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EFFECT OF DESICCATION AND STORAGE ENVIRONMENT ON LONGEVITY OF Ehretia cymosa THONN. SEEDS

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ABSTRACT

Globally, forestry faces challenges in the availability of seeds due to limited knowledge on seed handling of various species. Forestry seeds are constantly being reviewed and classified as either recalcitrant, intermediate, or orthodox based on their storage behavior. It is essential to understand the tree seed storage behavior to maintain seed viability and thus minimize seed losses. There is scanty literature combining factors of seed moisture content (6%, 9%, 12%, 15%, and 20%), seed storage temperature (20°C, 5°C and -20°C), seed storage duration (1, 4, 9 and 12 months), and germination in different sites with varying environmental variables. *Ehretia cymosa* is important in the Afromontane forestry landscape as a medicinal, rehabilitation, and conservation species. This study conducted desiccation and storage studies and their influence on the viability of *E. cymosa* seeds. The study sought to determine the optimum conditions for the storage of *Ehretia cymosa* that maintains viability. This study observed that *E. cymosa* dried to seed moisture content of 6%, stored for 12 months at 20°C and sown in the laboratory had the highest germination performance (27.6 ± 3.18%) ($p<0.05$). This confirms that *E. cymosa* seeds exhibit orthodox storage behavior. The authors recommend longer storage studies (>12months) to determine the actual longevity of the seeds of this species. The significance of these results would be useful for foresters and farmers that would need to use this species for various purposes.

Contribution/Originality: The paper's primary contribution is finding that *Ehretia cymosa* seeds exhibit orthodox storage behavior. This finding is important since it will improve the current practice of handling and storage of *E. cymosa* seeds to enhance viability and minimize seed losses.

1. INTRODUCTION

Trees seeds have in previous studies been classified as either orthodox, intermediate, or recalcitrant based on the moisture content level at storage [1, 2]. Orthodox seeds tolerate desiccation to a low moisture content of between 2-5% [3]. Intermediate category of seeds have been observed from other studies that they can be dried to certain levels, but not as low as orthodox species, and often observed to fail to survive sub-zero temperatures [4, 5]. Seeds that are not tolerant and cannot survive dehydration are categorized as recalcitrant [5, 6]. Variability in seed recalcitrance and desiccation tolerance has previously been studied in two ways: desiccation sensitivity and post-

harvest behavior [7-9]. There are challenges in clearly defining the seeds' storage behaviors of critical species leading to seed losses impacting seed availability [1, 10, 11].

There is scanty information on *Ehretia cymosa* Thonn. seed storage behavior. This species of the family *Boraginaceae* is distributed in parts of West Africa, Eastern Africa, and Northern Madagascar [12-15]. Past studies have focused on the medicinal values and distribution of *E. cymosa*, thereby highlighting a gap in seed collection and handling. There is scanty information focusing on desiccation tolerance and germination responses of *E. cymosa* [4, 16].

Ehretia cymosa has a crucial space in Kenyan communities for medicinal, conservation, and a species useful for rehabilitation of degraded areas [13, 17, 18]. For the species to be utilized, there is an expectation for the availability of seed, but this has been a challenge exacerbating the demand [17, 19]. These shortages can be associated with the limited information on its handling.

This study sought to assess the effect of desiccation and storage environment on the longevity of *Ehretia cymosa* seeds. The specific objectives were: i) to assess the effect of varying seed moisture content on germination *E. cymosa* seeds, ii) to determine the effect of storage temperature and seed moisture content on germination of *E. cymosa* seeds, iii) to determine the effect of seed moisture content and storage duration on germination of *E. cymosa* seed, and iv) to determine the effect of seed, storage temperature, storage period and site on germination of *E. cymosa* seeds. The findings from these objectives would guide organizations that collect, store, and distribute such seeds in a manner that maintains their quality.

2. MATERIAL AND METHODS

2.1. Study Site

The study was conducted between June 2009 and July 2010 at Kenya Forestry Seed Centre in Muguga, which is a Program of the Kenya Forestry Research Institute (KEFRI). The seeds were collected from the Thogoto forest in June 2009, which is the peak seeding season for the species [17]. Thogoto forest is situated within Kiambu County at Latitude -1.275495S Longitude 36.667082E and altitude 2,020 meters above sea level.

Seeds were collected from 5 randomly selected trees that had mature fruits. Mature fruits for *E. cymosa* turn color from green to orange-red [20]. Three kilograms of mature *E. cymosa* fruits were collected from each of the identified trees. The collected fruits were carried in cotton bags from the field and transported to the laboratory at the Kenya Forestry Seed Centre, Muguga, for processing.

2.2. Experimental Design

Fruits were taken from the bag and seeds extracted by squeezing the fruits by hand to remove the seeds, followed by surface drying the seeds with a dry towel [1]. A sample weighing 5g seeds was then subjected to moisture content test using an infrared moisture analyzer (Himmel-Changzhou scientific instrument). The moisture content (MC) value obtained was assigned as the initial moisture content (control) for this experiment [1, 20-22]. Seeds from the rest of the fruits were extracted by de-pulping (gently rubbing with hand to remove the fresh pulp). Seeds were cleaned further in running water to remove the excess mucilage and dried by gently rubbing with a towel to remove the excess water on the seed surface [1, 2]. One sub-sample of 2,000 seeds was randomly drawn from the extracted seeds to act as control with five other subsamples of 6,000 seeds each extracted for the five MC levels [20, 23].

This desiccation procedure involved the packing of five lots (MC: 20%, 15%, 12%, 9%, and 6%) of the seed samples in porous cloth bags (Table 1). These cloth bags were then put on blue silica gel and covered in a desiccator. During desiccation each bag was checked hourly by weighing and calculating using Equation 1: where Wt_s is seed weight at desired MC, Wt_i is the initial weight of seed, MC_i is the initial Moisture content, and MC_t is the targeted Moisture content [1, 20].

$$Wt_s = \frac{Wt_i*(100-MC_i)}{(100-MC_t)} \quad (1)$$

From each bag, the seeds were further split into three groups (of 2,000/group) for storage temperatures: minus 20°C, 5°C, and 20°C [10]. Each storage temperature group for each MC was further split into five storage period groups (400/group) for storage periods: 1, 2, 4, 9, and 12 months (Table 1). The seeds in each storage period group were packed in 75 airtight aluminum foil sachets for storage (25 @ minus 20°C, 25 @ 5°C and 25 @ 20°C).

Germination testing was conducted in both laboratory (Lab) and transparent glasshouse (G.H.) conditions. Each of the storage period groups after the expiry of the storage duration was split into two groups for the different germination sites: Lab and GH. The seeds in each site for each storage period group, for each storage temperature group and for each MC level, were finally divided into four replicates of 50 seeds each.

The seeds extracted earlier for the control were subjected to germination in 4 replicates of 50 seeds per replicate in both Lab and G.H. Seeds used for the control were germinated immediately when the targeted MC was attained.

During germination, all the seeds were placed in Petri dishes containing 1% Agar-agar solution and incubated in the germination chamber set at alternating temperatures 20°C and 30°C in 12 hours intervals [4, 24]. A seed was considered to have germinated when the radicle protruded. In the glasshouse (mean rH 53.8% and mean temperature 27.3°C) and seeds of *E. cymosa* were sown in germination boxes containing river sand that was sterilized by pouring a mixture of 450 ml, 3.5% concentration of Sodium Hypochlorite solution in 20 litres of water.

Germinated seeds were counted three times per week on Mondays, Wednesdays, and Friday by counting all seeds that had germinated cumulatively for 30 days or until there was no further germination taking place within three consecutive counts, whichever came first.

The experiment had four treatments; Desiccation (MC) with 5-factor levels (20%, 15%, 12%, 9%, and 6%); Storage temperature with three levels (Minus 20°C, +5°C, and +20°C), five storage levels (1, 2, 4, 9 and 12 months) and site with two levels, Laboratory (Lab) and Glasshouse (G.H.) (Table 1) with four replicates of 50 seeds per replicate (200 seeds for each of the 150 treatment level) totaling to 30,000 seeds.

Table-1. Experimental design.

Treatment	Levels				
Desiccation MC	6%	9%	12%	15%	20%
Storage temperature	Minus 20°C	+5°C	+20°C		
Storage period (months)	1	2	4	9	12
Site	Laboratory (Lab)		Greenhouse (G.H.)		

2.3. Data Analysis

The data were tabulated in a data-sheet in M.S. excel and analyzed for: effects of varying seed moisture content on germination, combined effects of storage temperature and seed moisture content on germination, combined effects of seed moisture content and storage duration on germination, and combined effects of seed storage temperature, storage period and experimental sites on germination of *Ehretia cymosa* seeds. This analysis was done with RStudio Version 1.2.1335. Post hoc analysis (Tukey's HSD) was used to determine the difference in means (95% CI) in the ANOVA with factors desiccation MC, storage temperature, storage period, and experimental site differences compared.

3. RESULTS

This study observed the mean germination was highest when *E. cymosa* seeds were dried to SMC 9% (18.0±0.77%) (Figure 1a). This performance was not significantly different from SMC 6% (P>0.05) but differed

from the rest (P<0.05), and the least performing was SMC 20% (7.8±0.74%) (Figure 1a). Mean germination of *E. cymosa* seeds under laboratory conditions outperformed the greenhouse condition, with 9% in the laboratory having the highest mean germination (20.0±1.07%) (Figure 1b).

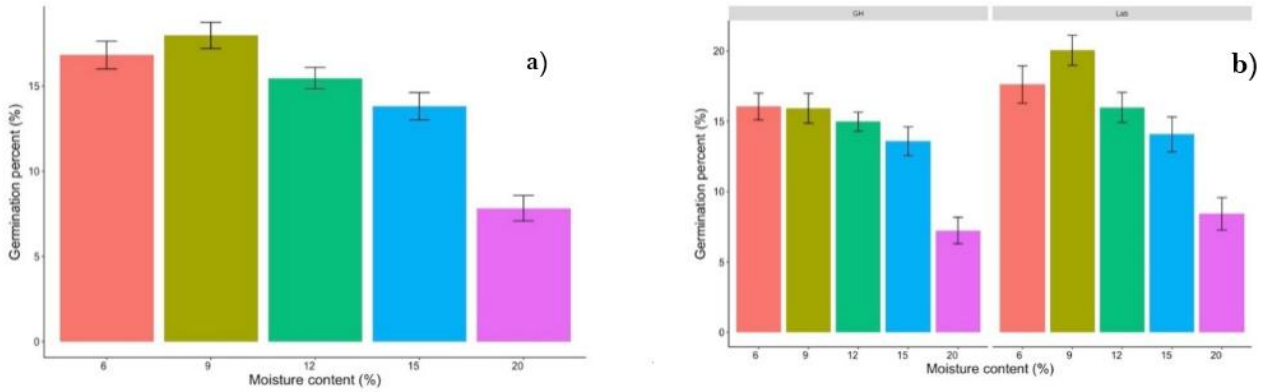

Figure-1. The effect of varying seed moisture content on mean germination of *E. cymosa* in both laboratory and greenhouse conditions (a and b) from each of the moisture content categories (6%, 9%, 12%, 15%, and 20%).

The highest mean germination percentage was observed from *E. cymosa* seeds dried to SMC 6% and stored in a 20°C environment (21.1±1.45%) (Figure 2a). Seeds stored at 20°C performed the best in germination for moisture content 6%, 9%, and 12% (Figure 2a). Seeds stored at 5°C performed highest in MC 15% and 20% (Figure 2a). Generally, the mean germination of seeds stored at 5°C and 20°C in the laboratory performed better than in the greenhouse (Figure 2b). It was also observed that in the laboratory seeds stored at 20°C performing better in MC 6%, 9%, and 12% while seeds stored in 5°C environment performed better in 15% and 20% (Figure 2b). The mean germination of seeds in the greenhouse gave the highest results on seeds that were immediately sown at SMC 21% (20.5±1.19%).

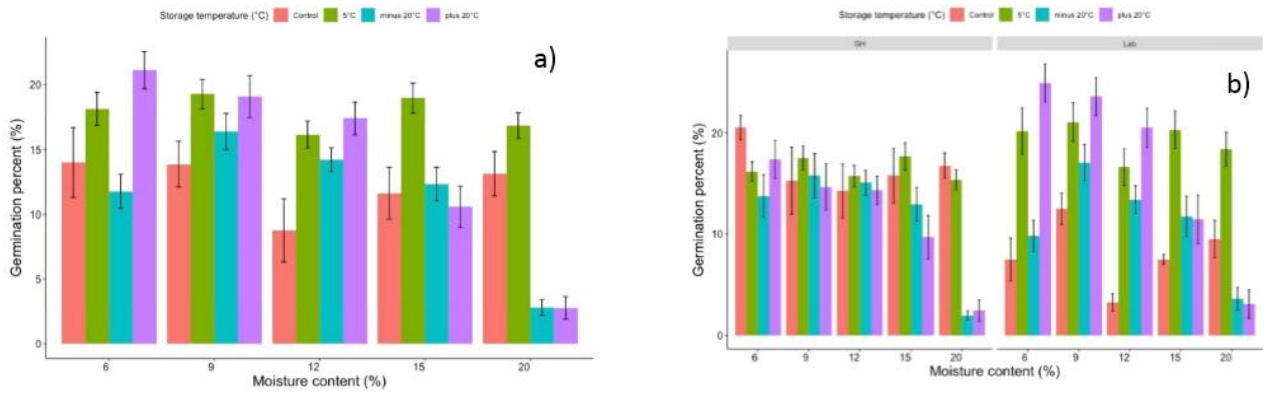

Figure-2. Germination performance of *E. cymosa* seeds stored in different temperatures (control, 5°C, 20°C and minus 20°C) after being dried to different moisture content (6%, 9%, 12%, 15%, and 20%).

The highest mean germination was observed from seeds with SMC 9% stored for 12 months (24.4 ± 1.79%) (p<0.05) (Figure 3a). Similar observations were seen in SMC 6% and 12% for SMC 15% and 20%, where the storage duration of 1 month resulted in the best germination (Figure 3a). Germination performance was highest under laboratory conditions; more so, the storage period of 12 months performed the best for SMC 6%, 9%, and 12%. Seeds stored for one month performed the best for the remaining SMC (15% and 20%) in laboratory conditions (Figure 3b).

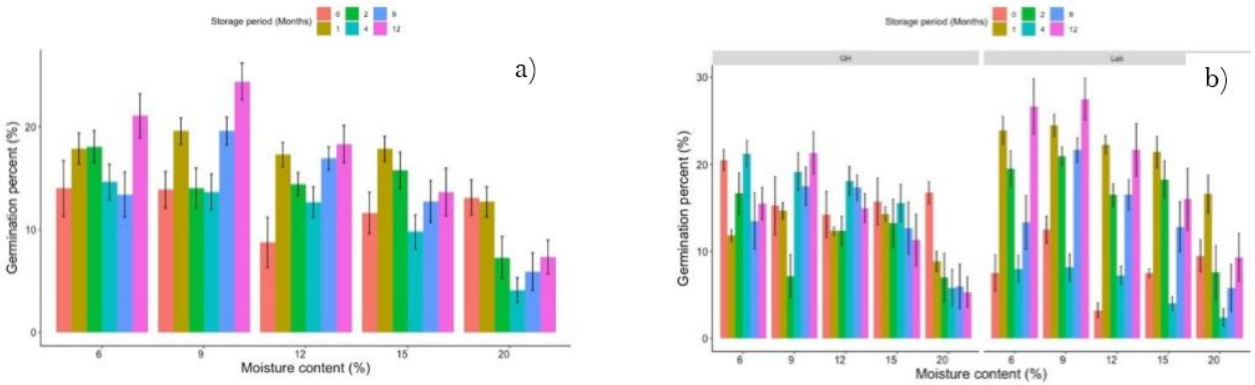

Figure-3. Effects of seed moisture content (6%, 9%, 12%, 15% and 20%) and storage duration (0, 1, 2, 4, 9 and 12 months) on germination of *E. cymosa* seeds.

The analysis for germination performance *E. cymosa* showed that seeds dried to SMC 6%, stored at 20°C for 12 months, and germinated in laboratory conditions (20-30°C) had the best germination performance (27.6±3.18%) ($p<0.05$), which was not significantly different from drying at 9% SMC ($p>0.05$) (Figure 4).

Figure-4. Combined effects of seed Moisture Content (6%, 9%, 12%, 15%, and 20%), storage temperature (5°C, 20°C and -20°C), storage period (0, 1, 2, 4, 9 and 12 months) and site (laboratory and greenhouse) on germination of *Ehretia cymosa* seeds.

4. DISCUSSION

Other studies focusing on the effect of moisture content on germination have shown lower SMC to be advantageous in the maintenance of seed viability [6, 25]. Lower seed moisture content in seed storage was shown to be beneficial by reducing lipid peroxidation of cell membranes and enhancing the activity of antioxidant enzymes after imbibition, thereby slowing the decline in viability [26, 27]. This study also agreed with other studies that lower SMC for orthodox seeds encourages germination performance.

Past studies on other species have shown that temperature and SMC affect seed viability of stored orthodox seeds as germination increases with a decrease in seed SMC and a decrease in storage temperature [3, 28]. This study's observation on the effect of storage temperature and SMC disagrees with past studies as for *E. cymosa* had better germination for lower SMCs and higher storage temperatures. The present study, however, shows that for *E. cymosa*, the germinability increases with a decrease in MC and an increase in storage temperature under laboratory conditions.

Previous studies on other species have shown that seeds with less moisture stored for longer periods resulted in higher mean germination [29, 30]. This study on *E. cymosa* seeds shows that there is no deterioration with longer storage duration (12 months). There are studies that have shown there are environmental effects that prevent seed deterioration in longer storage durations at higher temperatures [31, 32].

Few studies have focused on germination behavior under a combination of factors; seed MC, storage temperature, and duration, though on other species [7, 22, 23, 33]. This study looked at a combination of SMC, storage temperature, storage duration, and germination site conditions (temperature and relative humidity). The key factor of comparison was the two sites (greenhouse and laboratory conditions). The trends seen in the results section are similar to studies on other species focusing on low moisture content (<10%) and longer storage periods (12 months) [4, 6, 26]. The differences observed in this study came from storage and germination temperature that were higher compared to other studies for other species [10, 28].

5. CONCLUSION

This study observes that *E. cymosa* seeds had the highest germination when seeds were dried to 6% MC, stored at 20°C for 12 months, and germinated in the laboratory (relative humidity 70% and temperature range 20-30°C). This confirms that *E. cymosa* seeds exhibit orthodox storage behavior. The authors recommend longer storage studies (>12months) to determine the actual longevity of the seeds of this species.

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