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Selection of Suitable Biofertilizers for Production of Quality Seedlings of *Casuarina Equisetifolia* (Forst.) Using Decomposed Coir Pith Compost in Root Trainers

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ABSTRACT
A nursery experiment was conducted to assess the effect of bioinoculants (Frankia, Azospirillum and Phosphobacterium) on growth performance of Casuarina equisetifolia seedlings grown in decomposed coir pith as substrate in root trainers. Growth of C. equisetifolia seedlings was monitored for six months after inoculation with Frankia or Azospirillum or Phosphobacterium or combination of all the three inoculants. After six months, the plants were harvested and root and shoot length, collar diameter, root and shoot weight, nodule number and nodule weight were recorded. The total biomass was highest in Frankia + Azospirillum + Phosphobacterium (T_7) inoculated seedlings (48.23% increase over the control), followed by Frankia + Phosphobacterium (T_5) inoculated seedlings (41.73% increase over the control) and Phosphobacterium (T_3) inoculated seedlings (40.06% increase over the control). Further, the growth parameters and nutrient content significantly increased in all the treatments, compared to the control. Among the inoculated seedlings, Frankia + Azospirillum + Phosphobacterium (T_7) combination performed better. This was followed by Frankia + Phosphobacterium (T_5) combination and Phosphobacterium (T_3).
Key words: *Casuarina equisetifolia, Decomposed coir pith, Root trainers, Azospirillum, Frankia, Phosphobacterium, Seedling quality*

INTRODUCTION

Casuarina equisetifolia is a fast growing, multipurpose, actinorhizal tree capable of fixing atmospheric nitrogen in association with *Frankia*. These plants play an important role in increasing soil N content, land reclamation and agro forestry [1-3]. In addition, there is growing evidence on the beneficial effect of biofertilizers in increasing the growth and biomass of *C. equisetifolia* in nursery conditions [4-5]. Biofertilizers are increasingly used in agriculture and forestry because they are environment friendly and effectively supplement nutrients to the plants. Biofertilizers are known to increase the uptake of nitrogen, phosphorus and potassium along with production of growth hormones which enhance the growth and vigor of plants [6--7].

Azospirillum, an important associative symbiotic nitrogen fixing bacterium, fixes atmospheric nitrogen in soil [8], promotes seedling growth, biomass and nutrient uptake [9 -10, 4], tree growth [11], and also increases root biomass, root surface area, root diameter, density and length of root hairs [12]. The growth of *C. cunninghamiana* seedlings was stimulated when inoculated with *Azospirillum brasilense* [13].

Frankia is a widespread soil actinomycete, and has the ability to form nitrogen fixing nodules in non-leguminous plants called actinorhizal plants (including *C. equisetifolia*) [14]. *Frankia* inoculated seedlings of *C.equisetifolia* are known to have higher survival rate and grow more rapidly than uninoculated seedlings [15,4]. The solubilization of P in the rhizosphere is the most common mode of action implicated by plant growth promoting rhizobacteria (PGPR) that increase nutrient availability to host plants [16]. The plant growth promoting bacterial inoculants are used in many studies related to growth enhancement of *C. equisetifolia* [17].

Increasing interest in producing quality seedlings by the application of improved and modern nursery technique has been shown, in recent years [18]. Root trainer technology is used to raise the plants in forest nurseries [19]. Decomposed coir pith compost can be successfully substituted for soil or sand in conventional nursery mixtures [20]. Nowadays, use of sand in potting mixture is increasingly becoming difficult due to its non-availability and high cost. Decomposed coir pith compost, one of the waste materials that can be easily obtained from coir pith industries. Coir pith (100%) used as potting medium showed a spectacular increase in water holding capacity of potting mixture when tomato plants were grown on coir pith based potting mixture [21].

Decomposed coir pith used to raise seedlings is generally low in nutrients and microbial populations, which necessitated the use of large amounts of fertilizers in nurseries. However, the increased cost of seedling production and environmental concerns have forced to look for alternative strategies for seedling production of forest species in nurseries. This problem can be overcome by applying suitable microorganisms to improve the growth, biomass and nutrient content in *C. equisetifolia*. Hence the present study was undertaken to find out the compatibility of different microbial inoculants for the production of quality seedlings of *C. equisetifolia* in decomposed coir pith compost in root trainers.

MATERIALS AND METHODS

Study area

The experimental site - Plantation Division, Tamil Nadu Newsprint and Papers Limited (TNPL), Kagithapuram, Karur, Tamil Nadu, India - is located at 11˚3'10" N latitude and 77˚49'25" E longitude at an elevation of 150 m above msl. The maximum and minimum temperatures are 34˚C and 17˚C, respectively. Average annual rainfall is about 725 mm and relative humidity ranged from 37.9 to 69.3%.

Potting media and transplanting of seedlings

Healthy and uniform sized (about 5cm height) seedlings were transplanted to 300cc root trainers with decomposed coir pith as potting medium. The experimental setup was designed as Completely Randomized Block Design (CRBD) with 8 treatments and 40 replicates.

Physico-chemical analysis of Decomposed Coir pith

Apparent density, absolute specific gravity, maximum water holding capacity and volume expansion on wetting were determined by the method of Keen and Raczkowski [22] using brass cup. Analysis of lignin by Modified Klason Lignin assay, Cellulose by Updegraff method [23], N by Kjeldahl method [24], organic carbon by Walkey and Black method [25], P by Spectrophotometric method [26] and K by Flame photometric method [27] was carried out. Measurement of pH and EC was done by saturated paste extract method [28]. Physical attributes were determined in 3.7-liter containers (column height = 12.5 cm) using protocols from Ingram *et al.,* [29].

Isolation, mass multiplication and application of microorganisms

N-free semisolid malate medium (NFB) was used to isolate *Azospirillum* [30]. *Casuarina* roots were washed in sterile distilled water and in 25 mM phosphate buffer, pH 6.8, followed by three more washings in sterile distilled water [31]. The root samples were cut into pieces (5 to 8mm long) and placed in 10 ml serum vials containing 5 ml of NFB medium. Other vials containing NFB medium were inoculated with rhizosphere soil. The cultures were incubated at 32^0C for 24-72 h. White, dense, undulating pellicle formed just 1-3 mm below the surface of the medium was streaked on to Congo red plates and incubated at 32^0C for 72 h. After the incubation period, small scarlet colonies were observed, indicating the presence of *Azospirillum* sp. [32]. The isolated *Azospirillum* colonies were mass multiplied in nutrient broth.

Rhizosphere soil samples were collected from the plus tree of *C. equisetifolia.* The soil sample was diluted (10^{-3} to 10^{-}) and an aliquot (0.1ml) was spread on Pikovskaya's solid medium [33]. The plates were incubated for 27ºC for 3-5days. The colonies with clearing zones were counted and the results were expressed as log colony forming units (CFUs) per gram of soil. The PSB were mass multiplied in nutrient broth.

Frankia nodule suspension was prepared as per the method of Reddell *et al.,* [34]. *Frankia* nodules were collected from the plus tree of *C. equisetifolia*. The nodules were surface sterilized with 30% hydrogen peroxide for 20 min, followed by 70% alcohol treatment for 1 min and rinsed thrice in distilled water. Then they were ground in a pestle and morter and diluted with 2% sucrose solution.

Ten days after transplanting, 10ml each of *Azospirillum*, Phosphobacterium ($1x10^9$ cells/ml) and *Frankia* nodule suspension were applied to root trainers at 5cm depth near the root zone. Seedlings were watered to maintain 80% field capacity and kept in nursery conditions for 180 days.

Treatments

T_1- *Frankia*

T_2-*Azospirillum*

T_3-Phosphobacterium

T_4- *Frankia* + *Azospirillum*

T_5- *Frankia* + Phosphobacterium

T_6- *Azospirillum* + Phosphobacterium

T_7- *Frankia* + *Azospirillum* + Phosphobacterium

T_8- Control (Decomposed Coir pith alone)

Harvesting and morphometric measurement

Shoot length and collar diameter were recorded at monthly intervals up to six months. 180 days after transplanting, a total of 24 seedlings per treatment were randomly selected, harvested and determined the shoot and root length, collar diameter, nodule number and fresh weight. The seedlings were cut at collar region, dried at 70° C in hot air oven until the weight became constant.

Seedling survival percentage was calculated using the formula;

$$\text{Seedling survival percentage} = \frac{\text{Number of seedlings survived per treatment}}{\text{Total number of seedlings transplanted per treatment}} \times 100$$

Seedlings Quality Index was calculated using the formula of Dickson *et al.*, [35].

$$\text{Seedlings Quality Index (SQI)} = \frac{\text{Total dry weight (g/ plant)}}{\dfrac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \dfrac{\text{Shoot dry weight (g/plant)}}{\text{Root dry weight (g/plant)}}}$$

Microbial inoculation effect was calculated according to Muthukumar and Udaiyan [36].

$$\text{MIE} = \frac{\text{Dry weight of inoculated seedling - Dry weight of uninoculated seedling}}{\text{Dry weight of inoculated seedling}} \times 100$$

Nutrient Analysis

The oven-dried plant samples were ground to pass through a 0.5 mm plastic sieve before digestion. The dried plant material was ground in a mortar and pestle and total nitrogen content was estimated by the conventional micro-Kjeldahl method [37]. Total phosphorus was estimated by the method of Bartlett [26]. One gram of plant sample was digested with tri-acid mixture (HNO_3: H_2SO_4: $HClO_4$ in 9:2:1ratio) until it became colorless. After digestion, it was filtered and the volume was made up to 100 ml. Potassium in the extract was determined using a flame photometer as per the method of Jackson [27].

Estimation of total Chlorophyll and Protein content

Total chlorophyll content in leaf samples was estimated on fresh weight basis using the formula of Arnon [38]. The protein content was estimated by the method of Lowry *et al.*, [39].

Statistical Analysis

The data were statistically analyzed by one way analysis of variance (ANOVA) with SPSS and treatment means were separated using Duncan's Multiple Range Test ($P < 0.05$) [40]. Pearson's bivariate correlation analysis (SPSS version 11.5) was used to assess the relationship between the growth, biomass, nutrient content, total chlorophyll and protein content.

RESULTS

Chemical composition and physical properties of decomposed coir pith were displayed in (Table 1). Survival of *C. equisetifolia* was 100% in all the treatments including the uninoculated control (Table 2). Microbial inoculation resulted in significant increase in root length, shoot length and basal diameter in *C. equisetifolia*, compared to the uninoculated control. Triple inoculation involving *Frankia*, *Azospirillum* and Phosphobacterium (T_7) induced substantial increase in root shoot length, followed by dual and single inoculation. Significantly, there was not much difference in root length induction between the dual inoculants (*Frankia* + Phosphobacterium, *Frankia* + *Azospirillum* and *Azospirillum* + Phosphobacterium combinations). Among the single inoculants, *Azospirillum* stood first in inducing root length, and Phosphobacterium performed better in inducing shoot length. Within the dual inoculants, *Frankia* + Phosphobacterium combination induced least shoot length.

Nodule number was high with *Frankia*+ *Azospirillum* + Phosphobacterium inoculation (T_7), compared to single or dual inoculation involving *Frankia*. Within the dual inoculants, *Frankia* + *Azospirillum* combination resulted in higher nodule number.

Table 1: Chemical composition and physical properties of decomposed coir pith used for potting media for nursery experiments

Chemical Composition of decomposed coir pith		Physical properties of decomposed coir pith	
Lignin (%)	5.21	Bulk density (g/cc)	0.1025
Cellulose (%)	9.87	Particle density (g/cc)	0.3936
Organic carbon (%)	23.98	Porosity (%)	56.76
Nitrogen (%)	1.03	Maximum water holding capacity (%)	526.31
Phosphorous (%)	0.07	Volume expansion of 100ml (%)	22.92
Potassium (%)	1.23	pH	6.80
C:N ratio	23:1	EC	1.4 (mhos/cm)
Volume (mm)	0.55		

Table 2: Growth of *Casuarina equisetifolia* seedlings inoculated with bioinoculants in decomposed coir pith compost under nursery condition at 180 days after inoculation

Treatments	Seedling survival %	Root length (cm)	Shoot length (cm)	No of Nodules	Collar Diameter (mm)
T_1	100	18.07b	37.17b	4.69f	1.82b
T_2	100	20.36d	39.30c	3.22c	2.08d
T_3	100	19.23c	40.29d	3.07c	1.35b
T_4	100	20.47d	41.93e	4.07e	2.78e
T_5	100	21.14d	39.88cd	3.62d	2.75e
T_6	100	20.47d	42.12e	2.89b	2.59e
T_7	100	23.70e	45.87f	6.62g	3.35f
T_8	100	10.72a	29.10a	0.21a	1.04a

Means followed by a common letter(s) in the same column are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test)

Treatments : T_1 – *Frankia* ; T_2 – *Azospirillum* ; T_3 – Phosphobacterium ; T_4 - *Frankia* + *Azospirillum*; T_5 – *Frankia* + Phosphobacterium ; T_6 – *Azospirillum* + Phosphobacterium ; T_7 - *Frankia* + *Azospirillum* + Phosphobacterium ; T_8 – Control

Inoculation with *Frankia*, *Azospirillum* and Phosphobacterium (T_7) induced a significant increase in collar diameter, followed by *Frankia* + *Azospirillum* and *Frankia* + Phosphobacterium inoculations. Within the single inoculants *Azospirillum* performed better in increasing collar diameter.

Inoculation with *Frankia*+ *Azospirillum* + Phosphobacterium resulted in substantial increase in plant biomass over the uninoculated control or single inoculation or dual inoculation (Table 3). Within the dual inoculations, *Frankia* + Phosphobacterium inoculation performed better in plant biomass stimulation. Similarly, among the single inoculations, Phosphobacterium inoculation stood first in biomass stimulation in the host plant. While *Azospirillum* + Phosphobacterium and *Frankia*+ *Azospirillum* inoculation induced relatively lower root biomass, *Frankia* + Phosphobacterium induced higher root biomass. Similarly, within the single inoculations, root biomass was lower with *Frankia*, as compared to either *Azospirillum* or Phosphobacterium inoculation. In the case of shoot biomass, *Azospirillum* + Phosphobacterium combination performed better than *Frankia*+ *Azospirillum* and *Frankia* + Phosphobacterium combinations. Phosphobacterium inoculation resulted in maximum shoot biomass induction among the single inoculations.

Triple inoculation (*Frankia*+ *Azospirillum* + Phosphobacterium) resulted in greater accumulation of total chlorophyll and protein (58.56 and 74.85% increase over the control, respectively) in *C. equisetifolia*. Further, dual inoculation had an edge over the single inoculation in total chlorophyll and protein content of the host plant. Within the dual inoculants, *Frankia* + Phosphobacterium and *Azospirillum* + Phosphobacterium combinations induced higher total chlorophyll and protein content in the plant. Similarly, within the single inoculants, *Azospirillum* and Phosphobacterium performed better in inducing total chlorophyll and protein content in the plant (Table 4).

Table 3: Biomass of *Casuarina equisetifolia* seedlings inoculated with bioinoculants in decomposed coir pith compost under nursery condition at 180 days after inoculation

Treatments	Shoot dry wt (g/plant)	Root dry wt (g/plant)	Nodule dry wt (g/plant)	Total dry wt (g/plant)
T_1	4.733bc	1.710b	0.056d	6.500b
T_2	4.636b	1.870c	0.017ab	6.523b
T_3	5.126cd	1.863c	0.025bc	7.013bc
T_4	4.966bcd	1.623b	0.036c	6.626b
T_5	5.113cd	2.070d	0.028bc	7.213c
T_6	5.383d	1.643b	0.012a	7.040bc
T_7	5.860e	2.170d	0.0913e	8.120d
T_8	3.100a	1.093a	0.011a	4.203a

Means followed by a common letter(s) in the same column are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test) (wt-weight)

Treatments : T_1 – *Frankia* ; T_2 – *Azospirillum* ; T_3 – Phosphobacterium ; T_4 - *Frankia* + *Azospirillum*; T_5 – *Frankia* + Phosphobacterium ; T_6 – *Azospirillum* + Phosphobacterium ; T_7 - *Frankia* + *Azospirillum* + Phosphobacterium ; T_8 – Control

Table 4: Nutrient content and total Chlorophyll and Protein content of *Casuarina equisetifolia* seedlings inoculated with bioinoculants in decomposed coir pith compost under nursery condition at 180 days after inoculation

Treatments	N content (g/plant)	P content (g/plant)	K content (g/plant)	Total Chlorophyll (mg/g fresh weight)	Protein (mg/g fresh weight)
T_1	0.211c	0.0063b	0.0833b	1.14b	0.62b
T_2	0.180b	0.0064b	0.0933bc	1.36bc	0.71bc
T_3	0.170b	0.0096c	0.1240d	1.35bc	0.84c
T_4	0.180b	0.0052b	0.1010bc	1.36bc	0.87c
T_5	0.170b	0.0065b	0.0930bc	1.56c	1.23d
T_6	0.170b	0.0085c	0.108cd	1.46c	1.37d
T_7	0.220c	0.0145d	0.166e	1.81d	1.67e
T_8	0.120a	0.0031a	0.050a	0.75a	0.42a

Means followed by a common letter(s) in the same column are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test)

Treatments : T_1 – *Frankia* ; T_2 – *Azospirillum* ; T_3 – Phosphobacterium ; T_4 - *Frankia* + *Azospirillum*; T_5 – *Frankia* + Phosphobacterium ; T_6 – *Azospirillum* + Phosphobacterium ; T_7 - *Frankia* + *Azospirillum* + Phosphobacterium ; T_8 – Control

Triple inoculation enhanced the NPK content of the plant significantly as compared to the dual or single inoculation or uninoculated control (Table 4). Within the single inoculated plants, there was higher accumulation of nitrogen with *Frankia* and phosphorus and potassium with Phosphobacterium. However, the *Azospirillum* + Phosphobacterium combination expressed its superiority over *Frankia* + *Azospirillum* and *Frankia* +Phosphobacterium combinations in enhancing NPK content in the plant.

Positive correlation at 0.05 and 0.01 levels of significance was found for growth, biomass, nutrient content, total chlorophyll and protein content (Table 5).

Table 5: Pearson's correlation coefficients for growth, biomass, nutrient content, total chlorophyll and protein content

		shoot length	no of nodules	collar diameter	shoot dry weight	root dry weight	nodule dry weight	total dry weight	nitrogen content	phosphorus content	potassium content	total chlorophyll	total protein
root length		.968(**)	.837(**)	.836(**)	.954(**)	.902(**)	.520	.968(**)	.790(*)	.837(**)	.817(*)	.964(**)	.784(*)
shoot length			.820(*)	.826(*)	.972(**)	.811(*)	.539	.955(**)	.770(*)	.878(**)	.891(**)	.947(**)	.822(*)
no of nodules				.752(*)	.827(*)	.804(*)	.884(**)	.856(**)	.950(**)	.817(*)	.793(*)	.795(*)	.645
collar diameter			.		.773(*)	.663	.559	.768(*)	.645	.623	.652	.860(**)	.846(**)
shoot dry weight						.851(**)	.551	.987(**)	.800(*)	.910(**)	.879(**)	.941(**)	.840(**)
root dry weight							.569	.924(**)	.727(*)	.829(*)	.772(*)	.907(**)	.695
nodule dry weight								.590	.792(*)	.679	.683	.545	.505
total dry weight									.812(*)	.918(**)	.879(**)	.961(**)	.824(*)
nitrogen content										.782(*)	.705	.701	.551
phosphorus content											.970(**)	.878(**)	.811(*)
potassium content												.872(**)	.808(*)
total chlorophyll													.900(**)

**** & * Correlation is significant at the 0.01 & 0.05 level (n=24)**

Seedling quality index (Fig 1) also showed that Frankia + Azospirillum + Phosphobacterium inoculation (T_7) expressed its superiority over all other treatments. Further, Frankia + Phosphobacterium combination among the dual inoculants and Azospirillum among the single inoculants were responsible for better quality seedling production.

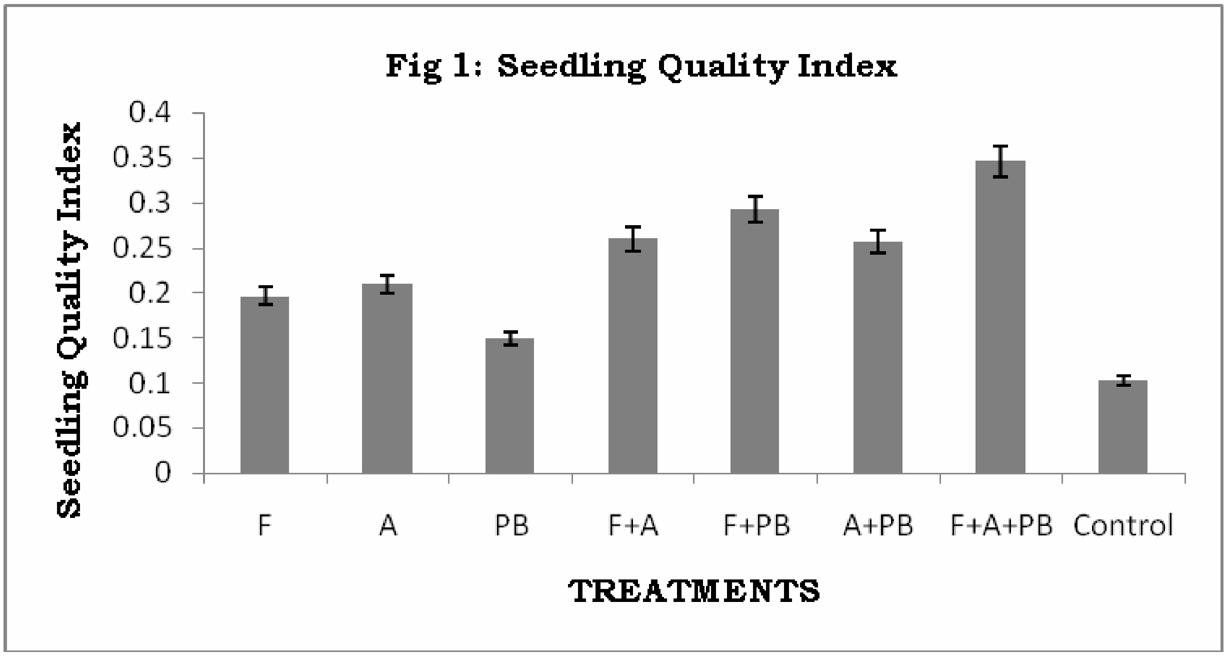

Fig 1 : Seedling quality index (SQI) of *Casuarina equisetifolia* inoculated with bioinoculants, grown in decomposed coir pith in root trainers at 180 days after inoculation

Treatments : T_1 – *Frankia* ; T_2 – *Azospirillum* ; T_3 – Phosphobacterium ; T_4 - *Frankia* + *Azospirillum*; T_5 – *Frankia* + Phosphobacterium ; T_6 – *Azospirillum* + Phosphobacterium ; T_7 - *Frankia* + *Azospirillum* + Phosphobacterium ; T_8 – Control.

The error bars represents the standard deviation values at 5% level of significance. Microbial inoculation effect indicated that *Frankia* + *Azospirillum* + Phosphobacterium inoculation (T_7) had a profound effect (about 50) on the plant, whereas all other treatments had a moderate effect (35 to 42) (Fig 2).

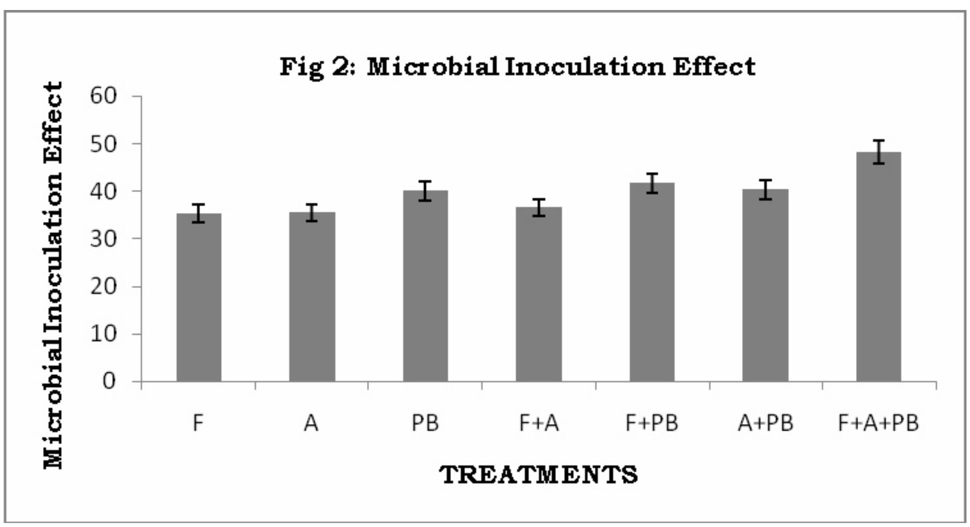

Fig 2 : Microbial inoculation effect (MIE) of *Casuarina equisetifolia* seedlings grown decomposed coir pith in root trainers at 180 days after inoculation

Treatments : T_1 – *Frankia* ; T_2 – *Azospirillum* ; T_3 – Phosphobacterium ; T_4 - *Frankia* + *Azospirillum*; T_5 – *Frankia* + Phosphobacterium ; T_6 – *Azospirillum* + Phosphobacterium ; T_7 - *Frankia* + *Azospirillum* + Phosphobacterium ; T_8 – Control.

The error bars represents the standard deviation values at 5% level of significance.

DISCUSSION

Potting media play a key role in small containers such as root trainers, as limited space is available to the plant for its development. Compost has a lot of air spaces which allow roots to grow quickly and abundantly [41]. With the aim of producing quality seedlings, in the present investigation we used decomposed coir pith compost as potting medium along with bioinoculants which enhanced the growth and biomass, nutrient content, protein and chlorophyll content of *C. equisetifolia* seedlings.

Casuarina seedlings grown in soil-based growth media inoculated with bioinoculants improved the growth and biomass of seedlings [16,42,4]. Similarly, in the present study seedlings grown in decomposed coir pith along with bioinoculants enhanced the growth and biomass of *Casuarina* seedlings. It shows that decomposed coir pith supports the growth of seedlings by providing nutrients, organic matter, carbohydrate, lignin and cellulose which can be easily hydrolyzed by microorganisms in the nursery condition.

Biologically active products, more appropriately called microbial inoculants, containing active strains of selective microorganisms like *Azospirillum*, Phosphobacterium and *Frankia* either singly or in combination, help plant growth by different mechanisms; among them biological nitrogen fixation and solubilization of insoluble phosphate are of considerable importance. Application of suitable biofertilizers to tree crops is the most convenient method to satisfy the nutritional requirement of plants. Biofertilizers enhanced the growth, biomass and nutrient content of several Shola tree species [9], *C. equisetifolia* [4], *Moringa oleifera* [43], *Acacia nilotica*[44], *Azadirachta indica* [45], *Delonix regia* [46], and *Erythrina indica* [47]. Similarly, in the present study inoculation of *C. equisetifolia* seedlings with *Frankia*+ *Azospirillum* + Phosphobacterium and grown in root trainer with decomposed coir pith significantly improved the growth, biomass and N, P and K content. Also, *Azospirillum* inoculated seedlings showed better growth and root biomass when compared to the control. The above results corroborate with earlier studies, which found a biomass increase of 90% [14] and 70% [48] in River Oak inoculated with *A. brasilense*. *Azospirillum* produces plant growth factors that cause the plant to produce more roots [49]. Decomposed coir pith has lot of air spaces which allows roots to grow quickly and abundantly. In this study, Phosphobacterium inoculated *C. equisetifolia* seedlings recorded increased growth and P uptake in relation to uninoculated control plants. The above results are in agreement with the observations made by Mohammed and Ramprasad [50] in *Eucalyptus camaldulensis*, Young [51] in *Leucaena leucocephala* and Rajendran *et al*., [4] in *C. equisetifolia*. This may be due to conversion of insoluble phosphorus to soluble form thus making it available for the growth of plants. Phosphobacterium also produces auxins and gibberellins, which may have favorable effects on plant growth.

Providing *Frankia* inoculum in early stages of plant development is an important aspect advocated for *Casuarinas* [52]. In the present study, plants grown in decomposed coir pith as a potting medium in root trainer and inoculated with *Frankia* had more nodulation and higher nodular biomass than uninoculated control seedlings. *Frankia* inoculated seedlings showed 35.33% increase in dry weight over the control seedlings. Similarly, Reddell [16] and Rajendran *et al*., [4] showed that artificial applications of nodule crush increased dry matter yield of *Casuarina*.

Increased growth, nodulation, shoot and root dry weight and nitrogen content in *C.equisetifolia* upon *Frankia* inoculation were studied in nursery conditions by Mwanza [53], Mansur and Baker [54] and Rajendran *et al*., [4]. In the present study, *Frankia* inoculated seedlings grown in decomposed coir pith media had better growth, nodulation and nutrient concentration over the control. This may be due to fixation of atmospheric nitrogen by *Frankia* in the host plant.

Among all the treatments, the combined inoculation of *Azospirillum*, Phosphobacterium and *Frankia* produced excellent growth, biomass and tissue nutrient content. The increase in height, diameter and dry matter of the *C. equisetifolia* seedlings after co-inoculation with all the three inoculants might be caused by the improved accumulation of nitrogen due to nitrogen fixation by *Frankia* [55] and *Azospirillum* [56] and Phosphorus solubilization by Phosphobacteria [57]. The total chlorophyll and protein contents were found to be higher in the seedlings inoculated with combined inoculation of *Frankia*+ *Azospirillum* + Phosphobacterium. Single inoculation with of *Azospirillum* also found to enhance the chlorophyll and protein content of the seedlings compared to uninoculated control seedlings. This increase is in agreement with the other findings [58-60,9] and this increase was attributed to the greater supply of nitrogen to growing tissues [61].

CONCLUSION

From the results, it is clear that decomposed coir pith compost can be substituted for soil or sand which are used in conventional nursery mixtures. It is suggested that the use of efficient biofertilizers – *Frankia*, *Azospirillum* and Phosphobacterium along with decomposed coir pith leads to increased growth, biomass and nutrient content of *C.*

equisetifolia in nursery conditions. Such seedlings can be easily transported and successfully transplanted without damaging the root system. These seedlings may perform better in nutrient impoverished soil.

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