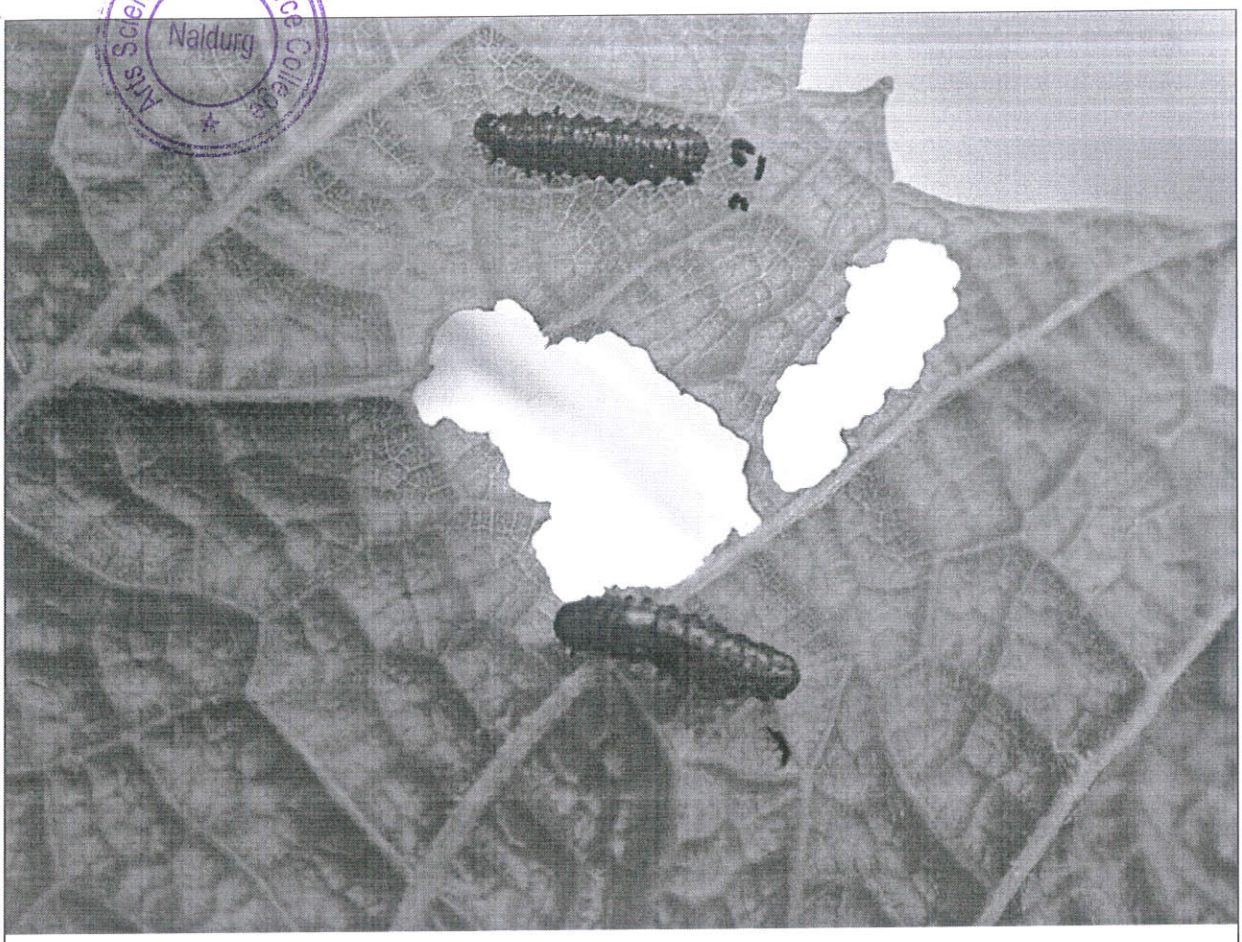

# Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS

Asian Journal of Plant Pathology

ISSN 1819-1541
DOI: 10.3923/ajppaj.2017.89.94

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# Research Article
# Prevalence and Effect of Mycorrhizae on Growth of *Pongamia pinnata* Nursery Plant

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## Abstract

**Objective:** This study was conducted to investigate the mycorrhizal colonization and biomass productivity of different sites of *Pongamia pinnata* nurseries plant. **Methodology:** The rhizosphere soil and root samples were collected from five different study sites. Indigenous AMF inoculums supplied 30 g per plant. After 90th days of treatment, plants samples were drawn and observations were recorded. **Results:** Percentage of Arbuscular Mycorrhizal (AM) infection, number of resting spores and AM fungi (AMF) species varies in different sites of nurseries. The AMF inoculums treatments promising data were observed almost in all study sites for 90th Days After Seedlings (DAS). This variation is attributed to various factors such as mycorrhizal status and biomass. *Glomus* species dominated in all sites followed by *Sclerocystis*, *Gigaspora* and *Acaulospora*. **Conclusion:** Mycorrhizae could contribute substantially to achieve better results for high oil seed production. When AMF are more colonized to plants then enhanced the biomass productivity.

Key words: *Pongamia pinnata*, AM infection, biomass production, DAS

Received: January 06, 2017 Accepted: March 04, 2017 Published: March 15, 2017

Citation: Mane Sudarshan Ramesh, Ganesh Mallikarjun Birajdar, Gavali Manaji Tanaji and Udhav Narba Bhale, 2017. Prevalence and effect of mycorrhizae on growth of *Pongamia pinnata* nursery plant. Asian J. Plant Pathol., 11: 89-94.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Karanj (*Pongamia pinnata* (L.) Pierre), a fast-growing oil-seed-producing tree legume has the ability to grow on wastelands. It belongs to Fabaceae family. Karanj tree is a wonderful tree almost like neem tree. It can be utilized for biofuel plantation on such lands. The pre-conditioning of young seedlings during the early stage of development with efficient Arbuscular Mycorrhizal Fungi (AMF) confers several benefits enhancing the possibility of their establishment in fields after out planting from nurseries. It has been found as a suitable option for biodiesel production[1]. It is a fast-growing nitrogen fixing tree legume with the potential for high oil seed production (seed contains 30-35% oil). *Pongamia pinnata* has the ability to grow on wastelands[2] and hence can be utilized for biofuel plantation on such lands (47.22 million ha)[3].

The AMF is ubiquitous group of fungi[4]. The AMF belongs to phylum Glomeromycota[5]. The general consensus is that AMF improve phosphate nutrition of legumes, which in turn enhances plant growth and nitrogen fixation[6]. The AMF have been shown to differentially colonize plant roots, causing a variety of effects on plant growth, biomass allocation and photosynthesis[7]. Increased access to low-mobility soil mineral nutrients has been considered to be the main beneficial effect of AMF on their host plants[4]. Though late, the importance of AM fungi in nursery management and in revegetation efforts of various types of lands has been realized and of late, it has become an integral part of all stages of afforestation programmes. Therefore, the present investigation was made to productivity and association of mycorrhizae on *P. pinnata* from different study sites.

## MATERIALS AND METHODS

**Sample collection sites:** The rhizosphere soil and root samples of Karanj (*Pongamia pinnata* (L.) Pierre) nursery plants were collected during February-May, 2015 in summer season from viz., Naldurg Andur, Jalkot, Osmanabad, Paranda and Kalamb for biomass production and arbuscular mycorrhizal fungal infection status. Ten different replications of rhizosphere soil and root samples were collected in separate ziplock polythene bags from each sites. Soil samples were used for isolation and identification of arbuscular mycorrhizal fungal spores and roots for assessment of percentage root colonization.

**Treatments of AMF inoculums to Karanj:** Inoculums of indigenous AMF maintained in greenhouse with Coleus (*Plectranthus scutellarioides*) as host (containing 270-300 spores/100 g soil) was applied 30 g per each plant after 15 days of seedlings of *Pongamia pinnata*. Seedlings without treatment of AMF are considered as control. The biomass and AMF infection data revealed after 90th days seedling (DAS). Seedlings were watered on alternate days and 10 mL of Hoagland solution twice in a week [Stock solution (1000 mL) ($NH_4H_2PO_4$, 1 mL, $KNO_3$, 6 mL, $Ca(NO_3)$ 2-4 mL, $MgSO_4$ 2 mL), micro-nutrients solution ($H_3BO_3$, 2.86 g, $MnCl_2$ 1.81 g, $ZnSO_4$, 0.22 g, $CuSO_4$, 0.08 g, $NaMoO_4$, 0.02 g)] to each pot, after 90th days of treatment[8]. Biomass, presence of arbuscules, vesicles, hyphal and Dark Septate Endophyte (DSE) and types of AMF species were monitored; then the root colonization index, i.e., percentage of root length colonized by AMF and spore population in 100 g of soil were calculated.

**Biomass production:** *Pongamia pinnata* treated and untreated plants, fresh weight of shoot and roots data were recorded. Shoots and roots were separated and oven dried at 60°C for 48 h for the determination of dry mass after recording their lengths[9]. Leaf area was measured by disc method. Fifty leaf disc of known size was taken from randomly selected leaves of the plant, discs and remaining leaf blades were oven dried and leaf area was calculated by using equation[10]:

$$\text{Area of total leaves (cm}^2\text{)} = \text{No of disc from total leaves} \times \text{Area of single disc}$$

**AMF spore density:** One hundred grams of rhizospheric soil was dissolved in 1000 mL of water and decanted through a series of 355-35 μm sieves[11]. Residues were filtered through Whatman filter paper No. 1 and all spores were counted under the stereo-zoom dissection microscope. Intact and healthy AM fungal spores were mounted in PVLG (Polyvinyl alcohol-lactoglycerol) with and without Melzer's reagent for identification using keys[5] and INVAM (http://www.invam.caf.wvu.edu). The AMF spores were identified up to species level.

**Assessment of percentage root colonization:** The roots were fixed in to 4% Formalin Aceto Alcohol (FAA). Fixed roots were washed to free FAA cleared in water. Roots are placed in glass vial with 10% KOH and stained in 0.05% trypan blue lactophenol to determine mycorrhizal colonization, these VAM fungal colonized segments studied. Then transferred the sieving on to a gridded petri plate and observed it under the binocular microscope 400X (Lawrence and Mayo LM-52-3521). The percent root colonization was measured by using the equataion[12]:

$$\text{Root colonization (\%)} = \frac{\text{No. of colonized segments}}{\text{Total No. of segments examined}} \times 100$$

**Statistical analysis:** Statistical analysis of the experiments were performed by using the method described by Mungikar[13].

## RESULTS

**Biomass production:** The present study, 10 parameters of biomass productivity was studied in *Pongamia pinnata* (Table 1). In this study, six replications were used. In biomass production, height of stem (121 cm), width of stem (5.2 cm), length of root (54.8 cm), the number of leaflets was observed more in Kalamb site (74), dry weight of shoot (18.21 g), fresh weight of shoot was found more in Kalamb site (44.7 g) and leaf area (2233.57 $cm^2$) were observed more in Kalamb sites. Fresh weight of root was increased in Osmanabad site (29.41 g) and less in Kalamb (21.6 g). After the AMF inoculums treatments promising data were observed almost in all study sites after 90th Days After Seedlings (DAS). Kalamb site was found good AMF inoculums source of soil.

**Mycorrhizal study:** Arbuscular Mycorrhizal Fungal (AMF) assessment of percentage root colonization and spore density was studied in *Pongamia pinnata* (Table 2). Spore density was found more in Andur site (446/100 g soil) and less than Jalkot site (63/100 g soil) followed by Andur site and Paranda site. After treatments of AMF inoculums considerably increased the AMF spore density per 100 g of soil in Paranda site i.e., 63 (334) followed by others. Among AMF spore, *Acaulospora rehmii*, *Glomus macrocarpum*, *Glomus geosporum*, *Sclerocystis* sp. were observed from all study sites (Fig. 1, 2). The AMF percentage of root colonization was increased in Kalamb site (91.66%), while less Paranda site (70.83%). The AMF root colonization types are Arbuscular (A), Vesicular (V), Hyphal (H), colonization and DSE was found in all sites.

## DISCUSSION

In the literature, there are several reports in which AMF inoculations decreased plant biomass but this decrease has often been found to be transient and reversed later, being followed by a positive growth response[14,15]. Results also suggested that the extent of growth depression or reduction in *P. pinnata* varied with inoculated AMF species, which can be related to the variations in carbon demand of different AMF species. It was suggested that such differences in growth response towards different AMF inoculants are directly related to the balance between benefits and costs of the symbioses[16]. It was examined the mycorrhization level of some important regional plants (*Azadirachta indica*, *Dalbergia sissoo*, *Dendrocalamus strictus*, *J. curcas*, *Leucaena leucocephala*, *Madhuca latifolia* and *P. pinnata*), which suggested that it was minimum in *P. pinnata*[17]. This could be due to the large seeds of *P. pinnata* and pointed out those plants with large seeds generally exhibit lower AMF densities. It was concluded that AMF inoculations should enhance biomass of *P. pinnata* only after depletion of metabolic reserves in its cotyledons and

Table 1: Impact of AMF treatment for biomass production in *Pongamia pinnata* from different study sites (90 DAS)

| | Sites | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | N | A | J | O | P | K | Mean±SD |
| Height of stem (cm) | 84 (105) | 67 (76) | 89.4 (91.1) | 97 (102) | 113 (115) | 121 (121) | 95.24±19.70 (101.67±16.33) |
| Width of stem (cm) | 2.5 (3.0) | 3.1 (3.6) | 2.6 (2.8) | 3.4 (4.1) | 4.7 (5.1) | 5.2 (5.4) | 3.54±1.12 (04±1.08) |
| Length of root (cm) | 36 (42) | 49.7 (50.0) | 31.5 (32.8) | 44.6 (45.11) | 49.2 (49.3) | 54.8 (56.33) | 44.3±8.91 (45.93±8.06) |
| No. of leaves | 8.0 (8.9) | 10 (11) | 4.0 (5.1) | 9.0 (9.33) | 13 (14) | 11 (16) | 9.17±3.07 (10.73±3.89) |
| No. of leaflets | 39 (41) | 31 (33) | 13 (15) | 51 (52.1) | 67 (67) | 74 (75) | 45.84±22.86 (47.19±22.20) |
| Fresh weight of shoot (g) | 29.4 (32.2) | 37.94 (40.11) | 31.61 (32.0) | 36.12 (36.77) | 41.3 (43.3) | 44.7 (44.0) | 36.84±5.77 (38.07±5.29) |
| Fresh weight of root (g) | 21.28 (23.55) | 26.17 (27.77) | 23.17 (27.0) | 29.41 (32.1) | 24.5 (25) | 21.6 (22.13) | 24.67±3.40 (26.26±3.55) |
| Dry weight of shoot (g) | 11.56 (11.75 | 15.27 (16.12) | 14.17 (15.11) | 13.97 (14.99) | 17.59 (18.51) | 18.21 (18.11) | 15.12±2.48 (15.77±2.47) |
| Dry weight of root (g) | 9.54 (10.1) | 12.2 (13.33) | 11.42 (13.0) | 10.62 (10.99) | 13.31 (14.0) | 10.96 (12.1) | 11.34±1.31 (12.25±1.49) |
| Total leaf area ($cm^2$) | 1417.90 (1473.11) | 1872.96 (1900.1) | 1729.42 (1799.11) | 1713.50 (1772.44) | 2157.52 (2200.11) | 2233.57 (2244.22) | 1854.16±303.96 (1898±288.87) |

Values means of three replications, values in parentheses are AMF treatment, N: Naldurg, A: Andur, J: Jalkot, O: Osmanabad, P: Paranda, K: Kalamb

Table 2: Arbuscular mycorrhizal fungal status in *Pongamia pinnata* different sites (90 DAS)

| | Sites | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | N | A | J | O | P | K | Mean±SD |
| AMF spore density (spore/100 g soil) | 413 (567) | 446 (669) | 63 (334) | 193 (550) | 286 (355) | 108 (499) | 251.50±157.90 (492.34±128.21) |
| Percentage of AMF root colonization | 75 (87) | 83.33 (86.33) | 87.05 (90.00) | 79.16 (82.11) | 70.83 (73.11) | 91.66 (92.22) | 81.16±7.73 (74.63±23) |
| Type of root colonization | HVA, DSE | | | | | | |
| Types of AMF species | *Acaulospora rehmii, Glomus macrocarpum, Glomus geosporum, Sclerocystis* sp. | | | | | | |

Values represent three replications, Values in parentheses are AMF treatment, A: Arbuscular, V: Vesicular, H: Hyphal, DSE: Dark septate endophytes

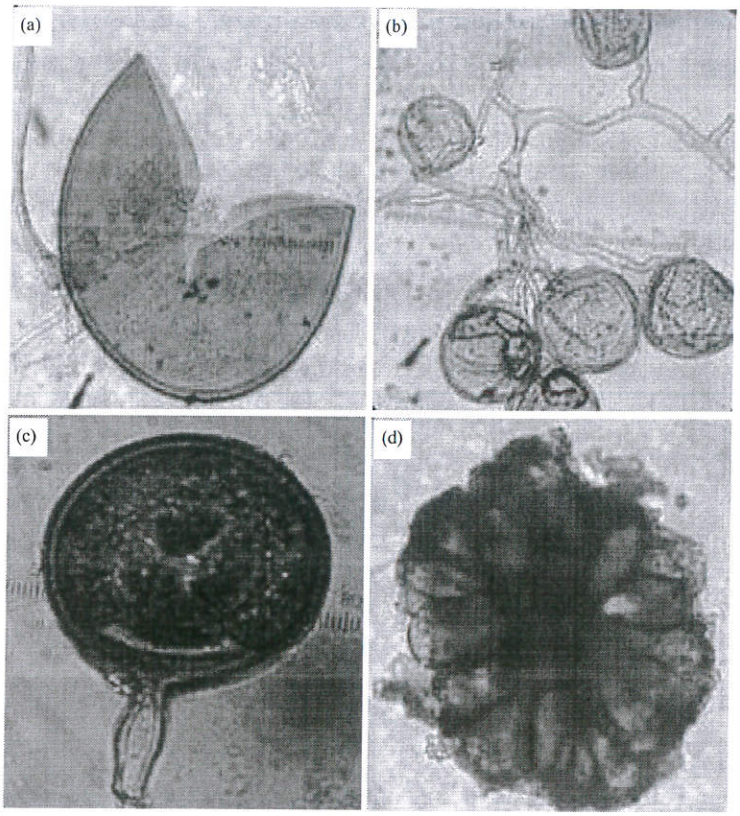


Fig. 1(a-d): Identified genera of AMF (400X), (a) *Acaulospora rehmii*, (b) *Glomus macrocarpum*, (c) *Glomus geosporum* and (d) *Sclerocystis* sp.

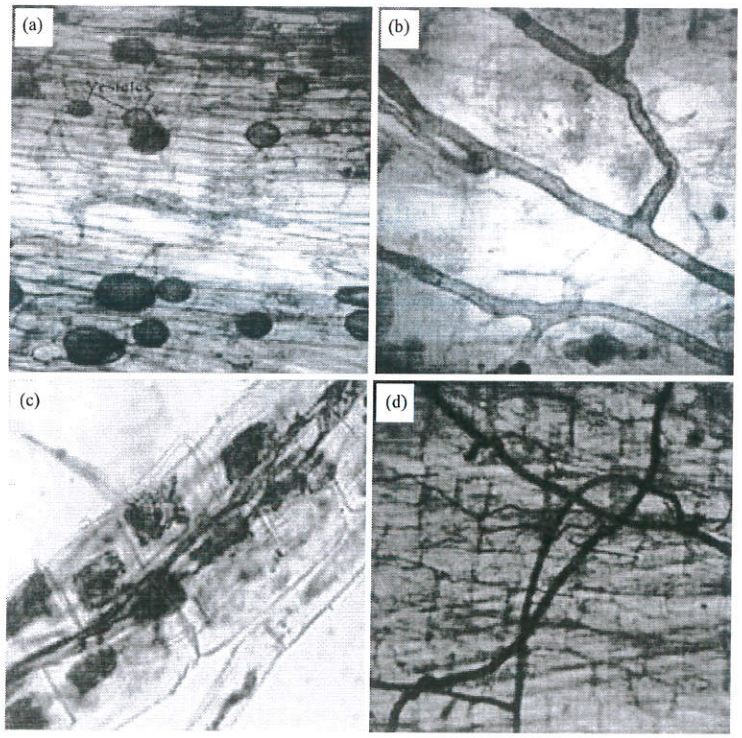


Fig. 2(a-d): Types of AMF colonization (400X), (a) Vesicles, (b) Hyphal, (c) Arbuscules and (d) DSE and hyphal

such mycorrhizal seedlings can be utilized for biofuel plantation[18,19]. It is relevant to mention that the possible synergistic effect would be the uptake by AM fungal hyphae and translocation into the plant of P released by PSB in soil[20-23]. Recently, the results showed that the combined inoculation of both PSB, AMF and rock phosphate produced vigorous plant growth of tree seedlings for quick planting[24]. It was reported that, *Azadirachta indica*, *Pongamia pinnata*, *Leucaena leucocephala* and *Acacia catechu* were most effective in catching mycorrhizae and can be used as the effective tool in rehabilitation of the degraded ecosystems[25].

## CONCLUSION

It was concluded that AMF inoculations should enhance biomass of *P. pinnata* only after depletion of metabolic reserves in its cotyledons and such mycorrhizal seedlings can be utilized for biofuel plantation. It was also discussed that AMF inoculations when amended enhanced biomass and AMF status of *P. pinnata* after 90 DAS of treatment. The importance of AM fungi in nursery management and in revegetation efforts has become an integral part of all stages of afforestation programmes. When AMF are more colonized to plants then enhanced the biomass productivity.

## SIGNIFICANCE STATEMENTS

- *Pongamia pinnata* is a good source of biofuel
- The AMF inoculations should enhance biomass of *P. pinnata* only after depletion of metabolic reserves in its cotyledons and such mycorrhizal seedlings can be utilized for biofuel plantation
- Increased access to low-mobility soil mineral nutrients has been considered to be the main beneficial effect of AMF
- The importance of AM fungi in nursery management and in revegetation efforts of various types of lands has been realized and of late, it has become an integral part of all stages of afforestation programmes

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