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Germination and initial seedlings growth response of *Ehretia serrata* in different Pre-sowing treatments

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ABSTRACT

The study was investigated to inspect the effect of different pre-sowing treatments on germination and initial growth performance of Ehretia serrata seeds at the nursery of Chittagong University, Bangladesh. Seeds were treated to six pre-sowing treatments e.g. Control (T_0), seeds immersed in hot water for 1 min (T_1), seeds treated with sandpaper rubbing (T_2), immersed in hot water for 30 sec (T_3), immersed in cold water 24 hours (T_4), immersed in H_2SO_4 for 30 sec (10% solution) (T_5). Forest topsoil and fine Sylhet sand were used as growing media. As seeds were very tiny in size, germination trays were used to make the experiment convenient. Germination percentage (78.43 %), germination uniformity (0.0235), and germination value (4.8630) were recorded highest in T_5 treatment (immersed in 10% H_2SO_4 and significantly ($P< 0.05$) different from other treatments. Maximum shoot height (11.34 cm) and root height (9.96 cm) were revealed in T_0 treatment. Finally, seeds immersed in H_2SO_4 for 30 sec (10% solution) were found suitable for maximum seedlings production. In the case of vigor seedling production, seedlings raised in forest soil were revealed suitable for Ehretia serrata.

Key Words: *Pre-sowing treatments, Germination, Seedling growth, Germination tray, Growing media and Germination value.*

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I. Introduction

World biodiversity is exhausting at an alarming rate because of human intrusions and environmental degradation, creating a high risk of disappearance (Rashid et al., 2014). Pitmen and Jorgenson (2002) mentioned that under the World Conservation Union (IUCN) Classification Scheme, half of the world's plant species may qualify as threatened with extinction. According to IUCN's Threatened Plants Unit about 60,000 plant species (25%) would turn into either extinct or rare within 2050 (Uberoi, 2010). Plant genetic variety is being disturbed in a dreadful way (Hossain et al. 2013). The genus *Ehretia* of the *Boraginaceae* family has nearly 50 species. *Ehretia serrata*. Roxb. (Local name: Kala-huja) is a medium-sized, deciduous tree with fluted stem, flowers numerous, white with an unpleasant smell. Fruit a drupe, small, globose or ellipsoid, 2-3 mm in diameter, produced copiously from the top branches, yellow or orange, or black when ripe. Each fruit contains 1-2 seeds (Das and Alam, 2001).

Flowering and fruiting occur from February to July. It is disseminated in tropical areas of Africa and Asia (Johnston, 1951). In Bangladesh, the species occurs naturally in the forests of Sylhet, Chattogram, and the Chittagong Hill Tracts, more commonly suburbs and city areas of Chattogram and Cox's Bazar (Das and Alam, 2001). The plants of the genus *Ehretia* are familiar for rich folkloric medicinal applications including in the cure of chest pains and abdominal (Mncwangi et al., 2012), toothaches, stomach diseases, diarrhea, cough, eczema, and syphilis (Torane et al., 2011).

Suitable nursery and plantation techniques, management systems of the species is pre-requisite to make a plantation program fruitful. Recently, 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 seedling growth performance of native tree species is crucial (Khurana and Singh, 2001; Smith et al., 2008). The selection of appropriate pre-sowing treatment is essential for quick and maximum seed germination (Thapa and Gautam, 2006). Hard coated seeds need more time to germinated and thus, direct sowing is not effective (Anon., 1972). Proper pre-treatments of seeds can stimulus germination time and germination process (Azad et al., 2006a; Azad et al., 2011; Azad et al., 2012). The effect of pre-sowing treatments on seed germination of few tropical forest tree species has been informed by several authors (Ahamed et al., 1983; Khan et al., 2001; Alamgir and Hossain, 2005; Azad et al., 2006b; Matin et al., 2006 and Haider et al., 2014). Therefore, an endeavor has been made to study the effect of pre-sowing treatments on seed germination to identify appropriate pre-sowing treatment for *Ehretia serrata*.

II. Materials and Methods

Study site

The present study was carried out during October 2018 to March 2019 in the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong, Chattogram, Bangladesh (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 as 32.60 °C and the minimum in January as 14.10 °C (Peel et al., 2007).

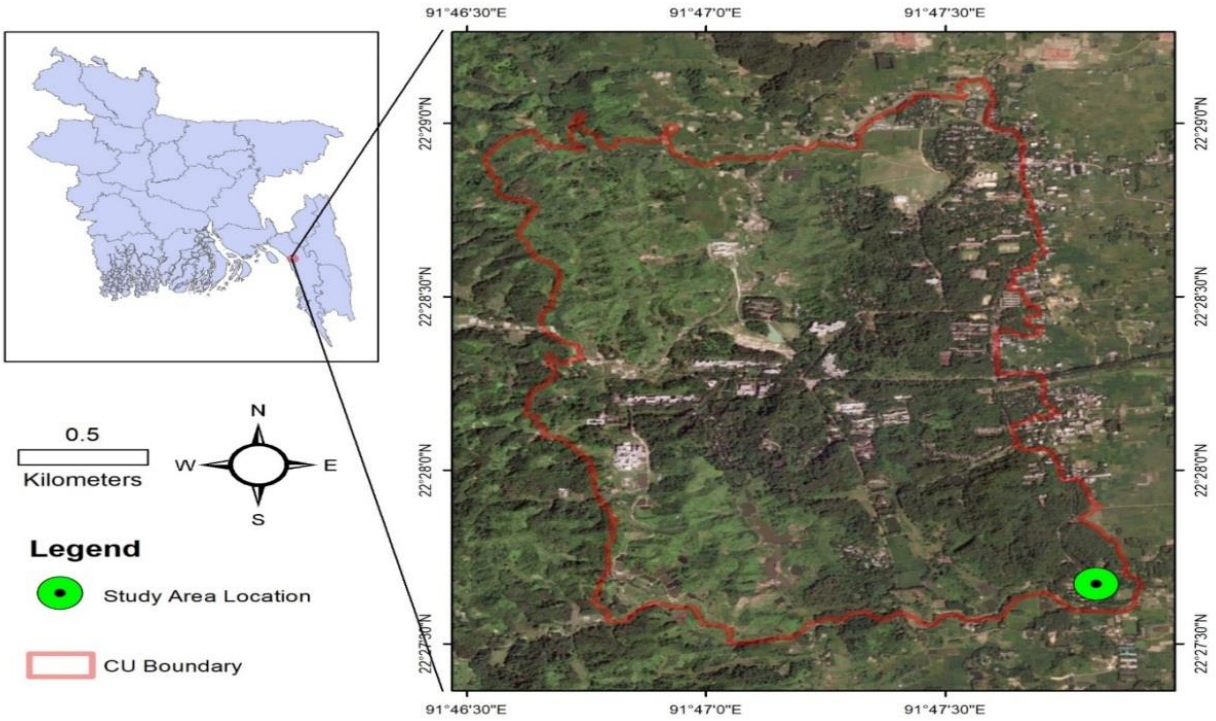

Figure 01. Map showing the location of nursery IFESCU (Institute of Forestry and Environmental Sciences, University of Chittagong)

Seed collection

E. serrata fruits were collected from Bohaddarhat, Bangladesh during October 2018. Phenotypic

characteristics of seeds were measured. Fruits were immersed in water for overnight and extracted seeds, dried in the open sun for three days. Randomly selected seeds length and width are respectively 3.22±0.07 mm and 0.0056±0.002 mm. About 178,000 seeds were found in one kg.

Experimental design

As the seeds were tiny (Figure 05) and hard to make the experiment convenient, seeds were sown in germination tray (Figure 06). The media of the germination tray was forest topsoil collected from the forest floor and fine Sylhet sand. The investigation is made up of six pre-sowing treatments with 3 replications (15 seed per replication) in a randomized complete block design. Forty-five (45) healthy seeds were chosen randomly from each treatment. Daily germination was recorded as soon as the seeds start germination. Seedlings raised in the germination tray were transferred to polybag (Figure 07) after 2 months 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. Six seedlings from each treatment were selected randomly and shoot height and root height of the seedlings were recorded. The pre-sowing treatments are :

T_0- Seeds without treatment and sown in germination tray (soil media), Control
T_1- Seeds immersed in hot water for 1 min and sown in germination tray (fine sand media)
T_2-Seeds treated with sandpaper rubbing and sown in germination tray (fine sand media)
T_3- Seeds immersed in hot water for 30 sec and sown in germination tray (fine sand media)
T_4- Seeds immersed in cold water for 24 hours and sown in germination tray (fine sand media)
T_5- Seeds immersed in H_2SO_4 for 30 sec (10% solution) and sown in germination tray (fine sand media).

Data collection and analysis

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

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

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

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

Germination energy (GE): It measured by computing the daily germination percentage of its peak time (Dwivedi, 1993).

Germination index (GI): According to (AOSA, 1983) GI was calculated using this formula:

$$\text{Germination index (GI)} = \frac{\text{No.of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No.of germinated seeds}}{\text{Days of first count}}$$

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:

$$\text{MGT} = \Sigma Dn\ \Sigma n$$

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).

Germination Uniformity (GU): It was calculated by using the formula:

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

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).

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

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).

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 the tables.

III. Results and Discussion

Germination performance

The germination performance of *E. serrata* seeds was affected by different pre-sowing treatments in this study. Seed germination starts first in T_5 (20th day) after the seed was sown and T_0 required maximum time (at 27th day) to initiate germination. Maximum germination percentage (78.43%) was recorded in T_5 (seeds immersed in H_2SO_4 for 30 sec) followed by 64.71% in T_3 (seeds immersed in hot water for 30 sec), 58.82 % in T_2 (seeds treated with sandpaper rubbing), and 52.94 % in T_4 (seeds immersed in cold water for 24 hours). Germination percentage was lowest (37.25 %) in T_0 (seeds with no treatment, or control) and significantly ($p<0.05$) different from other treatments. The minimum germination period (25.33 days) was found in T_3 and T_4 showed a maximum germination period (35.33 days) (Table 01).

Table 01. Germination response of *E. serrata* seeds in different pre-sowing treatments

Treatments	Germination start after (days)	Germination Period (days)	Germination Percentage (%)	Germination capacity
T_0	27	34.33 ab*	37.25 d	Poor
T_1	25	27.00 abc	45.09 cd	Poor
T_2	23	29.33 abc	58.82 bc	Average
T_3	24	25.33 c	64.71 ab	Average
T_4	25	35.33 a	52.94 bcd	Average
T_5	20	26.33 bc	78.43 a	Good

*Mean 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 (2.2137) was recorded in T_5 and no significant difference was observed among T_0, T_1, T_2, T_4, and T_5 treatments. Mean germination time was maximum (36.89) in T_0, slightly vary from T_4 (36.23) and lowest in T_2 (27.85). The highest germination uniformity (0.0235) and Germination value (4.8630) were recorded in T_5 treatment and significantly ($p<0.05$) different from other treatments. The maximum germination energy (21.57%) was found in T_4 and T_5 treatments and slightly vary from T_2 and T_3 (Table 02).

Table 02. Germination response of *E. serrata* seeds in different pre-sowing treatments

treatments	germination Energy (%)	Germination Index (GI)	Mean Germination Time (MGT)	Germination Uniformity (GU)	Germination value
T_0	11.76 c*	1.1177 a	36.89 a	0.0027 b	0.9600 d
T_1	11.76 c	1.1857 a	32.97 b	0.0060 b	1.4010 bc
T_2	15.69 ab	1.1430 a	27.85 c	0.0051 b	2.8520 bc
T_3	17.65 ab	1.2433 a	28.23 c	0.0142 ab	3.2830 ab
T_4	21.57 a	1.1880 a	36.23 ab	0.0040 b	1.9207 bcd
T_5	21.57 a	2.2137 a	26.47 c	0.0235 a	4.8630 a

*Mean 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).

Mean cumulative germination percentage

The cumulative germination percentage is the daily germination percentage for each treatment showed germination of T_5 treatment starts after 20 days of seed sown and rose rapidly and continued germination up to 78.43% within 20 days. In T_0 treatment, germination starts at 27th days, rose gradually, and reached 37.25% in 19th days. T_3 attained 64.71% germination percentage in the 43rd day of seed sown and remain fix up to the termination of the germination **(Figure 02)**.

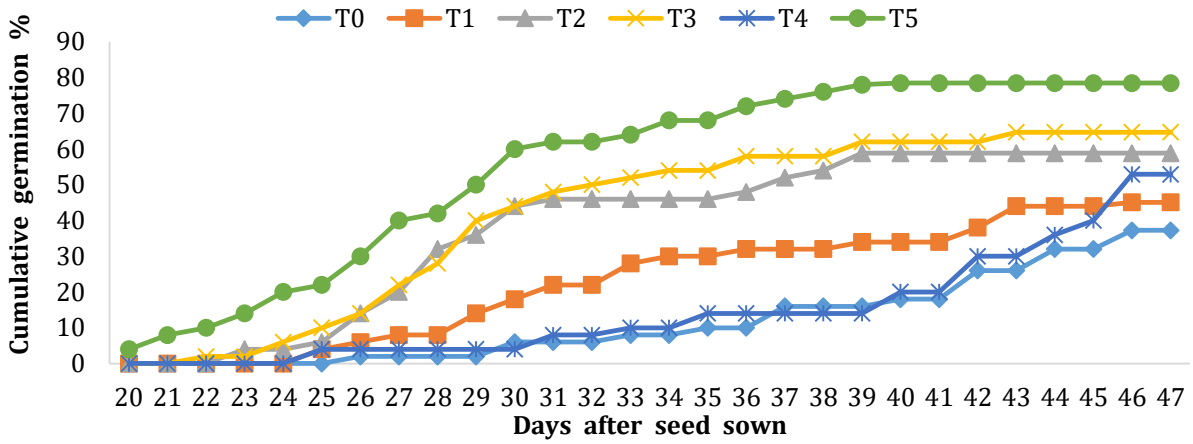

Figure 02. Cumulative germination percentage of *E. serrata* seedlings in different pre-sowing treatments

Growth performance of the seedlings

Different treatments affect the morphological growth of *E. serrata* seedlings. After 3 months of seed germination, the highest mean shoot height (11.34 cm) was recorded in T_0 (seed without treatment, control) and the lowest mean shoot height (5.65 cm) was observed in T_3 (seeds immersed in hot water for 30 sec). T_5 treatment attained mean shoot height (9.5 cm) after 3 months of germination (Figure 03). After 2 months of seed germination, maximum root length was found in T_0 (9.96 cm) and minimum in T_5 treatment (4.7 cm) (Figure 04).

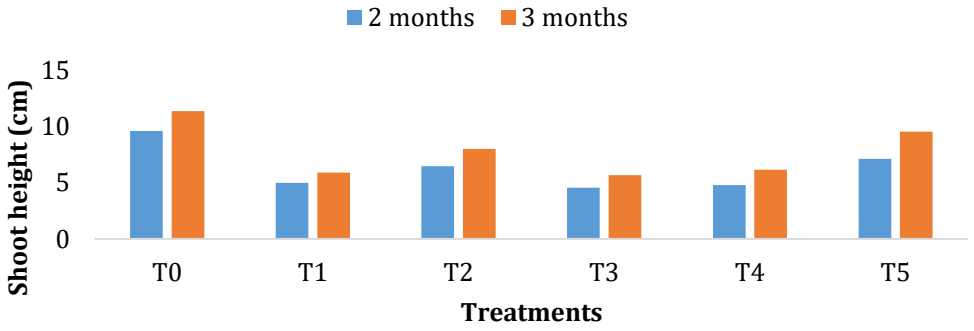

Figure 03. Growth of *E. serrata* seedlings in response to different pre-sowing treatments

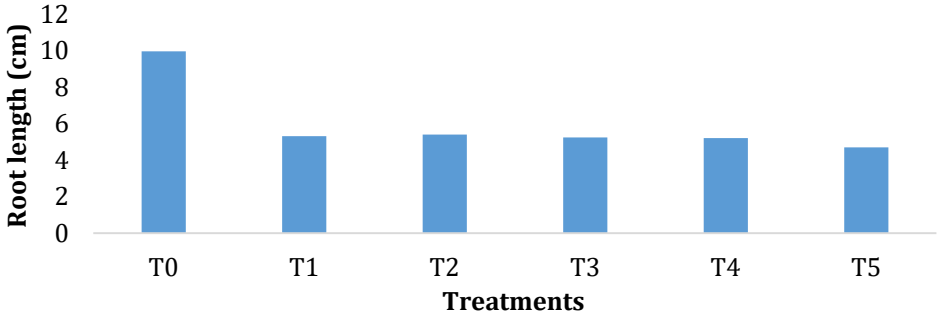

Figure 04. Root length of 2 months old *E. serrata* seedlings in response to different pre-sowing treatments

Discussion

Hard coated seeds are now and then impermeable to sufficient water and nuitrients for e vigor seedlings production . These seeds require suitable pre-sowing treatments for producing vigor seedlings for plantation in degraded forest land. Germination and seedlings growth performance of *E. serrata* was varied in different pre-sowing treatments. The seed of *Ehretia serrata* showed maximum germination percentage (78.43 %) in 30 seconds immersion in H_2SO_4 (10% concentration) treatment. Seeds without treatments (control, no treatment) revealed the lowest germination percentage (37.25 %). Considering the growth performance of seedlings, seeds without treatment, and sown in soil media (T_0) recorded the highest root and shoot height (Figure 03 and Figure 04). The soil was collected from the forest floor, and it provides sufficient nutrients to accelerate the seedlings' growth. On the contrary, fine sand media collected from Sylhet was devoid of nutrients. (Dey and Hossain, 2019) found that *Suregada multiflora* seedlings raised in sand media showed the highest germination and survival rate, but the growth rate was lowest as sand media failed to provide sufficient nutrients for growing plants. Seeds immersed in proper concentrated acid sometimes improves the germination rate (Hasnat and Hossain, 2018). Seeds immersed in diluted acid may accelerate the germination rate in a few hard seeds (Schmidt, 2000; Azad et al, 2010; Merou et al, 2011 and Azad et al, 2012). Hasnat and Hossain (2018) recommend 10% HCl and 10% H_2So_4 for 3 minutes to enhance germination and seedlings growth performance of Gutgutiya (*Protium serratum*). (Asiedu et al., 2012) revealed 90% concentrated H_2SO_4 is the suitable pre-sowing treatments for the germination of *Bauhinia rufescens* (Hasnat et al., 2016) revealed that soaking in cold water for 24 hours was the more effective in germination and vigor seedlings production of *Canarium resiniferum*. According to (Haider et. al, 2014) *Acacia catechu* obtained the highest germination percentage (80-81%) soaking in cold water for 24 hours. (Currie, 1984) reported that water, air and mineral nutrient availability of the growth medium is the most important physical factors affecting seedlings growth. So, tiny but hard-coated seeds of *E. serrata* influenced by acid treatments

Figure 05. Seeds of *E. serrata*

Figure 06. *E. serrata* raising in germination tray

Figure 07. Seedlings of *E. serrata* transferred in polybag

IV. Conclusion

It can be concluded from the study that seeds immersed in H_2SO_4 for 30 sec (10% solution) and sown in fine sand media are recommended for the best germination performance of *E. serrata*. After 2 months of germination, seedlings need to transfer to polybags filled with soil media.

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