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Effects of Seed Treatments on Germination and Seedling Growth Attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery

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Abstract: The study was conducted over a period of six months from January to June 2004 to explore the effects of different seed treatments on germination and seedling growth attributes of a popular medicinal plant, Horitoki (*Terminalia chebula* Retz) in the nursery of Chittagong University, Bangladesh. Seeds were subjected to six pre-sowing treatments *e.g.* control (T_0), depulped (T_1), depulped and soaked in cold water for 12 hours (T_2), depulped and soaked in cold water for 24 hours (T_3), depulped and soaked in cold water for 48 hours (T_4) and depulped and soaked in hot water for 2 minutes (T_5). The study revealed that depulping the fruits and soaking in water for various periods significantly enhanced seed germination and seedling growth. Seed germination started 29 days after sowing and continued up to 86 days. The highest germination percentage (66.7%) was observed in the fruit depulped and soaked in cold water for 48 hours (T_4) followed by 60% in the depulped seeds soaked in cold water for 24 hours (T_3). The lowest germination percentage (48.9%) was obtained from controlled seeds (T_0). The highest germination value (4.41), germination energy (58.9) and vigor index (5291) was also obtained in T_4, which was significantly ($p<0.05$) different from the control and other treatments. Shoot length, root length, collar diameter and leaf number followed the same trend of higher value for T_4, T_5 and T_3 respectively. Similar trend was also observed in shoot, root and total seedling dry weight. Therefore, pre-sowing treatment, T_4 (depulped seeds soaking in cold water for 48 hours) was more effectivein germination and production of quality seedling of *T. chebula* in the nursery.

Key words: Depulping, germination value, imbibition period, *Terminalia chebula* Retz., vigor index

INTRODUCTION

Plants have been playing an important role in the health care from the time immemorial. At present, more than 10,000 plant species are being used for medicinal purposes. Bangladesh is a country reported as storehouse of plants; about 37% of which have medicinal uses[15]. Although Bangladesh Forest Research Institute enlisted 220 species of medicinal plants in Bangladesh[3], Yusuf *et al.* reported that the number was 546 [23]. Among the species, Horitoki (*Terminalia chebula* Retz.) is considered as an important medicinal plant in the country.

Terminalia chebula Retz., locally known as Horitoki, belongs to Family Combretaceae, is a medium to large deciduous tree with short cylindrical bole, rounded and spreading crown. It is found in deciduous forests throughout the greater part of India, Myanmar, Sri Lanka, Pakistan and Bangladesh. In Bangladesh the species occurs naturally in the deciduous Sal forests[5] and in the hill forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet[6]. Fruit of *T. chebula* is a drupe, about 3 cm long, ellipsoidal, yellow to orange brown, glabrous, shining hard when ripe, stone very is thick, bony and rough[6,17]. The fruits are used for medicinal purposes in combination with *Emblica officinalis* and *Terminalia belerica*, under the name of "Triphola". Ayurvedic recipe 'Triphola' is successfully applied in a variety of ailments like constipation, acidity and even in eye troubles[2]. The fruit extract is said to remove toxins and various other undesirable accumulations from the body to improve digestion and assimilation. It is also beneficial for chronic lung diseases, skin diseases, eye disorders and conditions with raised cholesterol levels[12]. The timber is very hard, fairly durable and used for building constructions, agricultural implements, carts, furniture and other purposes[17].

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T. chebula is widely used for medicinal and other purposes in Bangladesh. However, people don't get interest in raising the seedlings of the species in nursery due to low germination percentage (around 50%) and more average time (up to 2-3 months) requirement for seed germination[17]. Low germination percentage as well as long time requirement is believed due to the hard seed coat and thick fleshy pulp of fruits. A considerable body of evidences suggested that germination of seeds with hard seed coat is enhanced by pre-sowing treatments[8,13,20,21]. If untreated, the drupes germinate slowly and irregularly[11]. This delayed and irregular germination of seeds in the nursery is a serious constraint of efficient nursery management and plantation establishment. Therefore much research have been carried out to develop effective seed treatments to remove the dormancy as well as to break the hard seed coat of different species in order to ensure faster and maximum germination[16,18,21]. However, literatures, which have examined the effect of seed treatments of *T. chebula,* are very scare. So, under the present circumstances the research work has been designed to explore the seed germination period, germination percentage and initial growth performance of *T. chebula* under a number of easily applicable low cost pre-sowing treatments.

MATERIALS AND METHODS

Study site and growing media: The present study was conducted over a period of six months from January to June 2004 in the nursery of Chittagong University, Bangladesh (lies approximately at the intersection of 91°50´E longitude and 22°30´N latitude). The soils used in the experiments were collected from the forest areas of the University Campus. Soils were sieved well (<3mm) and mixed with decomposed cow dung in a ratio of 3:1 and filled in polybags of 15 x 10 cm size.

Seed collection: Fruits of *T. chebula* were collected from the seed orchard division of Bangladesh Forest Research Institute (BFRI), Chittagong. The fruit of *T. chebula* is drupe and hence only one seed is present in each fruit[6]. All fruits were dried in sunlight and stored in airtight container till the treatments were applied. Uniform seeds were used for the treatments to reduce non-treatments variation since germination percentage and seedling vigor was found positively correlated with seed size[4,10].

Experimental design and treatment combinations: A Randomized Complete Block Design (RCBD) was adopted for the study. There were six treatments including control and 3 replications for each treatment. For the purpose

Fig. 1: Intact fruits (left) and depulped seeds (right) of *T. chebula*.

some seeds were depulped at two ends with sharp knife in such a way that the embryo was not damaged while others were kept intact for using as control (Figure 1). The pre-sowing treatments used in the experiment were:

T_0: control (intact fruits without depulping and soaking)
T_1: fruits were depulped but were not soaked in water
T_2: fruits were depulped and soaked in cold water for 12hours
T_3: fruits were depulped and soaked in cold water for 24hours
T_4: fruits were depulped and soaked in cold water for 48hours and
T_5: fruits were depulped and soaked in hot water (80°C to 100° C) for 2 minutes and were immediately washed in cold water.

Fifty seeds were tested for each replication (one seed in each polybag) to explore the effect of pre-sowing treatments on germination. So, a total of 150 seeds were used in each treatment. After germination, seedlings were allowed for growing to assess initial growth performance.

Assessment of seed germination pattern and percentage: The effects of pre-sowing treatments were assessed periodically by counting germinated seeds. The germination was recorded daily from the date of sowing and continued till the germination ceased. The seed germination criterion was visible protrusion on the surface of soil at least 0.5 cm of the cotyledon and hypocotyle of the seedlings. Daily germination percentages were summed up to obtain cumulative germination percentage for each pre-sowing treatment on each assessment date. Then seedlings were allowed to grow altogether under same environmental condition for three months.

Germination phase, germination energy and germination value: The imbibition period (number of days from sowing

to commencement of germination) was recorded. The germination energy, defined as the germination percentages when the mean daily germination (cumulative germination percent divided by the time elapsed since sowing date) reached its peak, was also determined. Germination energy is also a measure of the speed of germination and hence, it was assumed as a measure of the vigor of seedling it produced. In addition, germination value (GV) which is a composite value that combines both germination speed and total germination provides an objective means of evaluating the results of germination test was calculated using the formula of Djavanshir and Pourbeik[7] :

GV= (3DGs/N) *GP/10

Where,

GV = Germination value

GP = Germination percentage at the end of the test

DG = Daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing

3DGs = The total germination obtained by adding every DGs value obtained from the daily counts.

N = The total number of daily counts, starting from the date of first germination

10 = Constant

Growth performance: At the end of the experiment, all seedlings were measured for total height and collar diameter. Total number of leaf in each seedling was also counted. Five seedlings from each replication were randomly selected and uprooted very carefully to estimate the seedling biomass. The uprooted seedlings were then separated into leaves, shoot and root components and were dried in electric oven at 70°C until the constant weight was obtained for studying biomass productions in different pre sowing treatments.

Seedling Vigor Index: Vigor Index of the seedlings was calculated according to Abdul-Baki and Anderson (1973) as germination percent X seedling total length i.e. total shoot and root length[1].

Statistical Analysis: Data were statistically analyzed by using computer software Microsoft Excel and SPSS ver.10.00 to explore possible treatment variations. The Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were also used for the analysis.

RESULTS AND DISCUSSIONS

Seed Germination:

Germination period: Seed germination started 29 days after sowing and continued up to 86 days. Different treatments significantly affected the germination period for the species. The fastest germination *e.g.* least imbibition period (29 days) was observed in T_4 and delayed germination, *e.g.* highest imbibition period (45 days) was found in T_0 (Table 1).

Germination percentage: The highest germination percentage (66.7) was observed in T_4 (Depulped seeds soaked in cold water for 48 hours) followed by T_3 (60), which was significantly higher than control (T_0) and T_5. The lowest germination percentage (48.9) was recorded from control treatment (Table 1).

Germination value and germination energy: Germination value varied from 0.91 to 4.41 among the treatments. The highest germination value was found in T_4 (4.41) followed by T_1 and lowest was in T_5. Similarly, the highest germination energy (58.9%) was in T_4 followed by 53.3% in T_2 and lowest (41.1) was in T_5 (Table 1).

Germination pattern: Mean daily germination percent varied in different days in different treatments for *T. chebula* seeds. The highest mean daily germination percentage was observed 52 days after sowing in T_1 and T_3, 55 days in T_2, 57 days in T_4 and 86 days in T_0 and T_5 (Figure 2 and figure 3). Seed germination started 29 days after sowing and continued up to 86 days (Figure 4). The cumulative germination percent in treatment T_4 rose sharply from 28 day to 64 days after sowing the seeds and remained constant up to the end of germination test (90 days).

Fig. 2: Intact fruit (left) and germination of depulped seed (right) of *T. chebula* 50 days after sowing in the polybags.

Table 1: Imbibition period, germination period, germination percentage, germination value and germination energy of *T. chebula* seeds under different pre-sowing treatments.

Variables	Treatments					
	T_0	T_1	T_2	T_3	T_4	T_5
Imbibition period (day)	44.67 a* ± 2.02	37.0 b ± 0.0	34.0 bc ± 0.0	31.0 cd ± 0.0	29.0 d ± 1.0	42.33 a ± 2.67
Total germination Period (day)	86.0 a ± 0.0	53.66 d ± 1.67	64.0 c ± 0.0	67.0 bc ± 3.0	74.0 b ± 5.0	86.0 a ± 0.0
Germination (%)	48.9 b ± 2.22	56.7 ab ± 5.77	58.9 ab ± 4.0	60.0 ab ± 1.92	66.7 a ± 2.02	50.1 b ± 6.75
Germination Value	1.65	4.21	4.14	3.98	4.41	0.91
Germination Energy (%)	48.9	53.0	53.3	50.0	58.9	41.1

Note: * _ Means followed by the same letter (s) are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).
± indicates the standard error of mean.

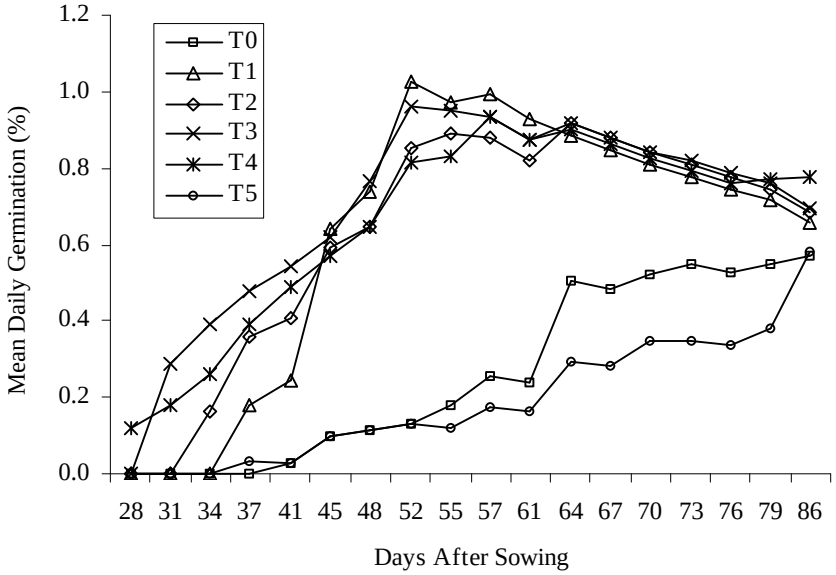

Fig. 3: Mean daily germination percent of *T. chebula* seeds under different treatments.

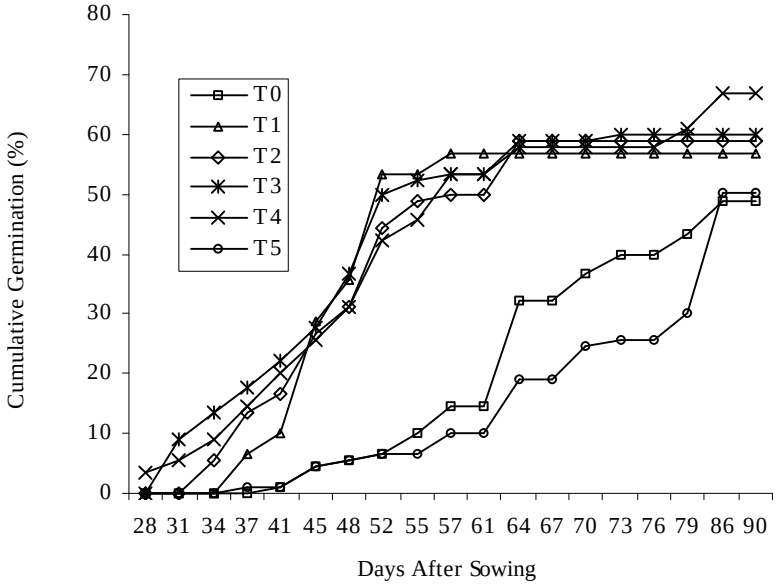

Fig. 4: Cumulative germination percent of *T. chebula* seeds under different treatments.

Table 2: Shoot length, root length, total length, number of leaf, collar diameter and vigor index of *T. chebula* seedlings grown under different treatments five months after sowing the seeds in polybags.

Variables	Treatments					
	T_0	T_1	T_2	T_3	T_4	T_5
Shoot length (cm)	14.67 c* ± 2.42	33.17ab ± 2.04	28.67bc ± 3.21	31.67 ab ± 4.37	38.67 a ± 4.25	32.67ab ± 1.33
Root length (cm)	26 b ± 1.52	30.66ab ± 6.35	28.17 b ± 0.92	30 b ± 1.52	40.67 a ± 1.45	32.67ab ± 3.52
Total length (cm)	40.67 c ± 2.49	63.83 b ± 8.39	56.83c ± 2.84	61.66 b ± 2.96	79.33 a ± 3.17	65.33ab ± 4.8
Leaf number.	24 b ± 3.21	51.67 a ± 2.84	50.33 ab ± 13.67	43.67 ab ± 3.17	53.66 a ± 6.43	47.33ab ± 9.49
Collar diameter (mm)	3.49 b ± 0.53	5.03 a ± 0.17	4.19 ab ± 0.6	4.83 a ± 0.2	5.41 a ± 0.17	5.57 a ± 0.1
Vigor Index	1989 c ± 5.53	3619 b ± 48.41	3347 b ± 11.36	3699 b ± 5.68	5291 a ± 6.40	3273 b ± 32.4

Note: * _ Means followed by the same letter (s) are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).
± indicates the standard error of mean

Growth performance: Shoot length of the seedlings developed under treatments was highest (38.67 cm) in T_4 followed by T_1, which was significantly higher than that of control and T_2 (Table 2). Mean root length of the seedlings was found highest (40.67 cm) in T_4 followed by T_5 and T_1 treatments. Highest collar diameter of *T. chebula* seedlings was found in T_5 (5.57 mm) followed by T_4 and lowest was in T_0. The leaf number significantly varied due to the treatments and found highest in T_4 (53.66) followed by T_1 and the lowest (24) was in control. Vigor index was highest (5291) in T_4, which was significantly higher than that of other treatments (Table 2 and Fig. 5).

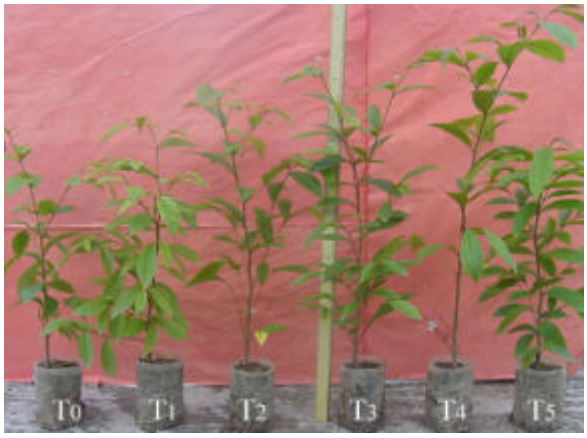

Fig. 5: Growth performance of *T. chebula* seedlings grown under different treatments five months after sowing the seeds in polybags.

Seedling biomass production: Leaf dry weight and shoot dry weight was highest (2.26 g and 1.53 g respectively) in T_4 while root dry weight was maximum (0.9 g) in T_5 (Table 3). However the total biomass per seedling was highest (4.61 g) in T_4 that was significantly different from control. The lowest leaf dry weight, shoot dry weight, root dry weight and total dry weight were observed in the T_0 (intact fruits without any treatment).

Generally the Legume seeds with hard seed coat were reported to enhance germination with pre-sowing treatments[8,9,13,14,21,21]. If untreated, the drupes germinate slowly and irregularly[11]. However, the findings of the present study show that depulped seeds of Horitoki (*T. chebula*) soaked in cold-water increased germination speed, germination percentage and seedling growth and biomass production in comparison to the control treatments. Jackson described that seeds soaking in water for 48 hours improved germination[11]. Luna described that fermentation of seed for three weeks by removing fruits' pulp and placing the seeds in between layers of straw in a tray having perforations at the bottom gives about 60% germination[17]. Again, clipping the seeds at the broad end in such a way that the embryo is not damaged, and such seeds are soaked in cold water for about 36 hours and sown in nursery beds under shade, provides about 80% germination. The results of the present study also supported by the findings of the many authors. For example Rashid *et al.* has shown that, whole fruits of *T. chebula*, pre-treated by soaking in water for 48 hours with successive treatment by 10 % sulfuric acid for 20 minutes showed up to 70 % germination[22]. A germination success of up to 50% was obtained when clean seeds (removing the dry pulp) were sown at BFRI[2]. Nainar *et al.* has shown that among the seed pre-treatments including mechanical scarification (MS), hot water treatment (with or without removing the testa) and sulfuric acid treatment (with or without breaking the testa), mechanical scarification gave the highest germination percentage (60%) in *T. chebula*[19].

Conclusion: For establishing nursery of a particular species for producing maximum number of quality seedlings with minimum cost, time and labor, the seed pre-treatments are required. Since the seed coat of *T. chebula*

Table 3: Leaf dry weight, shoot dry weight, root dry weight and total dry weight of *T. chebula* seedlings three months after germination.

Variables	Treatments					
	T_0	T_1	T_2	T_3	T_4	T_5
Leaf dry weight (g)	1.02 b* ± 0.25	1.96 ab ± 0.26	1.94 ab ± 0.65	2.16 a ± 0.14	2.26 ab ± 0.17	1.9 a ± 0.1
Shoot dry weight (g)	0.39 b ± 0.15	1.023 a ± 0.18	0.93 ab ± 0.34	1.27 a ± 0.043	1.53 a ± 0.19	1.36 a ± 0.07
Root dry weight (g)	0.36 b ± 0.14	0.60 ab ± 0.1	0.49 ab ± 0.23	0.55 ab ± 0.13	0.82 a ± 0.026	0.9 a ± 0.07
Total dry weight (g)	1.77 b ± 0.55	3.58 ab ± 0.55	3.37 ab ± 1.23	3.98 a ± 0.22	4.61 a ± 0.36	4.16 a ± 0.2

Note: * _ Means followed by the same letter (s) are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).
± indicates the standard error of mean

is hard, it takes more time to germinate with lower germination percentage in nursery establishment. The present study of pre-sowing treatments of seeds would prove itself potential in the practical fields. Among the treatments applied in the experiment for *T. chebula*, fruit depulping at two ends and soaking in cold water for 48 hours was found more effective in respect to faster germination, higher germination percentage, seedling growth, biomass production and seedling vigor index in comparison to control and other treatments. Since, depulping of fruits and soaking in cold-water for a particular period of time is easily applicable and cost effective, the treatment may be recommended for large-scale seedling production in the nurseries. However, depulping of dry fruits with knife is difficult and troublesome. Therefore depulping the fresh fruits by rotting in water may be an important aspect of future study to avoid the difficulties of depulping the dry fruits for better germination.

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