EFFECT OF MOISTURE CONTENT AND TEMPERATURE ON VIABILITY, VIGOUR AND LONGEVITY OF *CORDIA SINENSIS* Lam. SEEDS IN STORAGE

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DECLARATION AND RECOMENDATION

I understand that plagiarism is an offense and I therefore declare that this thesis is my original work and has not been submitted or presented to any other institution for any other award.

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DEDICATION

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LIST OF ABREVIATIONS AND ACRONYMS

%	Per Cent
ANOVA	Analysis of Variance
AOSA	Association of Official Seed Analysts
ASALs	Arid and Semi-arid Lands
DW	Dry Weight
FAO	Food and Agriculture Organization of United Nations
FW	Fresh Weught
FWB	Fresh Weight Basis
FPI	Forest Protection and Improvement
g	Gram
G.I.	Germination Index
GENSTAT	General Statistics
ISTA	International Seed Testing Association
IMC	Initial Moisture Content
KEFRI	Kenya Forestry Research Institute
KFSC	Kenya Forest Seed Centre
Kg	Kilogram
m	Meter
MC	Moisture Content
^{o}C	Degree Celcius
RH	Relative Humidity
SPSS	Statistical Package for the Social Sciences
TMC	Target Moisture Content

DEFINATION OF TERMS

Agroforestry is a collective name for land use systems and practices where woody perennials are grown on the same land management unit as agricultural crops and animals either in a spatial mixture or temporal sequence. There must be significant ecological and economic interactions between the woody and nonwoody components. (FAO, 2008)

Seed viability is the percentage of seeds in a seedlot, which germinate under the test conditions, or the number of seeds that germinate per unit weight of the seedlot (Walters, 1998).

Seed vigour is defined as "the Sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination (Walters, 1998

Seedlot is a quantity of seeds having uniform quality produced at a specific location and collected from a single crop in one harvest (Maua, 2004).

Orthodox seeds are seeds tolerant drying (desiccation) to low moisture content of less than 10%. They also tolerate storage at low temperatures, and can generally be stored for very long Periods (Roberts, 1973).

Recalcitrant seeds are seeds damaged by desiccation and those of tropical species may be damaged by exposure to low temperatures. They are said to be desiccation and chilling-sensitive (Roberts, 1973).

Intermediate seeds are seeds that do not fit into either of the above two categories. They can be desiccated, although not to such low levels as orthodox seeds, and they are often sensitive to chilling. They range between moisture contents of 10% to 30%, (Ellis, 1991）

Seed longevity is defined as seed viability after seed dry storage (storability) and, therefore, describes the total seed life span (Rajjou and Debeaujon, 2008).

In situ conservation has been defined as the conservation of whole ecosystems and natural habitats where wild or cultivated species are maintained and may continue to evolve (FAO, 2008)

Ex situ conservation maintains germplasm outside its original habitats, in the form of whole plants in botanical gardens and field genebanks, seeds as in seed genebanks, or certain other parts of the plant (FAO, 2008)

ABSTRACT

Cordia sinensis Lam. in the family *Boraginaceae* is a popular indigenous multipurpose tree species valued for fodder, fruit, structural timber and fuel wood. It is widely distributed in the arid and semi-arid lands (ASALs) of Kenya. Production of seedlings is through seeds. There are challenges acquiring viable and vigorous *Cordia sinensis* seeds for raising planting materials due to difficulties in post-harvest storage. In spite of the tree's exceptional multipurpose qualities and ability to grow in ASALs, very little effort has been put in improving the post-harvest storage of the seeds. Changes occurring in all seed 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 *C. Sinensis* seeds are not exceptional. Information on their seeds viabilty and vigour loss in storage is scanty and unrelable. It is against this background that this study investigated the effects of different moisture content (MC) and temperature regimes on viability and vigour loss of *Cordia sinensis* seeds in storage. The broad objectives of this study were to determine the rate of loss of viability, vigour and lonevity of *Cordia sinensis* seeds under varying moisture contents and temperatures regimes. The experiment combined five moisture contents (MC) (6%, 8%, 10%, 12% and 18%) and four storage temperature regimes (6^0C, 15^0C, 25^0C, and 35^0C) for a period of 150 days and stored seeds retrieved at interval of 30 days for viability, vigour and longevity tests. Data generated were subjected to analysis of variance (ANOVA) using general statistics (GenSTAT; 16:2013**).** The results revealed that *C. sinensis* seeds with lower moisture content of 6% stored across 6^0C, 15^0C, 25^0C and 35^0C temperature regimes maintained significantly higher viability, vigour and highest P_{50} (measure of longevity in days) compared to other seeds with higher moisture content stored across all temperatures regimes. However, seeds from both Marigat and Lodwar with highest MC (18%) stored across respective five-temperature regimes maintained lowest seed viability, vigour and lowest longevity ($P_{50)}$ as storage period progressed to 150 days. There was positive correlation between seed viabilty and seed vigour when both temperaures and MC decreased such that both viabilty and vigours increased. This suggests that decrease in storage temperatures and seed MC increased seed longevity, seed vigour and seed viability. Increases in seed viability, vigour and longevity, in storage was in the order of 6%>8%>10%>12%>18% MC and 6^0C>15^0C>25^0C>35^0 temperature over time. The study concluded that *Cordia sinensis* seeds stores best at 6% moisture contents at lower storage temperarure of 6^0C, again lower seed moiture contents of 6% stored relatively better compared to seed with 8%, 10%, 12% and 18% across all storage temperature of 6^0C, 15^0C, 25^0C, and 35^0C. Thus recommends to seed handlers to dry the *Cordia sinensis* seeds to 6% for higher longevity, vigour and viability.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Forests cover about 31% of the world's total land area with 7% designated as plantations and 93 % natural forests. Forest ecosystems remain essential refuges for biodiversity, and 12 % of the world's forestland is primarily for the conservation of biological diversity, forest products, food security and livelihoods (Campbell and Atkinson, 1989). Many residents in developing countries, including Kenya meet their nutrition, health and incomes from non-wood forest products (KFMP, 1994). The gazetted forest cover in Kenya has reduced from about 10% to less than 2% of the total land-size in the last three decades (Kenya Land Alliance, 2002; Wass, 1999; Ngece, 2003). Therefore, the need for more trees can not be over-emphasized.

Over the last 20 decades, Kenyas demand for forest seeds has increased due to government commitment to aforestation and need for rehabilitation of degraded lands (Muriuki and Jaenicke, 2001; Basweti *et a*l., 2001). The 2010 Constitution of Kenya advocates for a national tree cover of at least 10% of the total land area. The constitutions also inform and encourage the public to participate in the management, protection and conservation of indigenous plant genetic resources for a clean and health environment for every citizen. The Kenya vision 2030 and Farm Forestry Rules (Farm Forestry Rules, 2009), also provides synergy for increasing forest cover to 10% forest in Kenya. The contribution of forests and trees to meeting the present and future challenges of food security, poverty alleviation and sustainable development depends on the availability of planting materials. Therefore, the need for more trees seeds and developing short-term strategies and research agenda for providing planting materials in terms of seeds required in operational forestry cannot be overemphasized.

Most of forest tree species are managed in the wild, in natural ecosystems, or are at a very primitive stage of domestication compared to agricultural crops (Boyle and Sawyer., 1995). Forest tree genetic resources conservation ex-situ entails sampling and maintaining of wide genetic variation that resides within and among populations of selected target species. Substantial levels of human intervention practices are therefore required for ex-situ conservation, in the form of simple approaches including seed collection and storage (Elliot *et al.*, 1997: Elliot *et al.*, 1998).

Planting of indigenous tree species based on local seed sources is classified as *in-situ* conservation, because it involves growing trees in their natural environment. However, artificial establishment of plantations can expose trees to conditions that are very different from those under which they develop in the wild. Management of a forest sustainably ensures it has capacity to provide goods and services and does not diminish over time (FAO, 1993). It requires forest management practices that allow resources like seeds in forests be utilized sustainably. The sustainable forest management methods adopted must be appropriate to the physical, socioeconomic and institutional contexts (FAO, 1998). This also suggests that special attention must be given to conserving intraspecific genetic variation in peripheral or isolated populations, as they could possess features that will help to protect them from future climate change (Muller-Starck and Schubert, 2001).

Acceptable levels of seedling establishment are ensured by quantities of seeds, which can vary substantially across different biomes. Seed size, viability, germination and establishment rate parameters precisely do determine seeding rates (Gibson-Roy *et al.,* 2010). These parameters of seed quality are captured in the concept of "pure live seed" (a measure of the purity, viability and germination capacity of a seed batch (FAO, 1998). Quality seeds are collected at the point of natural dispersal to ensure that quality, desiccation tolerance (for orthodox seeds) and longevity are maximized (Hay and Probert, 2000). During collection a good separation distance between the mother trees is also important especially in natural forests, to ensure that one does not collect half-sibs which ultimately lead to inbreeding of the resultant population. About thirty mother trees with a spacing of about one hundred metres between them is recommended for a majority of species (Dawson and Were, 1998).

Many tropical forest species produce recalcitrant seeds (Saccade´ *et al.*, 2004), including many of the species important for timber. Recalcitrant seeds survive desiccation to relatively high moisture content (aften 30% MC depending on species) and die on storage at sub-zero temperature (Berjak and Pammenter, 2008). Based on seed response to desiccation lead to their seed storage behaviour categorized into three: orthodox, intermediate and recalcitrant. Orthodox seeds can be dried to low water contents (4-7%) with little effect on viability whilst recalcitrant seeds are killed by drying to moisture contents bellows 20-30% (Pritchard, 2004). Research reveals that Intermediate seeds can survive considerable levels of desiccation to levels approaching those of orthodox seeds (MC. 7-10%), but do not benefit from sub-zero temperatures (Ellis *et al.*,, 1990, 1991): However, forest species are

classified as: true orthodox for those tolerates drying below 10% moisture content and storage at subzero temperatures; recalcitrant tropical (could be stored in high relative humidity, with higher sensitivity to low temperatures and desiccation (Kapoor *et al.*, 2011)

Sourcing seeds of local provenance can be problematic, particularly in highly fragmented landscapes where small, remnant patches of vegetation separated by large areas of land exist. In these localized areas, the demand for seeds can easily exceed the supply and there may be some risks of detrimental effects on the viability of the source vegetation caused by overharvesting of seeds (Broadhurst *et al.*, 2008). Therefore, seed storage is a crucial link in forest afforestation and restoration chain.

Optimum handling and storage of orthodox seeds over many seasons allows practitioners to capitalize on high-seeding years, providing a resource for large restoration or tree planting projects. Maintaining careful control of the storage environment ensures seed viability and lifespan for many years. Seed viability in storage and the rate of seed deterioration investigation has been studied extensively (Kundu and Kachari, 2000; Walters *et al.*, 2005). Deterioration is increased death of an individual seed as deterioration proceeds (Tang *et al.*, 2000). Failure by seed to germinate indicates seed death and period until seed death occurs is the seed longevity (Hay *et al.*, 2003; Mollah *et al.*, 2002); Saccade´ *et al.*, 2000). Resumption of active growth of the embryo and the emergence of the young plant is referred to as seed germination (Tame, 2011).

Flexibility in the available storage conditions is preferable, and seeds should be stored under conditions, which increases their lifespan (longevity). Storage is preservation of viable seeds from the time of collection until they are required for sowing. However, when seeds are stored at high temperatures or high seed moisture levels, their viability and vigour tends to decline, which leads to seed deaths in storage caused by Deterioration (Kapoor *et al.*, 2011). Seed Deterioration may be as a result of active micro-organism in high temperature and moisture contents and improper handling. There is need to conserve seeds in a way that maintains their viability and vigor for the longest possible time from harvest to sowing in storage (Rajjou and Debeaujon, 2008). Therefore, appropriate storage condition slows Deterioration of seed in storage and maintains substantial high viability, vigour and longevity.

A possible mitigating factor against these seed viability loss, seed vigour loss and seed longevity

threats is to take trees seed to ex-situ condition, which is the core mission of storability, which simply can be defined as the incorporation of seed moisture contents and storage temperatures into storage conditions. The discipline involves technologies that play key roles in provision of seeds when required, maintaining of higher seed viability and vigour as well as adding seed longevity for long periods.

1.2 Problem Statement

The arid and semi-arid lands (ASALs), which support about 30 % of Kenya's total population, produces 50% of domestic animals and occupies 80% of the land has remained degraded with very low forest cover (KFMP, 1994). *C. sinensis* tree has the potential to grow in those areas to increase forest cover and rehabilitate degraded land. The tree has also multipurpose uses including the use for food, fodder and medicine. However, the propagation and multiplication of *C. sinensis* tree has remained low due to loss of seed viability, vigour and longevity of *C. sinensis* seeds when stored under unfavourable moisture content and temperature conditions. These challenges in *C. sinensis* seed storage compromises its contribution towards achieving 10% forest cover in land and national tree cover target by 2030 (vision, 2030)

Prediction of longevity of many agricultural seeds 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) and soybean (Agha *et al*., 2004). However, seeds for wild plants are poorly studied. There is scanty information available on the longevity of *C. Sinensis* seeds. Precisely, there is paucity of information on how seeds of the species stored under different moisture content and temperatures would lose both viability and vigour in storage. Therefore, an intervention is required on handling of *C. sinensis* seeds so as to prolong their shelf life and ensure seed stocks for rehabilitation of degraded land as well as for increasing Kenya's forest cover. It is against this background that the study seeks to investigate rate of loss of both seed viability and vigour when stored with different moisture contents at different storage temperatures.

1.3 Objectives of Study

1.3.1 Broad Objective

The broad objective was to determine the rate loss of seed viability, vigour and storage longevity period of *Cordia sinensis* seeds at different temperatures and moisture contents.

1.3.2 Specific Objectives

I. To determine the seed longevity period of *Cordia sinensis* in storage at varying moisture contents and at varrying temperatures conditions.
II. To determine the rate of loss of viability of *Cordia sinensis* seeds under varying moisture contents and temperatures regimes.
III. To determine the rate of loss of seed vigour *of C. sinensis* stored at varying moisture contents and at varrying temperatures conditions.
IV. To establish the correlation between seed viability and seed vigour *of C. sinensis* stored at varying moisture contents and temperature conditions

1.4 Research Hypothesis

$H_{01.}$ Different moisture content and temperature have no influence on seed longevity of C. *Sinensis* seeds on storage

$H_{02.}$ Different seed moisture contents and temperature have no effect on viability of *C. Sinensis* seeds on storage?

$H_{03.}$ Different moisture content and temperature have no influence on seed vigour *of C. sinensis* seeds on storage?

$H_{04.}$ There is no correlation between seed viability and seed vigour *of C. sinensis* seeds stored under varying moisture contents and temperature conditions

1.5 Justification

Proper handling procedures and storage methods for orthodox seeds allows conservernionists to maintain seed alife for decades and perhaps hundreds of years. By monitoring storage environments, practioners can ensure seed viability and vigour is preserved for longer period. This is desired because *in-situ* measures alone offer no guarantee for the conservation of plant genetic resources. The findings

of this research will contribute to the formulation/development of protocols of storing *C. sinensis* seeds for longer periods at optimum moisture content and temperature and will enable prediction of the rate loss of viability and vigour of *C. sinensis* seeds ex-situ. Seed researchers, merchants and seed dealers will apply these research findings in the management of seed stocks in ex-situ repositories.

1.6 Scope of Study

The study was restricted in Lodwar representing extreem dry area and marigat representing moderate dry areas. The choice of the two study sites was based on common occurrence of *C. sinensis* populations in both areas. Fruit collection from the field covered a period of two weeks of December 2015 and storage was for one hundred and fifty days. The collection factors mainly focused on tree distance from each other, the number of trees and equal quantity of fruit collected from each individual tree. The seed viability, vigour and longevity data collection were restricted to laboratory work only.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ecology of Cordia Sinensis

C. sinensis commonly known as Edome by Turkana and Sharamban by Kalenjin community is widely distributed in Middle East, Parkistan, India, Srilanka and Africa. In Kenya, the species is distributed in the arid and semi-arid areas particularly in former provinces of Coast, North eastern, and Eastern (Beentje and Ghazanfar, 2012). Precisely the species is well documented in former districts of Turkana, Baringo, Samburu, Garissa, Mbere, Kajiado, Tharaka, Kitui, and Machakos (Beentje and Ghazanfar, 2012). The tree grows up to 35 feet and rarely to less than 10 feet in height with a smooth bark and grey- white stem. Leaves are rough and sand papery. Flowers are cream, in terminal clusters. Mature fruits are yellow to orange-red, oval with a pointed tip, to 2cm long, containing about 4 seeds (Beentje and Ghazanfar, 2012; Beentje., 1994).

Cordia sinensis is a multipurpose tree used for timber, furniture, poles, construction, fuel wood, fodder, food (fruit), brewing local beer (fruit pulp), bee forage (Maundu *et al*., 1999): Maundu and Tengnas, 2005; Maua *et al.,* 2004). It is a prized species offering most of the characteristics generally sought for in trees for multipurpose use. These characteristics include; coppicing readily after pruning and pollarding, fast growth, its large fruit, twigs and leaves makes fodder for goats, cattle and sheep**.** The timber from the species is valuable, easy to work on, and strong (Maua *et al.*, 2004).

Possibilities of growing *Cordia sinensis* on a large scale and in plantations for commodity production like honey, gum and fodder, and for rehabilitation of degraded lands in dry lands is worth exploring. Collection of mature yellow, orange or bright red fruits (drupe) of *Cordia sinensis* seeds are from the crown in fruit forms that consist of a fleshy pulp enclosing the seed. The recommended stage for fruit collection is when the fruit are yellowish, orange or bright red (Maua *et al.,* 2004). Importantly seed dispersal is by animals. Birds, monkeys and human beings forage competitively on pulpy fruits thus promoting in-situ seed conservation.

2.2 Cordia Sinensis Seed Handling

The recommended seed extraction from fruit is to de-pulp the fruit while it is still fresh by gently rubbing on wire mess/screen and then, clean the seeds by washing in pressure running water to remove

mucilage and immediately rubbed with dry clean towel to remove water on the seed surface. Squeezing between hands for ripe fruit is also another extraction method. If the seeds are not for immediate planting, the current recommendation is that the seed should be dried to moisture content of around <10% and stored in a cold room (Albrecht, 1993)

C.sinensis seeds tend to have about 15,000 seeds in a kilogram (Maua *et al.*, 2004). Even after best extraction-method and storing conditions (temperatures and moisture contents), seeds eventually lose viability gradually (Schmidt, 2000). Storing and distribution of deteriorated seeds due to loss of viability is counterproductive as they hinder the number of seedlings to be raised hence affecting land area planted. The benefit of issuing seeds to clients with proper information on viability, storage, sources and seedlings vigour is meant to ease handling problems ((Schmidt, 2000; Albrecht, 1993)

2.3 Biology of Forest Seeds

Knowledge of seed biology is crucial for the proper handling of seeds, including their storage. With respect to handling, the term 'seed' usually refers to the unit extracted from the fruit and handled as a unit during storage, pre-treatment and sowing (Hong and Ellis, 1998）). Seed handling encompasses a series of procedures beginning with selection of the best seed source, through collection, processing, storage and pre-treatment to germination. Each link of this chain implies a potential risk of losing seed, and all links in the process are of equal importance (although they are not necessarily equally sensitive) (Schmidt, 2000). Improper seed handling during collection or processing kills the seed, whereby, even the best storage conditions will not bring it back to life. However, a handling procedure may become expensive if a certain loss cannot be tolerated during the process (Schwember and Bradford, 2005).

Three sequential phases of seed development are recognized (Xu and Bewley, 1991; Leprince *et al*. 1993; Bewley and Black, 1994). Firstly, following fertilization, rapid cell division and differentiation of the embryo occurs. This can be referred as the histo-differentiation phase. This is followed by a phase of cell expansion marked by an initial increase in fresh weight, dry weight and decrease in moisture content and reserve materials (proteins, lipids and carbohydrates) accumulate. Cell division is fully arrested during this phase. The end of maturation phase is signaled by the cessation of dry weight increase at the point referred to as mass maturity or physiological maturity (Ellis and Pieta Filho,

1992). This point coincides with the formation of an abscission layer between the parent plant and the vascular connection of the seed (Hay and Probert, 1995). Consequently, the final stage is often referred to as the post abscission phase during which the seeds of many species undergo maturation drying, as moisture is rapidly lost to the atmosphere.

2.4 Seed Storage

Newly collected fruits and seeds are particularly susceptible to damage, primarily because they often have relatively high moisture contents. Since loss and deterioration of seeds is irreversible, appropriate handling immediately after collection is crucial for high seed quality. Seed storage is the preservation of viable seeds from the time of collection until they are required for sowing. When seeds are sown immediately after collection, storage is not necessary. The best sowing date for a given species being raised in a nursery depends on (a) the anticipated date of planting which is dependent on climate, (b) the time needed in the nursery for planting stock of that species to reach the right size for out-planting. Rarely does seed sowing requirements coincide with the best time for seed collection

Seed storage facilities should be related to the amount of seeds and the period over which they are to be stored and remains viable between collections and sowing time. Again seed storage conditions lacks purpose if the seeds at the end of processing for storage are largely non-viable (Menttananda, 2001)
Seed quality is affected by collection and post-harvest handling prior to storage which tends to dictates seed longevity and the period for which seeds remain viable and vigourous in storage. General seed storage principles established for agricultural crops can also be applied to forest species (Gawrysiak-Witulska *et al.*, 2011). However, seed longevity even under identical processing and storage conditions is known to vary from species to species (Walters *et al.*, 2005). Wild plants are typified by developmental variation and long period of seeds on mother plant.

2.5 Seed Types

Today three major classes of seed are recognized based on seed storage behavior (Roberts, 1983), Orthodox seeds are dried down to a low Moisture contents (MC) of about 3-7% (wet basis) depending on oily content and can successfully stored at low or sub-freezing temperatures for long periods. *C. sinensis* belongs to class of orthodox (Bewley and Black, 1982). Recalcitrant seeds include a number

of large seeds that cannot withstand appreciable drying without injury (King and Roberts, 1979). Most short-lived recalcitrant tropical species are constituents of the moist tropical forests, where conditions conducive to immediate germination (high humidity and high temperature) are prevalent throughout the year (King and Roberts, 1979). Although increases in seed longevity of this magnitude have been achieved experimentally, some of the methods are expensive to apply and the effects on seed life are less dramatic than the effects of differences in temperature and humidity (Goldbach, 1979). Exclusion of oxygen will prevent aerobic, but not anaerobic, respiration, whereas reduced MC and temperature will decrease the level of both viability and vigour.

Past studies have shown that moisture content and temperature are the two most important conditions for seed storage conditions which should be manipulated to maximize seed longevity in hard coated orthodox species (FAO, 2014). Systematic predictions have been made of seed longevity under a range of temperature and MC for several agricultural crops (Guberac *et al.*, 2003). Few studies have focused on wild tree species (Muthoka, 2000). Therefore, *C. sinensis*, a wild plant species is poorly studied and there is need to develop prediction model to predict shelf life and prevents seed deterioration in storage

2.6 Seed Storage Categories

Most agricultural crops have seeds that can be dried and stored at low temperatures for years without losing their ability to germinate. These have been termed orthodox seeds, because they are considered the most usual and widespread type (Rajjou, 2008; Schmidt, 2000). However, many tree species, particularly in the tropics, have seeds that do not follow the orthodox rules. They are difficult to store because they do not tolerate drying and have therefore been termed recalcitrant seeds (Hoekstra *et al.*, 2001). Other seeds do not seem to fit into either of these two categories and are called intermediate seeds (Schmidt, 2000). Differences among species probably form a continuum from very orthodox to very recalcitrant seed. From a practical point of view, there are two factors that are critical for seed storage: seed moisture content and storage temperature (Thomsen, 2000).

An attempt has been made to develop a ‘low-input’ alternative to the conventional cold storage of seed. The technique is called ultra-dry storage and allows preservation at room temperature. It is

considered a useful low-cost option when no adequate refrigeration can be provided. On the other hand, it has been argued that drying seeds beyond critical moisture content may provide no additional benefit to longevity and may even accelerate seed ageing rates (Vertucci and Roos 1993; Walters and Engels, 1998). Research on various aspects of ultra-dry seed storage, including drying techniques such as sun/shade drying or vacuum/freeze drying and their applicability to a broader number of species should therefore be continued (Hay and Probert, 2000).

Selection of appropriate storage methods should be based on a range of criteria, including the biology of the species in question (Engels and Wood, 1999), practicality and feasibility of the particular method chosen, as well as the cost effectiveness and security afforded by its application (Maxted *et al.*, 1997).

Given the intensity of anthropogenic pressure and the importance of rehabilitating disrupted or degraded environments, in-depth research of forest species is warranted. Routine methods used for determination of seed quality and viability include germination testing and the tetrazolium test. Methods such as measurement of soak solution pH, electrical conductivity, and potassium content of leachate, all based on the permeability of the cell membrane system, are increasingly being employed in the assessment of seed vigour, as they are reliable and fast and can thus speed the decision making process. However, electrical conductivity testing, as applied to forest seeds, has yet to be standardized. Studies conducted so far have focused on assessment of seed soaking times (Keenan *et al.*,2012).

2.7 Stages of Seed Germination

Germination incorporates all events that commence with water uptake by quiescent dry seeds and terminate with elongation of the embryo axis (Bewery and Black, 1994). Penetration of the external seed structures surrounding the embryo by the radicle is the visible sign of germination. Germination starts when the dry seed imbibes water triggering biochemical processes, which result in protrusion of the radicle. The imbibition process by dry seed is tri-phasic: rapid water uptake resulting in weight increment (phase I); phase (II) is plateau phase characterized by physiological activities (protein synthesis, metabolism of stored reserves and enzymes synthesis) and phase (III) is characterized by further water intake and cell elongation that occurs after imbibition is complete (Bewery and Black, 1994). Seeds which do not imbibe most likely exhibit physical dormancy, as is common with in some

species of Fabaceae, Caesapinaceae and Mimosaceae (Turner, 2005; Baskin and Baskin, 2004).

Cordia sinensis trees flowers and produces many seeds once a year. However, seed have not been appropriately used by the local communities for rehabilitation of the degraded areas; this may be attributed to lack of temporary seed storage and handling methods (Maua *et al.,* 2004) Fresh seeds of many tropical tree species germinate readily during 14-21 days germination tests (Sautu *et al.*, 2006). In some tree species germination duration may be considerably long over one year to germinate while others 2.5 years to germinate (Sautu *et al.*, 2006).

Germination testing is designed to estimate the maximum number of seeds that will produce a normal seedling and to give results that can be replicated. Germination is the number of normal seedlings produced from 100 pure seeds expressed as a percentage or number of seedlings per given weight for chaffy seeds. In laboratories germination tests is run in cabinets or rooms that meet specific requirements for temperature and light control in order to make accurate and repeatable estimates. A normal seedling is that which has all the essential plant structures necessary for the plant to continue to grow normally under favorable conditions (ISTA. 1996).

A seed producer has the responsibility of supplying users with sufficient seeds for meeting the requirements for different purposes. On the other hand, the seed demand has to be realistic and over-supply has to be avoided since seeds are valuable, sometimes rare given productivity nature of some plants (Maua *et al.*,2004). The amount issued must be based on the weight of the seeds (in number of seeds per kilogram), purity, and their germination capacity (seed viability). The amount of seeds required to raise a given number of seedlings is then calculated using seed requirement model (Maua *et al.,* 2004).

Seedlings requirement model:

$$\text{Quantity required} = \frac{\text{No. Of seedlings}}{\%\ \text{purity} \times \%\ \text{germination} \times \text{No of seeds per kg}}$$

The number of seeds of *Cordia sinensis* in one-kilogram tends to be approximately 15000, with purity of 90% and germination percentage of 80% (Maua *et al.,* 2004).

2.8 Storage Temperature and Seed Viability

Temperature is one of the requirements that enable a seed to germinate (Bewley and Black, 1982). If temperature is not suitable, the germination of a plant (seed) is affected; when the temperature is low, the viability period will be longer as compared to when the temperatures are high. Although low temperature is generally preferred in order to reduce ageing and prevent insect and fungal activity, many species can be stored at ambient temperature for long periods provided their moisture content is low and they are free from insect and mould attack (Oskouei1 *et al.,* 2014). In absence of dormancy, orthodox seeds keep viability longer at low temperature, but since cold storage is expensive; in terms of establishment and operation, it may only be economical for a small portion of the total seed produced, are only stored for long-term (Schmidt, 2000).

Cold storage is mandatory if the seeds are likely to lose viability at ambient temperature, i.e. for short-term storage and sensitive seeds and any long-term storage. In Philippines, seeds *of Pinus metkusi* lose viability within eight months when stored at ambient temperature. At 2^0 C, they can be stored without significant loss in viability for up to 14 months (Schwember and Bradford, 2005). Hence, any storage beyond a few months of this species must be under reduced temperature. At least two species of *Eucalyptus*, *E. deglupta* and *E. microtheca* , have short viability under ambient conditions and must be stored at low temperature (3- 5^0C) to maintain viability beyond two years (Merritt *et al.,* 2003). Generally, the lower the storage temperatures, the longer the viability of stored seeds (Merrit *et al.,*2003). Most orthodox seeds maintain viability for decades under storage temperature of -10 to -15^0 C (Schmidt, 2000).

2.9 Seed Moisture Content

Like temperature, moisture content is also a basic requirement essential for a seed to germinate. The lower the moisture contents, the langer the period of seeds storage. In orthodox seeds, there are exceptions to these generalizations to recalcitrant seeds, which cannot withstand drying to relatively low moisture content. Most of the seeds that are recalcitrant must retain relatively high moisture content in order to maintain maximum viability. Moisture makes the seeds prone to microorganism attack, mould-growing (Weinberg, 2008; Schmidt, 2000). At high seed moisture content (over 30%), non-dormant seed may germinate and from 18% to 30% moisture content, rapid deterioration by

microorganisms may occur particularly in the presence of oxygen. Fungi can grow and destroy many seeds stored at 10% to 18% moisture content (Schmidt, 2000). Respiration can occur in seeds stored at moisture content in excess of 18% to 20% (Va´zquez-Yan˜es *et al.*, 2000). Moreover, in poor aeration, spontaneous heating can raise the temperature high enough to kill the seeds (Va´zquez-Yan˜es, *et al,* 2000). Below 8% to 9% moisture content, there is little or no associated insect activity. Seed dried below 4% to 5% moisture content although immune from attack by insects and fungi may deteriorate faster than seeds maintained at moisture content 1% to 2% higher (Va´zquez-Yan˜es, *et al,* 2000). The activities of the fungi and other contaminants of stored seeds are more strictly related to relative humidity of the inter seed themselves. This is because the moisture content of some seeds for example oil seeds may be different from others such as starchy seeds, even though the equilibrium relative humidity (R.H) is the same for both (Va´zquez-Yan˜es, *et al,* 2000).

Orthodox seeds conform to certain rules of the thumb that predict well the pattern of loss of viability in relation to storage environment. Thus, for each 2% decrease in seed moisture content, the storage life of the seed is doubled (Pritchard *et al.*, 2004)), and for each 5.6 degrees centigrade decrease in seed storage temperature, the storage life of the seeds is also doubled (Va´zquez-Yan˜es, *et al.*, 2000).

The arithmetic sum of the storage temperature in degrees (faren-height) and the percentage relative humidity (R.H.) should not exceed 100 units with no more than half the sum contributed by the temperature. Some expansion and qualification is required to accompany these rules of thumb. Calculation of seed moisture content is generally on a fresh weight basis according to International Seed Testing Association (ISTA) practices (ISTA, 2007).

$$\% \text{ M.C.} = \frac{\text{FW - DW}}{\text{FW}} \times 100$$

Where: M.C= Moisture content, FW= fresh weight of seeds, DW= dry weight of seeds,

2.10 Seed Metabolic Processes

Metabolic processes requires availability of water, as orthodox seeds dry during maturity and later on during processing, the available free water is lost. The little water left in the seeds 4-6% depending on desiccation rate is "bound" to macromolecules, thus. It is immobile and does not enter into chemical reactions. Respiration is significally reduced when moisture content has been lowered to below 18-20% (Pritchard *et al*. 2004). Hence, in dry seeds 3-7% there is practically no metabolism; the seed is alive without any measurable life manifestation (Bewley and Black, 1994). In desiccation sensitive (recalcitrant) seeds moisture contents is always high and the seeds are co-currently metabolically active (Probert and Hay. 2000)

As long as free water is available, metabolism is strongly related to temperature. If moisture contents and temperature are high, so is metabolism. Low temperature will drastically decrease metabolism but metabolic process will still continue as long as free water is available. Even when moisture content has declined below the level where metabolic activities have ceased, both temperature and moisture contents continue to influence seed longevity in storage through the ageing processes (Kozlowski, 1972; Schmidt, 2000). No matter how optimal storage conditions are, seeds will sooner or later die. Ageing denotes the progression of deteriorating events that take place within the seed and which ultimately lead to death of the seed**.** The effect of seed MC, *m* (% fresh weight), and storage temperature, *t* (°C), on seed viability, *v* (probits), with time, *p* (days), can be described by the seed viability equation of (Probert and Hay. 2000).

2.11 Measure of Seed Longevity

Plotting the results of serial germination tests of subsamples of a seed lot stored under controlled conditions provides a sigmoidal curve that conforms to a negative cumulative normal distribution (Tang *et al,* 2000). Individual seeds within a lot die at different times and the frequency distribution of deaths is normal. Therefore, it is not possible to obtain a single measure of the longevity of a seed lot. Thus, the time taken for germination to fall to 50% (p50) have been commonly used as a measure of longevity by many authors as it has the advantage of this period being the most accurately determined one (Probert, 2003, Muthoka, 2003, Tang *et a.l,* 2000). When the survival curves of different seed lots of a species are compared for storage under precisely the same conditions, longevity often differs,

whether measured as p50 or any other viability period.

2.12 Seed Quality

Seed quality is composed of its genetic, physical, physiological and sanitary traits (Marcos-Filho and MCDonald, 1998), which more or less affected by environmental condition during seed crop vegatation (MCDonald, 1998), seed processing (Siddique and Wright, 2003), seed storage conditions and storing period (Schwember and Bradford, 2010; Tang *et al,* 2000). Seed propagation remains the principal mode of propagation in tree silvulcuture in the temperate as well as in the tropical regions. Seed collection activities are often the most labour and cost intensive part of all the seed handling operations. However, optimum seed quality at the time of collection is a precondition for an overall high initial seed quality and subsequent longevity in storage (Merritt and Dixon, 2011). The period during which seeds remain viable will depend on their quality at the time of harvest, the treatment received between collection and storage, and the storage conditions (Walters, 2007, Rajjou and Debeaujon, 2008). The rate of aging depends on the seed moisture content, temperature, and initial seed quality (Walters, 1998; Walters *et al*., 2005).

Seed deterioration is defined as the loss of quality, viability and vigor either due to ageing or effect of adverse environmental factors. The rate of deterioration rapidly increases with increasing of either seed moisture content or temperature of storage (Bulkvic *et al.,* 2015). In any seed lot especially agricultural species, losses of seed vigour are related to a reduction in the ability of seeds to carry out all the physiological functions that allow them to perform (ISTA, 2009). Tree seeds are not exception, thus necessity for testing of seed vigour of *C. sinensis* in storage.

Seed germination depends on the interaction of the seed with the environment, and occurs under favourable conditions with the key environmental factors: water availability, appropriate temperature and in some cases light (Hartman, et *al*., 2007). However, propagation of a number of these dryland tree species are difficult due to problem of low seed germination. The low seed germination is due to seed coat. Most seeds consist of an embryo, surrounded by one or more covering layers. The covering layers consist of a living endosperm of one to several cell layers and a testa, which is mostly dead tissues that cause dormancy leading to poor growth potential (Dhupper, 2013).
Dormancy is an adaptation that ensures seeds will germinate only when environmental conditions are

favorable for survival (Luna, *et al*., 2009). Seed dormancy can be due to seed coat, embryo, or a combination of both. Seed coat imposed dormancy may be due to non-permeability to water and/or gases, mechanical prevention of radicle extension, or prevention of inhibitory substances from leaving the embryo. In embryo-imposed dormancy, there is usually a requirement for temperature and/or light treatment that must be satisfied naturally during a period of after-ripening. Seeds of most dryland tree species thus require treatments to overcome seed dormancy and hasten germination. The conditions necessary to allow seeds to break dormancy and germinate can be highly variable among species, within a species, or among seed sources of the same species (Coder, 1994). C. sinensis have seed coat dormancy thus requiring to be soaked in water to break the dormancy after storage (Maua et al., 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Seed Sources and Collection

C.sinensis seeds were collected from Lodwar, (Turkana County) and Marigat, (Baringo County). Generally yellow ripe fruits were collected from thirty trees within a distance of at least 50m in equal quantities to increase genetic diversity and good sample representation from each tree. The collected fruit were carried in sisal sacks from the field. The fruits were temporary stored for 2 days thinly spread in a room before being put in a sack again for transportation to the laboratory at Muguga for extraction.

3.2 Seed Extraction

The fruit containing the seeds were sampled by radomly taking one hundrend fruits as representative of the whole seed lot for initial moisture conten testing. The seeds were extracted by squeezing the fruit and rubbing derived seeds on a dry towel. The extracted seeds were tested for moisture content by subjecting two replicates of 5g for 130 degrees celcius for 5 hours according to internation seed testing association procedure for oily seeds (ISTA, 2007). The moisture contents obtained were used as the initial moisture contents for the experiment. To extract the remaining seeds, the Fruits were placed on a wire screen/mess and gentle rubbed with hand to remove the fresh pulp and reduce sticky mucillage. The extracted seeds were washed with water under pressure to remove mucilage (Hong and Ellis, 1996) before gently rubbing with towel to remove excess water on seed surface. To clean the seeds further, they were picked by hand and placed on dry cotton towels.

3.3 Seed Desiccation for Moisture Content Determination

The protocol developed by DFSC and IPGRI in 1999 was followed with certain modifications to determine the seed desiccation tolerance at each of the three-development stages. Seeds were dried in silica gel in a ratio of 1:5 and enclosed in 6 cm x 8 cm perforated nets to allow the easy separation of the small seeds from the silica during re-weighing. For each maturity stage, randomly selected seed samples were dried to four target moisture levels namely 12%, 10%, 8%, and 6%, from intial moisture

contents of 18% 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 103^0C according to International Seed Testing Association procedure for oily seeds (ISTA, 2007). The endorcarp made up almost 50% of the seed firmly binded round it thus both moisture contents testing and desication was done with endorcarp embided round the seed. The remaining seed lots were divided into five sub-samples, 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 $3000cm^3$ (30cm by 20cm by 5cm) rectangular box on 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 four moisture content levels; 6%, 8%, 10% and 12%. The seeds with initial moisture contents were retained as controls. The seeds were, constantly regenerated through drying above silica gel at 25°C in incubator using descation and storage protocal (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 terminted when it reached the weight corresponding to the final degree of 6%, 8%, 10% and 12% moisture for each treatment. After the seeds attained 6%, 8%, 10%, 12% and 18% moisture content, they were then, put in airtight glass virols prior to storage at 6^0C, 15^0C, 25^0C and 35^0C temperature, respectively.

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= ((100-IMC)/100-TMC)) *initial seed weight (g), where IMC = initial moisture contents and TMC= Target moisture contents. Desiccated seeds were subdivided into five equal parts and put in small glass vials and then replicated five times in storage condition in five temperature regimes of 6^0C, 15^0C, 25^0C, 35^0C for 150 days for retrieval for testing in intervals of 30 days until 150 days were complete.

3.4 Determination of Seed Viability

The standard germination test, which is considered the universal test for seed quality, evaluates the maximum potential of a particular seed lot under an ideal set of conditions (ISTA, 2007). 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, 2013).

Seeds were retrieved at intervals of 30 days and were subjected to germination assay where respective seeds stored at different moisture contents and temperatures were tested by germinating seeds in germination cabinet for computation to viability. The seeds were pretreated by subjected seed to soaking in cold water for 24 houres to break the dormancy (Maua, *et al.,* 2004). Four replicates of 50 pretreated intact seeds (with endocarp) were sown on 1% (w/v) agar (plain agar) in distilled water in 9cm Petri dishes. The dishes were then incubated in germination cabinets set at alternating temperatures $20/30^0$C. Light was applied for 8h/d during the warm temperature of 30^0C phase (ISTA, 1999). Germinated seeds were scored daily for up to 7 weeks. A seed was considered as normally germinated when the radicle protruded by 2–3cm. Since *C. sinensis* seeds are multigerm containing more than one embryo in some endocarp, the radicles from one edorcatp were scored as one germinant.

3.5 Determination of Seed Vigour

To assess vigour four hundred seeds with 6, 8, 10, 12, and 18% moisture contents which were retrieved from each of five temperature storage regimes of 6, 15, 25 and 35^0C in interval of 30 days and tested for vigour. Seeds (without endocarp) were sown on 1% (w/v) agar (plain agar) in 9cm Petri dishes and then incubated in germination cabinets set at alternating temperatures $20/30^0$C. Light was applied for 8h/d during the warm temperature phase (ISTA, 1999). Germinated seeds were scored daily for up to 7 weeks. A seed was considered as normally germinated when the radicle protruded by 2–3cm. Seed vigour was measured by Germination index (G.I.) which was computed using the following formula (Perry, 1984).

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

n = number of seedlings emerging on day “d”
d = day after planting

3.6 Determination of Seed Longevity

Not all seed 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. A lot or 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. In referring to storage life, lifespan, period of viability, or storage potential, we mean the length of time required for a certain percentage of the seeds to die or conversely for a certain percentage to live. Different percentages have been used for different purposes (Roberts, 1972).

The imporpotance of seed storage is to conserve seeds in a way that maintains their viability and vigor for the longest possible time from harvest to sowing (Hilli *et al.* 2003, Rajjou and Debeaujon 2008). Although seed longevity is an intrinsic characteristic that varies from species to species, (Walters *et al*. 2005), the period during which seeds remain viable will depend on their quality at the time of harvest, the treatment received between collection and storage, and the storage conditions (Walters, 2007, Rajjou and Debeaujon, 2008).

The P_{50} which is widely used as measure of longevity in many wild plant species (Probert, 2003; Muthoka, 2003) which is the time taken for seed viability to decline by 50% was used to measure *C. sinensis* seed longevity. The P_{50} was read directly from the viability graph by drawing a line along X-axis at 50% viability for Marigat and Lodwar in Turkana Counties respectively. The points of intersection where the line touched the viability graph was again drawn straight downward to touch the Y-axis where the time was read at point of intersection at Y-axis.

3.7 Data Analysis

Data was cleaned entered in Ms Excel spreadsheets. Preliminary and final data analysis was carried out using GENSTAT 16th edition statistical software. The GESTAT was chosed as it dealt with numerical data unlike the other two available test of EXEL which dealt with graphcal propotion of which it doesn't give clear inferential test out put while SPSS is for social work mostly used to analyse quistionares. ANOVA is used when checking significant difference between two or more treatments and data for variables must follow a normal distribution. Since the viability data was in percentage (propotion) was converted using ASIN 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%) were run to determine if there was a significant difference in the viability and vigour levels of different moisture contents of seeds collected in two sites (Marigat and Lodwar), stored at different temperatures and assessed in interval of 30 days. Vigour and viability data were square root transformed to meet model assumptions.

CHAPTER FOUR

4.0 RESULTS

4.1 Cordia Sinensis Seeds Longevity Assessed by P_{50} for 150 Days

Overall, seeds lost viability with increasing storage period. The time taken for viability to decline by 50% is widely used as a measure of longevity in many wild plant species (Probert, 2003; Muthoka, 2003). For both Marigat and Lodwar seedlots, the initial seed germination was approximately 86% and 82% and therefore P_{50} would be 43 % and 41% respectively. Essentially, P_{50} refers to the time, taken for viability to drop to 50% percent of the initial germination (Newton *et al.*, 2009). Seeds from both Lodwar and Marigat with 6% and 8% did not attain P_{50} even after storing for 150 days, while those with moisture content of 10, 12 and 18 % had P_{50} ranging between 29 and 6 days (Table 4.1). There seems to be variations between the two provenances on the time taken for at least 50% of the seeds to have germinated under storage. In particular, seeds from the Lodwar provenance were shorter-lived with P_{50} of 29 and 30 days. On the other hand, seeds from the Marigat provenance were longer lived with P_{50} ranging between 29 and 75 days (Table 4.1).

Table 4.1: Seed viability of *C. sinensis* assessed by P_{50} for seeds stored at different temperature (6^0C, 15^0C, 25^0C, and 35^0C) for 150 days and different moisture contents (6, 8, 10, 12 and 18 % f.w.b).

Moisture content	P_{50} viability at 6^0C Celcius		P_{50} viability at 15^0C Celcius		P_{50} viability at 25^0C Celcius		P_{50} viability at 35^0C Celcius	
	Lodwar P_{50} (days)	Marigat P_{50} (days)	Lodwar P_{50}(days)	Marigat P_{50}(days)	Lodwar P_{50}(days)	Marigat P_{50}(days)	Lodwar P_{50}(days)	Marigat P_{50}(days)
6%	N/A	N/A	N/A	75	40	50	30	32
8%	N/A	N/A	30	70	25	45	20	31
10%	30	60	25	45	17	33	15	28
12%	20	40	17	35	9	30	7	26
18%	15	23	9	15	6	15	5	9

4.2 C. Sinensis Seeds Viability Loss at 60C for 150 Days at varying MC

Overall, the two provenances in terms of moisture content and storage temperature were not significantly different. Whilst there was statistically significant difference in the moisture content (6%, 8%, 10%, 12% and 18%) and also storage temperature (6^0C, 15^0C, 25^0C, 35^0C) for seeds sourced from the two sites with p-value for both being <0.001 at <α=0.05.

The results revealed that seeds stored with 18% MC lost viability faster than those stored at 6% moisture contents in both Margat and Lodwar provenances (Figure 4.1). For Marigat and Lodwar seed lots stored at airtight containers for 150 days with a moisture content of 6^0C, viability was retained consistently at about 80% (Figure 4.1). In contrast, Margat, seeds at 18% MC and stored for150 days had the life span of seeds lost by 120 days while those for Lodwar at 12% MC lost viability over the same period of 120 days. However, differences in viability for both provenances were similar for other storage MC with viability ranging between 10 and 50% for seeds kept between 12 and 8 % moisture contents respectively. Typically results presented indicate that seed life spans increased with decrease in moisture content. Other researchers have found similar findings for other plant species. Pronyk *et al*. (2006) found that seeds of Canola increased in lifespan with decrease in MC. Canola seeds stored at 12MCs showed that germination decreased with storage time, temperature, and moisture content to 12%. After 56 days, germination of canola stored at 12% MC. wet basis and at 25- 30°C dropped from 88% to 73 %. The same value of 73% germination stored: at 12% MC. and at 30-35°C showed after approximately 27 days, at 14 % MC. and at 25-30°C showed after 29 days, at 14 % MC. and at 30-35°C showed after 12 days.

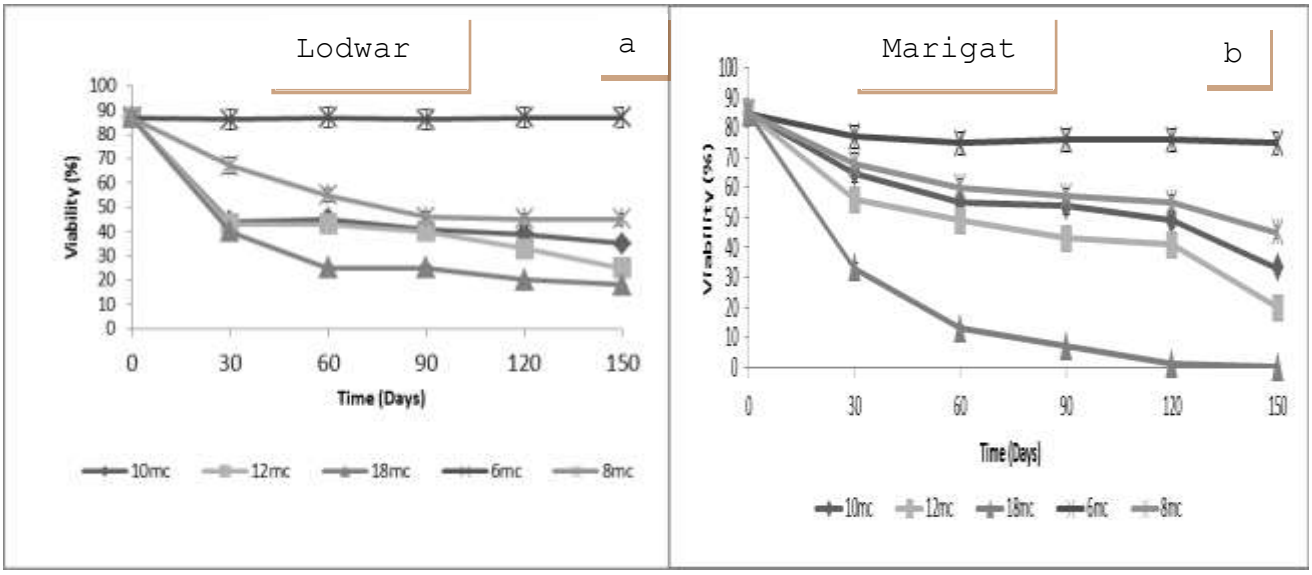

Figure 4.1: Seed viability at 6^0C for seeds from (a) Lodwar and (b) Marigat for 150 days

4.3 C. Sinensis Seeds Viability Loss at 15^0C for 150 Days at varying MC

Overall increase in storage moisture content combined with high temperature culminated to seeds losing viability rapidly in storage. In particular, seeds stored with 18% MC lost viability faster than seeds stored at 6% moisture contents in both study sites (Figure 4.2). For Lodwar and Marigat sites, seeds stored at 18% MC at 150 days had P_{50} of 30 days and eventually lost complete viability by 150 and 90 days respectively (Figure 4.2). On the other hand, Marigat seed lots with moisture content of 6 % and stored for 150 days did not attain P_{50} whilst seedlot from Lodwar recorded P_{50} at 140 days.

Survival curves demonstrating seed deaths with time showed similar trends for 10% MC and 12 moisture contents. Evidently, for the Marigat populations seed stored at 12 and m10 MC storage showed P_{50} which were similar at 30 and 32 days respectively. Except for seeds stored at 18 % moisture content, none of the seedlots for the moisture content ranging from 6 to 12% lost viability completely (Figure. 4.2).

Margat seedlots stored at 18% MC complete viability was lost at 150 days while by the 28^{th} of storage at least 50 % of the seeds had germinated. On the lower temperature of 6 % MC, viability was not lost completely in storage. However, the intermediate moisture contents of 8, 10 and 12 had viability

declining to 25 % at the end of the storage period. P_{50} for these intermediate moisture contents were 90, 45 and30 days for 8, 10 and 12% MC respectively. Viability was retained accordingly at 40% and 50% for 6% MC for both seedlots from Lodwar and Marigat (Figure 4.2). At half the storage period of 75 days, seeds with lower moisture contents of 6% had 55 and 60% viability for Lodwar and Marigat populations respectively. Seed lots with higher moisture content at 18% at half storage period of 75 days had 12 and 5% for Lodwar and Marigat respectively. However, for intermediate seed lots with moisture contents of 12, 10 and 8 % at 75 days has viability ranging between 25- 30 % and between 35- 50% for Lodwar and Marigat respectively (Figure 4.2). Findings reported here support work by Pronyk (2006) who found that seeds of Canola increased in lifespan with decrease in MC. Canola seeds revealed that germination decreased with storage time, temperature, and moisture content. After 56 days, germination of canola seeds stored at 12% MC. wet basis and at 25- 30°C dropped from 88% to 73 %. The same value of germination stored: at 12% *MC*. and at 30-35°C showed after approximately 27 days, at 14 % MC. and at 25-30°C showed after 29 days, at 14 % MC. and at 30-35°C showed after 12 days

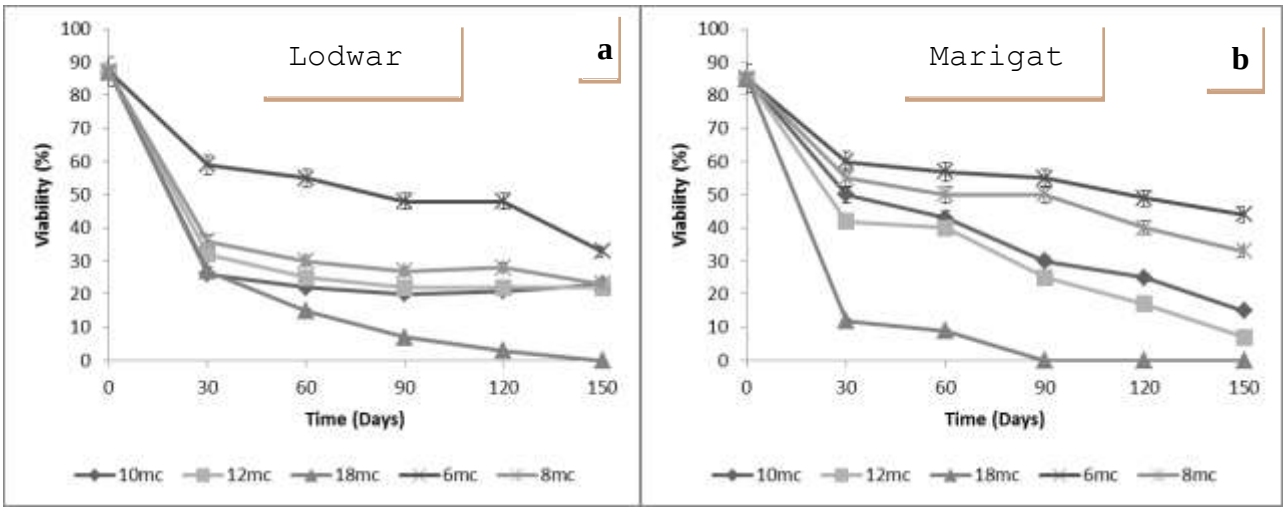

Figure 4.2 Seed viability at 15^0C for seeds from (a) Lodwar and (b) Marigat for 150 days

4.4 C. Sinensis Seeds Viability Loss at 250C for 150 Days at varying MC

The results demonstrate that further increase of temperature to 25^0C, seeds lost viability faster with increasing storage period. In overall, seeds lost viability with increasing storage period, moisture content and storage temperature. Specifically, the results indicate that seeds stored at 18% MC lost viability faster than those stored at 6% moisture contents in both study sites (Figure 4.3). For both

Lodwar and Marigat, seeds at 18% MC and stored for 150 days lost complete viability by 30 and 60 days respectively. In contrast, seed lots with moisture content of 6 % and stored for 150 days, retained viability to the end of storage period of 150 days to 20 and 35% respectively, while other intermediate seed lots at 8 and 10% MC for both study sites had a declined viability of 20 and 2% for seeds from Lodwar, 15 and 10% for seeds from Marigat. A similar trend was recorded for seeds with 12% MC for seed lots from both study sites (Figure 4.3). At half the storage period of 75 days, seeds lot at 6% from both Lodwar and Marigat had declined viabilty to 32% and 55% respectively from viability of approximately >82%. The seeds with highest MC of 18% from both study sites lost complete viability before half the storage period, at 75 days, while the seed lots at 12% moisture content from both Lodwar and Marigat lost viability to 10%. Similar trend of decline in seed viability were recorded for seeds with 8% and 10% MC to 13% and 30% viability for Lodwar and to 30% and 40% viability for Marigat respectively. At P_{50} seed lot at 6% MC had longevity period of 32 and 90 days for Lodwar and Marigat respectively, seeds at 18% MC from both study sites has same P_{50} of approximately 25 days. P_{50} for intermediate seed lot from both study sites with 8%, 10% and 12 % MC had longevity period of 32 days, 30 days and 28 days for seeds from Lodwar and 35 days, 30 days and 27 days for seeds from Marigat, respectively (Table 4.1). The study result presented suggests that seed life spans decreased with increase in moisture content at respective constant storage temperature. Trend reported here are similar to work by Pronyk (2006) that seeds of Canola increased in lifespan with decrease in MC.

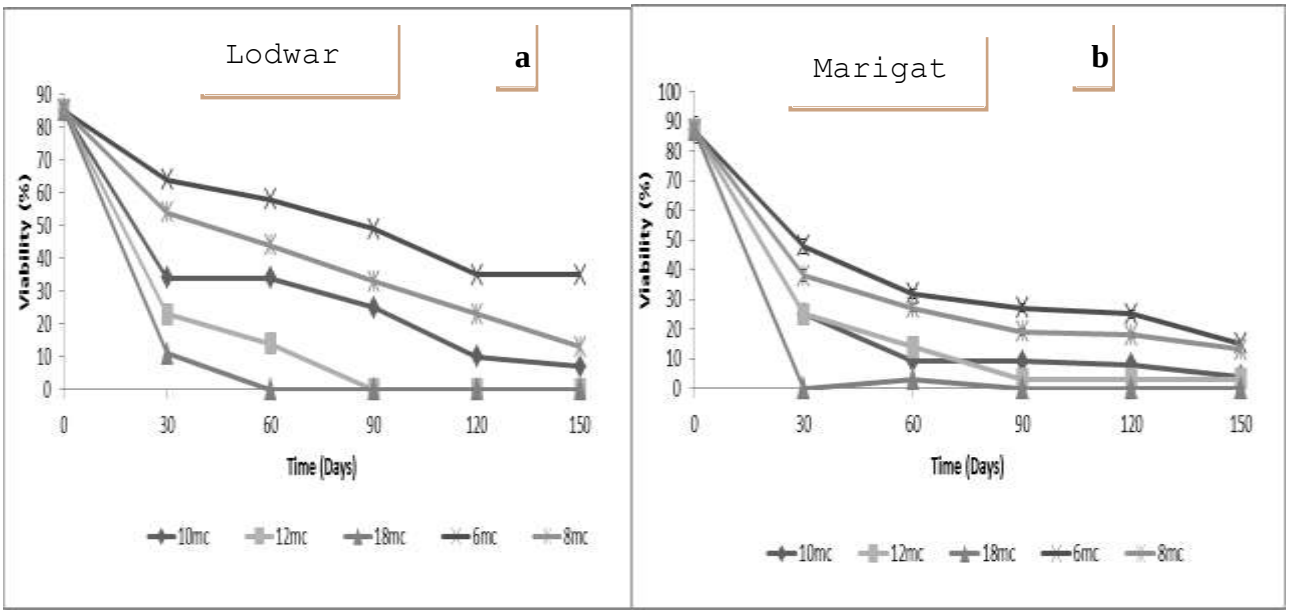

Figure 4.3: Seed viability at 25^0C for seeds from (a) Lodwar and (b) Marigat for 150 days

4.5 C. Sinensis Seeds Viability Loss At 350C for 150 Days at varying MC

Further increase of storage temperature to 35^0C, seeds lost viability faster with increasing storage period. Overall, seeds lost viability with increasing storage period and moisture content at elevated constant temperature of 35^0C. Results indicated that seeds stored at 18% MC lost viability rapidly than those stored at 6% moisture contents in both study sites (Figure 4.4). At $P_{50,}$ seed lots from both Lodwar and Marigat at lower moisture content of 6% had same longevity of 30 days while those at higher moisture content of 18% had a similar trend of P_{50} of 15 days for both Lodwar and Marigat. Lodwar intermediate seeds at 8, 10 and 12% MC had same P50 of 28 days while those for Marigat had P50 of 27, 28 and 29 days for 12,10 and 8% MC respectively. At half the storage period of 75 days, seedlots from Lodwar at 12 and 18% MC has lost complete viability while seed lots from Marigat has 2% and complete loss of viability, respectively (Figure 4.4). At lower MC of 6% seeds from both populations had lost viability to 20 and 30% for Lodwar and Marigat, respectively, while intermediate seeds lots at 8 and 10% MC at 75 days had declined seed viability to 2 and 1% for Lodwar and 15 and 9% for Marigat, respectively. At full storage period of 150 days, all seed lots from Lodwar had lost complete viability by 90^{th} day except those at 6% MC which has minimal viability of 2% while seeds from Marigat at 150 days' storage period at 10, 8 and 6% MC had viability of between 1 and 5% except at 12 and 18 % MC which lost complete viability also by 90^{th} day (Figure 4.4). Typically, results indicated that seed lifespans increased with decrease in moisture content and at constant storage temperature (figure 4.4). Findings reported here support work by Pronyk (2006) that seeds of Canola increased in lifespan with decrease in MC and Increasing temperature, however, reduces seed longevity (Walters *et al.*, 2005)

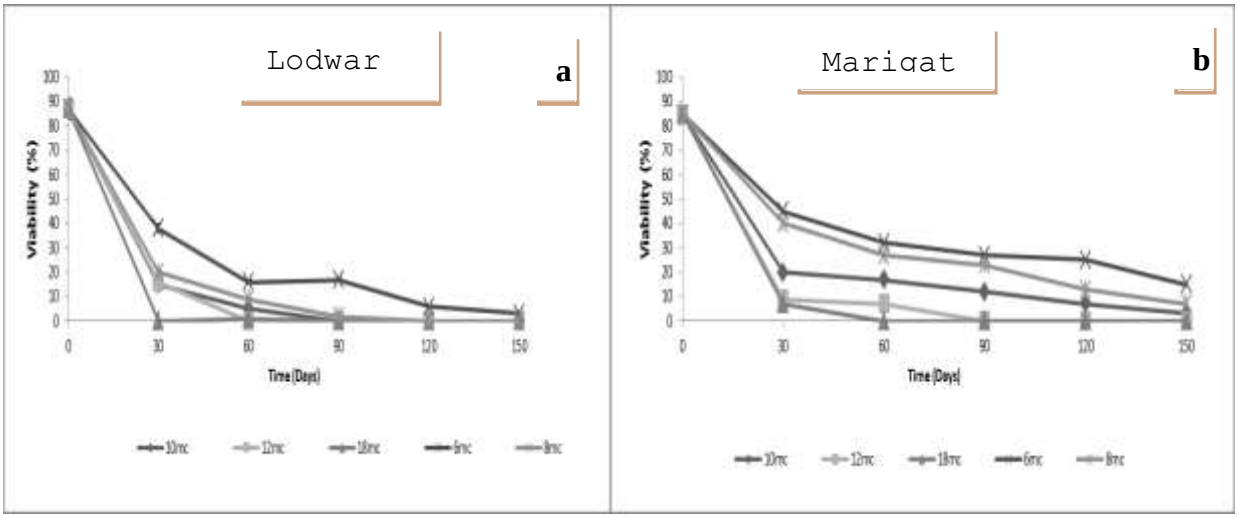

Figure 4.4: Seed viability at 35^0C for seeds from (a) Lodwar and (b) Marigat for 150 days

4.6 C. Sinensis Seeds Vigour Loss at 60C for 150 Days at varying MC

Overall, in comparison, the results from the two sites with respect to moisture content and storage temperature were not significantly different. Whilst there was statistically significant difference amongst the moisture content (6%, 8%, 10%, 12% and 18%) and also among storage temperature (6^0C, 15^0C, 25^0C, 35^0C) for seeds sourced from the two sites with p-value for both being <0.001 at$<\alpha=0.05$.

The average levels of vigour in 6^0C were not comparatively different between the two sites. What stands out is that 6% moisture content was the best followed by 8% with 18% being the worst. The levels are highest at the onset and lowest after 150 days for the whole storage period.

Overall, seed lost vigour with increasing storage time. For example, at 6^0C for 150 days, seed lost vigour from 3.5 to 1.5 (G.I) vigour for seeds at 6% MC from both Lodwar and Marigat, on the other hand seeds at 8%, 10%, 12% MC declined to approximately 1.4, 1.3, 1.2 G. I for seeds from Lodwar and Marigat at 6^0C for 150 days. However, seeds at 18% MC for 150 days at 6^0C lost highest vigour to approximately 1 G.I for seeds from both Lodwar and Marigat. All seed retained vigour after 150 days. However, viability decline was in the order with rest of MC as 6%>8>10>12>18 for both study sites (Figure 4.5). The results showed that seeds stored with 18% MC lost vigor faster than those stored at other moisture contents in both study sites, equally with shorter storage period compared to other seed lots (Figure 4.5).

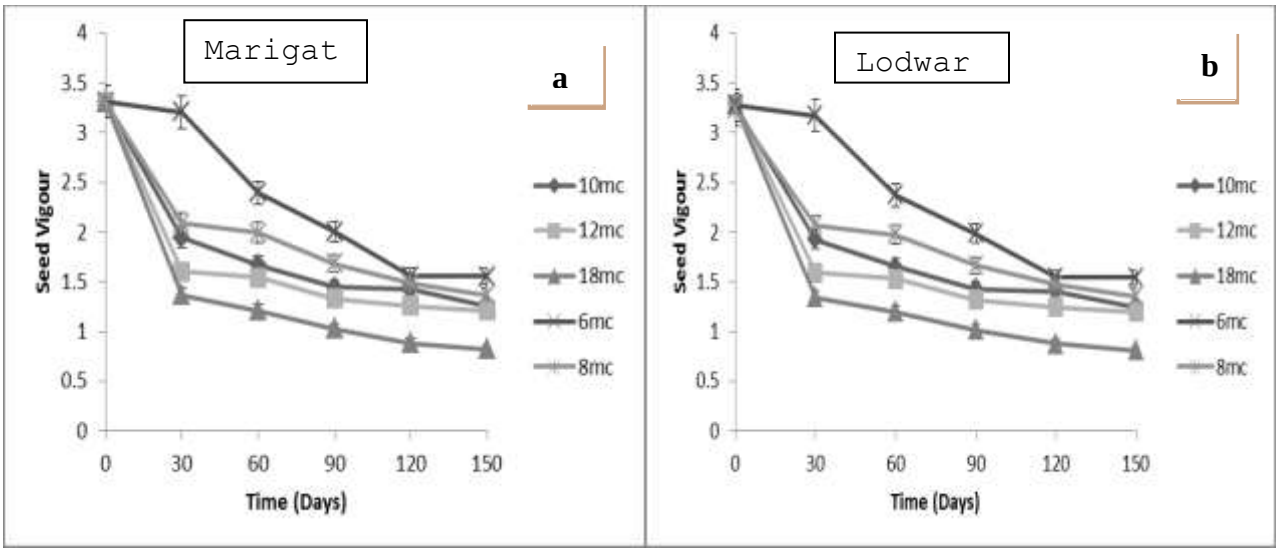

Figure 4.5: Seed vigour at 6^0C for seeds from (a) Marigat and (b) Lodwar for 150 days

4.7 C. Sinensis Seeds Vigour Loss at 150C for 150 Days at Varying MC

In higher storage temperature of 15^0C for seeds stored with same moisture contents of 6%, 8%, 10%, 12% and 18%, results of the seed vigour showed decrease of seed vigour as days progressed. For example, seeds with 6% moisture contents lost viability to 0.5 G. I in both study sites while 8%, 10%, 12% and 18%, lost vigour at varying time in the order of 6>8>10>12>18%. In general, seeds at all moisture contents lost vigour with time. However, seeds stored with 18% MC lost vigour faster than other seeds batches, while those with 6% MC lost least vigour with time (Figure 4.6). Seeds stored with 8%, 10%, and 12% MC had no significant difference in seed vigour between the seeds collected from Marigat and Lodwar sites (Figure 4.6). As at 6^0C, 15^0C and 25^0C, seeds stored at 15^0C there was significant seed loss of vigour for seed stored with MC of 8%, 10% and 12%, the seeds from both Margat and Lodwar lost vigour quickly as temperature increased to 15^0C (Figure 4.6). The seed loss of vigour was in the order with MC as 6>8>10>12>18% for seeds stored at 15^0C for both study sites (Figure 4.6).

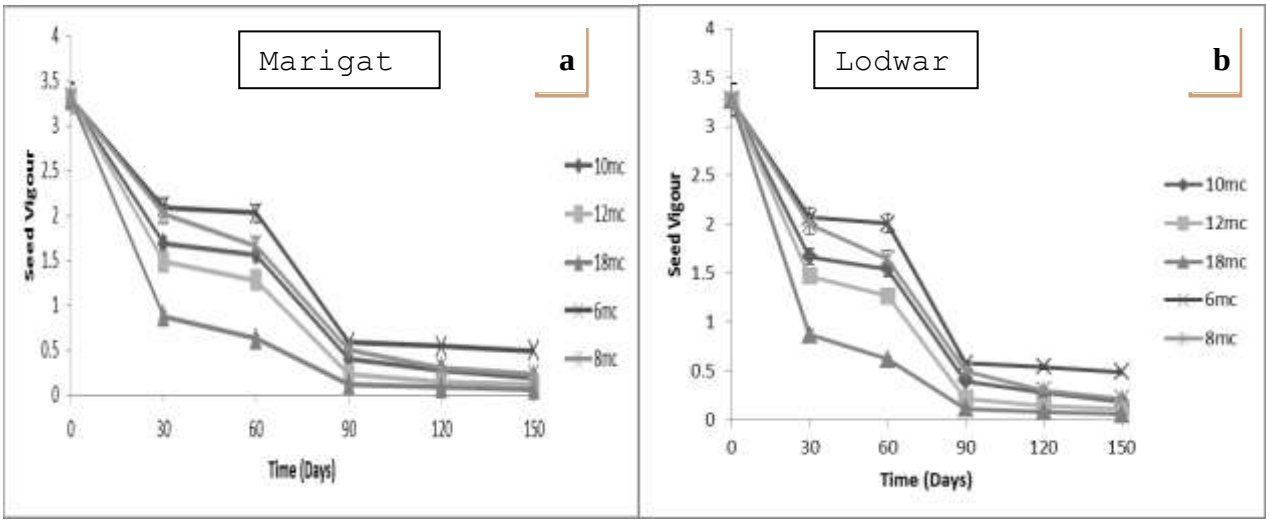

Figure 4.6: Seed vigour at 15^0C for seeds from (a) Marigat and (b) Lodwar for 150 days

4.8 C. Sinensis Seeds Vigour Loss at 250C for 150 Days at varying MC

In further increase of temperature to 25^0C for seeds stored with same moisture contents of 6%, 8%, 10%, 12% and 18%, results of the seed vigour showed that, in overall, seed vigour was not significantly difference for seeds obtained from both Marigat and Lodwar. In general, seeds at all

moisture contents lost vigour with time. However, seeds stored with 18% MC lost vigour faster at 90th day than other seeds batches, while those with 6% MC lost least vigour to 0.5 G. I with time (Figure 4.7). Seeds stored with 8%, 10%, and 12% MC had no significant difference in seed vigour between the seeds collected from Marigat and Lodwar sites (Figure 4.7). As at 6^0C and 15^0C, seeds stored at 25^0C had significant differences ($P<0.001$) loss of vigour for seed stored with MC of 8, 10 and 12% (Figure 4.7). The seed loss of vigour was again in the order with MC as 6%>8%>10%>12%>18% at 25^0C storage for both study sites (Figure 4.7).

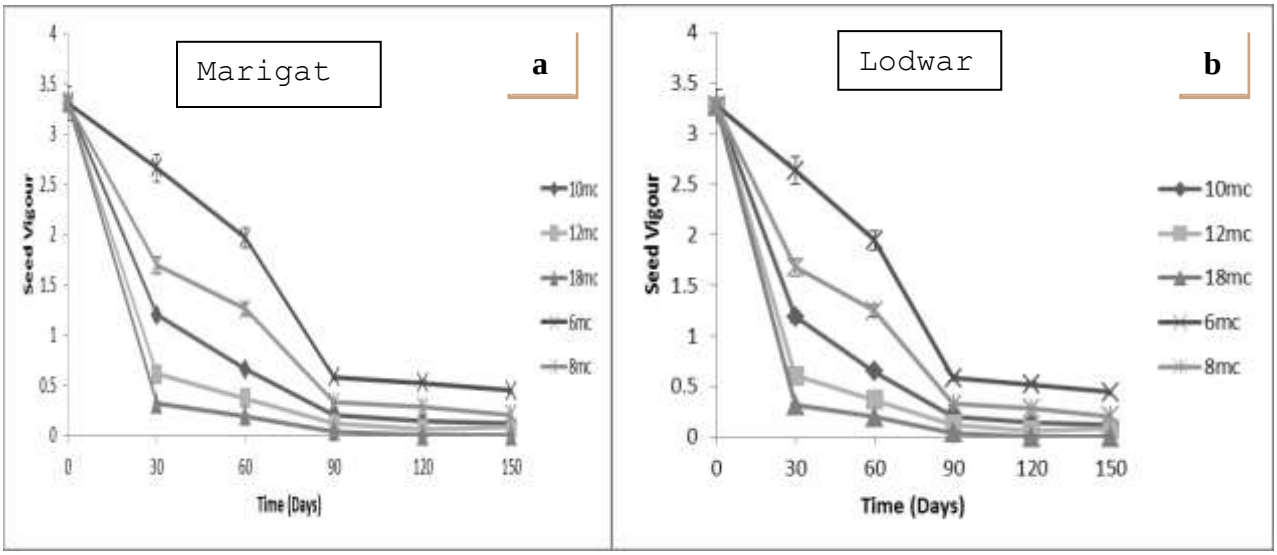

Figure 4.7: Seed vigour at 25^0C for seeds from (a) Marigat and (b) Lodwar for 150 days

4.9 C. Sinensis Seeds Vigour Loss at 350C for 150 Days at Varrying MC

In further increase of storage temperature to 35^0C the seed vigour levels decreased for both study sites as the days progressed and seed with 6% moisture content still had the highest vigour levels with seeds with 18% moisture content being the least in both study sites. The levels are not distinctly different in the two study sites. In general, seeds at all moisture contents lost vigour gradually. However, seeds stored with 18% MC lost vigour faster than other seeds batches, while those with 6% MC lost least vigour with time (Figure 4.6). Again seeds stored with MC of 6% had higher vigour over the entire study period with relative to other moisture contents for seeds collected from both Marigat and Lodwar. Seeds stored with 8, 10, and 12% MC had no significant difference in seed vigour between the two sites (Figure 4.6). Seeds stored at 15^0C had significant loss of seed vigour for seed stored with

MCs of 8, 10 and 12% (Figure 4.6). The seed loss of vigour was in the order with respect to MC as 6>8>10>12>18% at constant storage temperature of 35^0C for both study sites (Figure 4.6). In all moisture contents, there was a continual decrease in vigour levels as the days progressed. Vertucci and Roos (1990) propose that MC for maximum lifespan can be temperature dependent.

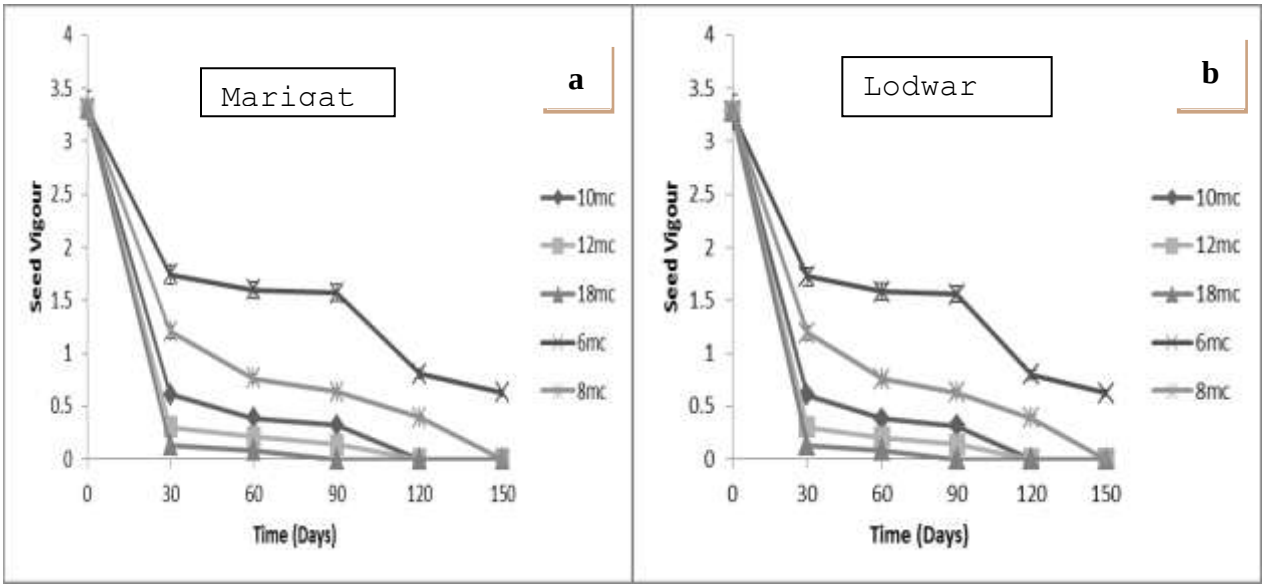

Figure 4.8: Seed vigour at 35^0C for seeds from (a) Marigat and (b) Lodwar for 150 days

4.10 Regression of Viability against Time, Moisture Content, Storage Temperature and Provenance

The results confirm that the predictors do influence the model (p-value<0.0001) when dependent (vigour) was regression against predictors: time (days), moisture content (%), storage temperature, site (Table 4.2).

The Model Summary of Regression on viability against time, moisture content, storage temperature and sites in Appendix 2, the independent variables (predictors: time (days), moisture content (%), storage temperature, site) explain 69.2% of the dependent variable (viability) in the model. The ANOVA (Table 4.2) shows that descriptive variables do have significant effect on the model.

The coefficients in Appendix 3 shows that all variables in the equation with exception of site (p-value=0.082) do influence the model based on the sig (significance test column in Table 4.3)

TABLE 4.2: Regression of Viability against Time, Moisture Content, Storage Temperature and Provenance

ANOVA[a]					
Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	154238.267	4	38559.567	135.396	0.000[b]
Residual	66925.983	235	284.791		
Total	221164.250	239			

a. Dependent Variable: Viability (%)

b. Predictors: (Constant), Time (Days), Moisture content (%), Storage temperature, Provenance

TABLE 4.3: The Coefficients Table on Viability against Time, Moisture Content, Storage Temperature and Provenance

Coefficients					
Model	**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
(Constant)	109.283	5.068		21.564	.000
Site	3.800	2.179	.063	1.744	.082
Storage temperature	-10.443	.974	-.385	-10.719	.000
Moisture content (%)	-8.092	.770	-.377	-10.505	.000
Time (Days)	-.376	.021	-.635	-17.700	.000

Viability=109.283 -10.443 Storage temperature – 8.092MC – 0.376Time

4.11 Regression of Time Verses Viability for Lodwar and Marigat Provenance

In figure 4.9 a) and b), the R^2 shows 27.1%, and 33% of the variation in seed viability in both Lodwar and Marigat is due to time taking into account predictors of moisture content and temperature at 6^0C storage temperature. The low R^2 may be due to extremes values brought about by different moisture content where seeds with 6% MC had higher viability points of more than 80% while seeds with 18% had lower viability points of less than 10%. The two graphs in figure 4.9 a) and b) exhibit a negative relationship where increase in storage days in a constant storage temperature of 6^0C is inversely propositional to viability over time for seed from both Lodwar and Marigat provenances (figure 4.9 a and b)

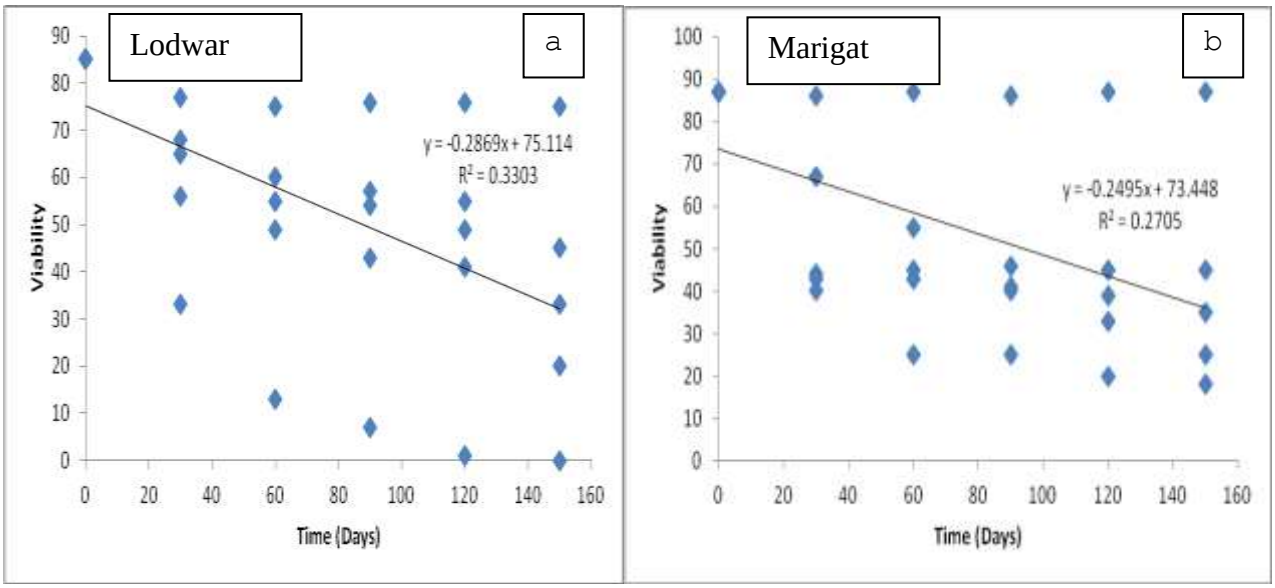

Figure 4.9: Regression of time verses viability of seeds stored at 6^0C and collected from a) Lodwar and b) Marigat

4.12 Regression Graphs of Time Verses Viability for Lodwar and Marigat Provenance

In regression of values of seed viability from both Lodwar and Marigat, the R^2 shows 30%, 50%, 55% and 56% of the variation in seed viability was due to time taking into account predictors of moisture content and temperature in respective storage temperature of 6, 15, 25 and 35^0C respectively figure 4.10, a), b), c) and d). The graphs exhibit a negative relationship where increase of storage time was inversely propositional to seed viability in the four-storage temperature regimes of 6, 15, 25 and 35^0C ((figure 4.10, a), b), c) and d)).

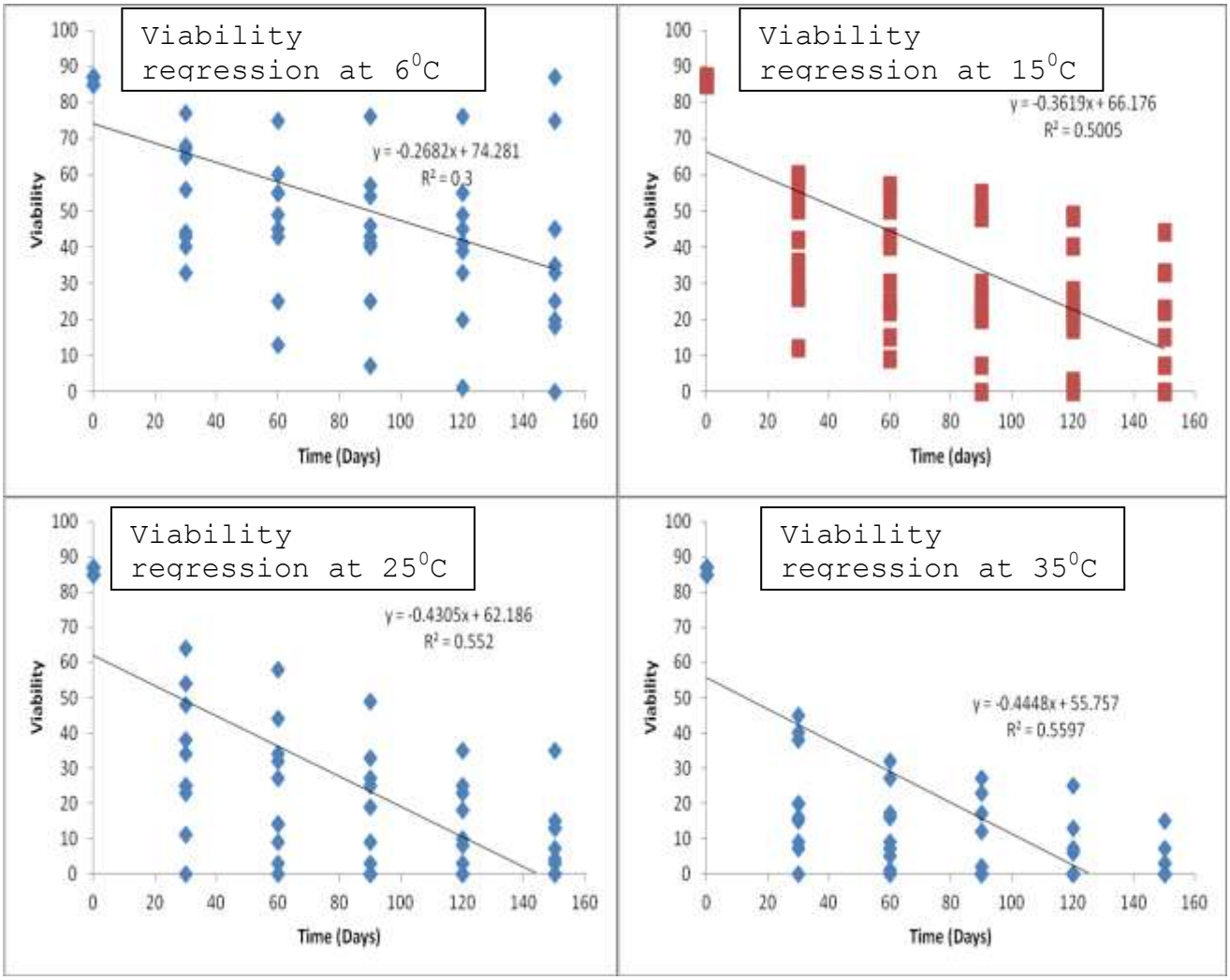

Figure 4.10: Combined regression for time versus viability of seeds stored at a) 6^0C, b) 15^0C, c) 25^0C, d) 35^0C collected from both Lodwar and Marigat

4.13: Regression of Vigour against Time, Moisture Content, Storage Temperature and Provenance

The results (Table 4.4) confirms that the predators' do influence the model (p-value<0.0001) when dependent (vigour) was regression against predictors: time (Days), moisture content (%), storage temperature, Provenance. The Model Summary of regression (Table 4.4) on vigour against time, moisture content, storage temperature and sites, the independent variables (predictors: time (days), moisture content (%), storage temperature, provenance) explain 71.1% of the dependent variable (vigour) in the model (Table 4.4) The coefficients results (Table 4.5) shows that all variables in the equation with exception of Site (p-value=0.835) do influence the model, this is proofed by the

(significance test column (Table 4.5)

Table 4.4: Regression on Vigour against Time, Moisture Content, Storage Temperature and Provenance

ANOVA[a]					
Model	**Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
Regression	223.008	4	55.752	148.086	.000[b]
Residual	88.474	235	0.376		
Total	311.482	239			

a. Dependent Variable: Vigour (germination index)

b. Predictors: (Constant), Time (Days), Moisture content (%), Storage temperature, Provenance

Table 4.5: Coefficients Model Summary of Regression on Vigour against Time, Moisture Content, Storage Temperature and Provenance

Coefficients					
Model	**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
(Constant)	3.833	0.184		20.801	0
Site	0.017	0.079	0.007	0.208	0.835
Storage temperature	-0.292	0.035	-0.287	-8.253	0
Moisture content (%)	-0.21	0.028	-0.261	-7.502	0
Time (Days)	-0.017	0.001	-0.752	-21.631	0

a. Dependent Variable: Vigour (germination index)

Vigour=3.833 - 0.292 storage temperature – 0.21MC – 0.017Time

4.14 Regression Graphs of Time Verses Vigour

Similar trend to that of viability in figure 4.10 a) and b) was exhibited by vigour in figure 4.11 a) and b) where the R^2 shows that, 50.8% and 62.3% of the variation in dependent of vigour is reduced by taking into account predictors of time, moisture content and temperature at constant temperature of 6^0C for both Lodwar and Marigat provenance. The graph exhibits a negative relationship where

increase of both moisture contents and storage temperature causes vigour to decrease over time

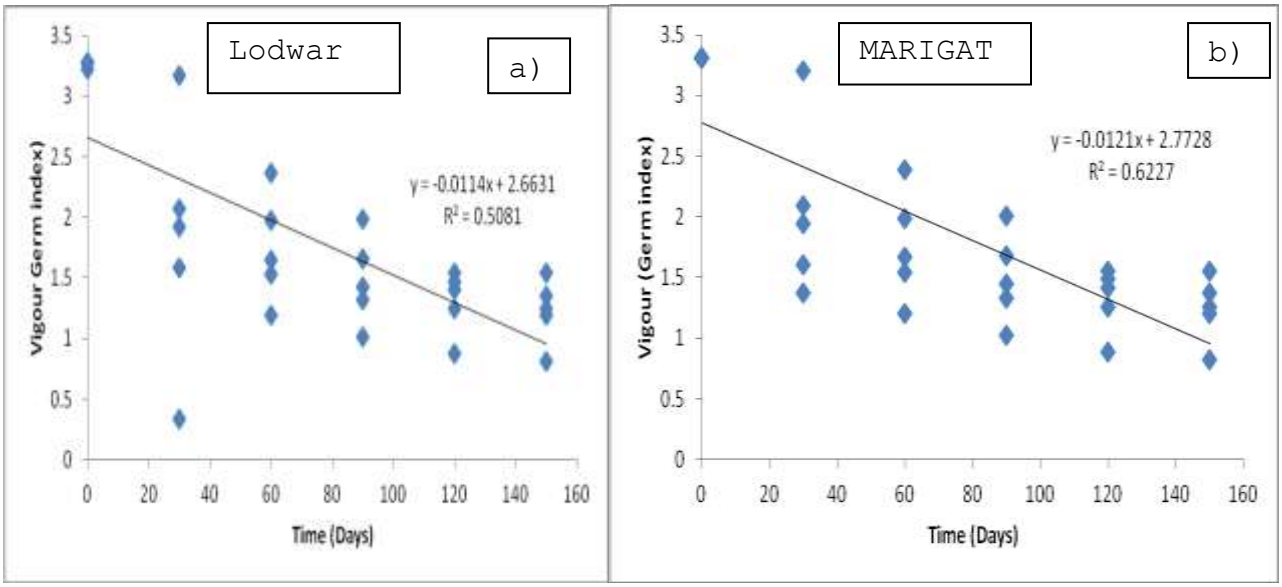

Figure 4.11: Regression for time verses vigour for seeds stored at 6^0C Collected from a) Lodwar and b) Marigat

4.15 Overall Regression Graphs of Time Verses Vigour for Lodwar and Marigat Provenance

In overall regression which tends to take averages values of Lodwar and Marigat provenance (figure 4.12. a), b), c) and d), the R^2 shows that, 56.2%, 78.8%, 64.6% and 55.7% of the variation of seed vigour is due to time taking into account predictors of moisture content and temperature in respective storage temperature of 6, 15, 25 and 35^0C, respectively. The graphs (figure 4.12. a), b), c) and d),) reveal that increase of both moisture contents and storage temperature caused decrease in viability over time. The regression summary (figure. 4.13) which again takes dependents (vigour) and against predators (time, moisture contents and temperatures) for the both Lodwar and Marigat sites reveals that increase of both moisture contents and storage temperature decreased vigour over time. The R^2 shows that 56.6% of the variation in seed vigour is also due to time by taking into account predictors (time, moisture content and temperature) (figure. 4.13).

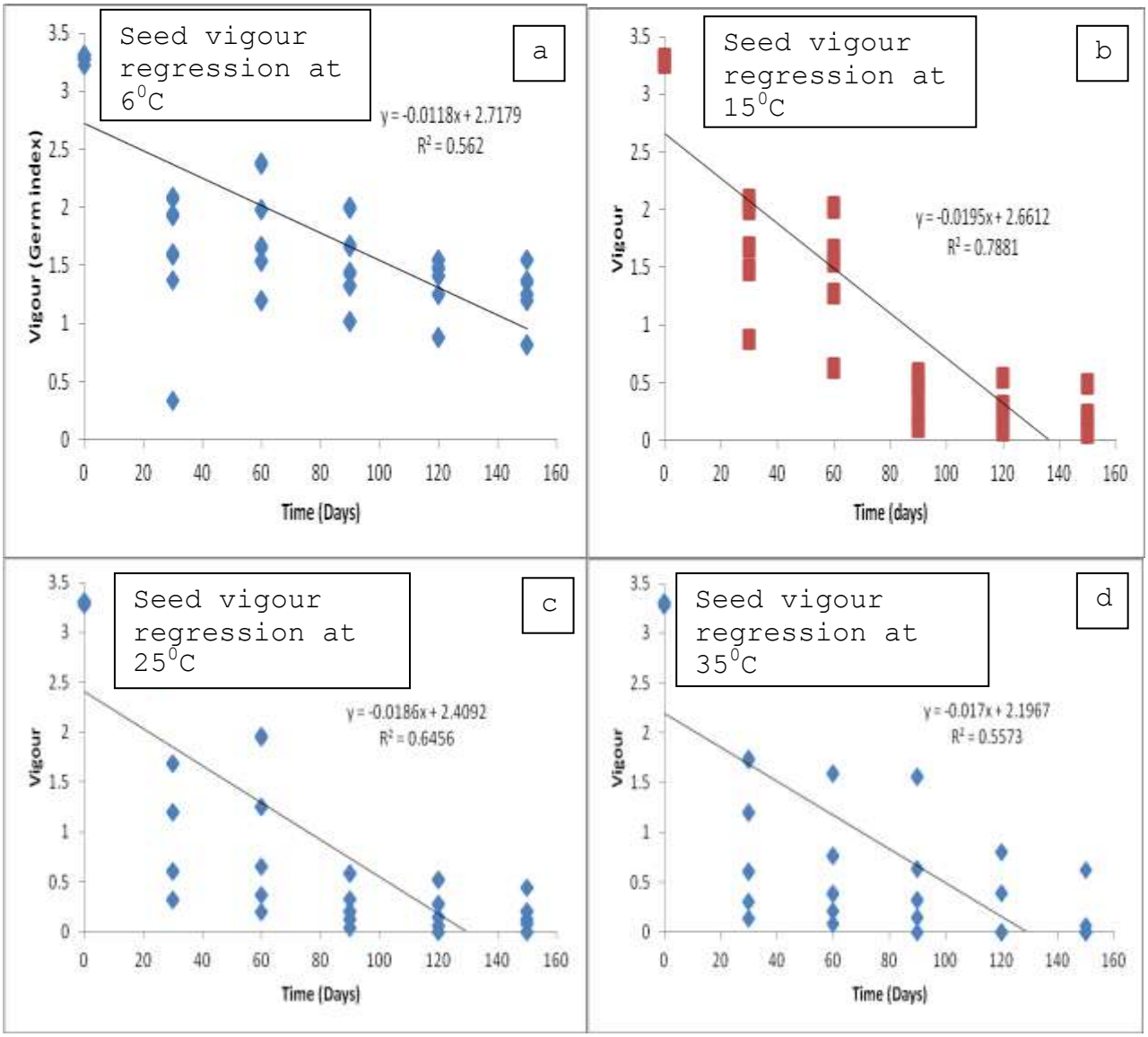

Figure 4.12: Valiet regression for time versus vigour of seeds stored at a) 6^0C, b) 15^0C, c) 25^0C, d) 35^0C from both Lodwar and Marigat combined.

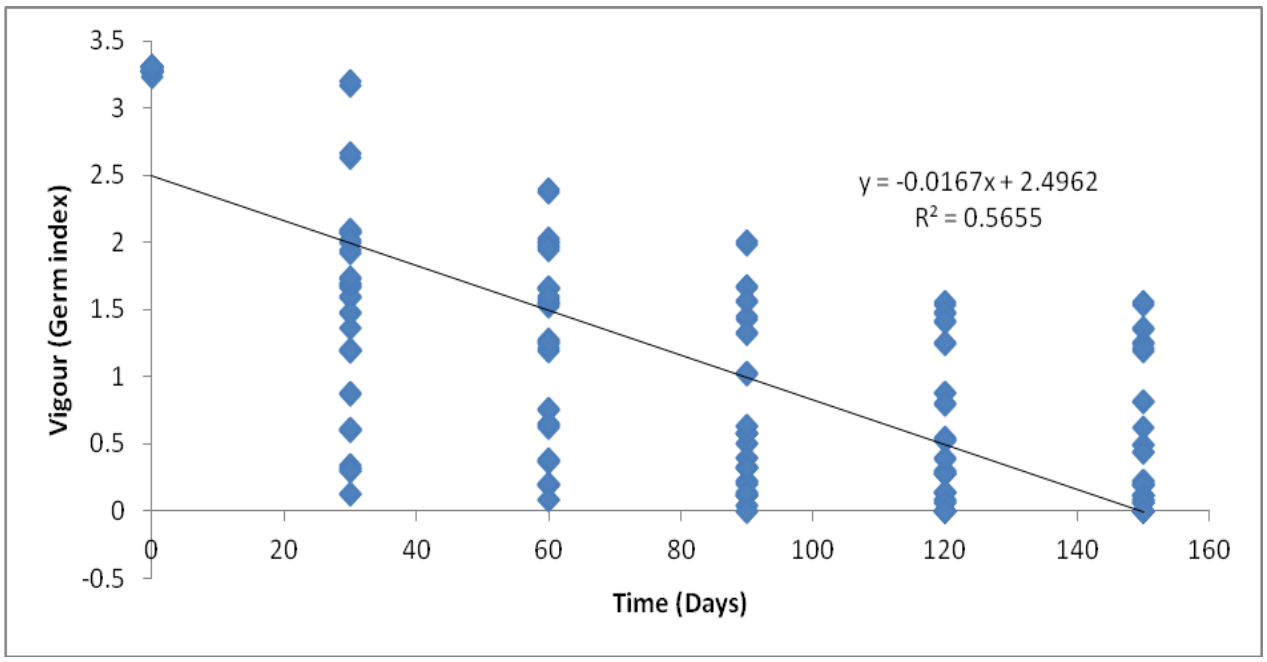

Figure 4.13: Regession summary of time verses vigour of seeds collected from both Lodwar and Marigat

4.16 Correlations between Viability and Vigour

The results revealed a strong positive correlation between viability and vigour (table 4.4). As viability declined with time equally the vigourity declined with time at respective moisture content and storage temperature. The results from both correlations vigour (0.919) and viability (0.919) are very close to one (1) (table 4.4) which suggests a very strong posive correlation between the viability and vigour.

Table 4.6: Correlations between viability and vigour

Correlations			
		Viability	Vigour
Viability	Pearson Correlation	1	0.919**
	Sig. (2-tailed)		0.000
	N	240	240
Vigour	Pearson Correlation	0.919**	1
	Sig. (2-tailed)	0.000	
	N	240	240

CHAPTER FIVE

5.0 DISCUSSION

5.1 Seed longevity

This study has investigated seed handling methods for *C. sinensis* by manipulating moisture content and temperature. Results presented here shows there are short lived and long-lived seeds. Seeds with low moisture content of 6% had the longest life compared to seeds with highest moisture content of 18% stored across all temperature regimes of 6^0C, 15^0C, 25^0C and 35^0C for 150 days.

For example, the results for seeds with 18%, 12%, 10%, 8% and 6% moisture content stored at 6^0C temperature revealed that with decrease of seed moisture content seed longevity increased. Seed longevity increased in the order with MC as 6>8>10>12>18% for both study sites. Low temperature promotes seed longevity, especially when the seed´s moisture content had been previously reduced. Under such circumstances, the metabolic activity of seed greatly decreases. Therefore, an adequate dehydration of the seeds is the first requirement for long-term storage (Pritchard *et al.,* 2004). Lastly, 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).

Again, the results for same seeds with 18%, 12%, 10%, 8% and 6% moisture content replicated in storage condition at elivated temperature of 15^0C, 25^0C and 35^0C revealed that with increase of storage temperature, seed longevity period reduced with time. Seed longevity reduction was in the order with MC as 6>8>10>12>18% in respective storage temperature. The results revealed that seed longevity reduction was influenced by moisture contents and storage temperature (table 4.1) thus agreeing with similar seed research (Pritchard *et al.,* 2004; Carvalho and Nakagawa, 2000).

5.2 Seed Viability

This study has investigated seed handling methods for *C. sinensis* by manipulating moisture content and temperature. Reduction in both moisture content and storage temperature resulted in increase in viability. Seeds with low moisture content of 6% had the highest viability compared to seeds with

highest moisture content of 18% stored across all temperature regimes of 6^0C, 15^0C, 25^0C and 35^0C for 150 days.

For example, the results for seeds with 18%, 12%, 10%, 8% and 6% moisture content stored at 6^0C temperature revealed that with decrease of seed moisture content seed viability increased. Seed viability increased in the order with MC as 6>8>10>12>18% for both study sites (Figure 4.1). This conforms to Orthodox seeds which conform to certain rules of the thumb that predict well the pattern of loss of viability in relation to storage environment, where for each 2% decrease in seed moisture content, the storage life of the seed is doubled (Walters *et al.,* 2005; Schmidt, 2000).

Results for same seeds with 18%, 12%, 10%, 8% and 6% moisture content stored at 15^0C revealed that with increase of storage temperature, seed viability reduced with time. Seed viability was in the order with MC as 6>8>10>12>18% for both study sites (Figure 4.2). This again conforms to Orthodox seeds to certain rules of the thumb that predict well the pattern of loss of viability in relation to storage environment, where approximately each 5.6 ^{0}C decrease in seed storage temperature, the storage life of the seeds is doubled (Walters *et al.,*2005).

Further, the results for the same seeds with 18%, 12%, 10%, 8% and 6% moisture content stored at further higher temperature of 25^0C revealed that increase of storage temperature, reduced seed viability levels as the days progressed, seed viability was in the order with MC as 6>8>10>12>18% for both study sites (Figure 4.3).

Further increase of storage temperature to 35^0C for same seeds with 18%, 12%, 10%, 8% and 6% moisture content revealed that with further increase of storage temperature, seed longevity period reduced further with a continuous decrease in seed viability levels as the days progressed, seed viability was in the order with MC as 6>8>10>12>18% for both study sites (Figure 4.4)

In general, loss of viability in relation to storage environment conforms to orthodox seeds rules of the thumb that predict well the pattern of loss of viability in relation to storage environment, where each 2% decrease in seed moisture content, the storage life of the seed is doubled (Walters *et al.*, 2005), for each 5.6 degrees' centigrade decrease in seed storage temperature, the storage life of the seeds is

doubled also (Walters *et al.*,2005). Determination of safe grain storage time is an answer to the following question: how long can grains of particular moisture content and temperature be stored without the risk of the quality deterioration (Ryniecki, 2006) of which *C. sinensis* seeds is not different. A related work on canola seeds revealed that germination decreased with storage time, temperature, and moisture content. After 56 days, germination of canola stored at 12% MC. wet basis and at 25- 30°C dropped from viability of 80% to 73%. The similar drop to 73% viability was showed: at 12%, MC. at 30-35°C after 27 days, at 14 % MC. at 25-30°C after 29 days, while at 14 % MC. at 30-35°C showed after 12 days (Pronyk *et al.*,2006). In another study *A. australis* in one study (dried to 6 % MC, then stored in sealed containers at 5° C) preserved viability for 6 years (79 % germination compared with the initial 88 %), while storage at below freezing temperature maintained a germination of about 60 % for up to 12 years (Preest, 1979). The same seed stored at higher MC or temperatures (15 – 20 % MC. or 15° – 20° C) had lost all germination power within 14 months.

C.sinensis seed batch with 18%, 12%, 10%, 8% and 6% moisture content stored at 6^0C, 15^0C, 25^0C and 35^0C temperature regimes confirmed that seed with respective moisture content is a very important factor to consider for long storage and viability as seed lost viability with increase of seed moisture contents in all storage temperature. Moisture content increase in seeds stored at constant temperature was verified to cause a decrease in longevity, in all temperatures (Walters, 1998; Walters *et al.*,2005). However, storage temperature in this study revealed also that it was equally important in seed longevity and seed viability as decrease of storage temperature increased seed longevity although with continuous decline in seed viability as day's progresses. *C.sinensis* seeds from MARIGAT with highest MC of 18% when stored for 150 days at temperature of 35^0C, 25^0C, 15^0C and 6^0C seeds viability reduced from 87% to zero (dead) in 30 days at 35^0C, from 87% to zero (dead) in 60 days at 25^0C, from 87% to zero (dead) in 90 days at 15^0C and from 87% to zero (dead) in 120 days at 6^0C with those from Lodwar following similar trend of reducing viability from 85% to zero (died) in: 60 days at 35^0C, from 87% to zero (dead) in 90 days at 25^0C, from 87% to zero (dead) in 90 days at 15^0C and from 87% to zero (dead) in 120 days at 6^0C when stored for 150 days. However, seeds from Marigat with lowest MC of 6% had all their seeds viable (alive) beyond 150 days respectively but with reduced viability to: 20% at 35^0C, 40% at 25^0C, 55% at 15^0C and 78% at 6^0C in 150 days. Seeds from Lodwar had similar trend, as the seeds remained viable (alive) beyond 150 days at 6% MC also but with a reduced viability at respective storage temperature from an initial viability of 85% to: 15% at 35^0C, 27% at 25^0C, 40% at 15^0C and 85% at 6^0C in150th day. Based on seed response to desiccation,

orthodox seeds can be dried to low water contents (<7%) with little effect on viability (Menttananda, *et al*., 2001) whilst intermediate seeds can survive considerable levels of desiccation to levels approaching those of orthodox seeds (MC. 10%), but exhibit a negative response to chilling at sub-zero temperatures, particularly -20°C (Ellis *et al.,* 1990, 1991). This is consistent with results from many orthodox seeds and *C. sinensis* is not different. 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 recommended *C. sinensis* be stored with low MC (<5-8) in cool dry place for short time and medium term periods but for long term be stored at sub-zero temperatures for many years with no significant loss of viability this suggests the seeds are an intermediate class

5.3 Seed Vigour

Scientists have known for years that seed longevity improves if seeds are dried to low water contents (Justice and Bass, 1978: FAO, 1994) and this certainty forms the basis of the FAO recommendation of *5+2%* water for safe storage of orthodox seeds (FAO, 1994). In most instances, the life span of seeds increases when they are dried to water contents as low as 5%. The results from the experiment for *C. sinensis* revealed seeds with low moisture content of 6% had the longest life with highest vigour compared to seeds with highest moisture content of 18% stored in same low temperature of 6^0C for same period of 150 days (Figure.4.5). The results further revealed that with decrease of seed moisture content both seed longevity period and seed vigour increased for seeds stored in constant storage temperature. Specifically, *C. Sinensis* seeds with 18%, 12%, 10%, 8% and 6% moisture content stored at constant temperature of 6^0C, 15^0C, 25^0C and 35^0 C confirmed that seed with respective moisture content is a very important factor to consider for long term storage and vigourity as seed lost vigourity with increase of seed moisture contents in all storage temperature (figures 4.5, 4.6, 4.7, and 4.8). Moisture content increase in seeds stored at constant temperature was verified to cause a decrease in longevity, in all temperatures (Walters, 1998; Walters *et al.*,2005). However, storage temperature in this study revealed that it was equally important in seed longevity and seed vigour which revealed that decrease of storage temperature increased seed longevity and prolonged seed vigourity. Similar study by Weinberg *et al*., (2008) examined the vigour of the maize (corn) stored in the self-regulated atmospheres in the sealed containers. They noticed that the vigor percentage decreased during the storage period, and decreased as the moisture content increased. With 18 % MC. and above the vigour decreased to zero after 35 days of storage. Another study Maua *et al.,(*2004) generalized that

C.sinensis seeds with less than 10% MC would store in cool dry place for short time and medium term periods, but no attempt to classify them to either orthodox or intermediate class was made, therefore this study classified *C. sinensis* as orthodox seeds which is in conformity to FAO (1994) that, Orthodox seeds will retain viability longer, when dried to a low moisture content (4 – 8 %) and then stored in a sealed container in cool dry storage. moisture content of 4 – 8 % is considered safe for most orthodox species; 5 % ± 1 % is recommended for long-term storage for genetic conservation. Scientists have known for years that seed longevity improves if seeds are dried to low water contents (Menttananda, *et al.,* 2001) and this certainty forms the basis of the FAO recommendation of *5+2%* water for safe storage of seeds (FAO, 1994). In most instances, the life span of seeds increases when they are dried to water contents as low as 5%

5.4 Correlation between Seed Viability and Seed Vigour

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). Decline in seed viability is linked to the moisture content of the seed (Vieira *et al*., 2001). Other studies have also documented similar initial seed vigor decline and a subsequent seed vigor increase for seed lots stored in continuous low temperature and low 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 *C. sinensis* reveals positive correlation between seed viability and seed vigour. The correlation figure of both viability (0.919) and vigour (0.919) were close to value of one (1) which suggests a very strong positive correlation (table 4.5). The positive correlation conforms to results test from this research for both viability and vigour, which revealed that decrease of seed moisture content, the seed longevity period was increased. The initial viability was approximately 86% and not 100%, which R^2 would have given 100%. This was difficult to explain this apparent anomaly unless one assumes that a certain percentage of the seed possibly were less mature (Austin, 1998) or of different genotype (Roberts, 1994) and were adversely affected by extraction method or storage temperature while the remainder was not.

CHAPTER SIX

6.0 CONCLUSION AND RECOMENDATIONS

6.1 CONCLUSION

This study showed that seed lonevity period increased with reduction of both seeds moisture contents and storage temperature. The seed with lowest moisture contents of 6% had the highest logevity period across all the storage temperature. However, P_{50} (period taken by seed to loss viabily by 50%) decreased with increase of storage temperature across all the seeds with different moisture contents.

The results further showed that, both seed moisture content and storage temperature influenced rate loss of both seed viability and vigour in storage in storage. Storing of seeds with 6, 8, 10, 12 and 18% MC across a contant temperature of 6, 15, 25 and 35^0C depicted that decreasing both seed moisture content and storage temperatures influenced both seed viability and vigour in storage. Therefore, MC and storage temperature are the two factors that influenced both seed viability and vigour. The study confirms that, the *C. sinensis* seeds are orthodox that stored long at 6% MC and with both high viabilty and vigour acros all the storage temperature.

There is continuum in longevity, viability and vigour decreasing as moisture content increases and temperature increases. The results revealed a strong positive correlation between viability and vigour. As viability declined with time, equally the vigourity declined with time at respective moisture content and storage temperature.

6.2 RECOMENDATIONS

The *Cordia sinensis* seeds be stored for long period of time for up to minimum of two years as to derive and use probert and Robert formulae in calculating longevity. The optimal and best storage condition of *Cordia sinensis* are moisture content of 6% and storage temperature of 6^0C for longer storage period, where cryopreservation is not available.

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APPENDICES

APPENDIX 1: MODEL SUMMARY OF REGRESSION ON VIABILITY AND VIGOUR AGAINST TIME, MOISTURE CONTENT, STORAGE TEMPERATURE AND SITE

Model Summary of Regression on Viability

Model Summary					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.835[a]	0.697	0.692	16.876	1.613

a. Predictors: (Constant), Time (Days), Moisture content (%), Storage temperature, Site
b. Dependent Variable: Viability (%)

Model Summary of Regression on Vigour

Model Summary[b]					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.846[a]	0.716	0.711	0.6135835	1.688

a. Predictors: (Constant), Time (Days), Moisture content (%), Storage temperature, Site
b. Dependent Variable: Vigour (germination index)

APPENDIX 2: LODWAR AND MARIGAT INTIAL MC

(a) Lodwar intial MC

M_1	M_2	M_3	% MC
98.57	103.57	102.613	19.14
99.35	104.35	103.484	17.32
Total			36.46
Average			18.23

Source: laboratory test (2015)

(b) MARIGAT intial MC

M_1	M_2	M_3	% MC
83.277	85.516	85.187	14.69406
95.586	97.114	96.893	14.46335
Total			29.15741
Average			14.57871

Source: laboratory test (2015)

APPENDIX 3: LODWAR AND MARIGAT DESICATION DATA

(a) Lodwar desication Data

Target MC (%) **(A)**	**IntialMC (%)** **(B)**	**Intial wt (gm)** **(C)**	**Derived wt (gm)** $\frac{\textbf{(100-B) X C}}{\textbf{(100-A)}}$
12	18.23	410	380.97
10	18.23	415	376.45
8	18,23	415	368.27
6	18.23	415	357.83

Source: laboratory test (2015)

(b) MARIGAT desication data

Target MC (%) **(A)**	**Intial MC (%)** **(B)**	**Intial wt (gm)** **(C)**	Derived wt (gm) $\frac{\text{(100-B) X C}}{\text{(100-A)}}$ **(D)**
12	14.49	172.010	163.038
10	14.49	172.013	159.417
8	14.49	172,675	156.55
6	14.49	172.021	152.623

Source: laboratory test (2015)

APPENDIX4: ANALYSIS OF VARIANCE FOR SEED VIABILITY

Variate: Total number germinated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MC_%	4	27433.6	6858.4	9.04	<.001
Residual	235	178321	758.8		
Total	239	205754.7			

Variate: Total number germinated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage_tempC	3	61481.6	20493.9	33.52	<.001
Residual	236	144273.1	611.3		
Total	239	205754.7			

Interactions

Variate: Total number germinated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MC_%	4	27433.6	6858.4	13.61	<.001
Storage_tempC	3	61481.6	20493.9	40.68	<.001
MC_%*Storage_tempC	12	6008	500.7	0.99	0.456
Residual	220	110831.4	503.8		
Total	239	205754.7			

APPENDIX 5: ANALYSIS OF VARIANCE FOR SEED VIGOUR

Variate: vigour

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
MC_%	4	1690.23	422.56	8.25	<.001
Residual	235	12041.15	51.24		
Total	239	13731.38			

Variate: vigour

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Storage_tempC	3	2206.55	735.52	15.06	<.001
Residual	236	11524.84	48.83		
Total	239	13731.38			

Interactions
Variate: vigour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MC_%	4	1690.23	422.56	9.84	<.001
Storage_tempC	3	2206.55	735.52	17.12	<.001
MC_%.Storage_tempC	12	384.51	32.04	0.75	0.705
Residual	220	9450.09	42.95		
Total	239	13731.38			

PLATES: *CORDIA SINENSIS* PICTURES

Plate 1: Germinating seeds of *Cordia sinensis*

Plate 2: (a) *Cordia sinensis* tree (b) *Cordia sinensis* mature fruit

Plate 3: Desicated packed seed for storage