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

R. Senthilkumar[1], R. Rajasekaran[2] and T. Selvaraj[3]

[1]PG and Research Department of Botany and Microbiology,
A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur - 613 503, India.
[2]Department of Microbiology, Marudupandiyar College, Thanjavur - 613 403, India.
[3]Department of Plant Sciences, Ambo College, Jimma University, Ethiopia, East Africa.

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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 mycorrihizal fungus (AM), Ectomycorrihizal 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 Ectomychrrhizae *P.albus* 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* basidiospore form shows the maximum growth in root (19.6) and shoot length (26.6) of *Casuarina junghuhniana Pisolithus albus* vermiculite form shows maximum length in both plants DAP shows second maximum *Frankia* inoculated plants shows maximum result in biomass of *Casuarina equisetaifolia* and its followed by *Pisolithus albus* vermiculite form for *Casuarina junghuhniana,* the *Pisolithas albus* inoculated plant tissues maximum results and its followed by *Pisolithas albus* spores. Rhizobus of *Causarina equisetifoloa* and *Casuarina junghuhniana* inoculated with VAM spores. Maximum ECM colonization observed in treatment inoculated *Pisolithus albus* sopre. 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 junghuhnian,a Casuarina equisetifolia, Pisolithus albus.*

Casuarinas are a distintictive group of angiosperms, belonging to the family Casuarinaceae. And these are the unique amongst the angiosperms and are placed in an order by

* To whom all correspondence should be addressed.
Mob.: +91-9842425623
E-mail: raskumar2008@yahoo.co.in

themselves, Casuarinales. Casuarinas are characterized by long needle like branch lets 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 *casuarina*s 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, and detect the effect of ECM 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]

1cm 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 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

age group plantation was collected to determine the spore population. Mycorrhizal spores were obtained by wet sieving and decanting method proposed by Gerdemaan and Nicholson[5]. 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.

Bioinoculam production N

In the experiment, AM fungi, Ectomycorrhizal fungi, *Frankia* and chemical fertilizer (DAP) were used.

AM Inoculam

AM inoculums were collected from Department of Microbiology, Tamilnadu Agricultural University, Coimbatore.

Ectomycorrhizal inoculam 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 Inoculam

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 .watering 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[4]

1cm 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.

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

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 Gerdemaan and Nicholson[5]. 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 endomycorrhozal 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 Casuarianaceae. 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 Villupuram 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 1. *Frankia* nodule bio-mass

S. No.	Age of Plants	Fresh weight of the nodule(gm)*	Dry weight of the Nodule(gm)*
1.	One	12.69	6.74
2.	Two	13.59	7. 86
3.	Three	20.97	15. 77
4.	Four	44.92	21. 65
5.	Five	45.36	23. 89
6.	Seven	24.63	14.68

* Mean of three replications

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

S. No	Treatments	*Casuarina equisetifolia*		*Casuarina junghuhniana*	
		Shoot Length (cm)*	Root Length (cm)*	Shoot Length (cm)*	Root Length (cm)*
1.	T_1	18.2	11.0	17.6	8.5
2.	T_2	21.0	17.5	22.3	8.8
3.	T_3	28.01	19.6	26.4	10.7
4.	T_4	22.3	13.1	26.6	14.7
5.	T_5	20.4	17.4	22.3	13.2
6.	T_6	21.7	18.6	22.86	9.6
7.	T_7	20.1	18.9	22.76	11.1
8.	T_8	20.4	19.4	23.5	14.0
9.	T_9	22.1	15.2	23.7	10.6
10.	T_{10}	27.7	18.6	25.16	14.6

* - Mean of three replications.
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.

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. No	Treatments	*Casuarina equisetifolia*				*Casuarina junghuhniana*			
		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	T_1	0.69	0.27	0.28	0.10	0.28	0.12	0.21	0.08
2	T_2	0.81	0.38	0.23	0.12	0.86	0.22	0.29	0.09
3	T_3	1.21	0.33	0.29	0.12	1.31	0.49	0.29	0.12
4	T_4	1.15	0.53	0.30	0.14	1.16	0.61	0.37	0.18
5	T_5	0.76	0.42	0.29	0.12	0.85	0.32	0.26	0.16
6	T_6	0.90	0.47	0.23	0.10	0.79	0.49	0.26	0.18
7	T_7	1.13	0.48	0.37	0.18	1.07	0.24	0.32	0.19
8	T_8	0.88	0.38	0.25	0.10	0.64	0.28	0.23	0.15
9	T_9	0.95	0.62	0.38	0.22	0.27	0.18	0.26	0.15
10	T_{10}	0.97	0.42	0.20	0.11	0.63	0.53	0.28	0.13

*- Mean of three replications.

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.

Table 4. Status of AM spore population in rhizosphere soil of C*asuarina equisetifolia* and *Casuarina junghuhniana* Seedlings treated with different bioinoculants in nursery

S. No	Treatments	No. of Spores*	
		Casuarina equisetifolia	*Casuarina junghuhniana*
1.	T_1	4	12
2.	T_2	72	80
3.	T_3	Nil	Nil
4.	T_4	Nil	Nil
5.	T_5	10	6
6.	T_6	14	22
7.	T_7	13	7
8.	T_8	15	17
9.	T_9	20	30
10.	T_{10}	17	13

*- Mean of three replication

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.

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 Fungi	% of AM & ECM infection	AM Fungi			ECM Fungi	% 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%

V – Vesicles, H – Hyphae, A – Arbuscles.

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 fraternal* 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 equietifolia* of six different age groups were collected, processed, isolated, quantified, and identified different AM fungi recorded in the rhizosphere of *Casuarina equisetifoliia* 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 rhiozosphere 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 multicaulae, 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 multicaulae* 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 6. Soil Nutrients status of potting media after inoculation of with AM fungi

S. No	Sample	Soil Parameters*					
		pH	EC(dsm-1)	O.C %	N	P	K
1.	Control	8.3	0.20	0.35	61	8	95
2.	*Casuarina equisetifolia*	8.4	0.14	0.38	63	9	110
3.	*Casuarina junghuhniana*	8.5	0.12	0.38	63	9	105

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

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 (T_3 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*, (T_4 treatment) *Pisolithus albus* vermiculite based vegetative mycelium inoculated seedlings had greater shoot

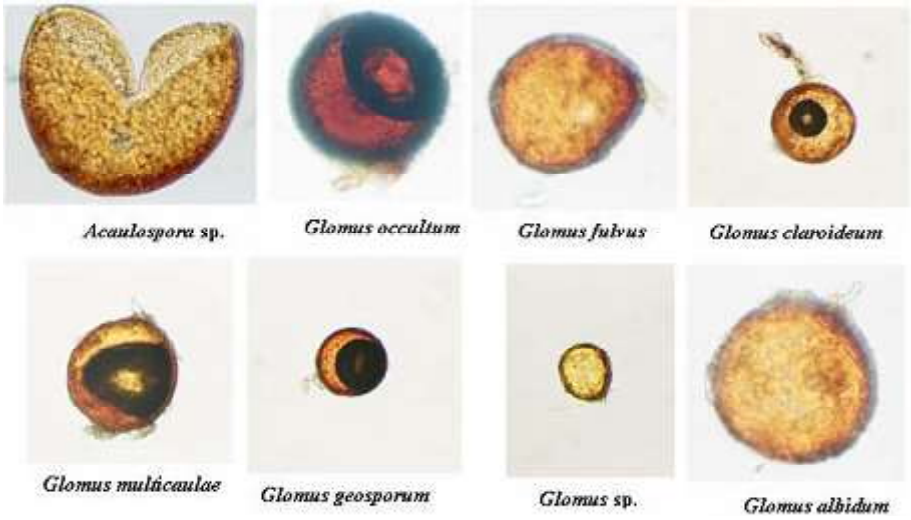

Fig. 1. AM fungi recorded from the rhizosphere of Casuarina equisetifolia

Fig. 2. VAM incoculam and beeds

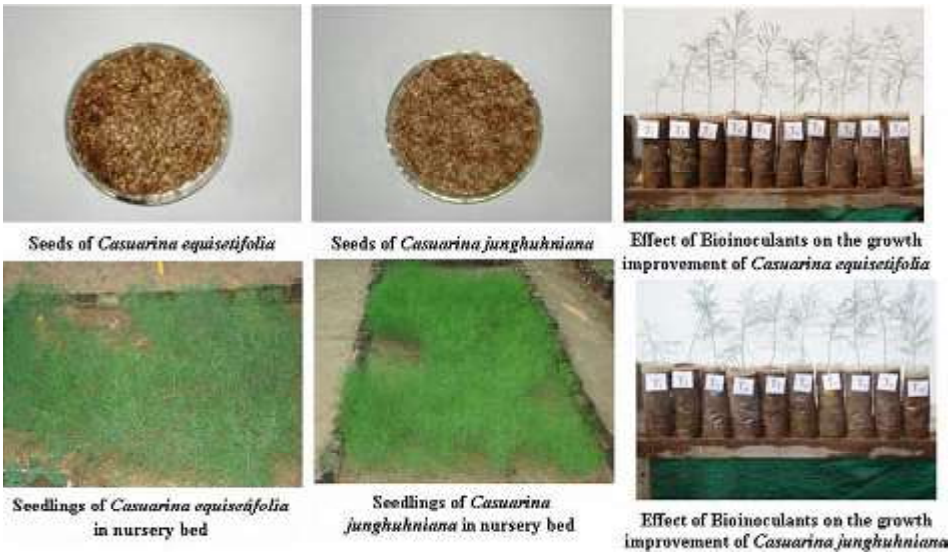

Fig. 3. Different kinds of bio-inoclants used in the nursery experiment

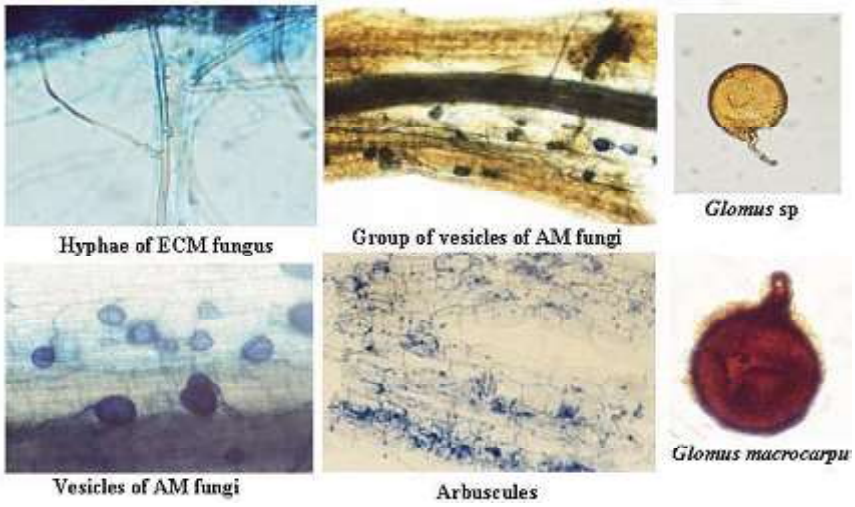

Fig. 4. Mycorrhizal structures observed in nursery experiment

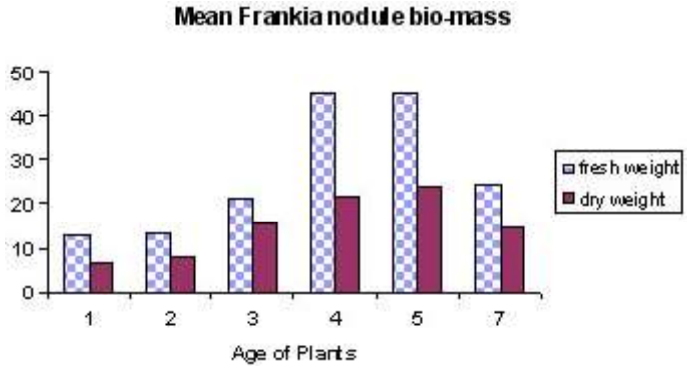

Fig. 5. Mean franika nodule bio-mass

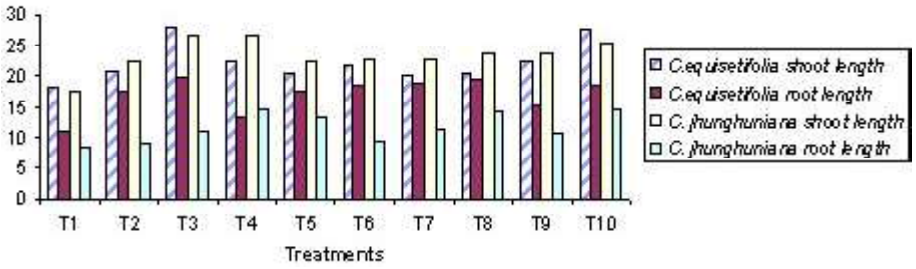

Fig. 6. Effect of Bioinoculants on the growth improvement of *Casuarina equisetifolia* and *Casuarina jhunghaniana*

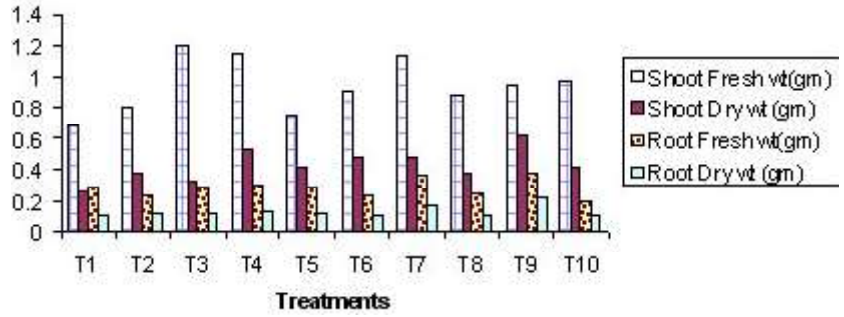

Fig. 7. Effect of Bioinoculants on the shoot and root biomass improvement of *Casuarina equisetifolia* in nursery experiment

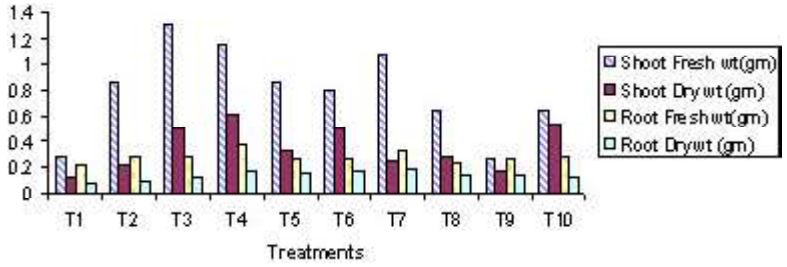

Fig. 8. Effect of Bioinoculants on the shoot and root biomass improvement of *Casuarina jhunghuiana* in nursery experiment

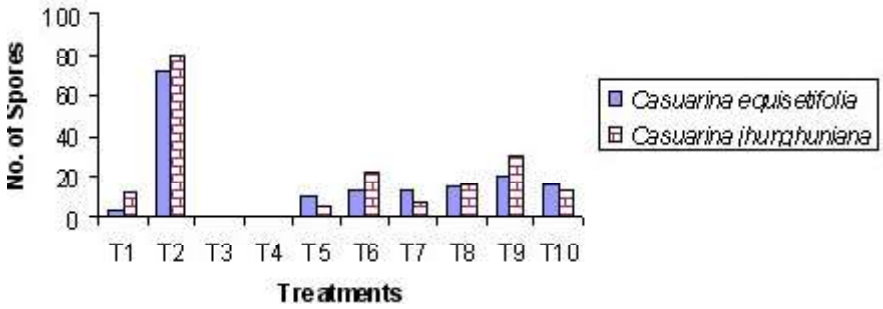

Fig. 9. Status of AM spore population in rhizosphere soil of *Casuarina equisetifolia* and *Casuarina jhunghuiana* seedings treated with different bioinoculants in nursery

and root lengths, followed by seedlings treated with chemical fertilizer (DAP) (T_{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_9 Treatment (*Frankia* nodules), inoculated seedlings of *Casuarina equisetifolia*, followed by the treatment T_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_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* 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_2) this is followed by both the seedlings treated with *Frankia* nodules (T_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_2 (AM fungi inoculated) of both tree species (85% and 80% respectively). Less percent colonization of AM fungi was recorded in the treatment T_1 (uninoculated control seedlings). It was also observed that ECM fungal colonization in the root Segments of both tree species in treatment T_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 rhizpoplane 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

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[6]. 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 burden[7]. 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 same[8]. After three to five years the infection is very high and the plants get high dry weight. In India[9] 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 ioculation. *Glomus mosseae* is predominant in chromium over burden area.

It's interesting to note that both percentage root colonization and AM spore colonization of *Casuarinia 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 spoil[10]. 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 world[11].

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* nodules) and chemical fertilizer (DAP). It was observed from the study that all the inoculated seedlings of both *C.equsetifolia 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.equsetifolia* 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[12]. 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[13]. 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 leucocethala* and resulted in fast and increased growth as compared to uninoculated plants [14,12] has screened several VAM fungi for their suitability to two slow growing tree species, *Accacia nilotica* and *Calliandra calothyrus* 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 had 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[15]

REFERENCES

1. Bentham. Plant taxonomy.In: Casuarina improvement and utility. ICFRE., 1873.
2. Costas Throdorou and Paul Reddel. *In vitro* synthesis of ectomycorrhizae on Casuarinaceae. *New phytologist*., 1991; (**118)** : 279-288.
3. Bagyaraj, D. J and Menge, J. A. Interaction between VA- mycorrhiza and *Azotobacter* and their effects on rhizosphere, micro flora and plant growth. *New phytol.,* 1978; **80:** 567-573.
4. Phillips, J .M and Hayman, D. S, Improved procedures for Clearing roots and staining parasitic and AM for rapid assessment of infection, *Trans. Br. Mycol. Soc*., 1970; **55**: 158-161.
5. Gerdemann, J .W and Nicolson, T .H Spores of mycorrhizal Endogone species extracted from soils by wet-sieving and decanting. *Trans. Br. Mycol*. *Soc*., 1963; **46**: 235-244.
6. Jasper, D. A., Robson, A. D. and Abbott, L. K. Revegetation in Iron-ore Mine nutrient requirements for plant growth and the potential role of Vesicular Arbuscular Mycorrhizal fungi. *Australian Journal of Soil Research*., 1988; **26**(3): 497-507.
7. Call, S.R and Davis, R. Revegetation of lignite burden soil-potential role of AM fungi. *Australian journal of botany*., 1998; **29**(6): 420-428.
8. Jasper, D. A., Robson, A. D. and Abbott, L. K. The infectivity of external hyphae of Vesicular Arbuscular Mycorrhizal fungi. *New phytologist*., 1989; **112**: 93-99.
9. Mishra,R.S and Patnaik,M.*Acacia nilotica* inoculated with Mycorrhiza-role on chromite mine spoil. *New phytologist*., 1991; **119**: 292-299.
10. Jasper, D. A., Robson, A. D. and Abbott, L. K. The effect of surface mining on the infectivity of Vesicular Arbuscular Mycorrhizal fungi. *Australian Journal of Botany*, 1987; **35(6)**: 641-652.
11. Mohan. V. Studies on ectomycorrhizal association in Pinus patula plantations in Nilgiri hills, Tamilnadu. Ph.D thesis, University of Madras, India. 1991; 260.
12. Reena, J. and Bagyaraj, D. J. Growth stimulation of *Tamarindus indica* by selected VAM fungi. *World Journal of Microbiology and Biotechnology.,* 1990; **6**: 59-63.
13. Selvaraj, M. Syamala, D., Arumugam, S. and Rao, M.V. Growth and productivity of AM inoculated tropical seedlings, *Indian Forester*, 1996; 1161-1167.
14. Bagyaraj, D. J., Byra Reddy, M.S and Nalini, P. A. *Forest ecol. Manage* ., 1989; **27**: 81-85.
15. Chen.Y.L and B. Dell. Effect of AM on *E. globulus*. *New phytology.,* 2000; **146**: 545-556.