Effect of combined treatment of Microbial Consortia and Microwave Energy on Germination of *Melia dubia* seeds

R.Ravi, T.Kalaiselvi and M.Tilak

Abstract— The germination of Melia dubia is enhanced by exposing the fruits to microwave energy of 2450 MHz. This technique provides a quick and effective method of treatment for the seeds having a hard impermeable seed coat. Physical and chemical seed treatments methods did not improve seed germination. So germination studies were carried out under laboratory conditions to find out the synergetic effect of microbial consortia and microwave treatment on seed germination. Exposing the fruits to microwave energy for 7.5 min followed by seed pelletization with selected microbial consortia recorded the highest germination percentage of 68% over control. This was followed by the treatment exposing seeds to microwave energy for 5 min + microbial consortium (Germination Percentage 52 %) and microwave energy for 10 min + microbial consortium (Germination Percentage 32 %). The control, microwave energy treatment at 2.5min and microwave energy treatment at 20 min had no germination during study period. There is a threshold value for the microwave energy dose, beyond which it may be non effective. The time taken for germination varies between 3 to 6 months normal conditions, but significant differences were noticed with days taken for initial germination. Among the treatments, microwave radiation for 7.5 min + microbial consortium was superior to all other treatments in terms of minimum time taken to initial germination

Index Terms— *Melia dubia* , Seed, Microwave, Microbial consortia, Germination, Root Length, Shoot length

1 INTRODUCTION

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 and it is common in moist deciduous forests of Kerala. It is also found in the tropical moist deciduous forests of Sikkim, Himalaya, North Bengal, Upper Assam and Khasi hills of Orissa.

The tree has been considered as the best alternate pulpwood species. Besides, it has also been extensively exploited by many other industries like match wood and medicine. Owing to its importance, this species has been identified as potential, commercially viable pulpwood species for ex-situ cultivation in Tamil Nadu. 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 [7]. Today, there are about 594 paper mills in India, with 34 considered as large companies. In the State of Tamil Nadu, there are 39 paper mills, including the Tamil Nadu Newsprint and Paper Ltd (TNPL) and Shesayee Paper Boards Ltd (SPB). 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 [5]. Inadequate raw materials and stringent forest policies have forced the wood based industries to become self-reliant in terms of acquiring their own raw materials. Hence, tree species *viz.,Eucalyptus*, *Casuarina* and Leuceana are promoted in large scale. In order to replace these exotic tree species, efforts have been made to identify indigenous tree species with superior quality pulps.

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Ltd (SPB). 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 [5]. Inadequate raw materials and stringent forest policies have forced the wood based industries to become self-reliant in terms of acquiring their own raw materials. Hence, tree species *viz.,Eucalyptus*, *Casuarina* and Leuceana are promoted in large scale. In order to replace these exotic tree species, efforts have been made to identify indigenous tree species with superior quality pulps. *Melia dubia* has been screened as one of the best alternate of pulpwood species [9]. Being an indigenous species it has great potential to meet the demands of pulpwood and other needs. Although regeneration of this species under natural conditions is very high, the seeds generally exhibited very low germination due to hard seed coat under laboratory conditions. Vegetative means of propagation is difficult . In view of this, studies on germination and establishing a practicable germination package assumes a greater significance

2. Material and methods

Collection of Fruits and Rhizosphere Soil

Fruits from phenotypically superior plus tree of *Melia dubia* were collected from State Horticulture Farm at Kallar and surrounding area. In order to isolate microbes associated with fruit surface, fruits with different stages of maturity *viz*, green, yellow, partially degraded and dried fruits were collected and culture were isolated by following enrichment culture technique. Rhizosphere soil samples were collected from soil adhering to the root system.

Description of Fruit

Melia dubia fruit is a drupe which is ellipsoid in shape with 1-6 seeds and it possess outer most skinny exocarp, followed by mesocarp and hard stony endocarp (spermosphere) and finally black coloured seeds in the locules

Seeds

Fresh seeds of *Melia dubia* were (both crown and ground collection) collected from various sources at Kallar. The fruits ripen during November to February. These seeds were used for the germination study.

Growth Medium

Growth medium used in this experiment was rhizosphere soil mixed with sand in the ratio of 1:1 (Rhizosphere: Sand). Rhizosphere soil collected from root zone of *Melia dubia* in State Horticulture Farm, Kallar

Physio-chemical properties of the soil used for germination of *Melia dubia*

i. Rhizosphere soil + Sand

Physio-chemical properties of the soil used for germination of *Melia dubia*

Treatment	pH	EC (dSm^{-1})
Sand + Rhizosphere	7.56	0.05

3. Development of Microbial Consortia

The selected isolates were mass multiplied in nutrient broth at room temperature till a population of 10^{10} / ml was achieved. Each multiplied inoculums was mixed with sterilized and neutral lignite at 30-40% moisture content. The cultures were carried at room temperature under aseptic conditions.

The selected *Azospirillum*, *Azotobacter*, *Berijerinckia* and Phosphate Solubilizer microbes were also mass multiplied and carrier based cultures were prepared.

Microbial Treatment of Seeds

The selected seeds were pelletized with 5 gram each of carrier based bacterial (4 isolates) and actinomycete's (1 isolate) cultures . The nursery mixture was incorporated with 5 gram each of *Azotobacter*, *Azospirillum* and *Beijerinckia*

Fruit Pre-Treatment on Seed Germination.

The seeds of *Melia dubia* were collected in bulk from phenotypically superior trees from various sources at Kallar and Thalavadi. The matured fruits of green in colour were subjected to the following pre-sowing seed treatments for improving its seed germination by exposing it to microwave energy (2450 MHz) and dominant microflora were isolated. The experimental design adopted was CRD in three replications at 25 fruits per treatment per replication. The treatment details are presented below

Notation	Treatment
T1	Microwave Energy in microwave oven for 2.5 min + microbial consortium.
T2	Microwave Energy in microwave oven for 5 min + microbial consortium.
T3	Microwave Energy in microwave oven for 7.5 min + microbial consortium.
T4	Microwave Energy in microwave oven for 10 min + microbial consortium.
T5	Microwave Energy in microwave oven for 15 min +microbial consortium.
T6	Microwave Energy in microwave oven for 20 min + microbial consortium.
T7	Control

Details of the various pre-treatments applied for *Melia dubia* seeds.

In both germination studies, the following parameters were recorded.

Days to Initial Germination

Days taken for initiating germination identified through visual hypocotyls bent observed for each of the treatment replication wise and the mean was expressed as days to initial germination.

Days for Completion of Germination

Days taken for completing germination (no further observation of germinating seedling) was observed for each of the treatment replication-wise and the mean expressed as days for completion of germination.

$$\text{Germination percentage (G \%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Germination Percentage

It is the percentage of seeds in a given sample, which have germinated within a given period (defined as the energy period) of 15 days. [3]

Thirty days after sowing, five seedlings were randomly selected from each treatment and the following seedling parameters were taken and the mean was worked out and reported.

Shoot Length

The distance between the tips of the primary shoot to the collar region was measured in centimeter as shoot length and expressed in cm.

RESULTS

Effect of Seed Pre-Treatments on Germination of *Melia dubia* (Rhizosphere Soil + Sand) in Plastic Tray Days to Initial Germination

Significant differences were noticed with days to initial germination. Among the treatments microwave radiation for 7.5 min + microbial consortium seed treatment was superior to all other treatments in terms of minimum time taken to initial germination (10.67days). Germination was completely absent when the seeds were exposed to microwave treatment + microbial consortia seed treatment for 2.5min. Similarly when the fruits are exposed to higher duration for more than 20min, then also there was no germination (Table I). This was followed by microwave radiation for 5 min + microbial consortium seed treatment (12.33 days). Microwave radiation for 10 min + microbial consortium seed treatment took 14 days for initial germination. The maximum of 20 days for initial germi-

nation was observed under microwave radiation in for 15 min + microbial consortium seed treatment. Germination was nil in microwave radiation for 20 min +microbial consortium seed treatment , microwave radiation in for 2.5 min + microbial consortium seed treatment and control (Table.I).

Days for Completion of Germination

Significant variations were observed among the evaluated seed treatments for the time taken to complete germination. The days taken for completion of germination were maximum of 44.67 days with microwave radiation for 5 min +microbial consortium seed treatment . The minimum days taken for completion of germination was recorded with seeds pre-treated with microwave radiation for 7.5 min +microbial consortium seed treatment (32.67days) which was significantly superior to all other treatments. This was followed by microwave radiation for 15 min +microbial consortium seed treatment (36.67 days) (Table I).

Germination Percentage

Variations noticed for germination per cent due to different pretreatment were highly significant. Germination percent recorded was the maximum (68 %) with microwave radiation in for 7.5 min +microbial consortium seed treatment. This was followed by microwave radiation for 5 min +microbial consortium seed treatment (52%) and microwave radiation for 10 min +microbial consortium seed treatment (32%). Germination percent recorded was the minimum (12%) with microwave radiation for 15 min +microbial consortium seed treatment . The control, microwave radiation for 2.5 min +microbial consortium seed treatment and microwave radiation for 20 min +microbial consortium seed treatment recorded the no germination during study period (Table. I).

Shoot Length

Among the seed pre-treatment, microwave radiation for 15 min +microbial consortium seed treatment recorded significantly the longest shoot length (21.38cm). This was followed by microwave radiation for 5 min +microbial consortium seed treatment (16.46cm) and the shortest shoot length of 12.98 cm was recorded under microwave radiation for 10 min +microbial consortium seed treatment (Table. I).

Treatments	Days to initial germination	Days for completion of germination	Germination (%)	Shoot length (cm)
T1	00.00	00.00	00.00	00.00
T2	12.33	44.67	52.00	16.46
T3	10.67	32.67	68.00	14.57
T4	14.00	36.33	32.00	12.98
T5	20.00	42.00	12.00	21.38
T6	00.00	00.00	00.00	00.00
T7	00.00	00.00	00.00	00.00
Mean	8.142	22.23	23.42	9.341
SE(d)	1.652	1.272	2.468	1.993
CD(0.05%)	3.544	2.729	5.295	4.275

Table I. Effect of Pre-Sowing Treatments on Germination

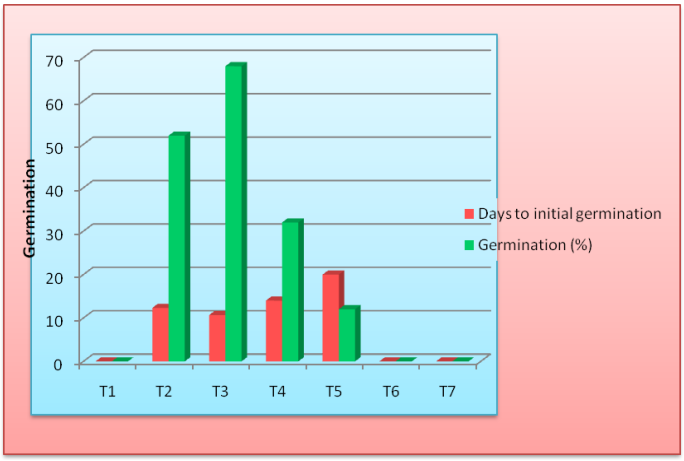

Fig.1 Effect of Pre-Sowing Treatments on Germination of *Melia dubia* Fruits (Rhizosphere Soil + Sand)

DISCUSSION

Melia dubia is one of the alternate indigenous pulp yielding tree species getting importance all over India particularly in Tamil Nadu. It is being promoted by both governmental and nongovernmental paper industries for pulp wood. Even-though, it is considered a one amongst the best, the lacuna with tree species is very poor seed germination. Studies carried out so for indicated that the germination under nursery condition ranges from 14 % to 28 % [10]. Even many physical and chemical used to induce and enhance seed germination were found to be futile. The germination could not be enhanced over 28% with existing practices.

As described in the materials and methods fruits of *Melia dubia* possess outer most pulpy exocarp followed by hard stony endocarp (spermosphere) and finally black coloured seed in the locules. Hence, it is assumed that these physical structures might inhibit seed germination. But, despite these physical barriers their natural regeneration or germination has been very good under the tree cover. As the plant grows through the soil conditions certain group of micro organisms dominate, established, become persistent and multiply in the rhizosphere soil. These rhizosphere microorganisms are mostly ten to hundred times greater than that of the normal soil.

The germination obtained with green fruits was only 20 % which is slightly higher than the reported value (14 %). Hence, to boost up the germination still more, green fruits were treated with microbial consortia and sown in nursery mixture containing *Melia dubia* rhizosphere soil. Before microbial treatment the fruits were exposed to microwave energy for different period. Among various exposure periods, treating fruits for 7.5 minutes, recorded maximum germination. The number of days taken for initial germination and mean germination time also got reduced due to exposure for 7.5. min. There was no germination in over (20 min) and under (2.5 min) exposure of fruits to microwave energy.

Even though many methods like saccarification with sand paper, acid and hot water treatments remove hardseededness by cracking the seed coat and allowing imbibitions, the methods did not work with the study material. In fact many authors [1,3] reported that the low seed germination in hard seed coated and physiologically dormented seeds could be overcome by various physical and chemical treatments. Microwave treatment for improving germination of hard seeded *Stylosanthes seabrana*.[1].Exposure of stylosanthes seeds to microwave energy partly reduced hardseededness and increased germination percentage. The mode of action of microwave treatment can be attributed to improved permeability of seed coat membranes to water and its distribution into the seeds during imbibition and to increased macromolecular hydration essential for the completion of the germination process. Thus the microwave treatment has been shown to cause physical aberrations and produce di - electric heating of seed coat. These changes have could have facilitated water imbibitions. The overexposure hambered seed germination (1). Germination of *Acacia longifolia* and *Acacia sophorae* was enhanced by microwave energy at 2450 MHz and it was found to be effective method for seed having an impermeable seed coat.[9].The reports of present study are in line with the findings of above authors. Similar to their report, the results documented in the current study showed that the seeds did not germinate when they were exposed to 20 mins. This indicates that there is a threshold period for exposing the seeds to energy, beyond which it may not be effective.

The novel finding in the present study is that, apart from energy therapy the seeds also require microorganisms for inducing and facilitating germination, since the uninoculated control recorded no germination. [6] . This is in line with earlier studies conducted in rhododendron [8] which reports that mixing of non rhizosphere soil with the rhizosphere soil of rhododendron in different ratios (1:1, 1:3 & 3:1) has affected the seed germination positively. Forest or non rhizosphere soil alone resulted in poor germination in comparision to mixed soil treatments. Sterilization of rhododendron rhizosphere soil resulted in decreasing the percent seed germination. Out of three ratios, highest seed germination was recorded in rhizosphere soil mixed with non rhizosphere soil treatment in 1: 1 ratio. [2,8]

In the early years of past decade, many investigating groups concentrated on nursery studies with rhizosphere soil as one of the substrates of container media. In such studies combinations of different types of soil, such as forest soil and non forest soil in different ratios have been evaluated for influencing seed germination and subsequent growth of important forest species. Rhizosphere soil mixed with non forest soil in 1:9 ratio improved seed germination and subsequent growth of *Cedrus deodara* and *Pinus wallichiana* under nursery conditions [4]. These results indicate that the presence of certain factors probably of microbial origin that could influence the seed germination.

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Reference

[1] Anand, A, S.Nagarajan, D.K.Joshi, A.P.S.Verma, A.Kar. (2008). Microwave seed treatment reduces hard seededness in *Stylosanthes seabrana* and promotes redistribution of cellular water as studied by NMR relaxation measurements. Seed Sci, Technol., 37:88-97.

[2] Durgapal,A, A.Pandey and L.M.S.Palni.(2002). The use of rhizosphere soil for improved establishment of conifers at nursery stage for application in plantation programmes. Journal of Sustainable Forestry. 15: 57-73.

[3] ISTA. (1999). International rules for seed testing. Seed Sci. & Technol. (Supplement Rules), 27: 25-30.

[4] Nguyen Tran,V.(1979).Effects of Microwave energy on the Strophile, Seed Coat and Germination of Acacia Seeds. Aust.J.Plant Physiol., 6:277-287.

[5] Parthiban, K. T. and M.Govinda Rao. (2008). Pulpwood based industrial agroforestry in Tamil Nadu-A case study, The Indian forester., 134:155-163.

[6] Singh.K.K, S.Kumar and A.Pandey. (2008). Soil Treatments for Improving Seed Germination of Rare and Endangered Sikkim Himalayan Rhododendrons. World Journal of Agriculture Science. 4(3): 288-296.

[7] Palsaniya D. R., S. K. Dhyani, R. K.Tewari, Ramesh Singh and R. S.Yadav. (2009). Marketing issues and constraints in agroforestry. In Agroforestry: Natural Resource Sustainability, Livelihood and Climate Moderation Eds: O.P. Chaturvedi, A.Venkatesh, R.S.Yadav, Badre Alam, R.P.Dwivedi, Ramesh Singh and S.K. Dhyani). Satish Serial Publishing House. India.563 –577.

[8] Smith, M.T and P.Berjak. (1995). Deteriorative changes associated with the loss of stored dessication-tolerant and dessication-sensitive seeds. In: Seed development and germination. Eds: Kigel.J. and Galilli, G., Marcel Dekker, New York. 701-746.

[9] Bharti, A.K, (2006). Screening alternate pulp wood species and establishment of tree improvement strategies for the screened species. M.Sc.(For.) Thesis, TNAU, Coimbatore, India.

[10] Camus,D. (2008). Standardization of nursery techniques for quality seedling production in *Melia dubia* Cav. Seedlings. M.Sc(For.)Thesis, TNAU,Coimbatore, India

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