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Synergistic potential of microorganisms (bio-fertilizer) on growth performance of *Meliadubia*

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ABSTRACT

The objective of the study was to test the synergistic effect of microorganisms (bio-fertilizer) on the growth of *Meliadubia*. For this study the bio-fertilizer *Azospirillum*, *Azotobacter*, Phosphobacteria, AM fungi, and Potash Mobilizer developed by Institute of Forest Genetics and Tree Breeding (IFGTB) Coimbatore, were used. The synergistic efficacy of bio-fertilizer was assessed in different forms *viz*., dual, triple, quadruple and pentuple. The growth parameters *viz.*, maximum shoot length (94.3 cm), root length (24.5cm), collar diameter (12.85mm), shoot and root dry weights (23.27g and 31.47g), total dry weights (54.73g) revealed plants receiving bio-fertilizer consortia (pentuple) performed well, which was further intrigued by the growth indices and quality indices. This study highlighted the potential efficacy of bio-fertilizer in combination i.e., triple, quadruple and pentuple was more efficient than single inoculants.

Key words: *Meliadubia, Microbial consortia, Bio-fertilizer*

Introduction

Meliadubia commonly known as Malabar Neem, a large deciduous and fast growing tree with wide spreading branches on a stout, straight tall bole acquires greater significance (Nair *et al*., 2005), geographically found in the tropical moist deciduous and semi evergreen forest of South and Central Western Ghats, Eastern Ghats and North East India upto an altitude of 1500-1800 meter (Pradeep, 2015). Otherwise, this species is commonly distributed in moist deciduous forests of Kerala, and is very popular in Southern states of India for its fast growth and wide adaptability in diverse edaphic and climatic conditions (Gupta, 1993 and Mabberley, 1997), which has created a huge demand in saplings among the plantation industries.

Establishment of a good forest plantation depends on high quality saplings (Duryea and Landis, 1984). A good quality sapling stock depends on quality soil or the potting amendment used as growing substrate, preferably by adopting an inexpensive and environmentally friendly method to meet the nutritional requirements for improved plant growth. Application of arbuscular mycorrhizal (AM) fungi is one such ecofriendly and inexpensive method, where the symbiotic association of soil microorganisms in the potting / polybag mixture may facilitate nutrient transfer from substrate to plant, thereby, can improve seedling growth, tree height, and plant yield (Smith, 2008; Kapulnik *et al*., 2010). Therefore, the application of most suitable growth promoting beneficial microorganisms becomes important in nursery condition, where the efficient use of beneficial microorganisms helps not only to achieve maximum growth, but also in economize production by decreasing fertilizer inputs and their run off (Marschner, 1995).

Meliadubia is one such fast growing species which needs regular / constant management inclusive of

nutrient availability being most vital. Therefore, the present experiment has been designed and carried out to test the efficacy of bio-fertilizeron seedling growth of *Meliadubia* in nursery condition.

Materials and Methods

An experiment was conducted to test the efficacy of bio-fertilizer on growth of *Meliadubia* saplings. This experiment was conducted in nursery located at Gottipura (Van Vignan Kendra of IWST). The details of the materials and methods followed are furnished below;

Collection of bio-fertilizer

Bio-fertilizer of *Azotobacter*, *Azospirillum*, Phosphobacteria and Potash mobilizer (Tricho-K) were procured from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. The samples were maintained at ambient room temperature *i.e*., 28±2^{0}C and used for further work.

Compatibility study

Compatibility study was carried out by streaking dual inoculants on solidified nutrient agar medium to find out compatibility among microorganisms. Primarily, each of the co-inoculated strains was grown in nutrient agar medium at 30 °C for at least 3 d and then streaked perpendicularly on freshly prepared nutrient agar medium; *i.e.*, after the first strain was allowed to grow at 30 °C for 3 d, the second strain was streaked at an angle of approx. 90° going outward from the emerged colonies of the first strain. The second colony was incubated at 30 °C for another 3 days (Islam *et al*., 2018).

Treatment details

T1 – Control
T2 – AM fungi
T3 – *Azospirillum*
T4 – *Azotobacter*
T5 – Phosphobacteria
T6 – Potash Mobilizer (Tricho-K)
T7 – AM + *Azospirillum*
T8 – AM + *Azotobacter*
T9 – AM + Phosphobacteria
T10 – AM + Potash Mobilizer (Tricho-K)
T11 – AM + *Azospirillum* + *Azotobacter* + Phosphobacteria
T12 – AM + *Azospirillum* + *Azotobacter* + Phosphobacteria + Potash Mobilizer (Tricho-K)

Effectiveness of the bio-fertilizer

Effectiveness of the bio-inoculant formulation was tested on growth of *Meliadubia* saplings at the intervals of 90 and 180 days under open nursery conditions (Monika singh *et al*., 2019).

Maintenance and observations

During the application of bio-fertilizer to the *Meliadubia* saplings, the saplings were evenly watered in a regular way to maintain the moisture at field capacity and other usual care was taken to protect the plants from pests and diseases up to 180 days, and the corresponding growth parameter readings were recorded (Monika Singh *et al*., 2019).

Plant biometric observations

Plant growth parameters like, shoot length, collar diameter, root length, shoot and root dry weight and total dry weight were recorded at 90 and 180 days after the application of bio-fertilizer.

1. Shoot length

The Plant height was measured from the base of the plant to the terminal growing point of the main stem and expressed in centimeters (cm).

2. Collar diameter

The collar diameter was measured with the help of digital verniercalliper and recorded in millimeter.

3. Root length

The saplings will be removed from poly bags without damaging the roots and the root length will be measured from the collar region to the tip of the root and expressed in centimeter (cm).

4. Shoot and root dry weight

The saplingsafter recording all the above observations will be separated into shoot and root. The shoot and root samples will be dried at 85 ^{0}C for 48 hours and the dry weights will be recorded when the constant weights obtained and expressed in grams (g) per saplings.

5. Total dry weight

Total dry weight will be calculated by summing up the dry weight of the shoot as well as the root and expressed in grams per seedling.

Total dry weight = Shoot dry weight + Root dry weight

Growth indices

1. Absolute Growth Rate (AGR)

It is the change in actual growth over time. AGR will be calculated by the following formula (Wareing and Philips, 1981) and expressed in mg/g/day.

$$AGR = \frac{W_2 - W_1}{t_2 - t_1}$$

Where, W_1 and W_2 are the means of plant dry weights at times t_1 and t_2

2. Relative Growth Rate (GRR)

Relative growth rate (RGR) will be calculated by using the following formula (Hoffmann and Poorter, 2002) and expressed in mg/g/day.

$$AGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where, In W_1 and In W_2 are the means of the natural logarithm transformed plant dry weights at times t_1 and t_2

3. Root shoot ratio

Root shoot ratio will be derived by using the following formula

$$\text{Root Shoot Ratio} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

4. Sturdiness quotient

Sturdiness quotient will be calculated by using the formula (Ritchie, 1984).

$$\text{Sturdiness quotient} = \frac{\text{Shoot length}}{\text{Collar diameter}}$$

5. Volume index

It will be determined by using the following formula (Kumaran and Surendran, 1999).

Volume Index = Diameter^2 (cm) × Height (cm)

Where, Height = (Root length + Shoot length)

6. Dickson's quality index (DQI)

It will be determined by using the following formula (Dickson *et al*., 1960)

$$\text{Quality Index} = \frac{\text{Saplings dry weight}}{\frac{\text{Height (cm)} + \text{Shoot dry weight (g)}}{\text{Diameter (mm)}} \quad \text{Root dry weight (g)}}$$

Microbial Inoculation Effect (MIE)

The microbial inoculation effect (MIE) will be calculated based on the formula of Bagyaraj (1992).

$$MIE = \frac{\text{Dry weight of inoculated plants} - \text{Means dry weight of uninoculated plants}}{\text{Dry weight of inoculated plants}}$$

Statistical analysis

The survival data from a laboratory experiment was statistically analyzed by Completely Randomized Design (CRD) and means were compared by Duncan's Multiple Range Test (DMRT) (Little and Hills., 1978).

Statistical analysis of the data obtained from nursery was done using factorial CRD and means were compared by DMRT.

Results and Discussion

An attempt was made to study the efficacy of bio-fertilizeron growth of *Meliadubia*s aplings under nursery condition.

Compatibility analysis of the microbial inoculants used in the formulation development

Before developing a formulation, compatibility study was done between all the said microbial bio-inoculants. It was observed from the study that, these microorganisms were compatible with each other and they did not show antagonistic interaction, rather synergistic interaction was observed during their growth (Fig. 1). Similar results were reported by Shanmugam *et al*. (2002) and Belkar and Gade (2012).

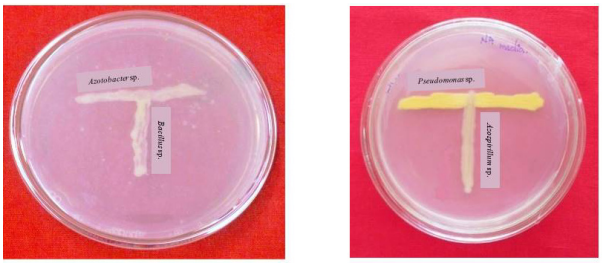

Fig. 1. Compatibility analysis among the bio-inoculants

Plant biometric observations

1. Shoot length, Collar diameter and Root length

The efficacy of bio-fertilizer was tested on shoot

length, collar diameter and root lengthof *Meliadubia* at the interval of 90 and 180 days after treatment, as depicted in Table 1.

The plant growth performance will be indicated by plant biometric observations like, shoot length, collar diameter and root length. In case of plants receiving bio-fertilizer, maximum shoot length was recorded in plants receiving bio-fertilizer consortia (44.63 cm and 94.30 cm) at 90 and 180 days respectively, and the least plant height of 16.17 cm and 64.67 were observed from the plants with control (Fig. 2). The same results were observed from the studies of Chavan *et al*., 2013 and Dhanya *et al*., 2019, the increase in shoot length may be due the presence of all essential nutrients in the microbial formulation which are in readily available form. This leads to increased uptake of nutrients which in turn enhances the height of saplings. Comparitive results have been presented by Atilla *et al*., (2014).

Plants treated with bio-fertilizer consortia, recorded maximum collar diameter (4.03 mm, and 12.85 mm) when compared to control (1.57 mm and 5.58 mm) at 90 and 180 DAS respectively. This study supports the investigations done by Patel and Suresh (2018) and Ramasamy (2009), who postulated that the combined application of Phosphobacteria, vermicompost and *Azospirillum* along with NPK enhanced the collar diameter (0.914 cm) of *Bixaorellana* saplings.

The significant differences in the root length of *Meliadubia* saplings were observed in the treated plants and control. Plants with highest root length

Fig. 2. Nursery experiment and Treatment difference

Table 1. Plant biometric observations

Sl. No.	Treatments	Shoot length		Collar diameter		Root length	
		90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS
1	T1	16.17^{f}	64.67^{d}	1.57^{d}	5.58^{d}	2.74^{g}	10.42^{g}
2	T2	$19.33e^{f}$	70.50^{cd}	2.09^{d}	7.02^{cd}	3.18^{fg}	12.3^{fg}
3	T3	19.50^{ef}	78.67^{bc}	2.43^{cd}	7.50^{bcd}	3.00^{fg}	13.15^{efg}
4	T4	21.50^{def}	79.83^{bc}	2.33^{d}	8.03^{bcd}	4.1^{ef}	11.33^{fg}
5	T5	25.83^{de}	80.17^{bc}	2.27^{d}	7.90^{bcd}	4.5^{e}	19.5^{bcd}
6	T6	19.00^{ef}	73.33^{cd}	2.57^{bcd}	8.17^{bcd}	5.12^{de}	15.42^{def}
7	T7	28.00^{cd}	78.33^{bcd}	2.12^{d}	8.75^{abc}	6.15^{d}	17^{cde}
8	T8	37.00^{ab}	88.17^{ab}	2.15^{d}	7.35^{cd}	8.00^{c}	19.67^{bc}
9	T9	36.00^{bc}	90.08^{ab}	3.69^{ab}	10.83^{abc}	8.03^{c}	22.25^{ab}
10	T10	35.50^{bc}	87.75^{ab}	2.46^{bcd}	8.17^{bcd}	7.81^{c}	19.50^{bcd}
11	T11	38.33^{ab}	89.43^{ab}	3.63^{abc}	11.33^{ab}	9.38^{b}	21.53^{ab}
12	T12	44.67^{a}	94.30^{a}	4.03^{a}	12.85^{a}	12.40^{a}	24.50^{a}
	CD @ 0.5	8.01	13.92	1.23	3.88	1.17	4.10

*DAS – Days after sowing

(12.40 cm and 24.50 cm) were observed in plants receiving consortium of bio-inoculants than the plants with control. This study supports the investigations done by Patel and Suresh (2018). The extend of increase in root length is due to the treatment T3: Vermicompost @ 20g/ seedling was found to be 30.8, 36.6 and 42.3 per cent over control at 45, 90 and 135 days respectively (Dhanya *et al*., 2019) and is in support with the results published by Bharadwaj *et al*., 2014.

Shoot dry weight, root dry weight and total dry weight

The efficacy of bio-fertilizer was tested on shoot dry weight, root dry weight and total dry weight of *Meliadubia* at the interval of 90 and 180 days after sowing is represented in Table 2.

In case of plants receiving bio-fertilizer, maximum shoot dry weight was recorded in plants receiving bio-fertilizer consortia (12.92 g and 23.27 g) at 90 and 180 days respectively, and the least shoot dry weight of 2.44 g and 11.40 g were observed from the plants with control. This study confirms the investigation of Chavan *et al*., 2013 and Sujatha and Manjappa, 2015.

Plants treated with bio-fertilizer consortia, recorded maximum root dry weight (14.50 g and 31.47 g) when compared to control (1.75g and 13.50 g) at 90 and 180 DAS respectively. This study supports the investigations done by Sujatha and Manjappa (2015) and Swaminathan (1995).

In case of plants with bio-fertilizer consortia showed highest mean (average) total dry weight *i.e*., 27.42 g and 54.73 g/plant than the plants which are kept as control (4.19 g and 24.90 g/plant) at 90 and 180 DAS respectively. The present study is in accordance with the study of Chavan *et al*. (2013) stated that the growth and biomass production was higher in the plants inoculated with bio-fertilizer compared to control. In General, among the bio-fertilizer, the better growth performance was observed in the plants inoculated with combination of *Azotobacter* + *Azospirillum* + Phosphate Solubilizing Bacteria.

Quality indices

1. Root shoot ratio

The maximum root shoot ratio was observed from the plants receiving consortium bio-inoculants (1.35) when compared to control and other single bio-in-

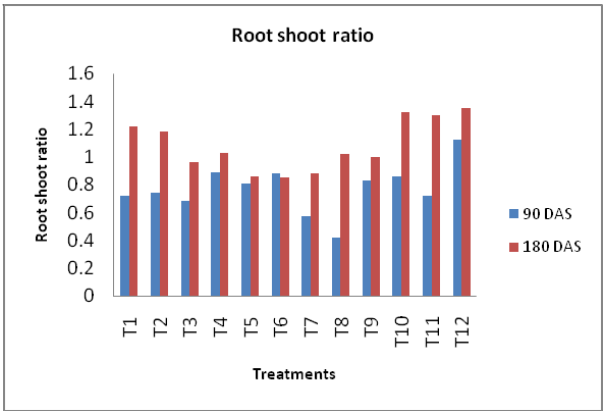

Fig. 3. Root shoot ratio

Table 2. Plant biometric observations (Cont…)

Sl. No.	Treatments	Shoot dry weight		Root dry weight		Total dry weight	
		90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS
1	T1	2.44^{g}	11.40^{e}	1.75^{e}	13.50^{d}	4.19^{g}	24.90^{e}
2	T2	3.30^{f}	12.73^{de}	2.43^{de}	15.03^{cd}	5.73^{f}	27.77^{de}
3	T3	3.10^{fg}	15.17^{cd}	2.12^{de}	14.6^{cd}	5.22^{fg}	29.77^{de}
4	T4	2.78^{fg}	16.73^{bcd}	2.49^{cde}	17.17^{bcd}	5.27^{fg}	33.90^{bcd}
5	T5	3.15^{fg}	17.43^{bcd}	2.55^{cde}	15.07^{cd}	5.70^{f}	32.50^{cde}
6	T6	3.07^{fg}	16.20^{bcd}	2.69^{cde}	13.73^{cd}	5.75^{f}	29.93^{de}
7	T7	5.27^{e}	19.38^{abc}	3.03^{cd}	17.03^{bcd}	8.30^{e}	36.42^{bcd}
8	T8	8.22^{d}	17.58^{bcd}	3.48^{c}	18.00^{bcd}	11.70^{d}	35.58^{bcd}
9	T9	9.03^{c}	21.47^{ab}	7.50^{b}	21.57^{bc}	16.53^{b}	43.03^{abc}
10	T10	8.21^{d}	18.87^{abc}	7.10^{b}	24.93^{ab}	15.30^{ac}	43.80^{abc}
11	T11	10.13^{b}	19.40^{abc}	7.32^{b}	25.25^{ab}	17.45^{ab}	44.65^{ab}
12	T12	12.92^{a}	23.27^{a}	14.50^{a}	31.47^{a}	27.42^{a}	54.73^{a}
	CD @ 0.5	0.80	5.36	1.01	9.35	1.09	11.82

*DAS – Days after sowing

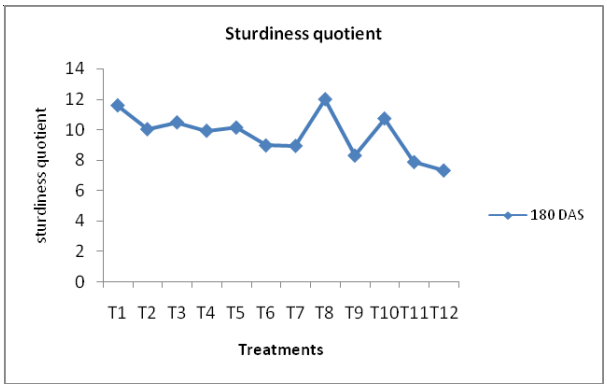

Fig. 4. Sturdiness quotient

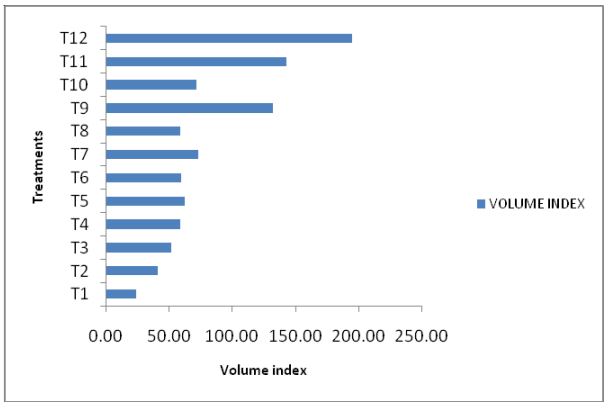

Fig. 5. Volume indices

Table 3. AGR and RGR

Sl. No.	Treatments	AGR	RGR
1	T1	0.18[a]	0.0077[a]
2	T2	0.24[a]	0.0076[a]
3	T3	0.27[a]	0.0084[a]
4	T4	0.32[a]	0.0090[a]
5	T5	0.30[a]	0.0084[a]
6	T6	0.27[a]	0.0080[a]
7	T7	0.31[a]	0.0071[ab]
8	T8	0.27[a]	0.0054[bc]
9	T9	0.29[a]	0.0096[c]
10	T10	0.32[a]	0.0081[c]
11	T11	0.30[a]	0.0085[c]
12	T12	0.35[a]	0.017[d]
	CD @ 0.5	NS	0.002

*NS – Non-significant

oculated plants. This investigation confirmed by the hypothesis of Aseri and Rao (2005).

2. Sturdiness quotient

A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. Highest sturdiness quotient was recorded in plants receiving bio-inoculant consortia (7.34) than control and other plants which were receiving single inoculants of bio-fertilizer. This result is in agreement with the findings reported by Patel and Suresh, 2018.

3. Absolute growth rate (AGR) and Relative growth rate (RGR)

The maximum AGR and RGR were observed from the plants receiving consortium bio-inoculants formulation *i.e.*, 0.35 g and 0.017 g respectively when compared to control (0.18 g and 0.0077 g). This investigation confirmed by the hypothesis of Hernández-Ramos *et al*. (2020).

4. Volume indices

In case of plants receiving bio-fertilizer, maximum volume index was recorded in plants receiving bio-fertilizer consortia (194.83) and the least volume index of 23.35 was observed from the plants with control.Several authors also evidenced similar findings as like Kumar (2007) in *Ailanthusexcelsa*, David camus (2008) in *Meliadubia*.

5. Dickson's quality index (DQI)

Dickson's quality index differed significantly due to influence of bio-fertilizer on biomass production. The maximum DQI was observed from the plants receiving consortium bio-inoculants (5.48) when compared to control and other single bio-inoculated plants. This investigation confirmed by the findings of Chavan *et al*., 2013 and they stated that the DQI was higher in T7 (0.59) treatment followed by T5

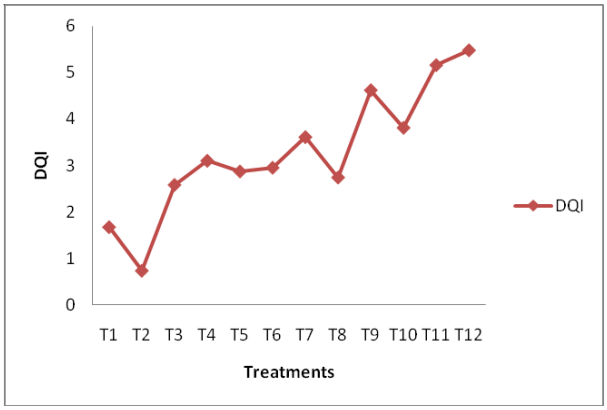

Fig. 6. Dickson's quality index (DQI)

and T6. The lowest Dickson's quality index was found in both control and T2 (0.44).

6. Microbial Inoculation Effect (MIE)

$$MIE = \frac{\text{Dry weight of inoculated plants} - \text{Mean dry weight of uninoculated plants}}{\text{Dry weight of inoculated plants}}$$

$$MIE = \frac{54.73 - 24.90}{54.73}$$

MIE = 54.50

MIE is very useful for the assessment of the extent to which introduced beneficial microbial inoculants compete with native endophytes (of potting / polybag mixture – unsterilized soil mixture) to bring about plant growth response. In this study, we can observe about 54.50% plant growth response of microbial inoculants over the un-inoculated plants (Bhagyaraj, 1992).

The application of bio-fertilizer significantly increased seedling growth attributes *viz*, shoot length, collar diameter, root length and biomass production. Hence bio-fertilizer can be used to get good quality saplings (Chavan *et al*., 2013). The combined inoculation of nitrogen fixing bacteria and P-solubalizing bacteria significantly increased the growth. In the present study consortium of bio-fertilizer enhanced the growth and improved the dry matter of *Meliadubia*. This might be due to the increased absorbing surface area due to extensive external network of mycelium produced by bio-fertilizer in association with the host root system.

Conclusion

In the present investigation the growth and biomass production was higher in the plants inoculated with bio-fertilizer consortia compared to control. The better growth performance was observed in the plants inoculated with combination of AM + *Azotobacter* + *Azospirillum* + Phosphobacteria + Potash Mobilizer bacteria. Therefore, triple, quadraple and pentuple treatments were on par with each other and performing significantly better than other single inoculated treatments and control.

Conflict of Interest

The research findings in this article do not have any conflict of interest.

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