Effect of Pre-sowing Treatments on Germination and Initial Growth of *Terminalia citrina*: A Medicinal Tree Species in Bangladesh

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Abstract

Terminalia citrina (Gaertn.) Roxb. *ex*. Fleming (local name-Hatiyal) is an important medicinal tree species naturally grown in Sal and hill forests of Bangladesh. An experiment was conducted at the Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh, to find out the effects of pre-sowing treatments on germination and vigor seedling production capability. Seeds were treated with six pre-sowing treatments, e.g. T_0-seeds without any treatment (Control), T_1-seeds soaking in normal water for 24 hours and sown in polybags (24º C), T_2-seeds soaking in normal water for 48 hours and sown in polybags (24º C), T_3-fruits sown in seedbed, T_4-seeds soaking in hot water for 1 minute and sown in propagator house (80º C), and T_5-seeds sown in propagator house. Highest germination percentage (95.83%), germination energy (37.5%), and germination value (1.0506) were found in T_5 treatment (seed sown in propagator house) and significantly (p<0.05) different from other treatments. Collar diameter and leaf number were recorded after three and four months of seed germination. After 3 months of the last germination, maximum shoot height (46.6 cm) was revealed in T_2 (seeds immersed in normal water for 48 hours and sown in polybags) treatment. Collar diameter (6.02 mm) and leaf number (12.6) were recorded highest in T_2 treatment. The lowest diameter (4.23 mm) and leaf number (5.8) were found in T_4 treatment (seeds soaking in hot water for 1 minute and sown in propagator house). Finally, seeds sown in the propagator house (sand media) revealed comparatively better germination behaviour but low growth performance. Seeds treated with normal water for 48 hours treatment revealed appropriate for vigour and quality seedlings production for *T. citrina*.

Keywords: Treatments, germination, vigour, seedlings, medicinal

1. Introduction

Medicinal plants are the life-saving elements of forest products and play a significant role in the health care of rural people all over the world. They offer essential raw materials for the production of conventional and modern medicines and important therapeutic agents (Ghani, 2003; Islam et al., 2016). As stated by World Health Organization (WHO), there are almost 21,000 medicinal plant species all over the world (Penso, 1980). In Bangladesh, around 500 plant species including trees, herbs, and shrubs used as medicinal plants because of their therapeutic properties (Ghani, 2000). In another study, Yusuf et al. (2009) documented 747 plant species have therapeutic properties in Bangladesh.

Terminalia citrina (Gaertn.) Roxb. ex. Fleming, belonging to Combretaceae family, a valuable medicinal tree of Bangladesh. The medium to large sized deciduous tree having very young shoots are shining, rusty hairy, soon glabrate. Bark light grey, vertically fissured, exfoliating in large flakes, inner bark light yellow, turning brown. Leaves sub-opposite, elliptic to oblong-lanceolate.

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Flowers small, dull-yellow, sessile in terminal spikes. Fruit a drupe, oblong-lanceolate, about 5 cm long, smooth, orange-yellow when ripe, obscurely 5-cornered when dry (Das and Alam, 2001). It is originated in deciduous forests throughout the greater part of India, Myanmar, Sri Lanka, Pakistan, and Bangladesh (Hossain et al., 2005). In Bangladesh, the species occurs naturally in the forests of Sylhet, Chattogram, Chittagong Hill Tracts, Dhaka, Mymensingh and also planted as avenue tree (Das and Alam, 2001). The fruits are used for medicinal purposes in combination with *Emblica officinalis* and *Terminalia bellirica*, known as the name of "Triphola". The Ayurvedic recipe 'Triphola' is effectively and widely used in various ailments such as skin diseases, eye disorders, constipation, chronic lung disease, acidity, digestion, and assimilation (Kanjilal et al., 1984; Ara et al., 1997). Seeds are used in stomach aches and intestinal diseases (Lev and Amar, 2000). The plants also used in asthma, diarrhea, anemia, arthritis, cough, cardiac disease, infections, hepatomegaly (Soe and Ngwe, 2004). The timber is very hard, fairly durable, and used for building structures, furniture, agricultural implements, carts, and other purposes. Because of the hard seed coat and thick fleshy pulp, germination percentage of seeds is low (<50%) and requires more time to germinate (up to 2-3 months) (Luna, 1996). Suitable nursery and plantation techniques, management systems of the species are pre-requisite to make a plantation program fruitful. Lately, interest in producing quality seedlings by application of upgraded and modern nursery techniques has augmented (Gera and Ginwal, 2002). In the case of region-specific biodiversity conservation and restoration plans, integrated information of the seed collection, storage, seed germination requirements, and seedlings growth performance of native tree species are crucial (Khurana and Singh, 2001; Smith et al., 2008), but most of the studies are concentrated on fast-growing species only. The selection of appropriate pre-sowing treatment is vital for quick and maximum seed germination (Thapa and Gautam, 2006). Hard coated seeds need more time to germinate and thus, direct sowing is not effective (Anon., 1972). Proper pre-sowing treatments of seeds can stimulate germination time and germination process (Azad et al., 2006; Azad et al., 2011; Azad et al., 2012). The effect of pre-sowing treatments on seed germination of a few tropical forest tree species have been informed by several authors (Khan et al., 2001; Haider et al., 2014; Nandi et al., 2020 and Dey et al., 2020). Therefore, an endeavour has been made to study the effect of pre-sowing treatments on seed germination to identify appropriate pre-sowing treatment for *Terminalia citrina*.

2. Materials and Methods

2.1 Study site

The study was carried out in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Chattogram (lies between 91°50´E longitude and 22°30´N latitude) (Hossain et al., 2005) (Figure 01). The climate is tropical monsoon with an average monthly highest temperature of 29.75° C and a monthly lowest of 21.24° C. The maximum temperature usually occurs in May at 32.60° C and the minimum in January at 14.10° C (Peel et al., 2007).

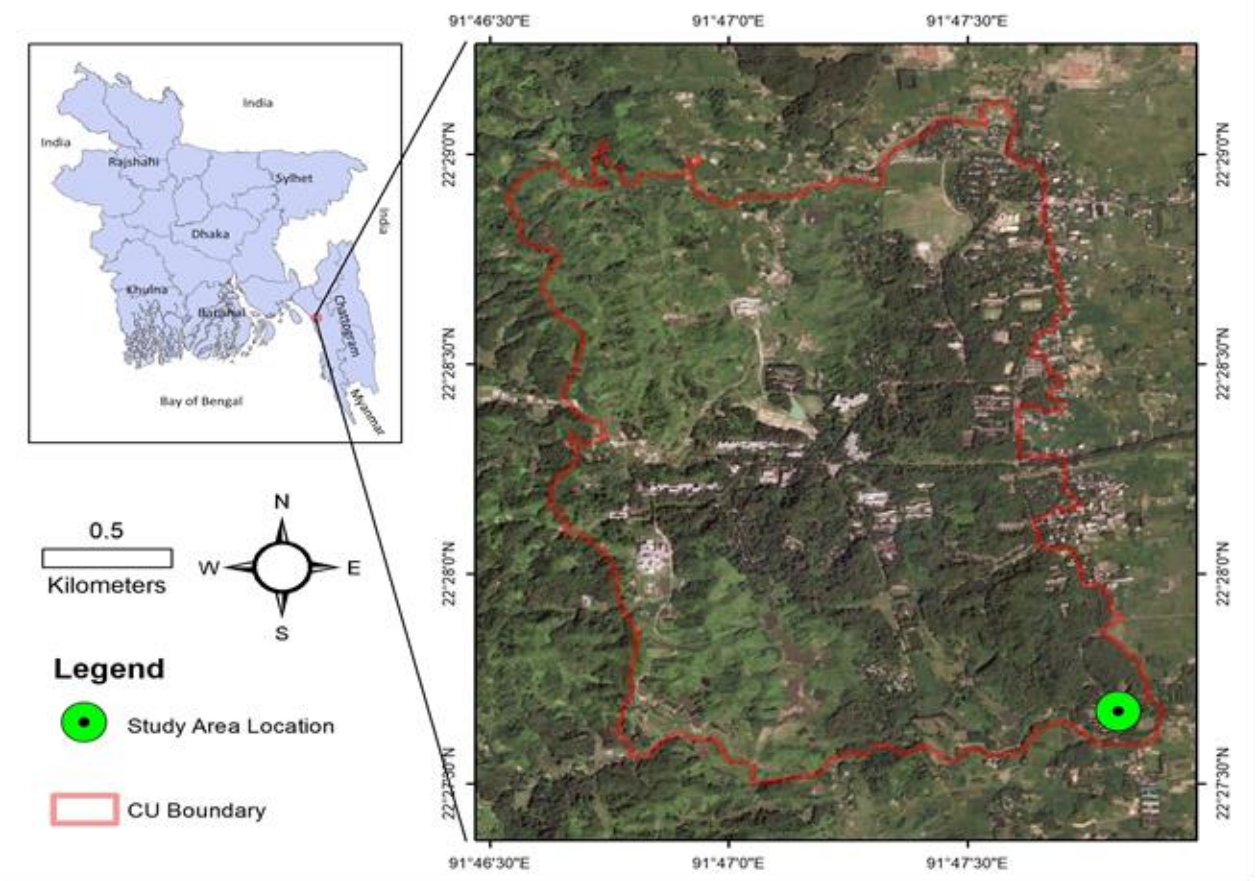

Figure 01. Map showing the location of University of Chittagong in Bangladesh

2.2 Seed collection

T. citrina fruits were collected from Kalurghat Forest Depot Area, Chattogram, Bangladesh during February 2019. Mature seeds were collected from selected plus trees. Seeds were extracted manually from mature fruits by depulping method. Then dried in the open sun for three days. Randomly selected seeds and fruits information like length, width and weight was recorded. Only healthy seeds were used for the experiment.

2.3 Experimental design

The soil used for filling polybags were collected from the forest floor, dried and sieved well (<3 mm) and mixed with decomposed cow dung in a ratio of 3:1. 15×10 cm size polybags were used for the experiment. The media used in the propagator house were fine Sylhet sand. Forest topsoil was used in the open nursery bed. The study was made up of 6 treatments (including control) with 3 replications (15 seed per replication) in a Randomized Complete Block Design. Forty-five (45) healthy seeds were chosen randomly for each treatment. Daily germination progress was recorded as soon as the seeds start germination. Seedlings raised in open nursery bed and propagator house were transferred to polybag after one month of the last germination of seeds. The pricked-out seedlings were kept in shade for 2 weeks and then transferred to sunlight. Proper care and maintenance were done regularly. At the end of two and three months of seed germination, three vigor seedlings from each replication were selected for measuring shoot height, collar diameter and leaf number of the seedlings.

The pre-sowing treatments are as:
T_0-Seeds without any treatment (Control)
T_1-Seeds soaking in normal water for 24 hours and sown in polybags (24º C)
T_2-Seeds soaking in normal water for 48 hours and sown in polybags (24º C)

T_3-Fruits sown in seedbed
T_4-Seeds soaking in hot water for 1 minute and sown in propagator house (80º C)
T_5-Seeds sown in propagator house

Figure 02. Fresh fruits of Terminalia citrina

Figure 03. Dry fruits of T. citrina

Figure 04. A seedling of T. citrina

Figure 05. *T. citrina* seedlings raised in polybag

2.4 Data collection and analysis
2.4.1 Germination percentage

The number of seeds out of 100 seeds from the starting of germination to the termination of germination (Kumar, 1999).

$$\text{Germination \% (GP)} = \frac{\text{No of seed germinated}}{\text{No. of seed sown}} \times 100 \quad (1)$$

2.4.2 Cumulative germination % (CGP)

It assessed at the end of seed germination by summed up daily germination (Hasnat et al., 2019).

$$\text{CGP} = \frac{\text{Cumulative number of seeds germinated}}{\text{Number of seeds sown}} \times 100 \quad (2)$$

2.4.3 Germination energy (GE)

It is measured by computing the daily germination percentage of its peak time (Dwivedi, 1993).
.4.4 Germination index (GI)

According to AOSA (1983), GI was calculated using this formula.

$$\text{GI} = \frac{\text{No.of germinated seeds}}{\text{Days of first count}} + \ldots + \frac{\text{No.of germinated seeds}}{\text{Days of first count}} \quad (3)$$

2.4.5 Mean germination time (MGT)

It calculates the rate and the time-spread of germination (Bewley et al., 2013; Soltani et al., 2015) and it should determine the time to half of the germination. The formula; MGT= $\Sigma Dn/\Sigma n$, (4) where, D=the number of days counted from the starting of germination, n=the number of seeds that were germinated on day D (Ellis and Roberts, 1981; Afzal et al., 2005).

2.4.6 Germination Uniformity (GU)

It was calculated by using the formula.

$$GU=\frac{\Sigma n}{(\Sigma(Fn-t)\text{^}2\times n)} \quad (5)$$

where, t is the time in days, beginning from day 0, the day of germination, and n is the number of seeds germinated at t and F are alike to MGT (Abdolahi et al., 2012).

2.4.7 Germination value (GV)

It was calculated by multiplication of the peak value of germination and mean daily germination (Hasnat et al., 2019).

GV=Peak value of germination×mean daily germination (6)

2.4.8 Germination capacity

It is the percentage of seeds germinated in an experiment from the starting to end. It was classified as follows: a) 90-100%-very good, b) 70-90%-good, c) 50-70%-average, d) 30-50%-poor, e) 20-30%-very poor, and f) less than 10%-extremely poor (Kumar, 1999).

2.5 Statistical analysis

All the recorded data were analyzed statistically by using computer package software SPSS ver. 23. Duncan's Multiple Range Test (DMRT) was employed to define the statistical significance and it was shown by different letters in different tables.

3. Results

3.1 Morphological features of seeds

The average length and width of fruits were found 2.412±0.051 cm and 1.054±0.013 cm respectively. Around 714 fruits were found per kg. The average length and width of seeds were 1.732±0.089 cm and 0.784±0.02 cm respectively. Around 2,173 seeds were found per kg (Table 01).

Table 01. Seed length, width and number of seeds per kg of *T. citrina*

	Length (cm)	Width (cm)	Weight/seed (g)	Number/kg
Fruit	2.412±0.051	1.054±0.013	1.4±0.045	714
Seed	1.732±0.089	0.784±0.02	0.46±0.02	2,173

The results indicated that three tree species had variable effects on the soil composition and it varied by the depth of the soil layer (Table 01). The data were expressed as the mean for soil depth, sampling site, and type of species.

3.2 Germination performance

The germination behaviour of *T. citrina* seeds was affected by different pre-sowing treatments in this study. Seed germination starts first in T_5 and T_4 (42th day) after the seeds was sown and T_3 required maximum time (47th day) to initiate germination.

Maximum germination percentage (95.83%) was recorded in T_5 (seeds sown in propagator house) followed by 75% in T_4 (seeds soaking in hot water for 1 minute and sown in propagator house), 58.3% in T_2 (seeds soaking in normal water for 48 hours and sown in polybags), and 33.3% in T_0 (control). Germination percentage was lowest (16.7%) in T_3 and significantly (p<0.05) different from other treatments. The minimum germination period (43.3 days) was found in T_4, T_5 respectively and maximum

germination period (54.3 days) was recorded in T_0 (Table 02).

Table 02. Germination response of *T. citrina* seeds in different pre-sowing treatments

Treatments	Germination start after (days)	Germination end after (days)	Germination period (days)	Germination (%)	Germination capacity
T_0	43	59	54.33 a*	33.33 c	Poor
T_1	44	72	52.00 a	20.83 c	Very poor
T_2	44	71	50.00 ab	58.33 b	Average
T_3	47	56	52.23 a	16.67 c	Extremely poor
T_4	42	57	43.33 b	75.00 b	Average
T_5	42	59	43.33 b	95.83 a	Very good

*Means followed by the same letter(s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

The maximum germination index (0.5569) was recorded in T_5 and significantly ($p<0.05$) different from other treatments. Highest germination energy (37.5%) was found in T_5 and lowest (16.7%) in T_1 and T_3. The germination uniformity revealed no significant difference among the treatments. Maximum germination value (1.0506) was recorded in T_5 which is significantly ($p<0.05$) different from other treatments and minimum (0.0387) in T_3. Mean germination time was maximum (56.67) in T_1, followed by T_2 (55.43) and lowest in T_5 but MGT showed no significant difference among the treatments (Table 03).

Table 03. Germination response of T. citrina seeds in different pre-sowing treatments

Treatments	Germination Energy (%)	Germination Index (GI)	Mean Germination Time (MGT)	Germination Uniformity (GU)	Germination value
T_0	20.83 ab*	0.0514 b	52.89 a	0.0007 a	0.1299 c
T_1	16.67 b	0.02999 b	56.67 a	0.0007 a	0.0477 c
T_2	25.00 ab	0.0889 ab	55.43 a	0.0012 a	0.3428 c
T_3	16.67 b	0.0259 b	51.67 a	0.0013 a	0.0387 c
T_4	29.17 ab	0.1302 ab	46.78 a	0.0013 a	0.7080 b
T_5	37.50 a	0.5569 a	46.73 a	0.0011 a	1.0506 a

*Means followed by the same letter(s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

3.3 Mean cumulative germination percentage

To obtain cumulative germination percentage for each treatment, daily germination percentages were summed. The cumulative germination of T_5 treatment starts after 42 days of seed sown and rose rapidly and continued germination up to 96 % within 60 days. In T_0 treatment, germination starts at 43rd day and reached 33.3% gradually. T_3 showed lowest cumulative germination percentage (16.7 %) (Figure 06).

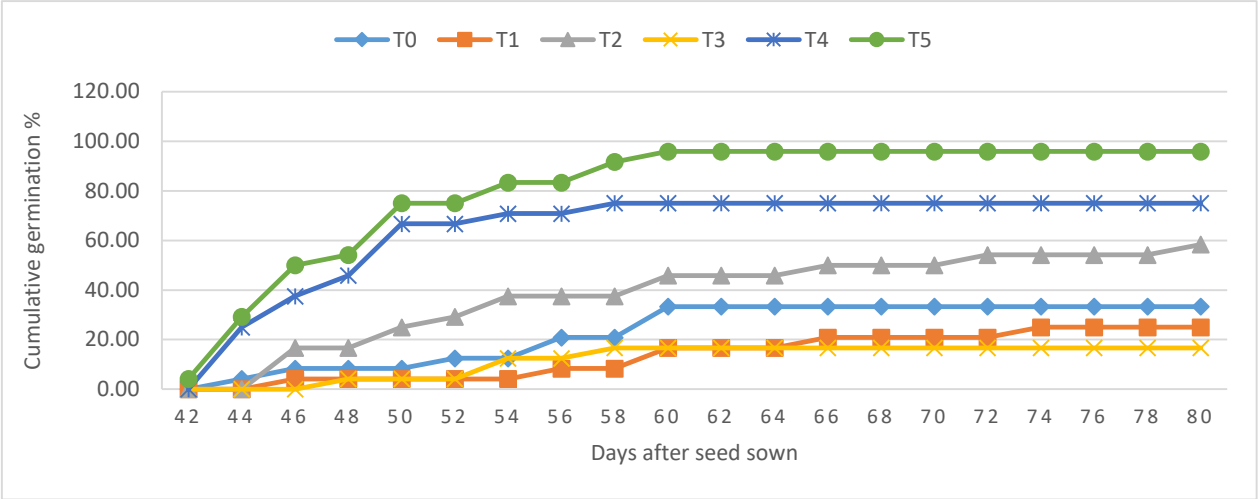

Figure 06. Cumulative germination percentage of *T. citrina* seedlings in different pre-sowing treatments

3.4 Growth performance of the seedlings

Different treatments affect the morphological growth of *T. citrina* seedlings differently. After 3 months of seed germination, the highest mean shoot height (46.6 cm) was recorded in T_2 (seeds soaking in normal water for 48 hours and sown in polybags) and the lowest (30.23 cm) was observed in T_4 (seeds soaking in hot water for 1 minute and sown in propagator house). T_0 treatment revealed 42.30 cm shoot height (Figure 07).

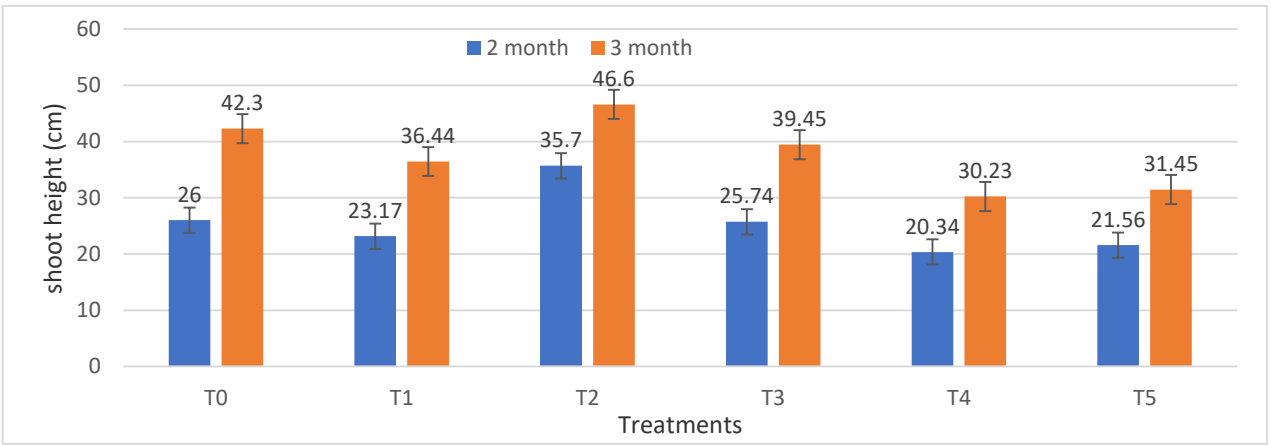

Figure 07. Growth performance of *T. citrina* seedlings in response to different pre-sowing treatments

Collar diameter and leaf number were recorded at 3-and 4-months old seedlings. The highest collar diameter (6.02 mm) attained in T_2 treatment (seeds soaking in normal water for 48 hours and sown in polybags) and lowest (4.23 mm) in T_4 treatment (seeds soaking in hot water for 1 minute and sown in propagator house). Maximum number of leaf (12.6) produced in T_2 and minimum (5.8) in T_4 treatment (Table 4).

Table 04. Mean collar diameter and leaf number of *T. citrina* seeds in different pre-sowing treatments

	Collar diameter (mm)		Leaf number	
Treatments	3 months	4 months	3 months	4 months
T_0	4.92±0.20	5.81±0.36	9.2±0.66	11.6±0.81
T_1	3.9±0.17	5.01±0.09	6.4±0.24	9.8±0.80
T_2	5.26±0.48	6.02±0.28	10.4±1.17	12.6±1.12
T_3	3.88±0.15	4.74±0.20	7.6±0.51	9±0.71
T_4	3.02±0.14	4.23±0.17	4.8±0.37	5.8±0.66
T_5	3.32±0.06	4.58±0.19	6.4±0.51	7.8±0.97

4. Discussion

The science of seed biology encompasses the development and physiology of seeds until they finally germinate or fail to do so (Schmidt, 2000). Germination and seedling establishment are critical stages that affect both quality and quantity of crop yields (Subedi and Ma, 2005).

The present findings of the study on *Terminalia citrina* found that seeds sown in propagator house provide the highest germination percentage (95.83%). Seeds soaking in normal water for 48 hours was 58.3% and in control 33.3% germination respectively. Highest germination energy (37.5%), germination value (1.0506), germination index (0.5569), and lowest mean germination time (46.73) observed in propagator house. The minimum germination period (43.3 days) was found in propagator house and maximum (54.3 days) recorded in control treatment. This study supports the findings of *Acacia* spp. where sand is a suitable medium for seed germination (ISTA, 1993). Sand as a germination substratum is preferred for large seed-producing tree species (Magini, 1962). *Lithocarpus elegans* showed the highest germination percentage in propagator house (Nandi *et al.*, 2019). Maximum shoot height (46.6 cm), collar diameter (6.02 mm), and leaf number (12.6) recorded in T_2 (seeds soaking in normal water for 48 hours and sown in polybags) treatment and T_4 (seeds soaking in hot water for 1 minute and sown in propagator house) showed the lowest performance. Dey and Hossain (2019) reported that *Suregada multiflora* seedlings raised in propagator house showed the highest germination and survival rate, but the growth rate was lowest as sandy media failed to provide sufficient nutrients for growing plants. According to Hossain et al., (2005) *T. chebula* fruits de-pulping at two ends and soaking in cold water for 48 hours treatments revealed the highest germination (66.70%) and growth performance. Whole fruits of *T. chebula* soaking in cold water for 48 hours with successive treatments by 10% dilute H_2SO_4 for 20 minutes revealed 70% germination (Rashid et al., 1990). Ara et al. (1997) showed that clean seeds revealed maximum germination (50%) and initial growth. Nainar et al. (1999) applied different pre-sowing treatments in *T. chebula* seeds and the highest germination percentage (60%) recorded in mechanical scarification treatment. Hasnat et al. (2019) revealed soaking in water at room temperature for 48 hours is the best treatment for *Castanopsis indica* species. According to Haider et al. (2014) *Acacia catechu* obtained the highest germination percentage (80-81%) soaking in cold water for 24 hours. So, from the study for *Terminalia citrina* species, sandy media is suitable to get maximum germination but not vigor seedlings production. For a successful plantation program, seedling's morphological features such as collar diameter, shoot height, leaf and node number, etc. should be considered. Hence, seeds soaking in normal water for 48 hours and sown in polybags is suggested for vigour seedlings production of *T. citrina* species.

5. Conclusion

Seed's pre-sowing treatments significantly affect the germination of *Terminalia citrina*. Seeds sown in the propagator house showed maximum germination percentages but lowest growth performance. In contrast, seeds treated with normal water for 48 hours and sown in polybags produced vigour and quality seedlings at the initial stage. The result of the present study recommends that nursery

owners or other seedling producer organizations treat *T. citrina* seeds with water for 48 hours at room temperature and sown in polybags in large-scale plantation programs.

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