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Effect of Pre-Sowing Treatments on Germination and Initial Seedling Growth of *Castanopsis Indica*- An Endangered Tree Species in Bangladesh

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Abstract

Castanopsis indica (Sil Batna) is an ecologically valuable multipurpose indigenous tree species of Bangladesh. Considering its high value but poor natural regeneration due to seed dormancy, the authors conducted an experiment at the Institute of Forestry and Environmental Sciences Chittagong University (IFESCU) during 2012-2013 to find out effective pre-sowing treatments. Ten pre-sowing seed treatments were applied on mature, even-sized seeds namely – control; sandpaper rubbing; nail clipping; immersion in normal water (at room temperature: 24°C) for 24 hrs., 48 hrs. and 72 hrs.; immersion in hot water (80°C) for 1 minute; soaking in 10% dilute H_2SO_4; soaking in 10% HCl for 5 minutes; and sowing in propagator house. Seeds sown after sandpaper rubbing at the distal end revealed best performances by providing highest germination percent (66.7%), germination energy (30%), germination index (0.17), germination rate (0.0145), germination value (30%) and plant percent (66.7%) within shortest period (38 days). The treatment also produced most vigor seedlings with 20.9 cm shoot height, 15 node number, and largest leaves (11.1 cm×2.9 cm). Hence, it is recommended to adopt sandpaper rubbing method for maximum germination and quality seedlings.

Key Words: *Castanopsis indica*, indigenous, pre-sowing treatment, germination, dormancy

Introduction

Indigenous trees are ecologically valuable as they conserve biodiversity and ecosystems as well as the beauty of landscapes. Native trees are more adaptive to local climatic conditions and soil, need less water, and help to maintain natural balance (DeFries et al. 2007). Fruits, seeds, tubers, etc. of native trees are edible to humans and local wildlife. Moreover, they provide habitat to the indigenous wild animals, birds, and useful insects. In contrast, exotic species directly or indirectly cause imbalance of soil moisture, nutrients and underground water in nature and thus pose serious threats to ecosystems (Heywood 1989; Prieur-Richard and Lavorel 2000; Dukes 2002; Weber 2003; Seabloom et al. 2006). Invasive exotics disturb soil and impose it to erosion, reduce soil fertility (Barua et al. 2001), create biotic disturbance, alter habitat structures, increase competition and change microclimates. They are responsible for reduction of native species richness and changes in functional processes like productivity, decomposition, hydrology, nutrient cycling, etc. (Mack et al. 2000). Biological invasions by exotic species have significant long-term effects on bio-

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diversity as well as global changes (D'Antonio and Vitousek 1992; Dukes and Mooney 1999; Lonsdale 1999; Mack et al. 2000; Naylor 2000; Fine 2002; Mooney and Hobbs 2002; Seabloom et al. 2006). Like climate changes, these have negative impacts on environment which is sometimes irreversible in hundreds to thousands of years (D'Antonio and Vitousek 1992).

Slowly degradable leaves of some exotic species pose a threat to the native flora. Having non-edible fruits and discomfort habitat they distract fauna. Some exotics suppress the highly ecologically valuable native tree species through their luxuriant growth (Hossain and Pasha 2001). The major concern is that exotics causing the extinction of threatened native species through competition, habitat alteration, and diseases (Vitousek et al. 1997; Gurevitch and Padilla 2004). *Castanopsis indica* is an ecologically important native tree and concomitantly vulnerable because of its high seed dormancy, less competitive nature and slow growth.

Castanopsis indica (Roxb. *ex* Lindl.) A. DC., commonly known as Shil Batna, is an evergreen tree growing in the natural forests of Bangladesh. It belongs to the Fagaceae family. Besides Bangladesh, it is found in mixed deciduous forests and evergreen forests of southern China, India, Nepal, Bhutan, Myanmar, Thailand, Laos, Vietnam. This plant is a medium to large-sized tree grow up to 30 meters height and bole generally formed straight with up to 100 cm in diameter. The tree is wild in nature. Bark brown or silvery grey in color with more or less fissured vertically. Branches arranged in ascending order and twigs are hairy at the young stage and glabrous when it matures. Leaves are simple, alternate, 6-20 by 3-9 cm, elliptic-oblong, acute, glabrous and shiny above, and densely hairy below. Leaf margin distinctly and sharply toothed except near the base. Flowers unisexual and grown in the same tree. Fruits densely clustered with 3-4 seeds and covered completely by long slender straight sharp spines of 2.5-4 cm lengths. Flowering occurs in April–May and fruiting time is August-September. Having yellow-brown or pinkish color with lustrous surface and hardy grain, wood of this plant is very attractive. The wood is hard and generally used for construction and furniture making due to its resistant nature to termites and insects. The wood can also be used for making charcoal. Moreover, the bark is a source of tannin and used as dye (Soepadmo et al. 2002). The more important product is Methanol extract of *C. indica* (MECI) leaves has significant effect on Ehrlich Ascites Carcinoma (EAC) cell. A dose of dependent manner can decrease the tumor volume and tumor weight, and elevate the life span of EAC tumor bearer (Dolai et al. 2012).

Despite its ecological, economic and medicinal value the species is becoming rare day by day in Bangladesh because of its high seed dormancy and poor natural regeneration. Though there is a number of studies on the best seed sowing techniques and germination of many species across the countries (i.e. Haider et al. 2014; Hasnat et al. 2017; Jannat et al. 2017; Hossain et al. 2018 etc.) and throughout the world (i.e. Krishnamoorthy et al. 2016; Karki et al. 2018; Manokari and Shekhawat 2018 etc.). Many of these studies are with the commercially important tree species. But there is a great dearth of information on the germination behavior and nursery growth of the rare native species like *Castanopsis indica*. Considering the conservation status, in the plantation initiatives, this tree species should be given priority as it is threatened in the wild. In this situation, the study was structured to find out the appropriate pre-sowing treatments for high germination rates and initial seedling growth in order to improve nursery techniques and conserve this rare native species having high ecological and economic value.

Materials and Methods

Seed sources and morphological features

Seeds of *Castanopsis indica* were collected from Dulhazara natural forests of Cox's Bazar, Bangladesh during August 2012. The mature, healthy and uniform sized seeds were collected for the experiment in order to reduce germination variation as seed size is effectively correlated with germination behavior and seedling vigor (Bonner 1987). The length, width, thickness, and weight of the collected seeds were recorded before sowing in the nursery. The average length and width of the seeds were 1.7 ± 0.06 cm and 1.5 ± 0.09 cm respectively. The number of seeds varied from 533 to 556 in one kg (Table 1).

Seed size and weight were measured in the "Seed Research Laboratory" of the Institute of Forestry and Environmental Sciences Chittagong University (IFESCU). Seeds were sown within three days after collection for better

Table 1. Seed length, width, and number of seeds per kg of *C. indica*

Parameters	Length (cm)	Width (cm)	Weight/20 seeds (g)	Seeds/kg	Weight/1,000 seeds (g)
Average	1.7±0.06	1.5±0.09	36.6±0.7	547±9.7	1,830±32.6
Range	1.6-1.8	1.4-1.7	36-37.5	533-556	1,800-1,875

±indicates the standard error of the mean.

germination results.

Study site and growing media

The experiment was conducted in the IFESCU nursery (22.4610°N, 91.7959°E), Chittagong, Bangladesh from August 2012 to March 2013. The sowing media was prepared with soil collected from the forest sites of the Chittagong University campus. After collection, the soil was dried and sieved well (< 3 mm) and mixed with decomposed cow-dung in a ratio of 3:1 to prepare media. Conventional polybags of 15 cm×10 cm (6"×4") were filled with the prepared growing media mixture. Another media with fine *Sylhet sand* [1] was used in the experiment for seed germination in propagator house[2].

Experimental design

Completely Randomized Design with three replications for each treatment was used in the experiment. The treatment combination contained ten pre-sowing methods. Due to scarcity of seed in the natural forests, each replication consisted of ten seeds in ten polybags. The ten pre-sowing methods in the experiment were: T_0: Seeds sown with no treatment (control), T_1: Seeds with sandpaper rubbing at the distal end T_2: Seeds nicked by nail clipper at the distal end, T_3: Seeds immersed in normal water (room temperature, 24°C) for 24 hours, T_4: Seeds immersed in normal water (room temperature, 24°C) for 48 hours, T_5: Seeds immersed in normal water (room temperature, 24°C) for 72 hours, T_6: Seeds immersed in hot water (boiled water) for 1 minute, T_7: Seeds soaked in 10% concentrated H_2SO_4 for 5 minutes, T_8: Seeds soaked in 10% concentrated HCl for 5 minutes, T_9: Seeds sown in propagator house. In treatments T_0, T_1, T_2, T_3, T_4, T_5, T_6, T_7, and T_8 seeds were sown in normal polybags with soil media, and in treatment T_9 seeds were sown in propagator house with *Sylhet sand* media.

Data collection and analysis

The number of seeds germinated in each treatment was recorded regularly. The starting and closing dates of germination and other germination parameters were also measured. Germination percentage estimates the viability of a population of seed. The number of seeds germinated at each day in each replication of treatments was counted to calculate germination percentage and cumulative germination percentage (Kumar 1999; Almodares et al. 2007). To determine the germination energy and germination energy period the peak time for daily germination percentage was assessed (Dwivedi 1993). Germination index (germination speed) (Brown and Mayer 1988) was calculated as the sum of the percentage of seeds germinated on each day divided by the number of days since the germination test started (Throneberry and Smith 1955; Allan et al. 1962; Maguire 1962; Kendrick and Frankland 1969; Bouton et al. 1976; AOSA 1983; Khandakar and Bradbeer 1983; Bradbeer 1988; Wardle et al. 1991). Germination index correlated with seed vigor.

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

$$\text{Cumulative germination \% (CGP)} = \frac{Cumulative\ Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} \times 100$$

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

[1] ***Sylhet sand***: River sand with higher proportion of silica found in Sylhet region, Bangladesh.

[2] **Propagator house:** Greenhouse is a structure, primarily of glass, in which temperature and humidity can be controlled for the cultivation or protection of plants. Propagator house is one type of greenhouse with a bed consists of *Sylhet sand* with controlled temperature and humidity.

Table 2. Effect of pre-sowing treatments on germination behavior of *C. indica*

Treatments	Germination start (day)	Cumulative germination (%)	Germination energy (%)	Germination energy period (day)	Germination index (GI)	Mean germination time (MGT)	Germination rate (R)	Plant percent (%)	Germination value (GV)	Germination capacity (GC)
T_0 (Control)	63	25^{b}*	8.4^{ab}	91^{cd}	0.03^{b}	174.60^{d}	0.0058^{b}	25^{b}	0.05^{a}	Very poor
T_1 (Sand paper)	38	66.7^{d}	30^{d}	28^{ab}	0.17^{d}	70.03^{b}	0.0145^{c}	66.7^{d}	0.53^{b}	Average
T_2 (Nicking)	79	43.3^{c}	15^{bc}	98^{cd}	0.04^{bc}	159.73^{cd}	0.0063^{b}	43.3^{c}	0.13^{a}	Poor
T_3 (Normal water, 24 hr.)	71	31.7^{bc}	13.4^{bc}	91^{cd}	0.03^{bc}	165.69^{cd}	0.0062^{b}	31.7^{bc}	0.08^{a}	Poor
T_4 (Normal water, 48 hr.)	41	46.7^{c}	28.3^{d}	112^{d}	0.05^{bc}	182.27^{d}	0.0055^{b}	46.7^{c}	0.18^{a}	Poor
T_5 (Normal water, 72 hr.)	69	43.3^{c}	23.4^{cd}	126^{d}	0.04^{bc}	182.13^{d}	0.0055^{b}	43.3^{c}	0.15^{a}	Poor
T_6 (Hot water, 1 min.)	-	-	-	-	0.00^{a}	0.00^{a}	0.0000^{a}	-	-	No germination
T_7 (H_2SO_4, 5 min.)	105	45^{c}	21.7^{cd}	98^{d}	0.04^{bc}	181.47^{d}	0.0055^{b}	45^{c}	0.15^{a}	Poor
T_8 (HCl, 5 min.)	69	48.3^{c}	16.7^{bc}	98^{d}	0.05^{c}	156.67^{cd}	0.0065^{b}	48.3^{c}	0.2^{a}	Poor
T_9 (Propagator house)	57	21.7^{b}	8.3^{ab}	63^{bc}	0.04^{bc}	145.08^{c}	0.0069^{b}	21.7^{b}	0.1^{a}	Very poor

*Means followed by the same letter(s) in the same column are not significantly different at $P<0.05$, Duncan's Multiple Range Test (DMRT).

Mean germination time (MGT) is defined as a measure of the rate and time-spread of germination (Bewley et al. 2013; Soleymani and Shahrajabian 2018). The rate of germination is the reciprocal of MGT (Mukarati et al. 2013). At the end of the experiment, the survived seedlings were counted to determine plant survival percent. Germination value was calculated by multiplying the peak value of germination (PV) and mean daily germination (MDG).

[3)]Mean germination time (MGT)$=\frac{\sum Dn}{\sum n}$

Rate of germination (R)$=\frac{1}{MGT}$

Plant % (PP)$=\frac{Number\ of\ surviving\ seedlings}{Number\ of\ seeds\ sown}\times 100$

Germination value (GV)=Peak value of germination (PV)×Mean daily germination (MDG)

Germination capacity was evaluated by calculating the percentage of total seed germinated in each treatment at the end of the experiment. The germination capacity was categorized by following Kumar (1999) where -i) 100-90% -very good, ii) 90-70% -good, iii) 70-50% -average, iv) 50-30% -poor, v) 30-20% -very poor, and vi) (<) 10% -extremely poor.

Growth performance was estimated by measuring shoot length, leaf length, leaf width, and node number. Five months old three vigor seedlings from each replication were selected for measurement. For collar diameter measurement seven months old vigor seedlings were selected. Shoot length was measured from the collar region to the shoot tip of the seedlings. Collar diameter was measured at the collar region, transitional zone between root and shoot of the seedlings with a Vernier caliper. The total number of nodes including both leaf fall scar and leaf present was counted

[3)]MGT=ΣDn/Σn, here n is the number of seeds, D is the number of days counted from the beginning of germination.

and average number was calculated. Leaf length and leaf width were also measured with scale.

Statistical analysis

Statistical analysis of data was done using the computer software package Statistical Package for the Social Sciences (SPSS). The analysis of variance (ANOVA) was studied by applying Duncan's Multiple Range Test (DMRT).

RESULTS

Seed germination behavior

Germination behaviors of *C. indica* were significantly affected by pre-sowing treatments. Seeds rubbed on sand-paper (T_1) germinated at first (38 days after seeds sowing). The longest time (105 days) was required by seeds soaked in 10% H_2SO_4 for 5 minutes (T_7). No germination observed in seeds soaked in hot water treatment (T_6) (Table 2). Significantly highest germination percentage (66.7%) found in T_1 (Fig. 1). The lowest germination percent (21.7%) observed in seeds sown in the propagator house (T_9). T_1 attained maximum germination energy (30%) with minimum germination period (28 days after germination started) while seeds soaked in normal water for 72 hours (T_5) taken longest germination period (126 days). The lowest germination energy (8.3% and 8.4% respectively) was found in seeds sown in T_9 and control (T_0). Germination index was highest (0.17) in T_1 and lowest (0.03) both in control and 24 hrs. normal water treatment. Mean germination time was highest (182.27) in 48 hrs. normal water treatment (T_4) and lowest (70.03) in T_1. Germination rate found highest (0.0145) in T_1 and lowest (0.0055) in T_4. Seedling survivability found the maximum (66.7%) in T_1. The lowest plant percentage (21.7%) observed in T_9. T_1 revealed significantly higher germination value (0.53) than any other treated seeds. Germination capacity was very poor in T_0 and T_9. Seeds with hot water treatment had no germination capacity (Table 2).

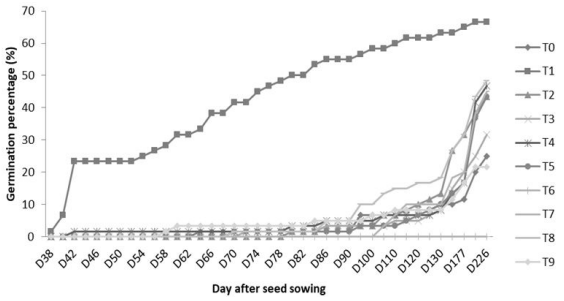

Fig. 1. Effect of pre-sowing treatments on cumulative germination percentage of *C. indica*.

Table 3. Effect of pre-sowing treatments on shoot height, node number, leaf length, leaf width and collar diameter of *C. indica* seedlings in the nursery

Treatments	After germination				
	5 months old seedlings				7 months old seedlings
	Shoot length (cm)	Node number	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)
T_0 (Control)	7.5^{b}*	9^{bc}	4.1^{b}	1.1^{b}	6^{d}
T_1 (Sand paper)	20.9^{e}	15^{e}	11.1^{d}	2.9^{d}	6^{d}
T_2 (Nicking)	12.8^{cd}	12^{de}	7.2^{bc}	2^{bc}	7^{d}
T_3 (Normal water 24 hr.)	10^{bc}	8^{bcd}	5^{bc}	1.4^{b}	5^{c}
T_4 (Normal water 48 hr.)	7.2^{b}	8^{cd}	5.2^{bc}	1.4^{b}	6^{cd}
T_5 (Normal water 72 hr.)	10.6^{bc}	7^{bcd}	4.9^{bc}	1.4^{b}	7^{d}
T_6 (Hot water, 1 min.)	-	-	-	-	-
T_7 (H_2SO_4, 5 min.)	10.6^{bc}	7^{bc}	4.2^{b}	1.2^{b}	6^{d}
T_8 (HCl, 5 min.)	15.5^{d}	12^{de}	8.3^{cd}	2.7^{cd}	7^{d}
T_9 (Propagator house)	19.3^{e}	3^{ab}	3.7^{b}	1.2^{b}	2^{b}

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

Growth performance of C. indica seedlings

Pre-sowing treatments significantly ($P < 0.05$) affect the growth performance of *C. indica* seedlings (Table 3). The results showed that seeds of T_1 demonstrated the highest shoot length (20.9 cm), node number (15), leaf length (11.1 cm) and leaf width (2.9 cm). Lowest collar diameter (2 mm) observed in T_9 while highest (7 mm) was both in Nicking (T_2) and Normal water 72 hrs. (T_5). Overall growth was highest in seedlings grown from seeds sown after rubbed with sandpaper (T_1) followed by seeds sown in HCl for 5 minutes (T_8) and seeds nicked at the distal end (T_3) (Table 3).

Shoot increment

After 8 months of seeds sowing, it was found that the shoot growth was comparatively higher in the months of April and May (Fig. 2). The experiment also showed that seeds in T_1 treatment attained highest mean shoot height (42.6 cm) and least shoot height (20 cm) was recorded in T_0. T_9 surprisingly revealed maximum growth between February and March (Fig. 2). This may because seedlings were grown in a controlled condition.

Discussion

The natural reproductive structure of *C. indica* is the seed. Dormancy due to hard coat is a common hindrance for seed germination naturally. In this study, both the morphological (length, width, and weight) and physiological (dormancy level and pre-sowing treatments) study of *C. indica* seed were done. The results of the study revealed that there is a vital role of pre-sowing treatments on *C. indica* seeds for enhancing germination and initial seedling growth (Fig. 3). Germination behavior varied among different pretreatment methods. Seeds were sown after application of sandpaper rubbing at the distal end (T_1) broken dormancy more effectively than any other pretreatment methods. Maximum germination percentage (66.7%), germination energy (30%), germination index (0.17), germination rate (0.0145), plant percent (66.7%) and germination value (0.53) found in T_1 than any other treatments. The treatment was found to be extremely effective as it makes the seed coat permeable for water entry and gaseous exchange that break the seed dormancy resulting enzymatic hydrolysis and transforming the embryo into a seedling (Alamgir and Hossain 2005; Ayisire et al. 2009; Azad et al. 2010b). The treatment suggests that dormancy in *C. indica* seeds is mainly due to hard coat which restricts the seed tes-

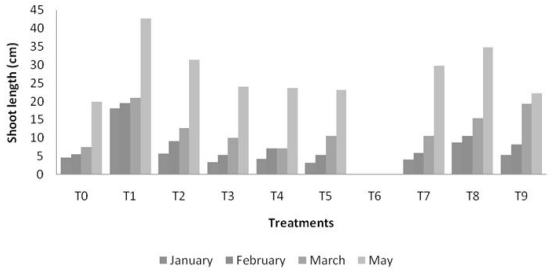

Fig. 2. Shoot height increment of *C. indica* seedlings with different pre-sowing treatments in the nursery at 8 months age.

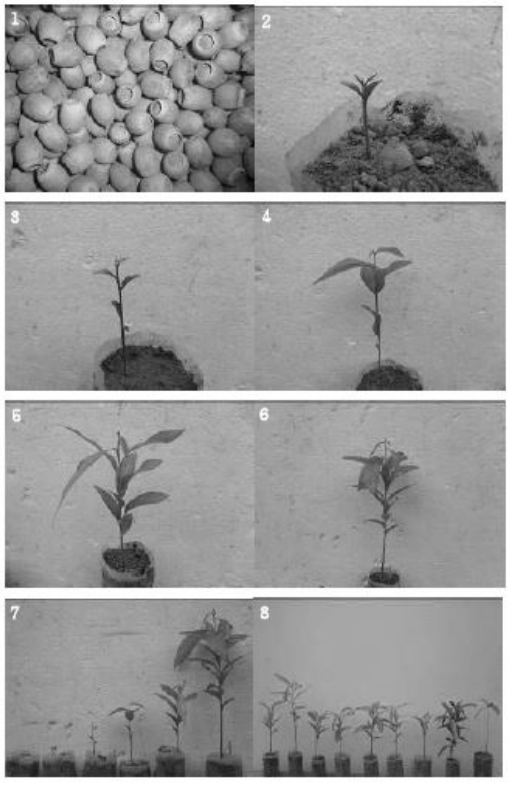

Fig. 3. [1] Seeds of Sil Batna (*Castanopsis indica*); [2]7 days old seedling after germination of Sil Batna (*C. indica*); [3]15 days old seedling; [4]45 days old seedling; [5]90 days old seedling; [6]180 days old seedling; [7]Seedling height increment at interval of 2, 7, 15, 45, 90 and 180 days at a glance; [8]Most vigor seedlings under different treatments (from left to right T_0, T_1, T_2, T_3, T_4, T_5, T_7, T_8, T_9).

ta permeable to water and gases required for germination process. Though nail clipping is a good method for hard-coated seeds, in *C. indica* sandpaper rubbing provided best results than nail clipping as it allows water and gases rather slowly but effectively. Sandpaper rubbing improved the germination percentage from no germination (0%) in control to 50% for *Garuga pinnata* and from 50% in control to 80% for *Vitex glabrata* as reported by Hossain et al. (2018). Hasnat et al. (2017) reported for *Canarium resiniferum*, germination percent improved from 3% (control) to 17% after rubbed in sandpaper. In that study, soaking in water at room temperature for 24 hours provided maximum (33%) germination percent. The pre-sowing treatments with abrasion to sandpaper, nail clipping, acid may result in non-uniform seed germination behavior and may produce heterogeneity in seedling growth leading to varying in vigor and size of the seedlings (Kumar1999; Schmidt 2000) which was also observed in the present study.

Seeds soaked in HCl for 5 minutes (T_8) emerged the second-best pre-sowing treatment method. This method produced a significantly higher germination percentage (48.3%). Sometimes soaking in acid enhances germination percentage. Whenever seeds soaked sufficiently in acid may boost germination rate up to 90% in some hard-coated seeds (Msanga 2000; Alamgir and Hossain 2005; Azad et al. 2010a; Merou et al. 2011; Azad et al. 2012). But, insufficient soaking may not effective enough. Moreover, the concentration of acid and time of exposure are very critical and vary from species to species. In this study only one concentration and time of exposure to the HCl was used. Soaking seeds in HCl soften the hard coat of seeds and make the seed coat permeable to water and air (Schmidt 2000; Azad et al. 2012). After the pretreatment when seeds are sown immediately they sucked water and uptake gas easily from the soil.

Hot water treatment is effective in some hard-coated seeds like *Momordica charantia* (Hsu et al. 2003); *Parkia biglobosa* (Aliero 2004). But it depends on the heat of water and duration of soaking in it. Treatment at 100°C may be too hot for some species even though for a very short duration (Clemens et al. 1977). Sudden soaking in boiled water (100°C) may soften seed coat and allow water to permeate the seed tissue that urges seed germination (Agboola 1991; Agboola and Adedire 1998; Sabongari 2001). But when seeds soaked in boiled water for more than 4 seconds, the embryo may be destroyed due to contact with excessive heat for long time (Aliero 2004).

Seedling growth especially shoots height and collar diameter was greatly boosted by sandpaper rubbing treatment on seeds. This differs from the findings of Hossain et al. (2018) and Hasnat et al. (2017) who reported normal water soaking as the best pre-sowing treatment for seedling growth of *Vitex glabrata*, *Garuga pinnata*, and *Canarium resiniferum*. However, in this study immerging in water at room temperature for 48 hours given 46.7% germination and proved as third best method for pre-sowing treatment of *C. indica* seeds which also showed promising growth of the seedlings. Soaking seeds in water at room temperature for 48 hours slowly soften seed coat and make it permeable to water. Thus, through imbibition process water leaches into seed and swell slowly.

Conclusion

Seed pre-sowing treatments significantly affect the germination of *C. indica*. Sandpaper rubbing at the distal end established as the best pre-sowing procedure followed by soaking in 10% concentrated HCl acid for 5 minutes treatment and water treatment at room temperature for 48 hours. Among all pre-sowing treatments, seeds sown after sandpaper rubbing at the distal end provided the maximum germination rate, highest seedling survivability and produced most vigor seedlings. The result of the present study recommends the nursery owners or other seedling producer organizations to apply sandpaper rubbing method for maximum germination and better seedlings, as it is safe, less costly and more effective than acid treatment.

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