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ORIGINAL ARTICLE

Application of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal (AM) fungi in the growth enhancement of *Meliadubia* in Nursery

Sangeetha Menon[1*] and Varadharajan Mohan[2]
[1]Department of Life Sciences, KristuJayanti College (Autonomous), Bengaluru,Karnataka.
[2]Forest Pathology Laboratory, Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu.
*Corresponding author email: sangeethamenon@kristujayanti.com

ABSTRACT
The success of forest plantations lies in the quality of planting stock used for raising them, as most of the plantation sites used are not fertile or sometimes degraded. Application of chemical fertilizers in forestry is economically expensive and also adversely affects the soil health in the long run. Use of bio-fertilizers such as the mycorrhizas, nitrogen fixers and phosphate solubilizerscan play a very important role to produce a healthy planting stock as they enhance nutrient acquisition by the vegetation, improve plant health and neutralize pesticides. Meliadubia Cav.is a large deciduous tree found in forests of Peninsular India, Kerala, North Bengal, Assam, Sikkim and Bhutan. The wood is mainly used in plywood industry for making wallboards, door panels, flooring, furniture, farm implements, boxes and catamaran building. *In this context, the present study was carried out to determine the effect of PGPR and AM fungi on M. dubia seedlings in nursery. Six PGPR isolates viz., Azotobacter chroococcum, Azotobacterpaspali, Azospirillum lipoferum, Azospirillum brasilense, Bacillus subtilis and Stenotrophomonas sp. and two species of AM fungi (Glomusaggregatum and Glomusclarum) isolated from the rhizosphere of M.dubia plantations in Tamil Nadu was used in the study. Application of the selected AM fungi and PGPR isolates was done individually and in combination and growth parameters were recorded after 180 DAI (Days After Inoculation). Significant findings were obtained.*
Key words: m*Meliadubia*, PGPR, AM fungi, Growth parameters.

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INTRODUCTION

Forests, the second largest land use in India next to agriculture regulate climate and water resources and serve as habitats for plants and animals. They play an important role in the well-being of humanity through opportunities for recreation, spiritual renewal and other services and are a source of essential goods such as wood, food, fodder and medicines. India's forests, being one of the hot spot of biodiversity in the world, possess high degree of endemism and have recently been subjected to various pressures mainly due to biotic factors. Agroforestry sector is one of the rapidly growing sector in India trying to overcome the problems in sustainable land management. Despite the increase in land value, forest area and quality has declined due to excessive use of wood for timber, fuel, illegal encroachments, forest fires, grazing, pests and diseases etc. Although exotic species of *Eucalyptus*, *Casuarina* and *Acacia* are planted in larger area, in the recent years, fast growing native trees has been promoted for agro forestry to generate higher income in the semi-arid regions. Among many native tree species, *Meliadubia*Cav. (Malabar Neem) is one of the economically important and fast growing ones identified by Indian Council of Forestry Research and Education for promoting among Indian farmers and state forest departments. *Meliadubia* is a large deciduous tree attaining a height of more than 30 m and a breast height diameter of 143 cm. It is commonly called as Malabar Neem and belongs to family Meliaceae. It is distributed throughout peninsular India, the Malay Peninsula and tropical Asia in moist deciduous, evergreen and semi-evergreen forests. Apart from its wood being used in plywood industry, *M. dubia* has a life cycle of 8 to 12

years and is getting popularized in domestic and global markets due to its fast growth nature, sturdiness and resistance to pests and insects. Also this tree plays an important role in carbon sequestration and mitigation of climate change impacts [1]. Bio-inoculants are capable of making afforestation programmes successful by improving planting stock and ultimately increasing productivity. Use of bio-inoculants in forestry is significant due to non-availability of large scale irrigation, fertilization and protection measures in plantations and cost and short supply of chemical fertilizers. Therefore, inoculating nursery seedlings with selective bio-inoculants holds promise for improving seedling quality, out planting performance and increased resistance to root diseases and climatic stresses in the field with the added benefit of reducing the cost of chemical fertilizers used in plantations. Since these bio resources represent a great diversity in chemical, physical and biological characteristics, their efficient use depends on identification of suitable type of bio-inoculants. Numerous studies have been documented on the potential benefits of various beneficial micro-organisms as bio-inoculants in agriculture. Over the years, the combined ability of these bio-inoculants has also been tested extensively for enhanced crop growth and yield [2, 3]. However, such reports on forestry tree species are scarce. Karthikeyan and Sakthivel [4] found that *A. chroococcum* produced significant quantities of IAA for root initiation and *A. chroococcum* inoculated stem cuttings had higher rooting and significant growth than IBA treated stem cuttings of *Eucalyptus camaldulensis*. Sangeetha *et al.* [5] studied the effect of *Azotobacter* sp. on the growth enhancement of *G. arborea* in nursery and found that inoculation of seedlings with *Azotobacter* sp. resulted in enhanced plant growth, biomass and seedling quality over uninoculated (control). However as per literature survey, no reports could be cited on the effect of PGPR on *Meliadubia* trees, which formed a base for the present study. This study was carried out to evaluate the study and elucidate the information on efficacy of bio-inoculants on the growth improvement of *Meliadubia* in nursery.

MATERIAL AND METHODS

Nursery experiments: Nursery experiments were conducted at Experimental Nursery, Forestry, Land Use, and Climate change (FLUCC) Division, IFGTB, Coimbatore, Tamil Nadu, India.

Potting medium: A mixture of solar sterilized sand: soil: farmyard manure in the ratio 1:2:1 was used as the potting medium. The soil was analysed for physico-chemical characteristics such as texture, pH, Electrical Conductivity(EC), bulk density, organic carbon, available Nitrogen(N), Phosphorus(P), Potassium(K) and micronutrients such as Copper(Cu), Zinc (Zn), Iron(Fe) and Manganese(Mn)in the soil and water testing lab, FLUCC Division, IFGTB, Coimbatore, Tamil Nadu, India (Table 1). *Raising and Transplantation of seedlings:* Healthy seeds of *Meliadubia* were obtained from Seed Division, Kerala Forest Research Institute (KFRI), Peechi, Kerala. The seeds were soaked in cool water overnight before sowing. The seeds were then sown by the method of layering in primary beds of 10 x 1 m size filled with 5 cm thick solar sterilized soil and sand in the ratio of 1:2. A thin layer of sterilized fine sandy soil was sprinkled to cover the seeds. Watering of primary beds was done with spray can twice a day. The germination started after 7 days and continued up to 21 days. A month old uniform size seedlings were immediately transplanted into the bags in nursery and were watered daily. The experimental design for nursery experiment was set up as designed in Table 2.

Bioinoculants used in the study: Six Plant Growth Promoting Rhizobacteria (PGPR) strains and two Arbuscular Mycorrhizal (AM) fungi were used in the study (Table 2). These bioinoculants were isolated in the previous study by the authors(6).Liquid formulations of PGPR *viz., Azotobacter, Azospirillum*and PSB were prepared aseptically in Jensen's Broth medium, Nitrogen free Bromothymol Blue (NFB) Broth Medium and Pikovskaya's Broth medium respectively. Liquid bioincoulants were diluted with sterile water to obtain a population of 10^8 cells/ml and were applied as 5ml/polybag to polythene bags (10 x 20 cm size) already filled with the potting medium up to 5cm below the top surface as per completely randomized design by layering inoculation technique. The soil based inoculum of Arbuscular Mycorrhizal (AM) fungi consisted of *Glomusaggregatum* and *Glomusclarum*at the rate of 12-15 spores per gram of soil. AM fungi were applied as 5gm/ polybag. The potting medium was again filled up over the bioinoculant layer. *Biometric observations:*Seedlings were harvested at 180 Days After Inoculation (DAI) and recorded growth parameters [15 seedlings from each treatment i.e., plants grown in sterilized potting media inoculated with different types of microbial bio inoculants (*Azotobacter, Azospirillum,* PSB, AM fungi)] such as shoot height, root length, collar diameter and shoot dry weight, root dry weight and total dry weight were recorded. Volume index (VI), Quality Index (QI), shoot: root ratios, Absolute Growth Rate (AGR), Relative Growth Rate (RGR), Persistence of PGPR and AM fungi and Microbial Inoculation Effect (MIE) were also calculated. The calculations were carried out as described by Sangeetha *et al.* (5).

Statistical Analysis: All data were subjected to analysis of variance and the significant difference among the means was compared by Duncan's Multiple Range Test (DMRT) at P=0.05 level using ANOVA by SPSS 20.0 version, statistical software (SPSS Inc.).

Table-1:Physico-chemical properties of sterilized potting medium

T	pH	EC (dSm^{-1})	BD (gm/cc)	OC (%)	Macro Nutrients (kg/ha)			Micro Nutrients (ppm)			
					N	P	K	Cu	Zn	Fe	Mn
Sandy loam	7.4	0.08	1.25	0.23	112.46	20.45	11.38	1.8	1.54	10.8	11.08

(T: Texture; EC: Electrical Conductivity; BD: Bulk density; OC: Organic carbon)

Table-2: Experimental design and treatment structure

Species : *M. Dubia*	
Number of Treatments	: 9
Number of Replicates/treatment	: 5
Number of plants per replicate	: 10
Design	: RBD
Experiment I	**Experiment II**
Single inoculation treatment	**Multiple inoculation treatment**
T1 – Control	T1 – Control
T2 – *Azotobacterchroococcum*	T2 – *Azotobacter + Azospirillum*
T3 – *Azotobacterpaspali*	T3 – *Azotobacter +* PSB
T4 – *Azospirillumlipoferum*	T4 – *Azotobacter +* AM fungi
T5 –*Azospirillumbrasilense*	T5 – *Azospirillum +* PSB
T6 – *Bacillus subtilis*	T6 – *Azospirillum +* AM fungi
T7 – *Stenotrophomonas sp.*	T7 – Phosphate Solubiliizing Bacteria (PSB) + AM fungi
T8 –*Glomusaggregatum*	T8 – *Azotobacter + Azospirillum* + PSB
T9 –*Glomusclarum*	T9 – *Azotobacter + Azospirillum +* AM fungi

RESULTS AND DISCUSSION

Effect of bioinoculants on growth parameters of M. dubia seedlings

In general, seedlings inoculated with combination of all bio-inoculants showed maximum values for the growth parameters at 180 DAI in *M. dubia seedlings.* Further, among the treatments involving bio-inoculants, dual combinations, especially the ones involving a N fixer and a P solubilizer/mobilizer were found to be better than single inoculations.

At 180 DAI (Single inoculation), *Glomus clarum* treated seedlings (T9) showed maximum shoot length, root length and collar diameter, followed by seedlings treated with *Glomus aggregatum* (T8). At 180DAI (multiple inoculation), maximum shoot length, root length and collar diameter was observed in seedlings treated with Azoto + Azosp + AM (T9) followed by Azoto + Azosp + PSB (T8)(Table 3).

Table-3: Effect of bioinoculants on shoot length, root length and collar diameter of *M. dubia*seedlings

T	SL(cms)		RL (cms)		CD (mm)	
	SI	MI	SI	MI	SI	MI
T1	17.20a	18.40a	22.54a	22.13	4.41a	5.28a
T2	27.96b*	30.47b	31.71b	33.32b	6.24b	7.07b
T3	28.12b	31.70c	30.61b	34.91c	7.34c*	8.33c
T4	28.90b	32.95d	33.30c	35.94c	7.68c	8.92d
T5	36.61e	36.79e	40.65f	40.68d	10.61ef	11.21e
T6	32.56c	38.59f	36.99d	42.29e	9.42d	12.86f
T7	34.74d	40.30g	38.86e	43.84f	10.22e	12.75f
T8	38.32f	42.12h	41.76f	45.52g	10.82f	13.60g
T9	40.02g	44.48i	43.89g	47.53h	11.48g	15.19h

**Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level.*
(T: Treatment; SL: Shoot Length; RL: Root Length; CD: Collar Diameter; SI: Single Inoculation; MI: Multiple Inoculation)

At 180 DAI (Single inoculation), seedlings treated with *G. clarum* (T9) showed maximum dry weight for shoot and root and total dry weight followed by *G. aggregatum* (T8). At 180 DAI (Multiple inoculations), short dry weight, root dry weight and total dry weight was observed maximum in seedlings treated with Azoto + Azosp + AM(T9) showed followed by Azoto + Azosp + PSB (T8)(Table 4).

At 180 DAI, in single inoculation, seedlings treated with *G. clarum*(T9) showed significant volume index, followed by *G. aggregatum*(T8). At 180 DAI (multiple inoculations), seedlings treated with Azoto + Azosp + AM (T9) showed maximum volume index followed by Azoto + Azosp + PSB (T8). At 180 DAI, in single inoculation, untreated seedlings (T1) and seedlings treated with *Stenotrophomonas* sp.(T7), *B. subtilis*(T6), *Azospirillum brasilense* (T5) and *Azospirillum lipoferum*(T4) showed significant quality index, but among them they were at par. At 180 DAI, in multiple inoculations, no significant difference was observed in quality index for all the treatments. At 180 DAI (single inoculation), seedlings treated with *Stenotrophomonas* sp.(T7) were sturdy the maximum followed by *B. subtilis*(T6),*Azospirillum brasilense*(T5),*G. clarum*(T9) and *G. aggregatum* (T8), but among them they were at par. At 180 DAI (Multiple inoculations), seedlings treated with Azoto + Azosp + AM (T9) showed maximum sturdiness quotient followed by Azosp + AM (T6)(Table 5).

At 180 DAI, in single inoculation, seedlings treated with *Stenotrophomonas* sp. (T7) showed significant shoot/root ratio followed by untreated seedlings (T1) and seedlings treated with *B. subtilis*(T6), but among them they were at par. At 180 DAI, in multiple inoculations, untreated seedlings (T1) showed significant shoot/root ratio followed by Azoto + AM (T4).In single inoculation, seedlings treated with *G. clarum*(T9) showed maximum AGR followed by *G. aggregatum* (T8). In multiple inoculations, seedlings treated with Azoto + Azosp + AM (T9) showed maximum AGR followed by Azoto + Azosp + PSB (T8)and PSB + AM (T7), but among them they were at par. In single inoculation, untreated seedlings (T1) showed maximum RGR followed by *G. clarum*(T9). In multiple inoculations, seedlings treated with Azoto + Azosp + AM (T9), Azoto + Azosp + PSB (T8), PSB + AM (T7) and untreated seedlings (T1) showed maximum RGR, but among them they were at par (Table 6).

Percent Microbial Inoculation Effect (MIE) on M. dubia seedlings

At 180 DAI, in single inoculation, maximum percent MIE was observed in treatment with *G. clarum*(T9)(78.68%), followed by *G. aggregatum*(T8)(76.38%) and *Azospirillumbrasilense*(T5) (72.80%) and *Stenotrophomonas* sp. (T7)(70.23%). At 180DAI, in multiple inoculations, maximum percent MIE was observed in treatment of combination of Azoto + Azosp + AM (T9)(79.55%) followed by Azoto + Azosp + PSB (T8)(77.94%), PSB + AM (T7) (76.30 %), Azosp + AM (T6) (74.10%) and Azosp +PSB (T5)(70.82%)(Fig.1).

Persistence of PGPR and AM fungiin treated soil of M. dubia seedlings

At 180 DAI in single inoculation, population of *Azotobacter*was found maximum in treatment with *Azotobacter chroococcum* (T2) followed by *Azotobacter paspali*(T3). At 180DAI, in multiple inoculations, *Azotobacter* population was recorded maximum in treatment of Azoto + Azosp(T2)and Azoto + PSB (T3), but among them they were at par. At 180 DAI in single inoculation, *Azospirillum* was found maximum in treatment with *Azospirillum brasilense*(T5)and *Azospirillum lipoferum*(T4), but among them they were at par. At 180 DAI in multiple inoculations, seedlings treated with combination of Azosp + PSB (T5)had maximum population of *Azospirillum*. At 180DAI in single inoculation, PSB was found maximum in treatment with *Stenotrophomonas* sp. (T7)followed by *Bacillus subtilis* (T6)(Table 7).

At 180 DAI, in single inoculation, AM fungal population was found maximum in treatment with *G. clarum*(T9)followed by *G.aggregatum*(T8). At 180 DAI, in multiple inoculations, AM fungal population was found maximum in seedlings treated with combination of Azoto + Azosp + AM (T9)followed by Azosp + AM (T6). At 180 DAI, in single inoculation, significant root colonization was observed in seedlings treated with *G. clarum*(T9)followed by *G. aggregatum*(T8). At 180 DAI, in multiple inoculations, maximum root colonization was observed in seedlings treated with combinations of Azoto + Azosp + AM (T9), Azosp + AM (T6), Azoto + AM (T4) and PSB + AM (T7), but among them they were at par (Table 7).

No comparable reports were found by the authors through their extensive literature survey in the tree species studied. , However, similar phenomenon has been reported earlier in related tree species belonging to same family like *Azadirachta indica* (7), *Chukrasia tubularis* [8], *Melia azedirach* [2] and *Coffea canephora* [9]. The findings of the study were in agreement with the findings of Seema *et al*. [10] who reported that AM fungi and *Azotobacter chroococcum* inoculation on *Gmelina arborea* resulted in improved growth and biomass. Zambrano and Diaz [11] reported a positive correlation between mycorrhizal colonization and plant height of *Gmelina arborea.*

Current study on the effect of AM fungi, nitrogen fixers and phosphate solubilizing bacteria on the growth and biomass production of *M.dubia* in nursery has thrown light on the identification of suitable combinations of different biofertilizers with synergistic interactions, for a given set of environmental conditions. By the application of such compatible combinations of bio fertilizers in the forest tree nurseries, the performance attributes of the seedlings can be improved, which in turn will increase the out planting survival rate and field performance. The overall outcome of the nursery experiment had shown a positive response of *M.dubia* seedlings to inoculation with different bio-inoculants as compared

to the uninoculated control. Statistically significant increase in plant growth parameters of *M.dubia*in nursery conditions, confirmed the positive effect of bio-inoculants, especially the triple inoculations of N fixing and P solubilizing / mobilizing bio-inoculants. These results were really interesting because in some of the previously reported trials with tropical trees, AM fungal inoculation failed to improve tree seedling growth in the nursery. Probably, in cases of co-inoculation of different tree species, selection of suitable N fixing and P solubilizing / mobilizing organisms might be very important parameters.

Table-4: Effect of bioinoculants on shoot dry weight, root dry weight and total dry weight of *M. dubia* seedlings

T	SDW (gm)		RDW (gm)		TDW (gm)	
	SI	MI	SI	MI	SI	MI
T1	2.64a	3.75a	1.87a	2.13a	4.51a	5.88a
T2	4.94b	6.08b	3.56b	4.57b	8.50b	10.65b
T3	5.97c	7.48c	4.89c*	5.60c	10.86c	13.09c
T4	6.53d	8.99d	5.01c	6.56d	11.54c	15.55d
T5	9.52f	10.82e	7.06e	9.33e	16.58f	20.15e
T6	7.35e	12.54f	5.23c	1.16f	12.58d	22.70f
T7	9.08f	13.23g	6.06d	11.58g	15.15e	24.81g
T8	10.36g	14.74h	8.73f	11.92g	19.09g	26.66h
T9	11.25h	16.35i	9.90g	12.40h	21.15h	28.75i

**Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level.*
(T: Treatment; SDW: Shoot Dry Weight; RDW: Root Dry Weight; TDW: Total Dry Weight; SI: Single Inoculation; MI: Multiple Inoculation)

Table-5: Effect of bioinoculants on the Volume Index, Quality Index and Sturdiness Quotient of *M. dubia* seedlings

T	VI		QI		SQ	
	SI	MI	SI	MI	SI	MI
T1	778.16a	1133.90a	1.65ab	1.91a	3.89c	3.47e
T2	2335.40b	3194.40b	1.60ab	1.63a	4.47d	4.31g
T3	3196.90c*	4630.00c	1.57ab	1.70a	3.85c	3.80f
T4	3676.70c	5481.80c	1.67b	1.74a	3.76bc	3.69f
T5	8765.20f	9740.00d	1.75b	1.67a	3.46ab	3.28d
T6	6180.00d	13406.00e	1.77b	1.77a	3.45ab	3.00ab
T7	7707.70e	13710.00e	1.82b	1.71a	3.40a	3.16cd
T8	9383.00f	16219.00f	1.63ab	1.83a	3.54ab	3.09bc
T9	11079.00g	21258.00g	1.38a	1.65a	3.48ab	2.93a

**Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level.*
(T: Treatment; VI: Volume Index; QI: Quality Index; SQ: Sturdiness Quotient; SI: Single Inoculation; MI: Multiple Inoculation)

Table-6: Effect of bioinoculants on the Shoot/Root Ratio, Absolute Growth Rate and Relative Growth Rate of *M. dubia* seedlings

T	SRR		AGR		RGR	
	SI	MI	SI	MI	SI	MI
T1	1.40de	1.75e	0.56a*	0.54a	0.15e	0.10d
T2	1.39cd	1.33cd	0.52a	0.61a	0.06abc	0.06a
T3	1.22ab	1.33cd	0.69a	0.76a	0.07bc	0.06ab
T4	1.30bc	1.37d	0.57a	0.84a	0.05ab	0.06a
T5	1.35cd	1.16a	0.55a	1.43b	0.03a	0.08bc
T6	1.40de	1.23b	0.68a	1.92c	0.06ab	0.09cd
T7	1.49e	1.14a	0.72a	2.37d	0.05ab	0.11d
T8	1.18a*	1.23b	1.52b	2.39d	0.09cd	0.10d
T9	1.13a	1.31c	2.01c	2.78e	0.11d	0.11d

**Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level.*
(T: Treatment; SRR: Shoot Root Ratio; AGR: Absolute Growth Rate; RGR: Relative Growth Rate; SI: Single Inoculation; MI: Multiple Inoculation)

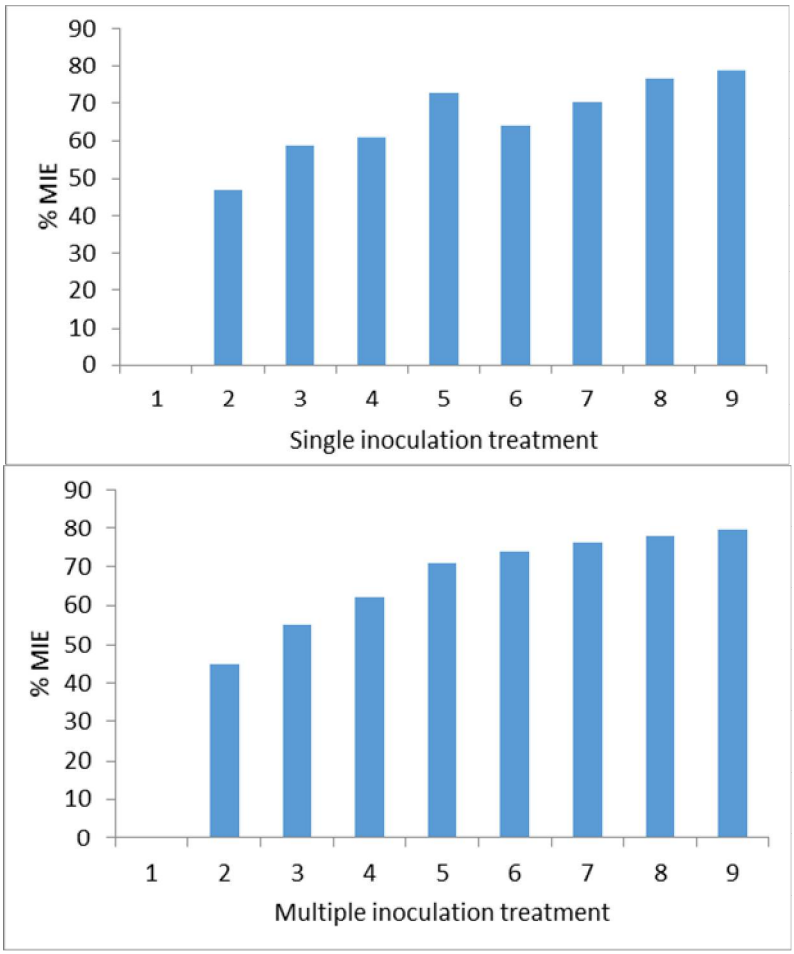

Fig. 1- Per cent Microbial Inoculation Effect (MIE) on *M. dubia* seedlings

Table-7: Persistence of PGPR and AM fungi in treated soil of *M. dubia*seedlings

T	*Azotobacter* sp.		*Azospirillum* sp.		Phosphate Solubilizing Bacteria (PSB)		AM Spore population		% root colonization by AM fungi	
	SI	MI	SI	MI	SI	MI	SI	MI	SI	MI
T1	2.00a	1.66a	5.00a	4.33a	4.00a	5.66a	33.66a*	64.33a	33.33a	38.00a
T2	20.66d	19.00d	8.00a	5.00ab	5.33ab	7.66a	35.00a	63.33a	38.66b	45.66b
T3	14.33c	19.00d	8.00a	6.00ab	7.66bc	6.33a	50.33ab	81.66a	46.00c	52.66c
T4	5.33ab*	6.33b	22.00b	22.00d	9.00c	6.33a	55.00b	139.00bc	46.00c	66.33d
T5	4.33ab	8.33bc	18.33b	27.00e	5.66abc	7.66a	68.33b	70.66a	47.66cd	50.00bc
T6	7.00b	8.00bc	6.66a	6.66ab	12.33d	26.33d	66.33b	156.00cd	52.33d	67.33d
T7	4.66ab	7.66bc	8.00a	8.00abc	16.00e	20.00c	69.00b	118.33b	52.66d	63.66d
T8	5.66ab	8.66c	6.66a	10.66c	5.00ab	11.66b	135.00c	84.00a	62.33e	50.33bc
T9	5.00ab	7.66bc	7.66a	8.33bc	8.33bc	8.00a	168.67d	172.67d	68.00f	68.33d

**Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level.*
(T: Treatment; SI: Single Inoculation; MI: Multiple Inoculation)

CONCLUSION

This study revealed that the co-inoculation of N fixing and P solubilizing/mobilizing organisms improved the plant growth (primarily seedling height, collar diameter and biomass) in *Meliadubia*. This combination worked out can be utilized for promoting plant growth in tropical region, concomitantly saving considerable amount of chemical N and P fertilizers under nursery and field conditions.

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