Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
8(1): 96–103 (2018) ISSN 2229-2225

www.creamjournal.org

Article

Doi 10.5943/cream/8/1/8

Increase in plant growth and biomass of *Casuarina equisetifolia* L. by incorporating three different fungi in the rhizosphere

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Ojha S, Arya A 2018 – Increase in plant growth and biomass of *Casuarina equisetifolia* L. by incorporating three different fungi in the rhizosphere. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 8(1), 96–103, Doi 10.5943/cream/8/1/8

Abstract

Casuarina is a multipurpose tree, used for fuel wood, land reclamation, dune stabilization, shelter belts and pulp production. Roots of the plant are associated with a number of microbes present in rhizospheric zone. They have the basic physiology, which helps in survival of the plant in diverse situations. These microbes often serve to colonize the plant, which grow in poor soils. Many species of *Casuarina* grow on soil with low fertility, *C. equisetifolia* thrives in sand dunes or near the seashore. The outstanding ability of various Casuarinas to grow vigorously on poor soils is partly due to their unusual symbiosis with an actinomycete *Frankia* that enables them to use nitrogen directly from the atmosphere. *Frankia* plays a key role on the growth of *Casuarina* besides that there are other useful bio-inoculants present around the roots. Seedlings of *C. equisetifolia* were grown in the botanical garden of The M.S. University of Baroda, Vadodara. These were inoculated with three different fungi i.e. *Aspergillus niger*, *Trichoderma viride* and *Chaetomium globosum* either individually or in combinations. Growth was recorded at regular intervals. Root length, shoot height, basal diameter, and biomass were recorded. Application of bio-inoculants resulted into increased seedling growth, improved quality and nutrient uptake. Nodules were also collected after harvesting and different characteristic were recorded. Nodules recorded from plants having *Aspergillus niger* were more in number and bigger in size than *Trichoderma viride* and *Chaetomium globosum*. Plants inoculated with *Aspergillus niger* and *Trichoderma viride* showed better seedling growth as compared to Ascomycetous fungus.

Key words – *Aspergillus niger* – Bio-inoculants-*Chaetomium* – fungi, Plant biomass – *Trichoderma viride*

Introduction

The genus *Casuarina*, with approximately 18 species growing naturally in Australia and islands of the Pacific, belongs to the family Casuarinaceae. *Casuarina* has multipurpose applications in agroforestry, silviculture, land reclamation and soil fertility improvement in the tropics and subtropics (Rao & Rodriguez 1995). Being termite resistant and long lasting, it finds use as supporting material in building construction. The wind blowing through the canopy produces a unique whistling sound, hence referred as “wind tree”. *C. equisetifolia* is an evergreen, monoecious tree 6-60 (35) m tall, with a finely branched crown. The highly regarded wood ignites readily when green, and ashes retain heat for long period. It has been called “The best firewood in

Submitted 9 August 2017, Accepted 18 January 2018, Published 13 February 2018
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the world" (Orwa et al. 2009) and produce high quality charcoal. The wood is also used to produce paper pulp. Root extracts are used for medical treatment of dysentery.

Soil sustainability is threatened by management methods including over cultivation, decreased or increased water uptake, under or over fertilization, non judicious use of biocides, failure in maintain soil organic content, and removal of natural vegetation. Such management practices may lead to change in physico characteristics of soil and thus affecting the soil sustainability (Bhattacharyya et al. 2016). Reliance on biological processes have been emphasized by Sanchez (1994) to minimize external inputs. This approach has been developed for better soil biota management using microsymbionts (Woomer & Swift 1994).

The outstanding ability of various *Casuarinas* to grow vigorously on poor soils is partly due to the unusual symbiont *Frankia*. It helps the plant to use nitrogen directly from the atmosphere. The rate of nutrient absorption and nutrient use efficiency: the efficiency with which various nutrients are utilized to produce dry matter are the two factors that control the rate of plant growth in low soil nutrient conditions (Koide et al. 2000). *Frankia* which plays a key role in the growth of *Casuarina*, there are other bio-inoculants present around the root, like mycorrhizal symbiont, fungi and bacteria which are very important and beneficial for the plant. Bio-inoculants can increase crop yield by 20-30%, by replacing chemicals N and P by 25%. These stimulate plant growth, activate soil biologically, restore natural fertility, and provide protection against drought at the same time prevent from soil borne diseases. There is some evidence indicating the importance of bioinoculants on *C. equisetifolia* growth under nursery (Rajendran 1993, Vasanthakrishna et al. 1994) and field conditions (Rajendran & Devraj 2004). Several bio-inoculants are widely used for the growth of various crops and trees having nitrogen fixing bacteria in soil (Krishnmoorthy 2002, Vijaya Kumari & Janardhanan 2003) In general, co-inoculation with *Frankia* and helper organisms such as strains belonging to the genera *Bacillus, Pseudomonas, Azospirillum, Phosphobacterium* and mycorrhizal fungi increased the nodulation capacity, biomass of host plants, as well as nutrient uptake (Rajendran 1993). Actinorhizal plants in association with arbuscular mycorrhizal (AM) fungi are responsible for enhanced plant growth under poor soil conditions (Wheeler et al. 2000). Mycorrhizae improve nutrient uptake especially phosphorus (P) of the host plants (Jakobsen 1999) and have a high nutrient mobilizing potential (Lapeyrie et al. 1991). From various studies, it can be stated that interaction of arbuscular mycorrhizal fungi and rhizosphere microflora of plant roots play an important role in enhancing plant growth (Amballa & Bhumi 2017).

Microbes play an important role in nutrient metabolism and uptake. They promote plant growth and suppress disease by their various activities (Prakash et al. 2014). Use of traditional and modern farm knowledge to enrich the soil organically and by the use of microbes is advocated by Ramesh et al. (2005). Rice grown organically has thicker cell walls and lower level of free amino acids than conventional rice (Kajimura et al. 1995). Free amino acids promote pest attack so this variety of rice was protected from many insect pests. Atmospheric N_2 fixation rates of Casuarinas are of the same order as those observed in legumes. Thus rates of over 100 kg N/ha/yr., which is approximately the amount of nitrogen added as fertilizer in intensive forest plantations have been reported for *C. equisetifolia*. In *Leucaena leucocephala*, an increase of 33.2% in plant height was observed following inoculation with phosphobacteria (Chiu 1990) and *Trichoderma* (Altomarne et al. 1991). As an eco-friendly approach the use of three fungal organisms on growth of *C. equisetifolia* was assessed.

Materials & Methods

The experiment was carried out in the green house of Botanical garden Department of Botany, The Maharaja Sayajirao University of Baroda, and Gujarat, India. The climate is tropical with an annual rainfall of 300 mm. The maximum and minimum monthly temperatures were 32-15 °C. Soil used was sandy-clay. Soil chemical analysis was done prior to experiments using standard procedures.

Isolation of Fungi

Isolation of fungi was done from random soil samples collected from rhizosphere of *Casuarina* growing in different pots using Agar Plate Method. Plates were prepared containing Potato dextrose agar medium. Soil dilution plate method was used. Different dilutions were taken in replicates and growth was observed after 7 days. Fungal Colonies appearing on plates were isolated and then further identification of pure cultures was done

5 Mass culture of Fungi

For obtaining mass culture, the fungi were inoculated in maize meal medium. In polypropylene bags, 50g sand, 25g of crushed maize and 80ml of distilled water was taken. Then the bags were autoclaved for 30min at 15lbs psi pressure and were then inoculated by desired fungi. Bags were kept for 10days at room temperature and in between they were shaken so that the fungus gets distributed equally inside the bags. Well grown fungal cultures were incorporated in the soil. Then seedlings of *C. equisetifolia* were planted in the soil. The pots were watered once in a day to maintain appropriate moisture and then the effect of fungi on plant biomass was recorded.

There were four different types of treatments tried. Which involved bioinoculants alone or in combinations. Uninoculated control sets were also maintained. Each treatment consisted of ten replicates and data were recorded after every 20 days. The experiment was performed in 16 pots. *C. equisetifolia* seedlings were used of almost 60 days with thick dark green colour shoots having side branches with average shoot length at the time of transplantation.

Biomass study

Various morphological parameters like shoot length, root length, diameter of stem at the base, fresh weight /plant (g), dry weight / plant (g), no. of nodules/plant, chlorophyll content (Sadasiva & Manickam 1996) were recorded at 20d intervals, till three months.

Soil Characteristics

Soil testing was carried out from the control and treated pots for NPK values. The samples were sent to Gujrat State Fertilizer and Chemicals Limited, India (GSFC). Available N was determined by alkaline permanganate method (Subbiah & Asija 1956). Electric conductivity and soil pH was determined by taking 1:5 soil-water suspension. Values of P and K are mentioned in kg/h.

Results and Discussion

It is evident from Table 1 and Fig. 1, that in comparison to control the plants with *A. niger* and *T. viride* showed enhanced growth of *C. equisetifolia* up to 120 days. Various kinds of rhizospheric microbes may impact with their growth promoting and antagonistic behaviour on plant growth. A combination of three bioinoculants did not provided better growth of plants. Treatment with *Trichoderma viride* increased the plant growth. The increased growth response might be due to direct effect of fungal organisms on the plant through the secretion of growth promoting metabolites or increased nutrient uptake (Chet & Baker 1981). Similar to present results an increased root and shoot length was observed in sesame plants, when seeds were treated with *Trichoderma* spp. (Shankar & Jeyarajan 1996).

Effect of root length and nodule formation in *Casuarina*

The Effect of different treatments on plant characters such as root length, number of nodules per plant, basal girth and number of branches were also recorded (Table 2). Number of nodules formed by *Frankia* were higher in treatment with *A. niger* than other two treatments, which may be due to enhanced nutrient supply. The positive effect of dual inoculation on growth and nitrogen fixation by *Frankia* with AM has been earlier reported by Sempavalan et al. (1995). Aqueous extracts of green phyloclades of *Casuarina cunninghamiana* tissue increased the *in vitro* growth of its diazotrophic microsymbiont *Frankia* (Zimfer et al. 2004).

Table 1 Effect of different treatments on the height (cm) of *Casuarina equisetifolia* after 20 days interval (mean value of three plants in cm).

Treatments	20 days (cm)	40 days (cm)	60 days (cm)	80 days (cm)	100 days (cm)	120 days (cm)
Control (No fungus)	80.5	81	82	87.5	89.5	90.1
C. equisetifolia + *A. niger*	73.3	74.3	77.3	84	89	93.2
C. equisetifolia + *T. viride*	83.3	86	86.3	90.2	95.5	98
C. equisetifolia + *C. globosum*	78.3	80	82	84.5	87.3	89
C. equisetifolia + *A. niger* + *T. viride* + *C. globosum*	78	74	75	77.5	79	81.5

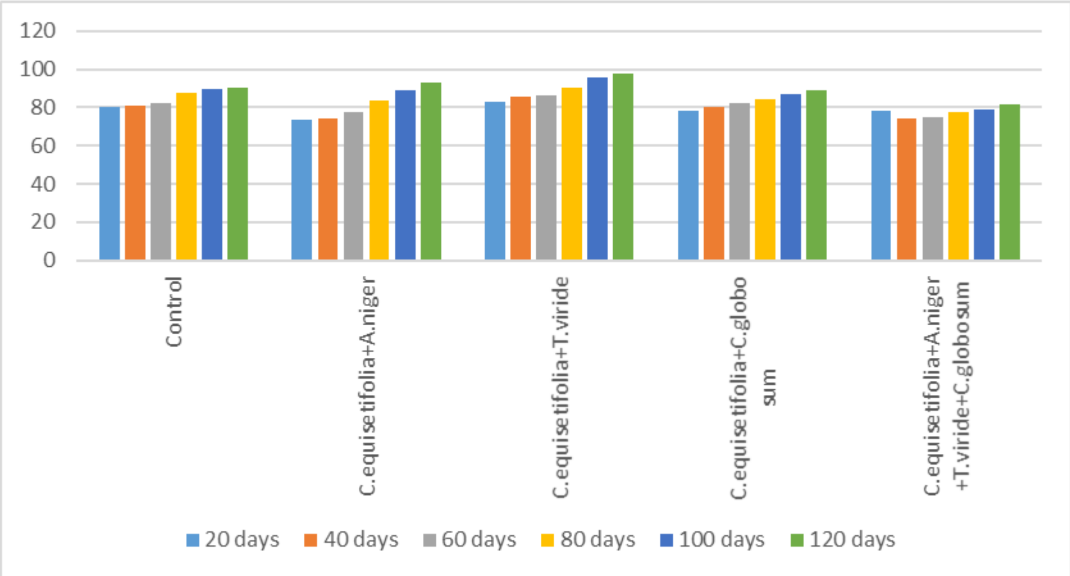

Fig. 1 – Effect of different treatments on the height of *Casuarina* plants.

The availability of phosphorus and nitrogen determines plant dependence on mycorrhizal fungi and synthetic nitrogen fixers (Rodriguez-Echeverria et al. 2007). The impact of rhizospheric organisms on plant community processes is a cumulative effect of microbe-plant and environmental factors (Reynold et al. 2003).

Table 2 Effect of different treatments on the root length and nodule formation in *Casuarina* plants.

Treatments	Root length (cm)	Number of nodules/plant	Basal girth(cm)	Number of branches/plant
Control	10	4	0.5	4
C. equisetifolia + *A. niger*	16	8	0.76	8
C. equisetifolia + *T. viride*	15	2	0.96	10
C. equisetifolia + *C. globosum*	10	2	0.61	8
C. equisetifolia + *A. niger* + *T. viride* + *C. globosum*	14	5	0.55	5

Effect of treatments on the plant biomass of *C. equisetifolia*

Different agronomical parameters were recorded such as root dry weight, shoot dry weight, root shoot ratio and total biomass. The results were analysed which shows that when *Casuarina* seedlings were treated with *A. niger* and *T. viride*, results were more promising (Table 3). Control recorded lowest root dry weight along with amendment of *C. globosum*. It also showed least shoot dry weight. Inoculated seedlings were having more biomass than uninoculated ones. Similar findings were observed by Gurumurthi & Rawat (1989), The use of *A. niger* was suggested by Vassilev et al. (2006) in the presence of Moroccan PR (phosphate rock) on the growth of white clover (*Trifolium repens)* Mittal *et al* (2008) used A. awamori and Richa et al.(2007) used A. niger to see the growth enhancement in *Zea mays. C. equisetifolia* accumulates 80% of biomass in the main stem and it can improve soil nitrogen content and have capacity to withstand various stresses. The biomass accumulation ratio is positively related with plant age (Srivastava 1995). There is need to commercialize the phosphate solubilizing fungi and it can be done by mass culture of pathogen free, high infectivity product with a higher self life.

The present study (Fig. 2) shows that the total chlorophyll content was almost same after twenty and forty days of plant growth. However, it was more in treatment with *C. globosum* after 40 days and it was more in soil treatment with *A. niger* after 20 days.

Table 3 Effect of different treatments on the increase in plant biomass of *C. equisetifolia*.

Treatments	**Root dry weight (gm)**	**Shoot dry weight (gm)**	**Total plant biomass (gm)**	**Root shoot ratio**
Control	2	18	20	0.11
C. equisetifolia + A. niger	7	52	59	0.13
C. equisetifolia + T. viride	8	50	58	0.16
C. equisetifolia + C. globosum	3	15	18	0.20
C. equisetifolia + A. niger + T. viride + C. globosum	3	18	21	0.16

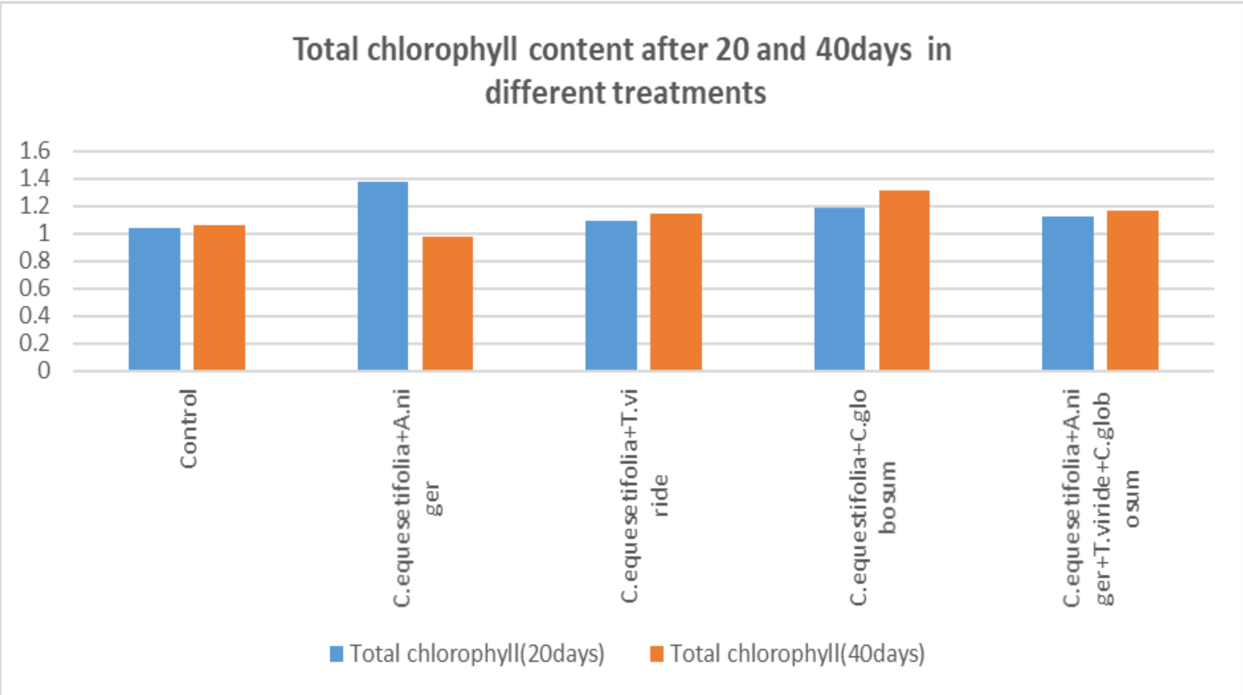

Fig. 2 – Total chlorophyll content in *C. equisetifolia* after 20 and 40 days.

Effect of different treatments on the nutrient content of soil

Physico-chemical properties of the soil used in the study were analyzed. The results are presented in Table 4. There were changes in total nutrient contents due to influence of three different treatments given to the soil of *Casuarina* pots. The table shows that, potassium and phosphate content were higher when treated with *Aspergillus, Chaetomium* and *Trichoderma.* The EC reduced in all the treatments and concentration of Potassium increased from 135kg/h to 160 in treatments with *A. niger* and *Trichoderma viride* and it was 240 kg/h in control pots. In different treatments the concentration of phosphate increased three times. Richardson (1994) and Richardson et al. (2009) described the role of microbes in the phosphorus availability.

Table 4 NPK analysis in soil samples taken from pots having *C. equisetifolia*.

	Initial (0 day)					Final (120day)				
	OC/TN	PHOSPHATE	POTASSIUM	pH	EC	OC/TN	PHOSPHATE	POTASSIUM	pH	EC
Control (soil)	0.59	4	135	8.09	0.44	0.37	10	240	8.39	0.35
Soil+Aspergillus niger	0.59	4	135	8.09	0.44	0.33	15	160	8.20	0.2
Soil+Trichoderma viride	0.59	4	135	8.09	0.20	0.34	12	160	8.16	0.21
Soil+ Chaetomium globosum	0.6	4	114	8.06	0.21	0.5	15	150	8.16	0.5

Conclusion

After 120 days of plant growth height was more in *T. viride* followed by *A. niger*. Number of root nodules were more in pots of *C. equisetifolia* supplemented with *T. viride* followed by *A. niger*. After 20 days of growth the chlorophyll content was more in pots supplemented with *Aspergillus niger*. The results after 40 days showed more amount of total chlorophyll in pots containing fungus *Trichoderma viride*. The tried fungi can be used as biofertilizers for improved growth of *C. equisetifolia.*

Acknowledgements

One of the authors Dr S. Ojha is thankful to DST, New Delhi for awarding women scientist fellowship to her. We thank Head department of Botany, M.S. University of Baroda for providing necessary laboratory facilities and to Head, Mycology dept. Agharkar Research Institute, Pune for confirming the identity of three fungi.

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