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Effect of seed size, pre-sowing treatments and potting mixture on seedlings growth character and biomass production under nursery conditions of *Terminalia chebula* Retz

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Abstract
The experiment conducted aims in improving seed germination, seedling growth and biomass production of *Terminalia chebula.* For the experiment, the depulped fruits were graded into three different sizes on the basis of length and were subjected to eight pre-sowing treatments and followed by transplanting seedlings in three different potting mixtures. It was evident from the study that large size seeds (L3) excelled in all germination, growth and seedling biomass parameters. Among treatments, maximum germination parameters were recorded from T8 (nicking at broad end then soaking in ordinary water for 36 hours). Among seed size and pre-sowing treatment combinations, most successful result was observed from large size seeds subjected to nicking at broad end then soaking in ordinary water for 36 hours (T8L3). Among three different potting mixtures, seedlings transplanted in the potting mixture M3 (Soil: Sand: FYM-1:2:3) exerted significantly maximum seedlings growth and biomass production under nursery conditions.

Keywords: Pre-sowing treatment, seed size, potting mixture, germination parameters, seedling growth, seedling biomass

Introduction
Terminalia chebula Retz. commonly known as ‘Chebula’ and ‘Manahi’ in Manipuri belonging to family 'Combretaceae' is an important medicinal tree distributed throughout India upto an elevation of 1600 m (Troup, 1921) [32]. Commercially, the most important product of this tree is the fruit which is known as ‘myrobalans’. This fruit is one of the most important component of ‘Triphala’ used in ayurvedic medicines and in ‘chayvanprash’ which is used as food supplement and tonic along with the fruits of *Terminalia bellirica* and *Emblica officinalis.* The species yields principal tannin of commercial importance. *T. chebula* are an important source of tannin (25-32%) (Hukkeri *et al.*, 2010) [14]. The unripe fruit is astringent and aperients in nature used in treatment of dysentery and diarrhoea. Moreover, the ripe fruit enriches the blood circulation, treats ophthalmia, spleen, piles, eyes, gums, paralysis, sore throat and strengthen nervous system (Jose and Jacob, 1998) [15]. Finely powdered dry fruits is used as dentifrice and coarsely powdered fruits for smoking in pipe for relieving asthma, ashes mixed with butter forms a good ointment for sores as well as fruit in combination with other drugs is prescribed for snake-bite (Hukkeri *et al.*, 2010) [14].

Despite having a variety of uses, chebula plants are under threats which are the main causes in vast decline of their population worldwide. The seeds of chebula shows low germination in the field due to poor germinative capacity exerted by presence of hard mesocarp in seed, thick shell (endocarp) and poor formation of kernel (Troup, 1921) [32]. Seed dormancy is another factor preventing seed germination even under the provision of favourable germination environment. Physical dormancy exerted in this species is caused by the seed coat impermeable to moisture which restricts moisture reaching the embryo and also preventing gaseous exchange. Considering the above constraints stated it is necessary to include varied seed sizes of the tree species before sowing in nursery. The fruit of *T. chebula* is botanically a drupe with hard endocarp requiring a long period of time for the seeds to germinate under optimum soil environment. Generally, raw fruits are utilized for pickle making (Devi, 2018) [10] and the left out fruits in natural population to a great extent are destroyed by rats, squirrels and

rodents, wherein all the above factors lead to poor natural regeneration of chebula in its natural population (Singh *et al.*, 2003) [28]. Pre-sowing treatment methods are employed for overcoming seed dormancy in many individual species and seed lots depending upon experiment, knowledge, practices and experience (Schmidt, 2000) [27]. Physical dormancy may be overcome by physical scarification of the seed coat by piercing, nicking, chipping, filing or burning with the help of knife, needle, hot wire burner, abrasion paper (Catalan and Macchiavelli, 1991) [6] or acid treatment (Kobmoo and Hellum, 1984) [19]. Seed treatments are practiced to ensure faster and uniform germination of seeds. Therefore, the study has been conducted to improve the germination and seedling vigour by giving varied treatments to the seeds and its potting mixture to overcome the poor germination constraints in this commercially important tree species.

Materials and Methods

The experiment on seeds and nursery technology of *T. chebula* was conducted during 2017-2018 in the laboratory of Department of Forestry and Environmental Science, Manipur University and field related experiments were executed in the nursery of Sadar East Range Forest, Central Forest Division, Manipur, respectively. The nursery is located at 24.83º N latitude and 93.94º E longitudes at an altitude of 810 m above mean sea level. The climate of the study site is sub-tropical in nature. The area receives an annual rainfall of approximately 2439.7 mm, most of which is experienced during June –July months. However, during the study period, the total rainfall was 304.3 mm.

Material collection

The seeds of *T. chebula* was collected from Andro; Imphal East; Manipur during November-December 2017. The fresh fruits of *T. chebula* were collected and sorted manually for defected and healthy fruits. The defected fruits were discarded and healthy ones were graded into three grades viz. small (<1.8 cm), medium (1.8-2.4 cm) and large (> 2.4 cm) sized seeds, respectively.

Pre-sowing treatments

To assess germination behavior and growth of the seedlings, the graded fruits were depulped and subjected to eight different pre-sowing treatments. The details of eight different treatments are given in table 1.

Table 1: Different methods of pre sowing treatments

Code	Treatments
T1	Control
T2	Immersion in ordinary water for 48 hours.
T3	Immersion in ordinary water for 72 hours.
T4	GA_3 of 0.3% for 8 hours followed by rinsing under running tap water.
T5	GA_3 of 0.3% for 16 hours followed by rinsing under running tap water.
T6	GA_3 of 0.3% for 24 hours followed by rinsing under running tap water.
T7	Conc. H_2SO_4 for 20 minutes followed by rinsing under running tap water.
T8	Nicking at broad end then soaking in ordinary water for 36 hours.

Potting mixture

Potting mixture used for raising the seedlings in this experiment consist of soil, sand and FYM in three different proportions as given in table 2.

Table 2: Potting mixture proportion

Code	Description	Ratio
M1	Soil: Sand: FYM	1:1:1
M2	Soil: Sand: FYM	1:2:2
M3	Soil: Sand: FYM	1:2:3

Germination studies

A sample of 120 seeds per treatment was taken for conducting the experiment and was sown in nursery beds.

Germination percent

Germination percentage was calculated by following formulae:

Germination (%) = (No. of seeds germinated / total no. of seeds sown) x 100

Germination capacity

Germination capacity was calculated as the cumulative number of seed germinated at the end of test period plus the number of un-germinated viable seed at the end of the test expressed in percentage (Paul, 1972) [25].

Germination energy

Germinating energy was calculated on the basis of percentage of total number of seeds that had germinated when the germination reached its peak generally taken as the highest number of germination in 24 hours period (Czabator, 1962) [8].

Seedling growth studies

For seedling growth studies, five randomly selected seedlings per replication were carefully uprooted without breaking the roots.

Shoot length (cm)

It was measured from leading shoot tip to the collar region of the seedling at ground level.

Root length (cm)

The length of tap root was recorded by placing it horizontally on the ground.

Collar diameter (cm)

Collar diameter of the seedling was measured by using a caliper.

Seedling length (cm)

Seedling length was recorded by using following formula:

Seedling length = Shoot length + Root length.

Seedling biomass studies

For seedling biomass studies, five randomly selected seedlings per replication were carefully uprooted without breaking the roots.

Fresh seedling weight (g)

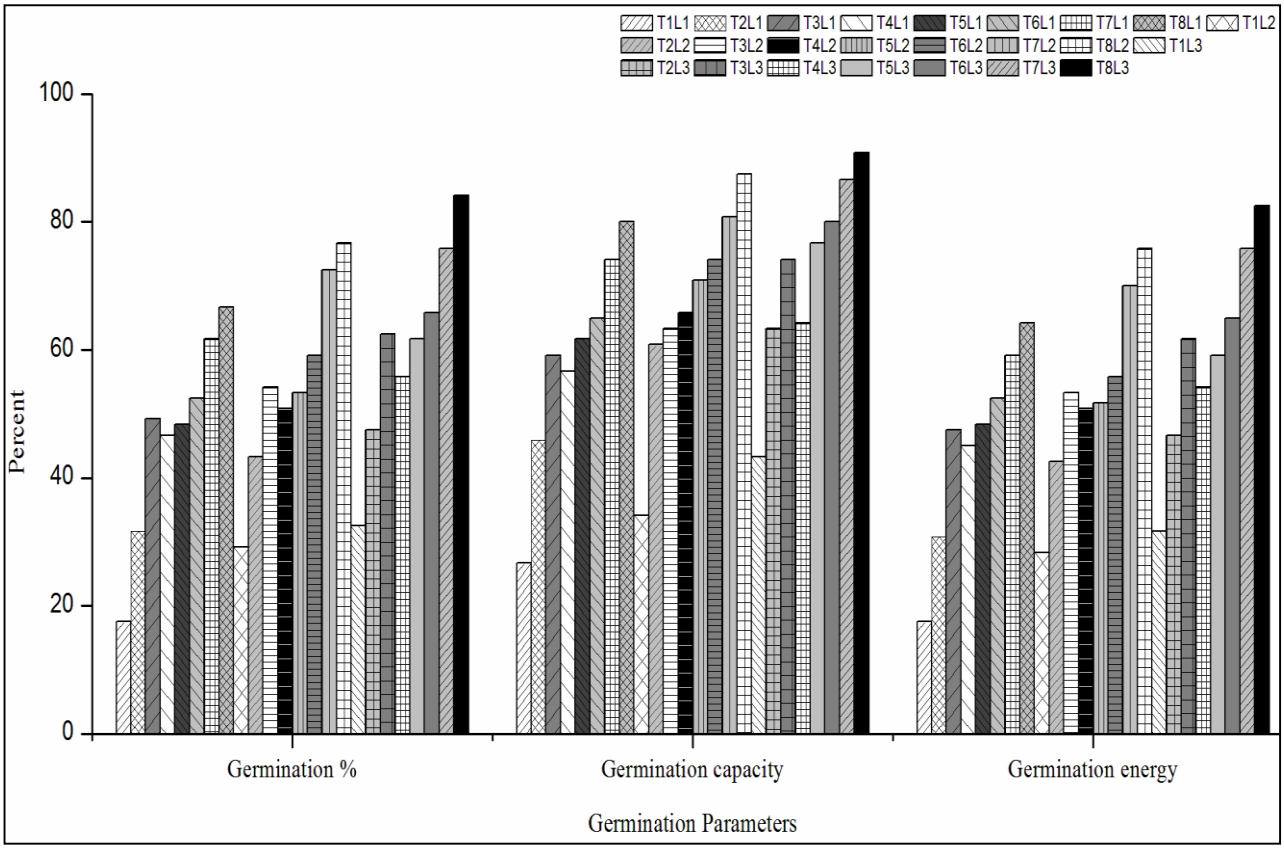
It was calculated as the sum of fresh root weight and fresh shoot weight.

Dry seedling weight (g)
It was calculated as the sum of oven dry root weight and oven dry shoot weight.

Root-shoot ratio
The ratio was worked out on oven dry weight basis by dividing the weight of oven dry shoot by the weight of oven dry root of each plant separately.

Results and Discussion
Effect of seed size and pre-sowing treatments on germination behavior under nursery conditions
A critical review of data in table 3 indicated that among the three seed sizes, large size seeds (L3) were recorded maximum germination percent (60.73%), germination capacity (72.40%) and germination energy (59.59%). Among the pre-sowing treatments, maximum germination percent (75.84%), germination capacity (86.11%) and germination energy (74.17%) were recorded in T8 (Table 3). The combined effect of seed size and pre-sowing treatments had a significant effect on germination behavior (Fig.1). The large size seeds (L3) subjected to nicking at broad end then soaking in ordinary water for 36 hours (T8L3) exhibited significantly maximum germination percent (84.17%), germination capacity (90.83%) and germination energy (82.50%).

The seed size is indicative of food reserve and energy level which give variable performance in germination and seedling growth in many forest species (Baldwin 1942, Kandya 1978, Wood *et al.* 1997) [4, 16, 35]. Greater stocks of food and energy in larger seeds provide more available energy to stimulate germination (Flint and Palmblad, 1978) [12]. The results were also in agreement with the findings of Ajiboye *et al.* (2014) [2] who reported that large sized seeds of *Prosopis africana* and *Dialum guineense* excelled over small size seed for all germination parameters. The results shows that the germination attributes were higher for larger and heavier seeds than for smaller seeds concur with the results of other workers in various species such as *Sapindus mukorossi* (Attri, 2011) [3]; *Eucalyptus citriodora* (Aguiar and Nakane, 1983) [1]; *Albizia lebbeck* (Kumar *et al.*, 2001) [20]; *Acacia Senegal* (Harsh *et al.*, 2004) [13] and *Jatropha curcas* (Singh and Saxena, 2009) [29]. Missanjo *et al.* (2014) [21] reported that nicked seeds exhibited the highest significant (P<0.001) performance for vegetative characteristics of height, root collar diameter, number of leaves and germination percentage compared to other pretreatments in *Acacia polyacantha*. Missanjo *et al.* (2013) [22] also reported that in combination of nicking and large size seeds produced the highest germination (100%) in *Albizia lebbeck* in which similar findings were observed during the study (84.17%). However, the treatments of nicking with medium and small sizes of seeds shows germination percent of 76.67% and 66.67% respectively (Table 4).



L: Seed size category, T: T: Pre sowing treatments

Fig 1: Interaction effect of pre-sowing treatments and seed size on germination behavior under nursery conditions

Effect of seed size and potting mixture on seedlings growth character under nursery conditions
A scrutiny of data in table 4 reflected that among three seed sizes, large size seeds (L3) were recorded maximum shoot length (19.27cm), root length (16.90cm), collar diameter (0.29cm) and seedling length (36.17cm). This may be ascribed to the fact that large size seed contained more nutrient reserve and energy pool which might have stimulated better seedling growth. The results are thus, in agreement with the findings of Singh and Saxena (2009) [29], who reported that

large size seeds produced maximum height and collar diameter in *Jatropha curcas*. Similar growth characters for larger size seeds has been reported by several researchers in many species during the study of shoot length and root length in *Azadirachta indica* (Uniyal *et al.,* 2007) [33], maximum height and collar diameter in *Buchanania lanzan* (Nandeshwar *et al.,* 2005) [23], root length and shoot length in *Hardwickia binata* (Ponnammal *et al.,* 1993) [26], seedling height in *Salvadora persica* and *Jatropha curcas* (Dagar *et al.,* 2004) [9].

Table 3: Effect of seed size and pre-sowing treatments on germination behavior under nursery conditions

Parameters	Germination percent	Germination capacity	Germination energy
Effect of seed size			
L1	46.77	58.65	45.63
L2	54.90	67.19	53.54
L3	60.73	72.40	59.59
Effect of pre sowing treatments			
T1	26.39	34.72	25.83
T2	40.83	56.67	40.00
T3	55.28	65.56	54.17
T4	51.11	62.22	50.00
T5	54.44	69.72	53.06
T6	59.17	73.06	57.78
T7	70.00	80.56	68.33
T8	75.84	86.11	74.17

T: Pre sowing treatments, L: Seed size category

Among the potting mixture, maximum shoot length (18.77cm), root length (16.33cm), collar diameter (0.31cm) and seedling length (35.10cm) were recorded in M3 (Table 4). Thus the increment in growth performance is attributed by macro and micro nutrients supplied by the organic manures applied thereby improving the physio chemical properties in growing media. Bali *et al.* (2013) [5] reported that optimum germination and growth for *Terminallia bellirica* was in silt loam soil + FYM + sunken beds combination. However, Thakur *et al*. (2000) [31] reported sand+soil+FYM as the best potting medium for development of healthy seedlings with nodulated roots and better growth in *Albizia lebbeck*. The combined effect of seed size and potting mixture had a significant effect on seedlings growth characters (Table 5). The maximum shoot length (22.80cm), root length (20.10cm), collar diameter (0.34cm) and seedling length (42.90cm) were observed in M3L3 (Soil:Sand:FYM in the ratio of 1:2:3 with large size seeds) (Table 4). The results find the support from the findings of Suresh *et al.* (2007) [30] who reported that maximum root and shoot length of *Sapindus emerginatus* seedling were seen in large size seeds sown in mixture of sand + soil + humus.

Table 4: Effect of seed size and potting mixture on seedlings growth character under nursery conditions

Parameters	Shoot length (cm)	Root length (cm)	Collar diameter (cm)	Seedling length (cm)
Effect of seed size				
L1	14.00	11.73	0.22	25.73
L2	16.03	13.40	0.26	29.43
L3	19.27	16.90	0.29	36.17
Effect of potting mixture				
M1	14.03	11.23	0.21	25.27
M2	16.50	14.47	0.25	30.97
M3	18.77	16.33	0.31	35.10

L: Seed size category, M: Potting Mixture

Table 5: Interaction effect of potting mixture and seed size on seedlings growth character under nursery conditions

Parameters	Shoot length (cm)	Root length (cm)	Collar diameter (cm)	Seedling length (cm)
M1L1	12.30	9.80	0.18	22.10
M2L1	14.10	12.10	0.21	26.20
M3L1	15.60	13.30	0.27	28.90
M1L2	13.50	10.10	0.21	23.60
M2L2	16.70	14.50	0.25	31.20
M3L2	17.90	15.60	0.31	33.50
M1L3	16.30	13.80	0.23	30.10
M2L3	18.70	16.80	0.29	35.50
M3L3	22.80	20.10	0.34	42.90

L: Seed size category, M: Potting Mixture

Effect of seed size and potting mixture on seedlings biomass production under nursery conditions.

The data in table 6 reveals that maximum fresh seedling weight (6.29g), dry seedling weight (5.15g) and root-shoot ratio (1.03) were recorded in large size seeds (L3). Greater dry weight of seedlings from large size seeds is in accordance with the findings for *Acacia nilotica* and *Albizia lebbek* (Khera *et al.,* 2004) [18], *Prunus jenkinsii* (Upadhaya *et al.,* 2007) [34], *Artocarpus heterophyllus* (Khan, 2004) [17]. Maximum root dry weight in *Parkia biglobosa* and total dry weight in *Albizia lebbeck* (Ebofin *et al.,* 2003) [11] were reported from seeds with large size. Among the potting mixture, maximum fresh seedling weight (6.68g), dry seedling weight (5.32g) and root-shoot ratio (1.02) were recorded in M3 (Table 6). Maximum dry seedling weight was seen in growing media consisting of soil+sand+FYM (2:1:1) for *Mangifera indica* seedlings (Parasana *et al.,* 2013) [24]. Chand *et al.* (2007) [7] also reported that *Terminalia tomentosa* seeds sown in soil medium consisting of soil, sand and FYM in the ratio of 2:1:1 resulted in maximum dry weight of shoot and total dry weight of seedlings. But from the experimental findings *T. chebula* did not behave in a similar manner as maximum seedlings biomass production was seen in M3 (Soil: Sand: FYM-1:2:3). In the interaction between seed size and potting mixture, maximum fresh seedling weight (7g), dry seedling weight (5.42g) and root-shoot ratio (0.98) were recorded in M3L3 (Table 7). The results find the support from the findings of Suresh *et al.* (2007) [30] who reported maximum dry root and shoot weight of *Sapindus emerginatus* seedling in large size seeds sown in mixture of sand + soil + humus. However, the study suggested that the most favourable potting mixture for *T. chebula* was found to be M3L3 (Soil: Sand: FYM in the ratio of 1:2:3 with large seed size).

Table 6: Effect of seed size and potting mixture on seedlings biomass production under nursery conditions

Parameters	Fresh seedling weight (g)	Dry seedling weight (g)	Root-shoot ratio
Effect of seed size			
L1	5.79	4.99	1.07
L2	6.08	5.06	1.00
L3	6.29	5.15	1.03
Effect of potting mixture			
M1	5.38	4.82	1.06
M2	6.09	5.06	1.03
M3	6.68	5.32	1.02

L: Seed size category, M: Potting Mixture

Table 7: Interaction effect of potting mixture and seed size on seedlings biomass production under nursery conditions

Parameters	Fresh seedling weight (g)	Dry seedling weight (g)	Root-shoot ratio
M1L1	5.26	4.80	1.07
M2L1	5.76	5.00	1.06
M3L1	6.35	5.17	1.07
M1L2	5.32	4.80	1.05
M2L2	6.21	5.02	0.95
M3L2	6.70	5.37	1.02
M1L3	5.57	4.86	1.04
M2L3	6.31	5.16	1.07
M3L3	7.00	5.42	0.98

L: Seed size category, M: Potting Mixture

Conclusion

The study suggests that the seeds that did nicking at broad end along with soaking in ordinary water for 36 hours outclassed all other treatments for all germination parameters under nursery condition. Further, the combinations of large sized seeds with nicking at broad end accompanied by soaking in water for 36 hours produced significantly maximum values in all germination attributes under nursery condition. The seedlings that are grown in potting mixture of soil, sand and farmyard manure in the ratio of 1:2:3 showed significantly better growth and biomass production than other potting mixtures used. Experiments on the combinations between different seed sized and different potting medium revealed that seedlings that raised from large sized seeds grown in potting medium of soil, sand and farmyard manure in the ratio of 1:2:3 produced significantly better growth and biomass production than other treatment combinations.

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