International Journal of Pharmacy and Biological Sciences-IJPBS™ (2021) 11 (3): 139-144
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Growth Performance of *Casuarina junghuhniana* Seedlings Inoculated with Biofertilizers

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Received: 12 Mar 2021 / Accepted: 6 Apr 2021 / Published online: 1 Jul 2021
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Abstract

Nursery experiments were conducted to select suitable biofertilizers to improve the growth and biomass of *Casuarina junghuhniana.* Two months old clones of *C. junghuhniana* were transplanted to polythene bags with a potting mixture of unsterilized substrate and inoculated individually and in combinations with *Azospirillum*, *Paenibacillus polymyxa* and AM fungus. Uninoculated plants were maintained as control. Shoot length, root length, basal diameter, root weight and shoot weight were recorded after six months. Results showed that the total seedling length and biomass were significantly increased in all the treatments compared with the control plants. The seedlings inoculated with the combined application of *Azospirillum + Paenibacillus polymyxa* + AM fungus produced the maximum growth and biomass.

Keywords

Biofertilizers, Growth, Biomass, *Casuarina junghuhniana*, Nursery condition.

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1. INTRODUCTION:

Casuarina junghuhniana Miq., belongs to the family Casuarinaceae, is a native species of Indonesia and Timor Leste [1]. In India, within two decades of its systematic introduction in 1996, *C. junghuhniana* proved to be more preferred species than widely cultivated *C. equisetifolia* [2], because it has recorded faster growth, with a short rotation period of 3-4 years both in coastal and inland areas [3]. In recent years, *C. junghuhniana* has gained popularity among farmers in South India due to its various desirable characters include cultivation practices and marketability. Also, the demand for quality planting stock of *C. junghuhniana* has increased due to attributes like tolerance to drought, blister bark disease and good coppicing ability [4].

Nowadays, the substrate used for the production of planting stock in modern nurseries in India are very low in nutrient content and has a very few beneficial microbial populations. Though the substrate is applied with nutrient solution, the quality of planting stock is very poor due to insufficiency of desired microorganisms, (as they are generally host specific) low level of the rate of mineralization and nitrogen fixation. As a result, the quality of the planting stock is very poor. These can be overcome by addition of good quality biofertilizers [5].

Biofertilizers are containing living cells of different types of microorganisms that have an ability to fix atmospheric nitrogen or solubilize insoluble phosphorus and make them available for crops [6]. They form not only a part of integrated nutrients but are of low cost, which is of immense help to the farming community. Having all these facts as research view, the present study was carried out to evaluate the impact of various biofertilizers on the growth and biomass of *C. junghuhniana* to produce good quality of planting stock in nursery condition.

2. MATERIALS AND METHODS:

2.1. Geographical profile of the experimental site

The present study was conducted in a private nursery located at Allinagaram of Theni district in Tamil Nadu, which is lies between 10.0090º N latitude and 77.4888º E longitude. The annual maximum and minimum temperatures of the study sites were 35º and 13º C, respectively. The mean annual rainfall was about 120 – 140 mm. The soil type was sandy clay (sand 74.6%: silt 10.4%: clay 15%) with a p^H of 7.6.

2.2. *C. junghuhniana* clonal material

Two months old clones of *C. junghuhniana* (Clone name: IFGTB-CJ-WB-5) were procured from Plantation Division, Tamil Nadu Newsprint and Papers Limited (TNPL), located at Kagithapuram in Karur district, Tamil Nadu. Clones were transplanted in a mixture of unsterilized Sand: Soil in the ratio of 1:1 w/w in 13 × 26 cm polyethylene bags.

2.3. *Azospirillum* and *Paenibacillus polymyxa*

The pure cultures of *Azospirillum* (*Azospirillum brasilense*) and *Paenibacillus polymyxa* with a population load of 10^9 and 10^8 Colony Forming Units/gram of peat soil respectively were obtained from the Department of Microbiology, Thiagarajar College, Madurai, Tamil Nadu. The cultures were subcultured in respective medium to prepare peat soil-based cultures for further studies.

2.4. AM fungus

AM fungus, *Glomus fasciculatum*, was isolated from the rhizosphere soil of already existing plantation of *C. junghuhniana.* It was multiplied in pot culture in the sterilized mixture of sand and soil (1:1 v/v) and maintained in the roots of *Sorghum vulgare* as the host plant. The inoculum contained extrametrical hyphae, chlamydospores and infected root segments were used for further study.

2.5. Treatments

In order to find out the suitable bioinoculants and their combinations to achieve maximum overall growth and minimize the cost of clone production, the following treatments were given.

T_1 – Control (Sand: Soil)
T_2 – *Azospirillum* (10g)
T_3 – *Paenibacillus polymyxa* (10g)
T_4 – AM fungus (*Glomus fasciculatum*) (10gm)
T_5 – *Azospirillum* + *Paenibacillus polymyxa* (1:1)
T_6 – *Azospirillum* + AM fungus (1:1)
T_7 – *Paenibacillus polymyxa* + AM fungus (1:1)
T_8 – *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (1:1:1)

2.6. Experimental design

The experiment was set-up in a completely randomized block design with 7 treatments involving bioinoculants individually or in various combinations, and uninoculated plants were kept as control. Each treatment consisted of 25 clones. Totally 200 clones were used for data collection. All the plants were kept under identical nursery condition up to 180 days.

2.7. Harvesting and measurement

180 days after transplantation, from each treatment, a total of 12 clones were randomly selected. Clones were carefully uprooted without disturbing the root system and shoot length, root length and basal diameter were measured. Then the clones were washed in the running tap water. Excess of water was wiped out by placing them between the folds of blotting paper. The clones were cut at collar region and fresh weight of shoot and root were estimated using top pan electronic balance. Then the cut portions were dried separately at 70° C in paper bags in hot air oven and biomass estimation (root and shoot dry weight) was carried out using top pan electronic balance.

2.8. Seedlings Quality Index

Seedlings Quality Index was calculated using the formula of Dickson *et al*. [7].

$$\text{Seedlings Quality Index (SQI)} = \frac{\text{Total weight (g/plant}^{-1})}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot weight (g/plant}^{-1})}{\text{Root weight (g/plant}^{-1})}}$$

2.9. Microbial Inoculation Effect (MIE)

Microbial inoculation effect was calculated based on the mycorrhizal inoculation effect proposed by Bagyaraj [8].

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

2.10. Statistical analysis

All the data were statistically analyzed by analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (P< 0.05) [9].

3. RESULTS:

This study presents the results on collar diameter, total height and biomass with responsible to find out the suitable combination of *Azospirillum*, *Paenibacillus polymyxa* and AM fungus to improve the planting stock of *C. junghuhniana* in nursery condition.

3.1. Growth performance of *C. junghuhniana* seedlings

3.1.1. Shoot length

Significant increase in shoot length was recorded in *C. junghuhniana* clones inoculated with different biofertilizers compared with control at 180 days after inoculation (Table 1). Analysis of growth data revealed that the combined inoculation of *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) was found to be most effective in increasing shoot length of clones followed by *Paenibacillus polymyxa* alone (T_3). These treatments recorded the shoot length of 45.1 and 42.5 cm respectively (Table 1).

Table 1: Effect of bioformulations on the growth of *C. junghuhniana* clone (180 days after inoculation)

Treatments	Collar diameter (mm)	Shoot length (cm)	Root length (cm)	Total length (cm)
T_1	$1.2^{a} \pm 0.414$	$32.9^{b} \pm 0.243$	$15.58^{a} \pm 0.512$	$48.48^{b} \pm 0.512$
T_2	$1.3^{a} \pm 0.541$	$29.2^{a} \pm 0.532$	$15.68^{a} \pm 0.541$	$44.88^{a} \pm 0.541$
T_3	$1.5^{b} \pm 0.335$	$42.5^{c} \pm 0.553$	$15.70^{b} \pm 0.841$	$58.20^{e} \pm 0.841$
T_4	$1.6^{c} \pm 0.246$	$40.9^{c} \pm 0.132$	$15.83^{b} \pm 0.320$	$56.73^{d} \pm 0.320$
T_5	$1.8^{d} \pm 0.231$	$39.5^{c} \pm 0.410$	$16.00^{b} \pm 0.231$	$55.50^{d} \pm 0.231$
T_6	$1.7^{c} \pm 0.442$	$38.4^{c} \pm 0.532$	$16.00^{b} \pm 0.520$	$54.40^{d} \pm 0.520$
T_7	$1.9^{d} \pm 0.551$	$35.7^{b} \pm 0.541$	$16.08^{b} \pm 0.320$	$51.78^{b} \pm 0.320$
T_8	$2.4^{e} \pm 0.552$	$45.1^{d} \pm 0.543$	$16.11^{b} \pm 0.478$	$61.21^{f} \pm 0.478$

3.1.2. Root length

Statistically there is no difference in the root length. Among all the treatments, inoculation with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) recorded maximum root length followed by *Paenibacillus polymyxa* + AM fungus (T_7). These treatments recorded 16.11 and 16.08 cm of root length respectively (Table 1).

3.1.3. Total length

Among all the treatments, inoculation with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) recorded maximum total length followed by *Paenibacillus polymyxa* alone (T_3). These treatments recorded 61.22 and 58.20 cm respectively (Table 1).

3.1.4. Collar diameter

Statistically, the results revealed that the treatments and their interaction were found to be significant in collar diameter (Table 1). The highest collar diameter was recorded in clones inoculated with combined inoculation of *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) (2.4 mm).

3.2. Biomass of *C. junghuhniana* seedlings

3.2.1. Shoot biomass

In case of shoot biomass, among all the treatments, inoculation with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) recorded maximum shoot biomass followed by *Paenibacillus polymyxa* + AM fungus (T_7). These treatments recorded 8.53 and 7.48 g/plant respectively (Table 2).

3.2.2. Root biomass

Higher root biomass was obtained in treated seedlings when compared to control (Table 2). *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) treated seedlings recorded higher root biomass (2.04 g/plant). It was followed by *Paenibacillus polymyxa* (T_3) and it was statistically on par with *Azospirillum* + AM fungus (T_6). They were recorded as 1.55 and 1.53 g/plant respectively.

3.2.3. Total biomass

The total biomass is highest in *C. junghuhniana* clones treated with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8). It was recorded as 10.57 g/plant. It was followed by 8.87 g/plant in clones treated with dual inoculation of *Paenibacillus polymyxa* + AM fungus (T_7) (Table 2).

Table 2: Effect of bioformulations on the biomass of *C. junghuhniana* clone (180 days after inoculation)

Treatments	Shoot dry weight (g/plant^{-1})	Root dry weight (g/plant^{-1})	Total dry weight (g/plant^{-1})
T_1	$4.63^{a} \pm 1.410$	$1.02^{a} \pm 0.365$	$5.65^{b} \pm 0.185$
T_2	$4.58^{b} \pm 1.250$	$1.12^{b} \pm 0.254$	$5.70^{a} \pm 0.541$
T_3	$6.46^{b} \pm 0.854$	$1.55^{c} \pm 0.541$	$8.01^{d} \pm 0.250$
T_4	$5.59^{bc} \pm 0.854$	$1.08^{a} \pm 0.652$	$6.67^{c} \pm 0.652$
T_5	$4.67^{ab} \pm 0.852$	$1.12^{b} \pm 0.541$	$5.79^{b} \pm 0.652$
T_6	$7.01^{b} \pm 0.410$	$1.53^{c} \pm 0.521$	$8.54^{e} \pm 0.520$
T_7	$7.48^{c} \pm 0.410$	$1.39^{c} \pm 1.410$	$8.87^{e} \pm 0.632$
T_8	$8.53^{d} \pm 0.410$	$2.04^{d} \pm 0.365$	$10.57^{f} \pm 0.251$

Values are Mean ± Standard Deviation; Means followed by a common letter(s) in the same column are not significantly different at the 5 % level by DMRT; $T_1 – T_8$: See subtitle 2.5.

3.3. Seedling Quality Index

Maximum quality seedling index (0.356) was obtained in seedlings treated with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) followed by seedlings treated with *Paenibacillus polymyxa* + AM fungus (T_7) (0.271). Among the single inoculations, *Paenibacillus polymyxa* (T_3), showed the highest (0.186) Seedling Quality Index (Fig. 1).

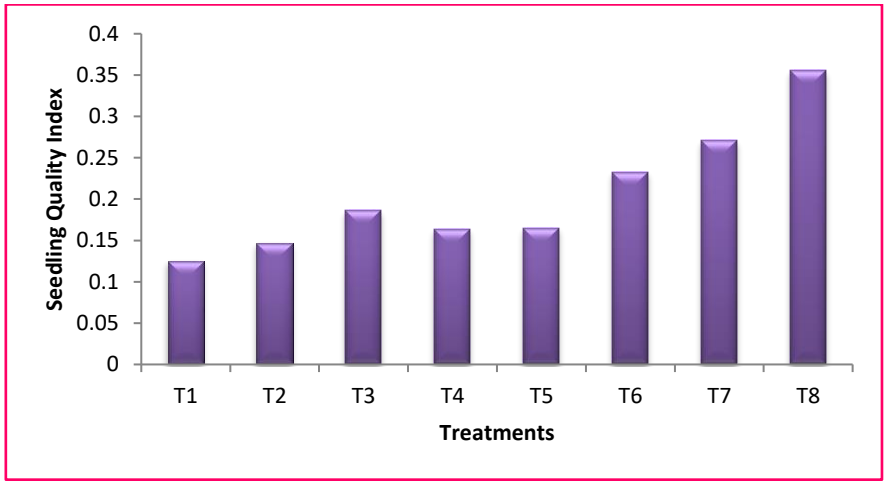

Figure 1: Seedling Quality Index of *C. junghuhniana* inoculated with different biofertilizers in Nursery condition

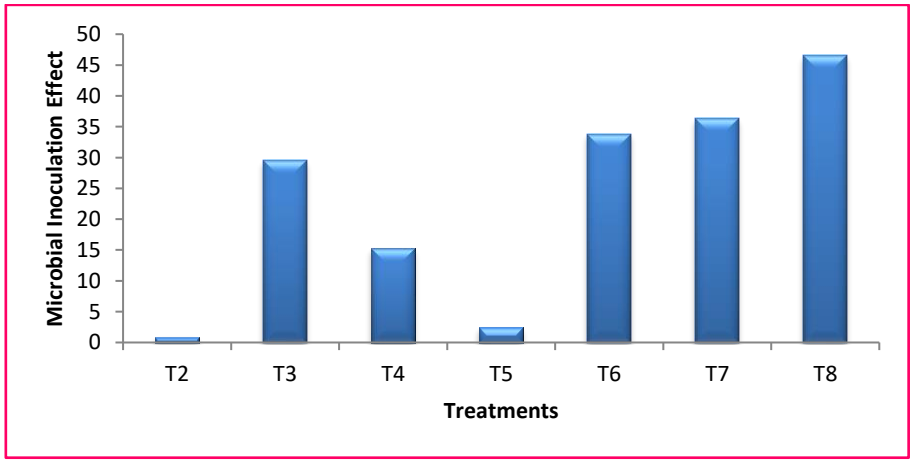

Figure 2: Microbial Inoculation Effect on *C. junghuhniana* inoculated with different biofertilizers in Nursery condition. $T_2 – T_8$: See subtitle 2.5.

3.4. Microbial Inoculation Effect

Microbial inoculation effect was highest in the seedlings inoculated with *Azospirillum* + *Paenibacillus polymyxa* + AM fungus (T_8) and followed by *Paenibacillus polymyxa* + AM fungus (T_7). It was recorded as 46.546 and 36.302 respectively (Fig. 2).

4. DISCUSSION:

The results of current research work reveal *Azospirillum* inoculated seedlings showed significant results when compared to the control. These results corroborated with earlier studies on quality seedling production of *Casuarina equisetifolia* [5], *Moringa oleifera* [10], *Acacia nilotica* [11], *Delonix regia* [12], and *Samanea saman* [13]. The better growth and root biomass may be due to increased root biomass and accumulation of nitrogen [14]. Further, *Azospirillum* also produced gibberellin and cytokinin like substances [15] which promote the growth of the seedlings.

In the present study the application of biofertilizers along with AM fungus showed improved growth, biomass and quality planting stock as already reported by Rajendran *et al.* [5] in *Casuarina equisetifolia* and this may be due to conversion of insoluble phosphorus to soluble form by AM fungus and thus making available intake by plants. The inoculation with AM fungi is also known to enhance plant growth by improving the mineral nutrient of the host plant [13].

The findings of present study also showed the combined inoculation along with *Paenibacillus polymyxa* influence the growth and biomass of *C. junghuhniana* clones. It is relevant to mention here that *Paenibacillus polymyxa* produce exopolysaccharides which protect the plants from pathogens and also cause root development which automatically leads to the better growth [16]. It was also reported that *Paenibacillus polymyxa* act as a helper bacterium in nitrogen fixation [17] and all these virtues of *Paenibacillus polymyxa* cause better growth of *C. junghuhniana*.

Overall, the combined inoculation of *Azospirillum* + *Paenibacillus polymyxa* +AM fungus produced excellent growth and biomass. The greater height, diameter and dry matter of the *C. junghuhniana* clones seedlings due to above mentioned traits of respective biofertilizers as already reported by several studies conducted for the quality seedling production of various plants include Casuarina [5,10-13,17].

5. CONCLUSION:

From this study, it was cleared that the use of combined inoculation of biofertilizers leads to increased growth and biomass of *C. junghuhniana* in nursery condition. Such seedling stocks may perform better growth and yield even in impoverished soil at farmland.

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