PHYSIOLOGY OF SEEDS OF *Ekebergia capensis* (Sparmm) DRIED TO VARIED MOISTURE CONTENTS AND STORED AT SELECTED TEMPERATURE REGIMES

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DECLARATION

DECLARATION BY THE CANDIDATE

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To all my family members for their support during the study period.

ABSTRACT

Ekebergia capensis is a popular indigenous multipurpose tree species valued for alternative medicine, furniture, light construction, poles and tool handles. Production of seedlings of this tree is through seeds. There are challenges acquiring viable and vigorous *E. capensis* seeds for raising planting materials due to difficulties in post-harvest storage. In spite of the tree's exceptional multipurpose qualities, very little effort has been put in improving the post-harvest storage of the seeds. Changes occurring in seeds during aging are significant as far as seed quality and longevity are concerned and are a consequence of the effects of different storage conditions and *E. capensis* seeds are not exceptional. In addition, information on the seed's viability and vigour loss in storage is scanty and unreliable. It is against this background that this study investigated the effects of drying to different moisture contents (MC) and storage temperature regimes on viability and vigour loss of *E. capensis* seeds for a varied period of time. This was achieved by determining the rate of loss of viability, vigour and longevity of seeds of *E. capensis* under varying moisture contents and temperatures regimes. The experiment comprised 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 and control with moisture content of 47 %. The stored seeds were retrieved at an interval of 30 days for viability, vigour and longevity assessment. Data generated were subjected to analysis of variance (ANOVA), regression and correlation analyses using GenSTAT- 16 software. Findings from the 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 viability, vigour and highest P50 (measure of longevity in days) compared to seeds with lower moisture content (25% or 15%) stored across all temperature regimes. In addition, seeds with 35% MC stored at 10 ^{o} C maintained highest longevity (P50) as storage period progressed to 90 days. There was a negative correlation between storage period and seed viability regardless of the storage temperatures and moisture content. Decrease in seed viability, vigour and longevity, in storage was in the order of 15%>25%>35% MC. The findings of this study showed that the critical % moisture content and storage temperature for maintenance of seed viability for a period of 30 days in seeds of *E. capensis* seeds is at 35% and 10 ^{o} C respectively. It is therefore recommended that *E. capensis* seeds should be dried up to a moisture content of 35% with storage temperature of 10 ^{o}C for 30 days for better longevity, vigour and viability.

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LIST OF ABREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CMC	Critical Moisture Content
DFSC	Danida Forest Seed Center
G. I	Germination Index
IMC	Initial Moisture Contents
IPGRI	International Plant Genetic Resources Institute
ISTA	International Seed Testing Association
KEFRI	Kenya Forestry Research Institute
LSMC	Lowest Safe Moisture Content
MC	Moisture Content
ND	Non-desiccated
RH	Relative Humidity
TMC	Targeted Moisture Contents
FAO	Food and Agriculture Organisation

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Seeds are the most convenient and successful way of storing the genetic diversity of plant species and for producing new plants routinely for agriculture and horticulture (Sehrawat, 2014; Shaban, 2013). 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 and Bassel, 2015).The importance of seed storage is to conserve seeds in a way that maintains their viability and vigour for the longest possible time from harvest to sowing (Hilli *et al.* 2003, Rajjou and Debeaujon 2008). However, conditions in which the seed is found during standard germination test are often in conflict with the conditions in the field, and therefore seed vigour test is necessary (Morab, *et al* 2015).

Seed storage and the ability to predict seed longevity must therefore not be underestimated. The critical moisture level to which mature embryos can be dried without inducing desiccation damage is generally species dependent and serves as a tool to define whether a seed is orthodox, recalcitrant or intermediate (Vertucci *et al*, 1995). Failure of a seed to germinate is the final outcome of a whole series of detrimental changes that together characterize seed storage (Martyn *et al.*, 2009). Due to the practical importance of maintaining seed stocks, much research has been undertaken to find the causes of deterioration in seed quality that often occurs during storage (Ndungu, 2018; Martyn *et al.*, 2009).

1.2 Statement of the Problem

Ekebergia capensis has versatile uses including its wide use in traditional medicine (Mairura, 2008). However, the propagation and multiplication of the tree has remained low due to limited knowledge on seed viability, vigour and longevity. It is suspected that the tree produces recalcitrant seed type and hence loses viability after maturity due to desiccation and /or un-favourable conditions for germination. This has led to its slow re-generation in nature. These challenges in *E. capensis* seed storage compromises its contribution towards achieving 10% forest cover in land and national tree cover target by 2030.

Prediction of longevity of many agricultural seed species using seed viability model exist (Tang *et al.,* 2000). Seed viability model for agricultural seeds species exist for wheat (Laca *et al.,* 2006), maize (Weinberg *et al.,* 2008 a) and soybean (Baek *et al.,* 2019). However, seed viability predictions for indigenous trees are poorly studied. There is scanty information available on the longevity of *E. Capensis* seeds. Precisely, there is paucity of information on how seeds of this species stored under different moisture content and temperature regimes would lose viability and vigour. Therefore, an intervention is required on handling of *E. capensis* seeds so as to prolong their shelf life and ensure seed stocks for increasing Kenya's forest cover and availability of useful tree species for purposeful planting by interested communities. It is against this background that the study seeks to investigate the viability, seed vigour and longevity of *E. capensis* seeds when stored with different moisture contents at different temperatures regimes.

1.3 Justification of the Study

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 *Ekebergia capensis* (Abiot *et al*., 2018, Orwa, *et al*.,2009). This plant is expansively used for treatment of various diseases such as dysentery, headaches, heartburn, chronic coughs, intestinal worms, diarrhea and cholera. The existence of this 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 this species in Kenya. However, studies on regeneration strategies cannot be conducted without information on the seed storage behavour of this species hence the study.

1.4 Objectives

1.4.1 Main objective

To determine the storage behavior of *Ekebergia capensis* seeds in order to provide information for developing a protocol for utilization in *ex-situ* regeneration and conservation.

1.4.2 Specific objectives

1. To determine the effect of varying moisture content and storage temperature regimes on viability of *Ekebergia capensis* seeds.
2. To determine the effect of varying moisture contents and storage temperature regimes on seed vigour of *Ekebergia capensis* seeds.
3. To determine the seed longevity of seeds of *Ekebergia capensis* after storage at various moisture contents and temperatures regimes.

1.5 Hypothesis

H_{01}: Different seed moisture contents and storage temperature regimes have no effect on seed viability of *E. capensis*.

H_{02}: Storage of fresh seeds of *E. capensis* at different moisture contents and temperature regimes has no effect on seed vigour

H_{03}: Storage of fresh seeds of *E. capensis* at different moisture contents and temperature regimes have no effect on seed longevity.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy and distribution of *Ekebergia capensis*

Ekebergia capensis is a tree that belongs to Melliacea (the Mahogany) family. It is commonly known as the Cape ash. This is a tree that grows up to 30 m high (Figure 2.1). It is known in some local languages as; *Araruet* (Kipsigis), *Arar* (Marakwet) *Mukongui* (Kamba), *Tido* (Luo), *Muchogomo* (Meru) (Kokwaro, 1976). The Nandi call it *Teldet*.

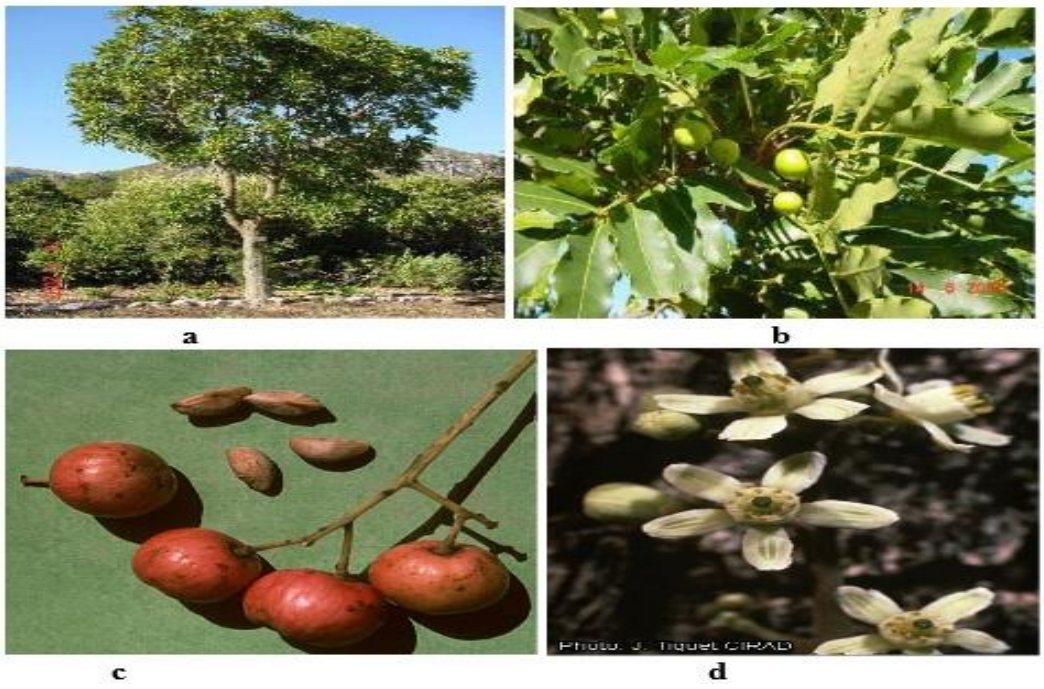

Figure 2.1 Photo of E. capensis Tree (a), leaves and fruits (b), ripe fruits and seeds (c) and flowers (d)

Source: (Mairura, 2008)

Ekebergia capensis is widespread, from Senegal east to Eritrea and Ethiopia, and south of Botswana, eastern South Africa and Swaziland (Arbonnier, 2004). In West Africa *Ekebergia capensis* occurs in dry forest and riverine forest on well drained soils. In East

and southern Africa, it is found in montane and riverine forest at 600–3000 M above the sea level, but also in savanna woodland and wooded grassland, and then often on termite mounds. It prefers deep sandy soils. The rainfall range is 750–2000 mm/year (Arbonnier, 2004).

2.2 Morphology of E. capensis

Bekele (2007) described *Ekebergia capensis* as an evergreen or sometimes semideciduous, dioecious, small to medium-sized tree up to 30 m tall; bole straight or sometimes crooked, branchless for up to 12 m, up to 100 cm in diameter, fluted or with short buttresses at base; bark surface smooth but in older trees often becoming rough and scaly, pale grey to dark grey or brownish grey, inner bark reddish, sometimes with white streaks; crown large and spreading or dense and rounded; twigs short-hairy, glabrescent, with conspicuous whitish lenticels, branchlets marked by circular leaf-scars (Bekele,2007).

Leaves arranged spirally, clustered in lax groups at ends of branches, imparipinnately compound with 3–7(–8) pairs of leaflets; stipules absent; petiole 2.5–10 cm long, swollen at base, rachis up to 25 cm long; petiolules 2–10 (–20) mm long; leaflets opposite or nearly so, elliptical to lanceolate or oblong-lanceolate, 3–13 (–14.5) cm × 1.5–6 cm, cuneate to rounded and asymmetrical at base, acute or shortly acuminate at apex, margin entire, papery to thinly leathery, short-hairy or glabrous below, pinnately veined with 10–15 pairs of lateral veins. Inflorescence an axillary panicle up to 20 cm long, densely short-hairy (Mairura, 2008).

Flowers unisexual, male and female flowers very similar in appearance, regular, (4–)5-merous, greenish white or pinkish white, fragrant; pedicel c. 2 mm long; sepals fused at

base, 1–3 mm long, short-hairy outside; petals free, 4–7 mm long, hairy outside; stamens with filaments fused into a cup-shaped tube, with usually 10 anthers inserted on the rim, ovary superior, almost globose, 2–5-celled, style 0.5–1 cm long, stout, stigma head-shaped; male flowers with rudimentary ovary, female flowers with smaller, non-dehiscing anthers. Fruit a globose to ellipsoid drupe 1–2(–3) cm long, pink to red-brown or deep red when ripe, with 2–4 stones, each stone usually containing 1 seed. Seeds with thin seed coat (Bekele,2007).

Seedling with epigeal germination; hypocotyl 3–5 cm long, epicotyl 6–8 cm long; cotyledons fleshy, c. 1.5 cm long; first 2 leaves opposite, imparipinnately compound with 1–2 pairs of leaflets (Bekele, 2007).

E. capensis is characterized by a rough light grey to almost black bark, with few buttress roots at the base. Bekele, (2007) further explained that the large glossy green leaves that are often tinged with a pinkish patch, or pink edges are pinnate. Also, the small sweetly scented flowers are white, occasionally also with pink tinge. They appear in loose sprays, in the months (September to November). A fleshy fruit containing four seeds appears seems inexperienced so turns bright red because it ripens in season.

2.3 Uses of *E. capensis*

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, toys, novelties, vats, food containers, boxes, crates, matches, turnery, veneer and plywood. It is also used as firewood and for

charcoal production (Komakech, 2018, Bekele, 2007). The bark, roots and leaves are widely used in traditional medicine (Komakech, 2018)

Bark decoctions, infusions and macerations are taken to treat inflammation, heartburn, dysentery, epilepsy. It is applied externally to ulcers, abscesses, boils, scabies, acne, pimples and itching skin (Bekele, 2007). A powder ready 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 and headache (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 (Murata *et al.*, 2008).

The wood is used by mid-wives in Zulu community to facilitate childbirth (van Wyk *et al.*, 1997). Decoctions of assorted components of *Ekebergia capensis* are used traditionally in Central Federal Democratic Republic of Ethiopia as an anthelmintic for the treatment of eutherian mammal. Bark and roots are used as ordeal poisons.

In southern Africa the bark has been used for tanning. The fruit is edible but usually not much liked. The foliage is browsed by livestock in the dry season. *Ekebergia capensis* is planted as an ornamental, particularly as a roadside tree, but also as a garden tree for its attractively coloured fruits and for shade (Bekele, 2007). The flowers are a source of nectar and pollen for honey bees (Van Wyk *et al.*, 1997).

2.4 Orthodox, Intermediate and Recalcitrant Seed Types

To be successful, storage conditions must maintain seed vigour and viability and ensure that normal seedlings are subsequently established under nursery conditions. Seed storage conditions ideally entail sub-zero temperatures and low relative humidity (RH), requiring the seed to have a low water content to prevent freezing damage (Pammenter and Berjak, 2013). Seeds that can be stored under these conventional conditions are referred to as 'orthodox', this term referring strictly to seeds whose storage life span can be predicted from seed water content and storage temperature (Roberts, 1973).

There is also a category of seeds referred to as 'recalcitrant' that are desiccation-sensitive, and so cannot be stored in the dry state, or at sub-zero temperatures. Many recalcitrant seeds (especially of tropical origin) are also chilling-sensitive, and should not be stored at extremely low temperatures (Roberts, 1973).

The terms orthodox and recalcitrant were introduced by Roberts in 1973, and have now come to refer to the different abilities of seeds to tolerate water loss or desiccation - although originally orthodox and recalcitrant were descriptors of storage behavior. It has since been suggested that there are many seeds that cannot be exclusively categorized, and which fall somewhere between desiccation sensitive and desiccation tolerant seeds (Berjak *et al*, 1994).

There are three categories into which seeds can be grouped according to their desiccation tolerance: orthodox, intermediate and recalcitrant. When orthodox seeds are mature, they have moisture contents lower than that facilitating germination (Daws *et al.*, 2004). This is a consequence of maturation drying, during which the loss of significant amounts of

water occurs in the final stage of their development, so that when such seeds are shed, they have a water content that is usually in equilibrium with the ambient relative humidity (Finch-Savage &Leubner-Metzger, 2006).

Orthodox seeds can be dried to a point where all tissue water is non-freezable, existing either as a 'glass' or bound to the surfaces of intracellular structures and macromolecules (Vertucci, 1990). This facilitates storage at sub-zero temperatures, for example -18°C, where they should remain viable (Daws *et al.,* 2004).

Intermediate seeds can be dehydrated to relatively low levels, but not to levels as low as those typical of orthodox seeds (Ellis *et al.,* 1990). The level to which intermediate seeds can be dehydrated makes conventional storage possible, but in some cases, they are sensitive to low temperatures even in the dehydrated state (Hong and Ellis, 1996). Those authors have suggested this dehydration-related chilling sensitivity to typify the seeds of some tropical species, which means that they must be stored at relatively high temperatures once they have been dehydrated. The relatively high temperatures, more than 15 ° C at which they have to be stored favour the activity of fungi and other microflora, leading to deterioration and death of the seeds (Bewley *et al.,*2012). Recalcitrant seeds are hydrated and metabolically active when they are shed, and in many cases, can germinate without additional water (King and Roberts, 1980). It is thought that the more actively metabolic a seed is when it is shed, the less water loss it tolerates (Berjak & Pammenter, 2007).

Plants producing recalcitrant seeds usually, but not exclusively, occur in humid, tropical environments where germination can be immediate, as the environment is usually

suitable for seedling growth throughout the year (Daws *et al.*, 2004). Recalcitrant seeds are damaged if water is lost, resulting in a loss in viability, although the degree of dehydration tolerated is a variable function of the species and the drying conditions (Berjak & Pammenter, 2007). Even at relative humidity that do not allow water loss, viability usually declines in a relatively short period of time - this is related to the onset of germination processes that require provision of additional water (Berjak & Pammenter, 2007). Recalcitrant seeds cannot be stored at sub-zero temperatures because of ice-crystal damage, these seeds are chilling sensitive (Daws *et al.*, 2004). Therefore, for seeds that are intermediate or recalcitrant in nature conventional seed storage cannot be used.

2.5 Long and Short-Term Storage of Recalcitrant Seeds

Conditions of low temperature and low relative humidity (RH) are the most useful for seed storage as metabolic rate is reduced, thus preventing or slowing deteriorative events (Wills *et al.*, 2016). Pammenter *et al* (2013) stated that cooling and drying mainly form the basis for long-term preservation of all seed types.

The long-term storage of orthodox seeds is relatively easy, as they should remain viable indefinitely under conventional seed storage conditions, but for recalcitrant and intermediate seeds, more complex techniques are necessary. Long-term storage of many recalcitrant seed species has been enabled by the development of a protocol that allows successful cryopreservation. Cryopreservation involves the very rapid freezing, in the present case of plant material, by plunging it into a cryogen; liquid nitrogen at the temperature of- 196 ° C often being used (Engelmann, 2004). In order for survival of the plant material, it must be dehydrated beforehand to a low enough water content, and must

be frozen sufficiently rapidly, to prevent crystalline ice from forming, as this is lethal (Berjak *et al.*, 1999).

It has been found that if water is removed rapidly there is little time for any damaging reactions to occur, and so a lot more water can be removed than if this were to be done slowly (Pammenter *et al.*, 2013). Most species of recalcitrant seed type are too large to be dried rapidly, and, although success with rapid drying of whole seeds has been achieved (Pammenter and Berjak, 1999), it is usual to remove the embryonic axis of a seed and to dehydrate it rapidly in a stream of air. This is called 'flash drying' (Berjak *et al.*, 1999).

The low water contents able to be achieved by flash drying, and the rapid freezing rate achieved by plunging into a suitable cryogen, minimize ice crystal growth so that it occurs at a sub-lethal level (Hajari, 2011). If excised zygotic embryonic axes or any other plant material besides seeds are cryopreserved, it must be borne in mind that *in vitro* methods need to be used to regenerate seedlings afterwards, as the natural food supply has been removed (Pence,2002). Pence (2002) defines *in vitro* techniques as those that utilize tissue culture methods in the maintenance, production or modification of plant material.

The long-term storage of seeds, or any plant material, is usually in aid of genetic conservation and is aimed at maintaining seed viability and genetic stability for decades or longer (Li and Prichart, 2009). Short-term storage of seeds is required for many practical purposes, and although conventional seed storage can be used for both long and short-term storage of orthodox seeds, cryopreservation is unsuitable for the short-term storage of recalcitrant seeds (Li and Prichart, 2009).

A simple reduction in temperature would be ideal for short-term storage, this is not suitable for all recalcitrant seeds. For example, recalcitrant cocoa seeds cannot be cooled to below 10 ° C without losing their germinability, so the maximum storage life that has been achieved is eight months with 24% germinability being retained (Bewley & Black, 2012). There are many similar examples of short-term storage of recalcitrant seeds during which many problems are encountered, and it seems that the only way in which to improve the methods currently in use is to gain a better basic understanding of these problems. Kioko *et al.* (2006) states that there is lack of information on viability and longevity of seed under different storage conditions in Kenya. Consequently, there is need for experimental data collection and evaluation on *E. capensis* seed storage behavior.

Germination of seed is a function of duration of storage, storage temperature and moisture content at storage (Handley *et al.,* 2005). Therefore, sensible management of storage temperature and storage length might maintain high germination of the seed. There is need to investigate and characterize specific storage conditions that are optimal for favourable germination percentages of *E. capensis* seed.

2.6 Growing *Ekebergia capensis*

Cultivation of indigenous plants is a proper way of maintaining and increasing the supply of useful plants products to the market (Mander *et al.,* 1996). Recent experience has shown that it is possible to cultivate numerous indigenous plant species in sophisticated agricultural systems. However, the feasibility of cultivating these plants in small-scale, low-input farming systems is unknown (Prinsloo and Street, 2007).

It is important to conserve medicinal plants by propagation and cultivation in a controlled environment. The solution for conservation is to develop protocols for cultivating valuable medicinal plants as small-scale farming crops (Van Staden, 1999). This enhances sustainable protection of biodiversity and also generates income for many through entrepreneurial planting of medicinal plants (Sparg, 2003).

Although micro-propagation protocols have been established for many indigenous medicinal species, these techniques are labour-intensive, costly and therefore only feasible for high-value species (Sparg,2003). Seed propagation is more promising and cost-effective for the mass production of seedlings (Zhou *et al.,* 2003).

E. capensis plant can be grown from freshly collected seeds. The fresh fruits mainly collected from grown method are soaked in water for a day and then scrubbed with a brush to remove the fleshy part which when left may form substrate that promote fungal or bacterial growth hence causing infections to the seed. The seeds are then sown in trays filled with river sand or normal potting soil, and planted not deeper than 5mm. They germinate in 4 - 8 weeks (Arbonnier, 2004). For large scale forest plantations, several nursery beds are raised on a hard-pan surface and loam soil mixed with sand is spread on to about 1cm high (Arbonnier, 2004). It can also be grown from cuttings (Zhou *et al.,* 2003). This is the fastest method of propagating this tree. Tip or hardwood cuttings can be planted in trays filled with river sand or can be planted directly into the ground as truncheons (Bekele, 2007).

E. capensis grows well when it is given lots of water, but can tolerate light drought and light frost, however, it is sensitive to heavy frost. A study conducted by Mairura (2008)

reported that the germination of *E. capensis* seed can be as high as 90% when ripe seeds are collected from the tree, but is usually up to only 50% when the seeds are collected on the ground beneath the tree. Soaking the seeds in water for one day and subsequent scrubbing with a brush promotes germination.

2.7 Seed Germination

Seed germination consists of the events that occur between a seed imbibing water and the emergence of the embryonic axis through its surrounding structures (Bewley, 2006). In practice, there a range of ways in which germination is measured ranging from radicle emergence (Steadman *et al.,* 2003; Ooi & Luo, 2006), often by a defined amount (Baker *et al.,* 2005; Hoyle *et al.,* 2008b), to complete emergence of both roots and shoots (Northam and Callihan, 1994; ISTA, 2009).

In order for germination to occur there are three basic requirements; water, oxygen, and a suitable temperature. For some species light may or may not be needed for germination to occur (Baskin and Baskin, 2001). In addition to these requirements for germination there are a range of phytohormones that can stimulate germination (van Staden *et al.*, 2000).

The first requirement for germination is a suitable seed moisture content (Baskin and Baskin, 2001). For laboratory testing, a range of moistened substrates such as paper, sand or organic matter (ISTA, 2009) or a medium containing water such as agar (Terry *et al.,* 2003) can be used to provide seeds with adequate moisture for germination.

Oxygen is required by germinating seeds for respiration. Whilst not normally a limiting factor in laboratory testing of seed germination, some seeds may have covering structures

that may prevent or limit the amount of oxygen getting to the embryo (Baskin and Baskin, 2001).

The precise temperatures required for seed germination is species specific, with each species having maxima and minima, above or below which no germination occurs (Côme and Corbineau,2006). The range of temperatures over which a species germinates is also be affected by the dormancy status of the seed (Vleeshouwers *et al.,* 1995). Whilst alternating temperatures can often produce better germination than constant ones (Baskin and Baskin, 2001), constant temperatures are often used successfully for laboratory germination (ISTA, 2009). Many species germinate equally well in light or in the dark. There are however some species that germinate better in the light and others that do better in the dark (Baskin and Baskin, 2001). For routine germination testing in the laboratory, it is recommended that seeds are exposed to light, unless there is evidence that it is inhibitory, as it allows for better germination (ISTA, 2009). Where artificial lights are used, the photoperiod is often alternating (Turner and Merritt, 2009).

2.8 Seed Storage Behaviour

Seeds can be classified into three different categories depending on their storage behaviour. According to Dickie and Pritchard (2002) seeds with orthodox storage behaviour make up about 90%, while 7% are recalcitrant and 3% are considered intermediate. It should be noted that this information is based on less than 2.5 % of all plant species, since tropical moist forest species are under-represented and that storage behaviour may vary within genera such as those for Acer, Magnolia and Ekebergia species (Dickie and Pritchard, 2002).

Orthodox seeds are best stored in a state of low moisture content and at low, generally subzero temperatures. Recalcitrant seeds species are usually found in tropical, sometimes in temperate, areas (Kolotelo *et al,* 2001). Their seeds are generally larger than orthodox ones, sensitive to low temperatures and must, to preserve their viability, be stored with high moisture content (Dickie and Pritchard, 2002). Since there are always exceptions a third "intermediate" storage category is recognized. Here different seed species show a continuum of storage behaviour from desiccation and/or low temperature sensitive to species tolerating quite low water potential and/or temperatures (Kermode and Finch-Savage, 2002).

2.9 Factors Affecting the Viability of Seeds in Storage

The length of time that a seed remains viable in storage is influenced by factors like moisture, temperature, and oxygen. In General, viability is retained better under conditions in which the seed metabolic activity is highly reduced, for instance, low temperatures and low moisture content, moreover it is shortened by higher oxygen pressure for many species.

Contrary to these generalizations are the recalcitrant seeds which must remain with relatively high moisture content to maintain maximum viability (Desai 2004). Both desiccation sensitive and desiccation tolerant seeds are damaged when stored at intermediate water potentials. However, the damage varies considerably depending on time among species and tissues (Hong *et al.,* 1998). Storage conditions that fluctuates like changing high/low RH make seeds loose viability fast while altering high/low temperatures at intervals during cold storage does not have any significant deleterious effects on viability (Bewley and Black, 2012).

2.9.1 Water Content

There are different ways in which plants cope with low water potential. Drought tolerant plants resist water loss by having outer coverings that is impermeable to water and also by reducing their surface area-to-volume ratios to avoid desiccation. They adapt to limited water availability while maintaining high internal water concentration. Desiccation tolerance plants resist water loss. Owing to protection mechanisms (Hong et al, 1998) such as a reversible cessation of metabolism enables seeds to survive in spite of water loss (Alpert and Oliver, 2002).

In non-dormant seeds, moisture content above 30% stimulates germination, while average water contents of 18-30% provides ideal conditions for faster deterioration caused by microorganisms. Seeds stored above 18-20% respire and if stored in poor ventilated area, they generate heat that can kill the seeds. Moisture contents of between 18-10% forms a limit for fungal growth and destruction of seeds while below 9-8%, there is very little or total absence of insect activity. Seeds with water content below 5-4% are totally immune to attack by insects and fungi but seed longevity is higher if they are stored at slightly higher moisture content (Bewley, Black, 2012). Metabolic activities of many seeds take place at a very low rate when maintained at 6% water content, and above 15% the metabolic activity of "dry" seeds may be substantial.

According to Kermode and Finch-Savage (2002) the ability to develop full tolerance to desiccation plants with recalcitrant seeds is not known whether it was lost, was never gained or is just not fully expressed in their genetic make-up. The desiccation sensitivity causes is still far from understood. Different ways and processes have been suggested to

be involved in the loss of viability of desiccation sensitive seeds and different processes may predominate in different moisture content regimes (Pammenter *et al.,* 2000).

Relatively high-water content in mature recalcitrant seeds is attributed to abbreviated maturation drying during seed development. The *ex-situ* storage of these recalcitrant seeds poses problems as during storage, drying them below critical moisture content (CMC) level induced cellular damage thus leading to rapid loss of ability to germinate. Therefore, the most important requirement in the preservation of recalcitrant seed is to determine their response to desiccation (Gold and Hay, 2007; Joseph *et al.,* 2011). Knowledge on the lowest safe moisture content (LSMC) or (CMC) of a species is necessary for planning and implementation of seed drying and storage in case of desiccation sensitive seeds (Martin *et al*., 2003; Joseph *et al*., 2011).

2.9.2 Temperature

Temperature of the seed another factor that determine their viability in storage (Desai 2004). Different plants have varying requirements for seed storage temperature (Desai 2004). Furthermore, in their findings, (Desai 2004). found out that high temperature drying is needed for the longevity of most seeds, while low moisture content and temperature between 0 and5 º C are the best conditions for storage of orthodox seeds. Generally, each 5.6 º C decrease in seed storage temperature increases twice the storage life of the seed (Bewley and Black, 2006; Desai 2004). For the case of recalcitrant seeds, no such general rule can apply, especially for tropical plant species where low temperature storage can be unsuitable because many are sensitive to chilling (Bewley and Black, 2012; Kermode and Finch-Savage, 2002). In most cases, combinations of time,

temperature and moisture content which lead to loss of viability of seeds during storage brings about some genetic damage in the survivors.

Temperature dictates the amount of water vapour the air can hold in the storage environment. Environments having higher temperatures has potential for holding more water vapour in the air than those in environments having low temperatures. If the water vapour of the environment is kept constant, the water content of a seed decreases at a rate of 1% with every 6.7 ^{o}C increase in temperature (Delouche *et al*, 1968). Temperature can influence the seed aging process by affecting the rate of some reactions making certain enzymes inactive (Vertucci and Roos, 1993). Using temperature isotherms, Vertucci and Roos (1993) pointed out that optimum moisture content for storage of seeds varies with storage temperature, thus cut-line temperatures for long term storage are those below the glass transition temperature. A study conducted by Bekele (2007) recorded 39% germination of *E. capensis* seeds after 9 months of storage at 4 ° C, and in a test in Ethiopia the germination rate of seeds kept in dry storage for 24 months was 4%. Bekele (2007)

2.9.3 Life Span

The period of time in which seeds can remain viable is genetically dictated. Environmental factors during growth and maturation, harvesting and post- harvesting handling and storage conditions affects on the lifespan of any seed, for example, whether the seed remains viable for the full period is determined by its genetic make-up or whether it loses its viability at some earlier stage (Desai and Mitchison., 1997).

Some seeds are genetically and chemically well adapted for longer storability than others under the same conditions e.g. *Canna spp, Lotus spp* and *Lupinus spp* while most species of agricultural plants are relatively short lived (Desai and Mitchison.,1997). Ageing and deterioration of (orthodox) seeds includes a wide range of degenerative activities that accumulate over time, causing loss of vigour and viability. These can be classified as physiological and biochemical activities. Physiological are reduction in rates of germination and seedling growth, increased number of morphologically abnormal seedlings, decreased ability to germinate when sown under stressful conditions, degrading of functional structures, and depletion of food reserves, increased metabolite and ion leakage and greater susceptibility of seedlings to disease causing micro-organisms (Bernal-Lugo *et al.,* 2000).

2.10 Research Gap

The present study focused on the storage potential of *E. capensis* seeds under natural ambient and -5 ^{o}C, 10 ^{o}C, 25 ^{o}C and moisture contents; 15, 25, 35%). Several workers (Patil *et al*., 1997; Abbas *et al.,* 2003; Radhamani *et al.,* 2003; Baxter *et al.,* 2004; Chin 1995) have already reported the recalcitrant behaviour of seeds of some indigenous trees such as *Havea brasiliensis, Theobroma cacao* and *Artocarpus heterophyllus* under natural drying or slow drying conditions. Attempts have not been made to unravel the duration in which *E. capensis* seeds can maintain high Viability and vigour when stored with different moisture content at varying temperature regimes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site Description

The experiments were conducted at the Kenya Forestry Research Institute (KEFRI) Seed Centre laboratory in Muguga. KEFRI is located 20 km South-East of Nairobi, Kenya. During the month of September 2016, fresh fruits containing seeds of *Ekebergia capensis* were collected from Ainabkoi, Sub-county Uasin Gishu County, (0° 0' 46" S, 0° 31' 12" N, 35° 18' 47" E, elevation, 2894 m above sea level and Cheborорwa, Marakwet West sub county, Elgeiyo Marakwet County located on latitude of (0° 56' 40.4" 0.9446° N, 35° 23' 32.9" 35.39 25° E), Elevation: 2303 M above sea level. (https://mapcarta.com/12745152.).

3.2 Sample Collection and Initial Processing

Freshly ripened and mature fruits of *Ekebergia capensis* were collected randomly from five trees in each collection areas by crown method and embryo maturity testing was done at the site (ISTA, 2009). When the fruits of *E. capensis* change in colour from green to reddish-brown and the mesocarp softens, it is believed to have matured, however, most of them attain physiological maturity when they are still green in colour and have hard mesocarp (ISTA, 2009). 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 (ISTA, 2009). Those seeds that had hard endocarp had attained physiological maturity. 3610 fruits were

then harvested, packed in perforated plastic bags and transported the same day to KEFRI seed center. In the laboratory, the fruits were subjected to post-harvest ripening to bring about uniformity in ripening and to further soften the fleshy part for easy depulping (ISTA, 2009). This was done by putting the fruits in sealed plastic containers and kept at temperatures slightly above room temperature until all the fruits softener. 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 (ISTA, 2009).

3.3 Seed Drying to Desired Moisture Content

Initial seeds weight was done and recorded following the protocol developed by DFSC and IPGRI in 1999. Ten seeds each weighing 5 grams were removed from the seed lot to be used as representative for testing initial seed moisture content. To do this, the ten seeds were divided into five samples each with two seeds and subjected to oven drying for 17 hours at 103 ^{o}C according to International Seed Testing Association procedure for seeds (ISTA, 2007). The seeds were then reweighed and average percentage moisture content calculated. The initial moisture content was found to be 47 %. The remaining seed lot was divided into four equal samples of 900 seeds each. Three of these samples were dried to three different target moisture contents namely 15%, 25% and 35%.

This was done by putting the seeds in perforated bags, weighed, and then placed in 3000 cm^3 (30 cm by 20 cm by 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. During drying, the seeds were constantly mixed with silica gel at 25 ^{o} C in an incubator using desiccation and storage protocol (Thomsen, 2000). The determined initial moisture contents (IMC), initial seed weight (ISW)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):

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

where IMC = initial moisture contents ISW = Initial Seed Weight and TMC= Target moisture contents.

To control the amount of absorbed water removed during drying and rehydration of the seeds, the samples were weighed periodically at interval of 15 - 30 minutes. The drying process was terminated when it reached the weight corresponding to the final degree of 35%, 25% and 15 % each for the three samples respectively. After the seeds attained required moisture content, each sample was further subdivided into three sub-samples of 300 seeds each. Each sample with respective moisture content were further sub-divided into three and stored in airtight glass vials prior to storage at +25 ^{o} C, +10 ^{o} C and -5 ^{o} C temperature, respectively. The stored seeds were retrieved at 30 days interval to determine germination percentage.

3.4 Determination of Seed Viability

Seeds were retrieved at intervals of 30 days for 3 months and subjected to germination assay where respective seeds with different moisture contents stored at various

temperature regimes were tested by germinating the seeds in agar media in germination cabinets inside the glasshouse having constant conditions for computation of germination percentage conditions (ISTA, 2007). Determination of Seed Vigour.

To assess vigour one hundred seeds each of control and those dried to 15%, 25%, and 35% moisture contents stored at -5 ^{o}C, 10 ^{o}C and 25 ^{o}C were retrieved at an interval of 30 days and tested for vigour. Germinated seeds were scored daily for up to 7 weeks and the data was used to calculate germination index. The environmental conditions for example light and germination medium were kept uniform for all the experiments A seed was considered to have germinated when the radicle and the plumule protrudes by 2–3cm. 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 seeds germinating on day "d"

d = days after planting

3.5 Determination of Seed Longevity

The P50 which 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% was used to measure *E. capensis* seed longevity. The P50 was read directly from the germination % graph by drawing a line along X axis at 50% germination 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.

3.6 Data Analysis

Raw data was entered in Microsoft Excel spreadsheet. Preliminary and final data analysis was carried out using GENSTAT® 16th edition statistical software. Since the viability data was in percentage (proportion) it was converted using Arc Sine (Y)1/2 for it to satisfy the assumptions of ANOVA. Descriptive charts were used to show seed life or shelf life and seed vigour. ANOVA (at α=0.05, level of confidence = 95%) was run to determine if there was a significant difference in the viability and vigour levels of seeds dried to different moisture contents and stored at different temperatures. Vigour and viability data were square root transformed to meet model assumptions. In addition, regression analysis was done to determine the relationship between germination percentage and storage period at different temperature and moisture content regimes.

CHAPTER FOUR

RESULTS

4.1 Effects of Varying Storage Temperatures and Seed Moisture Content (MC) on Percent Germination of *E. capensis* Seed at Varying Storage Durations.

4.1.1 Germination of *E. capensis* seeds of varied moisture contents stored at -5 ^{o}C for 30, 60 and 90 days

Before any treatment was applied, the seeds recorded 90 % germination (Figure 4.1). After 30 days of storage at -5 ^{o}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 4.1). Germination percentage of seeds with 25 % moisture contents was 50 % and seeds with 15 % moisture content did not germinate at all.

After 60 days of storage, there was a decrease in germination percentage in all the seeds (figure 4.1) 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 4.1).

After 90 days of storage, there was further decrease in germination percentage for all the seeds (Figure 4.1). The non-desiccated seeds had 51 % germination whereas the seeds having 35 % and 25% recorded germination percentage of 20 % and 5 % respectively (Figure 4.1). There was no germination with seeds stored at 15 % moisture content.

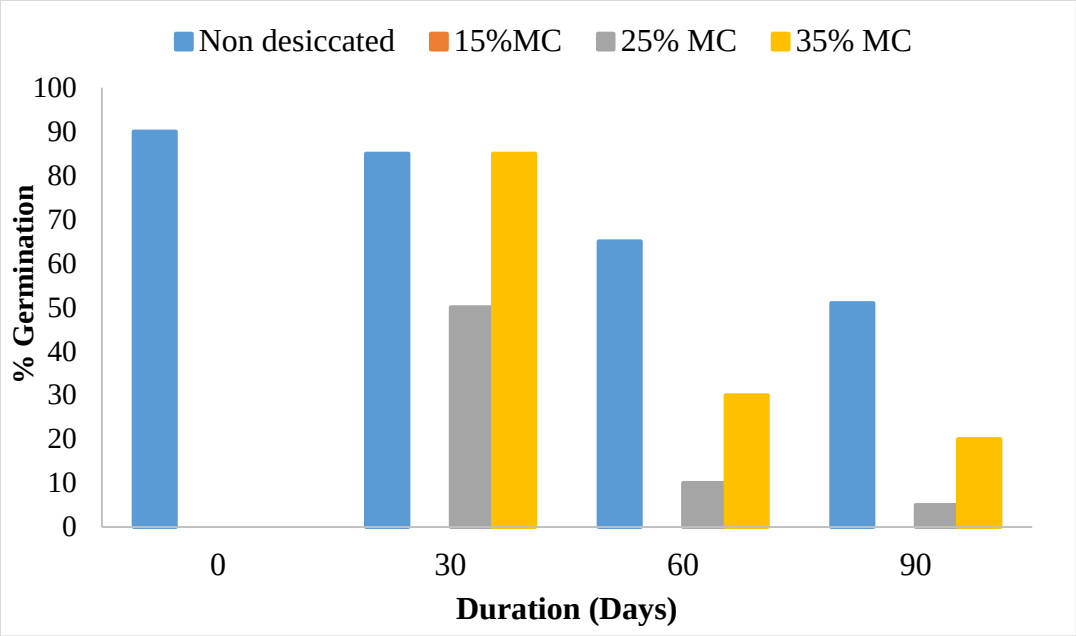

Figure 4.1 Percent germination of seeds with varying moisture contents after storage at -5 ^{o} C for 30, 60 and 90 days.

4.1.2 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 4.2). 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 ^{o}C. (Figure 4.2). However, seeds with 25% moisture content showed reduced germination percentage significantly to 45 percent (Figure 4.2). Seeds with 15% moisture content did not germinate at all after storage (Figure 4.2).

After 60 days of storage at 10 ^{o} C, seeds of *E. capensis* stored with varied moisture contents showed a general decrease in germination percentage. Non-desiccated seeds showed decrease in germination from 85 % to 78 % (Fig 4.2). Germination of seeds

stored with 35 % and 25 % moisture contents was 45 % and 15 % respectively (Figure 4.2). There was no germination of seeds stored with 15 % moisture contents (Figure 4.2).

The non-desiccated seeds (47 % MC) and seeds dried to 35 % stored for 90 days recorded a decreased germination percentage of 71 % and 40 % respectively as compared to when stored for 30 days and 60 days (Figure 4.2). However, it showed higher germination percentage than those stored with 25% and 15 % MC which was 10% and 0% respectively (Fig 4.2). Seeds stored at 15% MC recorded no germination after storage for 30, 60 and 90 days. (Figure 4.2) On the other hand, non-desiccated seeds recorded the highest germination percentage after storing for 90 days while the seeds stored with 25% MC for 90 days showed significantly reduced germination percentage. (Fig 4.2).

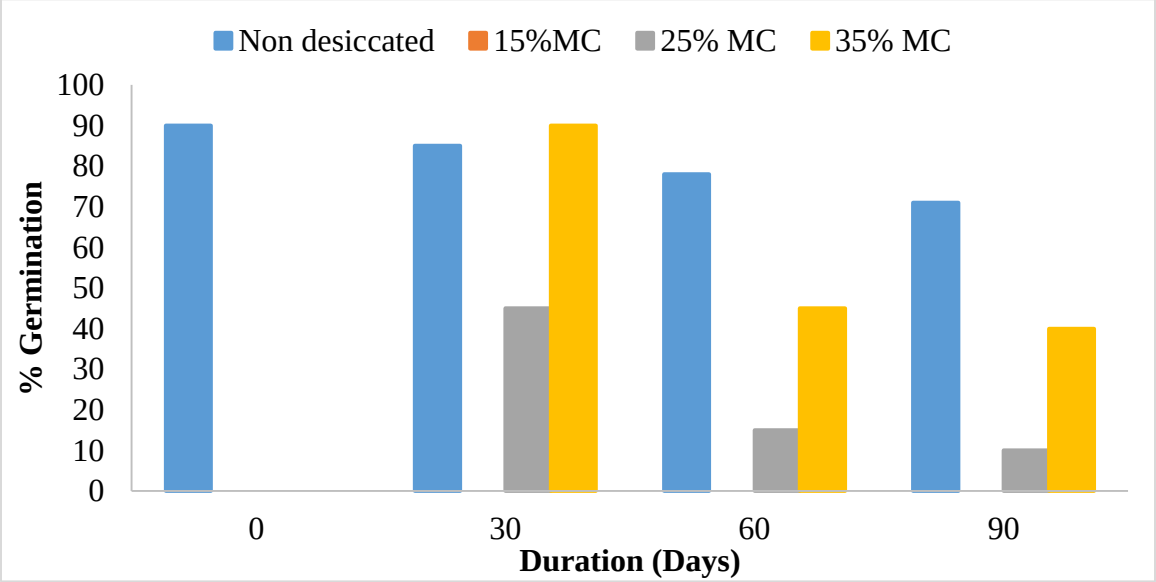

Figure 4.2 Germination percentage of E. capensis seeds of varied moisture contents stored at +10 ^{o} C for 30, 60 and 90 days.

4.1.3 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 4.3).

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 (Fig 4.3). 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 4.3). In contrast, seed lots with moisture content of 15 % maintained germination of 5% throughout the storage periods as shown in (Figure 4.3).

Results on the effect of main factors (storage temperatures, storage periods and percent moisture content) and their interactions on germination % of the seeds are presented in Table 4.1. The results indicated that storage period, % moisture content, storage temperature had significant effect ($p<0.05$) on germination %. In addition, storage period by % moisture content and storage period by temperature interactions showed significant effect ($p<0.05$) on germination % of the seeds (Table 4.1).

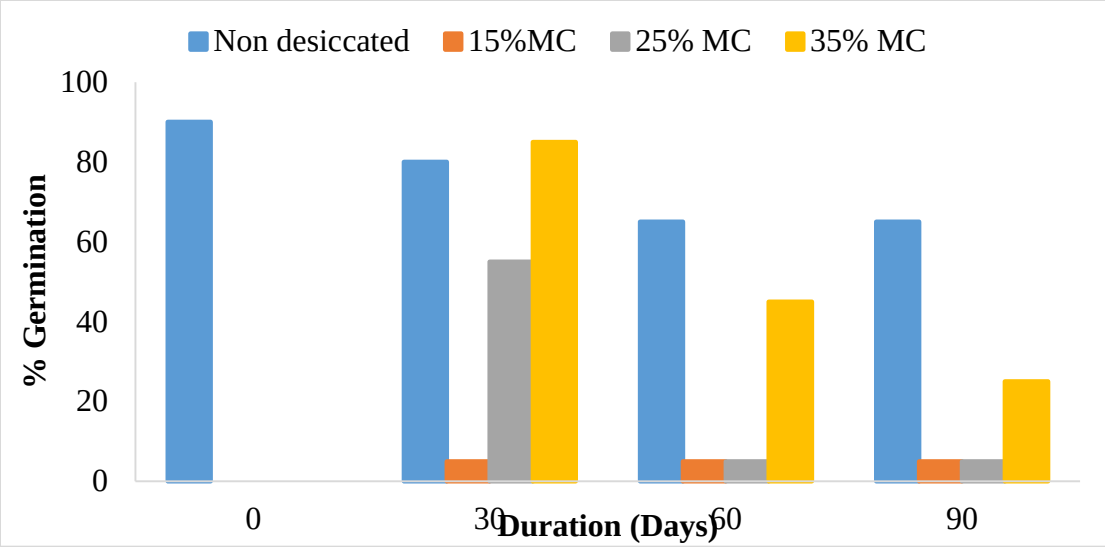

Figure 4.3: Percent germination of seeds with varying moisture contents after storage at 25 ^{o} C for 30, 60 and 90 days

Table 4.1 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.*

4.1.4 Regression analysis of seed germination against storage time, moisture content and storage temperature

Results in Table 4.2 indicate that seed storage moisture content and period had significant effect ($p<0.05$) on seed germination. However, insignificant relationship between storage temperature and germination % was reported.

Table 4.2 Regression of seed germination against storage time, moisture content and storage temperature

Coefficients[a]					
Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
Constant	5.556	12.650		.439	0.665
Moisture content	24.444	3.554	.735	6.877	0.000**
Temperature	0.833	3.554	.025	.234	0.817
Storage period	-14.722	3.554	-.443	-4.142	0.000**

a. Dependent Variable: Germination%

*** denotes significance at p<0.05*

Regression graphs in Figure 4.4 below shows the relationship of seed germination percentage and storage duration of seeds stored with varying moisture contents at various temperature regimes. R^2values of 51.1%, 36.8% and 48.9% were recorded for seeds stored at -5 ^{o}C, 10 ^{o}C and 25 ^{o}C in all the three moisture content regimes.

The graphs exhibit a negative relationship where increase in storage time was inversely propositional to seed germination in the three-storage temperature regimes (Figure 4.4)

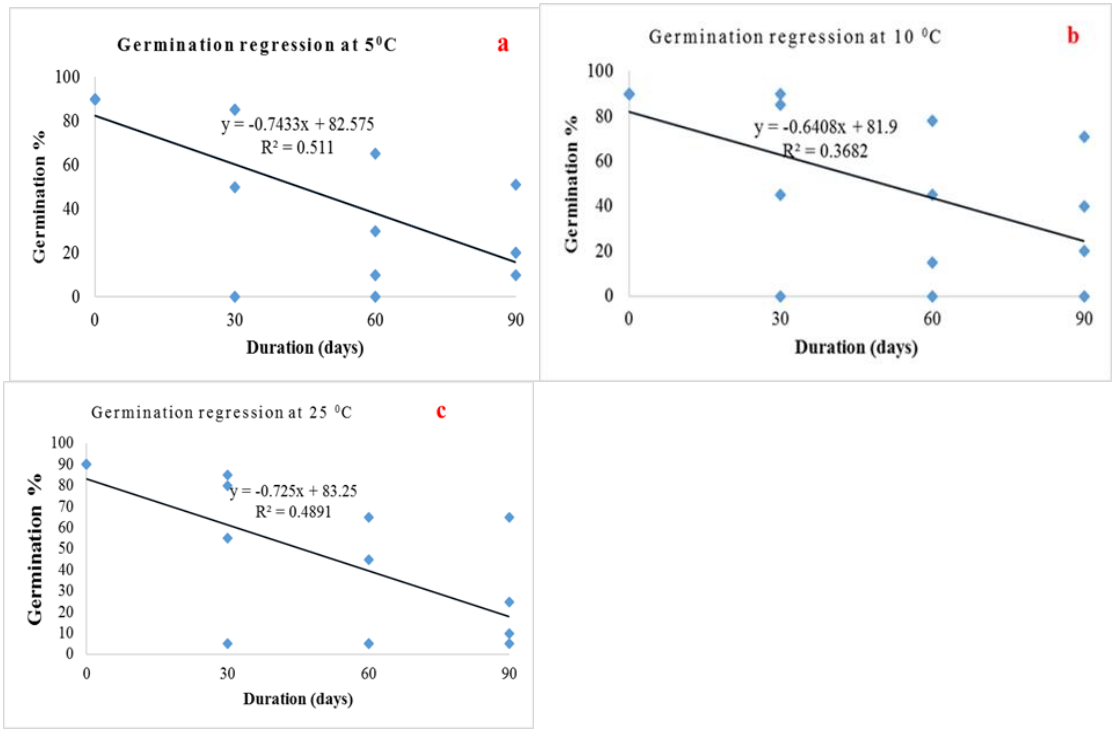

Figure 4.4 Regression graph for seed germination % versus storage duration of seeds stored with varying moisture contents at -5 ^{o} C (a), 10 ^{o} C (b) and 25 ^{o} C (c)

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

4.2.1 Seeds Vigour for seeds stored at -5 ^{o}C for 30, 60 and 90 Days at varying MC

4.2.1.1 Germination count per day (in weeks) for seeds stored at -5 ^{o}C for 30, 60 and 90 days at varying moisture contents.

There was a general decline in the number of seeds germinating per day across all the treatment as the storage period increased to 90 days (Table.4.3). The non- desiccated seeds and seeds with 35 % moisture content stored for 30 days germinated three weeks after planting with average germination count of 5.5 and maintained 8.5 after 6 weeks (Table 4.3). Germination begun after four weeks in the seeds stored for 30 days with 25 % moisture content, however, the seeds that were dried to 15 % moisture content did not germinate even after eight weeks.

After 60 days of storage, the non-desiccated seeds and the seeds stored with 35 % and 25 % moisture contents germinated after three weeks whereas those that were stored with 15 % MC did not germinate (Table 4.3). The mean germination count for the non-desiccated seeds stored for 60 days was 1, 3, 4.5, 6.5, 6.5 after 4^{th} 5^{th} 6^{th} 7^{th} and 8^{th} week respectively (Table 4.3). For the seeds with 35 % MC, the mean germination count was 1, 2.5, 3, 3, 3 after 4^{th} 5^{th} 6^{th} 7^{th} and 8^{th} week respectively (Table 4.3) while the seeds stored with 25 % MC had a mean germination count of 0.5 on the fourth week and 1 on the sixth week (Table 4.3). There was no germination in the preceding weeks. Seeds with 15% MC recorded no germination at all (Table 4.3).

Furthermore, one seed of the non-desiccated (47 % MC) seeds germinated after four weeks and increased to 4 and 5 after five and six weeks respectively (Table 4.3). The

seeds stored for 90 days with 35 % MC recorded an average germination count of 0.5, 1.5 and 2 after fourth, fifth and sixth week respectively (Table 4.3). In addition, the seeds stored with 25 % MC for 90 days only germinated on the fourth week with an average germination count of 0.5 (Table 4.3). Again, seeds with 15 % MC recorded no germination at all (Table 4.3).

Table 4.3 Germination count per day (in weeks) for seeds stored at -5 ^{o} C for 30, 60 and 90 days at varying % moisture contents.

Time taken to germinate	30 days Number germinating				60 Days Number germinating				90 Days Number germinating			
week	**ND**	**35%**	**25%**	**15%**	**ND**	**35%**	**25%**	**15%**	**ND**	**35%**	**25%**	**15%**
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	5.5	5.5	0	0	1	1	0.5	0	1	0.5	0.5	0
5	7	8	4.5	0	3	2.5	0.5	0	4	1.5	0.5	0
6	8.5	8.5	5	0	4.5	3	1	0	5	2	0.5	0
7	8.5	8.5	5	0	6.5	3	1	0	5	2	0.5	0
8	8.5	8.5	5	0	6.5	3	1	0	5	2	0.5	0

The average levels of vigour in -5 ^{o} C were comparatively different among moisture contents. Non-desiccated seeds showed decrease in vigour from 2.9 to 1.2 to 1.1 G. I by 30, 60 and 90 days of storage respectively (Figure 4.5). Seeds dried to 35% moisture content had the highest seed germination index followed by seeds with 25 % moisture content. Seeds with 15% moisture content recorded the lowest GI (Figure 4.5). Seed vigour levels were highest at the onset and lowest after 90 days for the whole storage period (Figure 4.5). Overall, the seeds lost vigour with increasing storage time. For example, the seeds with 15% MC stored for 30, 60 and 90 days at -5 ^{o} C completely lost vigour (0 G.I). 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 (Fig 4.5). Further decline in seed vigour 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 4.5). The results showed that seeds stored with 15% MC lost vigour

faster than those stored with higher moisture contents, equally with shorter storage period compared to other seed lots.

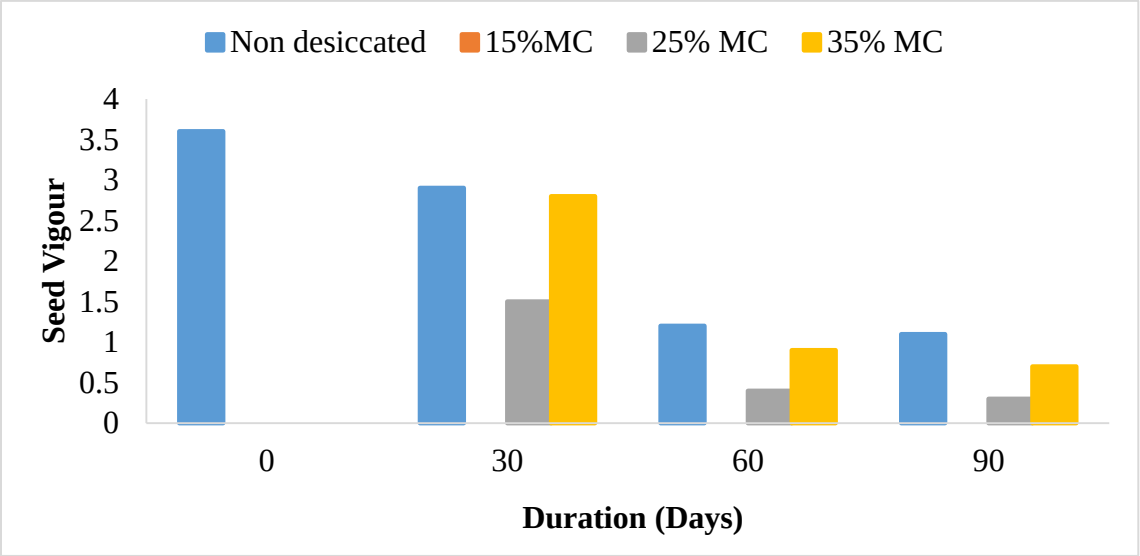

Figure 4.5 Seed vigour for seeds with varying moisture content stored at -5 ^{o} C for 30, 60 and 90 days

4.2.2 Seed Vigour for Seeds Stored at 10 ^{o} C for 30, 60 and 90 Days at varying Moisture contents

4.2.2.1 Germination count per day (in weeks) for seeds stored at 10 ^{o} C for 30, 60 and 90 days at varying moisture contents.

There was a general decline in the number of seeds germinating per day across all the treatments as the storage period increased to 90 days (Table.4.4). Germination begun on the fourth week in the non- desiccated seeds and seeds stored for 30 days with 35 % and 25 % moisture content with germination count of 2, 3.5, and 1.5 respectively. The germination count of the non- desiccated seeds increased to 5 and stopped at 8.5 on the fifth and sixth week respectively (Table 4.4). The seeds stored with 35 % MC and 25 % MC at 10 ^{o} C did not germinate anymore after five weeks but retained an average

germination count of 9 and 4.5 respectively. The desiccated seeds to 15 % moisture content and stored for 30 days did not germinate at all.

The non-desiccated seeds and the seeds stored with 35 % and 25 % moisture contents for 60 days at 10 ^{o}C begun germinating after 4, 3 and 4 weeks respectively with an average germination count of 4, 0.5, and 0.5 respectively whereas those that were stored with 15 % MC did not germinate (Table 4.4). The mean germination count for the non-desiccated seeds stored for 60 days at 10 ^{o}C was 5.5, 6.5, 7.5 and 7.5 after 5th 6th 7th and 8th week respectively (Table 4.4). For the seeds with 35 % MC, the mean germination count was 0.5, 1, and stopped at 4.5 after 4th 5th and 6th week respectively (table 4.4) whereas the seeds stored with 25 % MC at 10 ^{o}C resulted a mean germination count of 1 on the fifth week and stopped at 1.5 on the sixth week (Table 4.4). There was no germination on the other weeks. Seeds with 15% MC did not germinate (Table 4.4).

Furthermore, the non-desiccated seeds and seeds stored with 35 % MC at 10 ^{o}C for 90 days begun germinating on the 4th week with an average germination count of 5.5 and 2 respectively. (Table.4.4). Results further indicated that 6.5 of the non-desiccated seeds germinated on the 5th week and germination stopped on the 6th week with an average germination count of 7 (Table.4.4). On the other hand, germination count of seeds stored with 35 % MC at 10 ^{o}C for 90 days was 2on the 4th week, 3.5 on the 5th week and stopped germinating on the 6th week with an average germination count of 4. (Table.4.4). Seeds stored with 25 % MC recorded a lower germination count of 1 on the 6th week and there was no any germination thereafter. Seeds stored with 15 % MC at 10 ^{o}C for 90 days did not germinate (Table.4.4).

Table 4.4 Germination count per day (in weeks) for seeds stored at 10 ^{o} C for 30, 60 and 90 days at varying % moisture contents.

Time taken to germinate	30 days Number germinating				60 Days Number germinating				90 Days Number germinating			
Week	**ND**	**35 %**	**25 %**	**15 %**	**ND**	**35 %**	**25 %**	**15 %**	**ND**	**35 %**	**25 %**	**15 %**
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0.5	0	0	0	0	0	0
4	2	3.5	1.5	0	4	0.5	0.5	0	5.5	2	0	0
5	5	9	4.5	0	5.5	1	1	0	6.5	3.5	0	0
6	8.5	9	4.5	0	6.5	4.5	1.5	0	7	4	1	0
7	8.5	9	4.5	0	7.5	4.5	1.5	0	7	4	1	0
8	8.5	9	4.5	0	7.5	4.5	1.5	0	7	4	1	0

In higher storage temperature of 10 ^{o} C, the non-desiccated seeds and seeds stored with moisture contents of 35%, 25% and 15% recorded decrease of seed vigour as days progressed to 90 (Figure 4.6). The seeds showed a decreased G.I which in turn reflects the decrease in seed vigour. For example, seeds with 35% moisture contents showed decrease in vigour from 2.9 just after 30 days of storage to 1.2 and finally 0.9 G.I after 60 and 90 days of storage respectively (Figure 4.6). Similarly, seed vigour 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 4.6). It is worth noting that seed lots stored with 15% MC lost vigour just 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 10 ^{o} C (Figure 4.6)

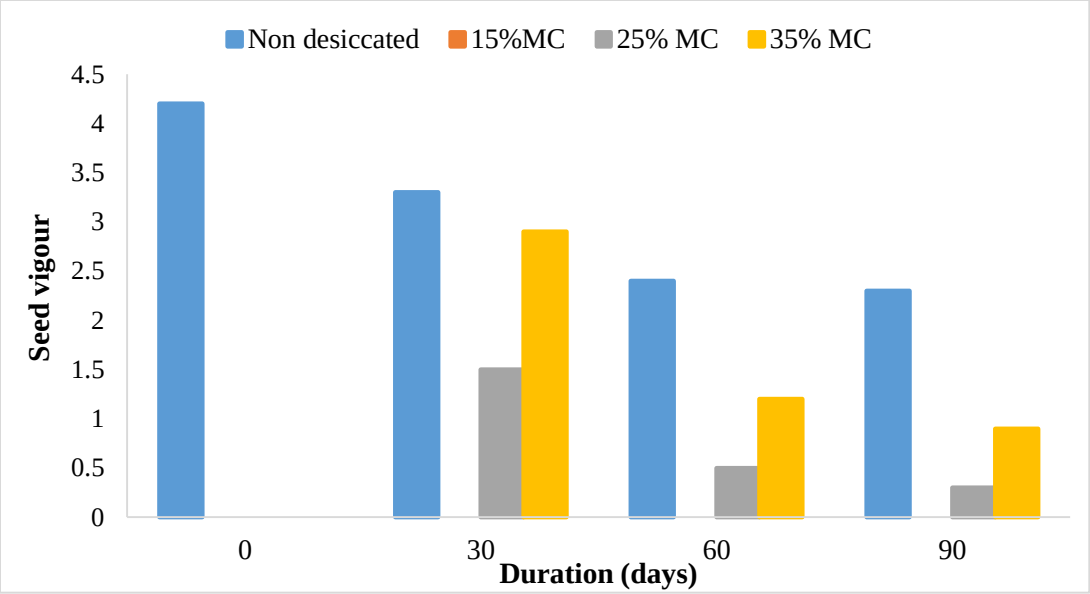

Figure 4.6 Effect of seed storage at 10 ^{o} C on seed vigour for non-desiccated seeds and seeds with 15%, 25% and 35% moisture content after 30, 60 and 90 days

4.2.3 Seed vigour for *E. carpensis* seeds with varying MC stored at 25 ^{o} C for 30, 60 and 90 Days.

4.2.3.1 Germination count (in weeks) for seeds with varying moisture contents stored at 25 ^{o} C for 30, 60 and 90.

There was a general decline in the number of seeds germinating per day across all the treatment as the storage period progressed to 90 days (Table.4.5). Germination of the non- desiccated seeds and seeds with 35 % and 25 % moisture content stored for 30 days at 25 ^{o} C begun on the fourth week with an average germination count of 4, 3.5, and 4 respectively (Table 4.5). The germination count of the non- desiccated seeds increased to 5 and stopped at 8 on the fifth and sixth week respectively. There was an increase in germination count to 6.5 on the 5th week for the seeds stored with 35 % MC. A further increase in germination count was noted and stopped at 8.5 on the 6th week. The seeds stored with 25 % MC stopped germinating after the 5th week with an average germination

count of 5.5 (Table.4.5). Contrary to the expected behaviour of the seeds with 15 % MC recording zero germination, these seeds recorded an average germination of 0.5 on the 3rd week after being stored at 25 ^{o} C for 30 days (Table 4.5). There was no germination recorded after the third week (Table 4.5).

The non-desiccated seeds and the seeds stored with 35 %, 25 % and 15 % moisture contents for 60 days at 25 ^{o} C begun germinating after 4, 3, 5 and 6 weeks with an average germination count of 2, 0.5, 0.5 and 0.5 respectively (table 4.5). The mean germination count for the non-desiccated seeds stored for 60 days at 25 ^{o} C increased to 5.5 on the 5th week and stopped at 6.5 on the 6th week (Table 4.5). For the seeds with 35 % MC, the mean germination count was 1.5, 3, 3, and stopped at 4.5 after 4th , 5th, 6th and 7th week respectively (Table 4.5) while the seeds stored with 25 % MC at 25 ^{o} C resulted to a mean germination count of 0.5 on the 5th week and there was no any other germination (Table 4.5). There was no more germination recorded in the seeds with 15% MC after it recorded an average germination count of 0.5 on the 6th week (Table 4.5).

Further, the non-desiccated seeds and seeds stored with 35 %, 25 % and 15 % MC at 25 ^{o} C for 90 days begun germinating on the 4th, 3rd, 5th and 6th week with an average germination count of 2, 0.5, 0.5 and 0.5 respectively (Table.4.5). Germination count of the non-desiccated seeds further increased to 4 during the 5th week and stopped germinating on the 6th week after a germination count of 6.5. Results further indicated that the seeds with 35 % MC showed an increase in germination count to 1.5, 3, 3 and stopped germinating at 4.5 on the 4th, 5th, 6th and 7th week respectively (Table 4.5).

The seeds stored with 25 % MC at 25 ^{o} C for 90 days stopped germinating after recording an average germination count of 0.5 on the 5th week (Table.4.5). On the other hand, germination count of seeds stored with 15 % MC at 25 ^{o} C for 90 days begun and stopped on the 6th week with an average germination count of 0.5 (Table 4.5).

Table 4.5 Germination count per day (in weeks) for seeds stored at 25 o C for 30, 60 and 90 days at varying % moisture contents.

Time taken to germinate	**30 days Number germinating**				**60 Days Number germinating**				**90 Days Number germinating**			
Week	**ND**	**35%**	**25%**	**15%**	**ND**	**35%**	**25%**	**15%**	**ND**	**35%**	**25%**	**15%**
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0.5	0	0.5	0	0	0	0.5	0	0
4	4	3.5	4	0.5	2	1.5	0	0	2	1.5	0	0
5	5	6.5	5.5	0.5	5.5	3	0.5	0	4	3	0.5	0
6	8	8.5	5.5	0.5	6.5	3	0.5	0.5	6.5	3	0.5	0.5
7	8	8.5	5.5	0.5	6.5	4.5	0.5	0.5	6.5	4.5	0.5	0.5
8	8	8.5	5.5	0.5	6.5	4.5	0.5	0.5	6.5	4.5	0.5	0.5

Further increase in storage temperature to 25 ^{o} C for seeds with moisture contents of 15%, 25% and 35% showed decrease in seed vigour with increase in storage period (Figure 4.7). 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 4.7). On the other hand, seeds stored with 15% MC lost vigour faster at 60th day than other desiccated seed batches, while those with 35% MC lost vigour from 2.3 to 1.4 to 0.8 G. I. (Germination Index) after 30, 60 and 90 days of storage respectively (Figure 4.7). Similarly, seeds stored with 25% MC showed a steady decrease in vigour from 1.7 to 0.2

to 0.1 after 30, 60 and 90 days of storage respectively (Figure 4.7). The loss of seed vigour was again in the order with MC as 35%>25%>15% at 25 ^{o}C storage (Figure 4.7).

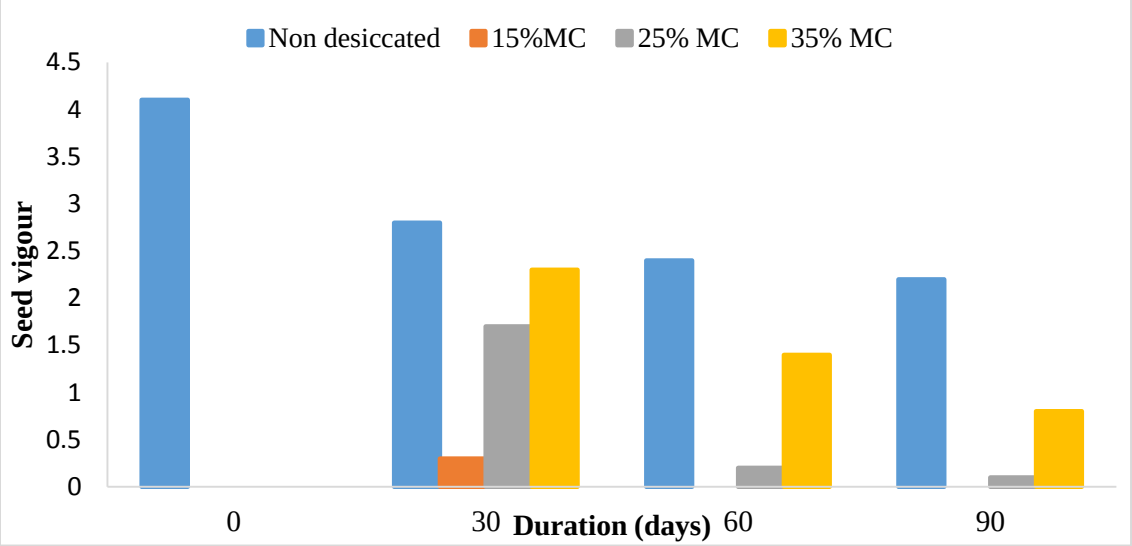

Figure 4.7 Effect of seed storage at 25 ^{o} C on seed vigour for non-desiccated seeds and seeds with 15%, 25% and 35% moisture content after 30, 60 and 90 days

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

The results shown on Table 4.6 indicate that the factors mainly moisture content and storage period influenced seed vigour significantly ($p<0.05$), however, temperature recorded insignificant influence ($p>0.05$) on seed vigour.

Table 4.6 Relationship between moisture content, temperature and seed vigour

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 vigour (G.I)

There was a negative co-relationship between storage period and seed vigour (Figure 4.8 a, b and c). Findings showed that seed vigour decreased with increase in storage period in all the three temperature regimes. The seeds stored at temperature of -5 ^{o}C, 10 ^{o}C and 25^{o}C, resulted to R^2 of 56.7%, 53.6% and 57.8% respectively (figure 4.8 a, b and c). The variation of seed vigour is due to time and taking into account variations in moisture content.

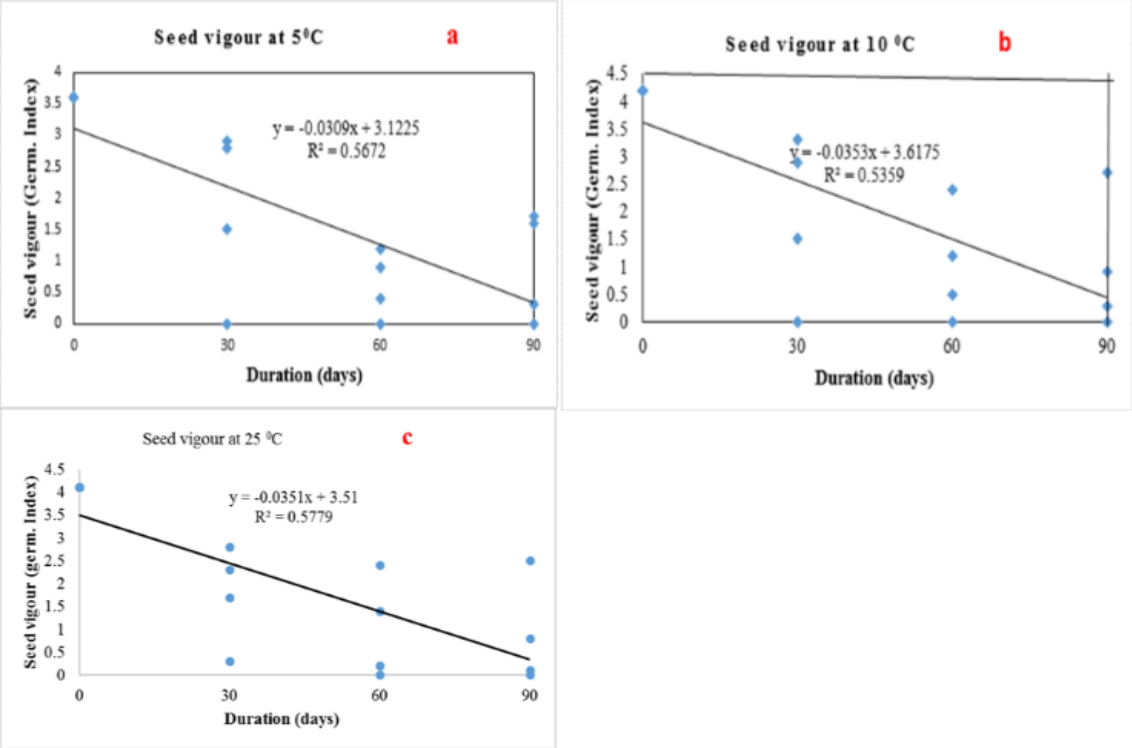

Figure 4.8 Regression for seed vigour versus storage duration of seeds stored at -5 ^{o} C (a), 10 ^{o} C (b) and 25 ^{o} C (c) for non-desiccated seeds and seeds with 15%, 25% and 35% moisture content after 30, 60 and 90 days

4.2.5 Correlations between seed germination % and Vigour

The results revealed a strong positive correlation between seed germination percentage and vigour (Table 4.7). 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 4.7) which suggests a very strong positive significant (p=0.000) correlation between the two parameters

Table 4.7 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).*

4.3 Determination of *E. capensis* Seed Longevity

Generally, results showed decrease in germination with increasing storage period. The initial seed germination was 90 % hence, P50 was 45 % (Table 4.8). Essentially, P50 refers 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 4.8). On the other hand, seeds with 35% MC were longer lived with P50 ranging between 50 and 60 days (Table 4.8).

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 4.8). In addition, seeds stored with 25% MC had P50 ranging between 30 and 32 days at 10 ^{o}C and 25 ^{o}C respectively (Table 4.8).

Table 4.8 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

CHAPTER FIVE

DISCUSSION

5.1 Effect of storage temperature and moisture content on viability of fresh seeds of *E. capensis*

Findings obtained in the present study indicated that both temperature and moisture content at which seeds of *E. capensis* were stored influenced the viability of the seeds just as in several other recalcitrant seeds (Willan, 1985) with high viability being retained longer in seeds stored at 10 ^{o} C after being dried to moisture content of 35%. Therefore, low storage temperature and high moisture content resulted in maintenance of seed viability. The optimum seed storage temperature of *Ekebergia capensis* for optimum seed germination was 10 ^{o} C. There was decrease in germination of the seeds when stored at 25 ^{o} C. This may be due to the detrimental effect of the high temperatures on seed reserves (Larcher 2003). In addition, the low germination percentage at 25 ^{o} C compared to 10 ^{o} C may be as a result of damage to the seed structure (Taiz and Zeiger 2009). Higher temperatures could actively alter enzyme activity and lowers the amount of amino acids available (*via* RNA synthesis), thus modifying metabolic reactions that lowers embryo development and hinder or lower seed germination, as reported by Larcher (2003), Marcos Filho (2005) and Taiz and Zeiger (2009).

This result shows that seeds of *Ekebergia capensis are* sensitive to storage temperatures and loss of moisture content. Results obtained in the present study agree with those reported by Hartmann *et al*. (1997a), where increase in storage temperature from 4 ^{o} C to 10 ^{o} C resulted in better germination of seeds of medicinal plants. Similarly, recalcitrant seeds of *Dioscorea dregeana* showed the highest percentage germination after storage at

10 ° C in comparison to lower temperatures examined (Kulkarni *et al*., 2007). In addition, *Tulbaghia alliacea* seeds showed highest percentage germination after transferring seeds from low storage temperature to a higher storage temperature (Kulkarni *et al*., 2005 *b*).

Appropriate storage temperatures and duration of storage of seeds have potential to conserve and improve seed quality and germination percentage (Mubvuma *et al.*, 2013). In contrast, Pradhan and Badola (2012) reported that recalcitrant seeds of *Swertia chirayita* showed higher percentage germination when stored at lower temperature of under 10 ° C. In an earlier study by Corbeneau and Come (1986), mature recalcitrant seeds of *Symphonia globulifera* stored in a wet medium at 15 ° C retained viability for up to two months, but when the temperature was reduced to 10 ° C or 12 ° C the seeds rapidly lost their viability. This indicates how carefully temperature must be controlled when storing recalcitrant seeds.

Results obtained in the present study indicate that drying fresh seeds of *E. capensis* to 35% moisture content can retain seed viability when stored at 10 ^{o}C for 30 days. If the storage is prolonged beyond this period there is significantly high loss of seed viability. On the contrary, the non-desiccated seeds maintained high percentage of seed germination after storage at 10 ^{o}C for 30, 60 and 90 days compared to the one stored at -5 ^{o}C. This may be due to energy of water which increases in response to increasing temperatures, hence stimulating an increase in diffusion pressure, which concomitantly increase the metabolic activity and lowers the internal potential of the seed, promoting increased absorption of water (Castro *et al*. 2004). Thus, hydration occurs more rapidly at higher temperatures through physical processes that could accelerate germination (Castro *et al.* 2004). This is supported by other researchers who reported that 40% seed moisture

is desirable for retaining good seed longevity. Another study by Pradhan and Badola (2008) recorded that seeds stored with 43% seed moisture resulted in 100% seed germination during the initial testing and above 50% seed germination in majority of population in the same species up to 18 months of storage. However, the present findings are contrary to those reported by McCormack (2004). The researcher stated that higher seed moisture (greater than 18%) results in loss of seed viability and vigour in the seeds of *Swertia. chirayita*. In addition, Bhatt *et al.* (2005) recorded low seed germination with the moisture content of 22% to 29% in freshly collected seeds of *Swertia angustifolia*. In another study, even after maintaining the moisture content of 15% to 21% in the domesticated recalcitrant seeds of *S. chirayita*, the study recorded low germination at the initial testing (Pradhan and Badola, 2010). This indicates that the critical seed moisture content for retention of viability varies for different recalcitrant seeds.

The moisture content of 35% was found to be critical moisture content for seeds of *E. capensis* since below this germination was greatly reduced or completely lost. Decline in seed viability is linked to the moisture content of the seed (Vieira *et al*., 2001). Pritchard *et al*. (2004) pointed out that desiccation sensitive seeds are killed by drying to water contents of between 20-30%. Berjak and Pammenter (1994) characterized recalcitrant seeds as desiccation sensitive with decreasing ability to withstand dehydration stress, to a maximum sensitivity even with the slightest loss of water. Critical levels of moisture content vary greatly among species and even among cultivars and seed lots. These results are in line with the finding of Merlin and Palanisamy (2000) who studied seed viability and storability of Jackfruit, Chacko, Pillai and *Garcinia gummi-gutta*. The longevity of seeds is affected by the reduction in moisture content below a critical value and the

estimates of critical moisture content vary considerably among species (Sanhewe and Ellis, 1996).

Most authors who studied seed behaviors of species within the family Meliaceae reported results showing desiccation sensitivity of the seeds which had high initial viability that gradually decreased during desiccation and the seeds are sensitive to higher moisture contents compared to seeds from trees of other families. Wang *et al.* (2004) reported that seeds of *Acmena acuminatissma* which belongs to the Meliaceae family are sensitive to desiccation below 40% moisture content. This result compares well with this research finding as it is shown that seeds of *E. capensis* are sensitive to desiccation below 35%. Baxter *et al.* (2004) assessed the responses of *Syzygium cuminii* seeds to desiccation. They reported that recalcitrant seeds of *S. cuminii* are desiccation sensitive as none of the seeds germinated below 16 %. In the current study, the seeds dried to 15 % moisture content lost viability after storage for 30 days irrespective of the storage temperature.

A study by Yousif *et al.* (2010) on seed germination of medicinal plants reported rapid loss of germination as a result of desiccation. Relatively high initial water content and intolerance to desiccation during storage clearly indicated that the storage behaviour of *E. capensis* seeds is recalcitrant. The results from this study are in agreement with (Abbas *et al.,* 2003; Radhamani *et al.,* 2003; Baxter *et al.,* 2004, 2012), who reported fresh mature Jamun seeds that were harvested with relatively high moisture content of (49.78%) resulted to 100% germination, however, the seed's embryo was killed when dried naturally (ambient conditions) to moisture content of (20%) within a period of 30 days of storage thus reducing the percentage germination.

The present study also recorded a reduction of germination percentage of 5% for *E. capensis* seeds with 15% moisture content stored at 10 ^{o}C. In contrast, Radhamani *et al.* (2003) reported CMC (27.6%) for recalcitrant seeds of *Syzygium cuminii*. This variation could be attributed to the different genetic backgrounds of the two tree species. The researcher proposed that water stress can take place in recalcitrant seeds with high moisture when stored, because recalcitrant seeds continuous to grow even at the end of their formation and maturation. However, they only lower their levels of metabolism. Thus, water stress in hydrated stored recalcitrant seeds comes up as a result of enhancement of cell division and expansion.

Desiccation sensitive or recalcitrant seeds are usually characterized by high critical moisture content (>30%) at the time of harvest (Baxter *et al.*, 2004). Although the value of LSMC varies with the physiological age and other characters of the seed, it is still considered important in defining and designing the short (ambient conditions) or long-term (freezing and cryo-temperature) storage protocols.

According to Maua *et al.* (2004), orthodox seeds can store for long period at low temperatures if their MC is low (<10), which is in contrast with this study which recommends *E. capensis* be stored with high MC (35%) or above at 10 ^{o}C for 30 days with no significant loss of viability. This suggests that the seeds of *E. capensis* is non-orthodox.

5.2 Effect of Storage Temperature and Moisture Content on the Vigour of *E. capensis* Seeds

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 +10 ^{o} C for the entire storage periods. A study by Liu *et al*, (2001) reported that recalcitrant seeds of Alexandra palm seeds only germinated within a narrow temperature range (20–30 ° C) hence agreeing with the high initial germination of the non-desiccated seeds in the present study.

Germination of the seeds stored with 15 % moisture content was seriously inhibited when it was stored at -5 ^{o} C and 10 ^{o} C for all the storage periods. This can be attributed to decreased seed water level which is a primary requirement for essential enzyme activation, substrate-breakdown, translocation, and utilization of reserve storage material (Aderounmu, and Asinwa, 2019).

The seeds tried to a moisture content of 35 % resulted to high average germination count spread through the 7 weeks after storage at 25 ^{o} C 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 (Scremin-Dias *et al*., 2006). Moisture content decrease in seeds stored at constant temperature was verified to cause a decrease in longevity in all temperature regimes (Walters.,2005).

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 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.

5.3 Determination of *E capensis* seed longevity

Not all seeds of species, cultivars, or individual seeds within a genetic group are destined to survive for the same period of time under a specified set of conditions (Shands *et al.*,1967). Sample of seeds does not die at one time, but the individual seeds making up the lot or sample die over a period of time (Shands *et al.*,1967). In referring to storage

life, lifespan, period of viability, or storage potential, it means the length of time required for a certain percentage of the seeds to die or conversely for a certain percentage to live. Scientists have known for years that seed longevity improves if seeds are dried to low water contents (FAO, 1994). Although seed longevity is an intrinsic characteristic that varies from species to species, (Walters 2005), the period during which seeds remain viable depends on their quality at the time of harvest, the treatment received between collection and storage, and the storage conditions (Walters and Koster, 2007, Rajjou and Debeaujon, 2008).

Results presented in this study showed variable longevity based on the treatments. Non-desiccated seeds maintained viability for longer periods than the desiccated seeds regardless of storage temperature. Among the dried seeds, seeds with moisture content of 35% had the longest life compared to seeds with lower moisture content of 25% and 15% stored across all temperature regimes of -5 ^{o} C, 10 ^{o} C and 25 ^{o} C for 90 days. Seed longevity decreased in the order with MC as control >35>25>15%. Low temperature promotes seed longevity, especially when the seed´s moisture content is high. Therefore, an adequate hydration of the seeds is the first requirement for long-term storage (Pritchard *et al.,* 2004). In addition, it is recommended to control the temperature of the storage environment in order to maintain the physiological quality of the seed (Carvalho and Nakagawa, 2000).

Reduction of temperature is the most convenient way of prolonging the storage lifespan of seeds, as it decreases metabolism and helps prevent water loss (Pritchard *et al.,* 2004). In addition, it is generally accepted that storage of recalcitrant seeds in the hydrated state at as low temperature as possible is the best way in which to retain viability in the short-

term storage (Berjak *et al*., 1989). A study conducted by Lewis (2002) 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. 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, hydration of the seeds is the first requirement for long-term storage (Pritchard *et al.*, 2004).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study showed that seed moisture content and storage temperature as well storage duration influenced seed viability as in;

1. Drying the seeds to moisture content below 35% MC significantly reduced percent germination. Furthermore, storing the seeds at -5 ^{o}C and +25 ^{o}C decreased seed germination regardless of the seed moisture content. Seeds of different moisture contents stored at 10 ^{o}C had higher seed viability compared to ones stored at -5 ^{o}C and +25 ^{o}C. The non-desiccated seeds had higher germination percentage than dried seeds to different moisture contents. Seeds dried to a moisture content of 35% had higher germination percentage than those with 25% and 15 % when stored at 10 ^{o}C. This study reports that, seeds of *E. capensis* are recalcitrant and that the critical storage temperature and moisture content is 10 ^{o}C and 35% respectively.

2. The vigour of *E. capensis* seeds decreased with decrease in moisture content and increase in storage duration. Seed vigour was completely lost for seeds dried to 15 % moisture content regardless of the storage temperatures. Increase in storage temperature lowered seed vigour of seeds stored with high moisture content over a longer period of time.

3. Seeds with higher moisture content that were stored at +10 ^{o}C remained viable for a longer period of time compared to those dried to 25 % and 15 % moisture contents.

Seed longevity period was however lowered after storage in higher temperatures. The non-desiccated seed had the highest longevity of 67 days after storage followed by seeds with 35 % moisture content which had longevity of 60 days. The seeds with 15% moisture content had the shortest longevity regardless of the storage temperature.

6.2 Recommendations

1. For maintenance of seed viability and vigour, *E. capensis* seeds, should be stored at 10 ^{o}C with 35% of moisture content.
2. Seeds with 35% moisture content can be stored for a period of 30 days at 10 ^{o} C without significant loss of viability and vigour.
3. Further studies to be done on other ways of propagating *E. capensis* tress.

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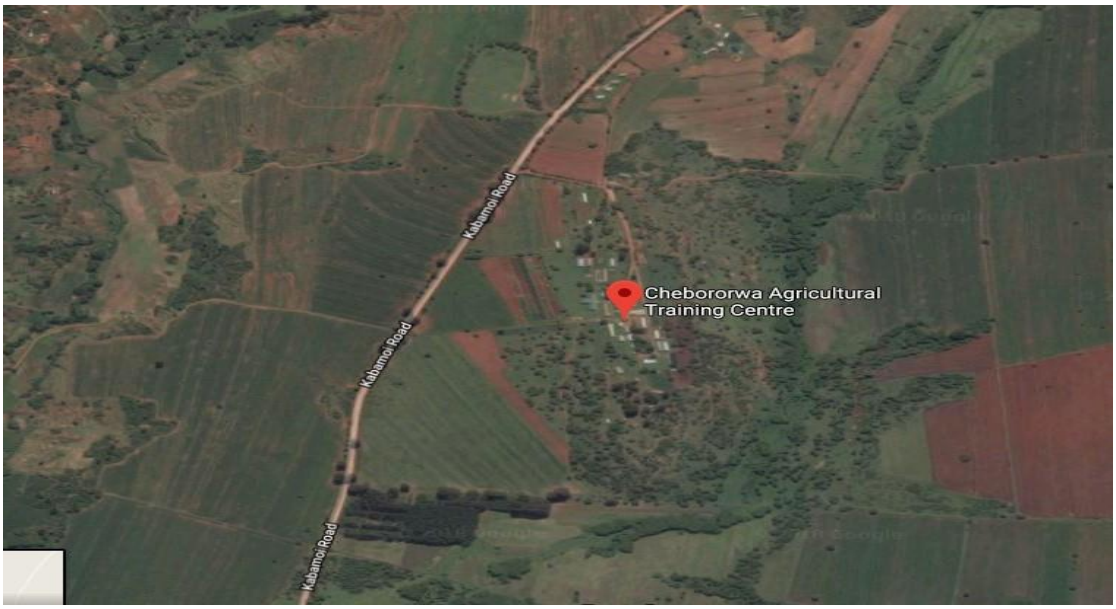
APPENDICES

APPENDIX I

Map of the study area



APPENDIX II

a) *E. capensis* Tree

b) *E. capensis* Fruits and Leaves

c) Seed Maturity Testing

d) KEFRI Seed Centre Lab

e) Post-harvest ripening of *E. capensis* Fruits

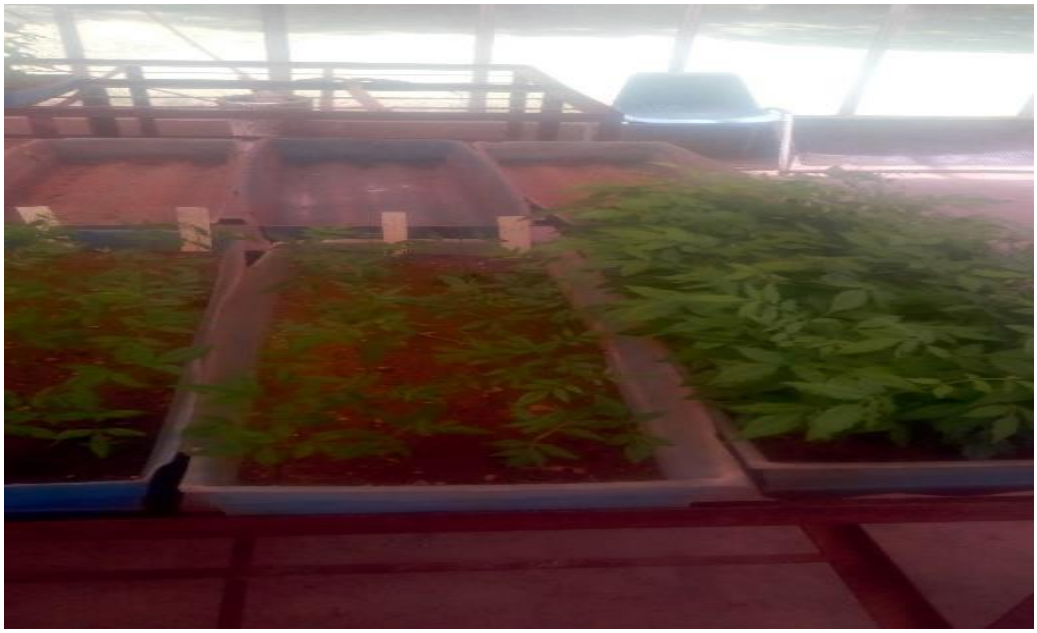
f) Seeds of *E. capensis*

g) initial and target seed weighing

h) Desiccation to target M.C

K

i) Planting E. capensis seeds stored in different treatments



j) germination of seeds stored under different treatments

Images a-j. Author, (2016)

APPENDIX III. Similarity Index/ Anti-Plagiarism Report

SC/PGB/066/14

ORIGINALITY REPORT

17%	11%	10%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.3bushketeers.co.za Internet Source	1%
2	Patrícia Pereira de Souza, Sérgio Yoshimitsu Motoike, Mychelle Carvalho, Kacilda Naomi Kuki, Eduardo Euclydes de Lima e Borges, Antônio Marcos Silva. "Storage on the vigor and viability of macauba seeds from two provenances of Minas Gerais State", Ciência Rural, 2016 Publication	<1%
3	mingaonline.uach.cl Internet Source	<1%
4	wrap.warwick.ac.uk Internet Source	<1%
5	REZAEI, Afsane, NASERY, Bahram, HEDAYATI, Mohamad Ali and HAGHVERDI, Katayoun. "Effects of Seed Moisture Content Decreasing on Germination Traits of Silver Fir (Abies alba Mill.)", Kastamonu Üniversitesi, 2012.	<1%