# APPLICATION OF BIO-FERTILIZERS FOR QUALITY SEEDLING PRODUCTION OF *AZADIRACHTA INDICA* A. JUSS

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## Introduction

*Azadirachta indica* A. Juss, commonly known as Neem, is a multipurpose tree including its medicinal and pesticidal uses. It is indigenous to South Asia. It possibly originated from Northern Myanmar and the Assam region of India and spread to Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia (NRC, 1992). It is also recorded as a wild tree in the Siwalik hills and in the dry forests of Andhra Pradesh, Tamil Nadu and Karnataka and spread all over India except in the foothills of the Himalayas (Mohan Ram and Nair, 1993). Around the world, the tree grows well in tropical and sub-tropical areas (Verkerk and Wright, 1993). It has a wide adaptability and establishes well in arid and semi-arid regions but the limiting factor is the slow growth of seedlings in the nursery. However, the use of bio-fertilizers such as Azospirillum, Phosphobacterium and Vesicular Arbuscular Mycorrhizal fungi (VAM) on *A. indica* can solve the problem of slow growth of seedlings. The asymbiotic dinitrogen fixing bacteria Azospirillum improve the plant growth and yield with Nitrogen fixation and growth promoting substances (Sumner, 1990). Phosphate solubilizing bacteria solubilize insoluble Phosphorus by producing organic acids, which are taken up by plants (Rodriguez and Fraga, 1999). Similarly VAM fungi enhance the uptake and translocation of P and N from the soil to the roots (George *et al.*, 1995). Further, very little information is available about the mycorrhizal status of *A. indica* and its response to various microbial inoculations (Phavaphutanon *et al.*, 1996). Therefore an experiment has been conducted on the influence of bio- fertilizers in *A. indica* with the following objectives.

1. To find out the effect of individual and combined bio-fertilizers : Azospirillum, VAM fungi and Phosphobacterium on the growth of seedlings.
2. To evaluate the quality of seedlings inoculated with bio- fertilizers.

## Material and Methods

The study was conducted at the Silviculture Nursery of Institute of Forest Genetics and Tree Breeding, Coimbatore (Tamil Nadu). The seeds of *A. indica* were collected in Coimbatore from a plus tree and used for this study. The seeds were soaked in water overnight and sown in nursery beds containing sand. Two weeks after germination the seedlings were transplanted into polythene bags (30 cm x 15 cm) containing 3 kg of field soil sterilized with 4 % of Formaldehyde solution.

The bio-fertilizers Azospirillum,

Phosphobacterium and VAM fungi were procured from Tamil Nadu Agricultural University, Coimbatore. A constant weight (15g) of individual or combined bio-fertililzer inoculum was applied to the seedlings in the polythene bags. Totally seven treatments were made and each treatment was replicated 15 times and a control was also maintained as shown in Table 1.

There were a total of 120 (8 x 15) seedlings set up in the nursery in a randomized block design. The seedlings were watered regularly and no nutrients were added.

After 120 days of treatment the plants were harvested and the growth assessments were made. All harvested plants after measurement were then oven dried at 70° C for 48 h for the determination of dry weight.

The seedling quality index (SQI) was calculated according to Dickson *et al.* (1960) by using the following formula :

$$SQI = \frac{\text{Total dry weight (g plant}^{-1})}{\frac{\text{Height (cm)}}{\text{Root collar dia.}} + \frac{\text{Shoot dry wt. (g plant}^{-1})}{\text{Root dry wt. (g plant}^{-1})}}$$

The microbial inoculation effect (MIE) was calculated based on the formula of Bagyaraj (1992) viz.:

$$MIE = \frac{\text{Dry weight of inoculated plants} - \text{Mean dry weight of uninoculated plants}}{\text{Dry weight of inoculated plants}} \times 100$$

Analysis of variance was performed on all data and the means were separated by using Duncan's Multiple Range Test (IRRISTAT, 1993).

## Results

*A. indica* seedlings varied in their response to inoculation with different

**Table 1**

*Details of treatments*

| Treatments | Quantity of inoculum (g) | | |
|---|---|---|---|
| | Single | Double | Triple |
| $T_1$ VAM | 15 | - | - |
| $T_2$ Azospirillum | 15 | - | - |
| $T_3$ Phosphobacterium | 15 | - | - |
| $T_4$ VAM + Azospirillum | - | 7.5 + 7.5 | - |
| $T_5$ VAM + Phosphobacterium | - | 7.5 + 7.5 | - |
| $T_6$ Azospirillum + Phosphobacterium | - | 7.5 + 7.5 | - |
| $T_7$ VAM + Azospirillum + Phosphobacterium | - | - | 5 + 5 + 5 |
| $T_8$ Control | - | - | - |

microbial bio-fertilizers and their combinations. The bi-fertilizer treated seedlings had greater plant height, stem girth, leaf number and root collar diameter than the un - inoculated seedlings (Table 2). Dual inoculation of bio-fertilizers (VAM & Azospirillum, VAM & Phosphobacterium, Azospirillum & Phosphobacterium) increased the plant growth over the control or the individual inoculations of VAM or Azospirillum or Phosphobacterium. Single inoculation of VAM fungi showed significant effect in root collar diameter. The combined inoculations of VAM fungi, Azospirillum and Phosphobacterium significantly increased the seedling growth over the control, individual and the dual inoculated seedlings. Similarly seedlings inoculated with bio-fertilizers showed higher biomass than the uninoculated control seedlings. Comparatively the combined bio-fertilizers, VAM, Azospirillum and Phosphobacterium inoculated seedlings had significantly greater biomass than other treatments. Further, reduced Root/Shoot ratio was found in all bio-fertilizer inoculated seedlings as compared to uninoculated control seedlings (Table 2).

The inoculation of VAM fungi, Azospirillum and Phosphobacterium improved the seedling quality as compared to uninoculated control and over other treatments (Fig. 1). The single VAM inoculated seedlings and VAM fungi with Phosphobacterium inoculated seedlings also showed improved seedling quality than other individual and dual inoculated seedlings (Fig. 1). The microbial inoculation effect ranged between 61.5 and 66.18% for single, 63.3 and 64.1% for double and 72.2% for triple bio-fertilizer inoculated seedlings (Fig. 2). Single inoculation of VAM fungi and combined inoculation of VAM, Azospirillum and Phosphobacterium showed higher percentage of microbial inoculation effect than other treatments and control seedlings (Fig. 2).

## Discussion

This study is a new attempt on the interaction of VAM, Azospirillum and Phosphobacterium in *A. indica*. The study shows the importance of bio-fertilizers for better growth and biomass of *A. indica* seedlings. The results of this study showed that the bio-fertilizers VAM, Azospirillum and Phosphobacterium might improve the plant growth through increased nutrients such as N and P (Safir *et al.*, 1972; Mosse *et al.*, 1973). Earlier reports on different crops also confirmed that these organisms improved the plant growth and biomass (Falik *et al.*, 1998; Negi *et al.*, 1990).

VAM fungi inoculated seedlings showed increased collar diameter due to the infection of VAM fungi in the host root system that extends the root collar maximum (Hooker *et al.*, 1992). The VAM association lowers the R/S ratio of plants as observed in this study. Since R/S ratios are lowered by nutrient application and reduced storage of secondary metabolites, the effect of VAM fungi on R/S ratios may also be due to nutrient accumulation (Hetrick, 1991).

Inoculation with Azospirillum and Phosphobacterium significantly increased the growth of seedlings of *A. indica*. The combined inoculations of Phosphobacterium and Azospirillum on crops like Sorghum (Alaguwadi and Gaur, 1992) and Bajra (Nirmala and Sundaram, 1996) gave significant increase in dry matter and yield over single inoculations. So, these results suggest the use of P solubilizing organisms

**Table 2**

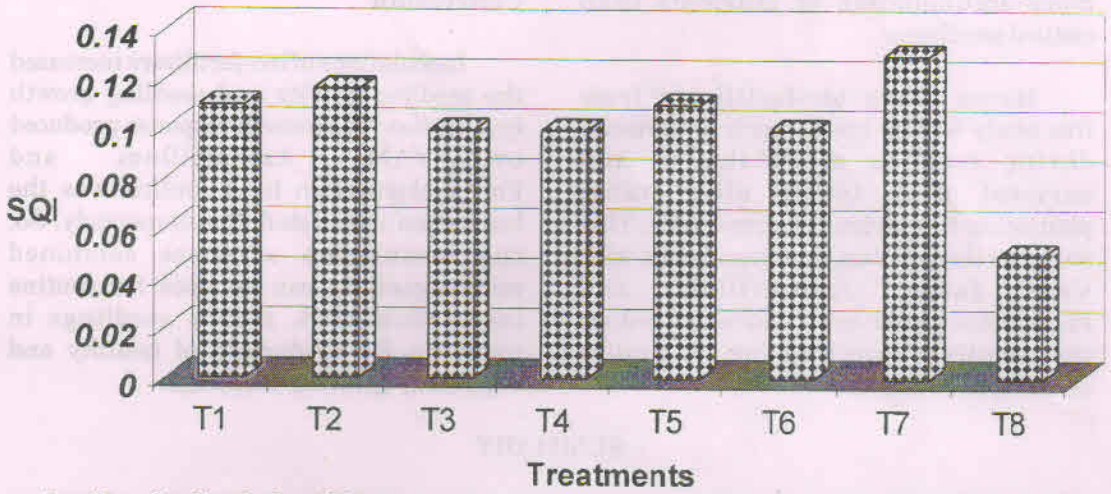
*Growth and biomass of Azadirachta indica seedlings inoculated with bio-fertilizers (Mean of fifteen replicates)*

| Sl. No. | Treatments | Shoot length (cm) | Root length (cm) | No. of leaves/ plant | Stem girth (cm) | Root collar dia. (cm) | Biomass (g/plant) | | R/S ratio |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Shoot (S) | Root (R) | |
| 1. | $T_1$ (VAM fungi) | 13.14 b | 17.14 b | 9.2 bc | 1.90 bc | 2.10 c | 1.51 d | 0.51 bc | 0.33 b |
| 2. | $T_2$ (Azospirillum) | 14.09 c | 17.24 bc | 8.8 b | 1.80 b | 1.93 b | 1.33 b | 0.49 b | 0.36 bc |
| 3. | $T_3$ (Phospho-bacterium) | 14.26 c | 17.98 bc | 9.6 bc | 1.88 bc | 2.04 b | 1.45 cd | 0.52 c | 0.35 bc |
| 4. | $T_4$ (VAM fungi + Azospirillum) | 15.67 d | 18.52 bc | 10.8 cde | 2.03 c | 2.14 c | 1.40 bcd | 0.53 c | 0.37 c |
| 5. | $T_5$ (VAM fungi + Phospho-bacterium) | 15.58 d | 18.33 bc | 11.8 de | 2.08 c | 2.25 c | 1.41 bcd | 0.54 c | 0.38 c |
| 6. | $T_6$ (Azospirillum + Phospho-bacterium) | 15.55 d | 18.62 c | 10.4 bcd | 2.06 c | 2.24 c | 1.37 bc | 0.54 c | 0.39 c |
| 7. | $T_7$ (VAM fungi + Azospirillum + Phospho-bacterium) | 20.39 e | 22.99 d | 12.6 e | 2.38 d | 2.67 d | 1.91 e | 0.61 d | 0.31 a |
| 8. | $T_8$ (Control) | 8.89 a | 10.09 a | 4.6 a | 1.49 a | 1.79 a | 0.47 a | 0.23 a | 0.48 d |

Means followed by same letters are not significantly different at 5% level by Duncan's Multiple Range Test.

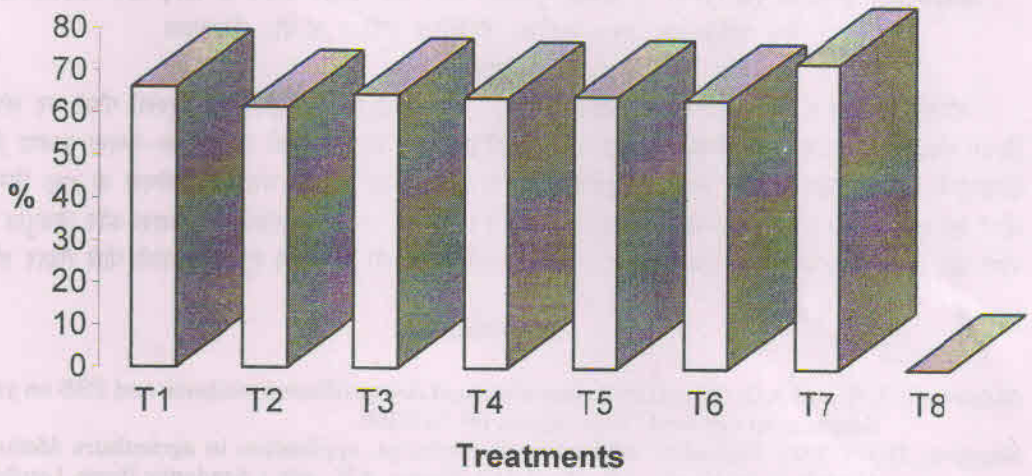
either alone or in combination in getting higher yield besides saving the high cost phosphate fertilizer in put. The greater biomass of bio-fertilizer inoculated seedlings is due to sufficient accumulation of nutrients during the growth of seedlings, which leads to improved seedling quality. The higher per cent of microbial inoculation in the VAM and combined bio-fertilizer inoculated seedlings is due to the microbes that improved the *A. indica* growth and biomass in the early phase of seedlings by colonization of roots which results in to

Fig. 1




Seedling Quality Index (SQI) in *Azadirachta indica* seedlings inoculated with bio-fertilizers

Fig. 2




Microbial inoculation effect of *Azadirachta indica* seedlings inoculated with bio-fertilizers

$T_1$: VAM fungi; $T_2$: Azospirillum; $T_3$: Phosphobacterium; $T_4$: VAM fungi + Azospirillum;
$T_5$: VAM fungi + Phosphobacterium; $T_6$: Azospirillum + Phosphobacterium;
$T_7$: VAM fungi + Azospirillum + Phosphobacterium; $T_8$: Control

more accumulation of nutrients than control seedlings.

Hence these bio-fertilizers from this study will be given much importance during seedling establishment and survival of *A. indica* after transplantation to nutrient stressed soils. This study further implies that inoculation with VAM fungi, Azospirillum and Phosphobacterium in *A. indica* can reduce the fertilizer requirement in quality seedling production.

## Conclusion

Inoculations of bio-fertilizers increased the seedling quality and seedling growth in *A. indica*. The growth response produced by VAM, Azospirillum and Phosphobacterium in *A. indica* was the best when inoculated simultaneously. So, the inoculation of these combined microorganisms can be used for routine inoculation of *A. indica* seedlings in nurseries for production of healthy and vigorously growing seedlings.

## SUMMARY

**An experiment was conducted on *Azadirachta indica* A. Juss inoculated with bio-fertilizers under nursery conditions. The bio-fertilizers VAM fungi, Azospirillum and Phosphobacterium were inoculated individually or in various combinations in sterile soil along with *A. indica* seedlings. Then the seedlings were harvested at 120 days after transplantation. The bio-fertilizer inoculated seedlings resulted in increased plant height, root collar diameter and biomass. The combined microbial inoculations resulted in quality seedlings in nursery.**

*अजेडिरेक्टा इण्डिका* ए० जुस्सो के अच्छी गुणवत्ता वाले पौधे तैयार करने के लिए जैव–उर्वरक देना

ए० कार्तिकेयन, एम० मारिया डोमिनिक सेवियो व बी० दीपाराज

सारांश

रोपणी दशाओं में जैव–उर्वरकों का टीका लगाए गए *अजेडिरेक्टा इण्डिका* ए० जुस्सो पौधों पर संपरीक्षण किया गया। वैम कवक, एजोस्पिरिलियम और फॉस्फोबैक्टीरियम जैव उर्वरकों का पृथक–पृथक अथवा विभिन्न संयोगों में लेकर क्लीव मृदा में लगे अ० इण्डिका के पौधों में टीका लगाया गया। प्रतिरोपण के 120 दिनों बाद पौधों को काट लिया गया। जैव–उर्वरकों का टीका लगे पौधों की ऊंचाई, मूलसंधि पर व्यास और जैवपुंज ज्यादा रहते पाए गए। मिलाजुलाकर जीवाणुओं का टीका लगाने से रोपणी में अच्छी गुणवत्ता वाले पौधे तैयार हो गए।

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