the compatibility of different biofertilizers and their augmentation effect on the production of quality seedling.

Material and Methods

Azadirachta indica fruits were collected from a single tree, located in Madurai, Tamil Nadu and seeds were separated, graded and uniform size was used for raising seedlings. Seeds were treated with biofertilizers and seedlings were raised in a mixture of unsterilize, Sand : Red Soil : Farm Yard Manure (2:1:1) in poly pots. Peat soil based culture of *Azospirillum (Azospirillum brasilense)* and *Azotobacter* with a population load of 10^{-9} and 10^{-8} colony forming units/gram of peat soil, respectively, were obtained from Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India. Seven days after germination in the poly pot, 10 grams of peat soil with culture of *Azospirillum* and *Azotobacter,* was inoculated by making holes in the root zone.

Arbuscular Mycorrhiza fungus, *Glomus fasciculatum,* was isolated and recorded as dominant species in the rhizosphere soil of *A. indica.* It was multiplied in pot culture in the sterilized mixture of Sand : Soil (1:1 v/v) and maintained in the roots of *Sorghum vulgare* as the host plant. The inoculum contained extramatrical hyphae, chlamydospores and infected root segments. Inoculum potentials were determined by the most probable number[11] and 12,500 infective propagules (10 gram of vermiculture based) were added in the root zones of each seedling.

Nursery experiment was conducted at the Forest nursery, Puducherry, India. The experiment was set up in a completely randomized design with eight treatments, such as T1- *Azospirillum*; T2- *Azotobacter;* T3 - *Arbuscular Mycorrhiza* (AM); T4- *Azospirillum* + *Azotobacter;* T5 – *Azospirillum* + AM; T6 – *Azotobacter* + AM; T7 – *Azospirillum* + *Azotobacter* + AM; T8 – Control (Sand : Red Soil : Farm Yard Manure alone), and 25 replicates. All the plants were kept under identical nursery condition up to 90 days.

Harvesting and measurement: Ninety days after transplanting from each treatment, a total of 12 seedlings were randomly selected, height and basal diameter were recorded. Seedlings were carefully uprooted without disturbing the root system and washed in the running tap water. Excess of water was wiped out by placing them between the folds of blotting paper. The seedlings were cut at collar region, dried separately at 70°C in paper bags in hot air oven and biomass estimation (root and shoot dry weight) was carried out using top pan electronic balance.

Extraction and estimation of chlorophyll pigments: Leaf samples were collected from each treatment and were used for chlorophyll-a and chlorophyll-b extraction[12]. One gram leaf material was ground in a chilled pestle and mortar in 80% Acetone and the homogenate sample was centrifuged at 3000 rpm for 2 min. Aliquots of 5 ml of 80% Acetone was added to the pellet and centrifuged twice till it becomes non green. The supernatants were pooled and protected from light prior to estimation of chlorophyll pigments. The absorbance of the extract was read at 645 nm and 663 nm. The chlorophyll content was calculated on a fresh weight basis using the formula[13].

Estimation of Protein[14] : 1g of leaf sample was cut into small pieces, ground well in a chilled pestle and mortar in 10 ml of Tris buffer (pH 7.5) and was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken and the pellet was discarded. To the supernatant 5ml of 10% Trichloro Acetic Acid (TCA) was added. The test tubes were shaken and kept in icebox for 2 hours. After this the extract in the test tubes were centrifuged at 6000 rpm for 10 minutes. The supernatant was removed; 5ml of 0.1N NaOH was added to the pellets. From this 1 ml of extract was taken and 4ml of alkaline mixture was added. The test tubes were kept in dark for about 15 minutes. 0.5ml of Folin Phenol reagent was added to this and kept in dark for 10 minutes. A blank was prepared with distilled water and reagents. The O.D. was measured at 620nm. The amount of protein in a given plant material was calculated by using a standard graph prepared with Bovine Serum Albumin (BSA).

Nutrient Analysis: Plant samples were taken for the bio-chemical analysis. The oven-dried plant samples were ground to pass through a 0.5 millimeter stainless steel sieve before digestion.

Estimation of total Nitrogen: 1g of plant sample was digested with concentrated sulphuric acid and catalyst (copper sulphate, potassium sulphate, ferrous sulphate and selenium powder). Digested samples were analyzed colorimetrically[15] using Kjeldahl auto analyser 1030.

Estimation of total Phosphorus: 1g of plant sample was digested with tri- acid mixture with HNO_3 : H_2SO_4 : $HClO_4$ in the ratio of 9:2:1 until it become colourless. After digestion it was filtered and the volume was made up to 100 ml. Phosphorus was estimated colorimetrically using a spectrophotometer[15].

Estimation of total Potassium: 1g of plant sample was digested with tri- acid mixture with HNO_3 : H_2SO_4 : $HClO_4$ in the ratio of 9:2:1 until it became colourless. After

digestion it was filtered and the volume was made up to 100 ml. Potassium in the extract was determined using a flame photometer[15].

Estimation of total calcium and magnesium: 1 g of plant sample was digested with tri-acid mixture with HNO_3 : H_2SO_4 : $HClO_4$ in the ratio of 9:2:1 until it became colourless. After digestion it was filtered and the volume was made up to 100 ml. Calcium and magnesium were determined by the Versenate method[15].

Statistical analysis: The data were statistically analyzed by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test ($P< 0.05$)[16].

Results and Discussion

Statistically, the result revealed that the treatments and their interaction were found to be non significant at $P<0.05$ (Table 1). However, highest collar diameter was recorded in the seedlings inoculated with combined inoculation with *Azospirillum* + *Azotobacter* + Arbuscular mycorrhizae (T7). It was recorded 6.430 % increase over control. Lowest collar diameter was recorded in uninoculated control (T8) seedlings.

Significant increase in shoot length was recorded in *Azadirachta indica* seedlings inoculated with different biofertilizers compared to control at 90 days after inoculation (Table 1). Analysis of data on seedling growth revealed that the combined inoculation of *Azospirillum*+ *Azotobacter* + AM (T7) was found to be most effective in increasing the growth of seedlings, followed by *Azospirillum*+ AM fungus (T5).Among all the treatments, inoculation with *Azospirillum*+ *Azotobacter* + AM (T7) recorded maximum shoot length followed by *Azospirillum*+ AM fungus (T5). These treatments recorded 10.08% and 5.74% increase over control, respectively. *Azospirillum* (T1) inoculated seedlings also showed higher shoot length and was statistically on par with other single inoculation of AM (T3).

Among all the treatments, inoculation with *Azospirillum*+ *Azotobacter* + AM (T7) recorded maximum total length (14.47%) followed by *Azospirillum*+ AM fungus (11.75%) (T5). *Azospirillum* (T1) inoculated seedlings also showed higher total length and was statistically on par with other single inoculation of AM (T3) and dual inoculation of *Azospirillum* + *Azotobacter* (T4) inoculated seedlings.

The data pertaining to shoot and root dry matter accumulation and total biomass are presented in Table 2. The result indicated significant responses in all treatments evaluated at 90 days after biofertilizers inoculation. The highest biomass in the shoot was recorded in seedlings inoculated with *Azospirillum*+ *Azotobacter*+ AM (T7). It was recorded 34.41 % increase over control and was followed by 29.78% increase with *Azotobacter* + AM (T6), which was statistically on par with *Azospirillum* (T1). *Azospirillum* (T1) was found to be more effective in producing shoot biomass than *Azotobacter* (T2) and AM fungi (T3).

Similarly, higher root biomass was obtained in treatment *Azospirillum* (T1) which was recorded 29.55% increase over control seedlings. *Azospirillum* + *Azotobacter* + AM (T7) inoculated seedlings also recorded higher root biomass and it was statistically on par with *Azospirillum*+ AM (T5), which were recorded as 22.78 (T7) and 21.35 % (T5).

The maximum total biomass was recorded in seedlings inoculated with *Azospirillum* alone (T1). It showed a 47.60 % increase over control. It was followed by 27.63% increase shown by inoculation with *Azospirillum*+ *Azotobacter* + AM (T7), *Azotobacter*+ AM (T6) and it was statistically on par with *Azospirillum*+ AM (T5). *Azospirillum* (T1) was more effective in producing total biomass when compared to other treatments (Table 2).

Nitrogen percentage concentration of *A. indica* seedlings inoculated with *Azospirillum*+ *Azotobacter*+ AM (T7) and *Azospirillum*+ AM (T5) was significantly higher than control values. The maximum nitrogen concentration (3.60%) was recorded in T7 treatment followed by T5 (2.63%). Minimum nitrogen concentration percentage was estimated in uninoculated control seedlings (Table 3).

The phosphorus percentage concentration was recorded in the seedlings treated with AM fungi + *Azospirillum* (T5) and was statistically on par with AM fungi (T3) and *Azotobacter* + AM fungi (T6) inoculation (Table 3).

The potassium, calcium and magnesium contents were maximum in the seedling treated with *Azospirillum*+ *Azotobacter* + AM (T7) (Table 3).

The total chlorophyll content was found to be maximum in the seedlings inoculated with *Azospirillum*+ *Azotobacter*+ AM (T7) (1.9194 mg/g fresh leaves) followed by *Azotobacter* + AM fungi (T6) (1.7035 mg/ plant) (Table 4).

Among all the treatments, protein content in tissue of neem seedlings were found to be maximum in the seedlings produced with combined inoculation of *Azospirillum*+ *Azotobacter*+ AM (T7) (0.07548 mg/plant), followed by dual inoculation of *Azotobacter* + AM fungi (0.06660 mg/plant) (Table 4).

A. indica seedlings inoculated with

Table 1. Effect of different biofertilizers on the growth of *A. indica* seedlings (90 days after inoculation).

Treatment	Collar diameter (mm)	Shoot height (cm)	Root length (cm)	Total length (cm)
T1	$3.47^{b} \pm 0.214$ (4.02%)	$38.49^{ab} \pm 0.214$ (3.77%)	$17.33^{a} \pm 0.512$ (22.82%)	$55.82^{a} \pm 0.512$ (9.03%)
T2	$3.36^{ab} \pm 0.261$ (2.25%)	$38.03^{ab} \pm 0.521$ (2.53%)	$14.18^{cd} \pm 0.541$ (0.49%)	$52.21^{cd} \pm 0.541$ (1.07%)
T3	$3.45^{b} \pm 0.365$ (3.697%)	$38.30^{b} \pm 0.651$ (3.26%)	$16.50^{bcd} \pm 0.841$ (16.40%)	$54.80^{bcd} \pm 0.841$ (7.03%)
T4	$3.24^{a} \pm 0.256$ (0.321%)	$38.81^{ab} \pm 0.145$ (4.63%)	$16.83^{d} \pm 0.320$ (19.28%)	$55.64^{ab} \pm 0.320$ (8.67%)
T5	$3.38^{ab} \pm 0.251$ (2.572%)	$39.22^{c} \pm 0.410$ (5.74 %)	$18.00^{d} \pm 0.231$ (27.57%)	$57.22^{d} \pm 0.231$ (11.75%)
T6	$3.48^{ab} \pm 0.241$ (4.180%)	$37.49^{d} \pm 0.521$ (1.07%)	$17.78^{abc} \pm 0.520$ (26.00%)	$55.27^{a} \pm 0.520$ (7.94%)
T7	$3.62^{de} \pm 0.251$ (6.430%)	$40.83^{cd} \pm 0.541$ (10.08%)	$17.78^{ab} \pm 0.320$ (26.00%)	$58.61^{ab} \pm 0.320$ (14.47%)
T8	$3.22^{ab} \pm 0.252$	$37.09^{a} \pm 0.542$	$14.11^{abc} \pm 0.478$	$51.20^{abc} \pm 0.478$

Table 2. Effect of different biofertilizers on biomass of *A. indica* seedlings (90 days after inoculation).

Treatment	Shoot dry weight (gram/plant)	Root dry weight (gram/plant)	Total dry weight (gram/plant)
T1	$2.875^{b} \pm 1.410$ (29.15%)	$1.359^{ab} \pm 0.365$ (29.55%)	$4.834^{a} \pm 0.185$ (47.603%)
T2	$2.346^{ab} \pm 1.250$ (5.39%)	$1.059^{ab} \pm 0.254$ (0.953%)	$3.705^{b} \pm 0.541$ (13.219%)
T3	$2.646^{bc} \pm 0.854$ (16.86%)	$1.071^{bc} \pm 0.541$ (2.097%)	$3.717^{bc} \pm 0.250$ (13.496%)
T4	$2.340^{bc} \pm 0.854$ (5.12%)	$1.061^{ab} \pm 0.652$ (1.143%)	$3.401^{bc} \pm 0.652$ (3.847%)
T5	$2.788^{bc} \pm 0.852$ (25.27%)	$1.273^{c} \pm 0.541$ (21.354%)	$4.061^{bc} \pm 0.652$ (23.060%)
T6	$2.889^{bc} \pm 0.410$ (29.78%)	$1.083^{bc} \pm 0.521$ (3.241%)	$4.072^{bc} \pm 0.520$ (24.335%)
T7	$2.992^{c} \pm 0.410$ (34.41%)	$1.288^{c} \pm 1.410$ (22.78%)	$4.180^{c} \pm 0.632$ (27.633%)
T8	$2.226^{a} \pm 0.362$	$1.049^{a} \pm 0.365$	$3.275^{a} \pm 0.251$

Figures in bracket give percentage increase over control. ± Standard deviation
Means followed by a common letter are not significantly different at the 5% level by DMRT.
Treatments : T1 – *Azospirillum*; T2 – *Azotobacter*; T3 – Arbuscular Mycorrhiza (AM); T4- *Azospirillum* + *Azotobacter*; T5 – *Azospirillum* + AM; T6 – *Azotobacter* + AM; T7 – *Azospirillum* + *Azotobacter* + AM; T8 – Control.

Table 3. Nutrient concentration of *A. indica* seedlings inoculated with different biofertilizers (90 days after inoculation).

Treatment	N%	P%	K%	Ca.me/100g	Mg me/100g
T1	$2.76^{a} \pm 0.235$	$0.090^{ab} \pm 0.584$	$1.37^{ab} \pm 0.541$	$0.096^{a} \pm 0.154$	$0.057^{f} \pm 1.652$
T2	$2.33^{a} \pm 0.256$	$0.103^{ab} \pm 0.541$	$1.30^{a} \pm 0.541$	$0.136^{b} \pm 0.541$	$0.034^{e} \pm 0.214$
T3	$2.20^{a} \pm 0.365$	$0.147^{bc} \pm 0.652$	$1.73^{cd} \pm 0.854$	$0.231^{d} \pm 0.698$	$0.009^{b} \pm 0.321$
T4	$2.37^{a} \pm 0.254$	$0.080^{a} \pm 0.410$	$1.50^{b} \pm 0.698$	$0.160^{c} \pm 0.584$	$0.024^{d} \pm 0.632$
T5	$2.63^{a} \pm 0.41$	$0.083^{a} \pm 0.265$	$1.43^{ab} \pm 0.541$	$0.160^{c} \pm 0.698$	$0.024^{d} \pm 0.425$
T6	$2.23^{b} \pm 0.652$	$0.123^{abc} \pm 0.254$	$1.70^{c} \pm 0.541$	$0.233^{d} \pm 0.415$	$0.020^{c} \pm 0.632$
T7	$3.60^{b} \pm 0.652$	$0.140^{c} \pm 0.651$	$1.87^{d} \pm 0.689$	$0.256^{e} \pm 0.245$	$0.020^{c} \pm 0.632$
T8	$2.23^{a} \pm 0.584$	$0.083^{a} \pm 0.265$	$1.30^{a} \pm 0.740$	$0.160^{c} \pm 0.254$	$0.006^{a} \pm 0.582$

± Standard deviation

Means followed by a common letter(s) in the same column are not significantly different at the 5% level by DMRT.

Treatments

T1 – *Azospirillum*; T2 – *Azotobacter*; T3 – Arbuscular Mycorrhiza (AM); T4- *Azospirillum* + *Azotobacter*; T5 – *Azospirillum* + AM; T6 – *Azotobacter* + AM; T7 – *Azospirillum* + *Azotobacter* + AM; T8 – Control.

Table 4. Impact of bioinoculants on Total chlorophyll and protein content (mg/plant) of *A. indica* seedlings.

Treatment	Protein (mg/g fresh weight)	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total Chlorophyll (mg/g fresh weight)
T1	0.03108	0.5148	0.3874	0.9022
T2	0.03996	0.5498	0.4145	0.5498
T3	0.04884	0.6657	0.5145	1.2155
T4	0.5328	0.7466	0.5874	1.3340
T5	0.06216	0.8274	0.679	1.5064
T6	0.06660	0.9433	0.7602	1.7035
T7	0.07548	1.0592	0.8602	1.9194
T8	0.02222	0.4151	0.3156	0.7307

Treatments

T1 – *Azospirillum*; T2 – *Azotobacter*; T3 – Arbuscular Mycorrhiza (AM); T4- *Azospirillum* + *Azotobacter*; T5 – *Azospirillum* + AM; T6 – *Azotobacter* + AM; T7 – *Azospirillum* + *Azotobacter* + AM; T8 – Control.

Phosphobacterium and AM fungi increased the plant growth and biomass[17]. In the present study also, dual inoculation of AM fungi with other biofertilizers influenced the growth and biomass. It is relevant to mention here that *Azospirillum* + AM fungi by virtue of its capacity to elaborate certain growth promoting substances like IAA and GA might induce the growth of *A. indica* seedlings[18]. Combined inoculation of *Azospirillum* + *Azotobacter* + AM produced excellent growth, biomass and tissue nutrient concentration. The greater height, diameter and dry matter of the *A. indica* seedlings due to co-inoculation of all the biofertilizers might be strongly improved by accumulation of nitrogen due to *Azotobacter*[19], *Azospirillum*[20] and phosphorus by AM fungi.

The total chlorophyll and soluble protein content was found to be maximum in the seedlings inoculated with *Azospirillum*. This increase is in agreement with other findings[21] and was attributed to the greater supply of nitrogen to growing tissues[22]. Similarly, increased chlorophyll and soluble protein content was also recorded in shola species with inoculation of *Azospirillum* + other biofertilizers[23].

It is inferred that under appropriate management the use of more efficient biofertilizers lead to an increased growth and biomass of *A. indica* seedling. The present study have clearly shown that the combined application of *Azospirillum* + *Azotobacter* + AM fungi might play a significant role in improving the growth response and nutrient uptake of *A. indica* seedlings, thereby producing good quality planting stock. These seedlings perform better growth, survival and more biomass production in nutrient impoverished soil.

References

1. Islam M S and Musa A M 1992, Neem ranks high in the Barind tracts of Bangaladesh. *Agroforestry Today* **4** 8.
2. Hedge D M and Durivedi B S 1994, Crop response to biofertilizers in irrigated areas. *Ret. News* **39** 19.
3. Jattan S S, Kumar Pujar S and Gbisht N S 1995, Preservatives in intensive management of Neem population. *Indian Forester* **121** 981-988.
4. Krishnamoorthy G 2002, *Agrolock Editor,* Usha Printers, New Delhi, April - June, pp. 22-24.
5. Vijayakumari B and Janardhanan K 2003, Effect of biofertilizers on seed germination, seedling growth and biochemical changes in Silk cotton. *Crop Res.* **25** 328-332.
6. Karthikeyan S, Anthoni Raj S and Prabakaran J 2003, Role of Biofertilizers in crop plants. *Agrobios Newsletter* **2** 11-12.
7. Somani L L, Bhandari S C, Sexena S N and Gulati I J 1990, *Phosphomicroorganisms - Biofertilizers* (Eds), Somani L L, Bhandari S C, Sexena S N and Vyas K K, pp. 271-294.
8. Asea P E A, Kucey R M N and Stewart J W B 1988, Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. *Soil Biol. and Biochem.* **20** 459-464.
9. Mohammad G and Ramprasad 1988, Influence of microbial fertilizer on biomass accumulation in polypotted *E. camaldulensis* seedlings. *J. Tropical Forestry* **4** 74-77.
10. Young C C 1990, Effects of phosphorus solubilizing bacteria and VAM fungi on the growth of tree species in subtropical - tropical soils. *Soil Sci. and Plant Nutrition* **36** 225-231.
11. Porter W M 1979, The most probable number method for enumerating infective propagules of vesicular - arbuscular mycorrhizal fungi in soil. *Australian J. Soil Res.* **17** 515-518.
12. Yoshida, Forno D A and Cock J H 1971, *Laboratory manual for physiological studies of Rice.* IRRI Publication, Philippines. pp 36-37.
13. Arnon D I 1949, Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaus. Plant Physical.* **124** 1-15.
14. Lowery O H, Rose brough N J, Parr A L and Randall R J 1951, Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193** 265-275.
15. Jackson M L 1973, *Soil chemical analysis,* Prentice Hall of India (Pvt) Ltd., New Delhi.
16. Duncan D B 1955, Multiple range and multiple f. tests. *Biometrics* **11** 1-42.
17. Kalavathy P, Santhanakrishnan P and Divya M P 2000, Effect of VA Mycorrhizal fungus and phosphorus solubilising bacteria in Neem. *Indian Forester* **18** 67-70.
18. Gaur A C and Rana 1990, *Phosphate solubilising microorganisms as biofertilizers.* Omega Scientific Publishers, p. 175.
19. Azcon R, Barea J M and Hayman D S 1976, Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate solubilizing bacteria. *Soil. Biol. Biochem.* **8** 135-138.
20. Gunjal S S and Patil P L 1992, Mycorrhizal control of wilt in *Casuarinas. Agroforestry Today* **4** 14-15.
21. McArthur D A J and Kawlis N R 1993, Influence of Vesicular arbuscular mycorrhizal fungi on the response of potato to phasphorus deficiency. *Plant Physiology* **101** 147-160.
22. Singh M, Jagadish Singh and Kalyan Singh 1983, Effect of phosphorous and biofertilizers on chlorophyll content of leaves and laghaemoglobin content of fresh nodules in Kharif grain legumes. *Indian J. Agro.* **28** 229-234.
23. Sekar I, Vanangamudi K, Suresh K and Suresh K K 1995, Effects biofertilizers on the seedling biomass VAM colonization, enzyme activity and phosphorous uptake in the shola tree species. *Myforest* **31** 21-26.