Effect of bioinoculants on biomass productivity under agroforestry systems

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Efficacy of native bioinoculants, viz. AM fungi and *Azotobacter*, separately as well as in combination was evaluated for enhancing biomass productivity of *Morus alba*, *Populus deltoides*, *Psidium guajava* and *Leucaena leucocephala* under different agroforestry models along with other plant species. The combination of all plant species tested was found to be favourable with respect to growth, yield and microbial population in soil. The combined inoculation of AM fungi and *Azotobacter* gave the best results. AM fungi were cultured on *Zea mays*, *Trigonella foenum-graecum*, *Ricinus communis*, *Vigna radiata*, *Solanum melongena* and *Lycopersicon esculentum*, wherein *R. communis* was found to be the best host plant.

**Keywords**: agroforestry, AM fungi, *Azotobacter*

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Land resources of India are under severe stress as per capita availability of land is on the decline with the growth of population. It has declined from 0.89 to 0.37 ha/person during 1951 to 1991 and further decline is projected to 0.19 ha/person in 2035\(^1\). It is, therefore, most important to develop strategies to cover each possible inch of land with some vegetation to meet the demands of growing population. Agroforestry, an efficient and integrated land use management system\(^2,3\), along with bioinoculants has great potential to optimise productivity in sustainable manner.

Mycorrhizal fungi are known to enhance soil productivity and fertility on sustainable basis\(^4\) by improving nutrient recycling\(^5\), biocontrolling of pathogenic diseases\(^6\) and synthesizing plant growth hormones\(^7\). The ability of *Azotobacter* to fix elemental nitrogen, and to synthesize vitamins, hormones and antibiotics, has been well-documented\(^8-10\). In India, since scanty information is available on the role of these bioinoculants with the tree species, the present work was undertaken. This paper deals with isolation and culture of native bioinoculants and evaluation of their effect on selected tree species under agroforestry models.

The spores of AM fungi, isolated\(^11\) from soil of Micromodel (field site), IIT, Delhi, were identified\(^12,13\) and surface sterilised with 200 ppm streptomycin and 2% (w/v) chloramines T for 10 min. They were then inoculated near the roots of *Trigonella foenum-graecum* (methi), *Lycopersicon esculentum* (tomato), *Solanum melongena* (brinjal), *Ricinus communis* (castor), *Vigna radiata* (moong) and *Zea mays* (maize) plants growing in sterilized soil pots. In each case, 20 replicates were taken and half of the pots were treated with hoagland nutrients solution\(^14\). The AM root infection of plants\(^15\) and AM spore count in the soil was monitored regularly. When AM spore count reached to 200 spores/100 g soil, the consortium of root based AM spores was used as inoculum for further studies.

*Azotobacter* was isolated from native soil and rhizosphere and total number of bacteria/g of soil was counted. Carrier (charcoal) based inoculum was prepared for further inoculation in selected plant/tree species.

The different types of agroforestry micromodels used in the study were as given below:

A. Horti-silvi model of *Morus alba* and *Leucaena leucocephala*—Twenty-four cuttings of mulberry/row (3 cuttings/kit) were planted in between *L. leucocephala* (stumps) plant rows. The distance between mulberry and *Leucaena* row was kept at 1½ m and plant-to-plant (mulberry) at 1 m. The mulberry row were treated with different bioinoculants, i.e. AM fungi, *A. chroococcum*, AM fungi + *A. chroococcum.*
B. Horti-flori model of *M. alba* and *Tegetes patula*—*T. patula*, treated with AM fungi and *Azotobacter*, was intercropped with mulberry in plots (6 m × 6 m) during November. The row-to-row (*M. alba* and *T. patula*) and plant-to-plant (*M. alba*) distance was kept at 1 m.

C. Horti-flori model of *Psidium guajava* and *T. patula*—The seeds of *T. patula* were sown during November, encircling guava plant (60-80 cm tall) already growing at field site. The treatments, viz. AM fungi, *Azotobacter* and *Azotobacter* + AM fungi were applied to *T. patula* seeds (25 seeds/treatment).

D. Agro forestry model of *Populus deltoides* and *Triticum aestivum*—The following combinations of *T. aestivum* and *P. deltoides* with different bioinoculants was used: 1, *T. aestivum* (alone) in plots with/without *A. chroococcum*; 2, *P. deltoides* alone in plot without any treatment; and 3, *T. aestivum* in plot (9 m × 9 m) with *P. deltoides* on boundaries treated with AM fungi, *A. chroococcum*, AM fungi + *A. chroococcum*. 

E. Silvi-horti model of *P. deltoides* and *Lagenaria siceraria*—Poplar plants (~100 cm shoot height) were planted with 1 m spacing. The seeds of *L. siceraria* were sown in between *P. deltoides* rows. Three different treatments, viz. AM fungi, *Azotobacter* and AM + *Azotobacter*, were given to vegetable seeds prior to sowing.

For *A. chroococcum* treatment, seeds were coated or cuttings were dipped in carrier based inoculum slurry, while the treatment of AM fungi was given by adding root based soil inocula. Uninoculated treatments served as controls. FYM/vermicompost (soil:vermicompost, 3:1) was used as organic manure. For all the models, data pertaining to plant growth parameters and microbial population, i.e. *Azotobacter*, total bacteria/g of soil and AM fungi (root infection % and spore count/100 g soil), were recorded.

Six AM fungi, viz. *Glomus mossae*, *G. microcarpum*, *G. macrocarpum*, *G. intraradices*, *Gigaspora margarita* and *G. heterogama*, were identified from the Micromodel soils. The consortium of these AM fungi was used in different agroforestry models. The AM root infection varied from 60 to 100% in different host plants (Table 1). The effect of Hoagland’s nutrient solution on AM root infection and spore count was found insignificant. *R. communis*, with 100% root infection was the best host plant (Fig. 1) followed by *T. graecum*, *Z. mays*, *S. melongena*, *V. radiata* and *L. esculentum*.

The isolated strain of *Azotobacter* was identified as *A. chroococcum* and the number of *A. chroococcum* and total bacteria per g of soil was recorded as 1-2 × 10^7 and 2 × 10^6, respectively. The number of *A. chroococcum* and total bacteria in Indian agricultural soils has earlier been reported upto 10^8/g soil, respectively. The associative and antagonistic action of soil microflora and soil characteristics influences the population of these microorganisms. The low number of *Azotobacter* and total bacteria in the present study may be due to alkaline (pH 8.6) nature of soil with low organic carbon content (0.3%).
In an agro-forestry model, the combination of *M. alba* and *L. leucocephala* showed good results (Table 2). *M. alba* survived and grew well with *L. leucocephala* stumps. The nitrogen fixing capability of *L. leucocephala* possibly promoted the growth of *M. alba*. The bioinoculants further enhanced the plants growth and microflora in rhizosphere soil. The highest growth was attained in Azotobacter + AM fungi treatment.

*T. patula* grew well with *M. alba* in a horti-flori system (Table 3). Inoculation of bioinoculants (Fig. 1) substantially increased the survival percentage (72-76%), plant growth and aerial biomass (395-510 g/plant), and flower yield (197-252 g/plant) of *T. patula*. However, the maximum effect was recorded with the treatment of *Azotobacter* + AM fungi.

*M. alba* has readily been infested by termites. On the other hand, *Tagetes* have biocidal values due to

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Survival (%)</th>
<th>AM infection (%)</th>
<th>AM spore count/100 g soil</th>
<th>Azotobacter cell count/g soil</th>
<th>Growth parameters/plant (mean values)</th>
<th>Total bacteria/g soil</th>
<th>Flowers yield/plant (g) (mean)</th>
<th>Total aerial biomass/plant (g) (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (FYM only)</td>
<td>65±10.0</td>
<td>65±12.0</td>
<td>42±7.0</td>
<td>0.7×10^5</td>
<td>180.2±5.6</td>
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<tr>
<td>AM fungi</td>
<td>87±7.0</td>
<td>80±10.0</td>
<td>65±8.0</td>
<td>2.0×10^5</td>
<td>212.2±4.5</td>
<td>2.7×10^6</td>
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<tr>
<td><em>Azotobacter</em></td>
<td>86±4.0</td>
<td>70±12.0</td>
<td>60±8.0</td>
<td>1.07×10^3</td>
<td>215.4±3.6</td>
<td>3.5×10^6</td>
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</tr>
<tr>
<td><em>Azotobacter</em> + AM Fungi</td>
<td>88±10.0</td>
<td>80±10.0</td>
<td>70±6.0</td>
<td>1.87×10^3</td>
<td>225.3±4.7</td>
<td>4.0×10^6</td>
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<tr>
<td>CD at 0.05%</td>
<td>8.29</td>
<td>2.30</td>
<td>7.71</td>
<td></td>
<td>9.33</td>
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<th>Total aerial biomass/plant (g)</th>
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</thead>
<tbody>
<tr>
<td>Control (FYM only)</td>
<td>58±5.0</td>
<td>52±12.0</td>
<td>50±10.0</td>
<td>1.2×10^5</td>
<td>82.5±5.3</td>
<td>2.0×10^6</td>
<td>120.4±17.5</td>
<td>245.0±15.4</td>
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<tr>
<td><em>Azotobacter</em></td>
<td>72±5.0</td>
<td>62±14.0</td>
<td>60±12.0</td>
<td>1.10×10^4</td>
<td>92.0±6.0</td>
<td>2.7×10^6</td>
<td>197.5±20.0</td>
<td>395.0±13.0</td>
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<tr>
<td>AM fungi</td>
<td>76±4.0</td>
<td>68±12.0</td>
<td>74±13.0</td>
<td>2.67×10^5</td>
<td>92.5±4.5</td>
<td>3.0×10^6</td>
<td>232.8±15.7</td>
<td>470.0±18.0</td>
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<tr>
<td>AM fungi + <em>Azotobacter</em></td>
<td>76±4.0</td>
<td>70±10.0</td>
<td>85±10.0</td>
<td>1.2×10^5</td>
<td>96.3±5.0</td>
<td>3.2×10^6</td>
<td>252.6±12.5</td>
<td>510.2±17.2</td>
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<tr>
<td>CD at 0.05%</td>
<td>1.15</td>
<td>3.26</td>
<td>11.19</td>
<td></td>
<td>11.75</td>
<td></td>
<td>6.30</td>
<td>4.42</td>
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presence of a number of bioactive components\textsuperscript{16} and, therefore, could have played some role in controlling termite infestation. Similarly, mulberry produces a number of allelopathic compounds\textsuperscript{17}; however, effect allelopathy was not observed in the combination.

The good growth of \textit{T. patula} with \textit{P. guajava} can also be seen from Table 3. The combination of \textit{A. chroococcum} + AM fungi resulted in the highest survival percentage (90\%), plant growth and aerial biomass (442 g/plant), and flower yield (220 g/plant). The guava crop is reported to face with bronzing of leaves, a common nutritional disorder due to phosphorus, zinc and potassium deficiencies\textsuperscript{18}. However, no such effect was observed in the present case probably because mycorrhizal fungi could have played a role in providing optimum amount of relatively immobile ions, specifically phosphate, Zn and Cu. Lintercropping of \textit{T. patula} promoted the growth of \textit{P. guajava} and enhanced soil fertility (Table 3).

In horti-silvi model of \textit{L. siceraria} and \textit{P. deltoides}, maximum yield of vegetable (2.5 kg/plant) was recorded with AM + \textit{Azotobacter} with maximum survival (85\%) of plants and maximum number of rhizospheric microflora (data not presented). The characteristics like straight bole, leaflessness during winter, multiple uses and compatibility with agricultural crops and high economic returns make popular versatile and most ideal tree species for planting on agricultural fields and fruit orchards. The inoculation of AM fungi and \textit{Azotobacter} further added importance of this tree in agroforestry.

The results of the present study on combined inoculation of AM fungi with Azotobacter are in accordance with the findings of previous workers\textsuperscript{19-22}. Crop productivity is usually limited by nitrogen and phosphorus availability, particularly in the tropic soils\textsuperscript{23}. These have been reported essential for plant establishment and growth especially under nutrient unbalanced conditions. The interactions of nitrogen fixers and AM fungi help in the uptake of tricalcium phosphate from soil\textsuperscript{24}. Moreover, biological active substances, such as plant hormones, vitamins, etc, by soil microorganisms, e.g. \textit{Azotobacter}, could stimulate the growth rate of AM fungi\textsuperscript{25}. The dual inoculation of these microorganism display synergistic effect resulting in maximum growth of plants through better N and P uptake\textsuperscript{26,27}. Thus, mycorrhizal fungi and nitrogen fixers can be regarded as alternative means for more rational agricultural/ agroforestry programmes in economizing nitrogenous anf phosphatic fertilizers.

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