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Effect of different pre-sowing treatments on viability, germination and seedling characters of Malabar neem (*Melia dubia* Cav.) fruits

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Abstract
Melia dubia is a multipurpose tree species having high commercial and economic importance. Due to its fast-growing ability, it is gaining popularity for generating income in a short span of time. The natural germination of the species is less than 25%. Therefore, alternate methods are needed to be found for enhancing the germination of fruits and seeds. This study evaluates the pre-sowing seeds treatments on viability, germination and seedling growth parameters of *Melia dubia* in laboratory conditions. *Melia dubia* fruits were treated with six pre-sowing treatments. The results revealed that cow dung treated fruits for seven days (T_1) had significantly enhanced germination. The maximum germination percentage (32.46) and viability percentage (45.71) was recorded in cow dung treated fruits for seven days (T_1) followed by use of bench vice after 100^0 C boiling of fruits for 24 hrs (T_4). Similar trends were observed in shoot length, root length and seedling dry weight. Hence, pre-sowing treatments was effective in enhancing germination and production of quality seedling of *Melia dubia*.

Keywords: *Melia dubia*, germination, seed treatment, viability

Introduction
The existing supply of raw material for wood-based industries in the country is lagging behind demand due to the excessive pressure of an ever-increasing population (Singh *et al*., 2020) [14]. To meet the need for wood, some short rotation fast-growing species are being pushed into degraded terrain, farm fields, and barren areas. The demand for raw wood by different industries increased from 52 million m^3 in 1998 to 95 million m^3 in 2010, and is projected to increase further to 153 million m^3 in 2020 (Vanam, 2019) [17]. Hence, *Melia dubia* can be an alternate source of raw material since it is a quick developing short rotation period tree. *Melia dubia* Cav. is a species of multipurpose tree commonly known as Malabaar Neem and a dicotyledons, perennial tree belongs to family meliaceae. It can be cultivated in a variety of agroforestry systems. The species is native to southern Asia and has been introduced to south Africa, middle east, America, Australia, Brazil and Southern Europe (Ram *et al*., 2014) [13]. It grows up to 6 to 20 m in height with spreading crown and straight clear bole of 10 m with tap root system. It grows in variety of soils such as deep fertile sandy loam soils and shallow gravelly soils (Nanda *et al*., 2021a, 2021b, Kumar *et al*., 2021) [10, 11, 9]. *Melia dubia* fruits are ovoid or ellipsoid yellow drupe with three to five seeds, but germination is less than 25% (Anand *et al*., 2012) [2]. *Melia dubia* is drought tolerant in nature. It grows in variety of soils such as deep fertile sandy loam soils and shallow gravelly soils. It grows well at annual temperature of 23-27°C and altitude up to 1800 m. It does well in moist regions, with annual rainfall ranging from 750-1500 mm (Goswami *et al*., 2020) [4]. *Melia dubia* (Malabar neem) possesses anti-cancer, anti-diabetic, anti-tumour, anti-inflammatory, antioxidant, antibacterial, anti-viral and fungicidal properties (Thangavel *et al*., 2019) [16]. Wood is moderately hard and 450 kg/m^3 in weight with sapwood and heartwood having grey and light red colour, respectively. *Melia dubia* under high density plantation, (2470-3950 trees/ha) can be planted in one hectare and the yield could be 75-95 tons per hectare from 5-6 years old plantation which gives better yield and quality of wood (Sinha *et al*., 2019) [15]. Its wood used in packing cases, agricultural implements, building purpose, fuel wood and leaves as fodder. Five-year-old trees gives better yield and quality of wood (Sinha *et al*., 2019) [15]. However, one of major constraints is its poor seed germination (Kumar *et al*., 2018) [8], therefore in this study, suitable pre-sowing applications to improve seed germination and quality seedlings are accessed.

Material and Methods

The experiment was laid out in Completely Randomized Design. The treatments comprised of [T_1: Cow dung slurry for 7 days, T_2: Mechanical scarification for 5 minutes, T_3: Use of bench vice, T_4: Use of bench vice after 100^0 C boiling, T_5: Conc. H_2SO_4 for 5 minutes, T_6: Fruit (Control)] each replicated four times. The experiment was conducted in the laboratory of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar (Haryana). The fruits were collected from plus trees of *Melia dubia* at Coimbatore (Tamil Nadu). In T_1, fruits were soaked in cow dung slurry for 7 days. Slurry was prepared by mixing of fresh cow dung with water. In T_2, Mechanical scarification was done by rotary coating machine. Seeds were subjected to rotary machine and grinded to remove or loose hard seed coat. It involved breaking and weakening the seed coat which makes seeds easily to germinate. In T_3, by application of bench vice, seed coat was nicked by pressing of hard coat of fruit. In T_4, boiling water treatment was given to seeds for 24 hours that are selected phenotypically to breaking seed coat. After that, seeds were subjected to bench vice to making nicking on seeds or loosen the seed coat. In T_5, concentrated H_2SO_4 treatment was given to seeds that was selected to enhance germination. In this treatment seeds were dipped in Concentrated H_2SO_4 for 5 minute and then directly washed with plenty of water. In T_6, whole fruit having hard seed coat was used for treatment. After the treatment, treated seeds were placed in sand method and put into germinator at 30 ^{0}C.

Viability

Seed viability refers to the capability of a seed to germinate and produce a normal seedling. Seed viability was determined through TZ test (tetrazolium test). Seeds were soaked in 0.1% solution of 2,3,5-triphenyl tetrazolium chloride in dark room at 30-35º C temperature for 24-48 hours at a pH of 6-8. Then seeds were washed with plenty of water. Viability of seed was visually judged on the basis of colour change in the embryo. The colour of embryo of viable seeds changed to bright red and dead or non- respiring seeds remain colourless. The colour of living seeds changed due to the reduction of colourless 2, 3, 5-tripheny tetrazolium chloride to red coloured formazan by the dehydrogenase enzyme activity of the living tissue (ISTA, 2011) [5]. The seed viability percentage was calculated as-

$$\text{Viability (\%)} = \frac{\text{Number of stained seeds}}{\text{Total number of seeds used}} \times 100$$

Germination

The number of normal seedlings was counted on the 30th day of germination in laboratory condition and pre-treated seeds were subjected for germination in a seed germinator which was maintained at 30^0C and 90% humidity. Germination percentage was calculated by using the following formula.

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

Vigour Indices

The sum total of those properties of the seed which determine the level of activity and performance of the seed lot during germination and seedling emergence.

Vigour index value was computed using the following formula suggested by Abdul-Baki and Anderson (1973) [1] and expressed as whole number.

i. Vigour Index-I

It was calculated by using following formula:

Standard germination (%) × Average seedling length (cm)

ii. Vigour index-II

It was calculated by using following formula:

Standard germination (%) × Average seedling dry weight (g)

Shoot length

Ten normal seedlings from four replications of each treatment were randomly selected. Shoot length was recorded by with the use of scale and average shoot length was calculated which is expressed in centimetre.

Root length

Ten normal seedlings from four replications of each treatment were randomly selected at the time of final count of standard germination. Root length was measured by with use of scale and average root length was calculated which is expressed in centimetres.

Seedling dry weight

Seedling dry weight was measured after the final count in the standard germination test (30 days). Seedlings were dried in a hot air oven. The dried seedlings of each replication were weighed and average seedling dry weight was calculated.

Statistical Analysis

The data was analysed statistically by using of model suggested by Panse and Sukhatme, (1967) [12].

Results and Discussion

Viability and germination

The perusal of data from Table 1 reveals that fruit viability and germination was significantly affected by all the treatments except concentrated H_2SO_4 for 5 minutes (T_5). The highest viability and germination (45.71% and 32.46%) were recorded in cow dung slurry treated fruits for seven days (T_1) which was significantly higher than rest of the treatments, followed by use of (T_4) bench vice after 100^0C boiling (22.91% and 14.12%) for 24 hours, use of (T_3) bench vice (20.78% and 12.18%), (T_2) mechanical scarification for 5 minutes (20.33% and 10.93%) and control (18.67% and 8.80%). The decrease in viability may be due to over-exposure of fruits to scarification treatments. Minimum fruit viability and germination percentage (9.66% and 5.00%) was recorded in treatment with conc. H_2SO_4 dipped fruits for 5 minutes. Sulphuric acid has a detrimental effect on seeds because of acids penetrates the seed via its exposed micropyle and end up damaging the seeds (Ells, 1963) [3]. Increased germination percent in cow dung manure treated fruits might be due to the removal, softening, damage and fermentation caused on the seed coat and increase permeability of water to fruits kernels and initial emergence of radicle. Any change occurred in the structure of fruit coat might made the entry of water in to the embryo and cotyledons. These results are in conformity with Krishna *et al.* (2011) [6] that maximum germination was possible when seeds were treated with cow dung. Similarly, Krishna *et al.* (2013) [7] reported maximum germination per cent in cow dung.

Table 1: Effect of pre-sowing treatments on viability, germination, shoot and root length of *Melia dubia* fruits

Treatment	Viability (%)	Germination (%)	Shoot length (cm)	Root length (cm)
T_1: Cow dung slurry for 7 days	45.71	32.46	15.52	6.78
T_2: Mechanical scarification for 5 minutes	20.33	10.93	11.89	4.78
T_3: Bench vice	20.78	12.18	12.24	5.28
T_4: Bench vice after 100^0C boiling for 24 hours	22.91	14.12	14.98	5.62
T_5: Concentrated H_2SO_4 for 5 minutes	9.66	5.00	7.93	2.46
T_6: Fruit control	18.67	8.80	9.83	3.69
C.D. at 5%	2.79	2.74	0.43	0.76

Shoot and root length

The data presented in Table 1 reveals that maximum shoot and root length was recorded (15.52cm and 6.78cm) in cow dung treated fruits for seven days (T_1) which was significantly higher than rest of the treatments, followed by (T_4) use of bench vice after 100 ^{0}C boiling for 24 hours (14.98cm and 5.62cm) and (T_3) use of bench vice (12.24cm and 5.28cm). This might be due to growth promotors and nutrients availability in cow dung. The results are in conformity with Krishna *et al*. (2011) [6] who reported that soaking of fruits of *Melia dubia* in cow dung, resulted maximum seedling parameters such as height, vigour index and dry weight. The minimum shoot and root length (7.93cm and 2.46cm) was observed in fruits dipped in concentrated H_2SO_4 for 5 minutes (T_5).

Table 2: Effect of pre-sowing treatments on seedling dry weight and vigour index-I and vigour index-II of *Melia dubia* fruits

Treatment	Seedling dry weight (g)	Vigour index-I	Vigour index-II
T_1: Cow dung slurry for 7 days	0.623	723.86	20.12
T_2: Mechanical scarification for 5 minutes	0.330	182.20	3.60
T_3: Bench vice	0.390	213.39	4.75
T_4: Bench vice after 100^0C boiling for 24 hours	0.478	295.10	6.77
T_5: Concentrated H_2SO_4 for 5 minutes	0.163	51.95	0.80
T_6: Fruit control	0.280	91.43	2.46
C.D. at 5%	2.79	37.63	1.20

Seedling dry weight

The data in Table 2 showed that seedling dry weight of *Melia dubia* was significantly affected by different pre-sowing treatments on fruit. Maximum seedling dry weight was recorded (0.623 g/seedling) in cow dung treated fruits for seven days (T_1) which was significantly higher than other treatments, followed by (T_4) use of bench vice after 100^0 C boiling for 24 hours (0.478 g/seedling) and (T_3) use of bench vice (0.390 g/seedling). The minimum seedling dry weight (0.163 g/seedling) was recorded in fruits dipped in conc. H_2SO_4 for 5 minutes (T_5).

Vigour Index-I and Vigour Index-II

Table 2 reflects that Vigour Index-I of *Melia dubia* seedling was significantly affected by different pre-sowing treatments of fruits. Maximum Vigour index-I was recorded (723.86) in cow dung treated fruits for seven days (T_1) which was significantly higher than other treatments, followed by (T_4) use of bench vice after 100 ^{0}C boiling for 24 hours (295.10), (T_3) use of bench vice (213.39) and control (91.43). The minimum Vigour index-I (51.95) was observed in fruits soaked in concentrated H_2SO_4 for 5 minutes (T_5). A similar trend was also observed for Vigour Index-II of *Melia dubia* by different pre-sowing treatments on fruit.

Conclusion

Based on one year study, it may be concluded that among all the pre-sowing treatments, cow dung treated fruits for 7 days proved better germination and viability per cent in laboratory. Similarly, seedling growth parameter such as shoot length, root length, vigour indices and seedling dry weight were observed higher in cow dung treated fruits for 7 days. Hence, this pre-treatment can be suggested to farmers to raise better germination and seedling growth of *Melia dubia*.

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Conflict of Interest

The authors declare no conflict of interest.

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