# ECONOMICS OF BIOFERTILIZER APPLICATION ON PRODUCTION OF PLANTING PROPAGULES OF TEAK IN A COMMERCIAL NURSERY

R.K. VERMA, JAMALUDDIN, V.S. DADWAL AND A.K. THAKUR

*Forest Pathology Division,*
*Tropical Forest Research Institute,*
*Jabalpur (Madhya Pradesh).*

## Introduction

Teak (*Tectona grandis* L.f.) is one of the most important timber yielding tree species of Asian countries particularly India, Indonesia, Myanmar and Thailand. Teak prefers dry tropical climate and its main growth period is restricted to the rainy season. Teak is generally propagated by seeds, which are sown on raised nursery beds (10m x 1m) during summer season. Teak seedlings after being kept for one year in the nursery are dug out and their root shoot cut into 10-15cm long pieces, which is generally called 'stump' and used as planting propagule of teak. The stumps are planted on onset of rainy season during the month of July for raising plantations of teak in India (Tewari, 1992). Thus the success of a commercial nursery of teak depends on production of stumps bed$^{-1}$ and diameter of stumps, as the planters prefer thicker stumps. To improve economics of stumps production by enhancing germination of teak seeds in commercial nursery earlier researchers have tried various techniques like weathering of seeds (Tewari, 1992), seed treatment with plant growth hormones and fungicides (Unnikrishnan and Rajeeve, 1990; Tiwari *et al.*, 2004).

Many bacteria and fungi are present in the soil and play an important role in growth of plants including teak. Till date no work have done to improve economics of production of planting propagules of teak by application of arbuscular mycorrhizal (AM) fungi and bacteria. However, initial works have been done on screening and application of AM fungi on teak (Durga, and Gupta, 1995; Verma and Jamaluddin, 1995; Verma *et al.*, 2001). Presence of *Azospirillum* in the endorhizosphere of teak roots (Verma, 2001) and other bacteria on the surface or in the cytoplasm of AM spores has been reported (Ho, 1988; Mosse, 1962; Tilak *et al.*, 1989; Varma *et al.*, 1981). In the rhizosphere of mycorrhizal roots (mycorrhizosphere) population of these bacteria enhanced (Ames *et al.*, 1984) indicating their role in the growth and development of the plants.

The present work is aimed to study the effect of application of AM fungi, phosphate solubilising bacteria (fluorescent *Pseudomonas*) and nitrogen fixing bacteria, *Azospirillum* on germination of seed and growth of teak seedlings. Economics of application of these biofertilizers on production of teak stumps in a commercial nursery is also worked out.

## Material and Methods

*Experimental Design* : The experiment was factorial (4 x 2); factor one was bacterial biofertilizers while factor two was AM fungi. The experiment was conducted in completely randomized design (CRD) with four replications. Different treatment combinations were as follows :

1. Control,
2. *Azospirillum*,
3. Phosphate solubilizing bacteria (PSB),
4. *Azospirillum* + PSB,
5. Arbuscular mycorrhizal fungi (AM),
6. AM + *Azospirillum*,
7. AM + PSB,
8. AM + *Azospirillum* + PSB.

*Raising of seedlings* : The seedlings were raised from locally collected seeds by Madhya Pradesh Forest Development Corporation at its commercial nursery, located at Belkund, Dhimarkheda, Katni. Seeds were sown @ 2 kg weathered teak fruits (2,600 fruits $kg^{-1}$, each fruit contains 2 seeds on an average) on 10m x 1m raised beds. The pH of nursery soil ranges from 6.9 to 7.5 and is classified as black cotton soil, which is amended with sand and loamy soil to improve the soil porosity. The microbial population of the experimental soil was determined before the experiment (Table 1).

*Preparation of inocula of AM fungi and Azospirillum* : An inoculum of AM fungi containing *Acaulospora laevis*, *A. scrobiculata*, *Glomus intraradices*, *G. etunicatum* and *Scutellospora pellucida* was multiplied in pot cultures using teak as trap plants in sand and soil in 1 : 1 v/v. The mycorrhizal roots of trap plants and rhizosphere soil were thoroughly mixed to get AM inoculum. *Azospirillum* was isolated from teak root collected from local teak plantation. The feeder roots were cut into 1 cm pieces, surface sterilized with 3% $H_2O_2$, for 5 minutes, washed with sterile water and suspended in 15 ml culture tube containing 5 ml semi-solid N free medium containing bromothymol blue and incubated at 30°C for a week. After 3 days the bacterium start to come out into the

**Table 1**

*Microbial population of nursery soil determined before start of the experiment in April 2002*

| Sl. No. | Micro-organisms | Population |
|---|---|---|
| 1. | *Actinomycete* (white colony) | 5.00 x $10^3$ c.f.u./ ml |
| 2. | *Aspergillus niger* | 0.71 x $10^3$ c.f.u./ ml |
| 3. | *Aspergillus* sp. | 1.00 x $10^3$ c.f.u./ ml |
| 4. | *Penicillium* sp. (white-pale wrinkle colony) | 0.33 x $10^3$ c.f.u./ ml |
| 5. | Sterile mycelium (gray-brown colony) | 1.14 x $10^3$ c.f.u./ ml |
| 6. | Fluorescent Pseudomonas | 0.50 x $10^3$ c.f.u./ ml |
| 7. | Other bacteria | 0.50 x $10^3$ c.f.u./ ml |
| 8. | Spores of AM fungi (*Acaulospora laevis*, *A. scrobiculata*, *Glomus etunicatum*, *G. mosseae* and *G. intraradices*) | 156 spores/50ml soil |
| 9. | Nematodes | 244/50ml soil |

medium through cut ends of the root segments and characteristic gray-white ring was observed below 2 mm from the surface with change in colour of medium, from green-yellow to blue after one week. The culture of PSB was prepared in King's medium. Two weeks old bacterial cultures were diluted with saline water to get $10^8$ bacteria $ml^{-1}$ before use.

*Inoculation* : Ten kg inoculum of AM fungi containing 79 infective propagules $ml^{-1}$ (Liu and Luo, 1994) was spread over 10m x 1m raised bed and irrigated immediately to save the inoculum from desiccation. The bacterial suspensions were also sprayed over the bed with the help of rose can.

*Recording of data* : Data on seed germination were recorded after two months of seed sowing and treatment of biofertlizers. Data on plant height, diameter and percentage of root length colonized was determined after five months. For collecting feeder roots for determination of root colonization by AM fungi the seedlings were uprooted with spade and the roots were washed with water carefully. The percentage of root length colonized was determined after staining roots (Koske and Gamma, 1989) by grid-line-intersect method (Giovanetti and Mosse, 1980). After one year the plants were uprooted and root-shoot (stumps) made after discarding cull seedlings. Thus the stumps produced per bed were counted.

*Calculations for economics of stumps production* : Economic benefit of biofertilizers was calculated considering increase in selling price of stumps in terms of increased production due to different treatments of biofertilizers. The profit is calculated after deducting the cost of biofertilizers as well as application cost. A profit index is calculated by combining percentage increase in selling price and percentage increase in collar diameter of seedlings.

*Statistical analyses* : The effect of treatments was analyzed by using a Linear Model ANOVA by using NH analytical software (Statistix, PC, DOS version 2.0, 1987). The means were compared using Duncan's multiple range test (DMRT) for each parameter if "F" value was significant.

## Results

*Germination* : After two months maximum germination was obtained in *Azospirillum* treatment (75.6% more) followed by *Azospirillum* and AM fungi combination (60% more) and *Azospirillum*, AM and PSB combination (46.7% more than control) (Table 2).

*Plant height* : Application of AM fungi alone or in combination with *Azospirillum* and PSB produced statistically the same height of plants, which is highest in AM + *Azospirillum* combination. Among non-AM treatments minimum plant height was in *Azospirillum* treatment, which is 19% less than control (Table 2).

*Diameter of seedlings* : Maximum diameter at collar region of seedlings was obtained in AM + *Azospirillum* treatment (11.8% more than control) followed by AM (6% more), AM + *Azospirillum* + PSB (4.8% more) treatment. *Azospirillum* alone treatment produced statistically less diameter (15.2% less as compared to control) (Table 2).

*Root colonization* : All treatment combinations receiving AM fungi produced significantly higher root colonization as

**Table 2**

*Interaction of biofertilizers on germination (%) of teak seed, height and diameter of seedlings raised on nursery bed (10m x 1m).*

| Biofertilizers application | Control | *Azospirillum* | PSB | *Azospirillum* + PSB |
|---|---|---|---|---|
| Germination after two months (%) : | | | | |
| Control | 4.5c | 7.9a | 6.3ab | 6.0bc |
| AM | 6.0bc | 7.2ab | 5.7bc | 6.6ab |
| No. of seedlings bed$^{-1}$ after two months : | | | | |
| Control | 470d | 820a | 656bc | 624bcd |
| AM | 476cd | 739ab | 616bcd | 683ab |
| Height after five months (cm) : | | | | |
| Control | 34.8bc | 29.2c | 34.6 | 38.5c |
| AM | 40.4ab | 46.3a | 46.2a | 45.6a |
| Dia. at collar region after five months (cm) : | | | | |
| Control | 1.44ab | 1.25b | 1.51a | 1.48ab |
| AM | 1.53a | 1.61a | 1.43ab | 1.49a |
| Root colonization by AM fungi (%) : | | | | |
| Control | 17.5cd | 30.0b | 10.0d | 20.0c |
| AM | 60.0a | 65.0a | 57.5a | 56.3a |

Values in columns and rows followed by the same letter are not significantly different at $p$=0.05.
AM= arbuscular mycorrhizal fungi, PSB= phosphate solubilising bacteria.

compared to any non-AM treatments being maximum in AM + *Azospirillum* treatment (2.7 times more to control). Maximum root colonization by native AM fungi was obtained in *Azospirillum* treatment (0.7 times more) (Table 2).

*Production of stumps* : After five months seedling number per bed was maximum in *Azospirillum* treatment followed by *Azospirillum* + AM fungi and in combined treatment (Table 3). Stumps produced after one year was also highest in *Azospirillum* treatment that was 32% more as compared to control (Table 3).

*Economics and profit index* : Maximum sale price bed$^{-1}$ was obtained in *Azospirillum* treatment, which was 35% more than control followed by

**Table 3**

*Economics of teak stump produced after one-year bed$^{-1}$ (10m x 1m) treated with biofertilizers ignoring the quality (dia.) of stumps.*

| Biofertilizers application | Control | *Azospirillum* | PSB | *Azospirillum* + PSB |
|---|---|---|---|---|
| Propagules (stumps) produced* (number) : | | | | |
| Control | 224.4b | 304.0a | 265.0ab | 281.0ab |
| AM | 283.0ab | 273.0ab | 283.0ab | 287.0a |
| Sale price of stumps** (A) (Rs.) : | | | | |
| Control | 404.0b | 547.2a | 477.0ab | 502.8ab |
| AM | 509.0a | 491.4ab | 509.6a | 516.6a |
| Cost of biofertilizers application*** (B) (Rs.) : | | | | |
| Control | 0.0 | 7.0 | 8.0 | 15.0 |
| AM | 52.0 | 59.0 | 60.0 | 67.0 |
| Net sale price (A-B) (Rs.) : | | | | |
| Control | 404.0b | 540.2a | 469.0ab | 491.3ab |
| AM | 457.4ab | 432.0ab | 449.5ab | 450.1ab |

Values in columns and rows followed by the same letter are not significantly different at $p$=0.05.
AM= arbuscular mycorrhizal fungi, PSB= phosphate solubilising bacteria.
*Cull seedlings excluded,
**Sale price @ Rs. 1.80 stumps$^{-1}$,
***Cost of biofertilizers, AM Rs. 50 bed$^{-1}$, *Azospirillum* Rs.5 bed$^{-1}$ and PSB Rs. 6 bed$^{-1}$ + application cost @ Rs. 2 biofertilizer$^{-1}$.

*Azospirillum*+AM +PSB (27.9% more) and AM+PSB treatment (26% more). The net profit (sale price – cost of biofertlizers including application cost) was significantly higher in *Azospirillum* treatment (33% more) as compared to control (Table 3). Maximum increase in collar diameter was in *Azospirillum* +AM treatment followed by AM and PSB, while maximum selling price was in *Azospirillum*, which was significantly different from the control. The maximum value of profit index was obtained in AM fungi along with *Azospirillum* treatment followed by AM treatment and combination of all of the biofertilizers (Table 4).

## Discussion

AM symbiosis is known to promote acquisition of mineral nutrients especially P by host plants (Bolan, 1991; Cooper and

**Table 4**

*Profit index of teak stumps produced after one year treated with biofertilizers.*

| Biofertilizers application | Control | *Azospirillum* | PSB | *Azospirillum* + PSB |
|---|---|---|---|---|
| Increase in collar dia. (%) (A) : | | | | |
| Control | 0.0ab | -16.4b | 4.4a | 1.6ab |
| AM | 5.9a | 9.5a | -6.3b | 2.5ab |
| Increase in selling price (%) (B) : | | | | |
| Control | 0.0b | 22.2a | 14.3ab | 15.8ab |
| AM | 11.1ab | 10.2ab | 8.6ab | 9.5ab |
| Profit index (A+B) : | | | | |
| Control | 0.0d | 5.8bcd | 17.4ab | 16.7ab |
| AM | 17.0ab | 19.7a | 2.3cd | 12.8abc |

Values in columns and rows followed by the same letter are not significantly different at $p$=0.05.
AM= arbuscular mycorrhizal fungi, PSB= phosphate solubilising bacteria.

Tinker, 1978; Lambert *et al.*, 1979) as a result of additional growth of AM hyphae that enlarges root-soil interface, facilitating efficient exploration of soil around the root (Jacobsen *et al.*, 1992; Li *et al.*, 1991).

Higher root colonization facilitated improved P-uptake that resulted into enhanced height and collar diameter in the plants having higher root colonization or treated with PSB. Development of mycorrhizae in feeder roots and spread of hyphae of AM fungi in the rhizosphere may help in tapping of additional nutrients from soil by mycorrhizal plants as compared to control.

Enhancement in growth of AM-treated seedlings may possibly also be due to mineralization of organic phosphorus by AM fungi (Jayachandran, *et al.*, 1992) and by *Azospirillum* (Seshadri *et al.*, 2000). Phosphate solubilising bacteria also mineralize insoluble phosphorus present in the soil and make available to plants for their growth and development.

Maximum number of seedlings bed$^{-1}$ was produced in *Azospirillum*, this may be due to production of some plant growth hormones by the *Azospirillum* (Bottini *et al.*, 1989, Janzen *et al.*, 1992). Seedlings produced in this treatment are comparatively shorter and thinner (Table 2) most probably due competition of seedlings among themselves for nutrients and space.

Enhancement in height and diameter of seedlings treated with *Azospirillum* is may be due to $N_2$ fixation by the bacterium, which was utilized by the teak seedlings. There is synergistic interaction in AM fungi and *Azospirillum* as both height and root colonization significantly. *Azospirillum* may be considered as arbuscular mycorrhizal helper bacteria. Many plant growth promoting rhizobacteria are also reported as mycorhization helper bacteria (Garbaye, 1994).

In the present study application of AM fungi along with *Azospirillum* produced the maximum profit besides AM fungi has also got other beneficial effects on plant growth, like disease resistance, drought tolerance, etc., therefore this treatment is recommended for production of propagules of teak in a commercial nursery.

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## SUMMARY

**Effect of application of biofertilizers, *Azospirillum*, phosphate-solubilising bacteria (PSB) and arbuscular mycorrhizal (AM) fungi was studied in a factorial experiment on production of planting propagules (stumps) of teak in nursery. Seed germination was maximum in *Azospirillum* treatment followed by its combination with AM and PSB after two months. Maximum height of seedlings was recorded in AM + *Azospirillum*, AM + PSB and AM+PSB+ *Azospirillum* combination after five months. Diameter of seedlings at collar region was maximum in AM, AM + *Azospirillum* and AM + *Azospirillum* + PSB combinations. The economics of biofertilizer application is also worked out. Application of biofertilizers increase the number of stumps produced per bed as well as the diameter of stumps. Therefore, an artificial profit index was calculated combining the both parameters to infer the real benefit of biofertilizers' application. Based on the profit index application of AM fungi along with *Azospirillum* was found the best treatment to produce quality planting propagules of teak in a commercial nursery.**