

Effect of Storage Condition and Duration on Germination Of Anisopterascaphula(Roxb.)Pierre Seed

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Abstract: Out of the four critically endangered species of Anisoptera genera, Boilam (*Anisopterascaphula Roxb.*) is at the highest risk of extinction in the IUCN Red List. It is critically endangered worldwide due to poor natural regeneration and short viability of seeds. A nursery trial was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chattogram to examine the scope of seed longevity in different storage conditions and durations and to evaluate the effect of storage condition and duration on germination of Boilam seed. Seeds were stored at six different storage conditions viz. open air (control), sand, chalk powder, normal refrigerator (0~4°C), saw dust and ash for different storage durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39 and 42 days. Storage condition, duration and their interaction were found significant on germination of seed. The highest germination rate (80%) is observed both at fresh sowing and in sand storage condition for 3, 6 and 9 days. Sand also showed highest (53.33%) germination up to 30 days duration of storage. It also prolonged the seed viability up to 39 days with 13.33% germinability.

Keywords: *Anisopterascaphula*, Boilam, germination percentage, seed, storage condition.

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I. Introduction

Bangladesh is a South Asian country that lies between 20°34' and 26°38'N latitude and 88°01' and 92°41'E longitude¹ enjoys a typical monsoon climate. Plenty of rainfall (3000 mm) with hot humid temperature blessed the southeastern part of the country (Chittagong, Chittagong Hill Tract and Cox's Bazar) with continuous high forests and many biodiversity hotspots composed of wide range of timber species. About 500 timber species grow in Bangladesh^{2, 3}. Among them, *Anisopterascaphula* (Family: Dipterocarpaceae) is the tallest, usually evergreen and often slightly buttressed with long cylindrical bole. The genera *Anisoptera* consists of ten species distributed from Chittagong (Bangladesh) to New Guinea including Malaysia, Myanmar, Thailand and Vietnam⁴. Eight out of the ten species under the genera are currently listed in the IUCN Red List (four species listed as critically endangered while others are considered endangered). Out of the four critically endangered species in the genera, *A. scaphula* is at the highest risk of extinction⁵. The main threats of this species are habitat loss, absence of mother trees for seed production and poor natural regeneration⁶. Moreover, the seeds are recalcitrant and cannot be stored for a long time. The fruits of this species consist of long wings that prevent the seeds from settling properly on soil for germination. The seed germination usually starts with developing the radicle from the wing side and later, plumule from the stalk end⁷.

Boilam (*Anisopterascaphula*) is a very tall resinous tree with a straight cylindrical bole of 30 – 40m and diameter of 100 – 150 cm. It is an evergreen tree species. It is found in Bangladesh, Malaysia, Myanmar and Thailand. In Bangladesh it occurs sporadically in the high forest of the Chittagong Hill Tracts and Chittagong, Moheshkhali islands and more common in Southern parts of Cox's Bazar, especially in Bhomarighona, Eidgor and Ukhiya. Boilam is commonly found in areas with maximum temperatures 33°C and minimum 20°C. Favorable annual rainfall is from 1,100 – 4,000 mm and humidity is high, ranging from 70-90 percent⁸.

Natural regeneration of Boilam is possible but due to lack of mother trees and profuse seeds, natural regeneration is not successful. Thick ground vegetation and heavy competition for growing space and light also retards the success of its regeneration and recruitment. Over exploitation due to illegal felling destroyed the seed trees almost completely. Moreover, the species does not bear seeds every year⁹ or consistently a bad producer of seeds. The ripening time is also very short, and hence any delay in seed collection causes complete loss of the seeds. This problem with seeds and their collection makes supply of sufficient seeds for plantation programme difficult. Hence the lack of sufficient quantity of quality seeds and seedlings invariably leads to a failure of establishing a large scale plantation programme.

Generally seedling is regenerated after storing of seed over variable period. Efficient storage of seeds is necessary to ensure continuous and cost effective supply of seedlings, which is a prerequisite for the success of any afforestation programme. Seed storage is also important for conserving the genetic resources which are ravaged by deforestation as well as by catastrophes such as forest fire, draught and floods. However, storage potential of tree seeds is highly species-specific and large variation has been encountered across the tree species. Based on the inherent storage potential, seeds are grouped into two main categories viz. recalcitrant and orthodox¹⁰. Recalcitrant (desiccation-sensitive) seeds are metabolically active when shed from the mother plant and possess relatively high moisture content. Even under ambient temperature and low relative humidity their post-harvest life is very short which also depends on the species. Since sensitive to desiccation, these seeds lose viability when their moisture content falls below 20 to 30%^{11,12}.

Boilam seeds are recalcitrant and need to sow immediately after collection of the fresh seed. Any kind of recalcitrant seed starts deterioration just after ripens. During collection, transportation, drying and storage of seeds must inevitably lead to some poor quality if they are not handled properly. Additionally, excessive dryness can render seeds more liable to cracking, brushing or abrasion on threshing. The effects of storage temperature and relative humidity have received more attention in the context of seed deterioration than have those of any other factors. The study of storage media and durations through germination tests is very time consuming requiring several weeks. In case of seed showing complicated dormancy, the tests may take even longer to accomplish. As the same time, it is indispensable to follow standard pattern to storage and testing seeds for germination in order to ensure uniformity and reproducibility of results. There are several seed storage methods which are used in agriculture crops. But a few of storage methods is found to use for forest tree seeds

The normal seed viability of Boilam is about 7 to 10 days. As Boilam seed is recalcitrant, it is imperative to find out a suitable storage method which can prolong its viability for a few days or weeks to raise seedlings in large scale in the nurseries. Therefore, the present study was undertaken to contribute in connection with developing a method for assessing the storage media and durations of Boilam seed that may provide reliable guidelines for seed storage.

II. Materials and Methods

Boilam seed storage experiment was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chittagong during May to August 2018. Mature fruits of Boilam were collected from the plus tree in the month of May. Fruits were de-winged manually and small, immature fruits and fruits that are infested by insects are discarded. Seeds of uniform size were sorted and used for the germination trials to reduce treatment variations on germination. Randomized Complete Block Design (RCBD) with three replications per treatment per day was adopted for the experiment to determine effect of storage conditions and durations of Boilam seed on germination.

Germination and viability test of fresh seed was conducted with four replications of 100 seeds each. Seeds were sown into moist sand bed and germination was calculated following standard method¹³. To examine the influence of storage conditions and durations a total of 7,560 seeds were taken and divided equally into six seed lots. Each lot has subjected to a specific storage condition as follows: open air/control, sand, chalk powder, refrigerator saw dust and ash. Seeds in all conditions were stored for different durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39 and 42 days. From stored seed, ten seeds with three replications were taken out after every three days and germination was tested by sowing in moist sand bed. Seeds were sown superficially (half buried) with 5 cm spacing in sand beds. Proper shade was provided until germination starts. Routine watering and weeding activities were carried out. From the date of sowing seeds, germination was monitored at every alternate days and it was continued until the germination ceased. Seeds were considered germinated when the cotyledons protruded from the seedbed surface. Germinated seeds were marked with small sticks to differentiate them from newly germinated seeds. Germination was observed on alternate days until completion.

The factorial experiment was followed as complete randomized block design with two factors - storage condition and duration. A variation (ANOVA) in germination potential under different conditions was analyzed using statistical package MSTAT.

III. Results and Discussion

Seed germination is the process, by which the dormant embryo wakes up, grows out of the seed coat and established itself as new independent seedlings (Fattah et al., 1976). Germination of Boilam seed was epigeous where radicle emerged by breaking through the dome-shaped structure of seed and moved downwards rapidly. Both fresh and stored seeds started germination within 10 to 15 days and completed within 40 days. The highest germination (80%) was recorded with both fresh seeds sown immediately after harvest and stored in sand for 3, 6 and 9 days. All seeds showed the same result for storage of three days. The seeds stored for 6 days, sand showed the highest (80%) germination (Fig. 2) followed by the seeds stored in control (73.33%), chalk powder (73.33%), saw-dust (66.67%), ash (66.67%) refrigerator (33.33) and stored for 9 days, sand showed the

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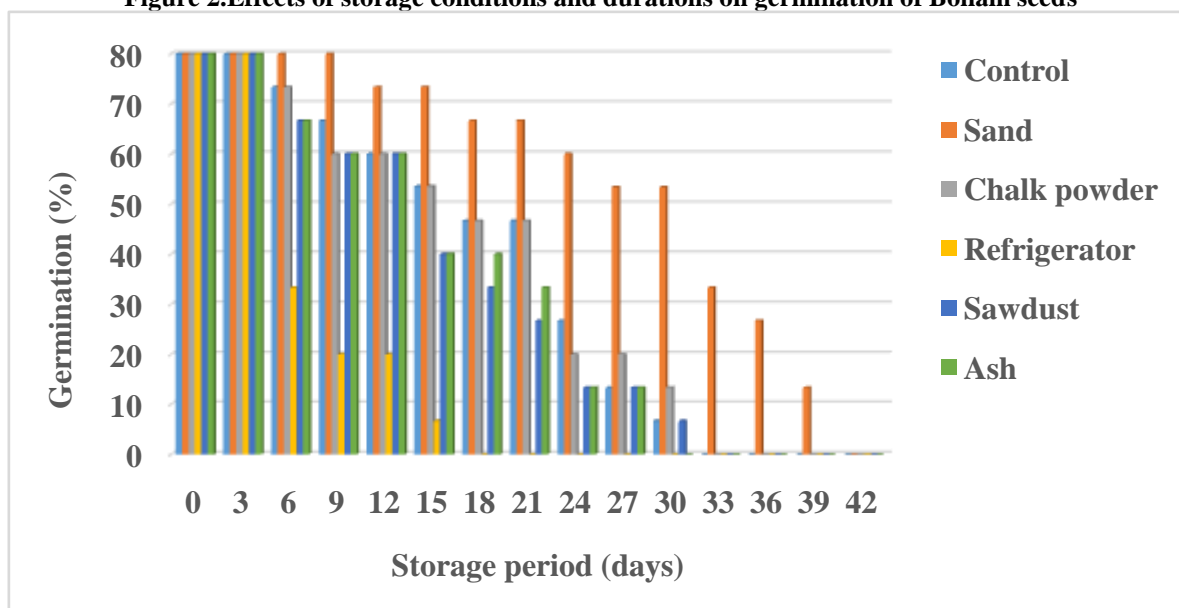
highest (80%) germination followed by control (66.67%), chalk powder (60%), saw-dust (60%), ash (60%) and refrigerator(20%). After 12 days of storage, sand showed 73.33% germination followed by control 60%, chalk powder 60%, saw-dust 60%, ash 60% and refrigerator(20). For 15 days of storage, sand showed 73.33% germination followed by control 53.33%, chalk powder 53.33%, saw-dust 40%, ash 40% and refrigerator(6.67%). After 18 days of storage, sand showed 66.67% germination followed by control 46.67%, chalk powder 46.67%, ash 40%, saw-dust 33.33%, although the seeds of refrigerator had no germination. For 21 days of storage, sand showed 66.67% germination followed by control 46.67%, chalk powder 46.67%, ash 33.33% and saw-dust 26.67%. After 24 days of storage, sand showed 60% germination followed by control, chalk powder 26%, 20%, and both ash and saw-dust 13.33% respectively. For 27 days of storage, sand showed 53.33% germination followed by chalk powder 20% and control, saw-dust and ash 13.33% respectively. After 30 days of storage, sand showed 53.33% germination followed by chalk powder 13.33%, both control and saw-dust 6.67% respectively. After the storage of 33, 36 and 39 days, the seeds of sand

Figure 1. A) Dewinged seeds, B) and C) Radicle and D) Seedlings of Boilam



showed 33.33%, 26.67%, 30% and 13.33% of germinability respectively, while others storage conditions showed no germination at all (Fig. 2). No more seeds were found with germinability after storage of 39 days. Present study revealed that storing seeds of Boilam in sand can prolong viability 13.33% up to 39 days. The results observed in declining trend on seed germination percent over the period was significant differences in the germination among the storage conditions and durations used in the experiment (Fig. 2).

Figure 2. Effects of storage conditions and durations on germination of Boilam seeds



For viability potential under different media and durations Analysis of variance (ANOVA) was done and F value was found highly significant (Table 1) within combination of the interaction of storage media and duration. Least Significant Difference (LSD) test were made for grading the values 14.155 at 5% and 18.709 at 1% level of those combinations more precisely. Germination level in earlier interval period of followed all storage media were found all most same and maximum. But emphasizing the prolonging period of viability in desired level, germination of seeds stored in sand after 18, 21 and 24 days were found statistically optimum and significant at 5% & 1% level than other conditions (Table 1). These results indicated that viability of Boilam seed is highly dependent on both storage conditions and durations which was in accordance with Manjkhola et al.¹⁴ and Panwaret al.¹⁵.

Seed germination of Boilam started 10 to 15 days after sowing and continued up to 40 days. The Germination started with the development of radicle from the winged side of the seed. Similar results were observed for *Aquilariacrassna* where seeds germinated within 9 to 15 days^{16,17}, *A. malaccensis*¹⁸ and for *Gyrinopswalla* Garten. within 7 to 14 days¹⁹. Germination was completed within 35 days, where Hossain et al.⁶ reported 46 days for different orientations and positions of Boilam seed.

The highest germination (80%) was recorded with both fresh seeds sown immediately after harvest and stored in sand for 3, 6 and 9 days. Hossain et al.⁷ obtained 65% germination at inverted half buried position. Tabin and Srivastava²⁰ recorded 92% and Adelinet al.²¹ recorded 70-80% germination in *Aquilariamalaccensis*. Bhat²² (2011) estimated maximum germination (80%) of *Calamusprasinus* seeds. However, Pandey and Khaton²³ recorded highest germination (80%) in *Sterculiaurens* seeds. Germination percentage varied from 6.67 to 66.67 among the treatments which is similar to Hossain et al.⁷.

Table 1. Two-way ANOVA for seed germination at different storage conditions in relation with storage periods

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Duration (D)	9	69875.556	7763.951	101.2689**	0.0000
Treatment (T)	5	38613.333	7722.667	100.7304**	0.0000
D x T	45	11031.111	245.136	3.1974**	0.0000
Error	120	9200.000	76.667		0.0000
Total	179	128720.000			

** Values are significant at p > 0.01 level

IV. Conclusion

Usually viability period of Boilam seeds remains 7 –10 days. The findings of the study indicates that sand medium prolongs seed viability of Boilam up to 39 days (13.33%). But it is not at desired level. The viability of Boilam remains up to 30 days (53.33%) considered at desired level. Seeds stored in sand condition can be suitable for prolong viability of Boilam seeds.

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