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Enhancing seed germination in *Altingia excelsa* Noronha. pre-treated by natural plant extracts under laboratory conditions at Pasighat in Arunachal Pradesh, India

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Abstract
Altingia excelsa Noronha. tall majestic tree, long straight cylindrical bole, belongs to the family Hamamelidaceae, classified as a valuable and durable hardwood timber species. Investigations were carried out on the effect of different pre-sowing treatment to get the improved germination rates under laboratory conditions in the year 2021. The experimental setup was a completely randomized design (CRD) constituting thirteen pre-treatments and four replicates. Among various treatments, soaking of fertile seeds in *Aloe vera* pulp extract 25% and 50% for 24 hours resulted into higher germination 64 to 66.33% followed by *Moringa oleifera* bark extract with 55% as compared with 24.67% for the control treatment. The findings indicate natural plant extracts concurred better response for production of seedlings with minimum cost and time for reforestation and afforestation programmes of the species.

Keywords: *Altingia excelsa*, pre-sowing treatments, germination, *Aloe vera*, *Moringa oleifera*

Introduction
Altingia excelsa Noronha. belonging to the family Hamamelidaceae, commonly known as jutuli (Assam, India); mala, rasamala, tulasan (Indonesia) and nantayok (Mayanmar) occurs naturally in tropical humid mixed hill and montane forests, distributed from the Assam (India) eastward through Myanmar to Southeastern China, Thailand, Cambodia, Laos, Vietnam, Malaysia and Indonesia (Phengklai 1985; Ferguson 1989; Ickert-Bond and Wen 2013) [35, 16, 23] is valued for its timber, aromatic resin, and the bark and roots being used in traditional Chinese medicine (Vink 1957; Zhang *et al*. 2003; Ickert-Bond *et al*. 2007) [49, 53, 22]. It is a tall majestic, high branched, emergent evergreen tree, with a dense crown and straight cylindrical bole attaining a height of about 50-60 meters and 150-185cm diameter (Soerianegara *et al*. 1994) [46]. Leaves alternate, elliptic to ovate to ovate-lanceolate, 6-12 cm long, 2.5-5.5 cm wide, with finely toothed margins and glabrous above. The tree is monoecious, bearing unisexual flowers and producing roundish light brown fruits, puberulous at the apex, woody heads, 0.9-1.1 cm diameter, stalks 2.0-3.0 cm long, a four-compartment capsule that opens at maturity during February (Phengklai 1985) [35]. Each of the cell contains one or two fertile seeds and upto 35 sterile seeds in each of the 4 compartments. The fertile seeds are flattened and surrounded by a narrow wing with contrast to the sterile seeds which are wingless, reduced sized and irregularly prismatical. The seeds contain oil and have sweetish scent which attracts animals and may contribute to the dispersal as well, by eating the seeds (Vink 1957; Pramono and Djam'an 2003; Zhang *et al*. 2003; Orwa *et al*. 2009; Ahmad *et al*. 2017) [49, 37, 53, 33, 1]. The leaves possess an agreeable aromatic odour and young flushes are also eaten as vegetable in West Java (Ochse and Brink 1931; Phengklai 1985) [31, 35]. The fragrant gum is produced after injury of the bark and sapwood which is used in perfumery, incense and for local use (Sakai and Rumbino 1987) [42] and in South East Asia, the plant has been used as tonic remedy for couch and chest complaints (Soerianegara *et al*. 1994; Wiart 2006) [46, 50]. However, the primary use of *jutuli* is for its wood, classified as a valuable and durable hardwood timber species that has a specific gravity of 0.81 being under 'strong' strength class (Anon. 1988; Febryano *et al*. 2021) [3, 14]. The constructional timber is obtained in large dimensions make it lucrative for trade and is used for bridges, buildings, many heavy constructions works like boat and ship building, flooring works and different kinds of construction and furniture making (Vink 1957; Anon. 1923; Rinyo *et al*. 2018) [49, 4, 41].

The tree is removed by selective cutting for obtaining commercial timber throughout many of the natural forests. This high-grading felling systems erodes and degrades the genetic resource which is not in the best interest of our natural resources (Vink 1957; Smallidge and Greason 1997; Kenefic 2014) [49, 45, 29]. To avoid the depletion of resources, *Altingia* regeneration should be promoted by enrichment planting (Soerianegara *et al*. 1994) [46]. Deb and Sundriyal (2007) [10] reported natural regeneration of *A. excelsa* arises in gaps and not in the understorey areas studied in Namdapha National Park, Eastern Himalaya, India. However, the natural regeneration takes place in disturbed habitats in forest and, on the other hand recalcitrant seeds are viable only for a short period (Vink 1957; Orwa *et al*. 2009; Pramono and Djam'an 2003) [49, 33, 37]. For the imminent availability of commercial timber, plantation programme has been employed by Indonesian Forest Service (Ferguson 1937; Vink 1957) [15, 49]. The success of plantation depends on the raising planting material, which requires stimulus pre-sowing practice to ample the germination process. The use of natural hormones available as coconut milk, honey, *Moringa*, *Aloe vera*, are economic, environmental friendly and can be used as a substitute for synthetic plant growth hormones (Phiri and Mbewe 2010; Emongor 2012; El-Sherif *et al.* 2017; Rajan and Singh 2021) [36, 13, 12, 38]. The use of efficient seed pre-treatment maximizes germination percentage and caters the availability of nursery planting stock for planting programmes to overwhelm the slow process of natural regeneration of many tropical tree species (Omokhua *et al*. 2019; Koirala *et al*. 2000; Alamgir and Hossain 2005; Thapa and Gautam 2006) [32, 30, 2, 48]. There is no published information on seed pre-treatment of *A. excelsa* and to fill the knowledge gap, this study was undertaken to investigate the appropriate pre-sowing treatments for satisfactory germination rates for seedling production in the nursery.

Materials and Methods

A study was undertaken in laboratory conditions at the College of Horticulture and Forestry, Central Agricultural University (I), Pasighat, East Siang District, Arunachal Pradesh, India situated at 28°04′43″ N latitude and 95°19′26″ E longitude with an altitude of 163 msl. to investigate the influence of different seed pre-treatment on the germination of *Altingia excelsa* Noronha. The mature fruits of *A. excelsa* was collected during the month of February 2021, from Leging village, Sile Korong area (28°5′41.63″N latitude; 95°17′26.52″E longitude at 243 msl) East Siang District, Arunachal Pradesh. The collected fruits were dried in shade for one week then threshed and sieved to discard husk and impurities. To get the fertile seeds, it is identified by its surrounded narrow wing which is flattened and light brown, found mixed along with the sterile prismatical seeds.

Extract Preparation: Extracts or juices of aloe vera, moringa oleifera, coconut milk and honey were prepared as follows:
Aloe vera: Fresh healthy *Aloe vera* leaves were collected for gel extraction, after the removal of its outer covering the pulp was sliced in small pieces and then blended for 3 minutes to make it uniform (Githiori *et al*. 2003) [19] and the mucilage was squeezed powerfully strained through a muslin cloth. The extracted filtered juice was obtained in a glass beaker and water extracts were obtained by diluting the juice with distilled water. (Anon. 1967; Hanafy *et al*. 2012; Zeljkovic *et al*. 2020) [5, 21, 52]. For obtaining 25% aqueous solution, pure aloe juice extract of 25 ml was dissolved in 75 ml of distilled water and similarly made for 50% solution which was immediately used for pre-treatments (Ramachandra and Rao 2008) [39].

***Moringa oleifera*:** Moringa oleifera bark extract was prepared from the scraped fresh bark from mature tree with the help of sharp knife, cleaned in running tap water and rinsed with distilled water. The bark was air dried in shade under controlled environmental conditions. The fully dried bark was grinded by an electrical grinder into fine powder. The powder was mixed with distilled water at a ratio of 1:10 (w/v). The mixture was shaken by an electrical stirrer for four hours, then mixture kept in dark at room temperature for 24 hrs. The extract was purified by filtering through Whatman No. 1 paper. The extracts served as the stock solutions (100%) for different treatment concentrations (Sarmin 2014) [43].

Coconut Milk: The preparation of pure coconut milk involved the liquid extract from crushed white fleshy coconut endosperm. The coconuts were cracked into pieces, deshelled and the brown skin was removed away to get the white kernel. The cleaned kernel was grated and the shredded pulp was blended and homogenized smoothly to a viscous slurry and was manually squeezed forcefully allowing the milk to filtered through a double layered cheese cloth getting only the clean coconut milk into a beaker. (Gapasin-Catada *et al*. 2016; Kate *et al*. 2017) [17, 27].

Honey: Honey solutions were prepared prior to the experiment by diluting the pure honey using sterile distilled water at different concentrations (v/v) and two concentrations of honey were prepared (25% and 50%) by dissolving the respective volumes of honey into corresponding volumes of sterile distilled water.
For different concentrations, the extracts (coconut milk, *Aloe vera*, *Moringa oleifera* bark) and pure honey was diluted as follows: 25% dilution (25ml extract: 75 ml water) and 50% dilution (50:50 extract: water). For germination parameters the seeds were subjected to thirteen treatments with four replications each and one hundred fertile seeds per replication was laid out in a completely randomized design (CRD) using standard methods. Fresh seeds were subjected to the following treatments: T_1 -control (untreated seed); T_2 - seeds soaked in coconut milk (25%) for 24 h; T_3 - soaked in coconut milk (50%) for 24 h; T_4 -soaked in *Moringa oleifera* bark extract (25%) for 24 h; T_5 -soaked in *Moringa oleifera* bark extract (50%) for 24 h; T_6 -soaked in honey (25%) for 24 h; T_7 - soaked in honey (50%) for 24 h; T_8 -soaked in *Aloe vera* pulp extract (25%) for 24 h; T_9 -soaked in *Aloe vera* pulp extract (50%) for 24 h; T_{10} - soaked in hydrogen peroxide (H_2O_2 1%) for 24 h; T_{11} - soaked in hydrogen peroxide (H_2O_2 2%) for 24 h; T_{12} -soaked in gibberellic acid (250 ppm) for 24 h; T_{13} -soaked in gibberellic acid (500 ppm) for 24 h. The plastic germination trays (48×35×8 cm) were filled with a mixture of soil, sand and farm yard manure in the ratio 3:1:1 and the fertile seeds were sown at a depth of 0.4 cm to 0.6 cm in the laboratory condition in the month of March, 2021. The trays were sprinkled water as per need to maintain adequate moisture content. Germination (epigeous) started from 8th day after sowing to a maximum of 30th day during the germination study and the data was recorded on daily basis until all germination process was over. The seeds were considered germinated when visible shoot was seen emerged on the

surface. The various germination parameters at the end of the experiment were calculated using the following methods:

Germination percentage: The germination percentage was calculated using the formulae (ISTA 2003) [25]:

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

Peak value, germination value and germination speed: Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test. Germination value is a composite value combining both germination speed and total germination providing an objective means of evaluating the results of germination test was calculated using the formula of Czabator (1962) [9].

Germination Value = Final DGS x Peak value; where DGS is (Daily Germination Speed)

$$\text{Germination Speed} = N_1/d_1+N_2/d_2+N_3/d_3+ \ldots\ldots\ldots\ldots + N_n/d_n$$

Where, N- number of germinated seeds, d- number of days.

Germination energy and energy period: Germination energy (GE) was calculated on the basis of percentage of total number of seed that had germinated when germination reached its peak, and the Energy Period was taken up to the day of peak germination (Seward 1980; Willan 1987) [44, 51].

$$GE = \frac{\text{Number of seed germinated upto the time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

Data analysis: The data obtained for germination parameters were statistically analysed using the analysis of variance (ANOVA) procedure for the significance of the treatments and the differences between the means were compared by Fisher's least significant difference test at 0.05 level following the model suggested by Panse and Sukhatme (1985) [34]. Result data lying beyond the range (in per cent) were transformed to arcsine-square-root-transformation values before statistical analysis, and the means of the germination parameters was tested using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez 1984) [20] to explore the significance level among the means of various treatments.

Results

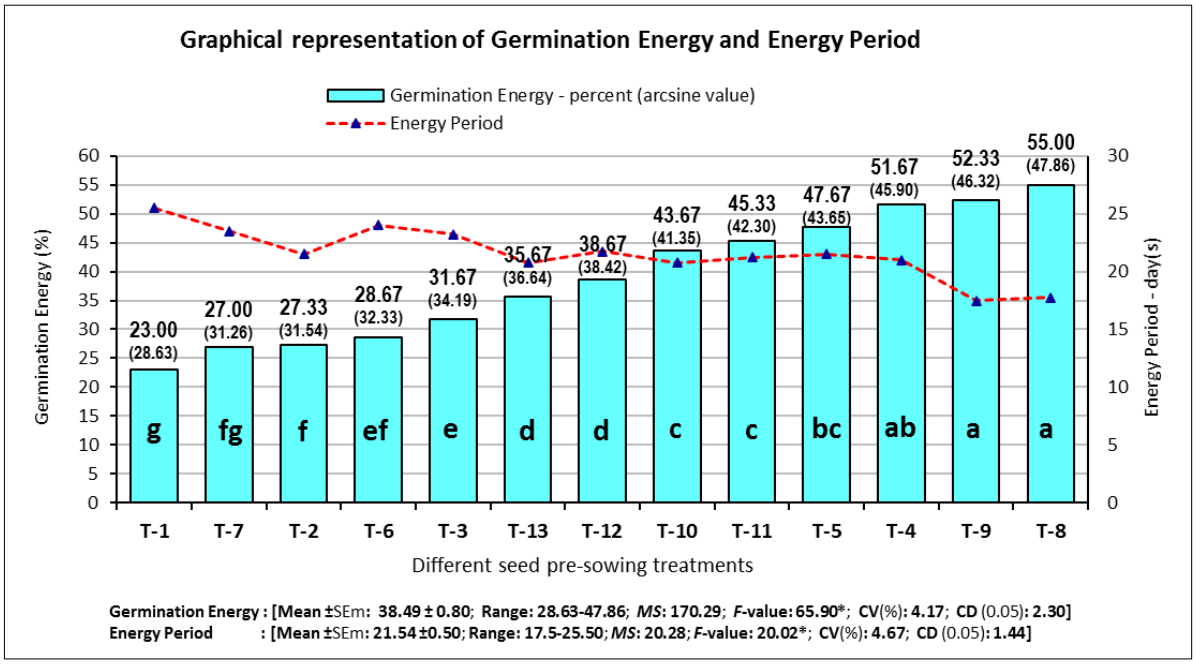
It is evident from the summary results (Table 1) for germination percentage, peak value, germination speed, germination value and energy period of *Altingia excelsa* Noronha. seeds was greatly influenced by various pre-treatments at laboratory conditions. Significant differences ($p \leq 0.05$) were observed among the different treatments for the germination parameters. The result of this study clearly showed variation in germination percentage ranged from 24.67 to 66.33%. However, the maximum percentage germination of seed treated with *Aloe vera* pulp extract T_8 achieved 66.33% and T_9 was on par at 64%, followed by T_4 (55%) seeds treated with *Moringa oleifera* bark extract and T_5 (52.33%), were significantly higher in comparison with other treatments. The percentage germination for control (T_1) partake the lowest of 24.67%. Germination value varied from 0.74 to 7.71 among the treatments, the highest germination value was found in T_8 (7.71) and T_9 (7.36) followed by T_4 (5.42). Similarly, the highest peak value and germination speed for treatments T_8, T_9, and T_4 were significantly greater than other treatments, with lowest value exhibited by T_1 (control) for all the germination parameters studied.

Table 1: Effect of seed pre-sowing treatment on germination parameters of thirteen treatments under laboratory conditions.

Treatments	Germination percent (arcsine value)	Peak value	Germination value	Germination speed
T_1	24.67 (29.75) ^{f}	0.90 ^{f}	0.74 ^{f}	0.94 ^{g}
T_2	31.00 (33.81) ^{e}	1.28 ^{e}	1.53 ^{e}	1.35 ^{e}
T_3	29.67 (32.97) ^{e}	1.26 ^{e}	1.50 ^{e}	1.31 ^{ef}
T_4	55.00 (47.86) ^{b}	2.46 ^{b}	5.42 ^{b}	2.69 ^{b}
T_5	52.33 (46.32) ^{bc}	2.24 ^{c}	4.52 ^{c}	2.34 ^{c}
T_6	30.00 (33.16) ^{e}	1.20 ^{e}	1.34 ^{ef}	1.22 ^{ef}
T_7	28.33 (32.13) ^{ef}	1.15 ^{e}	1.22 ^{ef}	1.15 ^{f}
T_8	66.33 (54.53) ^{a}	3.10 ^{a}	7.71 ^{a}	3.61 ^{a}
T_9	64.00 (52.51) ^{a}	2.99 ^{a}	7.36 ^{a}	3.45 ^{a}
T_{10}	48.33 (44.02) ^{c}	2.11 ^{c}	3.93 ^{c}	2.20 ^{c}
T_{11}	50.00 (44.97) ^{c}	2.13 ^{c}	3.95 ^{c}	2.24 ^{c}
T_{12}	41.33 (40.08) ^{d}	1.78 ^{d}	2.96 ^{d}	1.77 ^{d}
T_{13}	39.33 (38.81) ^{d}	1.72 ^{d}	2.73 ^{d}	1.69 ^{d}
Mean ± SEm (Range)	40.84±0.78 (29.7 54.53)	1.87±0.05 (0.90-3.10)	3.45±0.22 (0.74-7.71)	2.00±0.05 0.94-3.61
MS	267.99	2.01	21.34	2.95
CV (%)	3.84	5.90	12.72	5.81
F- value	108.83 *	165.13*	110.45*	219.49 *
CD ($p \leq 0.05$)	2.25	0.15	0.63	0.16

Note: Value denoted with the same letter(s) are not significantly different at $p \leq 0.05$ probability level according to Duncan's Multiple Range Test (DMRT). Values with (*) are significantly different at $p \leq 0.05$

Variation in germination energy with respect to energy period were elucidated in figure 1. to explore the speed of germination under different pre-sowing germination. The maximum germination energy 55%, 52.33% followed by 51.67% was observed in T_8, T_9, and T_4 respectively, and the minimum value exhibited by untreated seeds T_1 (23%). Energy period ranged between 17.5-25.50 days, displayed graphically (fig.1) for the maximum energy period (25.50) recorded in T_1 (control) appended with minimum germination energy, while minimum energy period was revealed in T_9 (17.50) and T_8 (17.75) with higher germination energy. This contrary trend of germination energy with regards to energy period was depicted significantly amongst the pre-sowing treatments ($p \leq 0.05$).



Note: Bars with same letter(s) for germination energy are not significantly different at $p \leq 0.05$ probability level according to Duncan's Multiple Range Test (DMRT).Values with (*) are significantly different at $p \leq 0.05$; ±SEm, standard error of the mean; *MS*, mean square value; *F*-value, ratio of variances (*Fisher* analysis of variance); CV, coffiecient of variation; CD, critical difference at 0.05 level, and the bars exhibit on percent value.

Fig 1: Germination energy (*Ascendlingly*), and energy period for different pre-treated seeds of *Altingia excelsa* Noronha. under laboratory conditions.

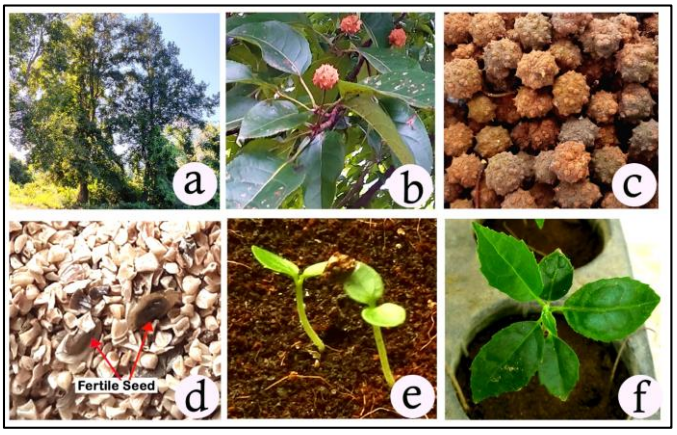


Plate 1: Adult individual of *Altingia excelsa* (a), twig with long peduncled fruit head (b), ripened woody fruit (c), both fertile and sterile seeds (d), germinated seedlings (e) seedling in root trainer (f).

Discussions

The investigation on the effect of different pre-treatment to get the improved germination rates in seeds of *A. excelsa* studied under laboratory conditions. Maximum germination was observed in T_8 (66.33%) and T_9 (64%) when seeds pre-treated with *Aloe vera* pulp extract at a concentration of 64-66%, this entail the vital role of leaf gel extract in boosting up germination rates, as a natural plant growth regulator. The findings are in line for seed germination in other species reported by several authors (Sumantra and Widnyana 2011; Imran *et al*. 2014; Zeljkovic *et al*. 2020) [47, 24, 52]. Secondly, application of *Moringa* bark extract in treatment T_4 and T_5 exhibited germination percentage of 55% and 52.33% respectively followed by T_{11} (50%) seed treated with 2% hydrogen peroxide. *Moringa* extract have been reported to improve seed germination (Phiri and Mbewe 2010; Basra *et al*. 2011) [36, 7] and stimulatory effect by hydrogen peroxide as an active oxygen source, promotes germination process (Kaur *et al*. 2020) [28]. From the results, it is evident that both *Aloe vera* pulp and *Moringa* bark extracts may be the reasonable natural substances to use as an alternative to synthetic hormones, while using oxidants such as hydrogen peroxide emerged the third best pre-sowing seed treatment method (Jann and Amen 1977) [26]. Furthermore, germination percentage varied between 28-31% when pre-treated by coconut milk and honey did not influence satisfactory germination rates. From DMRT, it was found that germination parameters were significantly different among the means of various treatments ($p \leq 0.05$). The peak value, germination value and germination speed followed the similar trend of results as revealed in germination percent for all the treatments (table 1). The higher germination value attained in T_8 (7.71) and T_9 (7.36) followed by T_4 (5.42) as the product of mean daily germination and peak value, and speed of germination reflected the measure of seed quality (Czabator 1962; Dunlap and Barnett 1983; Ginwal and Gera 2000) [9, 11, 18]. This is in accordance with the similar findings recorded by Asiedu *et al*. (2011) [6], Chettri and Singh (2022) [8] and Rinaldi *et al*. (2022) [40].

The results of germination energy and energy period shown in figure-1 determined maximum percentage in T_8, and T_9, followed by T_4 expressed within 17.50 to 21 days of energy period, attribute for those seeds which germinate rapidly and are likely to be capable of producing vigorous seedling in field conditions (Willan 1987) [51], whereas the extend energy period with lowest germination energy attained lower germination percent observed in T_1 (control). Hence, the present investigation may be useful for enhancing seed germination percentage of *Altingia excelsa* Noronha. pre-treated in *Aloe vera* pulp extract (25-50%) for 24 hours.

Conclusion

On the basis of the results, the study suggests that the fertile seeds of *jutuli* pre-treated with natural plant extracts viz., *Aloe*

vera pulp and *Moringa* bark extract under laboratory conditions concurred better response, which may be useful for producing maximum number of quality seedlings with minimum cost and time for reforestation and afforestation programmes undertaken for *Altingia excelsa* Noronha.

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