Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 3, No. 4, p. 39-45, 2013
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Seedling quality, biomass promotion and nutrient uptake potential of AMF, *Azotobacter* and *Pseudomonas* in *Azadiracta indica* under nursery condition

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Article published on April 25, 2013

**Key words:** Microbial inoculants, root infection, seedling quality, nutrient uptake.

**Abstract**

The efficacy potential of Arbuscular-Mycorrhizal fungi (*Glomus interaradics* and *Acaulospora scrobiculata*), Phosphorus Solubilizing Bacteria (*Pseudomonas straita*) and *Azotobacter (Azotobacter chroococcum*) were determined in *Azadirachta indica* seedling. Fifty gram AMF, 10g *Pseudomonas* and 10g *Azotobacter* were inoculated in rhizosphere of newly recruited seedlings individually or in integration. Seedling quality index enhanced significantly recorded maximum by 62.56% and seedling vigour by 63.85 percent with tripartite inoculation of AMF+PSB+*Azotobacter* over uninoculated seedling. Similarly, the seedling biomass was also enhanced substantially up to 72.42% with the same treatment. The biomass increment was also observed in seedlings inoculated with *AMF+Azotobacter* (60.0%), AMF+PSB (24.78%), and PSB+*Azotobacter* (17.60%) in comparison to control plants. The seedling dependency to microbial inoculants was found highest of 72.13% on AMF+PSB+*Azotobacter* followed with AMF+*Azotobacter* (60%). The microbial tripartite gave best results in production of superior quality planting material than with bipartite inoculation. The percent root infection by AMF recorded maximum 66.0% in seedling with AMF+*Azotobacter* followed with AMF+*Azotobacter* compared to control seedlings (7.66%). Similarly the integration of AMF+PSB+*Azotobacter* was found most effective in the uptake of nitrogen, phosphorus and potassium content which were increased by 42%, 105% and 137% respectively. The highest content of Fe (10.71 µg/g), Zn (0.18 µg/g) Mg (16.74 µg/g), Mn (0.98 µg/g) and Cu (0.12 µg/g) were analysed on inoculation with AMF, PSB and *Azotobacter*. The combined inoculation of AMF+PSB+*Azotobacter* was given best results in improving the quality of seedling, biomass and nutrient uptake of *Azadirachta indica* which has opened the new implications for their use in healthy seedlings production in nursery for the supply of ever increasing demands for afforestation and reforestation.

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## Introduction

Neem (*Azadiracta indica* A. Juss) is considered as sacred species by Indian's from ancient time. It is projected as an elegant species because of its pesticidal and medicinal virtues planted in large scale by community development and social forestry department. It is known to clean the environment of industrial and polluted area, conserve soil, augment aesthetic value, provide shelter in summer and improves physical and chemical properties of soil. The leaves and seed kernel of the species is being used as major ingredient in formulations against several insects of vegetable and field crops (Bramhachari, 2004). Moreover the leaves have good fodder value and the tender shoots are commonly used as tooth brush due to medicinal values of the species. In addition, it is worshiped by rural people represent goddess Durga and planted in front of their house. Due to these, there is an interest in society for raising Neem tree on bund of agriculture field, degraded forest area, near village, bund of ponds and on wastelands.

Since the tree is important for human and animal (Ambe, 2001) large scale afforestation is under taken every year to achieve plantation target but just after one season the majority of plants did not survive due to no use of fertilizers, use of poor quality planting materials, dependence on monsoon rain and non adoption of scientific method at the time of planting. Querejeta *et al.* (2003) also identified nutrient deficiency and water stress as major limiting factors for plant survival and growth after transplanting in tropical soils. Moreover presently the soil is becoming fragile by increased use of pesticides, pressure of population, management system and pollution unable to maintain its fertility and support plant growth. In this situation either regular fertilizer application and irrigation are required to maintain the growth or survival of plant or the production of seedlings of superior quality is required for reversing the current degradation of soil and forests (Pindi, 2011).

The microbial technology is emerging as new tools provides guarantee for production of quality nursery stock and highest survival in fields by reducing the transplanting sock and increase the nutrients uptake and water (Khalafallah and Abo-Ghalia, 2008). The technology consists plant growth promoting rhizotrophic microorganism bacteria and fungi that actively colonize plant root, fix atmospheric nitrogen, solubilize insoluble phosphate in soil and secretes some growth promoting substances for plants and capable of accelerating microbial transformation to facilitate more availability of nutrients, its uptake and enhance physiological process.

Naturally the plant interacts with many microorganisms AMF (Arbuscular Mycorrhizal Fungi), phosphorus solubiling microorganism, rhizobia and with other free living microorganisms (Miransari, 2011) to fulfill the requirements of nutrients and water apparent result is not seen because of the presence of low and ineffective microorganism in soil. Thus it is imperative to use the potential of soil microorganisms in production system by selecting their best combinations. It becomes thus possible to encourage healthy cultural systems without using chemical fertilizers in forestry and to sustain a better productivity and ecosystem conservation. From screening of literature it is also revealed that research on comparative study of different microbial inoculants in forest species is also not much available. In this context the present study was conducted to determine plant growth promoting potential of AMF, *Azotobacter* and Phosphorus solubilizing microorganism on the biomass and nutrient uptake of *Azadirachta indica* under nursery condition.

## Material and methods

*Study site*

The nursery soil used in experiment was sandy loam collected form pasture stand of State Forest research Institute campus, Jabalpur (India) lies between 23° 10'N and 79° 59'E. The soil was passed with 2mm size

sieve then filled in polythene bag of size 13cm X 23cm size.

Seed of *Azadirachta indica* collected from Katni provenance were sown in bags.

*Isolation and identification of AMF*

AMF (Glomus interaradics Schenck and Smith and Acaulospora scrobiculata (Trappe) Morton) isolated from rhizosphere soil of same provinance was isolated and multiplied in Panicum maximum grass for 8 months period in pot culture and used in this experiment.

*Inoculation of microbial culture*

AMF 50g consisted substrate, spores, infected root fragments and hyphae were placed in root region of recruit in each polythene bag at 5-10cm just below the soil surface. Non inoculated plants received the same amount of autoclaved soil with a 10 ml water extract of the soil by filtration as controls to balance composition of the microbial community (Jansa *et al*., 2005). The inoculum load calibrated by the MPN method was 0.17X $10^4$ per 25g of inoculum. Ten gm Phosphorus Solubilizing Bacteria (*Pseudomonas straita*) and 10g *Azotobacter chrococum*. (Inoculum load 2.17 X $10^6$) were also inoculated in seedlings as per experimental design.

*Experimental Design*

The experiment was arranged in Randomized Block Design with five replications contain ten seedlings in each replicate. The treatment was arranged as T0- Uninoculated control, T1-AMF, T2, PSB, T3- *Azotobacter*, T4-AMF+PSB, T5- AMF+*Azotobacter*, T6- PSB+*Azotobacter*, T7- AMF+PSB+*Azotobacter*.

*Data Record and observations*

After 150 days of inoculation four seedlings were selected randomly from each replicate for observing the biomass and AMF root infection in *A. indica*. Plant material was dried in an oven at 70°C for 7 days and weighed to determine biomass production of the seedling. The seedling quality is calculated as per formula given by Dickson *et al*., (1960) while seedling vigour of the seedling was determined by Qualls and Cooper (1968). The plant dependency to different microorganisms was calculated by expressing the difference between the total dry weight of treated plants by particular microorganism and the dry matter weight of non treated plants as a percentage of the total dry weight of treated plants according to Plenchette *et al*. (1983).

*Detection of AMF in soil and roots*

Percent root infection by AMF was calculated by cleaning the root fragments in 10% KOH and stained with 0.05% trypan blue according to the method of Phillips and Hayman (1970). The stained roots were examined under microscope to observe the presence of intracellular mycelium, vesicle and arbuscular in root pieces and calculated as length of cortical cells colonized (in mm) by the AMF fungi for each root segment and expressed as a percentage of total root length infected (Mc Gonigle *et al*., 1990).

*Analysis of nutrient content in plant*

Total N was analyzed by Auto Kjeltech, P by spectrophotometer and K by flame photometer as per Jackson (1973). The content of Zn, Cu, Mg, Fe and Mn were determined by means of an atomic absorption spectrophotometer GBC (John, 1970). All experiment data were statistically analysed by using SPSS Window software.

**Results and discussion**

*Effect on Seedling quality, Vigour index and Biomass*

The seedling quality, vigour and biomass production in *A. indica* inoculated with AMF, PSB and *Azotobacter* were compared to the control plants are given in Figure 1. Seedling quality index enhanced significantly and recorded maximum 62.56% with AMF+PSB+*Azotobacter*, 55.29 percent with AMF+*Azotobacter* and 54.21 percent with PSB+*Azotobacter* inoculation than control seedlings (Table -1). The vigour index of seedling was influenced maximum of 63.85 percent with the inoculation of AMF+PSB+*Azotobacter* over uninoculated seedling. The treatment of AMF either individual or in integration with other microbial

culture was found more effective on quality index, vigour index, root shoot ratio and biomass of the seedling as compared to PSB and *Azotobacter* inoculation.

The integration of tripartite microbial inoculants gave maximum result on all the parameter of the present study declined subsequently to bipartite and single microbe inoculation. The rhizotrophic microorganism viz. AMF, PSB and *Azotobacter* inoculated with *A. indica* made congenial environment, protected from diseases (Lax *et al*., 2011), increase absorptive capacity by AMF mycelium network (Hassan et al., 2012), compensated stress and drought resulted into enhanced uptake of nutrients which converted into higher biomass and improved the quality parameter of the seedlings. The same trend of influence was reflected in seedling biomass as observed in seedling quality index and vigour with AMF+PSB+*Azotobacter*. The biomass enhanced maximum 41.90 percent with AMF+PSB + *Azotobacter* followed with AMF*Azotobacter* and AMF+PSB respectively. Non-inoculated control plants exhibited very weak shoot growth and produced little biomass compared to the inoculated plants. The absence of the AMF in soil and root and other microorganisms is a factor that could limit seriously the growth and the development of control plants. Practically these conditions, the lack of growth with little biomass production in control plants could lead to a higher mortality of plants (Chen *et al*., 2008). It was also observed that the inoculation of tripartite microbial organisms influenced to higher biomass than bipartite and individual microbial organism.

The synergistic interaction effect of AMF+PSB+*Azotobacter* rendered higher benefit to seedlings rather competing by way of higher N fixation, solubilization of insoluble P and facilitated higher uptake of nutrients to seedling. These findings substantiate the reports of several researchers ( Zaidi, 2003, Roy and Srivastava, 2011, Raman, 2012). The dual inoculated plants also had positive effects on biomass and nutrients uptake as compared to single microbial inoculated plants and non inoculated control plants. *A. indica* was the most dependent tree for juvenile growth to different microbial inoculants ranged 12.31 to 72.13 percent.

However the plant depended maximum 72.13 percent on AMF + PSB + *Azotobacter* followed by AMF + *Azotobacter* (60 percent) and PSB + *Azotobacter* (45.90 percent) respectively (Figure 2).

**Table 1.** Effect of different microorganisms on quality parameters of *A. indica* seedlings. The data represent ± Standard Error (n=20).

| Treatments | Dickson Quality Index | Seedling Vigour index | Root Shoot Ratio |
|---|---|---|---|
| T0 | 0.76±0.03 | 32.30±3.22 | 0.213±0.006 |
| T1 | 0.93±0.04 | 52.45±3.50 | 0.550±0.004 |
| T2 | 0.88±0.01 | 46.80± 1.78 | 0.550±0.010 |
| T3 | 0.89±0.03 | 41.50±2.50 | 0.495±0.004 |
| T4 | 1.55±0.05 | 62.00±3.00 | 0.620±0.006 |
| T5 | 1.70±0.02 | 67..00±2.20 | 0.670±0.009 |
| T6 | 1.66±0.05 | 60.25±1.30 | 0.560±0.006 |
| T7 | 2.03±0.06 | 89.35±2.80 | 0.780±0.010 |

**Table 2.** Nutrient contents in *A. indica* seedlings as influenced by microbial inoculants.

| Treatments | N (%) | P (mg kg-1) | K (mg kg-1) |
|---|---|---|---|
| T0 | 0.152±0.005 | 10.60±2.50 | 137.12±6.50 |
| T1 | 0.182±0.004 | 20.20±3.00 | 179.97±5.56 |
| T2 | 0.176±0.004 | 19.82±0.55 | 171.40±6.35 |
| T3 | 0.193±0.002 | 19.18±0.40 | 175.69±4.30 |
| T4 | 0.209±0.004 | 20.10±0.80 | 257.10±9.00 |
| T5 | 0.210±0.003 | 21.20±0.28 | 287.10±6.50 |
| T6 | 0.201±0.004 | 19.90±0.12 | 201.40±6.50 |
| T7 | 0.216±0.005 | 21.78±0.11 | 325.60±6.11 |

The data represent ± Standard Error (n=5).

*Effect on AMF development in roots:* The mycorrhizal development in root observed higher in

inoculated seedlings than non inoculated seedling. The percentage AMF root infection was highest with AMF+*Azotobacter* followed in seedlings with AMF+PSB+*Azotobacter* (Figure 3).

The rate of AMF colonization was statistically at par for above treatments however it was lower as compared to bipartite inoculated seedling might be due the higher availability of soil P due to *Pseudomonas strait* as also profounded by other researchers (Smith and Smith, 2008, Ren *et al.*, 2012) that the increasing status of soil P limit the development of AMF in root.

The AMF inoculated seedling exhibited maximum of 87.87 percent improvement with AMF+*Azotobacter* followed with AMF+PSB+*Azotobacter* and AMF+PSB. AMF inoculated seedling exhibited 53 percent root infection as compared to uninoculated seedlings (7.5 percent) due to the presence of indigenous endophytes.

**Table 3.** Nutrient contents in *A.indica* seedlings influenced by microbial inoculants.

| Treatments | Nutrient content (µg dry weight g-1) | | | | |
|---|---|---|---|---|---|
| | Fe | Mg | Mn | Cu | Zn |
| T0 | 6.56±0.10 | 15.60±0.04 | 0.72±0.01 | 0.089±0.001 | 0.090±0.003 |
| T1 | 8.53±0.30 | 16.16±0.05 | 0.85±0.02 | 0.110±0.003 | 0.110±0.003 |
| T2 | 7.80±0.07 | 15.80±0.05 | 0.76±0.02 | 0.104±0.002 | 0.090±0.002 |
| T3 | 7.91±0.22 | 16.54±0.08 | 0.85±0.03 | 0.108±0.001 | 0.102±0.002 |
| T4 | 8.25±0.07 | 16.60±0.01 | 0.86±0.01 | 0.100±0.003 | 0.101±0.003 |
| T5 | 9.66±0.13 | 16.10±0.05 | 0.92±0.01 | 0.121±0.002 | 0.101±0.003 |
| T6 | 7.45±0.05 | 16.01±0.02 | 0.83±0.02 | 0.081±0.002 | 0.170±0.003 |
| T7 | 10.71±0.11 | 16.74±0.10 | 0.98±0.02 | 0.121±0.003 | 0.180±0.002 |

The data represent ± Standard Error (n=5).

*Effect on Nutrients uptake*

The nutrients contents of major and micro nutrients in seedlings were influenced significantly by interactive potential of microbial inoculants compared to the non-inoculated seedlings (Table 2). Seedlings inoculated with tripartite of AMF+PSB+*Azotobacter* exhibited highest improvement in N (42.0%), P (105%) and K (137%) contents followed by AMF+ *Azotobacter* (N 38.15%, P 100 % and K 109.37%) over uninoculated seedlings. The increment in NPK contents by AMF alone found 19.7%, 90.56% and 31.35% respectively. The inoculation of AMF and its interaction with other microbial inoculants was proved to be most effective in nutrients uptake in *A. indica* seedling in the present study. The higher nutrient content in inoculated seedlings might be due the increased extra N fixation by *Azotobacter*, higher P availability (Raman, 2012) and mobility of nutrients via mycelium network of the AMF resulted in to better nutrient and water uptake as also reported by Li *et al.* (2009), Kahiluoto *et al.* (2012). Similarly, the Fe content increased maximum of 63.26%, Mn (36.11%), Cu (35.95%) and Zn (100%) in seedlings inoculated with AMF+PSB+*Azotobacter* over uninoculated seedlings (Table 3). The AMF inoculated seedling developed strong symbiosis between host and fungus resulted in to mycorrhizal roots take up nutrients more efficiently than uncolonized root system. Similar findings were also reported by Smith and Read (2008), Kahiluoto *et al.* (2012).

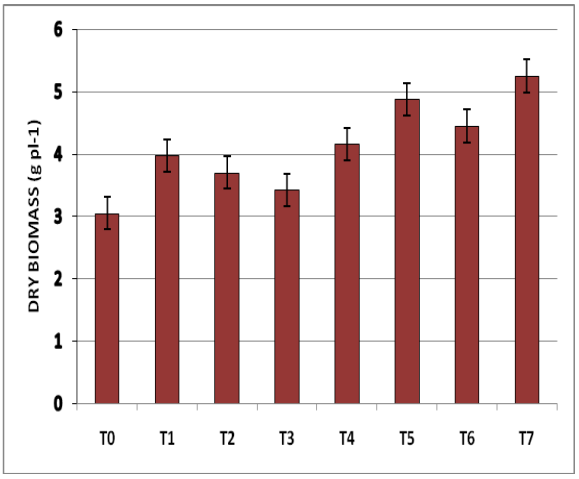


**Fig. 1.** Effects of AMF,PSB and *Azotobacter* on seedling biomass of *A. indica* seedlings under nursery condition.

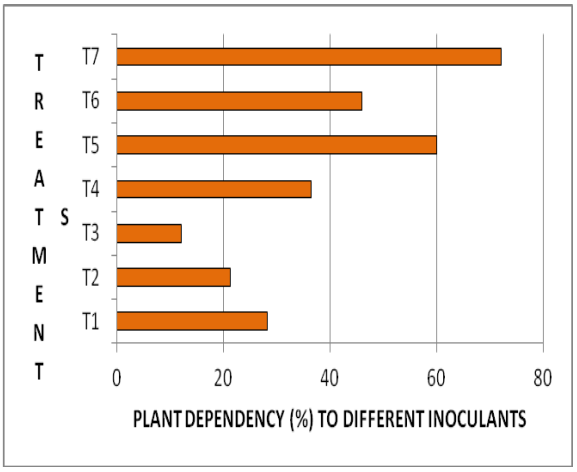


**Fig. 2.** Percentage Dependency of A. indica on different microorganisms under nursery condition.

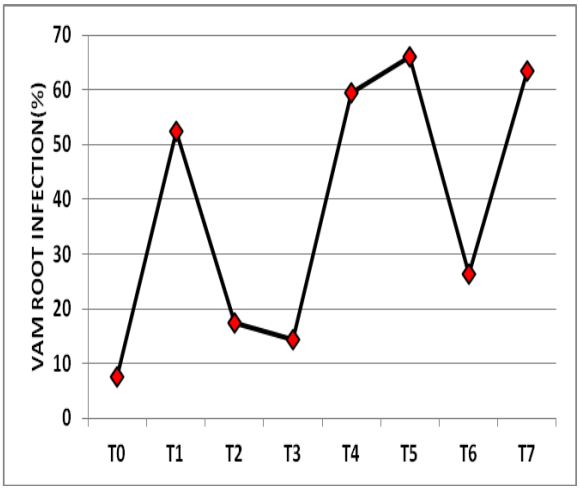


**Fig. 3.** AMF development in roots of A. indica as influenced by AMF, PSB and *Azotobacter* inoculation.

## Conclusion

The inoculation of tripartite microbial inoculants proved to be most effective in production of quality nursery stock with superior vigour and higher root shoot ratio. These seedlings attend good height with higher biomass due to more efficient uptake of soil nutrients due to AMF root development. *Azotobacter* and PSB also help seedling in mediating higher N and P availability in rhizosphere which the seedling uptake readily. The seedlings treated with bipartite and individual microbial organisms also found better as compared to uninoculated seedlings. Thus the present finding can be utilized in present production system for higher survival rate of plantations.

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