ScienSage

Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN
0976-9595
Short Communication

BIOFORMULATION FOR THE QUALITY SEEDLING PRODUCTION OF *AZADIRACHTA INDICA* A. JUSS. IN TROPICAL NURSERY CONDITIONS

J. Gunasundari*[1], K. Rajendran[2]

[1]*Department of Botany, The American College, Tallakulam, Madurai, Tamil Nadu, India*

[2]*Department of Botany, Thiagarajar College, Teppakulam, Madurai, Tamil Nadu, India*

**Corresponding author: jguna.rio@gmail.com*

Received: 18-07-2022; Accepted: 21-08-2022; Published: 30-09-2022

 https://doi.org/10.55218/JASR.202213815

ABSTRACT

Nursery experiments were conducted to select the suitable biofertilizers combination to attain quality seedlings of Neem. The biofertilizers such as *Azospirillum*, *Paenibacillus* and AM fungus (*Glomus fasciculatum*) were inoculated by separately and various combinations with each other at time of seed sowing. Shoot length, root length, collar diameter, and shoot and root dry weight were recorded on 80 days after treatment. Results showed that the total length of seedlings and biomass were significantly increased in the seedlings treated with combined inoculation of *Azospirillum* + *Paenibacillus* + AM fungus when compared to control. Among the individual inoculation, *Paenibacillus* (T_1) showed better response than other individual inoculation. Within double inoculations, *Azospirillum* + *Paenibacillus* (T_4) was superior when compared with other double inoculations. In general *Azospirillum* and its combination with other biofertilizers had more root length and biomass than other treatments.

Keywords: Biofertilizers, Neem, Nursery condition, Quality seedling production.

1. INTRODUCTION

Increasing pressure on the demands for timber, fuel, fodder and other medicinal and insecticide properties have led to an emphasis on research on Neem trees. *Azadirachta indica* A. Juss., commonly called Neem, is a fascinating multipurpose tree belongs to the family Meliaceae and it is native to India [1]. To increase the productivity, technological and biological inputs play the vital role of quality seedling production and increase the growth and biomass in the field conditions. Biological inputs not only increase the productivity but also increase the soil fertility. Biofertilizers play the imperative part in the establishment of good quality seedlings through an increase N_2 fixation by *Azospirillum*, phosphate solubilization by *Paenibacillus* and helping the phosphorus uptake by AM fungi [2].

The soil used in tropical nurseries like Madurai for the production of planting stock is very low in nutrient content and microbial population [3]. The quality of seedling is very poor due to insufficiency of desired microorganisms (most of the microorganisms are host specific) and the rate of mineralization and nitrogen fixation is very low. As a result, the quality of the seedling is very poor. This problem can be overcome by providing suitable biofertilizers. It has been already reported that the use of biofertilizers results in better growth and nutrient uptake in many tree species viz. *Acacia nilotica* [4], *Casuarina equisetifolia* [5-8], *Delonix regia* [9], *Erythrina indica* [10], *Feronia elephantum* [3], *Jatropha curcas* [11] and *Moringa oleifera* [12]. In this context, only few works have been carried out on *Azadirachta indica* [13-18], hence the present study was undertaken to find out the compatibility of different biofertilizers and their augmentation on quality seedling production in *Azadirachta indica*.

2. MATERIAL AND METHODS

2.1. Experimental site

Experiment was conducted at District Forest Department nursery (9.9383° N latitude and 78.1395° E longitude) of Madurai in Tamil Nadu. The experiment was set up in a Completely Randomized Block Design with 8 treatments and three replicates. Each replication comprised of 25 seedlings.

2.2. Seeds

Seeds were collected from the single plus tree grown at Thirumangalam (9.8216° N latitude and 78.9891° E longitude) of Madurai District in Tamil Nadu and the seeds were separated, graded and only the seeds in uniform size were used for raising seedlings.

2.3. *Azospirillum*, *Paenibacillus* and AM fungus

Lignite based culture of *Azospirillum* and *Paenibacillus* with a population load of 10^{-8} and 10^{-9} cells/g of lignite soil respectively were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The AM fungus, *Glomus fasciculatum* was multiplied in pot culture in the sterilized mixture of sand: soil (1:1 v/v) and maintained in the roots of *Sorghum vulgare* as the host plant.

2.4. Nursery medium

Potting mixture consisted of unsterilized sand: red soil: farm yard manure (2:1:1 v/v). At the time of seed sowing the following biofertilizers (Treatments) were added below the surface level.

T_1 - *Azospirillum* (15 g)
T_2 - *Paenibacillus* (15 ml)
T_3 - AM fungus (15 ml)
T_4 - *Azospirillum* (7.5 g) + *Paenibacillus* (7.5 ml)
T_5 - *Azospirillum* (7.5 g) + AM fungus (7.5 ml)
T_6 - *Paenibacillus* (7.5 ml) + AM fungus (7.5 ml)
T_7 - *Azospirillum* (5 g) + *Paenibacillus* (5 ml) + AM fungus (5 ml)
T_8 - Control

2.5. Harvesting and measurement

After 6 months, in each treatment an average height and basal diameter of 12 seedlings selected were noted and carefully uprooted for the estimation of root and shoot dry weight.

Nitrogen was analyzed colorimetrically using Kjeldahl analyzer-1030 and Phosphorus was estimated using a spectrophotometer by Bray P_2 method [19].

2.6. Statistical analysis

All the data were statistically analysed by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test ($P< 0.05$) [20].

3. RESULTS

3.1. Shoot and root length

Significant increase in shoot and root length was recorded in *Azadirachta* seedlings inoculated with different biofertilizers when compared to control at 180 days after inoculation. Analysis of growth data revealed that the combined inoculation of *Azospirillum* + Paenibacillus + AM fungus (T_7) was found to be most effective in increasing the growth of seedlings. Among all the treatments, inoculation with *Azospirillum* + *Paenibacillus* + AM fungus (T_7) recorded maximum shoot length (81.3 cm) followed by T_4 seedlings inoculated with *Azospirillum* + *Paenibacillus* (75.3 cm). Among the individual inoculation, *Azospirillum* (T_1) showed higher shoot length (56.8 cm) and statistically on par with *Paenibacillus* (T_2) and dual inoculation of *Paenibacillus* + AM fungus (T_6) inoculated seedlings with the shoot length of 55.1 and 55.6 cm respectively. In case of root length, *Azospirillum* and its combinations with other biofertilizers had more root length than other treatments. Statistically there is no much difference between the treatments except control (Table 1).

Table 1: Effect of different biofertilizers on the growth and biomass of *A. indica* seedlings

Treatment	Collar diameter (mm)	Shoot height (cm)	Root length (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight (g/plant)
T_1	2.45^{c}	56.8^{c}	49.0^{b}	8.00^{bc}	5.84^{b}	13.84^{b}
T_2	2.15^{c}	55.1^{c}	47.0^{b}	8.95^{c}	5.40b	14.35^{b}
T_3	1.80^{b}	46.7^{b}	50.5^{bc}	7.73^{b}	6.43bc	14.16^{b}
T_4	3.22^{d}	75.3^{e}	51.5^{bc}	11.40^{f}	6.57bc	17.97^{d}
T_5	3.16^{d}	66.8^{d}	52.6^{c}	10.20^{e}	7.00d	17.20^{c}
T_6	2.99^{cd}	55.6^{c}	50.5^{bc}	9.65^{d}	7.35d	17.00^{c}
T_7	3.59^{de}	81.3^{f}	52.1^{c}	12.60^{g}	8.26e	20.86^{e}
T_8	1.17^{a}	38.0^{a}	40.0^{a}	6.75^{a}	4.35a	11.10^{a}

Means followed by a common letter are not significantly different at 5% level by DMRT.

3.2. Collar diameter

Among all the treatments, seedlings inoculated with *Azospirillum* + *Paenibacillus* + AM fungus (T_7) recorded maximum collar diameter (3.59 mm). Among individual inoculation, *Azospirillum* (T_1) showed higher collar diameter (2.45 mm). Within double inoculations, *Azospirillum* + *Paenibacillus* (T_4) was superior (3.22 mm) when compared with other double inoculations (Table 1).

3.3. Total biomass of seedling

Maximum biomass (20.86 g/plant) was recorded in seedlings inoculated with *Azospirillum* + *Paenibacillus* + AM fungus (T_7). It was followed by T_4 seedlings (17.97 g/plant). Among single inoculation, *Azospirillum* (T_2) and AM fungus (T_3) were the more effective in producing seedling biomass than *Azospirillum* (T_1) (Table 1).

3.4. Nutrient concentration and nutrient uptake

3.4.1. Nitrogen

Nitrogen percentage concentration of *A. indica* seedlings inoculated with biofertilizers had significantly increased over control (Table 2). The highest nitrogen concentration (2.10%) was estimated in seedlings inoculated with *Azospirillum* + *Paenibacillus* + AM fungus (T_7) followed (2.0%) by double inoculation of *Azospirillum* + *Paenibacillus* (T_4). Statistically there is no significant difference between *Azospirillum* (T_1) and *Paenibacillus* + AM fungus (T_6) (Table 2).

Table 2: Biomass, nutrient concentration and nutrient uptake of *A. indica* seedlings

Treatment	Biomass (g/plant)	N (%)	P (%)	N uptake (mg/plant)	P uptake (mg/plant)
T_1	13.84^{b}	1.94^{bc}	0.06^{a}	0.217^{c}	0.007^{b}
T_2	14.35^{b}	1.84^{bc}	0.08^{b}	0.210^{c}	0.009^{b}
T_3	14.16^{b}	1.60^{b}	0.11^{c}	0.182^{b}	0.013^{c}
T_4	17.97^{d}	2.00^{d}	0.18^{e}	0.307^{e}	0.028^{e}
T_5	17.20^{c}	1.85^{bc}	0.08^{b}	0.267^{d}	0.012^{c}
T_6	17.00^{c}	1.98^{c}	0.13^{cd}	0.285^{d}	0.018^{d}
T_7	20.86^{e}	2.10^{d}	0.20^{ef}	0.384^{f}	0.037^{f}
T_8	11.10^{a}	1.34^{a}	0.06^{a}	0.095^{a}	0.004^{a}

Means followed by a common letter are not significantly different at 5% level by DMRT.

3.4.2. Phosphorus

The phosphorus content was highest (0.20%) in the seedlings treated with *Azospirillum* + *Paenibacillus* + AM fungus (T_7) followed by *Azospirillum* + *Paenibacillus* (T_4) and *Paenibacillus* + AM fungus (T_6) with the phosphorus content of about 0.18 and 0.13% respectively. Among single inoculations, AM fungus (T_3) had more phosphorous content than the rest (Table 2).

4. DISCUSSION

In the present study, the height, diameter and dry matter of combined inoculated seedlings were significantly improved. The increase of growth may be attributed to improved uptake of N, P, K, Ca and Mg. *Azospirillum* inoculated seedlings had shown better growth and root biomass when compared to control. These results are corroborated with the earlier report of increased the shoot length, root length and total dry weight and quality seedlings of *Azadirachta indica* [15].

Many researchers proved that *Paenibacillus* promotes plant growth on cucumber [21], pepper [22] and sesame [23]. It was explored that the mode of action of PGPR-mediated plant growth promotion, including that mediated by *Paenibacillus,* has been investigated and found that direct plant growth promotion via bacterial secretion of mimic phytohormones and bacterial nitrogen fixation and indirect plant growth promotion via PGPR suppression of plant pathogens that cause plant diseases [24].

From the outcome of the present experiment, it can also be inferred that AM fungi inoculation in unsterile soil definitely boosted the growth of the seedlings, as previously reported from other plant species [25, 26]. In general, it had been observed that significant growth and nutrient uptake efficiency were obtained when the number of infective propagules and/or spore density of AM fungi were high in the soil [27].

Collectively, biologically active products, more appropriately called microbial inoculants, containing active strains of selective microorganisms like *Azospirillum*, *Paenibacillus* and AM fungi either alone or in combination with each other helps in increasing the plant growth by biological nitrogen fixation and phosphate solubilization.

5. CONCLUSION

Further studies are needed to find out the impact of combined inoculation of *Azospirillum* + *Paenibacillus* + AM fungus on growth and yield of *Azadirachta indica* under field condition.

Conflict of interest

The authors have declared that there is no conflict of interest.

6. REFERENCES

1. Tewari DN, Monograph on Neem (*Azadirachta indica* A.Juss.), International Book Distributors, Dehradun. 1992.
2. Wong PP, Sternberg NE. *Appl. Environ. Microbiol.*, 1979; **38:**1189-1191.
3. Mohan E, Rajendran K. *Int. J. Curr. Microbiol. App. Sci.*, 2014; **3(7):**103-116.
4. Rajendran K, Jeyashree R. *J. Non-Tim. For. Prod.*, 2007; **14(1):**512-514.
5. Rajendran K, Sugavanam V, Devaraj P. *J. Trop. For. Sci.*, 2003; 15(1):82-96.
6. Rajendran K, Devaraj P. *Biomass Bioenergy*, 2007; 26(3):235-249.
7. Saravanan TS, Rajendran K, Santhaguru K. *Asian J. Exp. Biol. Sci.* 2012; **3(4):**752-761.
8. Uma M, Saravanan TS, Rajendran K. *J. Trop. For. Sci.*, 2014; **26(1):**125-133.
9. Meenakshi Sundaram M, Santhaguru K, Rajendran K. *Asian J. Biochem. Pharm. Res.*, 2011; **1:**99-107.
10. Rajendran K. *J. Biomet. Biostat.*, 2012; **3(2):**134-140
11. Kannan K, Rajendran K. *Int. J. Curr. Res. Aca. Rev.*, 2015; **3(2):** 92-103.
12. Kasthuri Rengamani S, Jothibasu M, Rajendran K. *J. Non-Tim. For. Prod.* 2006; **13(1):**41-46.
13. Shivaa MK, Vanangamudi K, Mani G. *Biotech. Agri. Indus. Environ.*, 2002; 91-101.
14. Vijayakumari B, Janardhanan K. *J. Trop. For. Sci. (Malaysia)*, 2004; **16(4):**477-480.
15. Meenakshi Sundaram M, Rajendran K. *J. Non-Tim. For. Prod.* 2007; **14(4):**255-260.
16. Alagesaboopathi C, Rajendran K. *J. Phytol. Res.*, 2009; **22:**125-130.
17. Banerjee K, Gadani MH, Srivastava KK, Neelam Verma, Jasrai, YT, Jain NK. *Braz. J. Microbiol.*, 2013; **44(2):**587-593.
18. Mesquita FO, Cavalcante LF, Filho FXO, Rodrigues RM, Campos VB, Souza JKC. *Com. Sci.*, 2019; **10(1):**45-53.
19. Jackson ML. Soil chemical analysis, Printice hall of India (Pvt) Ltd., New Delhi. 1973.
20. Duncan DB. *Biometrics*, 1955; **11(1):**1-42.
21. Ryu CM, Kim J, Choi O, Park SY, Park SH, Park CS. *J. Microbiol. Biotechnol.*, 2005; **15:**984-991.
22. Hahm MS, Sumayo M, Hwang YJ, Jeon SA, Park SJ, Lee JY. *J. Microbiol.*, 2012; **50:**380-385.
23. Ryu CM, Kim J, Choi O, Park SY, Park SH, Park CS. *Biol. Cont.*, 2006; **39:**282-289.
24. Jeong H, Choi SK, Ryu CM, Park SH. *Front. Microbiol.*, 2019; **10:**467-471.
25. Michelsen A. *For. Ecol. Manag.*, 1993; **59:**193-206.
26. Vasanthakrishna M, Bagyaraj DJ, Nirmalnath PJ. *For. Ecol. Manag.*, **68:**399-402.
27. Reena J, Bagyaraj DJ. *World J. Microbiol. Biotechnol.*, 1990; **6:**59-63.