Effect of Biostimulants (Azospirillum, Pseudomonas and Bacillus) on the growth and disease suppression of neem Azadirachta indica (A) juss.seedlings

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Abstract

Nursery experiments were conducted to select the suitable biostimulants and its combination to produce quality seedlings for agroforestry and farm forestry plantation. The biostimulants such as Azospirillum, Pseudomonas and Paenibacillus were inoculated by individually and it combinations at time of seed sowing. Shoot length, root length, collar diameter, and shoot and root dry weight were recorded on 180 days after inoculations. Results showed that the total length and biomass were significantly increased in the seedlings inoculated with combined inoculation of Azospirillum, Pseudomonas and Paenibacillus when compared to control. Among the individual inoculation Azospirillum (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: "Biostimulants", "Biofertilizers", "Azospirillum", "Pseudomonas" and "Bacillus".

Introduction

Plant microbe interaction develop virtuous microbiome niche around the plant. The beneficial microbes include plant growth promoting microbes of fungi (endophytes and mycorrhiza), azospirillum, Pseudomonas, Bacillus and rhizobacteria. These microbes play a multi-functional role in plant growth and development thru several mechanism like nutrient exchange, secretion of various bioactive compound1-aminocyclopropane-1-carboxylate (ACC) deaminase, sugars, siderophores, volatile organic compound, enzymes, exopolysaccharide and phytohormones like abscisic acid, gibberellin and cytokinin. These compounds act as a biostimulants and regulating physiological progressions in plants and stress mitigation (Abdel Latef et al., 2020; Atouei et al., 2019; Ansari et al., 2023). Bio stimulants are synthetic or natural substances that can be applied to plants and trees to enhance their growth through improved tolerance to abiotic stresses and increase yield and quality and it protect from pathogenic infections (Du Jardin, 2015). Biostimulants could be classified in many ways such as mode of action and its active ingredient, action in the plants or physiological response with plant (Bulgari et al., 2020). According to Lopes et al., 2021 to surge global agriculture production with the inoculation of plant growth promoting microbes is a advantageous technique for improving seed quality, crop productivity and food quality in more sustainable way (Abhilash et al., 2016; Mimmo et al., 2018; Asghari et al., 2020; Etesami, 2020).

Farmers adopting various techniques 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 and maintenance the soil fertility. Soil microorganisms are pivotal agents in providing growth promoting substances and helping to improve the soil health through ecosystem services(Bender et al., 2016), for instance by driving nutrient cycling, transformation of organic materials, enhancing plant productivity and helping to control against soil-borne diseases(Pieterse et al., 2016) According to Wong and Stenberg, 1979, bioinoculants are play the imperative part in the establishment of good quality seedlings through an increase N2 fixation by Azospirillum, phosphate solubilization by Paenibacillus and helping the phosphorus uptake by AM fungi.

The soil used in tropical nurseries like Madurai for the production of planting stock is very low in nutrient content and microbial population (Mohan and Rajendran, 2014). 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. Casuarina equisetifolia (Saravanan et al., 2012; Uma et al., 2014), Delonixregia (Meenakshisundaram et al., 2011), Erythrina indica (Rajendran, 2012), Feronia elephantum (Mohan and Rajendran, 2014) and Jatropha curcas (Kannan and Rajendran, 2015). It was already recorded that Azospirillum and AM fungi improve the growth and biomass Azadirachta indica seedlings by (Shivaaet al., 2002; Vijayakumari and Janardhanan, 2004; Meenakshisundaram and Rajendran, 2007; Alagesaboopathi and Rajendran, 2009; Banerjee et al., 2013; Mesquitaet al., 2019). However, response of Paenibacillus in association with other biofertilizers to be studied. Hence, the present study was undertaken to find out the compatibility of different biofertilizers combination with biostimulants (Azospirillum, Pseudomonas and Bacillus) and their augmentation on quality seedling production in Azadirachta indica in tropical nursery condition in southern part of Tamil Nadu, India.

Materials and methods

Experimental site soil

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.

Experiment was conducted at department nursery (9.9383° N latitude and 78.1395° E longitude) of Madurai in Tamil Nadu, India. The experiment was set up in a Completely Randomized Block Design with 8 treatments and three replicates. Each replication comprised of 25 seedlings using 25 × 30 × 30 cm poly pot in identical environment nursery condition.

The soil had a pH of 6.75, a total Carbon content of 3.52 g/kg, a total N content of 0.72 g/kg, and available P, K contents of 88.78, 380.35 mg/kg, respectively Azospirillum, Paenibacillus and Pseudomonas Azospirillum, Paenibacillus and Pseudomonas were isolated from the rhizosphere soil collected from the plantation and multiplied in the Department of Agricultural Microbiology, The American College, Madurai in the followed methods

Azospirillum

N-free semisolid Malate medium (NFB) was used to isolate Azospirillum (Dobereiner et al., 1976). 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 (Baldani and Dobereiner, 1980). 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 320C 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 320C for 72 h. After the incubation period, small scarlet colonies were observed, indicating the presence of Azospirillum sp. The isolated Azospirillum colonies were mass multiplied in nutrient broth (Rodriguez Caceres, 1982).

Pseudomonas fluorescens

Pseudomonas fluorescens was isolated by the serial dilutions and pour plate method using Kings B medium (King et al., 1954). 1mL of soil suspension from aliquot dilutions (105 to 108) was aseptically added. Twenty ml of sterilized, melted and cooled medium was added and poured in each petri plate and incubated at 28+ 2oC for 24 h. Well, separated individual colonies with yellow-green and blue white pigments were marked and detected by viewing under UV light. The individual colonies were pickup with sterilized loop and transferred on to fresh Kings B medium. The plates were incubated at 28+2oC for 24 h. The single colonies developed were transferred to Kings B medium slants and the pure cultures so obtained were stored in refrigerator at 4oC and mass multiplied for further use.

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.

T1 – Azospirillum
T2 – Paenibacillus
T3 – Pseudomonas
T4 – Azospirillum + Paenibacillus
T5 – Azospirillum+ Pseudomonas
T6 – Paenibacillus +Pseudomonas
T7 – Azospirillum + Paenibacillus+Pseudomonas
T8 – Control

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.

Nutrient Analysis

Nitrogen was analysed by calorimetrically using Kjeldahl analyzer-1030 and Phosphorus was estimated using a spectrophotometer by Bray P2 method (Jackson, 1973).

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) (Duncan, 1955).

Results

Collar diameter

Among all the treatments, seedlings inoculated with Azospirillum + Paenibacillus + Pseudomonas (T7) recorded maximum collar diameter (3.59 mm). It was calculated that 66.76 % increased over control. Among individual inoculation, Azospirillum (T1) showed higher collar diameter (3.21 mm). Within double inoculations, Azospirillum + Paenibacillus (T4) was superior (3.26 mm) when compared with other double inoculations (Table 1). It was recorded 62.88 % increased over control.

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 + Pseudomonas (T4) was found to be most effective in increasing the growth, biomass and quality of seedlings. Among all the treatments, inoculation with Azospirillum + Paenibacillus recorded maximum shoot length (78.6 cm) followed by T5 seedlings inoculated with Azospirillum + Paenibacillus (69.8 cm). It was recorded 41.47 and 34.09 % increased over control. Among the individual inoculation, Azospirillum (T1) showed higher shoot length (64.8 cm) and statistically on a par with Paenibacillus (T2). Seedlings inoculated with Paenibacillus + Pseudomonas (T6) and triplicate inoculation of Azospirillum + Paenibacillus + Pseudomonas showed 17.26 and 22.56 % increased over control

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	3.21^c (62.30)	64.8^c (29.02)	49.0^b (11.22)	8.43^{bc} 17.31	5.84^b 15.23	14.27^b 16.60
T_2	2.45^c 50.61	58.1^c 20.82	47.0^b 7.44	8.95^c 22.12	5.25b 5.71	14.2^b 16.19
T_3	1.98^b 38.89	49.7^b 7.45	50.5^{bc} 13.86	8.73^b 20.16	6.43bc 23.01	15.16^b 29.50
T_4	3.26^d 62.88	78.6^e 41.47	46.5^{bc} 6.45	11.45^f 39.12	6.57bc 24.68)	18.02^d 33.96)
T_5	3.16^d	69.8^d	49.5^c	10.28^e	7.00d	17.28^c

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	(61.71)	34.09	12.12	32.19		45.21
T_6	2.99^{cd}	55.6^{c}	50.6^{c}	10.65^{d}	7.35d	18.00^{c}
	59.53	17.26	14.03	34.55	32.65	33.89
T_7	3.64^{de}	59.4^{f}	52.1^{c}	12.60g	8.26e	20.86^{e}
	66.76	22.56	16.50	44.68	40.07	75.29
T_8	1.21^{a}	46.0^{a}	43.5^{a}	6.97^{a}	4.95a	11.9^{a}

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

Treatments: T_1 – Azospirillum; T_2 – Paenibacillus; T_3 – Pseudomonas; T_4 – Azospirillum + Paenibacillus; T_5 – Azospirillum + Pseudomonas; T_6 – Paenibacillus + Pseudomonas; T_7 – Azospirillum + Paenibacillus+Pseudomonas; T_8 – Control.

Total biomass of seedling

Seedlings inoculated with Azospirillum + Paenibacillus + Pseudomonas (T7) recorded maximum biomass (20.86 g/plant) was recorded 22.56 % increased over control. In the case of double inoculation seedlings inoculated with Azospirillum + Paenibacillus (T4) it was statistically on a par with seedlings inoculated with Paenibacillus + Pseudomonas It was recorded 33.96 and 33.89 % increased over control. Among single inoculation, Azospirillum (T2) and Pseudomonas(T3) were the more effective in producing seedling biomass than Azospirillum(T1) (Table 1).

Nutrient concentration and nutrient uptake

Nitrogen

Nitrogen percentage concentration and nitrogen uptake of A. indica seedlings inoculated with biofertilizers had significantly increased over control (Table 2). The highest nitrogen concentration (2.10%) Nitrogen uptake (0.384mg/plant) was estimated in seedlings inoculated with Azospirillum + Paenibacillus + Pseudomonas(T7). It was recorded 34.0 and 76.26 % increased over control. It was followed (2.0%) by double inoculation of Azospirillum + Paenibacillus (T4). Statistically there is no significant difference between Azospirillum (T1) and Paenibacillus + Pseudomonas(T6) (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	14.27^{b}	1.94^{bc}	0.07^{a}	0.217^{c}	0.007^{b}
	16.47	(25.77)	(11.1)	56.22	42.85
T_2	14.2^{b}	1.84^{bc}	0.08^{b}	0.210^{c}	0.009^{b}
	16.05	(20.6)	(22.2)	54.76	55.56
T_3	15.16^{b}	1.70^{b}	0.10^{c}	0.182^{b}	0.013^{c}
	21.38	(13.4)	(33.3)	47.80	69.23
T_4	18.02^{d}	2.00^{d}	0.18^{e}	0.307^{e}	0.028^{e}
	33.85	(28.8)	(77.7)	69.05	85.71
T_5	17.28^{c}	1.85^{bc}	0.08^{b}	0.267^{d}	0.012^{c}
	31.02	(21.1)	(22.2)	64.41	66.67

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T_6	18.00^{c} 33.8	1.98^{c} (27.8)	0.13^{cd} (50)	0.285^{d} 66.67	0.018^{d} 77.78
T_7	20.86^{e} 42.86	2.10^{d} (34.0)	0.18^{ef} (77.7)	0.384^{f} 75.26	0.037^{f} 89.18
T_8	11.92^{a}	1.44^{a}	0.04^{a}	0.095^{a}	0.004^{a}

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

Treatments: T_1 – Azospirillum; T_2 – Paenibacillus; T_3 – Pseudomonas; T_4 – Azospirillum + Paenibacillus; T_5 – Azospirillum + Pseudomonas; T_6 – Paenibacillus + Pseudomonas; T_7 – Azospirillum + Paenibacillus+Pseudomonas; T_8 – Control.

Phosphorus

The phosphorus percentage concentration and phosphorus uptake was highest (0.18% and 0.037mg/plant) in the seedlings treated with Azospirillum + Paenibacillus + Pseudomonas (T7) and it was statistically on a par with seedlings treated with Azospirillum + Paenibacillus (T4). It was recorded 77.7 % and 85.71% increased over control. Seedling treated with double inoculation of Azospirillum + Pseudomonas(T5) showed higher Phosphorus uptake and it was recorded 0.012mg/plant and it was recorded 66.67 % increased over control . Among single inoculations Pseudomonas(T3) had more phosphorous content than the rest (Table 2).

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 and P. 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 (Meenakshisundaram and Rajendran, 2007). It is also estimated that increased the root length and total dry weight of root were higher in the IAA, IBA when A. brasilense was incorporated with neem seedlings (Fallik et al., 1989). The combined inoculation of A. brasilense and Paenibacillus polymyxa may produce the large quantity of gibberellin and cytokine than the monocultures and it has revealed that the interaction between rhizosphere may significantly induced the auxiliary metabolism in plants (Cacciari et al.,1998)

Many researchers proved that Paenibacillus promotes plant growth on cucumber (Ryu et al., 2005), Pepper (Hahm et al., 2012) and Sesame (Ryu et al., 2006). 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 (Jeong et al., 2019).

Pseudomonas inoculated neem seedlings had improved growth and higher root production This may be the mechanism of PGP usually affects root hair development, resulting in structurally improved rooting systems. In addition, there is evidence that strains of Pseudomonas can produce the phytohormone indole acetic acid (IAA) (Otieno et al.,2013). P.

fluorescens is a bacterium that is capable of producing compounds that can facilitate the process of phosphate release in the soil (Widnyana and Javandira 2016).This bacterium also produces metabolites which act as regulators of plant growth (Attarzadeh et al.,2019). There is no disease found in duration in the experimental it may due to PGP Pseudomonas strains have been shown to have the ability to induce disease resistance in pearl millet as well as increase biomass under greenhouse and laboratory conditions (Jogaiah et al.,2010)

Conclusion

Collectively, biologically active products, more appropriately called microbial inoculants, containing active strains of selective microorganisms like Azospirillum, Paenibacillus and Pseudomonas either alone or in combination with each other helps in increasing the plant growth by biological nitrogen fixation and phosphate solubilization. Further studies are needed to find out the impact of combined inoculation of Azospirillum + Paenibacillus+ Pseudomonas on growth and yield of Azadirachta indica under field condition.

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