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Enhancement of Germination and Growth of *Melia dubia* Cav. by Microbial Consortia

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Abstract: *Melia dubia commonly known as "Malai Vembu" belongs to the family Meliaceae. It is a deciduous and fast growing tree with straight bole. It is indigenous to the western ghats of southern India. Owing to its importance, this species has been identified as potential, commercially viable pulpwood species for ex-situ cultivation in Tamil Nadu. Although regeneration of the species under natural conditions is very high, the seeds generally exhibited very low germination due to hard seed coat under laboratory conditions. Based on observation, it has been learnt that the seed germinates very well in the tree rhizosphere. None of the physical and chemical treatments tried facilitated seed germination. Hence experiments were carried out to find the microbiological and physical agents that would improve germination and reduce the mean germination time. Accordingly two enrichment culture studies were carried out to isolate microbes facilitating Melia dubia seed germination. In the first enrichment technique, the rhizosphere soil of melia was enriched with exocarp (spermosphere) and different sources of microbial inoculum such as elephant dung, goat pellet, cow dung and termite mound. Second set of enrichment was done by mixing the rhizosphere soil with endocarp (spermosphere) and various sources of microbial inoculum as mentioned above. From above enrichment studies, four bacteria and one actinomycete's isolates were obtained from rhizosphere soil enriched with spermosphere and elephant dung. The isolated strains were mesophilic with pH requirement of 8.0. Among various sources of carbon, sucrose was recorded to be the most preferable source of carbon. Henceforth, germination studies were carried out with different grades of fruits viz., green, yellow, dried and decayed fruits in the rhizosphere soil of Melia dubia. The results indicated that green fruits are best suited for germination. It had recorded greater germination (20%) over other grades.*

Keywords: *Melia dubia, Germination, Rhizosphere, Microbial inoculums.*

INTRODUCTION

Melia dubia is one of the alternate indigenous pulp yielding tree species getting importance all over India. It belongs to the family Meliaceae and commercially known as Malabar Neem and is locally called as Mala-vembu. It is a large deciduous and fast growing tree with wide spreading branches, straight and tall bole. It is indigenous to the Western Ghats of southern India and is common in moist deciduous forests of Kerala (Gamble, 1922). The current production of raw materials for pulp and paper is 2.76 million tonnes, against the demand of 5.04 million tonnes, a shortfall of 45 percent. The projected demand by 2020 is 13.2 million tonnes, which is still more staggering (Palsaniya *et al*., 2009). Today, there are about 594 paper mills in India, with 34 considered as large companies. The demand for wood-based products in Tamil Nadu is 8-10 lakh tonnes of wood pulp per year, which is greater than the 4 lakh tonnes that are currently available (Parthiban and Govinda Rao, 2008). *Melia dubia* has been screened as one of the best alternate of pulpwood species (Bharti, 2006). It has been promoted by both governmental and nongovernmental paper

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industries for pulp wood. Even though, it is considered as one amongst the best, the lacuna is very poor seed germination. The most constraining factor in establishing a successful plantation is very poor seed germination (Nair *et al*., 1991). Fruits of *Melia dubia* possess outer most skinny exocarp succeeded by pulpy mesocarp and finally hard stony endocarp. Thus, the seeds are surrounded by three different layers. These physical structures might inhibit seed germination. Despite these physical barriers, their natural regeneration or germination has been good under the tree cover. Moreover mixing the rhizosphere soil with nursery media and allowing it to stand for three to four months even for a year has been found to induce germination. Perhaps, some factor of microbial origin could induce and facilitate germination of *Melia dubia* under natural conditions. Specifically seed coat associated or spermosphere microbes could be of great use as seed germination inducers. Keeping all these in view, experiments were carried out and the results obtained are presented below.

MATERIALS AND METHODS

The materials used and the methods followed in conducting the various experiments in current investigation are summarized.

Enumeration of Total Aerobic Heterotrophic Microbes from Rhizosphere and Spermosphere

The total aerobic heterotrophic microorganisms, diazotrphs, phosphate solubilizers, cellulose and lignin degraders present in rhizosphere soil and fruits were enumerated on solid media using the serial dilution and plating technique of Parkinson *et al*. (1971). One gram of the representative soil sample was taken and serial dilutions were carried out in sterile water.

Enrichment Culture Technique

In order to isolate microbes capable of inducing seed germination of *Melia dubia,* the rhizosphere soil of Melia mixed with different sources of microbes and green fruit outer skinny exocarp and hard stony endocarp (spermosphere).

T_1 - 500 g Rhizosphere soil + 50 g cow dung + 50 g endocarp of *Melia dubia*

T_2 - 500 g Rhizosphere soil + 50 g goat pellet + 50 g endocarp of *Melia dubia*.

T_3 - 500 g Rhizosphere soil + 50 g elephant dung + 50 g endocarp of *Melia dubia*.

T_4 - 500 g Rhizosphere soil + 50 g termite mount + 50 g endocarp of *Melia dubia*.

T_5 - 500 g Rhizosphere soil + 50 g fungal garden + 50 g endocarp of *Melia dubia*.

T_6 - 500 g Rhizosphere soil + 50 g cow dung + 50 g exocarp of *Melia dubia*.

T_7 - 500 g Rhizosphere soil + 50 g goat pellet + 50 g exocarp of *Melia dubia*.

T_8 - 500 g Rhizosphere soil + 50 g elephant dung + 50 g exocarp of *Melia dubia*.

T_9 - 500 g Rhizosphere soil + 50 g termite mount + 50 g exocarp of *Melia dubia*.

T_{10} - 500 g Rhizosphere soil + 50 g fungal garden +50 g exocarp of *Melia dubia*.

T_{11} - Control (500 g Rhizosphere soil)

The above mentioned mixtures were prepared in poly pots and incubated for 2 months with 30-40% moisture content. The soil sample were collected after 2 months and subjected to following microbial analyses.

1. Total aerobic heterotrophic bacteria by using Nutrient agar medium.
2. Actinomycetes by using Kenknight's agar medium.
3. The diazotrophs *viz.,Azospirillum, Azotobacter* and *Beijerinckia* by using Waksmann No. 77 medium, *Beijerinckia* medium and 'N' free Malic acid medium respectively.
4. Phosphate solubilizing microorganisms by using Sperber's Hydroxy Apatite Medium.
5. Cellulose degraders by using Han's Medium.
6. Lignin degraders by using Lignin degrading medium.

RESULTS

Bacterial Population as Influenced by Exocarp of *Melia Dubia*

Total aerobic heterotrophic bacteria, actinomycetes and phosphate solubilizing microbial population are given in Table 1. The results indicated that the

Table 1
Microbial Population of Exocarp of *Melia dubia* as Influenced by Various Substrates (60 DAE)

T	Bac × 10^8 cfu g^{-1}	Acti × 10^2 cfu g^{-1}	PSM × 10^3 cfu g^{-1}	Azot × 10^4 cfu g^{-1}	Azos × 10^4 cfu g^{-1}	Beij × 10^4 cfu g^{-1}	Cell × 10^5 cfu g^{-1}	Lig × 10^5 cfu g^{-1}
T_1	37.00	63.00	1.000	1.660	10.33	24.33	0.660	1.000
T_2	20.66	44.33	0.660	4.660	12.33	31.33	0.000	0.660
T_3	25.66	69.66	2.330	2.330	16.33	18.33	1.000	0.660
T_4	30.00	53.67	1.000	4.330	13.00	14.66	0.330	0.330
T_5	46.66	74.66	1.000	4.000	54.00	14.00	0.660	1.660
T_{11}	33.00	63.33	0.660	4.660	37.33	14.33	0.660	1.000
Mean	30.05	64.83	1.722	3.500	24.83	20.16	0.611	1.055
SE(d)	6.585	20.14	1.863	1.677	5.174	4.455	0.577	0.693
CD (0.05)	14.34	NS	NS	NS	11.27	9.707	NS	NS

T – Treatment : Bac – Bacteria ; Acti – Actinomycetes : PSM – Phosphate Solublising Microorganism : Azot – Azotobacter : Azos – Azospirillum : Beij – Beijerinckia : Cell – Cellulose : Lig – Lignin

population of bacteria, actinomycetes and PSM varied in different enrichment treatments. The bacterial population of rhizosphere was positively influenced when it was incubated with exocarp (46.66×10^8 cfu/g of soil) . This was followed by enrichment of rhizosphere soil with exocarp and elephant dung (37×10^8 cfu/g of soil) but other sources of microbial inoculums reduced the rhizosphere bacterial load.

Rhizosphere Actinomycetes Population as Influenced by Exocarp of *Melia Dubia*

Actinomycetes of various enrichment subtrates revealed that inoculation of rhizosphere soil with exocarp recorded greatest actinomycetes population (74.66×10^2cfu/g of soil) enrichment with cow dung and termite mound reduced actinomycetes population (Table 1).

Rhizosphere Phosphate Solubilisers Population as Influenced by Exocarp of *Melia Dubia*

Apart from germination inducers microbes enhancing nutrient uptake also isolated and evaluated. The phosphate solubilizing microbial population of rhizosphere and the exocarp presented in the table 1. Phosphate Solubilizing Microbial count was observed to be the same for various enrichment techniques except the treatment receiving the goat pellet as the source of micro organisms. Goat pellet enrichment increases the phosphate solubilizer.

Diazotrophic Bacterial Population as Influenced by Exocarp of *Melia Dubia*

Azotobacter is a free living aerobic nitrogen fixing bacteria. The population was unaltered due to various enrichment techniques except the treatments receiving elephant dung and coat pellet as the source of inoculum. Elephant dung treatment has reduced the *Azotobacter* count by 50%. The cultural and morphological analyses indicated that the isolate found in various treatments as *Azotobacter chroococcum*. The results are given in table 1. *Azospirillum* is yet another diazotrophic microaerophilic and associative dinitrogen fixer. Rhizosphere soil with exocarp enrichment scored maximum population (54×10^4cfu/g of soil) over rhizosphere soil (37.33×10^4cfu/g of soil). But various sources of microbial inoculm reduced the population drastically and recorded lower population over control. Still they were statistically significantly different (Table 1). *Beijerinckia* is a free living acidophilic dinitrogen fixing bacteria. Its population in rhizosphere escalated due to cow dung (31.33×10^4cfu/g of soil) and elephant dung (24.33×10^4cfu/g of soil) application over rhizosphere soil sans enrichment (14×10^4cfu/g of soil) (Table 1).

Cellulose and Lignin Degraders Population as Influenced by Exocarp of *Melia Dubia*

The cellulose and lignin degraders population remained same for various enrichment substrates.

Maximum cellulose degrading microbial load of 1.0 × 10^5cfu/g of soil was observed in the rhizosphere soil enrichment with goat pellet and exocarp (Table 1). While cellulose degrades where not present in cow dung enrichment soil. Rhizosphere soil enriched with exocarp scored maximum value for lignin degraders (1.660 × 10^5cfu/g of soil). The qualitative analysis indicated *Penicillium* and *Aspergillus* as dominant lignin degraders associated with exocarp enriched treatment.

Rhizosphere Bacterial Population as Influenced by Endocarp of *Melia Dubia*

The results of number of heterotrophic bacteria and actinomycetes present in the rhizosphere and spermosphere of *Melia dubia* are present in the table 2. The rhizosphere had 21.33 × 10^8 Cfu/g of soils bacterial cells in a gram of soil. When the soil received the endocarp, the population had enhanced to 46 × 10^8 Cfu/g of soils which was double the content of rhizosphere.

The quantitative analysis of bacteria indicated the presence of *Bacillus* as the dominant genus in all the kinds of enrichment treatments. But the rhizosphere soil with endocarp additionally had yellow pigmented colonies. The *Bacillus sp.* presented in various enrichment were morphologically different. Hence, all these were pooled together and used for germination studies.

Rhizosphere Actinomycetes Population as Influenced by Endocarp of *Melia Dubia*

As in the case of bacteria, the rhizosphere enriched with endocarp hastened the growth of actinomycetes (133.6 × 10^2 cfu/g of soil). All the treatments (Table 2) recorded more number of actinomycetes than the rhizosphere soil (83.66 × 10^2 cfu/g of soil). The qualitative analysis showed the presence of *Streptomyces* in all the treatments.

Rhizosphere Phosphate Solubilizing Microbial Population as Influenced by Endocarp of *Melia Dubia*

The results of phosphate solubilising microbes are given in table 2. The phosphate solubilising population has reduced when different sources of inoculums were introduced as enrichment substrates. But the reduction in population was not statistically significantly different. Except elephant dung inoculation, other substrates reduced the phosphate solubilizers.

Diazotrophic Bacterial Population as Influenced by Endocarp of *Melia Dubia*

Nitrogen fixing microbes viz, *Azotobacter*, *Azospirillum* and *Beijerinckia* associated with endocarp in rhizosphere soil were analysed and presented in the table 2. Of various treatments, rhizosphere soil mixed with endocarp recorded maximum number of *Azotobacter* (7.33 × 10^4cfu/g

Table 2
Microbial Population of Endocarp of *Melia dubia* as Influenced By Various Substrates (60 DAE)

T	*Bac* × 10^8 *cfu* g^{-1}	*Acti* × 10^2 *cfu* g^{-1}	*PSM* × 10^3 *cfu* g^{-1}	*Azot* × 10^4 *cfu* g^{-1}	*Azos* × 10^4 *cfu* g^{-1}	*Beij* × 10^4 *cfu* g^{-1}	*Cell* × 10^5 *cfu* g^{-1}	*Lig* × 10^5 *cfu* g^{-1}
T_1	32.66	91.33	4.000	5. 330	17.00	10.33	1.33	5.00
T_2	19.66	119.3	2.330	5.330	23.33	17.66	1.00	2.00
T_3	32.33	102.3	3.000	5.000	27.00	9.330	1.00	1.33
T_4	32.00	111.3	3.330	3.660	37.00	4.000	1.33	3.00
T_5	46.00	133.6	2.660	7.330	70.66	27.00	2.00	1.00
T_{11}	21.33	83.66	4.000	4.000	43.00	18.33	1.00	2.00
Mean	30.66	106.9	3.222	5.111	36.33	15.00	1.277	2.388
SE(d)	4.593	16.31	1.763	1.539	5.453	3.760	0.720	1.122
CD(0.05)	10.01	NS	3.843	NS	11.88	8.195	NS	2.445

T – Treatment : Bac – Bacteria ; Acti – Actinomycetes : PSM – Phosphate Solublising Microorganism : Azot – Azotobacter : Azos – Azospirillum : Beij – Beijerinckia : Cell – Cellulose : Lig - Lignin

Table 3
Effect of Different Grades of Fruits on Germination of *Melia dubia* fruits in Rhizosphere soil

Grades of seeds	*Days to initial germination*	*Days to complete germination*	*Germination %*
Green (Mature)	48.00	88.00	20.00
Yellow (Colour)	38.66	96.67	11.67
Dried fruits	22.66	118.3	15.00
Decayed fruits	21.00	21.00	5.000
Mean	29.80	77.58	12.08
SE (d)	6.411	7.020	3.330
CD (0.05%)	14.78	16.19	7.680

of soil). Even though the rest of the treatments had enhanced the *Azotobacter* population, the enhancement over control (rhizosphere soil) was not statistically different. The population has reduced in termite mound received enrichment technique. In case of *Azospirillum* and *Beijerinckia*, addition of various substrates reduced the population except endocarp received treatment. The enrichment of rhizosphere soil with endocarp scored maximum *Azospirillum* (70.66×10^4 cfu/g of soil) and *Beijerinckia* (27×10^4 cfu/g of soil) over rhizosphere soil (43×10^4 cfu/g of soil and 18.33×10^4 cu/g of soil).

Cellulose and Lignin Degraders Population as Influenced by Endocarp of *Melia Dubia*

Population of cellulose and lignin degraders as influenced by various enrichment substrates was studied and the results are given in the Table 2. The cellulose degrading microbial population developed due to the enrichment of the rhizosphere soil with endocarp was 2×10^5 cfu/g of soil. The population remained either constant or slightly increased due to introduction of other substrates (Table 2). But the tannic acid utilizers population reduced due to the introduction of endocarp and goat pellet. The rhizosphere soil enrichment with elephant dung and endocarp scored maximum value for lignin degraders (5×10^5cfu/g of soil).

Effect of Different Grades of Fruits on Germination in Rhizosphere Soil

Based on observation it was felt that the seed germination might vary with the grade of fruit usedfor germination. Accordingly germination studies were carried out with different grades of fruits under laboratory conditions. Maximum germination of 20% was observed in green fruits. The minimum germination of 5% was due to decayed fruit. The observations *viz.*, the number of days taken for first germination, the completion of germination and germination percentage etc., are tabulated in table 3. The green fruits took 48 days to initiate and 88 days to complete the germination. Even though decayed fruits took minimum germination period of 21 days, the germination percentage was quite low and there was no further germination. Hence, green fruits were chosen for further germination studies.

Days to Initial Germination

Significant differences were noticed with days to initial germination. Among the different grades of fruits, decayed fruits treatment was superior to all other treatments in terms of minimum time taken to initiate germination (20 days). This was followed by dry fruits (21 days). The maximum of 48 days for initial germination was observed for green fruits.

Days for Complete Germination

Significant variations were observed among the evaluated seed treatments for the time taken to complete germination. The number of days taken for completion of germination was maximum of 118.33 days in dried fruits. In fact decayed fruits took only 21 days to initiate and complete germination but germination percentage was very low.

Germination Percentage

Variations noticed for germination percentage due to different grades of fruits were highly significant. Germination percentage recorded was maximum (20%) in green fruits. This was succeeded by dried fruits (15%) and yellow fruits (11.9%). It was found that green fruit has high germination percentage but takes long time to complete germination.

DISCUSSION

The soil biotic and abiotic factors are important as they are common to influence the distribution, occurrence, characteristics association and

quantification of microorganisms. The litter, root exudates and sloughed off root tissues and other plant products regulate the growth of soil microorganisms around the root zone. Hence, to isolate the microorganisms facilitating seed germination, different enrichment culture techniques were followed and the results are discussed here.

To isolate microbes capable of softening exocarp and endocarp (spermosphere) so as to induce germination, the rhizosphere soil along with exocarp or endocarp was enriched with different sources of microbial inoculum *viz.*, cowdung, elephant dung, goat pellet and termite mount. In both the enrichment culture techniques, mixing rhizosphere soil with either exocarp (spermosphere) or endocarp (spermosphere) induced bacterial growth in a greater manner. It is twice the quantum of rhizosphere population. Among the sources of microbial inoculum in the exocarp (spermosphere) enriched soil, elephant dung substantially improved bacterial population over other sources and rhizosphere soil. On the contrary, endocarp (spermosphere) enriched rhizosphere soil with all different sources of microbial inoculum recorded more number of bacteria over rhizosphere soil. In case of exocarp (spermosphere) enrichment with various sources of microorganisms like cow dung, goat pellet and termite mound infact reduced the bacterial population. This could be attributed to the bacterial inhibitory/antagonistic activity of substances in above said microbial sources.

Even the microbes in various sources might compete with the rhizosphere and exocarp (spermosphere) associated bacterial flora for nutrients and thus reduced the rhizosphere bacterial load. On the other hand, the bacterial load of rhizosphere soil + endocarp (spermosphere) received treatment enriched with microbial sources like elephant dung, termite mound and goat pellet scored greater value for bacteria. Only enrichment with cow dung reduced the bacterial population. The synergistic effect of metabolites produced during enrichment might be the cause for enhancement of bacteria over control and cowdung treatment.

Qualitative and quantitative analyses of bacterial population indicated that incorporation of exocarp (spermosphere) changed the quantitative nature of rhizosphere. Morphologically, three different kinds of *Bacillus* were recorded in exocarp (spermosphere) incorporated enrichment technique. The elephant dung received enrichment technique harbored yet another morphologically distinct bacillus over control. The qualitative analysis further revealed that the *bacillus* observed in both exocarp and endocarp (spermosphere) enrichment was one and the same.

Hence one *bacillus* from control, two strains from exocarp induced and one from elephant dung enriched treatment were screened and used for further studies. In case of actinomycetes, the influence of various sources of microbial inoculam and exocarp (spermosphere) treatment on actinomycetes population was quite negligible. In variably, all the treatments possessed Streptomycetes as the dominant genus. But variation in population was significantly different. But endocarp (spermosphere) enrichment had significantly influenced the actinomycetes and hence one actinomycetes strain was screened out and used for preparing microbial consortium. The domination of actinomycetes in the endocarp (spermosphere) indicates that the hard endocarp (spermosphere) might consisting of recalcitrant material that can be utilised mostly by actinomycetes. As the cell wall of plant parts dominated by macromolecules such as cellulose and lignin, the microbes utilizing cellulose and lignin too were isolated from enrichment samples. *Aspergillus* and *Penicillium* were the dominant, lignin and cellulose degraders observed in the study.

Apart from fruit coat softening microorganisms, microbes imparting nutrional effect *viz.*, diazotrophs and phosphate solubilisers were also documented. Various phosphate solubilisers obtained from both enrichment culture techniques were of fungi. Leaving aside the fungal cultures dominant bacterial flora obtained from enrichment studies and diazotrophs *viz.*, Azospirillum, Azotobacter and Beijerinckia were cultured and developed as inoculants and used for germination studies. The result revealed that the bacteria capable of softening

fruit coat can be obtained from enrichment of rhizosphere soil with exocarp (spermosphere). Based on observation and records, it has been felt that even the kind or grade or maturity stage of the fruit might control the germination. To investigate the same, seed germination experiment with different grades of fruits (green, yellow, dry and decayed) was carried out under laboratory conditions. As assumed, green fruits recorded maximum germination percentage over other grades. Germination was quite poor in yellow and dry fruits. The results showed that the germination reduced with drying or aging of the fruit. Hence, moisture content as well as physiological stage of the fruit might decide the germination. The reason for the least germination in decayed fruit might be lack of storage material for supporting germination. Studies carried out for *Acacia longifolia* and *Acacia sophorae* the low imbibitions in seed coat as the germination inhibition so that the present study is in the conformity with the respect of Nguyen tran, 1979. Spermosphere microbial composition of different grades of fruits assayed to compare the microflora obtained from enrichment culture techniques showed that the bacteria and actinomycetes documented were morphologically similar. Hence the aerobic heterotrophic bacteria, actinomycetes and different nitrogen fixers and phosphate solubilsiers isolated from exocarp (spermosphere) enrichment technique were developed as microbial cultures and used in germination studies.

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