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Germination and Initial Growth Performance of *Aphanamixis polystachya* (Wall) Parker -A Threatened Medicinal Tree Species in Bangladesh

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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 *Aphanamixis polystachya* seeds at the nursery of Chittagong University, Bangladesh. Seeds were treated to six pre-sowing treatments e.g., Control (T_0) , seeds immersed in normal water for 48 hours (T_1) , seeds immersed in normal water for 24 hours (T_2) , seeds immersed in hot water for 1 minute (T_3) , seeds sown in propagator house (T_4) , seeds sown in an open nursery (T_5) . Germination percentage, Germination index, Germination value, shoot length and leaf number were assessed for 2- and 3-months old seedlings in the nursery. Highest germination percentage (85.71%), germination energy (33.33 %), and germination value (1.188) were found in T_4 treatment (seed sown in propagator house) and significantly ($P< 0.05$) different from other treatments. Maximum germination index (0.2690), germination uniformity (0.0113), and minimum germination start time (16 days) observed in T_4 treatment. After 3 months of the last germination, maximum shoot height (21.94 cm) and leaf number (8.2) revealed highest in T_2 (seeds immersed in normal water for 24 hours) treatment. Finally, seeds sown in propagator house (sand media) were revealed comparatively better germination behavior than other treatments. Seeds treated with normal water for 24 hours were found suitable for vigor seedlings production for *Aphanamixis polystachya*.

Keywords: Pre-sowing treatments, Germination, Propagator house, Germination value, Vigor seedlings.

Introduction

Medicinal plants are the life-giving elements of forest products and play a substantial role in the health care of rural people all over the world. They provide us vital therapeutic agents and raw materials for the manufacture of traditional and modern medicines (Ghani, 2003; Islam *et al*., 2016). According to WHO (World Health Organization), there are approximately 21,000 medicinal plant species all over the world (Penso, 1980). About 500 plant species including trees, herbs and shrubs in Bangladesh used as medicinal plant due to their therapeutic properties (Ghani 2000). Yusuf *et al*., (2009) recorded 747 plant species in Bangladesh have therapeutic properties.

Aphanamixis polystachya (Wall) Parker, belonging to Meliaceae family, a valuable medicinal plant of Bangladesh. Large evergreen tree, with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8m in width (Fabricant and Farnsworth, 2001). Vernacular names used in Bangladesh are Pitraj, Baiddiraj, Bajor, Bajor, Pitti, Royna, Amoora, Tiktaraj (Uddin and Hassan, 2018). Flowering and fruiting occur in February-May. Fruit a capsule, obovoid, 2-4 cm in diameter, yellowish at first, pink or red at maturity. Seeds 1-3, cover with brownish-red or orange oily aril (Hossain *et al*., 2017). The seed has rich oil content which is non edible but a future source for biodiesel (Ferdous *et al*., 2013). It occurs naturally in Bangladesh, India, Pakistan, Sri Lanka, Nepal, Bhutan and Myanmar. In Bangladesh, it grows in the forests of Chattogram, Cox's Bazar, CHT, Gazipur, Sherpur, Tangail and Sylhet districts. According to Rai, (2014) *A. polystaycha* is an endangered tree species.

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A. polystachya root and bark used in abdominal complaints like enlargement of liver, glands and spleen disorders and corpulence (Apu *et al*., 2013b). Seeds have refrigerant, anthelmintic activities, laxative and used against the diseases of the blood and scale back muscular pain (Apu *et al*., 2013b). Oil of the seeds is used to treat rheumatism and jointly has pesticidal appeal. Bark and seeds of the plant are beneficial for ulcer treatment (Hossain *et al*., 2009). The tree plays very important role for various fields such as stabilization, commercial planting, erosion control, roadside plantation, environment management, shade tree and urban greening (Orwa *et al*., 2009).

This species has huge medicinal, economic and ecological value but due to degradation of forest and insufficient natural regeneration the species is becoming rare in Bangladesh. However, there are many researches to find out the best pre-sowing technique of many species (Hasnat *et al*., 2017; Nandi *et al*., 2020; Dey *et al.,* 2020 etc.). But information of seed pre-sowing treatment and initial seedling growth performance of *Aphanamixis polystachya* for ensuring their early and successful germination is absent, but this is very essential for efficient nursery management and plantation establishment of this species. In this circumstance, the study was conducted to find out the suitable pre-sowing treatments for maximum germination rates and initial seedling growth in order to improve nursery techniques and conserve this rare native species having high medicinal, ecological and economic value.

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 1). 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).

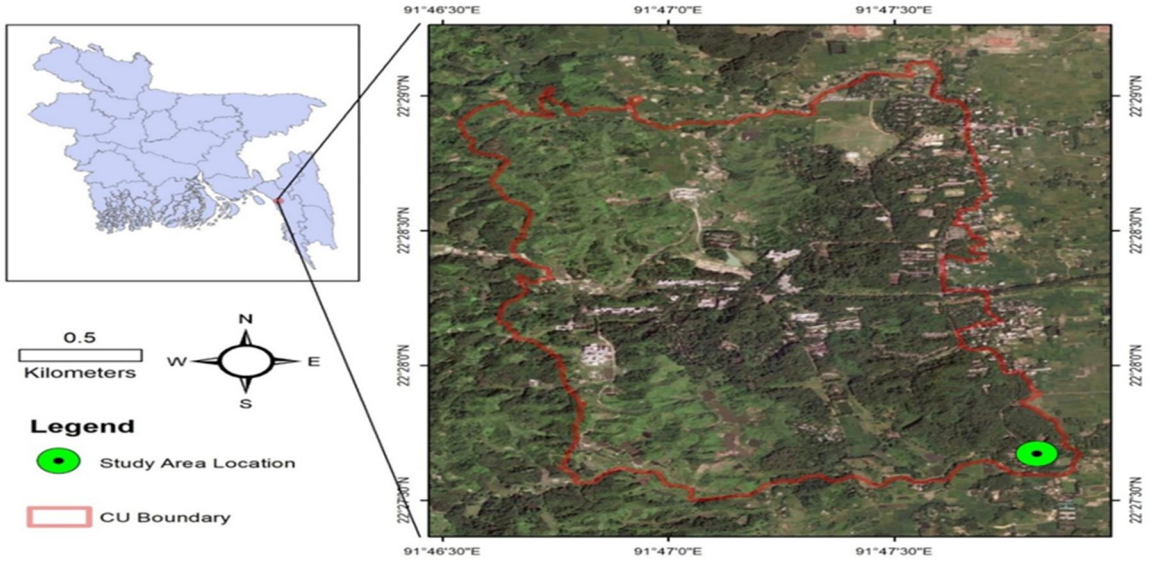

Fig. 1: Map showing the location of nursery of IFESCU (Institute of Forestry and Environmental Sciences, University of Chittagong)

2.2. Seed collection

Aphanamixis polystachya fruits were collected from University of Chittagong, Chattogram during March 2019. Phenotypic characteristics of fruits and seeds were measured. Seeds were extracted from fruits, dried in the open sun for three days. Randomly selected seeds information was recorded.

2.3. Experimental design

The soil used for filling polybags were collected from the forest floor, dried and sieved well (<3mm) 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. Collected seeds were sown in respectively prepared polybag, open nursery bed and propagator house. The study was made up of 6 treatments 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 1 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. Shoot height and leaf number of the seedlings were recorded after 2nd and 3rd month of last germination. The pre-sowing treatments are as:

T_0- Seeds sown in soil filled polybags without treatment (control)
T_1- Seeds soaking in normal water for 48 hours and sown in polybags (room temperature 24ºC)
T_2- Seeds soaking in normal water for 24 hours and sown in polybags (room temperature 24ºC)
T_3- Seeds soaking in hot water for 1 minutes and sown in polybags (boiled water)
T_4- Seeds sown in propagator house, and
T_5- Seeds sown in an open nursery bed

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).

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

2.4.2. Cumulative germination % (CGP)

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

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

2.4.3. Germination energy (GE)

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

2.4.4. Germination index (GI)

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

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

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:

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

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).

2.4.6. 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).

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

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 the different tables.

3. Results

3.1. Morphological features of seeds

The average seeds length and width was found 1.612±0.04 cm and 1.506±0.03 cm respectively. About 629 seeds were found per kg (Table 1).

Table 1: Seed length, width and number of seeds per kg of *A. polystachya* seeds

	Length (cm)	Width (cm)	Weight/seed (g)	Seeds/kg
Average	1.612±0.04	1.506±0.03	1.59±0.08	629

±Indicates the standard error of mean.

Date of germination starts and ends varied among different treatments. Germination starts after 16 days in T_4 treatment and T_0 required maximum time (22 days) to initiate germination. Highest germination percentage (85.71%) was found in T_4 and lowest 57.14% in T_1 and T_5. T_4 was significantly ($p<0.05$) different from other treatments. Lowest germination period (20.33 days) recorded in T_4 treatment followed by 21.67 days in T_2 but there was no significant difference among treatments (Table 2).

Table 2: Effect on germination behavior of *A. polystachya* seeds in different pre-sowing treatments

Treatments	Germination starts after (days)	Germination end after (days)	Germination period (days)	Germination (%)	Germination capacity
T_0	22	35	25.33 a*	61.90 b	Average
T_1	21	31	26.33 a	57.14 b	Average
T_2	20	31	21.67 a	71.43 b	Good
T_3	18	30	23.00 a	66.66 b	Average
T_4	16	31	20.33 a	85.71 a	Very good
T_5	18	32	23.00 a	57.14 b	Average

***** 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).

Maximum germination energy (33.33 %) was revealed in T_4 and T_2 and minimum germination energy (23.81%) in T_0 and T_3. Highest germination index (0.2690) was found in T_4 and there was no significant difference among treatments. Highest germination value (1.188) was recorded in T_4 treatment and significantly ($P< 0.05$) different from other treatments. Mean germination time found minimum in T_4 (23.16), slightly vary from T_2 and T_5 and maximum in T_1 (28.27) (Table 3).

Table 3: Effect on germination behavior of *A. polystachya* seeds in different pre-sowing treatments

Treatments	Germination Energy (%)	Germination Index (GI)	Mean Germination Time (MGT)	Germination Uniformity (GU)	Germination value
T_0	23.81 a*	0.1619 a	27.53 ab	0.0040 b	0.5661 b
T_1	28.57 a	0.1449 a	28.27 a	0.0029 b	0.5303 b
T_2	33.33 a	0.2048 a	24.83 bc	0.0049 b	0.8213 ab
T_3	23.81 a	0.1854 a	25.81 ab	0.0054 b	0.7444 ab
T_4	33.33 a	0.2690 a	23.16 c	0.0113 a	1.188 a
T_5	28.57 a	0.2061 a	24.50 bc	0.0055 b	0.5794 b

*****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).

To obtain cumulative germination percentage for each treatment, daily germination percentages were summed. Cumulative germination of T_4 starts after 16 days of seed sown and rose rapidly and continued up to 85.71% within 31 days. After 22 days of seed sown, T_0 treated seeds starts germinated and achieved 61.90% germination percentage (Fig. 2).

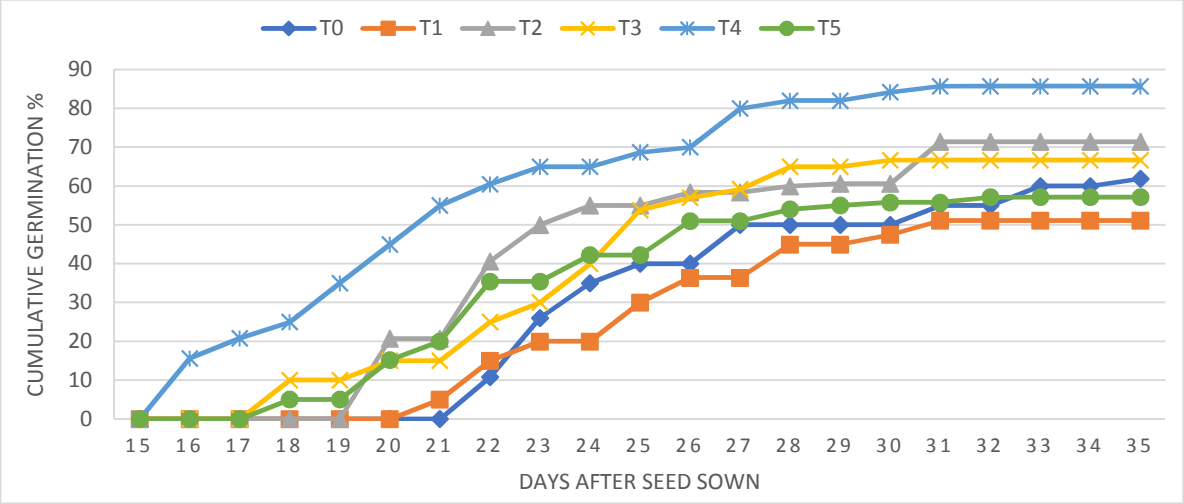

Fig. 2: Cumulative germination percentage of *A. polystachya* under different pre-sowing treatments

Shoot height was recorded at 2 and 3months old seedlings. The highest mean shoot height (21.94 cm) attained in T_2 treatment (soaking in cold water for 24 hours and sown in polybags) and lowest (17.84 cm) in T_5 treatment (Seeds sown in seedbed). Mean maximum number of leaf (8.2) produced in T_2 and minimum (5.8) in T_4 treatment (Table 4).

Table 4: Mean shoot height and leaf number of *A. polystachya* seeds in different pre-sowing treatments

Treatments	Mean shoot height (cm)		Leaf number	
	2 months	3 months	2 months	3 months
T_0	15.2±1.15	18.46±0.99	5.6±0.6	6.2±0.97
T_1	15.7±0.44	19.5±0.71	7±1.30	7.6±0.51
T_2	19.1±0.87	21.94±0.59	7.6±0.244	8.2±0.58
T_3	18±1.44	21.76±1.19	6.6±0.4	7±0.45
T_4	16.9±0.23	18.1±0.26	4.8±0.26	5.8±0.26
T_5	14±0.67	17.84±0.62	6±0.55	7.4±0.68

Fig. 3: Seeds of Aphanamixis polystachya

Fig. 4: Germination *A. polystachya* in propagator (fine Sylhet sand).

Fig. 5: *A. polystachya* seedlings raised in propagator house.

Fig. 6: *A. polystachya* seedlings raised in polybag

Discussion

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

The present findings of the study on *Aphanamixis polystachya* found that seed sown in propagator house (T_4) provided highest germination percentage 85.71% and control (61.90%). Highest germination energy (33.33 %), germination value (1.188), germination index (0.2690), germination uniformity (0.0113), and lowest germination start time (16 days) observed in T_4 treatment. According to Saboo *et. al*., (2014), *A. polystachya* seed germination ranges from 80-90% in 20-22 days after sowing and seed loses its viability quickly in 2-3 months. Present study supports Nandi *et al*., (2019) who found germination percentage was found highest (100%) in seeds sown at propagator house for a native tree species *Lithocarpus elegans.* This study supports the findings of *Acacia* spp. where sand is a suitable medium for seed germination (ISTA, 1993). Nandeshwar and Patra (2004) found that soil, sand and compost in the ratio of 1:1:2 is the best for growth and survival of *Acacia catechu* seedlings. *Suregada multiflora* showed maximum germination and survival percentage in propagator (Dey and Hossain, 2019). Sand as a germination substratum is preferred for large seed producing tree species (Magini, 1962). In case of, mean shoot height and leaf number T_2 (seeds soaking in normal water for 24 hours) revealed maximum and seed sown in open nursery bed showed lowest mean shoot height and seed sown in propagator house showed lowest leaf number. As sand media does not contain nutrients, so it failed to show best growth performance. So, after attaining certain height (1 or 2 months after germination) seedlings need to transfer polybag which contain soil and cow dung mixture 3:1. It will help to produce vigor seedlings for large scale plantation. Hasnat *et al*., (2017) revealed that seeds soaking in water for 24 hours was the more effective in germination and vigor seedlings production of *Canarium resiniferum* species. So, from the study for *A. polystachya* species, propagator house is recommended to get maximum germination and seeds soaking in normal water for 24 hours for vigor seedlings production.

4. Conclusion

Seed pre-sowing treatments significantly affect the germination of *Aphanamixis polystachya.* Seeds sown in propagator house in controlled environment and sand media showed maximum germination behaviors followed by seed treated with normal water at room temperature for 24 hours. After attaining certain height seedlings need to transfer from propagator to polybag with soil and cow dung media to get vigor and maximum seedlings for afforestation and reforestation programs.

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