AFRICA ENVIRONMENTAL REVIEW Journal
ISSN: 1727-8341

AER Journal Volume 3, Issue 2, pp. 212-223, 2019

RESEARCH ARTICLE
Available Online at *http://www.aer-journal.info*

Effects of Varying Storage Conditions on the Vigour of Fresh Seeds of *Ekebergia capensis*

R. L. Kibet[1*], E. J. Too[1], A. Omari[2] and M. Meso[3]
[1*]*Department of Biological Sciences, University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya;* limoreubens@gmail.com
[1]*Department of Biological Sciences, University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya*
[2]*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*

Abstract

Ekebergia capensis is a popular indigenous tree valued for its medicinal uses. However, it is an endangered tree species because of overexploitation and its slow regeneration rate in nature. Production of this tree is through seedlings from seeds with desirable vigour. There is limited knowledge on post-harvest storage of seeds of Ekebergia capensis without significant loss of seed vigor. This study was conducted in order to establish optimum storage conditions and period of storage of fresh seeds. This study investigated the effects of different moisture contents (MC) and storage temperature regimes on seed vigour loss of E. capensis seeds for a period 90 days. The fresh seeds were dried 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 stored seeds were retrieved at an interval of 30 days for vigour tests. Data analysis was carried using GLM statistical Model (GenSTAT.16) version. Findings from current study revealed that E. capensis seeds with higher moisture content of 35% stored across the tested temperature regimes viz: -5 º C, 10 º C and 25 º C maintained significantly higher vigour compared to other seeds with lower moisture content (25% and 15%) stored across all temperature regimes. Furthermore, seeds with 35% MC stored at 10 º C retained the highest vigour as storage period progressed to 90 days. A positive correlation existed between seed vigour and MC. Seed vigour decreased in the order of 35%>25%>15% MC. This study recommends that fresh seeds of E. capensis can be desiccated to a moisture content of 35 % and stored at 10 º C for 30 days without significant loss of vigor.

Keywords: *Ekebergia capensis*, Seed Vigour, Moisture Content, Storage Temperature, Storage Period

INTRODUCTION

The earth's biodiversity is being lost at an unprecedented rate due to human activities that degrade or encroach on habitats. Indigenous trees are part of this biodiversity and are endangered owing to overexploitation and climate change (Zedan, 2005). According to Paton *et al.* (2008), approximately 20 to 40% of medicinal plants are in danger of extinction. In addition, a report by Shaw (2015) indicated that destruction of rainforests and other natural habitats has affected the discovery of herbal medicine since the current extinction rates are at least 100 to 1,000 times higher than the natural background

regeneration rates. There is need therefore to conserve, propagate and sustainably use these tree species that are threatened by extinction, including the *Ekebergia. capensis* tree species (Orwa *et al*., 2009). *Ekebergia capensis* has versatile uses including its wide use in traditional medicine (Mairura, 2008). The bark, roots and leaves are widely used in traditional medicine. Bark decoctions, infusions and macerations are taken to treat gastritis, heartburn, dysentery, epilepsy, gonorrhoea and as vermifuge, and are applied externally to ulcers, abscesses, boils, scabies, acne, pimples and itching skin (Bekele, 2007). A powder prepared with the bark is sniffed against headache, colds and sinusitis. A root decoction is taken as a diuretic and to treat kidney problems, dysentery, heartburn, headache and respiratory complaints (Koch *et al.*, 2005; Kamadyaapa *et al.*, 2009). The root is chewed as an expectorant. Charred pulverized roots are sniffed for treatment of headache and blocked nose. Leaf macerations are used internally or externally to treat headache, fever, cough and skin complaints, and they are taken as a vermifuge (Murata *et al.*, 2008). The wood is locally valued for furniture, and it is also used for light construction, poles and tool handles (Prota, 2019). It is suitable for light flooring, joinery, interior trim, ship building, vehicle bodies, sporting goods, veneer and plywood. It is also used as firewood and for charcoal production **(**Komakech, 2018; Bekele, 2007). However, the propagation and multiplication of the tree has remained low due to loss of seed vigour when stored under unfavorable moisture content and temperature conditions. The situation is worsened by the fact that, in its natural habitat, seed maturity coincides with dry spells. These challenges in *E. capensis* seed storage and slow natural regeneration compromises its contribution towards achieving 10% forest cover in land and national tree cover target by 2030. While genetics govern the maximum time period for which a seed can remain viable in storage, the storage conditions ultimately determine the extent to which that storage potential is realized (Savage & Bassel, 2015; Crawford & Monks, 2009).

The prediction of viability of many agricultural seed species using seed viability model exist such as in 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 paucity of information on how seeds of the species stored under different moisture content and temperature regimes would retain high vigour. Therefore, an intervention is required on handling of *E. capensis* seeds so as to prolong their shelf life without losing vigour and ensure enough quality seed stocks for increasing Kenya's forest cover and availability of useful tree species for purposeful planting by interested communities. Although a lot of research has been done on the different species of medicinal plants worldwide, very little has been done on regeneration of the endangered herbal species both by natural and artificial means, not only in Kenya but also in other countries as well. According to International Union for Conservation of Nature and the World Wildlife Fund, over 10,000 species of medicinal plants are threatened with extinction (Bentley, 2010). These species have been severely depleted due to extensive utilization without counter efforts to restore them.

Among the endangered herbal plants in the world, the most prominent ones are *E. capensis* (Abiot *et al.,* 2018; Orwa *et al*., 2009), *Warburgia salutaris* (Maroyi, 2013), *Curtisia dentata* (Scott-Shaw, 1999), among others. The existence of *E. capensis* tree species and its importance is being threatened due to environmental degradation and encroachment of previously protected areas due to high population growth. Currently, there are no studies that have been conducted to find out the regeneration rates of these species in Kenya. However, studies on regeneration strategies cannot be conducted without information on

the seed storage behaviour of this species. Results from current study will provide information on the seed storage behaviour of *E. capensis* and aid other researchers in an attempt to come up with proper storage conditions that can sustain seed viability and vigour over a longer storage span.The present study therefore aimed at assessing the seed vigour of fresh seeds of *E. capensis* after storage at varying moisture contents and temperature regimes.

MAREIALS AND METHODS

Site Description

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 Ainabkoi 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).

Sample Collection and Initial Processing

Freshly ripened and mature fruits of *Ekebergia capensis* were collected randomly from several populations by crown method and embryo maturity testing was done at the site (ISTA, 2012). When the fruits of *E. capensis* changed in colour from green to reddish-brown and the mesocarp softens, it was believed to have matured, however, most of them attain physiological maturity when they are still green in colour and have hard endocarp. Therefore, maturity testing was necessary as the initial step in the experiment. 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 attained physiological maturity. In the laboratory, the fruits were subjected to post-harvesting ripening to bring about uniformity in ripening and to further soften the fleshy part for easy depulping (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 for Desired Moisture Content

The protocol developed by DFSC and IPGRI in 1999 was followed with certain modifications to determine the seed desiccation. Seeds were dried 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. The remaining seed lots were divided into three subsamples, put in perforated paper bags, weighed immediately and subjected to a desiccation process.

After seed initial moisture content was assessed, the seeds were divided into five equal lots for desiccation and weighing. The seeds were put in perforated bags, weighed,

and then placed in 3000 cm^3 (30 cm x 20 cm x 5 cm) rectangular boxes with thinly spread silica gel. The seeds were again thinly spread and covered with one layer of silica gel (non-destructive method) on top before replacing the box lid. The seeds were then desiccated to three moisture content levels; 15%, 25% and 35%. Some seed lots were not desiccated and hence formed the control. The seeds were, constantly regenerated through drying above silica gel at 25°C in incubator using desiccation and storage protocol (Thomsen, 2000). To control 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 -15ºC, +10ºC and +25ºC temperature regimes.

The initial weight in each bag was recorded; 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):

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

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 –5ºC, +10ºC and +25ºC for 30, 60 and 90 days. The seeds were retrieved after an interval of 30 days and determination of seed vigour was done.

Determination of Seed Vigour

To assess seed vigour four hundred seeds with 15%, 25%, and 35% moisture contents which were retrieved from each of temperature storage regimes of -5ºC, 10ºC and 25ºC were sown in rectangular plastic trays inside glass house. Germinated seeds were scored daily for up to 7 weeks. A seed was considered as normally germinated when the radicle protrudes by 2–3 cm. Seed vigour was measured by Germination index (G.I.) which was computed using the following formula (Perry, 1984).

$$G.I = \left\{\frac{n}{d} + \cdots \frac{n}{d}\right\}$$

Where n = number of seedlings emerging on day "d"

d = days after planting

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 Vigour of *E. capensis* Seeds

Seeds Vigor for seeds stored at -5 º C for 30, 60 and 90 Days at varying moisture contents

The seeds with varying moisture contents exhibited different vigor as evidenced by the germination indices. Seed vigor decreased progressively for all the seeds. Non-desiccated seeds showed decrease in vigor from 2.9 to 1.2 to 1.1 G. I by 30, 60 and 90 days of storage respectively (Figure 1). On the other hand, the GI of seeds with 35% MC declined from 2.8 to 0.9 after 30 and 60 days of storage respectively (Figure 1). Further decline in seed vigor to 0.7 G. I was recorded after 90 days of storage. Seeds with 25% MC showed a steady decrease in

vigour from 1.5, 0.4 and 0.3 G. I after 30, 60 and 90 days of storage respectively (Figure 1). What stands out is that among the dried seeds, seeds dried to 35% moisture content had the highest seed germination index followed by seeds with 25 % moisture content. Seeds with 15% moisture content lost seed vigor after storage for 30 days.

Seed vigor levels were highest at the onset and lowest after 90 days for the whole storage period (Figure 1). Overall, the seeds lost vigor with increasing storage time.

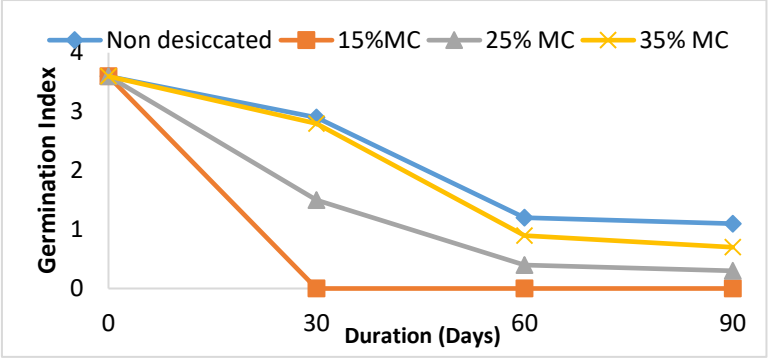

Figure 1: Seed Vigor for Seeds with Varying Moisture Content Stored at -5ºC for 30, 60 and 90 Days.

Seed Vigor for seeds stored at 10 ºC for 30, 60 and 90 Days at varying moisture contents

At higher storage temperature of 10º C, the non-desiccated seeds and seeds stored with 35% moisture content recorded higher germination indices than when stored at -5ºC (Figure 2). However, the seed vigor decreased progressively as the storage duration increased. Seeds with 35% moisture content showed decrease in vigor from 2.9 after 30 days of storage to 1.2 and finally 0.9 G.I after 60 and 90 days of storage respectively (Figure 2).

Vigor of seeds stored with 25% MC decreased from 1.5 to 0.5 and finally to 0.3 G. I after 30, 60 and 90 days of storage respectively (Figure 2). It is worth noting that seed lots stored with 15% MC lost vigour after 30 days of storage. For non-desiccated seeds, seed vigour decreased from 3.3 to 2.4 G. I after 30 and 60 days of storage respectively, before a very minimal decrease to 2.3 G. I after 90 days storage. The seed loss of vigour was in the order with MC as 35>25>15% for seeds stored at 10º C (Figure 2).

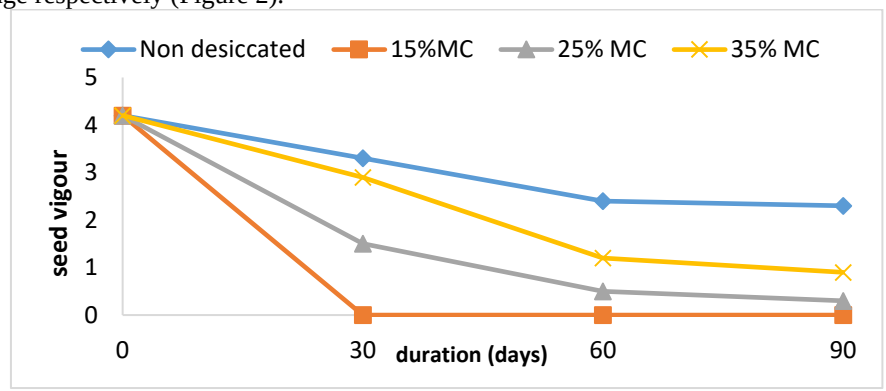

Figure 2: Effect of Seed Storage at 10ºC on Seed Vigor for Non-Desiccated Seeds and Seeds with 15%, 25% and 35% Moisture Content after 30, 60 and 90 Days.

Seed vigour for E. capensis seeds with varying MC stored at 25°C for 30, 60 and 90 Days

Non-desiccated seeds and seeds with 35% moisture content stored at 25°C had lower seed vigor than the same lot stored at -5°C and +10°C after storage for 30 days (Figure 3). Precisely, non-desiccated seeds recorded a definite decrease in seed vigour from 2.8, 2.4 to 2.2 (G. I) after 30, 60 and 90 days of storage respectively (Figure 3). On the other hand, seeds stored with 15% MC lost vigour after 60 days of storage. Seeds with 35% MC decreased vigour from 2.3 to 1.4 to 0.8 Germination Index after 30, 60 and 90 days of storage respectively (Figure 3). Similarly, seeds stored with 25% MC showed a steady decrease in vigor from 1.7 to 0.2 to 0.1 after 30, 60 and 90 days of storage, respectively (Figure 3).

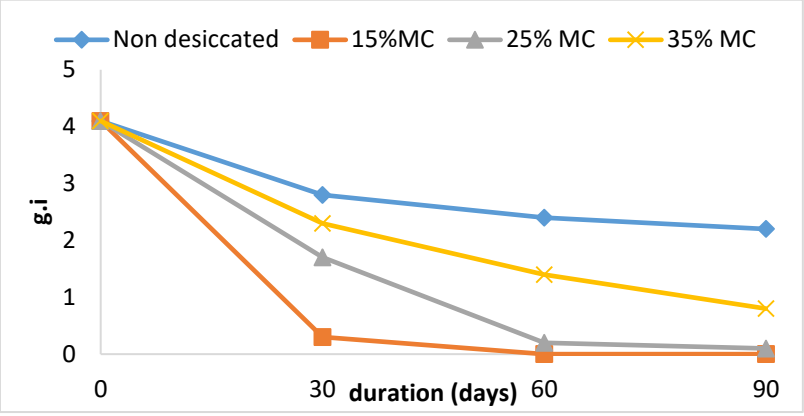

Figure 3. Effect of storage at 25° C on seed vigor for non-desiccated seeds and seeds with 15%, 25% and 35% moisture content after 30, 60 and 90 days.

Regression analysis of moisture content, storage temperature and storage period versus seed vigour for non-desiccated seeds and seeds dried to 35%, 25% and 15% moisture content

The results shown on Table 1 indicate that the factors mainly moisture content and storage period influenced seed vigour significantly ($p<0.05$), however, storage temperature recorded insignificant influence ($p>0.05$) on seed vigor.

Table 1: Relationship between Moisture Content, Temperature and Seed Vigor

Coefficients[a]					
Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
Constant	.259	.371		.699	.491
Moisture content	.811	.104	.755	7.787	.000**
Temperature	-.044	.104	-.041	-.427	.674
Storage period	-.494	.104	-.460	-4.747	.000**

a. Dependent Variable: Seed vigor (G.I)

The graphs (figure 4 a, b and c) reveal that duration of storage recorded negative relationship with seed vigour. Findings showed that seed vigour decreased with increase in storage period in all the three temperature regimes. The seeds stored at

temperature of -5ºC, 10ºC and 25ºC, resulted in R^2 of 56.7%, 53.6% and 57.8% respectively (figures 4 a, b and c). The variation of seed vigour is due to time and taking into account variations in moisture content.

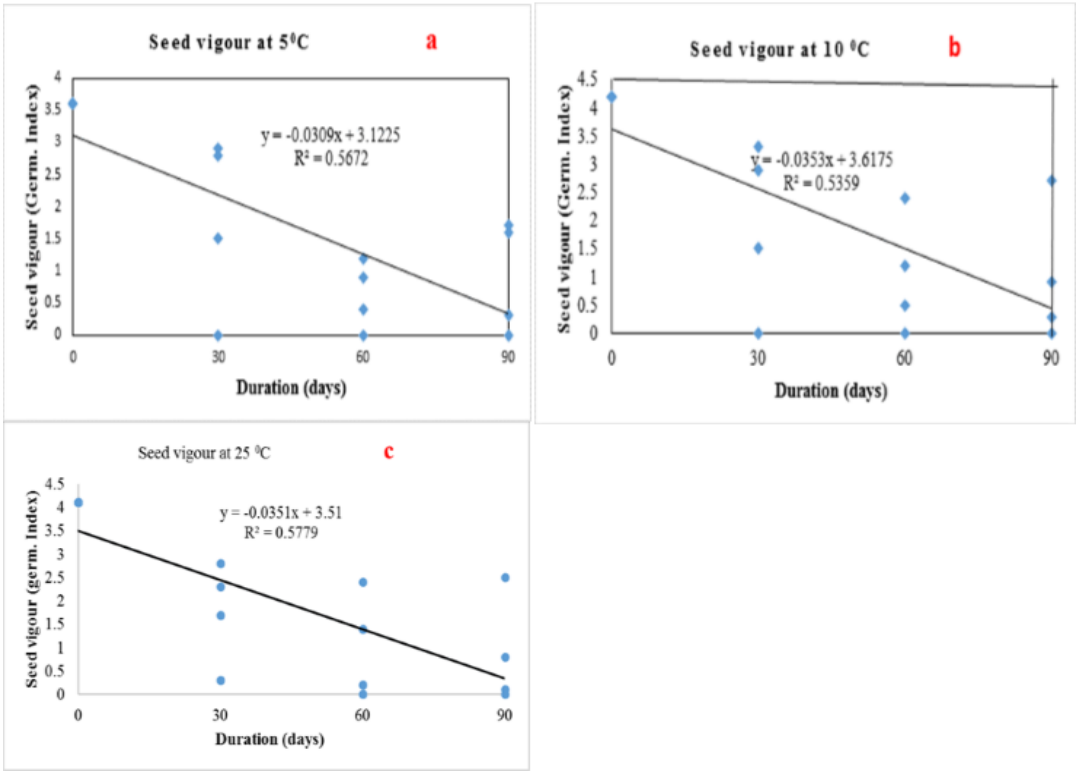

Figure 4: Regression Graphs for Seed Vigor versus Storage Duration of Seeds Stored at -5ºC (a), 10ºC (b) and 25ºC (c) for Non-Desiccated Seeds and Seeds with 15%, 25% and 35% Moisture Content after 30, 60 and 90 Days.

Correlations between Seed Germination % and Vigor

The results revealed a strong positive correlation between seed germination percentage and vigor (Table 2). As germination % declined with time equally, the vigour declined with time at respective moisture content and storage temperature. The correlation coefficient for both seed germination % and vigour was 0.953 which is very close to one (1) (Table 2) which suggests a very strong positive significant (p=0.000) correlation between the two parameters.

Table 2: Correlations between Seed Germination % and Vigour

Correlations

		Seed vigour	% Germination
Seed vigour	Pearson Correlation	1	0.953**
	Sig. (2-tailed)		0.000
	N	400	400
% Germination	Pearson Correlation	.953**	1
	Sig. (2-tailed)	.000	
	N	400	400

***. Correlation is significant at the 0.01 level (2-tailed).*

DISCUSSION

Seed vigour could be considered as independent attributes of physiological ability to germinate above or below optimal temperatures, and other aspects of tolerance to stresses (Marcos-Filho, 2015). Deterioration starts before seed harvest and continues during the harvest, processing and storage periods. The final stage of this deterioration is death of the seed. Nevertheless, seeds lose vigour before they lose the ability to germinate (Sivritepe, 2012). Seed vigour is a measure of accumulated damage in seed as viability declines (Luo *et al*., 2015). For seed vigour to be calculated, daily germination count was done for all the treatments.

Germination count of *E. capensis* seeds varied with varying moisture contents and temperature regimes. There was high germination count in the non-desiccated seeds stored at +10ºC for the entire storage periods. This contrasts a study by Liu *et al.* (2001) who reported that recalcitrant seeds of Alexandra palm seeds only germinated within a narrow temperature range (20–30°C). Germination count was lower in the desiccated seeds 35% MC and below throughout the storage period compared to the non-desiccated seeds.

Daily germination and seedling average counts were seriously inhibited in the seeds with 15% MC at -5ºC and +10ºC stored for 90 days. This observation was supported by (Taiz & Zeiger, 2009) who reported that there could be decrease in the rate of metabolic reactions at a temperature of 20ºC and below, affecting the essential processes that initiate germination in recalcitrant seeds of jamun. However, the desiccated seeds to MC of 15 stored at 25ºC for 90 days recorded germination of 5%. This can be attributed to increase in temperatures which elevated the metabolic activity of the seed thus promoting absorption of water (Castro *et al*., 2004).

The desiccated seeds to a moisture content of 35% resulted to high average germination count spread through the 7 weeks after storage at 25ºC for 30 days as compared to the non-desiccated seeds stored at the same temperatures and storage period which stopped germination after 5 weeks. The low germination in the non-desiccated seeds could result from fungal attack during water imbibition, even resulting in failure to germinate. The longer the period it takes for germination to stop in the nursery bed, the lower the G.I thus lower vigour.

The results from the current study for *E. capensis* revealed that seeds with high moisture content of 35% and above had higher vigour compared to seeds with moisture content of 15% stored at the same temperature of 10ºC for same period of 90 days. *E. capensis* seeds with 15%, 25% and 35% moisture content stored at varying storage temperatures of -5ºC, 10ºC and 25ºC confirmed that seed with respective moisture content is a very important factor to consider for long term storage and vigourity as seed lost vigour with decrease of seed moisture contents in all storage temperature.

Moisture content decrease in seeds stored at constant temperature was verified to cause a decrease in longevity in all temperature regimes (Walters, 2005). However, storage temperature in this study revealed that it was equally important in seed longevity and seed vigour which revealed that decrease in storage temperature increased seed longevity and prolonged seed vigour. Similar results were obtained in a study by Weinberg *et al.* (2008 b) who examined the vigour of maize (corn) stored in self-regulated temperature cabinets in sealed containers. Another study by Lewis (2002) showed that *Ekebergia capensis* seeds stored with the endocarp were able to survive for 12 weeks at 6°C without losing any germination capability. However, when *E. capensis* seeds were stored with the endocarp at 3°C the germination achieved after 8 weeks was 40% and the seeds that did not germinate became over-run with fungi, suggesting that the seeds were very debilitated. This supports the view that each

recalcitrant seed species has its own threshold before injury is incurred, as a result of either desiccation or chilling (Pammenter, *et al.,* 1994). This indicates that it is impossible to arrive at a general optimum storage temperature for all recalcitrant seed species; each one must be individually tested. It seemed that the endocarp played an important role in maintaining the water content of the *E. capensis* seeds which is important in maintaining seed vigour (McDonald, 1999), and protecting the seed from fungal contamination.

Other studies have also documented similar initial seed vigour decline and a subsequent seed vigour increase for seed lots stored in continuous low temperature and high relative humidity environments (Krueger *et al*., 2012). The reason for this fluctuation is still unknown. The statistics analysis for correlation between seed viability and seed vigour of *E. capensis* revealed positive correlation between seed viability and seed vigour. The correlation figure of both viability (0.953) and vigour (0.953) were close to value of one (1) which suggests a very strong positive correlation. The positive correlation conforms to results test from this research for both viability and vigour, which revealed that increase of seed moisture content, the seed longevity period was increased.

CONCLUSION AND RECOMMENDATION

The study showed that, both seed moisture content and storage temperature influenced seed vigour in storage. Storing of seeds with 15, 25 and 35% MC across a constant temperature of -5, 10 and 25ºC depicted that decreasing both seed moisture content and storage temperatures influenced seed vigour in storage. Therefore, MC and storage temperature are the two factors that influenced seed vigour. The study confirms that, the *E. capensis* seeds are recalcitrant that stored long at 35% MC and with both high viability and vigour across all the storage temperature.

The findings further revealed that there is continuum decrease in vigour as moisture content decreases. Thus, concluding that there is a strong negative correlation between vigour and percentage moisture content. As moisture content declined with time, equally the vigourity declined at respective storage temperature.

The study also showed that seed vigour increased with increase in seeds moisture contents and storage temperature. The seed with highest moisture contents of 35% had the highest vigour across all the storage temperature. However, it decreased when storage temperature was increased to 25ºC with different moisture contents. Based on the findings obtained in the present study, the following recommendations were made:

- For maintainace of seed vigour, *E. capensis* seeds, should be stored at 10ºC with 35% of moisture content.

ACKNOWLEDGEMENT

The authors wish to thank KEFRI for providing the facility to conduct the research. The farmers who provided the fruits from which the seeds were extracted are greatly acknowledged.

REFERENCES

Abbas, F. A., IsHak, J. A., Kreuze, J. F., Johansson, A., Mukasa, S. B., Tairo, F. & Valkonen, J. P. T. (2003). Some molecular characteristics of three viruses from SPVD-affected sweet potato plants in Egypt. *Archives of Virology*, *148*(12), 2449-2460.

Abiot, Y., Moriyasu, M., Ichimaru, M., Kato, A., Mathenge, S. G., Nganga, J. N. & Juma, F. D. (2018). Absolute configurations of two acyclic triterpenoids from Ekebergia capensis. *Phytochemistry*, *52*(8), 1593-1596.

Agha, S. K., Oad, F. C. & Buriro, U. A. (2004). Yield and yield components of inoculated and un-inoculated soybean under varying Nitrogen levels. *Asian J. Plant Sci., 3*(3), 370-371.

Alpert, P. & Oliver, M. J. (2002). Drying without dying. *Desiccation and survival in plants: drying without dying.* Wallingford: CABI Publishing.

Arbonnier, M. (2004). *Trees, Shrubs and Lianas of West African dry zones*.

Baxter, K. N. (2004). *The genus Syzygium: Syzygium cumini and other underutilized species*. CRC Press.

Bekele, T. (2007). Floristics and phytogeographic synopsis of a Dry Afromontane coniferous forest in the Bale Mountains (Ethiopia): implications to biodiversity conservation. *SINET: Ethiopian Journal of Science*, *30*(1), 1-12.

Bentley R. (Ed.). (2010). *Medicinal plants.* London: Domville-Fife Press.

Castro, R. D., Bradford, K. J. & Hilhorst, H. W. M. (2004). *Embebição e reativação do*

Crawford, A. & Monks, L. (2009). The road to recovery: The contribution of seed conservation and reintroduction to species recovery in Western Australia. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation*, *17*(4), 15.

Desai, A. & Mitchison, T. J. (1997). Microtubule polymerization dynamics. *Annual review of cell and developmental biology*, *13*(1), 83-117.

Gold, K. & Hay, F. (2007). Equilibrating seeds to specific moisture levels. *Technical Information Sheet_09, Royal Botanic Gardens Kew, UK*.

Hong, T. D. & Ellis, R. H. (1996). *A protocol to determine seed storage behaviour* (No. 1). Bioversity International. https://mapcarta.com/12745152.(Accessed on 25.03.2019).

ISTA (2012). *International rules for seed testing, edition 2012*. Int. Seed Test. Assoc., Bassersdorf, Switzerland

Kamadyaapa, D. R., Gondwe, M. M., Moodley, K., Musabayane, C. T. & Ojewole, J. A. (2009). Cardiovascular effects of Ekebergia capensis Sparrm (Meliaceae) ethanolic leaf extract in experimental animal paradigms. *Cardiovascular Journal of Africa*, *20*(3), 162.

Kermode, A. R. & Finch-Savage, B. E. (2002). Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. *Desiccation and survival in plants: Drying without dying*, 149-184.

Kirsten O., Ulrich, V., Morten, G., Kyvik, J. O. & Michael, B. R. (1999). The inheritance of migraine with aura estimated by means of structural equation modelling. *Journal of Medical Genetics*, *36*(3), 225-227.

Koch, H. M., Bolt, H. M., Preuss, R., Eckstein, R., Weisbach, V. & Angerer, J. (2005). Intravenous exposure to di (2-ethylhexyl) phthalate (DEHP): Metabolites of DEHP in urine after a voluntary platelet donation. *Archives of Toxicology*, *79*(12), 689-693.

Kokwaro, J. O. (1976). Medicinal plants of east Africa.

Kolotelo., Sisodia, A., Padhi, M., Pal, A. K., Barman, K. & Singh, A. K. (2001). Seed Priming on Germination, Growth and Flowering in Flowers and Ornamental Trees. In *Advances in Seed Priming* (pp. 263-288). Springer, Singapore.

Komakech, A. J., Dalahmeh, S., Tirgani, S., Niwagaba, C. B. & Ahrens, L. (2018). Per- and polyfluoroalkyl substances (PFASs) in water, soil and plants in wetlands and agricultural areas in Kampala, Uganda. *Science of the Total Environment*, *631*, 660-667.

Krueger, K., Goggi, A. S., Mullen, R. E. & Mallarino, A. P. (2012). Phosphorus and potassiumfertilization do not affect soybean storability. *Journal of Agronomy*, *104*, 405-414.

Laca, A., Mousia, Z., Díaz, M., Webb, C. & Pandiella, S. S. (2006). Distribution of microbial contamination within cereal grains. *Journal of Food Engineering*, *72*(4), 332-338.

Lang, Q. & Wai, C. M. (2001). Supercritical fluid extraction in herbal and natural product studies—a practical review. *Talanta*, *53*(4), 771-782.

Lewis, E. J. (2002) A study of some chilling responses of recalcitrant seeds of Avicennia marina (Forssk.) Vierh. and Ekebergia capensis Sparrm.

Liu, W. J., Tan, J. W. & Bai, K. J. (2001). Microclimate edge effects within and between *Shorea chinensis* fragments in Xishuangbanna. *Acta Phytoecologica Sinica 25*, 616–622 (*in Chinese with English abstract*)

Luo, Y., Guan, Y. J., Huang, Y. T., Li, J., Li, Z. & Hu, J. (2015). Single counts of radicle emergence provide an alternative method to test seed vigour in sweet corn. *Seed Science and Technology*, *43*(3), 519-525.

Mairura, F. S. (2008). Ekebergia capensis Sparrm. In Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Eds.). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Accessed 25, March. 2019.

Martin R. B. & White, P. J. (2003). Calcium in plants. *Annals of Botany*, *92*(4), 487-511.

Maroyi A. (2013). *Warburgia salutaris* (Bertol. f.) Chiov.: A multi-use ethnomedicinal plant species. *J Med Plant Res*, *7*, 53–60.

McDonald, B. A., & Linde, C. (1999). The population genetics of plant pathogens and breeding strategies for durable resistance in capensis seeds *Euphytica*, *124*(2), 163-180.

Murata, T., Miyase, T., Muregi, F. W., Naoshima-Ishibashi, Y., Umehara, K., Warashina, T. & Ishih, A. (2008). Antiplasmodial triterpenoids from Ekebergia capensis. *Journal of natural products*, *71*(2), 167-174.

Pammenter, N. W., Berjak, P., Farrant, J. M., Smith, M. T. & Ross, G. (1994). Why do stored hydrated recalcitrant seeds die? *Seed Science Research*, *4*(2), 187-191.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). *Agroforestree Database: A tree reference and selection guide* (Version 4.0). Kenya: World Agroforestry Centre.

Pammenter, N. W. & Berjak, P. (2013). Physiology of desiccation-sensitive (recalcitrant) seeds and the implications for cryopreservation. *International Journal of Plant Sciences*, *175*(1), 21-28.

Patil, S. G., avishankar, G., Hebbara, M. & Dasar, G. V., (1997). Conjunctive Use of Saline Water on Growth and Yield of Cotton. *Karnataka Journal of Agricultural Sciences*, *18*(2), 302-305.

Paton, A. J., Brummitt, N., Govaerts, R., Harman, K., Hinchcliffe, S., Allkin, B. & Lughadha, E. N. (2008). Towards Target 1 of the Global Strategy for Plant Conservation: a working list of all known plant species—progress and prospects. *Taxon*, *57*(2), 602-611.

Perry, D. A. (1984). Commentary on ISTA Vigour Test Committee Collaborative Trial. *Seed Sci.Technl.,12*, 301-308.

Prota, A., Bossio, A., Fabbrocino, F., Monetta, T., Lignola, G. P., Manfredi, G. & Bellucci, F. (2019). Corrosion effects on seismic capacity of reinforced concrete structures. *Corrosion Reviews*, *37*(1), 45-56.

Radhamani., S. P., Radhamani A., Rajendra, M., Michael, S., & Yule, L. (2003). Virus-induced gene silencing. In *Plant Functional Genomics*, pp. 287-293. Humana Press.

Savage, W. E. & George, W. B. (2015). Seed vigour and crop establishment: Extending performance beyond adaptation. *Journal of Experimental Botany*, *67*(3), 567-591.

Scott-Shaw, C. R. (1999). *Rare and threatened plants of KwaZulu-Natal and neighbouring Regions*. Pietermaritzburg: KwaZulu-Natal Nature Conservation Service.

Shaw, K. (2015). Encouraging and enabling a science-based approach to ecological restoration: an introduction to the work of the Ecological Restoration Alliance of Botanic Gardens (ERA). *Sibbaldia: The Journal of Botanic Garden Horticulture*, *13*, 145-152.

Sivritepe, H. O. (2012). Assessment of seed vigour. *Alatarım, 11*(2), 33-44.

Taiz, L. & Zeiger, E. (2009). *Fisiologia Vegetal* (4th Ed.). Porto Alegre: Artmed.

Tang, Y., Sheng, C., Cao, M. & Zhang, J. (2000). Viable seeds buried in the tropical forest soils of Xishuangbanna, SW China. *Seed Science Research*, *10*(3), 255-264.

Thomsen, S. J., Markham, K. R., Andary, C. & Cardon, D. (2000). The photostabilities of naturally occurring 5-hydroxyflavones, flavonols, their glycosides and their aluminium complexes. *Journal of Photochemistry and Photobiology A: Chemistry*, *136*(1-2), 87-91.

Van Wyk, B. E., Oudtshoorn, B. V. & Gericke, N. (1997). *Medicinal Plants of South Africa*. Briza.

Vertucci, C. W. & Roos, E. E. (1993). Theoretical basis of protocols for seed storage II. The influence of temperature on optimal moisture levels. *Seed Science Research*, *3*(3), 201-213.

Walters, D. R. (2005). Are plants in the field already induced? Implications for practical disease control. *Crop Protection*, *28*(6), 459-465.

Weinberg, H. E., Peter, R., Stamp, P. & Hund, A. (2008). Genetic diversity of Swiss maize

(Zea mays L. ssp. mays) assessed with individuals and bulks on agarose gels. *Genetic Resources and Crop Evolution*, *55*(7), 971-983.

Zedan, H. (2005). The role of the convention on biological diversity and its protocol on biosafety in fostering the conservation and sustainable use of the world's biological wealth for socio-economic and sustainable development. *Journal of Industrial Microbiology and Biotechnology*, *32*(11-12), 496-501.