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Effect of Pre-sowing Treatments on Seed Germination and Seedling Growth of *Canarium resiniferum*, A Rare Native Tree of Bangladesh

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Abstract

Dhup (*Canarium resiniferum*) is an economically and aesthetically important rare native tree species of Bangladesh. In natural condition 78.5-98.7% seeds do not germinate due to inhibition by hard seed coat, seed predation and unfavorable environmental conditions. A study was conducted in the Seed Research Laboratory and nursery of Institute of Forestry and Environmental Sciences, Chittagong University, Bangladesh in 2013 to find out appropriate pre-sowing treatments for maximizing germination and initial seedling growth. Eleven pre-sowing treatments were provided in both the seeds sown in polybags and seeds sown in propagator house. Results revealed that, germination started at first (after 20 days of seed sown) in seeds immersed in water at room temperature for 24 hours and germination completed within 38 days. Significantly higher ($p<0.05$) germination percentage (33%), germination energy (16.7%), plant percent (33%) and germination value (0.4) was found with seeds immersed in water at room temperature for 24 hours. Seedling height measured at three, four and five months after the seed germination in case of seeds treated by immersion in water for 24 hours was also greater than others. Therefore, pre-sowing treatment by immersion in water at room temperature for 24 hours was more effective in germination and production of quality vigor seedlings of Dhup.

Key Words: *Canarium resiniferum*, pre-sowing treatments, seed, germination, seedling growth

Introduction

Canarium resiniferum Brace *ex* King (Family – Burseraceae), locally known as Dhup, is a native rare tree species of Bangladesh (Khan et al. 2001). It is a lofty, evergreen tree with tall cylindrical bole, often buttressed trunk supporting its large canopy. The plant is naturally grows in the moist semi-evergreen forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Rangamati and Sylhet districts in Bangladesh (Das and Alam 2001). Flowering and fruiting time varied from June to December. Fruits are drupe, with about 3.4-5.0 cm length, 1.5-2.8 cm width and 60-85 fruits found per kg weight. Seeds are pointed at both ends of 3-4.8 cm long, 1-2 cm width, trigonous, bony, 3-celled but usually containing single embryo and 190-345 seeds found per kg weight. Generally, 1-2% seeds found with double embryos.

Dhup is an economically and aesthetically important tree species (Johnsingh 2006). It is a Non-Timber Forest Product (NTFP) from which resins are collected (GEF 2009). Resins are collected from mature plants (Rathor and Rathor 2013). The plant is also used locally for medicine, to

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make wax, varnish, incense, turpentine, producing veneer and plywood. Fruits are also eaten by the birds (Molur et al. 2001). According to Red Data Book of Bangladesh (Khan et al. 2001), conservation status of Dhup is data deficient and needs field survey to assess the status for conservation program.

The main threat is, in natural condition 78.5-98.7% seeds of Dhup (*C. resiniferum*) do not regenerate due to hard seed coat, seed predation and disturbed environmental conditions that are not favourable to seed germination (Velho et al. 2012). But, no planting stocks are available for reforestation or enrichment plantation and no propagation techniques are recommended yet for improvement of the germination (Molur 2001). Moreover, seed coat, structural differences and anatomy directly influences the seed germination (Silva et al. 2012). Duke and Polhill (1981) reported that cotyledons of legumes can vary by morphology and function as well as in terms of their exposure at germination. To improve germination percentage and to save this economically valuable plant species from becoming extinct nursery techniques with appropriate pre-sowing treatments is the only way (Alamgir and Hossain 2005). There are some studies done by Ali et al. (1997), Haider et al. (2014), Hossain (1995) on pre-sowing treatment effects of several plantation tree species in Bangladesh. But no studies about pre-sowing effects on germination and initial seedling growth of Dhup conducted. The main purpose of this study was to find out the appropriate pre-sowing treatments that maximize total germination and produce more vigour seedlings.

Materials and Methods

Study site, seed collection, handling, and growing media

The experiment was conducted in the Seed Research Laboratory, propagator house and nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU), Bangladesh. Fruits of *C. resiniferum* were collected from Adampur natural forest at Moulavi Bazar district of Bangladesh in October, 2012. Seeds were extracted manually before sowing by de-pulping mature fruits in order to enhance germination. Brownish seeds were then dried for four days under open sun in order to reduce the moisture. Only healthy seeds were sown in polybags (15 cm×10 cm) and propagator house (a plastic-coated chamber that maintains on average 35°C temperature inside) for experiment. The media of the polybags was mixture of topsoil collected from forest floor, and cow dung in the ratio of 3:1. The media used in propagator house was fine Sylhet sand where only T_{10} was conducted considering the whole setting as a treatment.

Experimental design and treatment combination

Eleven pre-sowing treatments with three replications for each were set in the experiment. For justification of seed germination behaviour and seedling growth performances of the treated seeds a sample plot was designed with non-treated seeds called control. A completely randomized design was used. The pre-treatment methods were as follows:

T_0: De-pulped seeds without any treatment sown in polybag (Control)
T_1: Seeds with pulp and without any treatment sown in polybag
T_2: Seeds scarified with sand paper at distal end of the seed
T_3: Seeds treated with nicking/nail clipping at distal end of the seed
T_4: Seeds immersed in water for 24 hours at room temperature
T_5: Seeds immersed in hot water (at boiling point) for 1 minute
T_6: Seeds immersed in 10% concentrated H_2SO_4 for 3 minutes
T_7: Seeds immersed in 10% concentrated H_2SO_4 for 5 minutes
T_8: Seeds immersed in 10% concentrated HCl for 3 minutes
T_9: Seeds immersed in 10% concentrated HCl for 5 minutes
T_{10}: Seeds sown in propagator house (fine Sylhet sand)

Pre-sowing treatment procedure

Sand paper treatment: Thirty randomly selected healthy seeds were manually rubbed with sand paper at the distal end. *Nail clipping:* Thirty randomly selected seeds were mechanically nicked at the distal end. *Water treatment at room*

temperature: Thirty seeds were put in a beaker and soaked for 24 hours in water at room temperature. *Hot water treatment:* Thirty seeds were put in a beaker and soaked into boiled water (immediately after reaching boiling point) for 1 minute. *Acid treatment:* Randomly selected seeds were put into four separate beakers with thirty seeds in each beaker, then 10% concentrated H_2SO_4 and HCl were added to the beakers separately and left for 3 and 5 minutes respectively for both acids. After immersion, the solution was drained off and repeatedly rinsed in running tap. Seeds of control and all the treatments (T_1-T_9) were sown in polybag except T_{10} (seeds sown in fine sand) which is conducted in propagator house at comparatively higher temperature (35°C temperature).

Seed germination behaviour measurement

The germination behaviour was recorded by daily counting of germinated seeds from the date of seed sown to the end of seed germination. Germination percentage and cumulative germination percent were calculated following Kumar (1999). When the mean daily germination reached to its peak the germination percentages was determined to assess the germination energy (Dwivedi 1993). Seedlings survived at the end of the experiment were counted to determine survival percent. By multiplying peak value of germination (PV) and mean daily germination (MDG), the germination value was found. The experiments with T_0-T_9 treatments were conducted in the normal atmospheric temperature where mean annual temperature, humidity and rainfall are 25.7°C, 78.04% and 2,794 mm respectively (Climate-data 2016).

Seedling vigour measurement

At the end of three months of seed germination, three vigour seedlings from each replication were selected for measuring seedling height. Height measurement continued up to five months of seed germination. Shoot height was measured from collar region to the tip of the seedlings.

Statistical analysis

Data were statistically analysed by using the computer software package SPSS and data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT).

Results and Discussion

Germination period

Germination behaviour of *C. resiniferum* seeds was significantly affected by immersion in water at room temperature, hot water, chemical scarification and manual scarification. All the 10 treatments had varying impacts on germination. Germination was started earlier (20 days after sowing) in seeds treated by immersion in water at room temperature for 24 hours, whereas seeds sown in propa-

Table 1. Germination parameters of Dhup (*C. resiniferum*) seeds under different pre-sowing treatments

Treatments	Germination start (day)	Germination end (day)	Germination percentage (%)	Germination energy (%)	Plant percent (%)	Germination value
T_0 (Seed only)	27	39	20^{abc}*	10^{abc}	20^{abc}	0.2^{a}
T_1 (Whole fruit)	-	-	-	-	-	-
T_2 (Sand paper)	27	34	17^{abc}	10^{abc}	17^{abc}	0.1^{a}
T_3 (Nicking)	26	30	20^{abc}	13.3^{bc}	20^{abc}	0.2^{a}
T_4 (Cold water, 24 hr.)	20	38	33^{c}	16.7^{c}	33^{c}	0.4^{a}
T_5 (Hot water, 1 min.)	43	43	3^{ab}	3.3^{ab}	3^{ab}	0.01^{a}
T_6 (H_2SO_4, 3 min.)	27	39	33^{c}	16.7^{c}	33^{c}	0.3^{a}
T_7 (H_2SO_4, 5 min.)	29	50	17^{abc}	10^{abc}	17^{abc}	0.1^{a}
T_8 (HCl, 3 min.)	27	35	30^{bc}	16.7^{c}	30^{bc}	0.3^{a}
T_9 (HCl, 5 min.)	24	34	27^{abc}	10^{abc}	27^{abc}	0.4^{a}
T_{10} (Seed in propagator house)	56	64	10^{abc}	10^{abc}	10^{abc}	0.02^{a}

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

gator house took 56 days for initiation of germination. Germination of seeds continued up to 38 days in T_4 (seeds soaked in water at room temperature for 24 hours), but it took 64 days to complete germination in T_{10} (seeds sown in fine sand at propagator house). Chemical scarification has almost no effect on the period of germination initiation as it was comparable to that of control (27 days). So, it is clear that immersion in water at room temperature for 24 hours reduced the seed dormancy period. The results are in line with Hossain et al. (2005) which reported germination enhancement of *Terminalia chebula* when seeds were soaked in water at room temperature for 48 hours. In that study seeds of *T. chebula* which were morphologically similar to *C. resiniferum* started germination in 29 days and continued up to 86 (Table 1).

Germination percentage

Germination percentage of *C. resiniferum* varied significantly with the treatments. The study revealed that significantly highest (33%) germination percentage was observed in T_4 (immersion in water for 24 hours) followed by 30% in both T_6 (seeds immersed in 10% concentrated H_2SO_4 for 3 minutes) and T_8 (immersed in 10% concentrated HCl for 3 minutes) (Table 1). Germination percentage was least (3%) in T_5 (seeds immersed in hot water for 1 minute) and no germination (0%) was found in T_1 (whole fruits without any treatment sown in polybag). Hossain et al. (2005) reported highest germination percentage (66.7%) for *T. chebula* when fruits were de-pulped and soaked in water at room temperature for 48 hours and lowest germination (48.0%) in control. Azad et al. (2011) estimated maximum germination (83%) for *Acacia auriculiformis* in hot water treatment, while 52% in water treatment.

Germination energy

Germination energy was calculated to explore the speed of germination under different pre-sowing germination treatments. It varied from 3.3% to 16.7% among the treatments. Highest germination energy (16.7%) was calculated for T_4, T_6 and T_8 followed by T_3 (13.3%). Germination energy also showed that only 10% seeds were germinated by peak germination period in seeds treated with sand paper at distal end (T_2), immersed in 10% concentrated H_2SO_4 for 5 minutes (T_7), sown in propagator house (T_{10}) and control. In a similar study Hossain et al. (2005) reported maximum (58.9%) germination energy in *T. chebula* seeds soaked in water at room temperature for 48 hours.

Germination value is closely related to survival of seedlings in the field conditions. But, in this experiment germination values found for different treatments were lower and not significant. Seeds soaked in water at room temperature for 24 hours and seeds immersed in 10% concentrated HCl for 5 minutes (T_9) treatments were represented by maximum germination value (0.4) (Table 1). On the other hand, seeds immersed in hot water for 1 minute showed

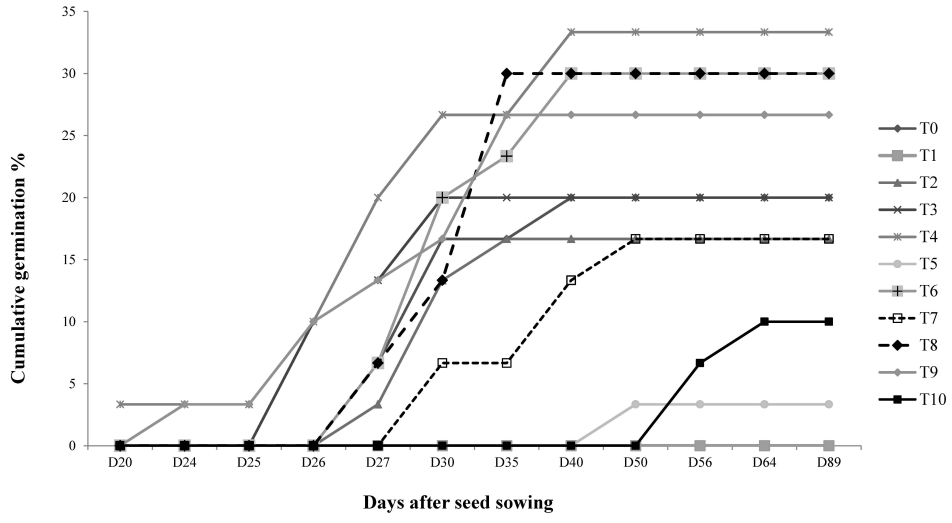

Fig. 1. Cumulative germination percentage of Dhup (*C. resiniferum*) under different pre-sowing treatments *[Here, T_0=Seeds only, T_1=Whole fruit, T_2=Seeds rubbed in sand paper, T_3=Seeds nicked by nail clipper, T_4=Seeds soaked in water at room temperature (24 hr). T_5= Seeds soaked in hot water (1 min), T_6=Seeds soaked in H_2SO_4 (3 min), T_7= Seeds soaked in H_2SO_4 (5 min), T_8=Seeds soaked in HCL (3 min), T_9=Seeds soaked in HCL (5 min) and T_{10}=Seeds sown in propagator house]*.

minimum (0.01) germination value. Hossain et al. (2005) also showed that *T. chebula* seed soaked in water at room temperature for 48 hours affects germination value.

Pattern of germination

Daily germination percentages were summed up to obtain cumulative germination percentage for each pre-sowing treatments on each assessment date. Cumulative germination percentage varied with the treatments. Germination in T_4 started after 20 days of seed sown, rose slowly and continued germinated up to 33% within 38 days. In T_3 and T_8 germination rose sharply from day 26 and reached it's highest (20% for T_3 and 30% in T_8) on day 35 and remained constant up to the end of the germination (Fig. 1).

Shoot growth

In the nursery stage, initial growth of the seedlings was observed to evaluate the effect of the pre-sowing treatments on growth and establishment of the seedlings. For each pre-sowing treatments, shoot growth was recorded at the age of 3rd, 4th and 5th months after completion of seed germination. Seeds soaked in water at room temperature for 24 hours produced vigour and straight seedlings with shoot height of 9.8 cm, 13.0 cm and 26.7 cm at the end of 3, 4 and 5 months respectively (Fig. 2). The growth parameter (shoot height) was also affected by T_6 as it produced shoot of 20.2 cm height followed by T_3 (17.6 cm) which were significantly higher than control (12.1 cm). The shoot length found in T_2 (10.9 cm) and T_{10} (6.8 cm) were lower than that's of control (12.1 cm). Hossain et al. (2005) also showed that pre-sowing treatments have significant impact on initial seedling growth, i.e. shoot length.

Discussion

Effective pre-sowing treatments like sand abrading and leaching by water enhance seed germination of hard coated seeds (Brown 1972; Villiers 1972; Bewley and Black 1982; Doran et al. 1983; Napier 1987; Palani et al. 1995; Schmidt 2000; Anand et al. 2012). Soaking *C. resiniferum* seeds in water at room temperature for 24 hours provided highest germination (33%) which supports the findings of Bebawi and Mohamed (1985) and Some et al. (1989) who reported highest germination rate of some tropical hard coated seeds by soaking in water at room temperature for 24 hours.

In the present study, seeds of *C. resiniferum* sown with pulp showed no germination at all in comparison to de-pulped seeds (33% germination) treated by immersing in water for 24 hour at room temperature (T_4). The result supports the findings of Schaefer (1989) who found seeds of some tropical and sub-tropical species completely failed to germinate without extraction from their fleshy fruit pulp. Heat damage occurred in *C. resiniferum* and germination rate was found only 3% which supports the reports of Schmidt (2000) that seeds with heat sensitive embryos may be killed by boiling water treatment.

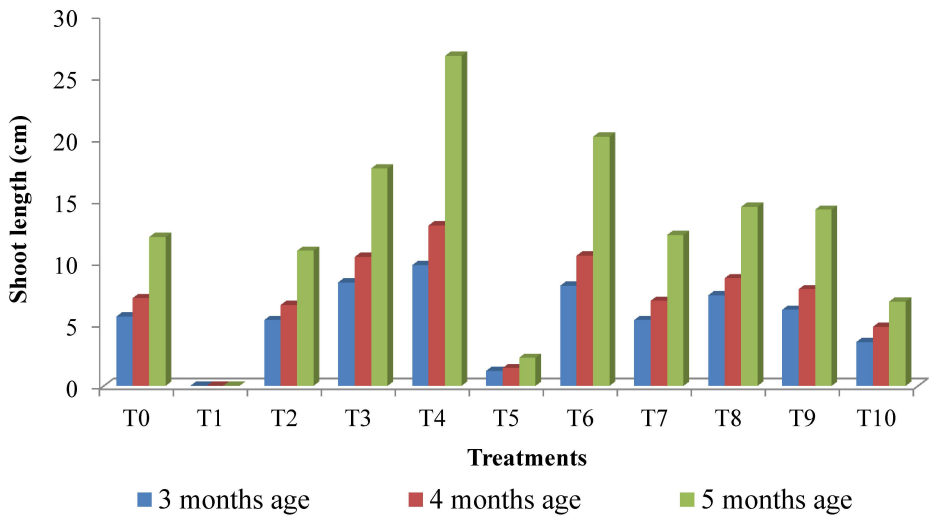

Fig. 2. Initial growth performance in terms of shoot length of Dhup (*C. resiniferum*) seedlings at different age in the nursery.

C. resiniferum immersed in 10% concentrated H_2SO_4 for 3 minutes (T_6) provided good germination percentage (30%) which supports the findings that a higher concentration sulphuric acid improves germination of hard coated seeds reported by Laurent and Chamshama (1987). Kumar (1999) and Schmidt (2000) mentioned that pre-sowing treatments with abrasion to sand paper, nail clipping, acid may result in non-uniform seed germination behaviour and may produce heterogeneity in seedlings which may lead to varying in vigour and size of the seedlings which was also observed in the present study.

Conclusion

Pre-sowing treatments may be useful for producing maximum number of quality seedlings with minimum cost, time and labour. Since the seed coat of *C. resiniferum* is hard and attack by insect pests resulting in poor regeneration in the natural condition as well as in the nursery. Though resulted germination percentage is lower in case of *C. resiniferum* the present study with pre-sowing treatments would prove itself potential in the practical fields. Among the treatments, seeds soaking in water at room temperature for 24 hours was found more effective in respect to faster germination, higher germination percentage and seedling growth of *C. resiniferum* in comparison to control and other treatments. Population of the species is greatly reduced in both the natural environment and homesteads; hence it needs immediate and effective conservation initiatives. Since, the seed treatment of soaking in water at room temperature for 24 hours is easily applicable and cost effective; the treatment may be recommended for large-scale seedling production in the nurseries for further conservation initiatives through plantation establishment.

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