Effects of Storage Moisture and Temperature Conditions on The Longevity of Seeds of *Ekebergia Capensis* Sparrm

Limo K. Reuben [1*], Too J. Emily [1], Omari Amuka[2] and Meso Michael[3]
[1]Department of Biological Sciences University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya

[2]Department of Department of Applied Sciences, Maseno University, P.O BOX 333, Maseno, Kenya

[3]Kenya Forestry Research Institute, Department of Seed Technology, P.O. Box, 20412-00200, Nairobi, Kenya

***Corresponding author's Email Address:** limoreubens@gmail.com

Abstract

Ekebergia capensis is an indigenous tree valued for its medicinal uses. However, it is facing extinction due to excessive utilization without counter efforts to restore them, and its slow regeneration rate in nature. Generation of this tree is mainly through seedlings from physiologically mature seeds. There is limited information on post-harvest storage of seeds of Ekebergia capensis. This study was carried in order to find out the optimum storage conditions and duration of storage of E. capensis seeds that enhance long shelf-life. This study investigated the effects of different moisture contents (MC) and storage temperature regimes on seed longevity of E. capensis seeds for a period 90 days. The seeds were desiccated to three moisture contents (MC) (15%, 25% and 35%) and three storage temperature regimes (-5 º C, 10 º C and 25 º C) for a period of 30, 60 and 90 days. The seeds in storage were retrieved at an interval of 30 days for seed longevity tests. Data analysis was carried using GLM statistical Model (GenSTAT.16) version. Findings from this study showed that E. capensis seeds having higher moisture content of 35% stored in all the three temperature regimes viz: -5 º C, 10 º C and 25 º C maintained significantly higher shelf life compared to other seeds with lower moisture content (25% and 15%) stored across all the temperature regimes. Furthermore, seeds with 35% MC stored at 10 º C retained viability and vigour for the longest period of time as storage period progressed to 90 days. A positive correlation existed between seed longevity and MC. Seed shelf life decreased in the order of 35%>25%>15% MC. This study recommends that seeds of E. capensis can be dried to a moisture content of 35 % and stored at 10 º C for 30 days without any significant loss of the seed germination qualities.

Keywords: *Ekebergia capensis*, seed longevity, moisture content, storage temperature, storage period

INTRODUCTION

The Earth's biodiversity is being lost at a very high rate due to climatic change and human activities that degrade and/or encroach on habitats. This biodiversity includes indigenous trees which are being threatened with extinction due to over-utilization and climate change (Zedan, 2005). According to Paton *et al.*, (2008), approximately 20 to 40% of indigenous medicinal plants are in danger of extinction. Furthermore, Shaw (2015) reported that deforestation and destruction of natural habitats has negatively affected the discovery of herbal medicine since the current extinction rate is approximately 1,000 times higher than the natural regeneration rates in the wild. The herbal health remedies and other importance from these endangered medicinal plants

are also threatened, examples are those found in *Ekebergia capensis* (Orwa, *et al*.,2009).

The species has essential uses ranging from traditional medicine, furniture to firewood (Komakech, 2018, and Bekele, 2007). Macerations from the bark is applied externally to ulcers, abscesses, and boils, scabies, acne, pimples and itching skin (Bekele, 2007). In traditional medicine; decoctions from the stem bark, infusions and macerations are taken as a remedy for gastritis, dysentery, heartburn, epilepsy, and gonorrhoea (Mairura, 2008; Komakech, 2018). The bark is dried and powder is prepared from it then sniffed to treat headache, colds and sinusitis. A decoction from the root is taken as a diuretic and to treat kidney problems, dysentery, heartburn, headache and respiratory disorders (Koch *et al.,* 2005; Kamadyaapa *et al.,* 2009). The root is chewed as an expectorant (Kibet *et al.,* 2020). Charred pulverized roots are sniffed against headache and nose blockage. Macerations from the leaves are used externally to treat headache, fever, cough and skin problems, and they are taken as a vermifuge (Murata *et al.,* 2008). The wood is valued in carpentry, and it is also used for light construction, poles and tool handles (PROTA, 2019). It is also an important source of fuel i.e. used as firewood and for charcoal production. All this importance is being threatened because the propagation and multiplication of this tree is very low due to very short shelf life of the seeds when stored under unfavourable moisture content and temperature conditions. These possess a challenge in that the seeds after maturity are physiologically destroyed as it waits for favourable conditions for regeneration in the wild. The seed maturity in its natural habitat coincides with the dry spell of October and November; this dry spell desiccates the seeds thus negatively influencing their shelf life and affects the ex-situ conservationists (Kibet *et al.,* 2020). These challenges in *E. capensis* seed storage and slow natural regeneration down folds its contribution towards achieving 10 % forest cover in land and national tree cover target by 2030. Genetics dictates the longevity of seeds in storage, however, the storage conditions finally determine the degree at which that storage potential is achieved (Savage and Bassel, 2015 and Crawford and Monks, 2009). The prediction of longevity of several agricultural seed species using seed viability model on seeds such as wheat and soybean crops (Tang *et al.,* 2000; Laca *et al.,* 2006; Weinberg *et al.,* 2008; Agha *et al.,* 2004). However, seeds for wild plants are poorly studied. There is very little knowledge on how seeds of these species stored under different moisture content and temperature regimes would enhance seed longevity.

Among the endangered medicinal plants in the world, the common are: *E. capensis* (Orwa, *et al*., 2009; Maroyi, 2013; Abiot *et al.* 2018), Warburgia *salutaris*, *Curtisia dentata* (Scott-Shaw, 1999), among others. The presence of *Ekebergia capensis* and its importance is being put at risk of extinction due to environmental degradation and encroachment of previously protected areas due to population increase (Kibet *at al.,* 2020). Currently, there is no scientifically documented information regarding its regeneration in Sub Saharan Africa. However, information on regeneration can easily be established through the knowledge on seed storage behaviour of this species.

MATERIALS AND METHODS

Sample Collection and initial processing

The experiment was conducted at the Kenya Forestry Research Institute (KEFRI) Seed Centre laboratory in Muguga. Fresh ripe fruits containing seeds of *E. capensis* were collected from Ainapkoi Sub-County,Uasin Gishu County, Kenya (0° 0' 46" S, 0° 31' 12" N, 35° 18' 47" E, 2894 M, Above Sea Level (http//:www.mapcarta.com/12745152.).

The study used experimental design where freshly ripened and mature fruits of *Ekebergia capensis* were collected randomly from several populations in the field by crown method and embryo maturity testing was done at the site (ISTA, 2012). This was done by identifying and picking 10 fruits having similar physical maturity characteristics (size and colour) in every population. The fruits were then cut cross-sectionally through the mesocarp into the endocarp using a sharp scalpel. The seeds that had hard endocarp had, presumably, attained physiological maturity. In the laboratory, the fruits were subjected to post-harvest ripening (ISTA, 2012). This was done by putting the fruits in sealed plastic containers and kept at temperatures slightly above room temperature until all the fruits had softened.

After attaining the desired softness, the fruits were removed and placed on a wire mesh screen and the seeds extracted by depulping. Depulping was done by hand-rubbing the fruits on the raised wire mesh screen. The wire mesh screen allows the fleshy part (exocarp and mesocarp) to be filtered out thus remaining with the seeds on it. The freshly extracted seeds were then washed in running water to remove mucilage and placed on a blotter sheet to remove any excess water.

Seed desiccation to required moisture content

The researcher utilized DFSC and IPGRI (1999) protocol with slight modifications to determine the seed desiccation of *Ekebergia capensis* seeds. The seeds were desiccated in silica gel in a ratio of 1:5 and enclosed in 6 cm x 8 cm perforated nets to allow easy separation of the small seeds from the silica during re-weighing. Randomly selected seed samples were dried to three target moisture contents namely 15%, 25% and 35%, from initial moisture contents of 47% using the method described in the DFSC/IPGRI protocol (1999). Two samples of seeds weighing 5 grams were removed from the extracted seed lot as representative and tested for initial moisture content by subjecting the seeds to oven drying for 17 hours at 103 ^{o}C according to International Seed Testing Association procedure for seeds (ISTA, 2007). The endocarp made up almost 50% of the seed firmly bounded round it thus both moisture contents testing and desiccation was done with endocarp imbibed round the seed (Ndung'u, 2018). The remaining seed lots were divided into three subsamples, put in perforated paper bags, weighed immediately and subjected to a desiccation process.

After initial assessment of the seed moisture content, the seeds were divided into five equal lots for desiccation and weighing. They were then put in perforated bags, weighed, and then placed in 3000 cm^3 (30cm x 20cm x 5cm) rectangular boxes with thinly spread silica gel to enable slow desiccation of *Ekebergia capensis* seeds. Thereafter the seeds were then desiccated to attain three moisture content levels: 15%, 25% and 35%. The control set was not desiccated. Using desiccation and storage protocol while maintain drying above silica gel at 25 ° C seeds were constantly monitored and removed from the boxes after attaining the required moisture content (Thomsen,2000). To regulate the amount of absorbed water removed during drying and rehydration of the seeds, the sub-samples were weighed periodically at interval of 15 - 30 minutes. The desiccation process was terminated when it reached the weight corresponding to the final degree of 15%, 25% and 35 % moisture for each treatment. After the seeds attained 15%, 25% and 35 % moisture content; they were then, put in air-tight glass viols and stored at -15 ^{o}C, +10 ^{o}C and +25 ^{o}C temperature regimes. The initial weight in each bag was recorded and the determined initial moisture contents (IMC) and targeted moisture contents (TMC) were used to calculate the corresponding targeted seed weight. The equation used to obtain the desired values was adopted (Kirsten *et al*., 1999):

$TMC = (\frac{100-IMC}{100-TMC})$ *initial seed weight*

where IMC = initial moisture contents and TMC= Target moisture contents.
Desiccated and non-desiccated seeds were subdivided into nine equal parts and put in small glass viols. Each of the subsamples were then replicated twice and stored under three temperature regimes of –5 ° C, +10 ° C and +25 ° C for 30, 60 and 90 days. The seeds were retrieved after an interval of 30days and determination of seed longevity was done.

Determination of Seed Longevity
Half-viability period (P50) is widely used as measure of longevity in many wild plant species (Probert, 2003; Muthoka*et al*, 2003), which is the time taken for seed viability to decline by 50%. The initial germination percentage of the untreated seeds was taken as the reference point. The P50 was read directly from the germination % graph by drawing a line along X axis at 50% germination (initial) on the Y axis. The points of intersection where the line touched the % germination graph was again drawn straight downward to touch the X-axis where the time was read at point of intersection at X-axis.

Data Analysis
Data was entered in Microsoft Excel spreadsheet. Preliminary and final data analysis was carried out using GENSTAT 16th edition statistical software. ANOVA (at α=0.05), were run to determine whether significant difference existed in seed vigour levels for seeds dried to different moisture contents and storage at varying temperatures. Vigour data were root transformed to meet model assumptions.

RESULTS AND DISCUSSION

Effect of storage temperature and moisture content on longevity of *E. capensis* Seeds

Germination of *E. capensis* seeds of varied moisture contents stored at -5 ° C for 30, 60 and 90 days
Before any treatment was applied, the seeds recorded 90 % germination (Figure 1). After 30 days of storage at -5 ° C, the seeds with different moisture contents had varied germination percentage. The non-desiccated seeds (control) together with the seeds with 35 % moisture content had the same germination percentage (85 %) (Figure 1). Germination percentage of seeds with 25 % moisture contents was 50 % and seeds with 15 % moisture content did not germinate. After 60 days of storage, there was a decrease in germination percentage in all the seeds (figure 1) regardless of the treatment. Non-desiccated seeds had 65 % germination while seeds with 35 % and 25 % moisture contents had 30 % and 10 % germination respectively. There was no germination with seeds having 15 % moisture content (Figure 1). At 90 days of storage, there was further decrease in germination percentage for all the seeds (Figure 1). The non-desiccated seeds had 51 % germination whereas the seeds having 35 % and 25% recorded germination percentage of 20 % and 5 % respectively (Figure 1). There was no germination of the seeds that were stored at 15 % moisture content. This is contrary to some scientists who have known for years that seed longevity improves if seeds are dried to low water contents (FAO, 1994).

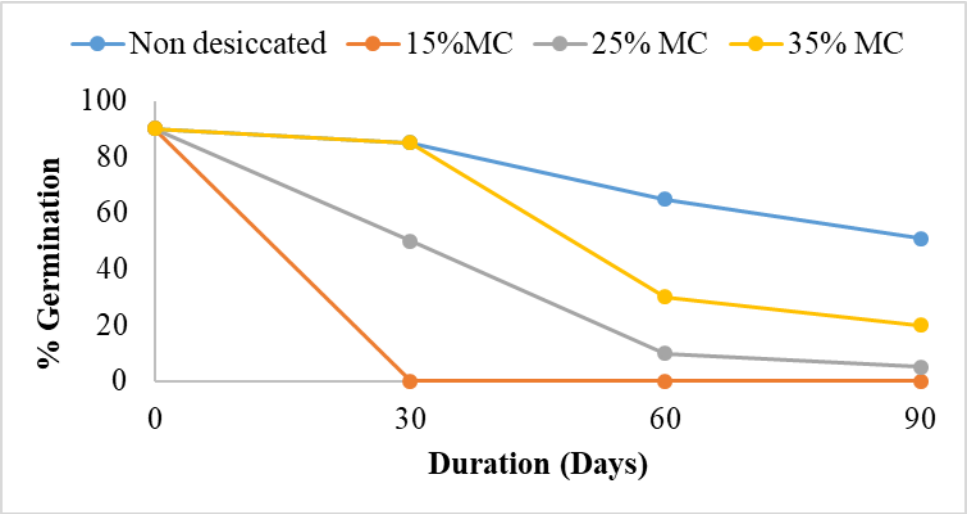

Figure 1: Percent germination of seeds with varying moisture contents after storage at -5 º C for 30, 60 and 90 days

Germination of *E. capensis* seeds of varied moisture contents stored at +10 º C for 30, 60 and 90 days

There was a slight decline of germination percentage of non-desiccated (47 % MC) seeds compared to seeds with 35% moisture content after storage for 30 days (Figure 2). The non-desiccated seeds dropped in germination percentage from initial 90 % to 85% whereas the seeds with 35 % moisture content maintained a germination percentage of 90 % after storing for 30 days at +10 ºC (Figure 2). This is in line with Pritchard *et al.*, (2004), who pointed out that adequate hydration of recalcitrant seeds is the first requirement for long-term storage.

However, seeds with 25% moisture content showed reduced germination percentage significantly to 45 percent (Figure 2). Seeds with 15% moisture content did not germinate at all after storage (Figure 2).

After 60 days of storage at 10 º C, the seeds stored at varied moisture contents showed a general decrease in germination percentage. Non-desiccated seeds showed decrease in germination from 85 % to 78 % (Figure 2). Germination of seeds stored with 35 % and 25 % moisture contents was 45 % and 15 % (Figure 2). There was no germination of seeds stored with 15 % moisture contents (Figure 2). This is contrary to a study conducted by Lewis (2002) who indicated that *Ekebergia capensis* seeds were able to survive for 12 weeks at 6 °C without losing any germination capability. However, the moisture content of the seeds was not indicated. The non-desiccated seeds (47 % MC) and the seeds dried to 35 % stored for 90 days had decreased germination percentage of 71 % and 40 % as compared to when stored for 30 days and 60 days (Figure 2). However, there was a higher germination percentage than those stored with 25% and 15 % MC which was 10% and 0% respectively (Figure 2). Seeds stored at 15% MC did not germinate after storage for 30, 60 and 90 days. (Figure 2) On the other hand, non-desiccated seeds showed the highest germination percentage after storing for 90 days while the seeds stored with 25% MC for 90 days had significantly reduced germination percentage (Figure 2).

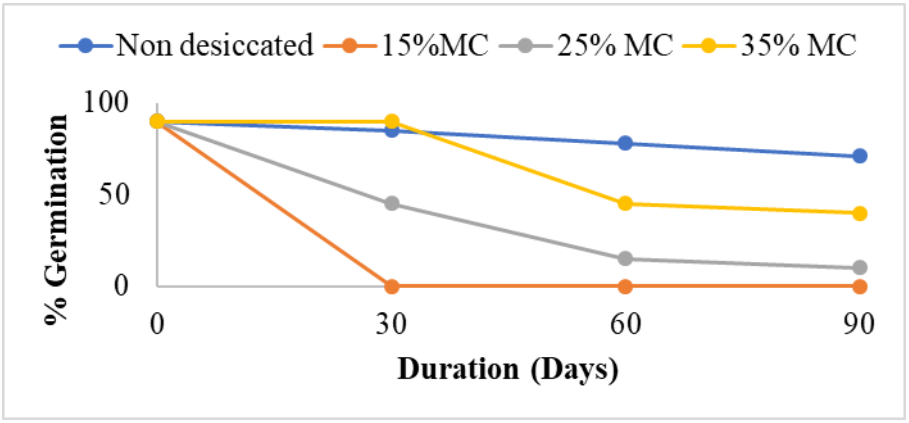

Figure 2: Germination percentage of E. capensis seeds of varied moisture contents stored at +10 º C for 30, 60 and 90 days

Germination percentage of *E. capensis* seeds of varied moisture contents stored at +25 º C for 30, 60 and 90 days

The results showed that further increase of temperature to 25 º C, reduced germination percentage as the storage period increase. Non-desiccated seeds showed high germination percentage of 80% after 30 days of storage. However, reduced germination to 65% was maintained after storing the seeds for 60 and 90 days (Figure 3). Findings further showed that seeds with 15% MC showed lower germination percentage than those with 35% and 25% moisture contents and the non-desiccated. Seeds with 35% MC and stored for 30, 60 and 90 days recorded germination of 85%, 45% and 25% respectively (Figure 3). Seeds with 25% MC recorded germination of 55% after 30 days of storage and maintained germination of 5% after 60 and 90 days of storage (Figure 3).

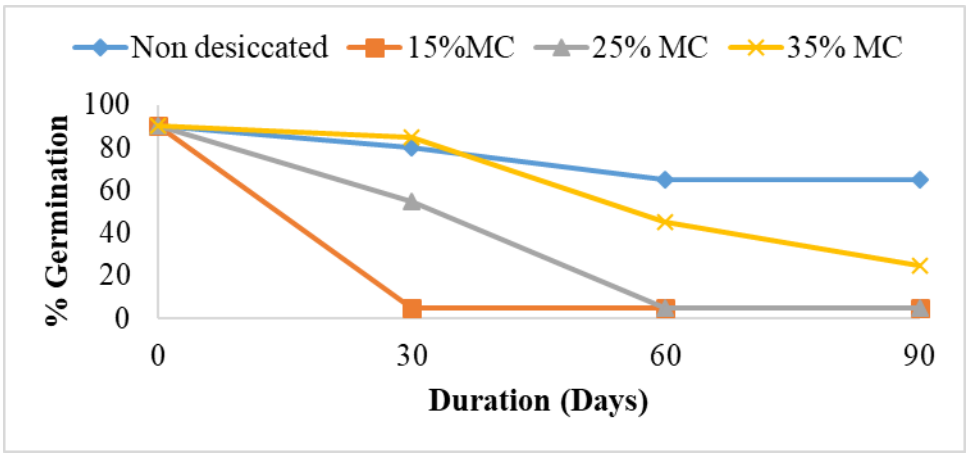

Figure 2: Percent germination of seeds with varying moisture contents after storage at 25 º C for 30, 60 and 90 days

The ANOVA table below provides a summary of main factors affecting germination of *Ekebergia capensis.* The results show that moisture content and storage period had an influence on seed germination $p<0.001$, also storage temperature at $p<0.025$. The interaction between moisture content and storage period, moisture content, storage period and storage temperatures had influence on seed germination with $p<0.005$). However, interaction between moisture content and storage temperature had no significant influence on seed germination $p>0.123$. (Table 1)

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Table 1: ANOVA showing the effect of main factors (%moisture content, storage temperature, and storage period) and their interaction on seed % germination of Ekebergia capensis seeds

	Germination (%)	
Source	***F***	***Sig***
Storage period (SP)	34.28	0.001**
% moisture content(%MC)	45.11	0.001**
Storage temperature (ST)	3.95	0.025**
SP x % MC	7.83	0.001**
% MC x ST	1.9	0.123
SP x ST	1.4	0.022**
SP x% MC x ST	2.55	0.051

*** denotes significance at p<0.05.*

Generally, there was decrease in germination with increase in storage period. The initial seed germination was 90 % hence; P50 was 45 % (Table 1). Essentially, P50, which is to the time, taken for viability to drop to 50 % percent of the initial germination. Seeds with moisture content of 15 % were shorter-lived with P50 ranging between 14 and 12 days (Table 1). On the other hand, seeds with 35% MC were longer lived with P50 ranging between 50 and 60 days (Table 1).

Seeds dried to 35% MC stored at 10 ^{o} C recorded the longest period of viability of 60 days while shortest viability period of 12 days was recorded in seeds stored with 15% MC at 25 ^{o} C (Table 1). In addition, seeds stored with 25% MC had P50 ranging between 30 and 32 days at 10 ^{o}C and 25 ^{o}C (Table 2).

Table 2: Seed longevity (P50) of E. capensis seeds stored at different temperatures (-5 ^{o} C, 10 ^{o} C and 25 ^{o} C) for 90 days Storage temperature

	-5^{o}C	**10^{o}C**	**25^{o}C**
Moisture Content	**P_{50}(Days)**	**P_{50}(Days)**	**P_{50}(Days)**
15% MC	14	13	12
25% MC	30	30	32
35% MC	50	60	55

Overall, the results of the current study reveal that seed longevity was influenced by moisture contents and storage temperature thus agreeing with similar seed researchers (Pritchard *et al.,* 2004; Carvalho and Nakagawa, 2000) who pointed out that low temperature improves seed longevity, more so when the seed´s moisture content is very high. Therefore, increased hydration of the seeds is the first requirement for long-term storage (Pritchard *et al.,* 2004).

CONCLUSION AND RECOMMENDATION

The study confirms that, the *E. capensis* seeds are recalcitrant due to its ability to retain viability for a longer period of time when stored with high moisture content (35 %) as

compared to the one stored with lower MC (15 %). The results further revealed that there is continuum decrease in seed shelf life as moisture content decreases. Thus, concluding that there is a strong negative correlation between the seed longevity and percentage moisture content.

Results from current study will provide information on the seed storage behaviour of *E. capensis*. This will aid other researchers in the process of coming up with ideal storage conditions that can increase the seed longevity of this species. For longer seed shelf life; the seeds of *E. capensis* should be stored at 10 ^{o}C with 35% moisture content.

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