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Effect of pre-sowing treatment on seed germination and seedlings growth of *Sapindus mukorossi* Gaertn. - an important medicinal plants in Bangladesh

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ABSTRACT

Germination behavior of Sapindus mukorossi Gaertn. under six different pre-sowing treatments and seedling growth performance in nursery and field conditions were investigated. The treatments were i) soaked in hot water (100^{0}C) for 10 second, ii) soaked in cold water for 12 hours, iii) soaked in cold water for 24 hours, iv) soaked in cold water for 36 hours, v) seeds scratched on the cemented floor and vi) control. Growth performances were determined by transferring the young seedling having 4-6 leaves at the age of 25-30 days, from germination bed to polybag filled with soil-cow dung mixture and followed by outplanting in the field at one year age. Germination percentage was significantly ($p \leq 0.05$) enhance by pre-sowing treatment in hot water (100^{0}C) for 10 seconds in comparison to other treatments. The survival percentage of seedling was highest (89%) in the field after one year at 2.0 m x 2.0 m spacing and average height was 94.32 cm after 2 years of outplanting. Pre-sowing treatment of seeds in hot water (100^{0}C) for 10 second for nursery raising and one year old seedlings for outplanting at 2.0 m x 2.0 m spacing in the field were found suitable for successful plantation for the species.

Key words: *Pre-sowing treatment, germination behavior, survival percentage and growth performance*

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I. Introduction

Medicinal plants are used as a source of drugs for the treatment of various health disorders all over the world from ancient times to the present day. A total of 250,000 species of flowering plants are referred to as medicinal plants. The World Health Organization (WHO) enlisted some 21,000 medicinal plant species (Penso, 1980). Ghani (2000) reported that about 500 plant species in Bangladesh identified as medicinal plant because of their therapeutic properties. Yusuf et al. (2009) reported 747 plant species have therapeutic properties.

Sapindus mukorossi belongs to the family *Sapindaceae*, an important medicinal plant in Bangladesh. It is commonly known by several names such as soapnut, soapberry, washnut, ritha and aritha. This

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is fairly large, deciduous tree with a straight trunk up to 12 meters in height with a globose crown and rather fine leathery foliage. Bark is dark to pale yellow, fairly smooth, with many vertical lines of lenticels and fine fissures exfoliating in irregular wood scales. The blaze is 0.8-1.3 cm, hard, not fibrous, pale orange brown, brittle and granular. Leaves are 30-50 cm long, alternate, per pinnate; common petiole very narrowly bordered, glabrous; leaflets 5-10 pairs, opposite or alternate, 5-18 by 2.5-5 cm, lanceolate, acuminate, entire, glabrous, often slightly falcate or oblique; petioles 2-5 m long. Inflorescence is a compound terminal panicle, 30 cm or more in length, with pubescent branches. Flowers are about 5 mm across, small, terminal, polygamous, greenish white, sub sessile, numerous, mostly bisexual, sepals 5, each with a woolly scale on either side above the claw. Fruits are globose, fleshy, 1-seeded drupe, sometimes two drupels together, about 1.8-2.5 cm across dry fruit (Chopra and Ghosh, 1946).

Sapindus mukorossi is well known for its folk medicinal values (Sharma et al., 2011). Pericarps of *S. mukorossi* have been traditionally used as an expectorant as well as a source of natural surfactant (Kasai et al., 1986). Due to the presence of saponins, soapnut is well known for its detergent and insecticidal properties and it is traditionally used for removing lice from the scalp. The fruits are of considerable importance for their medicinal value for treating a number of diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis (Kirtikar and Basu, 1991). The powdered seeds are employed in the treatment of dental caries, arthritis, common colds, constipation and nausea (Dhar et al., 1989). The seeds of *S. mukorossi* are used in Ayurvedic medicine to remove tan and freckles from the skin. It cleanses the skin of oily secretion and is even used as a cleanser for washing hair as it forms a rich, natural lather. The leaves are used in baths to relieve joint pain and the roots are used in the treatment of gout and rheumatism. The species *S. mukorossi* have become rare in Bangladesh due to habitat destruction and over exploitation. Thus, it is necessary to revamp the species in the country through plantation program.

For successful plantation program, proper nursery and plantation techniques and management systems of the species is pre-requisite. Owing to the hard covering of the seeds, their germination is slow, and therefore, direct sowing is not successful (Anon., 1972). Poor seed germination of ritha seed is one of the prime causes hinders the cultivation of the plants and deferred nursery establishment. Seed treatments can influence seed germination rate and germination process (Azad et al., 2006a; Azad et al., 2011 and Azad et al., 2012). The effect of pre-sowing treatments on seed germination of some tropical forest tree species has been reported by a number of authors (Ahamed et al., 1983; Matin and Rashid, 1992; Koirala et al.; 2000; Khan et al., 2001; Alamgir and Hossain, 2005; Azad et al., 2006b; Matin et al., 2006 and Haider et al., 2014). According to Thapa & Gautam (2006) identification of suitable pre-sowing treatment is necessary for quicker and higher seed germination. Therefore, an attempt has been made to study the effect of pre-sowing treatments on seed germination in order to recommend suitable pre-sowing treatment for *S. mukorossi*.

II. Materials and Methods

Study area: The study was carried out in the nursery of Bangladesh Forest Research Institute (BFRI), Chittagong, Bangladesh over a period of three years from July 2011 to July 2014. Geographic position of the study area is situated between 22^{0}22·27″ and 22^{0}29·0″ North latitude and 91^0 46·30″ and 91^0 46·30″ East longitudes. The climate of the study area is tropical in nature and characterized by hot humid summer and cool dry winter. The maximum and minimum temperature in the area varies from 28.3-31.9^{0}C and from 15.2-25.2^{0}C (Hossain and Arefin, 2012). Mean annual rainfall is around 3000 mm mainly occurred from June-September.

Seed collection and growing media: The seeds were collected from 15-20 years old mature healthy trees from BCSIR Laboratories, Chittagong in July. After de pulping seeds were dried in the sunlight for 1-2 days and healthy dried seeds were used for the experiment. The number of seeds per kilogram was 140-150. The germination trial was carried out by sowing seeds in germination trays filled up with soil mixed with decomposed cow dung at the proportion of 3:1 by volume. Seeds were sown in the tray at the depth of 0.5-1.0 cm.

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Experimental Design and pre-sowing treatments: Experiment was conducted in Complete Randomized Design (CRD) with three replications. To determine the effect of pre-sowing treatment on seed germination and seedling growth attributes, six different treatments were applied. The treatments were i) Soaked in hot water (100^0C) for 10 second, ii) Soaked in cold water for 12 hours, iii) Soaked in cold water for 24 hours, iv) Soaked in cold water for 36 hours, v) Seeds scratched on the cemented floor and vi) Control (seeds were sown without any treatment). In each replication 30 seeds were sown and total of 540 seeds were used for the germination trial. The trays were kept under nursery shade for three days and then exposed to partial sunlight. Watering was carried out through hand spray when necessary.

Assessment of seed germination and initial growth performance of seedlings: The effects of pre-sowing treatments on germination of seeds and seedling growth were explored periodically through counting the germinated seeds and assessing initial growth performance of seedlings. Cumulative germination was recorded after three days intervals of sowing and continued up to ending the germination (101 days after sowing the seeds). Germination phase like imbibition period was determined by counting the number of days required for the commencement of germination from the day of sowing and germination period was the number of days required for completion of germination from sowing the seeds. For assessing the growth performance, all seedlings were measured for above ground height and number of leaves was counted at the age of one month. Ten seedlings from each replicate, thus 30 seedlings from each treatment were randomly uprooted and measured for total length (root length and shoot length separately) for the assessment. Seedling vigor index (VI) was calculated according to (Abdul-Baki & Anderson, 1973) as the germination percent multiplied by total length of seedling (*i.e.* sum of shoot and root length).

Assessment of seedling growth performance in the nursery and the field: To determine the seedlings growth performance in the nursery and the field, healthy seeds were first sown in the nursery bed with soaked in hot water for 10 second. When the seedlings were about 25-30 days old (with 4-6 leaves), they were transferred to the polybags (23 cm x 15 cm in size) filled with soil mixed with cow dung (3:1). The polybags were kept under full shade for one week and then placed under direct sunlight and allowed them to grow there. When the seedlings were about one year old, 225 seedlings were out planted in the field at the beginning of the monsoon (June-July). Equal number of seedlings (225), were allowed to grow in the nursery for one year more. Data on shoot length, root length and leaf number of these seedlings were also recorded at three months, six months, twelve months and twenty four months after transferring them in polybags. Seedlings in the field were planted at 1.5 m × 1.5 m, 2.0 m × 2.0 m and 2.5 m × 2.5 m spacing at Hinguli Research Station, Chittagong, Bangladesh. 25 seedlings were planted in each replication, thus in 3 replications total 75 seedlings were planted for each treatment. The soil was sandy-loam with a pH 5.7-6.0. Average rainfall of the area was about 3200 mm and average maximum and minimum temperature was 34.7°C and 20.7°C respectively. Weeding was done at every four months in first year and at every six months in second year in the field. Survival percentage of the planted seedling in the field was determined one year after planting and height of each plant were recorded at six months, twelve months and twenty four months after planting.

Data analysis: Data were analyzed with computer software Microsoft XL to determine the significant ($p \leq 0.05$) variations among the treatments. Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) were carried out to analyze the data.

III. Results and Discussion

Seed germination and initial growth performance of seedlings

Pre-sowing treatment influence the germination period and germination percentage of *Sapindus mukorossi* seeds. The seed soaking in hot water for 10 second, showed highest germination (72%) and occurred between 34-78 days after sowing (DAS). Seed scratched on the floor showed 60% germination between the periods of 38-90 DAS. Seeds soaked for 36 hours in cold water showed 56% germination between the periods of 39-91 DAS. Seeds soaked in cold water for 12 hours

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showed 56% germination between 42-92 DAS. Seeds soaked in cold water for 24 hours showed 55% germination between 40-92 DAS. The lowest 48% germination was recorded for control between 48-98 DAS (Figure 01). The germination percentage in the seeds treated in hot water for 10 second, is significantly ($p<0.05$) higher than the other treatments, but there were no variation between seeds treated in cold water treatment 36 hours,24 hours,12 hours and seeds scratched on the cemented floor.

Soaking the seeds in water helps in softening the seed coats, removal of inhibitors and reduces required time for germination and enhances germination percentage (Hartman et al., 2007). Gupta 2003 reported that overnight soaking of *Rauwolfia serpentina* seeds in cold water offered increased germination (86%) against control (62%). *Acacia catechu* seeds showed better germination (80%) against control (62%) when the seeds are soaked in cold water for 24 hours (Haider et al., 2014). The outcomes of the present study is nearly analogous to those reported in the earlier studies mentioned here.

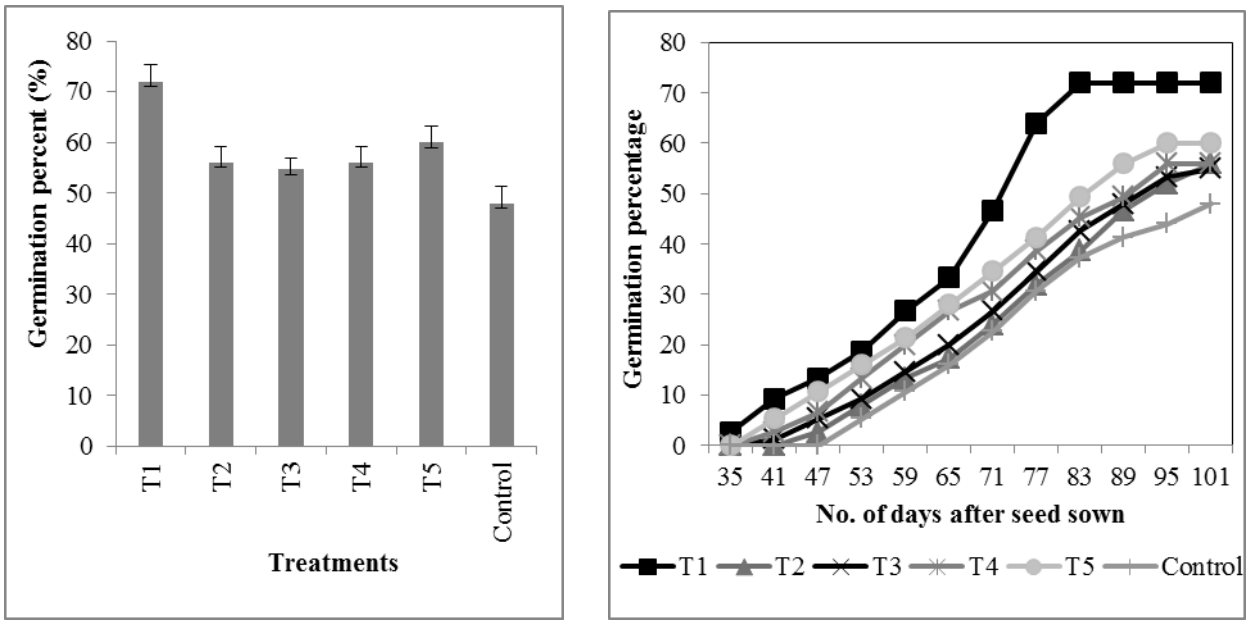

Figure 01. Germination percent (%) of *Sapindus mukorossi* with standard deviation (left) and trend lines showing progress of germination under different treatments (right) [T1= soaked in hot water for 10 seconds, T2 = soaked in cold water for 12 hours, T3 = soaked in cold water for 24 hours, T4 = soaked in cold water for 36 hours, T5 = scratched in cement floor].

Seedlings growth performance in nursery condition

The early growth performance of *Sapindus mukorossi* seedlings influenced to some extent with pre-sowing treatment. The shoot length, root length, leaf number and vigor index are shown in Table 01. The maximum root length (6.1 cm) and shoot length (9.3 cm), leaf number (5.2) and vigor index (1109) was marked with seeds treated in hot water for 10 seconds followed by seeds treated in cold water for 24 hours, seeds scratched on the floor, seeds soaked for 12 hours and 36 hours. The lowest length of root (5.4 cm) and shoot (8.5 cm), leaf number (4.5) and vigor index (667) were observed seeds with control. However, there was no significant variation in growth performance among the treatments at $p \leq 0.05$. Similar results were reported by several authors and mention that hot water treatment enhances the seed germination and seedlings growth performance in the nursery condition. Azad et al. (2011) mentioned that germination percentage, seedlings growth including root, shoot and total length of *Acacia auriculiformis* increased significantly with pre-sowing treatment especially by hot water treatment.

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The vigor index of the seedlings in the study was increased remarkably from 667 in control to 1109 in the treated seeds soaked in hot water for 10 seconds (Table 01). The vigor index usually depends on germination percentage and seedlings length. The study reveals that there was marginal variation of seedlings length among the treatment but the germination percentage with seeds soaked in hot water for 10 seconds was much higher than the other treatment which leads the vigor index considerably higher in the seeds soaked in hot water for 10 seconds than other treatment.

Table 01. Initial growth performance of *Sapindus mukorossi* seedlings germinated from different treatments one month after germination.

Growth parameters / Treatments	Root length (cm)	Shoot length (cm)	Leaf number (Nos.)	Vigor index
Soaked in hot water (10 sec.)	6.1 ± 1.10^{a}	9.3 ± 2.48^{a}	5.2 ± 0.63^{a}	1109^{a}
Soaked in cold water (12 hours)	5.6± 0.26^{a}	8.7 ± 1.57^{a}	4.6 ± 0.70^{ab}	801^{ab}
Soaked in cold water (24 hours)	5.9 ± 0.22^{a}	9.0 ± 0.32^{a}	5.05 ± 0.18^{ab}	820^{ab}
Soaked in cold water (36 hours)	5.5± 0.08^{a}	8.4 ± 2.51^{a}	4.6 ± 0.52^{ab}	778^{ab}
Scratched on the floor	5.6 ± 0.84^{a}	8.9 ± 2.37^{a}	4.9 ± 2.57^{ab}	870^{ab}
Control	5.4 ± 1.70^{a}	8.5 ± 0.80^{a}	4.5 ± 0.53^{b}	667^{b}

Treatment values associated with same letters indicates no significance difference among the treatments at $p \leq 0.05$; ± indicates standard error of mean

Seedlings growth performance in nursery condition

The experiment reveals that the germination percentage of *Sapindus mukorossi* seeds treated in hot water for 10 seconds (100^{0}C) was higher than that of other treatments (Figure 01). The initial growth performance of these seedlings including seedling length and vigor index were also higher than those in the other treatments (Table 01). Therefore, we sow only the seeds treated in hot water for 10 seconds in seed beds for assessing the seedlings growth performances in the nursery and in the field. Nine hundred seeds were sown in three blocks considering as replication of the nursery bed for the purpose. The germination percentage (73±1.2) was almost similar to the previous experiments. One month old seedlings having 4 - 6 leaves were transferred in the 15 cm x 23 cm sized polybags filled with soils mixed with cow dung (3:1) and allowed them to grow there. After one year of transferring the seedlings in the polybags, 225 seedlings were outplanted in the field (Hinguli Research Station). Rests of the seedlings were grown in the nursery for another year more. The seedlings mortality in the nursery bed, during and after transferring the seedlings to the polybag was about 2-4 percent which is very insignificant. Growth variation of seedlings was observed in the nursery in relation to age and the results are presented in Table 02.

Table 02. Seedlings growth performance of *Sapindus mukrossi* at different age (up to 24 months) in the nursery

Age of seedlings (month)	Survival %	Average length of roots (cm)	Average length of shoot (cm)	Average number of leaves per seedling
3	98	9 ± 2.79	15.5± 2.50	13± 3.27
6	98	13 ± 2.62	20 ± 5.18	20 ± 8.53
12	96	16± 5.86	33 ± 7.80	32 ± 6.98
24	96	22 ± 3.27	64 ±3.31	61 ± 8.64

The seedlings attained 15.5 cm height with average root length 9 cm and 13 number of leave in three months. The seedling achieved a height of 20 cm with 13 cm root and 20 number of leaf at six months. The average height 33 cm with 16 cm root and 32 number of leaf was recorded at 12 months. The seedlings touched 64 cm height with 22 cm root and 61 number of leaf at twenty four months (Table 02).

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Seedling survival and growth performance in the field

One-year old **s**eedlings of *Sapindus mukorossi* raised in the polybags were out planted in the field. Survival was recorded at 12 months and seedlings growth performances were determined at 6, 12 and 24 months after planting in the field and shown in Table 3. Survival percentage varied from 84-89 with an average of 87 among the treatments at 12 months after planting. The seedlings height varied from 39.4 - 42.3 cm at six months, 71.6 - 75.02 cm in one year and 84.8 - 94.3 cm in two years among the treatments.

The height growth of the seedlings was higher (94.32 cm) in 2.0 m x 2.0 m spacing in two years, and almost similar in 2.5 m x 2.5 m spacing (94.2 cm) and lowest (84.8 cm) in 1.5 m x 1.5 m spacing (Table 03). The variation of the height growth in the seedling may be due to the microclimatic condition between the spacing. The survival percentage and height growth of the seedlings in the field was satisfactory in 2.0 m x 2.0 m and 2.5 m x 2.5 m spacing. Considering the above mentioned facts and comparatively less land requirement, 2.0 m x 2.0 m spacing may be considered for planting of one year old seedlings in the field. Similar report was made by Haider et al. (2014) and mentioned that *Acacia catechu* seedlings showed satisfactory growth performance when they are planted at 2.0 m x 2.0 spacing at the age of six months, in the field. On the other hand, seedlings height in the nursery was low (64 cm) in comparison to the field condition (94.2) at two years age. Moreover, it was not realistic to maintain the seedlings in the nursery after two years of transferring in polybags due to some inevitable circumstances.

Table 03. Survival percentage and seedlings growth performances of *Sapindus mukrossi* in different spacing at Hinguli Research Station after out planting

Age of seedling / Spacing used	Survival % at 12 months	Average height (cm) at		
		6 months (cm)	12 months (cm)	24 months (cm)
1.5 m × 1.5 m	84 ± 2.30^{a}	39.45±6.86^{a}	71.63±2.98^{a}	84.80±7.86^{a}
2.0 m × 2.0 m	89 ± 2.30^{a}	42.32±8.68^{c}	75.02±10.56^{b}	94.32±4.47^{b}
2.5 m × 2.5 m	88 ± 1.33^{a}	41.11±4.52^{b}	74.56±8.39^{b}	94.20±4.69^{b}

Means followed by same letter (s) are not significantly different at p≤ 0.05, according to Duncan's Multiple Range test (DMRT), ± indicates the standard error of the mean.

IV. Conclusion

Owing to hard seed coat *Sapindus mukorossi* seeds usually exhibit slow and poor germination rate. The pre-sowing treatment of seed influences the germination percentage under nursery condition. Seeds start germination after 34 days of sowing and complete within 101 days. Maximum germination and highest initial growth performance was perceived in seeds treated with hot water (100^{0}C) for 10 seconds which was much higher than other treatments. Pricking of the seedlings at 25-30 days after germination from nursery bed to polybag ensures minimum mortality. Survival of the seedlings (89%) and growth performance of the seedlings in the field was satisfactory after out planting one year old seedlings at 2.0 m x 2.0 m spacing. Therefore, pre-sowing treatment of seeds with hot water (100^{0}C) for 10 seconds is suitable for seedling raising in the nursery and one year old seedlings at 2.0 m x 2.0 m spacing may be suggested for plantation program.

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