Studies on Beneficial Microflora and their Effect on Growth Improvement of Casuarina species

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Use of bioinoculants in agriculture horticulture and forestry greatly improve the survival and growth of plant species. The inoculated plants get increased tolerance to adverse soil ph, heavy metals, drought etc. The bioinoculants also help the plant for increased root development and enhances the biomass and yield. In the presence study the status of beneficial organisms like Arbuscular mycorrhizal fungus (AM), Ectomycorrhizal fungi (EMF) and Actinomycetes was investigated in the different age groups of Casuarina equisetifolia Plantations in Tamilnadu. Among different age group of the root samples screened, maximum percent root colonization of Arbuscular Mycorrhizal (AM) fungi was recorded in the root samples of one year old plantation (92%) this is followed by root sample of three year old plantation (67%). Minimum percent colonization was observed in four year old plantation sample (37%). The moderate percent colonization of AM fungi was seen in five and six year old plantation samples. No ectomycorrhizal fungal structures were recorded in the root samples of different age groups of plantations screened. Among different age group of the samples screened, maximum number of AM fungal spores (72/100 g soil) was recorded in the rhizosphere of one year plantation this is followed by two year old plantation (51/100g soil). The minimum number AM fungal spores was recorded in the rhizosphere of three year old plantation (33/100g soil). The C. equisetifolia and C. junghuhniana inoculated with various inoculants such as VAM, and Ectomycorrhizae Palbus and Laccaria fratera in various forms such as vermiculate form, basideospore form alginate bead form other then this also inoculate with Frankia. Chemical fertilizers DAP in IFGTB nursery. Pisolithus albus basidospore form shows the maximum growth in root (19.6) and shoot length (25.8) of Casuarina junghuhniana Pisolithus albus vermiculate form shows maximum length in both plants DAP shows second maximum Frankia inoculated plants shows maximum result in biomass of Casuarina equisetifolia and its followed by Pisolithus albus vermiculate form for Casuarina junghuhniana, the Pisolithus albus inoculated plant tissues maximum results and its followed by Pisolithus albus spores. Rhizobus of Casuarina equisetifolia and Casuarina junghuhniana inoculated with VAM spores. Maximum ECM colonization observed in treatment inoculated Pisolithus albus spore. The study shows the bioinoculants treated plant shows greater improvement when compared with chemical fertilizer and we are in the need to create on awareness on use of biofertilizer among the former to improve the growth Casuarina and other tree crops.

Key words: VAM, Casuarina junghuhniana, a Casuarina equisetifolia, Pisolithus albus.

Casuarinas are a distinct group of angiosperms, belonging to the family Casuarinaceae. And these are the unique amongst the angiosperms and are placed in an order by
themselves, Casuarinales. Casuarinas are characterized by long needle like branchlets divided into regularly spaced nodes. At each of the nodes a ring of teeth like structure which are reduced leaves. The flowers in casuarinas are unisexual. Most of the species are dioeciously as well as monoecious. Casuarina trees which mainly occur in tropical, subtropical and temperate coastal regions as well as in the arid lands\(^1\). The Casuarinas produce high quality fuel wood. These plants are capable of stabilizing sand dunes and eroding hill slopes. Now a day Casuarinas is mainly cultivated in barren and polluted areas and its mostly wanted plants for paper production. It has major role in protection of coastal areas from tsunami like disasters.

Now days we are in the need to develop social forests to maintain and increase the forest area to preserve the ecological balance of our earth. Other than this for our fuel demands also requires the afforestation programmes to increase the forest area. Aforestation programmed organized by government of India mainly encourages the farmers to cultivate the casuarinas because of their adoptability to Indian soil condition and its high demand and recommended the species such as *Casuarina equisetifolia* and *Casuarina junghuhniana*\(^2\). For the better growth of plants needs the nutrients such as nitrogen, phosphorus, potassium and some microelements such as magnesium, manganese calcium and etc, Barren lands and polluted areas won’t satisfy the nutrient level required for plant growth\(^3\). Application of chemical fertilizer to such a vast forest area needs more economy and also affects the environment. Hence it’s not recommendable. So we are in the need of remedy to the above problem.

The present study to investigate the status of the beneficial microbes AM, ECM, *Frankia* and their effect on growth enhancement of Casuarinas are very limited. Hence in the study an attempt was made to investigate the occurrence and distribution of AM and ECM fungi and *Frankia* in the casuarinas plantation. To isolate the status of beneficial microbes in the casuarinas plantations, and to study the effect of AM fungi on growth improvement of *Casuarina equisetifolia* and *Casuarina junghuhniana* seedlings in nursery, finally analysis the effect of *Frankia* on growth improvement of *Casuarina equisetifolia* and *Casuarina junghuhniana* seedlings in nursery.

**MATERIAL AND METHODS**

**Culture media used for the study**

The Potato dextrose agar medium (PDA), and Modified Melin Norkrans medium (MMN)

Culture media were used to find out the growth of various isolates of Ectomycorrhizal fungi.

**Sample collection from Casuarina plantation**

The soil, root and nodule samples were collected from the Casuarina plantations of age groups one, two, three, four, five and seven from Chinnathachur village belong to Villupuram District. The soil samples were collected in polythene bags and kept in refrigerator at 4. Then the root were washed in tap water and adhered soil particles were removed and kept in FAA solution. The root nodules washed in tap water, and placed in zip – lock bags.

**Root clearing method for am fungi association**\(^4\)

1 cm long root segments were first washed thoroughly in tap water and then placed in 10% KOH and heated to 90°C for 15 – 30 minutes. Then they were washed in water and acidified with 5N HCL for 5 -10 minutes. The root bits then stained with tryphan blue in lactophenol for 15 -30 minutes and the excess stain was removed and the root bits along with clear lactophenol for observation.

**Estimation of am root colonization**

AM infected root samples were cut into smaller segments of 1 cm length, clear and stained and the % of root colonization was estimated.

\[
\text{% of AM colonization} = \frac{\text{Number of AM positive segments}}{\text{Total segments scored}} \times 100
\]

The cleaning and staining of non pigmented roots were done following the Phillips and Hayman, Method\(^4\).

**Soil analysis**

**AM Spore count**

Hundred grams of rhizosphere soil of all

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\(^{1}\) Chinnathachur village belong to Villupuram District. The soil samples were collected in polythene bags and kept in refrigerator at 4.

\(^{2}\) Chinnathachur village belong to Villupuram District. The soil samples were collected in polythene bags and kept in refrigerator at 4.

\(^{3}\) Chinnathachur village belong to Villupuram District. The soil samples were collected in polythene bags and kept in refrigerator at 4.

\(^{4}\) Chinnathachur village belong to Villupuram District. The soil samples were collected in polythene bags and kept in refrigerator at 4.
age group plantation was collected to determine the spore population. Mycorrhizal spores were obtained by wet sieving and decanting method proposed by Gerdenman and Nicholson. Soil was suspended in 1 liter of water thoroughly shaken with glass rod and after allowing heavy particles to settle. The suspension was passed through a series micro test sieves with decreasing pore size (750 µm, 425 µm, 125 µm, 45 µm). The residue collected from 250 µm, 125 µm, and 45 µm sieves filtered through filter paper was spread over the Petri plate and observed under stereo microscope for AM spore count.

Bioinoculum production

In the experiment, AM fungi, Ectomycorrhizal fungi, Frankia and chemical fertilizer (DAP) were used. AM inoculums were collected from Department of Microbiology, Tamilnadu Agricultural University, Coimbatore.

Ectomycorrhizal inoculum production

ECM fungi Pisolithus albus and Laccaria fraterna were used in various forms and the inoculums preparation were made by using appropriate techniques.

Basidiospore inoculum

Fruit bodies of ECM fungi such as Pisolithus albus and Laccaria fraterna were collected from Eucalyptus plantations at Coimbatore and Ootacamund respectively. The dried fruit bodies were crushed and basidiospores were extracted and stored in Zip - lock polybags under refrigerator.

Vermiculite Based Inoculum

The pure cultures of ECM fungal isolates of Pisolithus albus, Laccaria fraterna were obtained from Forest Pathology Lab., IFGTB for the production of mycelial inoculums with vermiculite as carrier material.

The Petriplates containing PDA with antibiotics (streptomycin) were prepared, young growing mycelia (10 days old) disc of ECM were cut with 5mm cork borer and inoculated in the flask of 1000ml containing vermiculate and MMN medium (350ml). The flasks were kept for incubation at 27°C and were checked periodically for the growth of fungus for a period of about 12 days. The mycelium continues to grow in substrate and was ready to use after appropriate period of incubation. If the culture is to be inoculated to the plants before use, it is recommended to remove non assimilated nutrients in the solution by leaching.

Alginate bead inoculation

This technique was developed recently and employees submerged cultivation procedures followed by immobilization in calcium alginate gel. Immobilization procedures can preserve physiological properties of mycorrhizal roots. The pure culture of selected ECM fungi were obtained from Forest Pathology Lab, IFGTB.

Mycelium of selected fungus were harvested and washed in sterile water then kept in saline suspension, 9gm of fresh mycelium was prepared and fragmenting during 6-7 sec in a blender at 3600 rpm in 150 ml distilled water. The mycelial suspension mixed with 2% Sodium alginate and the mixture thus dipped in to 0.1M cacl2 solution to form calcium alginate beads with mycelium can be used as inoculums.

Frankia inoculums preparation

Frankia nodules were collected from Casuarina fields and crushed in to powder by using mortar and pestle before the nodules were cleaned by using tap water.

Soil management and assay

Soil used in the present study is the mixture of sand and soil in the ratio of 2:1 and it was sterilized by autoclaving at 15lbs for 1 hour for about 3 times in alternate day’s .the soil type, chemical nature, EC and macro elements (NPK) were analyzed in soil testing laboratory.

Raising of seedling

Healthy seeds of Casuarina equisetifolia, C. junghuhniana obtained from Seed Technology Lab, IFGTB, Coimbatore. The seeds were surface sterilized in 1 – 2 % hypochlorite for 5 min and washed with distilled water (38 x 27.5cm) plastic trays were filled with 3-5cm of sterilized sand. after that seeds were sown on the trays, a thin layer of sterilized sandy soil were spread t cover the seeds watered with spray carried out twice a day for first 15 days then once in a day for 15 days germinated seeds attain 6-8 cm height in 30days after that the seedlings were transferred one per bag in to polythene bags (13 ×20.5cm)

Application of inoculants in nursery

In this study we have taken 180 plants of Casuarina equisetifolia and 180 plants of
Casuarina junghuhniana for 10 treatments and 18 plants per treatment. And its divided further to three replications i.e., six plants per replications.

T1 - It serves as a control no inoculants were added.
T2 - In this treatment each seedling s inoculated with 10 gm of AM fungi
T3 - 5gm P. albus spores were inoculated
T4 - 10 gm P. albus vermiculite were inoculated
T5 - 10 beads of P. albus were inoculated
T6 - 5gm Laccaria fraterna spores were inoculated
T7 - 10gm Laccaria fraterna vermiculite were inoculated
T8 - 10 Beads of Laccaria fraterna were inoculated
T9 - 5gm of Frankia were inoculated
T10 - 0.25 gm of DAP were inoculated

**Assessment of effect of inoculants on Casuarina species**

**Growth measurements**
1. The root and shoot heights of the plants were measured before transplanting. After transplanting the height of the shoot were measured and tabulated up to the period of 3 months.
2. The height measurements are tabulated

**Weight**
Two plants from each replication of all treatments harvested and the harvested plants were collected to the laboratory and the root and shoot portions were cutter separately. The fresh weight of the roots and shoots were taken and tabulated, then the roots and shoot were kept in oven at 80°C for 24 hours. Then the dry weights were measured and tabulated

**Root clearing method for am fungi association**
1 cm long root segments were first washed thoroughly in tap water and then placed in 10% KOH and heated to 90°C for 15 – 30 minutes. Then they were washed in water and acidified with 5N HCL for 5 -10 minutes. The root bits then stained with tryphan blue in lacto phenol for 15 -30 minutes and the excess stain was removed and the root bits along with clear lacto phenol for observation.

**Estimation of am root colonization**
AM infected root samples were cut into smaller segments of 1 cm length, clear and stained and the % of root colonization was estimated.

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\% \text{ of AM colonization} = \frac{\text{Number of AM positive segments}}{\text{Total segments scored}} \times 100
\]

**Soil analysis**

**AM Spore count**

Hundred grams of rhizosphere soil of each replication were collected in all the treatment to determine the spore population. Mycorrhizal spores were obtained by wet sieving and decanting method proposed by Gerdemaa and Nicholson. Soil was suspended in 1 litre of water thoroughly shaken with glass rod and after allowing heavy particles to settle. The suspension was passed through a series micro test sieves with decreasing pore size (750 µm, 425 µm, 125 µm, 45 µm). The residue collected from 250 µm, 125 µm, and 45 µm sieves filtered through filter paper was spread over the Petri plate and observed under stereo microscope for AM spore count.

**Nutritional analysis**

Soil type, chemical nature, macro elements (NPK) and EC assessments were done by adopting standard methods in Soil testing laboratory, Agricultural office, Villupuram.

**RESULTS**

**Field investigation**

Mycorrhizal fungi play a critical role in an uptake of nutrients from the soil especially phosphorous and other essential elements for the growth of plants. There are two major types of mycorrhizal fungi viz., ectomycorrhiza and endomycorrhizal fungi. Among endomycorrhizal fungi arbuscular fungi are widely distributed in varied ecosystem in rainforest, shrubs, savannahs, grasslands, and sand dunes, arid and semiarid deserts. The ectomycorrhizal fungi mostly associated with woody plants specially gymnosperms and certain angiosperm members including Casuarina belonging to family Casuarinaceae. There are limited reports available on the status of mycorrhizal and other beneficial microorganism in association with Casuarina equisetifolia in India. Hence the attempt was made to investigate the status of mycorrhizal fungi in association with Casuarina equisetifolia plantation of different age group.
Observation of mycorrhizal fungi in the roots of *Casuarina equisetifolia*

The Root Sample of six different age groups of *C. equisetifolia* were collected from farmers field at Vilupuram district, Tamil Nadu and the root samples were processed and examined for the presence of vesicles, hyphae and arbuscules. Among different age group of the root samples screened, maximum percent root colonization of Arbuscular Mycorrhizal (AM) fungi was recorded in the root samples of one year old plantation (92%) this is followed by root sample of three year old plantation (67%). Minimum percent colonization was observed in four year old plantation sample (37%). The moderate percent colonization of AM fungi was seen in five and six year old plantation samples. No ectomycorrhizal fungal structures were recorded in the root samples of different age groups of plantations screened.

Among the AM fungal structures recorded in the root samples, both vesicular and hyphal structures were very common in all the root samples of different age groups of the casuarinas plantation studied. The arbuscular structures were observed only in the root samples of one and two year old plantation.

**AM structures in the root structures of *Casuarina equisetifolia***

Vesicular and hyphal structures of AM fungi were recorded in most of the root samples screened. Vesicles are sub globosed to irregular

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Age of Plants</th>
<th>Fresh weight of the nodule(gm)*</th>
<th>Dry weight of the Nodule(gm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>One</td>
<td>12.69</td>
<td>6.74</td>
</tr>
<tr>
<td>2.</td>
<td>Two</td>
<td>13.59</td>
<td>7.86</td>
</tr>
<tr>
<td>3.</td>
<td>Three</td>
<td>20.97</td>
<td>15.77</td>
</tr>
<tr>
<td>4.</td>
<td>Four</td>
<td>44.92</td>
<td>21.65</td>
</tr>
<tr>
<td>5.</td>
<td>Five</td>
<td>45.36</td>
<td>23.89</td>
</tr>
<tr>
<td>6.</td>
<td>Seven</td>
<td>24.63</td>
<td>14.68</td>
</tr>
</tbody>
</table>

*Mean of three replications*

**Table 2. Nursery Experiments. Effect of Bioinoculants on the growth improvement of *Casuarina equisetifolia* and *Casuarina junghuhniana***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatments</th>
<th><em>Casuarina equisetifolia</em></th>
<th><em>Casuarina junghuhniana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot Length (cm)*</td>
<td>Root Length (cm)*</td>
</tr>
<tr>
<td>1.</td>
<td>T₁</td>
<td>18.2</td>
<td>11.0</td>
</tr>
<tr>
<td>2.</td>
<td>T₂</td>
<td>21.0</td>
<td>17.5</td>
</tr>
<tr>
<td>3.</td>
<td>T₃</td>
<td>28.01</td>
<td>19.6</td>
</tr>
<tr>
<td>4.</td>
<td>T₄</td>
<td>22.3</td>
<td>13.1</td>
</tr>
<tr>
<td>5.</td>
<td>T₅</td>
<td>20.4</td>
<td>17.4</td>
</tr>
<tr>
<td>6.</td>
<td>T₆</td>
<td>21.7</td>
<td>18.6</td>
</tr>
<tr>
<td>7.</td>
<td>T₇</td>
<td>20.1</td>
<td>18.9</td>
</tr>
<tr>
<td>8.</td>
<td>T₈</td>
<td>20.4</td>
<td>19.4</td>
</tr>
<tr>
<td>9.</td>
<td>T₉</td>
<td>22.1</td>
<td>15.2</td>
</tr>
<tr>
<td>10.</td>
<td>T₁₀</td>
<td>27.7</td>
<td>18.6</td>
</tr>
</tbody>
</table>

* - Mean of three replications.

T₁ - Control, T₂ - VAM, T₃ - *Pisolithus albus* spores, T₄ - *Pisolithus albus* vermiculite,
T₅ - *Pisolithus albus* beads, T₆ - *Laccaria fraterna* spores, T₇ - *Laccaria fraterna* vermiculite,
T₈ - *Laccaria fraterna* beads, T₉ - *Frankia*, T₁₀ - DAP.
in structures. Arbuscular structures were recorded in the root samples of one and two year old plantations.

**Observation of AM fungal propagules in the rhizosphere of *Casuarina equisetifolia***

The rhizosphere soil samples were collected under the root zone of *Casuarina equisetifolia* in six different age groups. The samples are analyzed and recorded AM propagule population. The results on the maximum number of AM fungal spores in the rhizosphere of *Casuarina equisetifolia* of six different age groups. The study revealed that all the soil samples of different age groups had AM fungal

| Table 3. Effect of Bioinoculants on the shoot and root biomass Improvement of *Casuarina equisetifolia* and *Casuarina junghuhniana* in nursery experiments |
|---|---|
| **S.** | **Treatments** | **Casuarina equisetifolia** | **Casuarina junghuhniana** |
| No. | Shoot | Root | Shoot | Root |
| | Fresh wt (gm)* | Dry wt (gm)* | Fresh wt (gm)* | Dry wt (gm)* | Fresh wt (gm)* | Dry wt (gm)* | Fresh wt (gm)* | Dry wt (gm)* |
| 1 | T₁ | 0.69 0.27 | 0.28 0.10 | 0.28 0.12 | 0.21 0.08 |
| 2 | T₂ | 0.81 0.38 | 0.23 0.12 | 0.86 0.22 | 0.29 0.09 |
| 3 | T₃ | 1.21 0.33 | 0.29 0.12 | 1.31 0.49 | 0.29 0.12 |
| 4 | T₄ | 1.15 0.53 | 0.30 0.14 | 1.16 0.61 | 0.37 0.18 |
| 5 | T₅ | 0.76 0.42 | 0.29 0.12 | 0.85 0.32 | 0.26 0.16 |
| 6 | T₆ | 0.90 0.47 | 0.23 0.10 | 0.79 0.49 | 0.26 0.18 |
| 7 | T₇ | 1.13 0.48 | 0.37 0.18 | 1.07 0.24 | 0.32 0.19 |
| 8 | T₈ | 0.88 0.38 | 0.25 0.10 | 0.64 0.28 | 0.23 0.15 |
| 9 | T₉ | 0.95 0.62 | 0.38 0.22 | 0.27 0.18 | 0.26 0.15 |
| 10 | T₁₀ | 0.97 0.42 | 0.20 0.11 | 0.63 0.53 | 0.28 0.13 |

* Mean of three replications.  
T₁ – Control, T₂ – VAM, T₃ – *Pisolithus albus* spores, T₄ – *Pisolithus albus* vermiculite,  
T₅ – *Pisolithus albus* beads, T₆ – *Laccaria fraterna* spores, T₇ – *Laccaria fraterna* vermiculite,  
T₈ – *Laccaria fraterna* beads, T₉ – *Frankia*, T₁₀ – DAP.

| Table 4. Status of AM spore population in rhizosphere soil of *Casuarina equisetifolia* and *Casuarina junghuhniana* Seedlings treated with different bioinoculants in nursery |
|---|---|
| **S.** | **Treatments** | **No. of Spores*** |
| No. | | *Casuarina equisetifolia* | *Casuarina junghuhniana* |
| 1. | T₁ | 4 | 12 |
| 2. | T₂ | 72 | 80 |
| 3. | T₃ | Nil | Nil |
| 4. | T₄ | Nil | Nil |
| 5. | T₅ | 10 | 6 |
| 6. | T₆ | 14 | 22 |
| 7. | T₇ | 13 | 7 |
| 8. | T₈ | 15 | 17 |
| 9. | T₉ | 20 | 30 |
| 10. | T₁₀ | 17 | 13 |

* Mean of three replication  
T₁ – Control, T₂ – VAM , T₃ – *Pisolithus albus* spores, T₄ – *Pisolithus albus* vermiculite,  
T₅ – *Pisolithus albus* beads, T₆ – *Laccaria fraterna* spores, T₇ – *Laccaria fraterna* vermiculite,  
T₈ – *Laccaria fraterna* beads, T₉ – *Frankia*, T₁₀ – DAP.

Table 5. Percentage of Arbuscular Mycorrhizae and Ecto Mycorrhizae Infection on Casuarina equisetifolia and C. junghuhniana

| S. No | Treatments | Casuarina equisetifolia | | | Casuarina junghuhniana | | |
|-------|------------|-------------------------|-------|-----------------------|-------|
|       |            | Am Fungi                | ECM & | % of AM ECM infection | Am Fungi | ECM & | % of AM ECM infection |
|       |            | V | H | A | | V | H | A | | |
| 1.    | $T_1$      | + | + | - | - | + | - | - | - | 9% | + | - | - | - | 4% |
| 2.    | $T_2$      | + | + | + | - | + | + | + | - | 85% | + | + | + | - | 80% |
| 3.    | $T_3$      | - | - | - | + | - | - | - | + | 81% | - | - | - | + | 72% |
| 4.    | $T_4$      | - | - | - | - | - | - | - | - | Nil | - | - | - | - | Nil |
| 5.    | $T_5$      | + | - | - | - | - | - | - | - | 15% | - | - | - | - | Nil |
| 6.    | $T_6$      | + | + | - | - | - | - | - | - | 20% | + | - | - | - | 15% |
| 7.    | $T_7$      | + | - | - | - | + | - | - | - | 17% | + | - | - | - | 10% |
| 8.    | $T_8$      | + | - | - | - | + | - | - | - | 17% | + | - | - | - | 14% |
| 9.    | $T_9$      | + | - | - | - | + | - | - | - | 23% | + | - | - | - | 10% |
| 10.   | $T_{10}$   | + | + | - | - | + | - | - | - | 17% | + | - | - | - | 17% |

$T_1$ – Control, $T_2$ – VAM, $T_3$ – *Pisolithus albus* spores, $T_4$ – *Pisolithus albus* vermiculite,
$T_5$ – *Pisolithus albus* beads, $T_6$ – *Laccaria fraterna* spores, $T_7$ – *Laccaria fraterna* vermiculite, $T_8$ – *Laccaria fraterna* beads, $T_9$ – *Frankia*, $T_{10}$ – DAP.
spores in the rhizosphere but variation in the number of spores. Among different age group of the samples screened, maximum number of AM fungal spores (72/100g soil) was recorded in the rhizosphere of one year plantation this is followed by two year old plantation (51/100g soil). The minimum number AM fungal spores was recorded in the rhizosphere of three year old plantation (33/100g soil).

**Distribution of am fungi in the rhizosphere of Casuarina equisetifolia**

The rhizosphere soils under the root zone of *Casuarina equisetifolia* of six different age groups were collected, processed, isolated, quantified, and identified different AM fungi recorded in the rhizosphere of *Casuarina equisetifolia* collected from different age groups. It was observed that eight different AM fungi belong to two genera viz., *Acaulospora* and *Glomus* were recorded (Fig. 1). Among these genera, the genus *Glomus* was found predominant. Among the different species of genus *Glomus geosporum* was found predominant and it was found in the rhizosphere all the six different age groups of *Casuarina equisetifolia*. Among the rhizosphere soil samples of six different age groups of *Casuarina equisetifolia* plantation processed. The rhizosphere of two year age group of *C. equisetifolia* had seven different AM fungi viz., *Acaulospora sp, Glomus sp, Glomus claroideum, Glomus fulvus, Glomus geosporum, Glomus multicauleae, Glomus occultum*. This is followed by rhizosphere of seven year age group had five different AM fungi viz., *Acaulospora sp,Glomus claroideum,Glomus fulvus,Glomus geosporum,Glomus multicauleae* and the rhizosphere of one year age group had four different AM fungi viz., *Glomus sp, G. albidium, G. claroideum* and *G. geosporum*.

**Status of Frankia nodule population in plantation of Casuarina equisetifolia**

Root nodules of *Frankia* were collected from six different age groups of *Casuarina equisetifolia* plantations at random sampling method. Fresh weight and dry weight of these nodules collected from different age groups of *Casuarina equisetifolia* were done (Table-1 and Fig. 5). The results indicated that maximum fresh and dry weight of *Frankia* nodules was recorded from the sample 5 year old *Casuarina equisetifolia* plantation, four year old plantation. Low fresh and dry weight of the nodules was observed in one year and two year old plantation. It was also observed that the nodule population was increased when the age of the plantation increased up to five years. While, seven years old plantation had reduction in population.

**Nursery experiment**

An experiment was conducted to study the effect of different bioinoculants on growth enhancement of in nursery. The bioinoculants such as AM fungi, ECM fungus (*Pisolithus albus* basidiospores, vermiculite based, vegetative mycelium, and alginate bead inoculums), ECM fungus (*Laccaria fraterna* basidiospores, vermiculite based, vegetative mycelium, and alginate bead inoculums), *Frankia* nodules and chemical fertilizer (DAP) were used in the nursery experiment. All these bioinoculants and chemical fertilizers were inoculated to fifteen days old seedlings grown in poly bags. The seedlings in bags without application of bioinoculants and

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>pH</th>
<th>EC(dsm-1)</th>
<th>O.C %</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>8.3</td>
<td>0.20</td>
<td>0.35</td>
<td>61</td>
<td>8</td>
<td>95</td>
</tr>
<tr>
<td>2.</td>
<td><em>Casuarina</em> <em>equisetifolia</em></td>
<td>8.4</td>
<td>0.14</td>
<td>0.38</td>
<td>63</td>
<td>9</td>
<td>110</td>
</tr>
<tr>
<td>3.</td>
<td><em>Casuarina</em> <em>junghuhniana</em></td>
<td>8.5</td>
<td>0.12</td>
<td>0.38</td>
<td>63</td>
<td>9</td>
<td>105</td>
</tr>
</tbody>
</table>

* E.C. Electrical conductivity
O.C. Organic carbon;
P-Phosphorous;
N- Nitrogen;
K-Potassium

*Mean of three replication;*
chemical fertilizers are kept as control. The seedlings were harvested and recorded various growth parameters such as shoot length, root length, fresh and dry weights, AM fungal spore population and percentage of mycorrhizal colonization.

**Shoot and root length**

Data on shoot and root lengths of both *Casuarina equisetifolia* and *Casuarina junghuhniana* is represented in Table 2, Fig. 2 and Fig. 6. Maximum shoot and root length was recorded in (T₃ treatment) *Pisolithus albus* basidiospore inoculated seedlings of *Casuarina equisetifolia*, this is followed by the seedlings inoculated with (DAP) chemical fertilizer. In case of *Casuarina junghuhniana*, (T₄ treatment) *Pisolithus albus* vermiculite based vegetative mycelium inoculated seedlings had greater shoot length.

![Fig. 1. AM fungi recorded from the rhizosphere of Casuarina equisetifolia](image1)

![Fig. 2. VAM inoculum and beads](image2)
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**Fig. 3.** Different kinds of bio-inoculants used in the nursery experiment

**Fig. 4.** Mycorrhizal structures observed in nursery experiment

**Fig. 5.** Mean Frankia nodule bio-mass

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Fig. 6. Effect of Bioinoculants on the growth improvement of Casuarina equisetifolia and Casuarina jhunghiana

Fig. 7. Effect of Bioinoculants on the shoot and root biomass improvement of Casuarina equisetifolia in nursery experiment

Fig. 8. Effect of Bioinoculants on the shoot and root biomass improvement of Casuarina jhunghiana in nursery experiment

Fig. 9. Status of AM spore population in rhizosphere soil of Casuarina equisetifolia and Casuarina jhunghiana seedings treated with different bioinoculants in nursery

and root lengths, followed by seedlings treated with chemical fertilizer (DAP) (T\textsubscript{10}). In general all the bioinoculant treated seedlings had better shoot and root length as compared to uninoculated control seedlings.

**Shoot and root biomass**

Data on fresh and dry weights of shoot and root of both *Casuarina equisetifolia* and *Casuarina junghuhniana* is represented in Table 3, Fig. 3 and Fig. 7. The results indicated that the shoot and root dry weights was maximum in T\textsubscript{9} treatment (*Frankia* nodules), inoculated seedlings of *Casuarina equisetifolia*, followed by the treatment T\textsubscript{4} (*Pisolithus albus* vermiculite based vegetative mycelium).

In case of *Casuarina junghuhniana*, maximum shoot and root dry weight was recorded in seedlings of *Casuarina junghuhniana*, inoculated with *Pisolithus albus* vermiculite based vegetative mycelium (T\textsubscript{4}) this is followed by seedlings inoculated with *Pisolithus albus* basidiospore inoculum.

**Status of am spore population in the rhizosphere soil sample of Casuarina equisetifolia and Casuarina junghuhniana seedlings treated with different bioinoculants.**

Data on AM fungal spore population in the rhizosphere of both *Casuarina equisetifolia* and *Casuarina junghuhniana* seedlings treated with different bioinoculants in the nursery experiment is presented in Table 4, Fig. 4, Fig. 8 and Fig. 9. It was found that AM spore population was maximum in the rhizosphere of *Casuarina equisetifolia* and *Casuarina junghuhniana* seedlings particularly AM fungus treatment (T\textsubscript{2}) this is followed by both the seedlings treated with *Frankia* nodules (T\textsubscript{9} treatment). In some of the treatments like basidiospore inoculums and vermiculite based inoculums of *Pisolithus albus* did not reveal AM fungal spores in the rhizosphere of both the tree species.

**Percentage colonization of AM, ECM fungi on Casuarina equisetifolia and casuarina junghuhniana seedlings**

Data on percent root colonization of both AM and ECM fungi in 10 different treatments of *Casuarina equisetifolia* and *Casuarina junghuhniana* root samples collected from the nursery experiment is presented in Table 5. It was observed on the investigation that the maximum percent colonization of AM fungi was found in treatment T\textsubscript{7} (AM fungi inoculated) of both tree species (85% and 80% respectively). Less percent colonization of AM fungi was recorded in the treatment T\textsubscript{1} (uninoculated control seedlings). It was also observed that ECM fungal colonization in the root Segments of both tree species in treatment T\textsubscript{3} (*Pisolithus albus* basidiospore inoculated).

**Soil nutrient status of nursery potting mixture**

Data on the soil nutrient status of the potting media used in the nursery experiment is presented in Table 6. The rhizosphere soil of control (uninoculated) and AM fungal inoculated seedlings of both the tree species were analyzed in the soil testing laboratory, Agricultural Department at Villupuram, Tamil Nadu. It was found that all the parameters such as pH, organic carbon, Nitrogen, Phosphorus and Potassium were less as compared to AM treated potting media of both the tree species. The electrical conductivity was more in the rhizosphere of uninoculated controlled potting medium.

**DISCUSSION**

The rhizosphere and rhizoplane are in habitat by a wide range of micro organism that carries out activities, which are of great relevance to plant growth. Among these the micro organism which from relationship form the Endorhizosphere are well placed to influence plant behavior doing so, they become an integral part roots and in consequence considerably modify the activities of these absorbing organs of the various plants-microbe interaction. The most prevalent and the wide spread type of association is the “Mycorrhiza”. Although seven types of mycorrhizae are recognized (Ectomycorrhizae, Vesicular Arbuscular, Ericoid, Arbutoid, Monotropoid, Orchid and E – strain mycorrhizas) the most prevalent and wide spread type of mycorrhizae in plant kingdom is the Vesicular Arbuscular mycorrhizae. More than 80% of the plant species including most agricultural, horticultural, plantation crops.

Arbuscular mycorrhizal (AM) fungi can be found nearly all eco system through out the world. The occurrence of Arbuscular Mycorrhizal (AM) association in natural eco system is

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currently of great interest because of the role played by AM fungi in plant species in natural eco system in India is reported, only a few percentage of the total flora. So mycorrhizae surveys in diverse localities are needed to ascertain the functional status and mycorrhizal dependence of a plant species. When natural eco system are converted into agro eco systems a notable change in the composition and richness of AM fungal populations have been observed. In the present investigations an attempt was made to study the status on the occurrence and distribution of a AM fungi in association with Casuarina equisetifolia in different age group of plantations available in Villupuram district, Tamil Nadu. It was observed from the study that all the root samples and rhizosphere soil samples collected from different age groups pf plantation had AM fungal colonization in the roots and AM fungal spores in the soil samples but variation in numbers. Maximum percent colonization was observed in younger age level plantation as compared to older age plantation. Similarly the AM fungal spore population was found maximum in younger age level plantation as compared to older age level plantation. These findings are in accordance with findings made by other researchers on different plant species in various eco systems. The studies on revegetation of Iron ore mining soils with Accacia pyrifolia, Triodia pungiens, A.anura inoculation with Glomus species resulted in up to 70 %. Increases in dry matter production at low rates of phosphorus application. Study on lignite over burden. The plant species such as Side Oats, Indian grass and Klew grass were inoculated with AM fungi (Glomus fasiculatum and Gigaspora margarita) in containerized system and transplanted into the lignite over burden7. Plants inoculated with VAM fungi had greater survival percentage; ground mass, high level of nitrogen and phosphorus in above ground mass. The study conducted in bauxite mine soil with Eucalyptus marginata and Acacia pulchella. Infectivity of VAM is very low in the initial stage and also the growth rate plants are also the same8. After three to five years the infection is very high and the plants get high dry weight. In India9 this type of work in chromite mine spoil. Growth, nodulation and total nitrogen content of Accacia nilotica was observed due to all types of VAM inoculation. Glomus mosseae is predominant in chromium over burden area.

It’s interesting to note that both percentage root colonization and AM spore colonization of Casuarina equisetifolia in different age level plantation are positively correlated. The variation in percent colonization in roots and AM fungus spore population in the soil may be due to adoptic condition of the plantation sites. Jasper et al., noticed that, top soil disturbances decreases the number of spores and number of spore types. Studied the Acer pseudoplatanis stand growing on the two mines spoil9. The quantity as well as species diversity and viability of AM fungi population were disturbed.

Total of eight different fungi belongs to two different genera viz., Acaulospora and Glomus were recorded in that the genus Glomus was found predominantly in the rhizosphere of Casuarina equisetifolia in different age plantation. Among the different species of the genus Glomus, Glomus geosporum was recorded in the rhizosphere of Casuarina equisetifolia in all the six different age level of plantation. Similar observation also made by other researches on the different plant species. In India and other part of the world10.

Nursery Experiment was conducted to study the effect of different bio inoculants (AM fungi, Ectomycorrhizal fungi, P. albus and L. fraterna of Basidiospore, vermiculite and alginate bead inoculums and Frankia nODULE) and chemical fertilizer (DAP). It was observed from the study that all the inoculated seedlings of both C. equisetifolia and C. junghuhniana had better growth performance when compared to uninoculated control seedlings.

An attempt was also made to investigate the persistence of inoculated AM and ECM fungi in the roots and rhizosphere soils of all the treatments. It was recorded that AM spores population was found maximum in the rhizosphere soil sample of C. equisetifolia and C. junghuhniana seedlings especially AM fungus treatment (T2). This is followed by both the seedlings treated with Frankia nodules (T9 Treatment). Maximum percent colonization of AM fungi was found in treatment T2 (AM fungi inoculated) of both the tree species. It was also observed that ECM fungal colonization in the root segments of both the tree species treatment T3 (P.albus Basidioapore inoculated) similar
type of findings made by different on various plant species in different part of the world. The growth stimulation of *Tamarindus indica* by selected VAM fungi\(^2\). The results of their study indicate that inoculated plants had greater plant height, leaf number, stem girth, biomass, phosphate and zinc content. Also they have observed that number of VAM spores in the soil, percent root colonization and external hyphae where higher and the seedlings responded best to inoculation with VAM fungi with *Gigaspora margarita* followed by *Glomus fasiculatum*. Study the growth response of the tamarind seedling. They have observed the response of plant growth, biomass and phosphorus uptake was not significant in individual inoculation of all VAM fungi inoculated tamarind seedlings\(^3\). In the present study the response to plant growth, biomass and percent colonization of VAM fungi in inoculated plants were greater than control due to the mixed culture of both *Glomus fasiculatum* and *Glomus microcarpum*. Studied the growth response and efficiency of VAM fungi on *Ailanthus excelsa*, *Azadirachta indica* and *Parkinsonia aculeate* to inoculation with *Glomus fasiculatum*. Inoculation of soil with VAM fungi increased in dry matter yield of *Leucaena leucocephala* and resulted in fast and increased growth as compared to uninoculated plants \(^4\).\(^5\) has screened several VAM fungi for their suitability to two slow growing tree species, *Acacia nilotica* and *Calliandra calothyrsus* may noted that inoculated seedlings had greater length height leaf number, stem girth, biomass and phosphorus and zinc content. The effect of dual inoculation of VAM fungi and *Rhizobium / Frankia* and their efficiency on the various tree species been investigated by several researches. That dual inoculation *Acacia auriculiformis* with VAM fungi and *Rhizobium* resulted in greater number of nodules, seedling weight, uptake of nitrogen, and phosphorus and acetylene reduction than when these inoculants were used singly\(^6\).

**REFERENCES**