EFFECT OF HARVESTING TIME AND POST-HARVEST HANDLING ON QUALITY OF PUMPKIN SEED (*Cucurbita pepo L.*).

TARUS WINNIE JEBET

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DECLARATION

Declaration by the candidate

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Tarus Winnie Jebet Signature…………………… Date………………….

AGR/ PGC/17/12

Declaration by the supervisors

This thesis has been submitted with our approval as University Supervisors.

Professor Julius Ochuodho Signature………………….. Date ………………….

University of Eldoret

Dr. Nicholas Rop Signature…………………… Date ……………………..

University of Eldoret

DEDICATION

This thesis is dedicated to my parents, siblings and husband for their love, patience, kind heartedness and gentle encouragement which helped me get through this thesis writing process.

ABSTRACT

Pumpkin fruits are usually harvested by rural farmers purposely for food after which the extracted seed is used for planting, yet the age of fruit at maturity may not coincide with the seed quality. This study was done to determine the effects of harvesting time and post harvest handling of pumpkin fruit on seed quality. The field experiment was carried out in one season of the year 2015 in three sites (UOE, KTTI and AIC Cheptebo) of the North-Rift region of Kenya. Randomized Complete Block Design was used, laid out in split plot, where the main plot was the type of pumpkin and the sub plot was the harvesting stage. Pumpkin fruits were harvested at four stages (30, 40, 50 and 60 Days after Anthesis). Seeds were extracted then fermented for a period of 0, 2 and 4 days. They were then sun-dried until constant weight was obtained, then a thousand seed weight was determined. The dried seeds that had been harvested at 60 DAA were stored where they were produced (UOE, KTTI and AIC Cheptebo) for a period of three months. Seed testing for germination percentage, electrical conductivity and seedling height was done in the seed science laboratory using Completely Randomized Design. Data was obtained on Germination percentage, Seedling height, TSW, Electrical conductivity and Seedling growth rate. The data obtained was subjected to ANOVA using General Linear Model procedure for (SAS, Version 9.1.3). The treatment means for each test was compared by protected Fischer's test Least Significant Difference (LSD) at $P \leq 0.05$ level of probability. The seeds from fruits harvested at 60 DAA and fermented for a period of four days, from Cheptebo were significant on a thousand seed weight, germination and vigour at $P \leq 0.05$. Germination percentage was 90.9% for the round type and 87.6% for the oval type and TSW was 39.45 g for the round type and 29.03 g for the oval type. Electrical conductivity was lowest for seeds harvested from Cheptebo at 60 DAA. The seeds that were fermented for a longer period (4 days) gave higher germination percentage of 90% for the round type and 86% for the oval type compared to those fermented for 2 days and those not fermented. Seeds stored at UOE and KTTI for a period of three months were significant at $P \leq 0.05$ compared to those stored in Cheptebo. There was no significance at $P \leq 0.05$ between the two pumpkin types. The conclusion of this study showed that the round-shaped pumpkin fruits harvested at 60 DAA from AIC Cheptebo gave the highest Thousand Seed Weight, seed germination and vigour. The seeds fermented for a period of four days and stored in KTTI at 22^{o}C showed high TSW, seed germination and vigour. It is recommended that pumpkin fruits should be planted in relatively warm areas (30-38^{o} C), like Cheptebo and harvested at 60 DAA. The seeds should be fermented for four days, dried and stored in cooler room temperatures (18-24^{o} C) at the highlands (Eldoret and Kaiboi) and further selection and breeding should be done on pumpkin local types.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC:	African Inland Church
ASAL:	Arid and Semi Arid Lands
BC:	Before Christ
BCE:	Before Common Era
DAA:	Days After Anthesis
FAO:	Food and Agriculture Organization
GDP:	Gross Domestic Product
GOK:	Government of Kenya
KALRO:	Kenya Agricultural Livestock Research Organization
KNBS:	Kenya National Bureau of Statistics
KSHS:	Kenya Shillings
KTTI:	Kaiboi Technical Training Institute
LH:	Low highland
LM:	Low midland
MOA:	Ministry of Agriculture
TSW:	Thousand Seed Weight
UN:	United Nations
UNDP:	United Nations Development Programme
UOE:	University of Eldoret
WFP:	World Food Programme

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CHAPTER ONE

INTRODUCTION

1.1Background of the Study

Pumpkin (*Cucurbita pepo L.*) is an emerging important exotic vegetable in Kenya and is thought to have originated from North America (Mexico) between 7000 and 5500 BC (Robinson and Decker-Walters, 1997). It is a fruity vegetable belonging to the family of cucurbitaceae; and has been cultivated for more than 10,000 years in different locations of the world. The yield of pumpkin fruits and seeds varies with climate, variety/cultivar, production system, weed competition, plant diseases and management practices. Pumpkins have been cultivated for nutritional and medicinal/pharmacological applications; some of the pharmacological benefits include ant diabetic, antihypertensive, antibacterial, intestinal and anti-inflammatory effects (Bwade and Aliyu, 2012). The pumpkin seeds are highly nutritious since they have minerals (Mg, Ca, Zn, P and Fe), proteins, lipids, fibres and carbohydrates (Kwiri et al., 2014). Pumpkins have several uses, great diversity in their cultivars and the potential to be cultivated in a wide range of environments (Ondigi et al., 2008).

The reliance on few staple foods like maize causes low levels of food diversity. This results in food insecurity due to low yields caused by poor soil quality and unreliable rainfall. Pumpkin is regarded as a traditional crop, yet it has a potential as food and vegetable for human consumption and commercial purposes. Pumpkin is a warm-season crop, hence grows best during hot weather, whereby, seeds can germinate at 15 ^{0}C but germinate best at 29-32 ^{0}C. Pumpkins grow best at a temperature of 23-29 ^{0}C day and 15-21 ^{0}C at night. Growth stops at temperatures below 10^{0}C and maturity can be delayed for several days at temperatures below 5 ^{0}C (Bannayan et al., 2017). Low temperatures have adverse effects on flowering and fruit set. Pumpkins are monoecious plants. Normally, several male flowers form before female flowers develop. During periods of low temperatures, (below 22 ^{0}C), most pumpkin cultivars respond by producing male flowers only, yet male flowers do not form fruits.

Pumpkins are relatively deep rooted and can tolerate dry conditions. They grow well on sandy-loam soil. They are good rotational crops with other vegetables. Pumpkins are mostly grown at a spacing of 2m by 2m. Large varieties can yield up to 9Kg per fruit. Hand weeding and shallow cultivation is done during weeding so as to prevent the destruction of many fine roots near the soil surface. Pumpkins are usually cross pollinated by insects, especially, bees (OMAFRA, 2009).

The seeds' physical and physiological characteristics, such as size, moisture, dry matter content, germination capacity and vigour change during seed development and maturation. In the orthodox seeds, last stage of seed development and maturation is marked by dehydration, in such a manner that during the reserve deposition phase, there is also accumulation of potentially protective molecules, especially *lea* (late embryogenesis accumulated) proteins and soluble sugars, such as sucrose, raff nose, and stachyose. Their function is to prevent the damage caused by reduction of moisture in the seed tissues (Vidigal *et al*., 2009).

On the other hand, pumpkins are usually attacked by insects, like the cucumber beetle. Temperature is the main factor which enhances the activity and behaviour of many insects. Temperature helps in the distribution and development of insects as well as their growth (Aldigail, 2013).

Utilizing improved postharvest practices often results in reduced food losses, improved overall quality and food safety, and higher profits for growers and marketers. It is estimated that 9 to 16 percent of the product is lost due to postharvest problems during shipment and handling (Hailu et al., 2015). Mechanical injury is a major cause of losses. Many of these injuries cannot be seen at the time that the product is packed and shipped, such as internal bruising in tomatoes. Other sources of loss include over-ripening, senescence, the growth of pathogens and the development of latent mechanical injuries. Chemicals have been widely used to reduce the incidence of postharvest diseases. Although effective, many of these materials have been removed from the market in recent years because of economic, environmental, or health concerns (Armachius, 2017). According to Emadi and Kosse (2007), mechanical damage could result from loading of agricultural materials during harvest, transportation, and storage; these loads are either compressive, tensile, shear, or impact in nature. The result of mechanical damage is generally to rupture or damage cells. Furthermore, greater damage to agricultural materials is done by the same absorbed energy when products are loaded slowly as observed in storage or during material handling. Proper postharvest processing and handling is an important part of modern agricultural production. Postharvest processes include the integrated functions of harvesting, cleaning, grading, cooling, storing, packaging, transporting and marketing. The technology of postharvest handling bridges the gap between the producer and the consumer.

1.2 Statement of the Problem

Pumpkin is neglected in formal research and therefore pumpkin seed certification is missing. Rural farmers usually harvest the fruits of pumpkin purposely for food after which they use the extracted seed at planting yet the fruit age at maturity may not coincide with seed quality. Production of high quality seed in Cucurbits depends on the fruit age at harvest and even the fruit pre-storage condition and duration. Fruits of different physiological maturity are found on the same plant, thus making it difficult to tell the right fruit harvest time yet the seed germination mainly depends on its physiological maturity. The maximum seed germination and vigour may not coincide with the maximum dry matter accumulation which is an aspect of physiological maturity as reported for wheat, tomato, pepper and eggplant. Therefore there is need to find the correct harvesting stage in which seed quality is good. This study therefore sought to determine the physiological maturity and proper post harvest handling so as to improve seed quality of pumpkin seeds (*Cucurbita pepo L*.).

1.3 Justification of the study

Pumpkin production can enhance food security when there is reduced production of staple foods like maize and potatoes. Therefore, the utilization and improvement of productivity through cultivation of under-utilized crops such as pumpkin would help reduce genetic erosion of the crops. In addition, pumpkins have been cultivated for nutritional and medicinal/pharmacological applications; crushed, fresh seeds inhibit overactive bladder and cardiovascular diseases (Gamonski, 2012).

High quality pumpkin seed gives optimum stands and yields through early planting, reduced seeding rates, and drill planting. Pumpkin seed of high quality produces strong seedlings that grow faster than less vigorous ones, are more tolerant to adverse conditions in the seedbed, and are better able to resist diseases. Overall, the study helped to develop proper post harvest handling of cucurbit seeds and to understand the right harvesting stage when physiological maturity was at its peak for better seed quality and food security.

1.4. Research Objectives

1.4.1 Broad objective

The study aimed at determining the effect of harvesting time and proper post harvest handling on seed quality of pumpkin (*Cucurbita pepo L.*).

1.4.2. Specific objectives

1. To determine the effect of time of harvest on seed quality of pumpkin (*Cucurbita pepo L.*).
2. To evaluate the effects of post-harvest handling practices on pumpkin seed quality.
3. To evaluate the effect of production sites on pumpkin physiological maturity and seed quality.
4. To determine the effects of fruit shape on pumpkin physiological maturity and seed quality.

1.5. Hypotheses

1. H_1: There is a significant difference between time of harvest and pumpkin seed quality.
2. H_1: There is a significant difference between post-harvest handling practices of pumpkin and seed quality.
3. H_1: There is a significant difference among agro-ecological zones and seed quality of pumpkin.
4. H_1: There is a significant difference between pumpkin fruit shape and seed quality of pumpkin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

2.1.1 Pumpkin origin and its importance

Pumpkin remnants were first found in Florida in 10,000 BCE, Southern Mexico in 8000 BCE and in Illinois around 5000 BCE. Cultivation in Mexico then spread to South-western United States by 1500 BCE (Deyo and O'Malley, 2008).

Pumpkin fruit is a big, round, pear-shaped, cylindrical berry which is hard on the outside. It is filled with pulp, with seeds inside. After hollowing it out, the fruit can be used for decoration during Halloween. Pumpkin pulp is used as an addition to meet and in making jam. The flowers can be used to make tasty salads. The pumpkin fruits grown for seeds have to reach full maturity before the seeds are extracted for drying, cleaning, sorting, transporting and processing (Kaliniewicz, 2014).

Pumpkin fruit has been found to be rich in carbohydrate, protein and antioxidant activities. Antioxidants are required to boost the human body immunity against cancer and other deadly human diseases. They are also rich sources of vitamin A present as beta-carotene, unsaturated fatty acids and high amounts of amino acids arginine, aspartame and glutamic acid (Usha *et al*., 2010).

The pumpkin seeds are good sources of phytonutrients and contain fibre and beta-carotene, which is an anti-oxidant that helps with immunity, skin and eye cancer prevention. They are sources of vitamin K, vitamin C, Magnesium, Potassium, Manganese and Vitamin A (Szalay, 2017). Pumpkin seeds can also help in combating symptoms of overactive bladder and can modulate several cardiovascular risk factors.

The seed oil in the pumpkin has antioxidants that can ease inflammation (Gamonski, 2012).

2.1.2 Morphology and ecological adaptations of pumpkin

Pumpkins are hairy climbers with simple or branched lateral tendrils, yellow or whitish unisexual flowers, inferior ovary with parietal placentation and large seeds. Cucurbits are most diverse in tropical and sub-tropical regions (Mathew et al., 2013). They have strong, round stems and the fruit stalk is angular and flares out on all sides, where it joins the fruit. They have a hard rind. Pumpkins are vigorous, prostrate, annual vines with an extensive root system. They are able to put down peg roots to support the plant and their tendrils twine around other plants to prevent them from being blown out by strong winds. They are monoecious, that is, they have separate male and female flowers on the same plant which enhances cross pollination by especially bees (Napier, 2009).

Pumpkins can be cultivated at an altitude of 500m above sea level and in dry and intermediate ecological zones. The soil should be well-drained with a high amount of organic matter having an optimum pH range of about 5.5 - 7.5. Regular watering is important up to seed germination and weeding by hand should be done at three weeks after germination (Encyclopaedia Britannica, 2007). Pumpkins can tolerate harsh environmental conditions, can resist several pests and can yield relatively good returns with minimal fertilizer inputs (Kwiri et al., 2014).

2.1.3 Pumpkin growth and development

Interactions between genotype (cultivar traits), environment (climate and soil conditions) and management influence the crop growth and yield. Climate is the main factor that controls agricultural production. Therefore, crop growth and yield are affected by climatic variations, such as temperature and precipitation. Usually, an increase in temperature causes an increase in the developmental rate. The developmental rate then slows down with extremely high temperatures (Bannayan et al., 2017).

Pumpkins produce both male and female flowers on the same plant. The environmental and management factors usually affect the ratio of male to female flowers. Temperature, light, humidity and soil moisture are major determinants of female and male flowers. The first flowers on the vine are male after which three to four female flowers appear. The environmental condition of high temperatures, low humidity and long-day length encourages the development of male flowers. Female flowers develop at low temperature, high humidity and short day length (Napier, 2009). Pumpkins weight range from less than 0.5 kg to more than 50 kg and traditional varieties weigh between 2.7 kg and 8.2 kg. Moreover, pumpkin production is less labour intensive and more profitable compared to yam and many other staple (Oloyede *et al*., 2013).

2.1.4 Pumpkin fruit and seed quality

Fruit quality is determined by several characteristics, such as, colour, nutrient content, sweetness, flavour and texture. Colour and nutritional value are controlled by the carotenoid content, with a deep yellow or orange coloured fruit being desirable (Wyatt et al., 2015).

The seed quality aspects include; seed vigour, seed health and physiological quality. After seed physiological maturity stage, a series of physiological and biochemical alterations start. This results in gradual reduction of germination percentage and causes the death of seeds (Silva et al., 2017).

Pumpkin seeds can be dried to very low moisture content of about 5.3 ± 0.3% then stored for very long periods without spoiling. This is because they become unsusceptible to microorganism attack. Pumpkin seeds tend to have higher seed oil content compared to other oil seeds. They have quality edible seed oil and an ash content which is important for poultry and cattle feeding material (Ardabili et al., 2011).

2.1.5 Physiological Maturity on Seed Quality

Physiological maturity is that stage in which seeds reach their maximum dry matter accumulation, germination and vigour values. Identifying the physiological maturity stage is important so as to obtain seeds with high physiological and sanitary potential. Practical recognition of seed physiological maturity for cucurbitaceous species has been performed based on number of days after anthesis. The number of days after anthesis is the sure way of identifying the stage of maximum seed quality of cucurbits. Seeds of pumpkin present a high moisture content level during and after they reach physiological maturity, therefore, slow drying allows the development of tolerance mechanisms to water removal, thus better quality seeds. Quick drying of seeds with high moisture content may cause oxidative processes, hence seed quality deterioration (Silva et al., 2017).

Seeds that are harvested early have been found to be immature and poorly developed, therefore, are poorly stored compared to seeds harvested at physiological maturity (Singh and Lachanna, 2005). At physiological maturity, seeds had maximum viability and vigour. Attainment of physiological maturity is a genotypic character which is influenced by environmental factors, therefore, harvesting of seed at optimum stage of seed maturation is essential to obtain better seed quality.

Any delay in harvest from the point at which the seed becomes independent of the mother plant can cause seed contamination by pathogens leading to a decline in germination percentage from 100% to 50% (Nerson, 2007). On the other hand, drying to very low water content of (1 - 2%) is harmful to seeds as it reduces their storability.

Singh and Lachanna, (2005) asserted that one of the problems that cause a decrease in seed performance after physiological maturity is variability in dry and humid periods with high temperatures during the final phase of seed maturation. This predisposes the seed to an increase in deterioration rates and the occurrence of seed coat injury as a consequence of cotyledonary expansion and contraction after a series of moistening and drying cycles.

Higher vigour seeds were obtained from fruits harvested at 60 or 70 DAA, and storage of such fruits before seed extraction could not improve their performance. Fruits harvested at 50 DAA and stored for at least 15 days improved seed quality to the vigour level similar to those harvested at 60 or 70 DAA (Vidigal *et al*., 2009).

High quality seeds perform well in the field, ensuring optimum stand under a wide range of environmental conditions (Dayal, 2014). The maturity stages have been found to induce changes in moisture percentage, physical and chemical properties of the seed during their growth and development. The rate of decline in vigour increases with increase in relative humidity, temperature and seed moisture after the seed attains physiological maturity. Consequently, physiological maturity and harvest time are distinct events. Seed maturation is a process characterized as an organized sequence of several physiological events culminating at physiological maturity, while harvest time is a decision based on technological and economic parameters associated with seed yield or quality (Wang and Sun 2007). Physiological maturity can be determined on an individual seed or plant community basis. Individual seeds in the plant community usually reach physiological maturity at different times making definitions of physiological maturity on a community basis less precise. The determination of physiological maturity usually requires differing measurements of seed dry weight during the seed filling period which is often affected by sampling variation. This makes it difficult to determine the precise time of physiological maturity, that is, to identify the moment when the transfer of plant reserves to the seed ceases (Egli, 2008).

2.2 Harvesting of pumpkins

Pumpkin farmers are often faced with variations in fruit physiological maturity due to cross pollination by insects. Pumpkins are ready for harvest when the stalk becomes cracked and corky. The rind should also be hard for thumb or finger nail entry. The rind colour should also turn from being glossy to being dull. The pumpkins do not store well, if harvested immaturely (Napier, 2009).

2.2.1 Post-harvest Management of pumpkin

Soil adhering on the ground spot area of the fruit is removed with a soft cloth or washed using sodium hypochlorite; where 3 litres of sodium hypochlorite is mixed with 100 litres of water. This is effective against decay organisms (Technical Bulletin, 2004). Grading for uniformity of appearance is very important to meet market requirements. There are three established size categories (small, medium and large) depending on fruit weight.

Cucurbits are fleshy and berry-like in structure and have a relatively thick rind. They can weigh between 9 kg to 227 kg. Fruit shape varies from round to oval to slightly flat; and the rind colour varies from green to blue-green to tan. The fruit should be free from any noticeable skin blemishes; whereby the rind should not be discoloured or have any surface mould growth (Yudin and Schlub, 2014).

The optimum temperature for pumpkin storage is 12^0 C. Sound fruit can be stored under this temperature for up to 14 days without significant loss in quality. Fruits should never be stored below 5 ^{0} C because they are susceptible to chilling injury (Brew et al., 2006).

The optimum relative humidity for storage and transport of pumpkins is between 85% and 90%. This R.H range minimizes post harvest weight loss and avoids the growth of surface moulds. Pumpkins stored at R.H above 90% will result in increased decay. Storage at R.H below 80% will result in drying out of the flesh and adverse textural changes. Cucurbits have an extended storage life if stored at temperatures of about 10 ^{0}C (Ridgen, 2016).

Cucurbit seed development goes on even after the fruit is removed from the vine. Germination rates increase if the harvested fruits are stored for one week before extracting the seeds. Fermentation is then done to remove the persistent placental material encasing pumpkin seeds. Water is mixed with seeds and left for 1 to 2 days at a temperature of 20-35 ^{0}C. Fermentation is complete when the seeds settle to the bottom of the container and the placental material floats (ECPGR, 2011).

2.2.2 Appropriate Post-Harvest Handling to Improve Seed Quality

Postharvest handling is the stage of crop production immediately following harvest, including cooling, cleaning, sorting and packing. The instant a crop is removed from the ground, or separated from its parent plant, it begins to deteriorate. Post-harvest handling comprises the various technologies and practices undergone by the farmer, farmers' groups or cooperatives and/or agribusiness companies, from the field to the plate, to handle pumpkin production immediately following harvest, up to its final destination, such as storing, transport, cleaning, sorting, processing and packing. The instant the pumpkins are harvested, it begins to deteriorate. Postharvest handling aims at slowing this decaying process for the best quality for the consumer, either as fresh or dry produce, or as ingredients in a processed food product. The most important goals of post-harvest handling are keeping the product cool, to avoid moisture loss and slow down undesirable chemical changes and avoiding physical damage such as bruising or cutting, to delay spoilage (Basu, 2005).

Sanitation is also an important factor, to reduce the possibility of pathogens that could be carried by fresh produce, for example, as residue from contaminated washing water. After the field, post-harvest processing is usually continued in a packing house. This can be a simple shed, providing shade and running water, or a large-scale, sophisticated, mechanized facility, with conveyor belts, automated sorting and packing stations (Kitinoja and Al Hassan, 2012). In mechanized harvesting, processing may also begin as part of the actual harvest process, with initial cleaning and sorting performed by the harvesting machinery.

Initial post-harvest storage conditions are critical to maintaining quality. Each crop has an optimum range for storage temperature and humidity. Also, certain crops cannot be effectively stored together, as unwanted chemical interactions can result. Various methods of high-speed cooling, and sophisticated refrigerated and atmosphere controlled environments, are employed to prolong freshness, particularly in large-scale operations. Regardless of the scale of harvest, from domestic garden to industrialized farm, the basic principles of post-harvest handling for most crops are the same: handled with care to avoid damage (cutting, crushing, and bruising), cooled immediately and maintained in cool conditions, and culled (Kader, 2005).

Increasing emphasis on higher value farm products to meet the changing diets of urban consumers has focused renewed attention on post-harvest systems, while unacceptably high losses due to poor handling and lack of appropriate infrastructure have reduced economic benefits to small producers. Post-harvest activities are an integral part of the pumpkin production system, and the aim is to promote best practices for post-harvest handling and management along the entire pumpkin supply chain, focusing on a broad spectrum of operations and stakeholders in traditional and modern marketing systems. The ultimate goal of the system is to deliver high quality, safe pumpkins to consumers (Ganpat, 2013).

Obtaining the optimum postharvest quality of pumpkin actually begins very early in the farm planning process. The effects of pre-harvest factors on postharvest quality are often overlooked and underestimated. However, many of the decisions that we make during pumpkin production can greatly influence the postharvest quality of pumpkins. It is critical to remember that pumpkin quality is only maintained postharvest it is not improved during the harvest and storage processes. Thus, it is of utmost importance to consider the pre-harvest factors that allow us to maximize the quality of the pumpkin going into storage (Shewfelt and Prussia, 2003).

Quality cannot be improved after harvest, only maintained; therefore it is important to harvest pumpkin at the proper stage and size and at peak quality. Immature or over mature produce may not last as long in storage as that picked at proper maturity. Cooperative Extension Service publications are an excellent source of information on harvest maturity indicators for pumpkin. Harvest should be completed during the coolest time of the day, which is usually in the early morning, and produce should be kept shaded in the field. Produce should be handled gently (Oswell *et al.,* 2007). Crops destined for storage should be as free as possible from skin breaks, bruises, spots, rots, decay, and other deterioration. Bruises and other mechanical damage not only affect appearance, but provide entrance to decay organisms as well.

Historically, production of pumpkin has received the vast majority of attention in development efforts. Increasing yields, planting improved seed and growing new pumpkin are all important, but these improvements will be inefficient whenever food losses remain high during postharvest handling (Adebooye *et al.*, 2003). High levels of postharvest losses represent an enormous waste not only of food, but also of the land, water, fertilizers and human labor that went into producing the food. Handling practices can also affect postharvest quality. Produce that has been stressed by too much or too little water, high rates of nitrogen, or mechanical injury (scrapes, bruises, abrasions) is particularly susceptible to postharvest diseases. Poor post-harvest handling and inadequate on-farm storage facilities have been singled out as some of the major bottlenecks affecting the quality and quantity of pumpkin produced in the country.

2.3 Pumpkin seed storage

A delayed harvest of fruits reduces the physiological quality of seeds. Physiological quality of seeds harvested at 49 DAA, can be maintained during six months of storage. After seed physiological maturity, a series of physiological and biochemical alterations starts. This results in the gradual reduction of germination and cause death of seed. The incidence of fungal diseases is lower in slowly dried seeds, regardless of the storage time. Seed contamination can occur in the production field itself as well as the long exposure time of seeds to humidity during drying and storage. This can lead to the deterioration of physiological quality, germination loss, rotting and mycotoxins production. Cucumber seeds stored at room temperature (25 ^{0}C) had lower disease incidence compared to those stored at 35 ^{0}C and 45 ^{0}C (Silva *et al*., 2017).

The longevity of dry-stored pumpkin seeds depends on seed moisture contents, storage temperature and relative humidity, oxygen presser and to some extent on the integrity of the seed coat at the time of harvest and subsequent operations. Generally, storage temperature and relative humidity are the two most important factors governing seed longevity. Germination percentage and rate of germination is higher in seed lots stored for one or two years, compared with those stored for three or four years. A temperature level of 5 ^{0}C is low enough to slow down the biochemical and physiological processes, which can lead to seed deterioration. Improving the storage conditions can prolong seed longevity (Alhamdan, 2011).

Wilson (2005) asserted that seed moisture level is a decisive factor affecting seed quality. The proper seed moisture level for harvest is not that for safe seed storage. Thus, seed drying to a certain level is very crucial operation, should be performed very well in terms of drying rate and level, depending on the seed kind in concern.

Seed moisture level plays an important role in maintaining seed quality during storage period. Seeds are harvested as soon as possible following physiological maturity with a moisture level neither too high nor too low to avoid any damage to the seed coat.

Storage of pumpkin seeds at high moisture level is detrimental because it increases the activity of hydrolyzing enzymes and germination may occur in the store. At high moisture content, seeds are subjected to attack by fungi. High moisture is more detrimental, when oxygen is not readily available, as the products of anaerobic respiration such as: ethanol and acetaldehyde are toxic to the seed. At high moisture, the respiration rate of the seeds and the associated organisms increases, and then the temperature around the seeds increases too.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Field experiment.

3.1 Site selection and description.

The selection of the study site where the research was conducted constituted the first task of fieldwork preparation. This was done through the review of available reports and consultation with farmers and other stakeholders working within AIC Cheptebo farm (0° 26′ 34″ North and 35° 36′ 52″ East), Kaiboi Technical Training Institute farm (00° 19′ 00″ North and 35° 10′ 00″ East) and University of Eldoret farm (0° 31' 0'' North, 35° 17' 0'' East) respectively of North Rift Region of Kenya.

The experimental sowing design used in the field was Randomized Complete Block Design laid out in split plot, where the whole plot was pumpkin type and the sub-plot was the harvesting stages (30, 40, 50 and 60 DAA) and was blocked by the three sites (UOE, KTTI and AIC Cheptebo). Two local pumpkin seeds from round shaped fruit characterized by bulged seeds and oval-shaped fruit characterized by relatively flattened seeds obtained from the farmers were planted during the rainfall season (from May to August 2015).

Each plot from the three selected sites measured (8m by 12 m) replicated three times. The plots in the three ecological zones were cleared by slashing, followed by primary cultivation by hand digging using a *jembe*. Secondary tillage was then done to the medium tilth.

To obtain vigorous seedlings, then good yield, Diammonium phosphate fertilizer at a rate of 9.6 Kg/ha was used at planting. Two seeds were planted per hole at a spacing of 2 m by 2 m due to the spreading growth habit of pumpkin after which thinning was done two weeks after germination. Therefore, there were 24 plants per plot. The weeds were controlled by uprooting from three weeks after sowing then regularly done after every four weeks until harvesting.

The various ecological conditions from the three sites were also determined, including soil properties and climatic conditions (amount of rainfall, temperature). For soil analysis, 25ml of deionised water was added to10±0.1g of soil then the mixture was stirred for 10minutes and allowed to stand for 30minutes then stirred again for 2minutes. The pH of the soil suspension was then measured (Okalebo *et al*., 2002).

3.2 Experimental method and design

3.2.1 Experimental method

The pistillate flowers that were tagged at anthesis were 150. The fruits which developed from them were manually harvested at 30, 40, 50 and 60 days after anthesis (DAA).

The second part of the experiment was then carried out at the University of Eldoret in the seed science laboratory whereby the pumpkin seeds from the harvested fruits of each age (30, 40, 50 and 60 DAA) were manually extracted then the seeds having pulp were mixed with 250 ml of water and put in sealed plastic tins for a period of 2 and 4 days at room temperature so as to remove the seed pulp around the seed. Those seeds that were not fermented were a control treatment. After fermentation, the seeds were thoroughly washed with tap water (pH 6.8) and then sundried at ambient air (22-32°C) for a period of one week until constant weight. Seed moisture content was then determined by the high constant temperature oven (130°C, 1 hour) method according to ISTA methods (ISTA, 1999). The extracted seeds were then stored where they were produced under room temperature conditions, that is, in Kaiboi (22 ^{0}C), UOE (27.7 ^{0}C) and Cheptebo (38 ^{0}C) at a storage duration of 90 days after harvest.

3.2.2 Experimental design

The experimental sowing design used in the field was Randomized Complete Block Design laid out in split plot, where the whole plot was pumpkin type and the sub-plot was the harvesting stages (30, 40, 50 and 60 DAA) and was blocked by the three sites (UOE, Kaiboi and Cheptebo).

The seed testing was done at the laboratory using Completely Randomized Design.

The experiment was carried out in the seed science laboratory, using a substratum made of sand. A sub-sample of 100 well-mixed pure seeds from each treatment were planted in seed germination boxes at 25 seeds per replicate. The germination boxes were filled with moist sand and levelled using a scraper. The seedbed was then loosened using a rake, then a counting board containing the seeds placed above the seedbed and the lower plate removed to release the seeds uniformly. After which the planted seeds were carefully covered with loose moist sand.

3.2.3 Treatment arrangement

The experimental treatments of seeds being performed were: four fruit ages (30, $\mathbf{H_1}$, 40, $\mathbf{H_2}$, 50, $\mathbf{H_3}$ and 60, $\mathbf{H_4}$, DAA); two pumpkin types (round fruited, $\mathbf{V_1}$ and oval fruited, $\mathbf{V_2}$) blocked by three sites (AIC Cheptebo, $\mathbf{S_1}$, KTTI, $\mathbf{S_2}$, UOE, $\mathbf{S_3}$). Then three storage conditions (KTTI, **r.t.p1**, UOE, **r.t.p2** and AIC Cheptebo, **r.t.p3**) by three fermentation durations (0, $\mathbf{Fd_1}$ 2, $\mathbf{Fd_2}$ and 4, $\mathbf{Fd_3}$ days) was performed.

3.3 Laboratory seed testing

The Pumpkin seeds were tested for physical and physiological seed quality so that they could be characterized and rated. Thus, the following tests were performed:

3.3.1 Determination of Thousand Seed Weight (TSW)

From the working sample of 1000 seeds, ten replicates, each of 100 seeds were counted at random by hand. Each replicate was then weighed in grams and the result expressed to one decimal place (ISTA, 1999).

3.3.2 Germination test

Four replicates of 25 seeds from each lot were placed in sand moistened with distilled water and whose particles could pass through a mesh having sieves of 0.8 mm width and retained on a sieve having meshes of 0.05 mm width. The sand was then sterilized to kill pathogens and its pH value ranged from 6.0-7.5. The sowed pumpkin seeds were then incubated at 25 °C. The first count was done at four days after sowing and the last count eight days after sowing (ISTA, 1999). The results were expressed as a percentage of normal seedlings and the average percentage calculated to the nearest whole number.

3.3.3 Electrical conductivity test

The electrical conductivity of the seed leachate was determined according to (ISTA, 1999) rules. Four sub-samples of 50 seeds of each cultivar were weighed and put into plastic cups with 250 ml of distilled water, and placed in the incubator at 25^0C. After 24 hours, the electrical conductivity of the seed leachates was determined using an EC meter. The EC meter was cleaned by dipping it into distilled water and then wiped dry using a tissue paper after every sub-sample measurement. The weight of the seeds was then divided by the reading of the EC meter and recorded as μsg^{-1}

3.3.4 Rate of seedling growth

The measurement of seedling height was done from the fourth day from sowing to the tenth day from sowing so as to show the rate of growth.

RSG= Day 4$(\frac{x1+x2+x3+x4+x5}{5})$, ……, Day 10 $(\frac{x1+x2+x3+x4+x5}{5})$

Where x is the plant height measured at random

3.4 Data collection and analysis

The pumpkin fruits were harvested then the seeds were extracted and the seed colour and a thousand seed weight were used to determine morphological maturity. Half of the extracted seeds were then fermented and stored at room temperature conditions of Kaiboi, Cheptebo and UOE. All seeds were tested for germination, viability and growth vigour. Growth vigour score was evaluated based on the length of the seedling in millimetres. The final count included strong normal seedlings, weak normal seedlings, abnormal seedlings, and dead seeds. Seed testing was done using Completely Randomized Design shown by the following statistical models:

Model 1: $Y_{ijkl}= \mu +V_i +H_j +VH_{ij} +S_k + VS_{ik} + R_l +\sum_{ijklm}$

Where; Y denotes the dependable variable (seed quality)

$\boldsymbol{u}$ is the general mean

V_i is the effect due to Variety i

H_j is the effect due to Harvesting stage j

VH_{ij} is the effect due to the interaction of i^{th} Variety and j^{th} Harvesting stage

S_k is the effect due to site k

SV_{ik} is the effect due to the interaction of i^{th} Variety and k^{th} site

R_l is the effect due to replication l

$\sum_{ijklmn}$ is the random error effect

Model 2: $Y_{ijklm}= \mu +V_i +S_j+ FD_k + SC_l + R_m + \sum_{ijklmn}$

Where; Y denotes the dependable variable (seed quality)

$\boldsymbol{u}$ is the general mean

V_i is the effect due to Variety i

S_j is the effect due to Site j

FD_i is the effect due to fermentation duration i

SC_j is the effect due to storage condition j

R_m is the effect due to replication m

$\sum_{ijklmn}$ is the random error effect

The data obtained for each parameter was subjected to ANOVA using General Linear Model procedure for (SAS, Version 9.1.3). The treatment means for each test was compared by protected Fischer's test Least Significant Difference (LSD) at $P \leq 0.05$ level of probability. Standard errors of means were presented in tables and figures throughout the study.

CHAPTER FOUR

RESULTS

4.1 Soil pH analysis

Soil pH for the three sites was done, whereby UOE had (4.96), Kaiboi (5.4) and Cheptebo (6.9).

4.2 Pumpkin leaf and fruit morphology

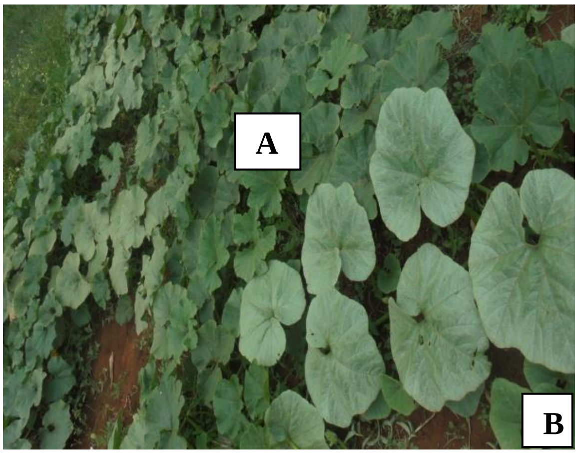

Figure 1: Leaf morphology of the two pumpkin types. (A) Oval type; (B) Round type. (Source: Author, 2015).

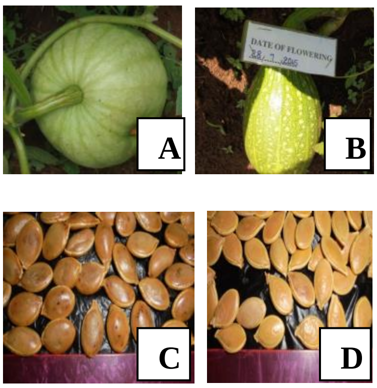

Figure 2 : Fruit morphology of the two types pumpkin; (A) Round Fruited and (B) Oval Fruited) (C) Seeds of the Round fruit and (D) Seeds of Oval fruit harvested at 60 DAA. (Source: Author, 2015).

4.3 Effect of harvesting stage on seed quality aspects

4.3.1 Effect of different harvesting stages on a thousand seed weight

The different harvesting stages of pumpkin fruits at different sites showed a significant difference ($p<0.05$). Harvesting at 60 DAA from Cheptebo resulted in the highest a thousand seed weight of 39.45 g and 29.03 g for round type and oval type respectively and was significantly different from 50 DAA, 40 DAA and 30 DAA but there was no significant difference between the harvesting stage of 40 DAA and 30 DAA. The two local types of pumpkin showed no significant difference (Table 1).

Table 1: **A thousand seed weight in grams (g) of pumpkins grown in UOE, Kaiboi and Cheptebo, Kenya, 2015.**

		HARVEST (DAA)			
SITE	TYPE	30	40	50	60
	ROUND	4.03±0.22d	6.30±0.47c	9.05±0.89b	25.95±1.02a
UOE	OVAL	2.45±0.44d	5.65±0.31c	8.35±0.52b	21.43±3.39a
	ROUND	6.55±1.80d	10.50±1.02c	15.80±0.37b	28.58±1.58a
KAIBOI	OVAL	4.15±0.66d	7.43±0.36c	10.85±0.48b	24.20±2.22a
	ROUND	7.83±0.52d	12.40±2.16c	27.00±0.62b	39.45±2.28a
CHEPTEBO	OVAL	5.93±0.28d	8.85±1.37c	22.80±2.55b	29.03±1.11a

Means with different letters within the row are significantly different at $P \leq 0.05$; ±SD n=4.

DAA; Days After Anthesis.

4.3.2 Effect of different harvesting stages on seed germination

The different harvesting stages of pumpkin fruits from different sites showed a significant difference at ($P \leq 0.05$) level of probability (Appendix 2). Harvesting at 60 DAA resulted in the highest germination percentage and was significantly different from 50 DAA, 40 DAA and 30 DAA but there was no significant difference from the harvesting stage of 40 DAA and 30 DAA. The three sites, Cheptebo, Kaiboi and UOE showed a significant difference at $P \leq 0.05$ level of probability, whereby, seeds from Cheptebo gave the highest germination percentage. The two types of pumpkin showed no significant difference (Figure 3) in germination percentage.

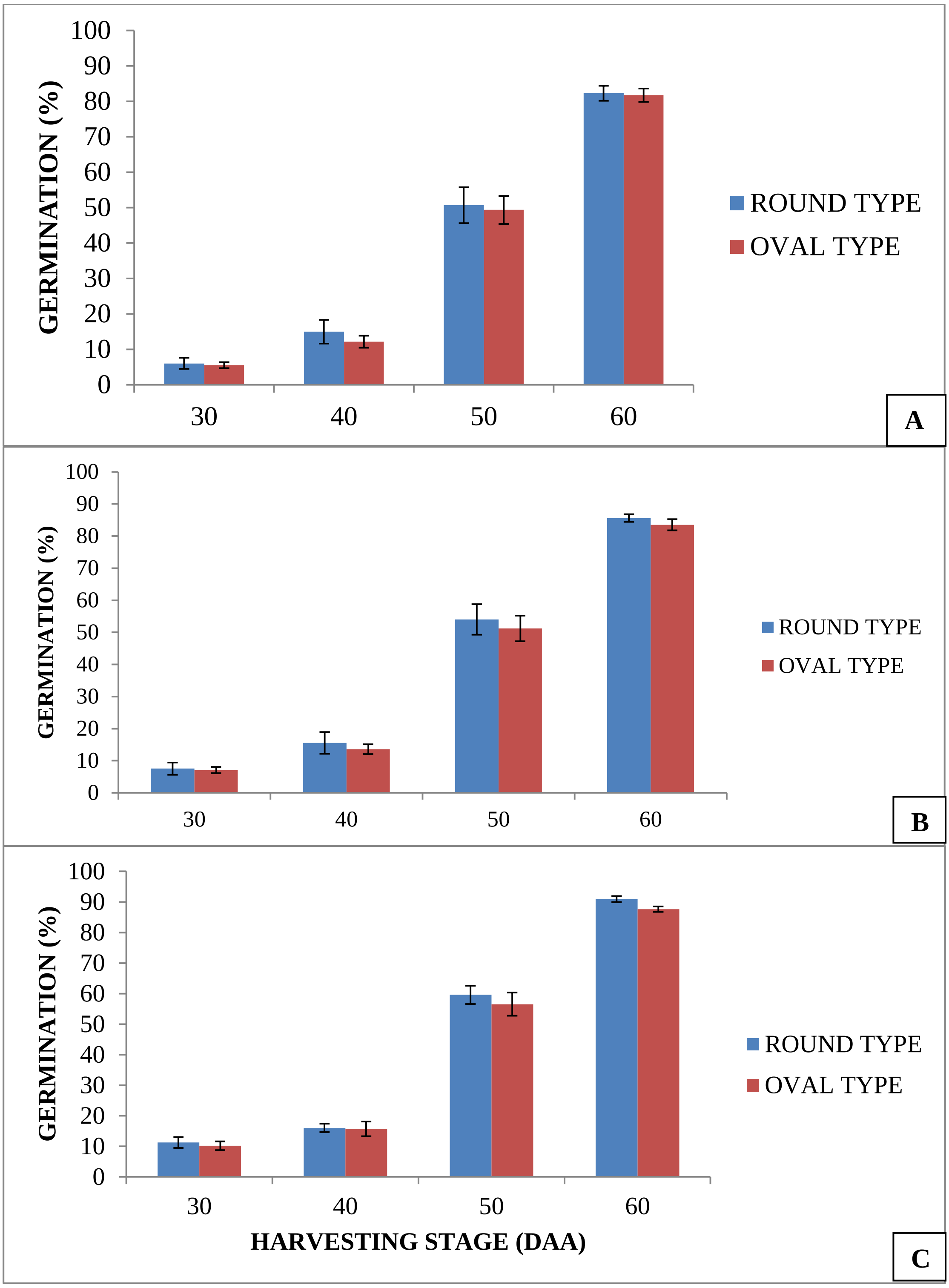

Figure 3: The germination percentage of pumpkin seeds obtained from fruits harvested from UOE (A), Kaiboi (B) and Cheptebo (C) respectively. Error bars represent Standard Error (SE) n=15.

4.3.3 Effect of different harvesting stages on pumpkin seed vigour (electrical conductivity).

The electrical conductivity at different harvesting stages of pumpkin fruits was significant at ($P \leq 0.05$) level of probability (Appendix 4). Harvesting at 60 DAA resulted in the lowest level of electrical conductivity which was significantly different from 50 DAA, 40 DAA and 30 DAA. The two local types of pumpkin showed no significant difference. Pumpkin fruits harvested from UOE, Kaiboi and Cheptebo showed significant difference on seed vigour at ($P \leq 0.05$) level of probability (Appendix 4). Pumpkin fruits from AIC Cheptebo gave seeds that had the highest seed vigour (low electrical conductivity) (Table 2).

Table 2: Electrical conductivity (μsg^{-1}) of seeds harvested from UOE, Kaiboi and Cheptebo, Kenya, 2015.

		HARVEST (DAA)			
SITE	TYPE	30	40	50	60
	ROUND	61.3±5.24a	59.3±4.7ab	48.0±2.31bc	46.3±2.33c
UOE	OVAL	65.3±3.29a	62.0±4.51a	51.3±0.88b	49.7±0.88b
	ROUND	58.7±4.91a	56.7±4.37ab	46.0±2.31bc	44.3±2.33c
KAIBOI	OVAL	62.3±2.9a	59.7±4.84a	49.0±0.58b	47.3±1.2b
	ROUND	56.3±5.24a	55.7±3.85a	43.7±2.03b	42.7±2.6b
CHEPTEBO	OVAL	60.7±2.96a	57.7±4.84a	46.0±0.58b	45.3±1.2b

Means with different letters within the row are significantly different at $P \leq 0.05$; ±SD n=4.

DAA; Days After Anthesis.

4.3.4 Effect of different harvesting stages on pumpkin seed vigour (Seedling height).

The seed vigour at different harvesting stages of pumpkin fruits was significant at ($P \leq 0.05$) level of probability (Appendix 3). Harvesting at 60 DAA resulted in the highest seedling height which was significantly different from 50 DAA, 40 DAA and 30 DAA. The two local types of pumpkin showed no significant difference. Pumpkin fruits harvested from UOE, Kaiboi and Cheptebo showed a significant difference on seed vigour at ($P \leq 0.05$) level of probability. The interactions between harvest*type and site*type was highly significant at ($P \leq 0.05$) level of probability (Appendix 3). Pumpkin fruits from Cheptebo gave seeds that had the highest seedling height (Figure 4, 5).

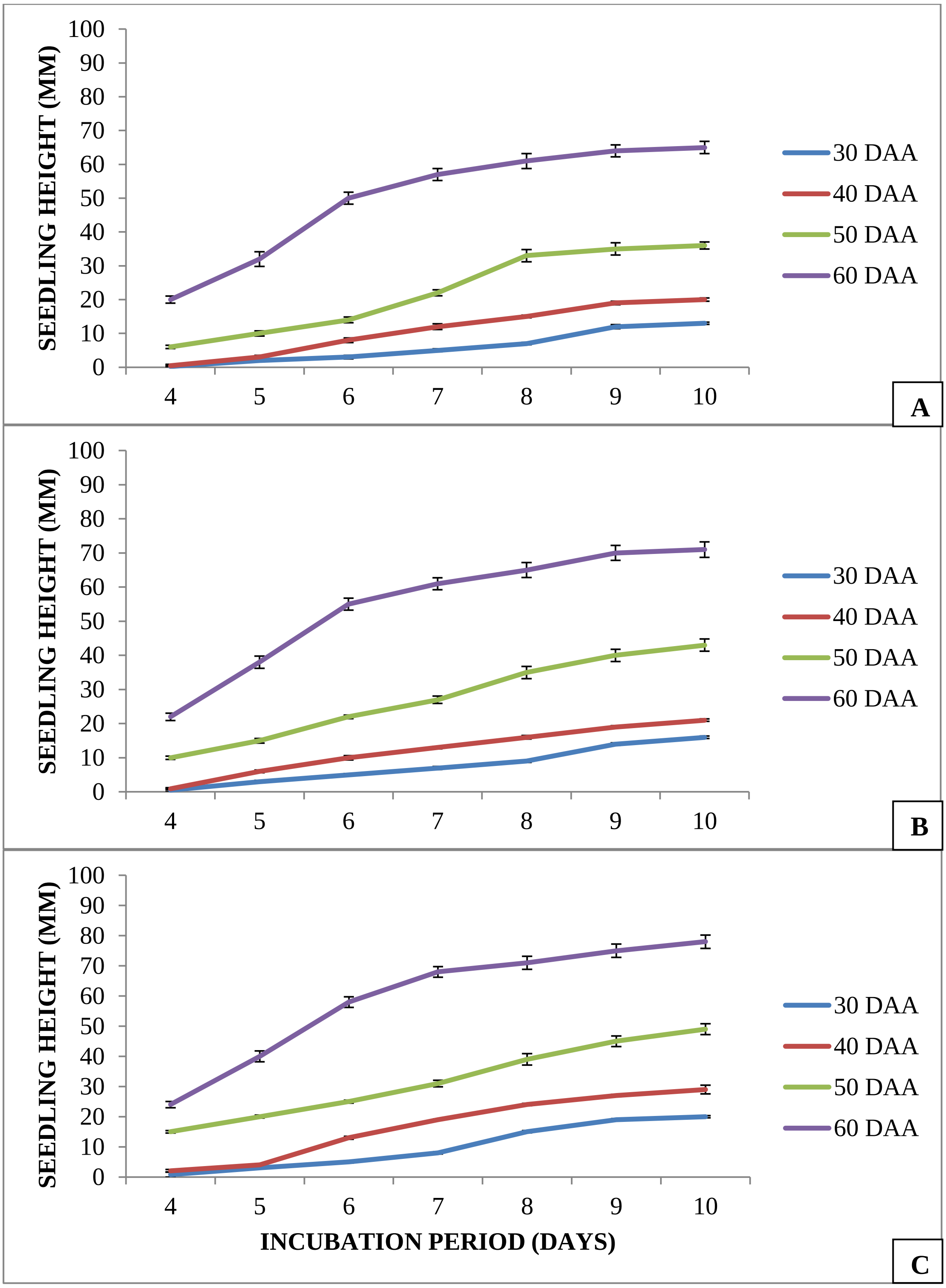

Figure 4: The seedling height of round type of pumpkin harvested at 30, 40, 50 and 60 DAA from UOE (A), Kaiboi (B) and Cheptebo (C). Error bars represent SD, n=5.

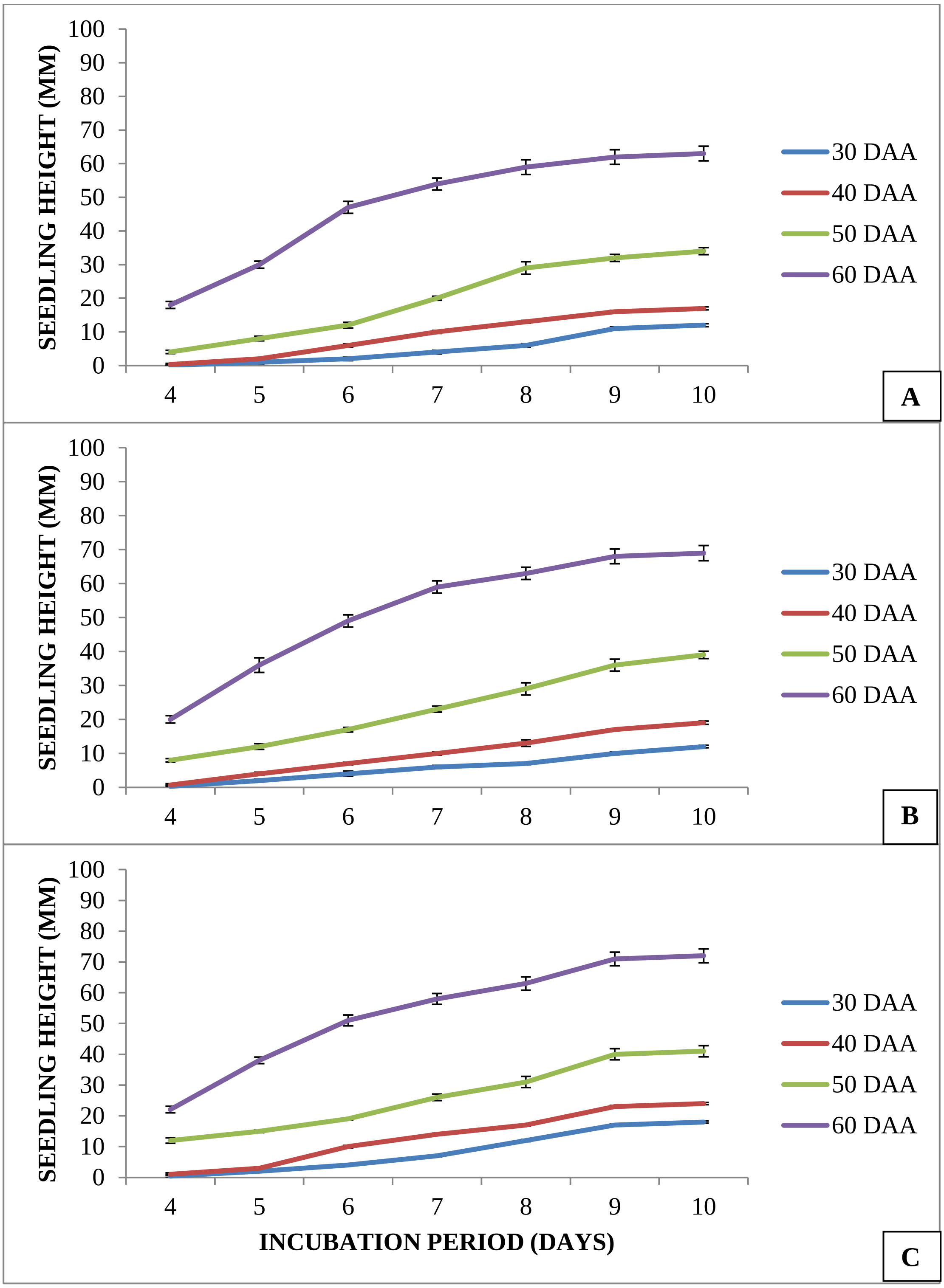

Figure 5: The seedling height of oval type of pumpkin harvested at 30, 40, 50 and 60 DAA from UOE (A), Kaiboi (B) and Cheptebo (C). Error bars represent SD, n=5.

4.4 Effect of seed fermentation duration on seed quality

4.4.1 Effect of seed fermentation duration on seed germination

Germination percentage of seeds fermented for zero, two and four days was significant at $P \leq 0.05$ level of significance. The seeds fermented for a longer period of time (four days), showed a higher germination percentage of 90.2% for the round type and 87% for the oval type, compared to those seeds that were fermented for a period of 2 days and those not fermented (control) which showed a low germination percentage of 39.7% for the round type and 32.7% for the oval type. The seeds extracted from fruits harvested at UOE, Kaiboi and Cheptebo showed a significant difference in germination percentage at $P \leq 0.05$ level of significance (Appendix 6), with seeds from Cheptebo showing the highest seed germination percentage compared to those harvested from UOE and Kaiboi. There was no significant difference between the two pumpkin types (Figure 6).

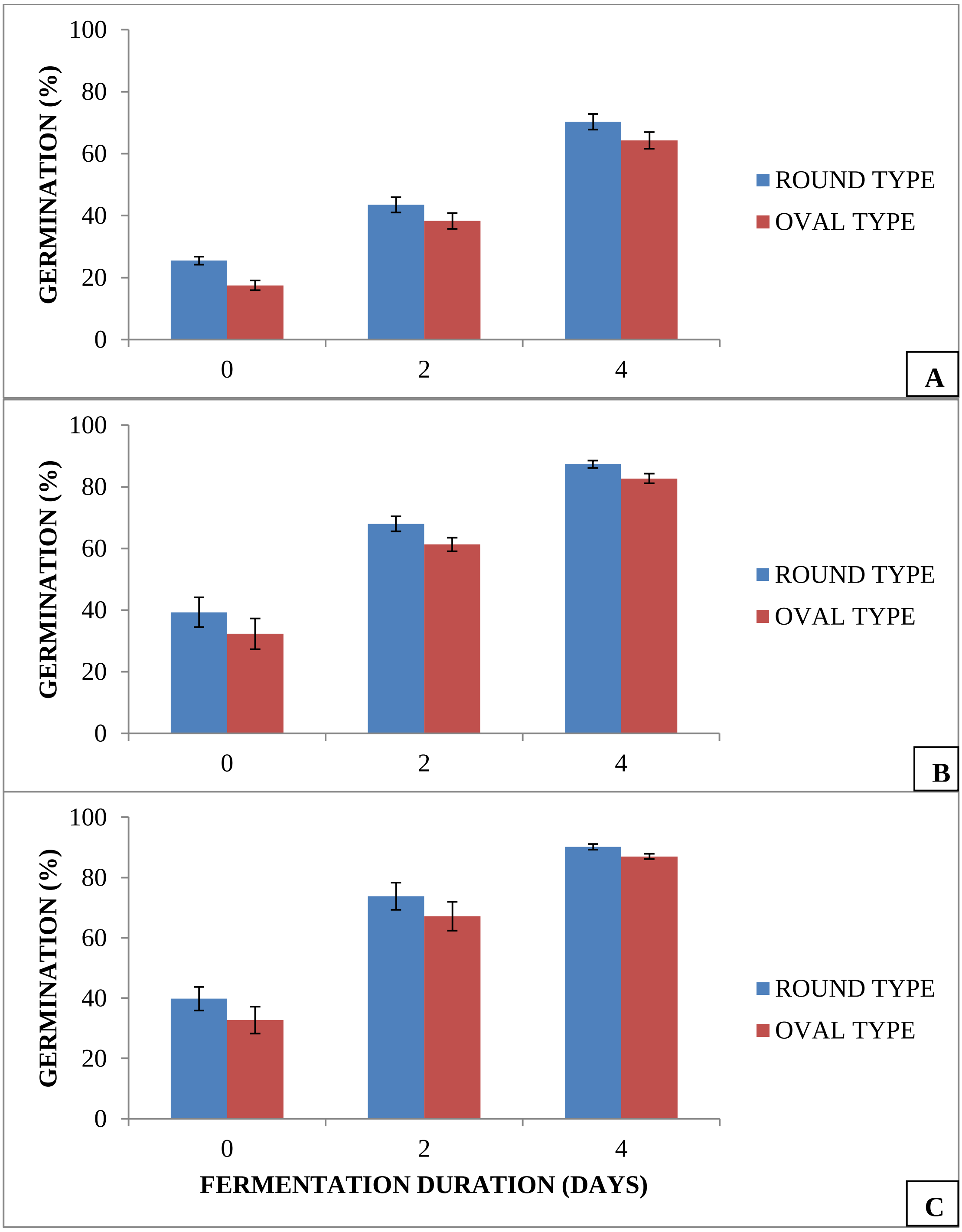

Figure 6: **Germination percentage of seeds harvested at 60 DAA and fermented for 0, 2 and 4 days. The seeds were extracted from fruits harvested at UOE (A), Kaiboi (B) and Cheptebo (C) respectively. Error bars represent Standard Error (SD), n=12.**

4.4.2 Effect of fermentation duration pumpkin seed vigour

The seedling height for seeds fermented for two and four days was significant at $P<0.05$ level of significance. The seeds that were fermented for a longer period (4 days) showed a higher seedling height which was significantly different from seeds not fermented and those fermented for a period of two days. The seeds extracted from fruits harvested at UOE, Kaiboi and Cheptebo showed a significant difference at $P<0.05$ level of significance (Appendix 7), with seeds from Cheptebo showing the highest seedling height compared to those harvested from UOE and Kaiboi. The round and oval types of pumpkin showed no significant difference (Figure 7, 8).

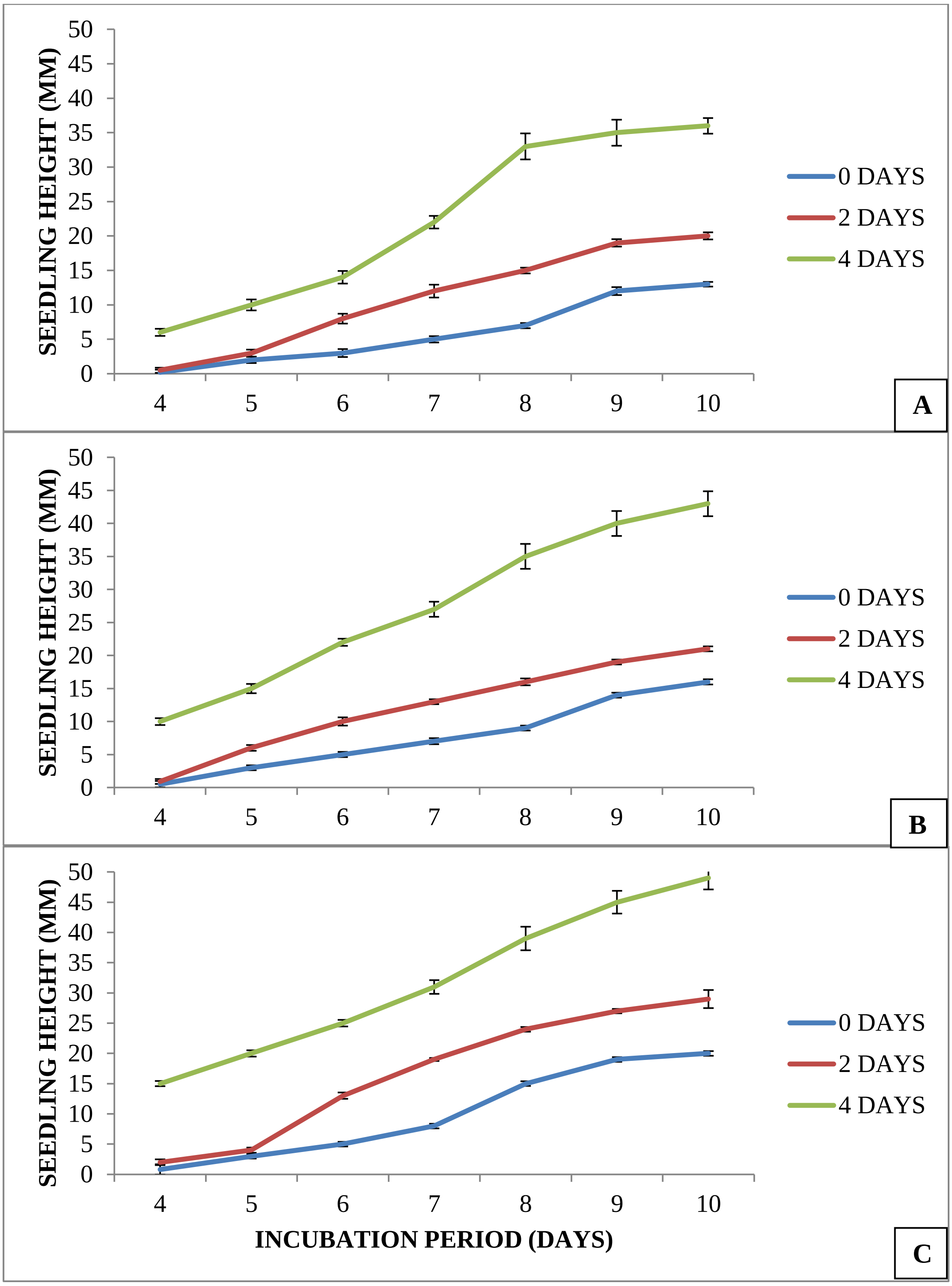

Figure 7: The seedling height of round type of pumpkin harvested at 60 DAA from UOE (A), Kaiboi (B) and Cheptebo (C) then fermented for 0, 2 and 4 days. Error bars represent Standard Deviation (SD), n=5.

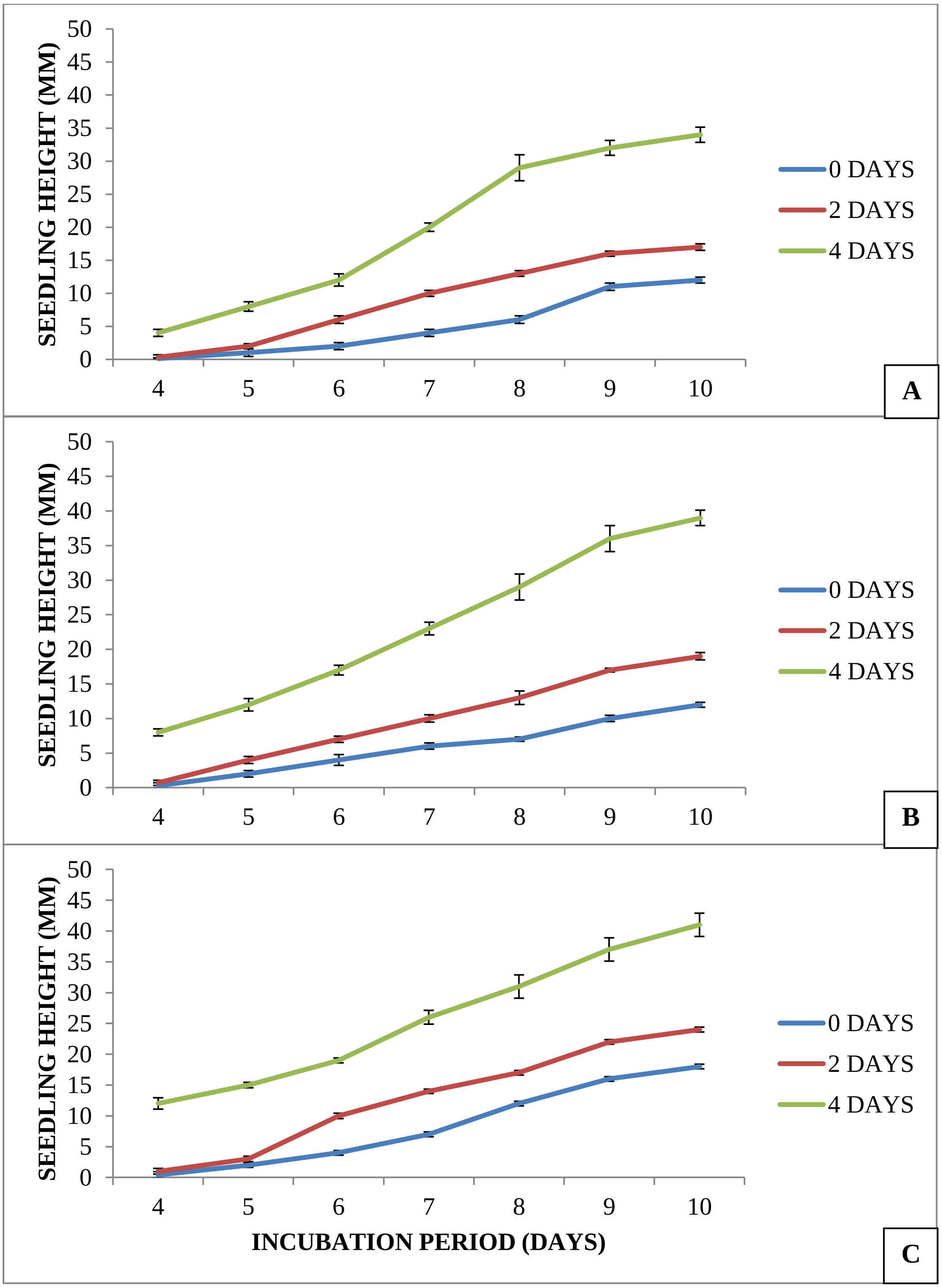

Figure 8: The seedling height of oval type of pumpkin harvested at 60 DAA from UOE (A), Kaiboi (B) and Cheptebo (C) then fermented for 0, 2 and 4 days. Error bars represent Standard Deviation (SD), n=5.

4.5 Effect of storage temperature on seed quality

4.5.1 Effect of storage condition on seed germination percentage

Pumpkin seed germination percentage for seeds stored at UOE, Kaiboi and Cheptebo was significant at ($P \leq 0.05$) level of probability. The seeds stored in Kaiboi showed a higher germination percentage of 82.6% for the round–fruited type and 77.6% for the oval-fruited type compared to the storage conditions at Cheptebo which resulted in the least germination percentage of 53.3% for the round fruited type and 50.1% for the oval-fruited type. The seeds stored in UOE showed a germination percentage of 67.9% for the round type and 62.3% for the oval type. The two types of pumpkin showed no significant difference at $P \leq 0.05$ level of significance (Appendix 8).

4.5.2 Effect of storage condition on seedling height.

The seedling height at different storage conditions was significant at $P<0.05$ level of probability (Appendix 9). Storage of seeds at Kaiboi (22^0C) showed the highest seedling height which was significantly different from storage in UOE (27.7^0C) and Cheptebo (38^0C) where they were produced. The two local types of pumpkin showed a significant difference in seedling height with the interaction between storage condition and pumpkin type being highly significant at $P \leq 0.05$ level of significance (Figure 9).

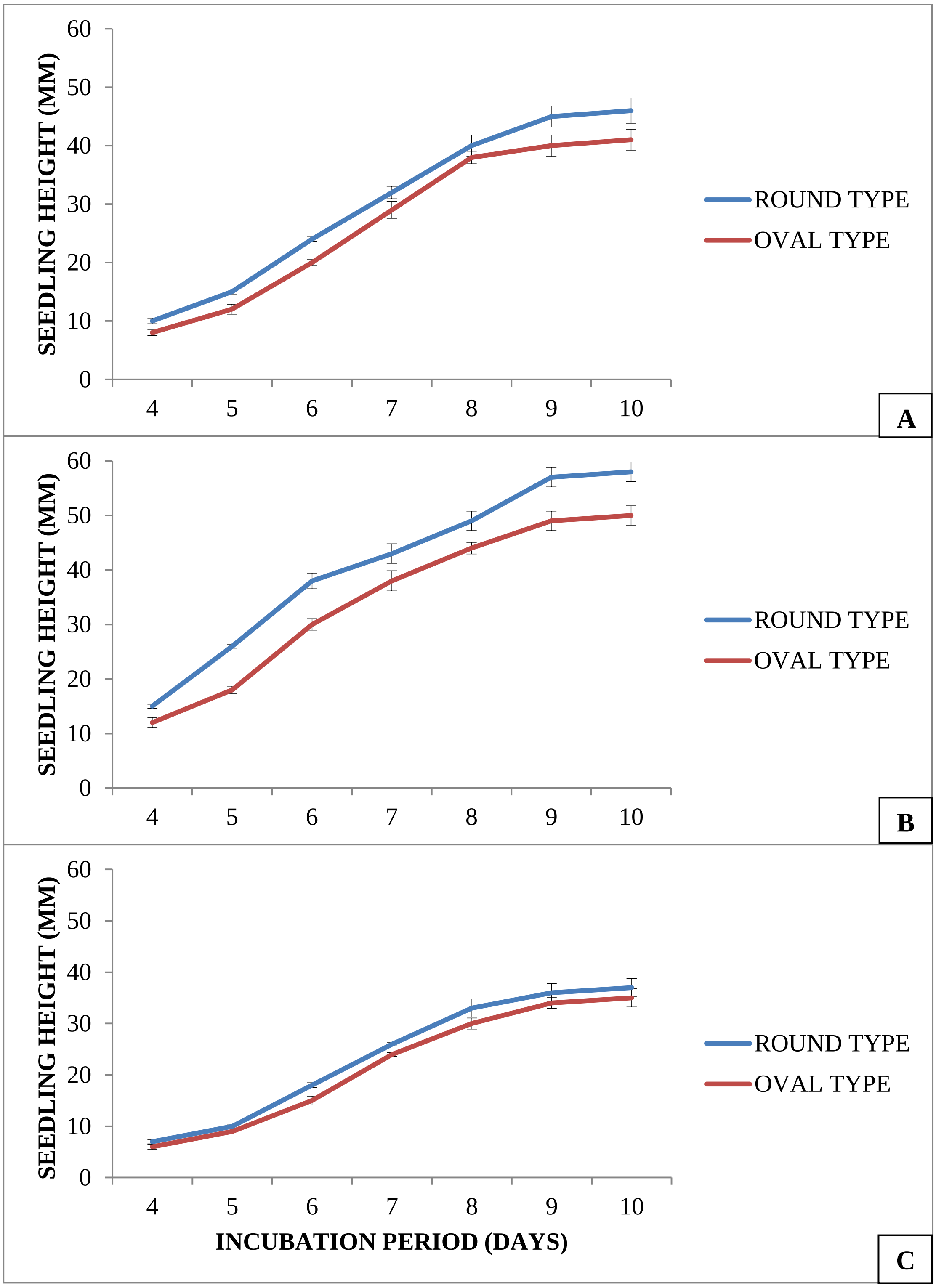

Figure 9: The seedling height of pumpkins harvested at 60 DAA and stored at UOE (A), Kaiboi (B) and Cheptebo (C) respectively for a period of three months. Error bars represent the standard error of means.

CHAPTER FIVE

DISCUSSION

5.1 Physiological maturity effect on seed quality

Germination percentage, Thousand Seed Weight and vigour results from Figure 3, Table 1 and Table 2 respectively, show that seeds harvested at 60 DAA gave the highest germination percentage, TSW and vigour compared to those harvested at 30, 40 and 50 DAA. Those seeds harvested at 30 DAA gave the lowest germination, TSW and vigour. These results are in conformity with those of Marcos 2005 who found out that early stages of seed development entails a slow dry mass accumulation, in which there is a lot of cell division and expansion responsible for setting up the appropriate structure for receiving substances that are transferred from the mother plant. The next phase is then characterized by dry mass accumulation which increases rapidly until it reaches maximum.

According to the results in Table 1 and 2, seeds harvested at 60 DAA showed the highest TSW of 39.5 g for the round type and 29 g for the oval type and the lowest seed electrical conductivity of 42.7 and 45.3 μsg^{-1} for the round and oval type respectively. These results agree with those of Acacio *et al*. (2015), who found out that at physiological maturity, the seed reaches the maximum dry weight and has the highest seed vigour because there is proper structuring of the seed cell membranes, thus a slow release of leachates.

On the other hand, the water content is one of the indicators of physiological maturity, but it is not a proper indicator due to the genetic and environmental conditions in the field (Franquera, 2015).

5.2 Effect of fruit age on seed quality aspects

There was a significant increase in the dry weight of pumpkin seeds from 30 to 60 DAA. The dry mass at 30 DAA was 4 and 2.5 g for the round and oval type respectively while at 60 DAA, the seeds had a higher dry matter accumulation of 39.5 and 29 g for the round and oval type respectively (Table 1). These results agree that maximum dry weight occurs when the seed reaches physiological maturity and that the seed dry mass is considered as one of the surest measures of seed maturity (Costa *et al*., 2006). This increase in seed weight also caused an increase in seed germination percentage due to the increase in seed embryo reserves from seeds extracted from fruits harvested at 60 DAA compared to those extracted from fruits harvested at 30 DAA.

The seed electrical conductivity was high at 30 DAA which was 61.3 and 65.3 μsg^{-1} for the round and oval type respectively, compared to the seed electrical conductivity at 60 DAA which was 42.7 and 45.3 μsg^{-1} for the round and oval type respectively. These results indicate that initially, seeds had lower physiological quality, thus releasing high amounts of leachate due to the poor structure and selectivity of the membranes hence a lower rate of seed vigour. Later there was a lower release of solutes as a result of proper cell membrane structuring as the physiological maturity approached and this led to a higher rate of seedling growth, thus, high seed vigour (Acacio *et al*., 2015).

5.3 Effect of the environmental factors on seed quality aspects

The effect of the site of production was significant. Seeds from Cheptebo gave the highest germination percentage, TSW and Vigour of 92%, 39.5g and 45μsg^{-1} respectively compared to those seeds from Kaiboi and UOE.

According to Maity and Chakrabarty (2013), the reduction in seed germination and vigour varies due to the different growing seasons because of the changes in temperature and relative humidity conditions during vegetative, reproduction and harvesting periods. Seed quality then is modified by the environment during seed development and maturation. Adverse weather conditions have an effect on the seed quality aspects (Demir *et al*., 2008). In mustard seeds for example, there is accumulation of soluble sugars through starch syntheses activity and Late Embryogenesis Abundance (LEA) proteins when approaching maturity. The solutes, enzymes and proteins contribute to grain development, followed by storage longevity and desiccation tolerance, especially in cereals (Farooq *et al*., 2014).

Crops grown under open-field conditions are usually exposed to abiotic and biotic stress factors which deter productivity and quality. The growth, development and post-harvest quality of any crop depends on the interaction between plant genetics and the environmental conditions in which they are grown. Environment is the overall of all the external conditions that influence the growth and development of plants (Olasoji *et al*., 2011). The crops have to be adapted to the region in which they are produced so as to be profitable. The environmental factors, such as temperature, light intensity and relative humidity influence crop growth and development. Temperature is the main regulator of crop development processes. Higher temperatures have more influence on the net photosynthesis than lower temperatures. Relative humidity reduces evaporation loss from plants hence maximum nutrient utilization. It also causes the maintenance of cell turgidity which helps in enzyme activity, leading to a higher yield. Cucumber and cluster bean had higher yields in open-field than in the shade net because these crops required more light-intensity and high temperature for better growth and development (Rajasekar *et al*., 2013).

5.4 Effect of storage condition on seed quality aspects

Pumpkin seed germination percentage and seedling growth rate was significant at P≤0.05, with seeds stored in Kaiboi showing the highest germination percentage of 82.6% and 77.6% for the round and oval types respectively, compared to the seeds stored in UOE and Cheptebo. These results are similar to those of Sharma (2011) who found out that the reduction on seed germination can be caused by stressful storage condition of high temperature. Amount of seed solute leakage increased with increase in temperature. Maximum seed leakage is responsible for the weak integrity of the seed coat membrane thus, the vigour level is decreased. Low temperature slows down the biochemical activity which reduces the loss of potassium leachate ion, caused by seed coat degradation.

Seed deterioration usually occurs during storage, which causes reduced germination percentage and seed vigour. Storage temperature and moisture content are important factors that affect seed viability during storage. Reducing the storage temperature and moisture content of seed causes an increased period of seed viability, while prolonged storage period reduces seed viability and seed vigour. Storage under unfavourable conditions decreases the seed vigour of seeds. Seeds must then be stored properly so as to maintain the standardized levels of seed vigour and germination. The storage period can vary from 6 months if planting is to be done the following season or even for more months. During storage, seed quality can remain at the initial level for a short period and then start declining to unacceptable levels of seed quality (Naguib *et al*., 2011).

Seed deterioration rate depends greatly on the storage condition, that is, temperature, relative humidity and seed moisture content. High temperature, relative humidity and moisture in the storage environment are principal factors involved in seed quality deterioration (Khalequzzaman *et al*., 2012). High temperatures increase the reduction rates by affecting enzymes that are involved in reactive oxygen, scavenging and repair. Low relative humidity keeps seed moisture content low, thus slowing seed deterioration. Lower temperature stabilizes the seed membranes, hence low loss of electrolytes from the seed, because membrane integrity influences leachate loss. Lower temperature also slows the rate of loss in seed viability. At higher temperatures, there is decrease in seed germination percentage over time (Mbofung *et al*., 2013).

Storage temperature of up to 5^0C is low enough to slow down the biochemical and physiological processes which causes seed deterioration. The rate of seed deterioration is slow if the seeds are stored at a lower relative humidity and temperature. More moisture allows them to respire, thus shortening the storage life due to spoilage through extraneous water and metabolic waste produced in respiration. Seed vigour decreased and electrolyte leakage increased from 6 months of storage according to Alhamdan, 2011.

5.5. Effect of fermentation duration on seed quality aspects

The germination percentage and seedling growth rate for seeds fermented at different durations was significant at $P \leq 0.05$, with the seeds fermented for a period of four days giving the highest germination percentage of 90%, compared to those seeds fermented for two days, or not fermented. This is in concurrent with Yao *et al*. (2012) who found out that fermentation improves seed germination and vigour since all the fermented seeds showed better germination and vigour, compared to the unfermented seeds. Meredith (2014) found out that the fermentation process breaks down the gelatinous material that encases the seed. These jelly sacks contain a germination inhibitor which needs to be removed so as to boost seed germination percentage. Fermentation is also helpful in eliminating seed-borne diseases.

The rate of fermentation depends on the concentration of microorganisms, cells, cellular components and enzymes, pH and temperature for anaerobic respiration. Pumpkin seeds are usually harvested when wet and they need to be cleaned by washing the seeds so as to separate them from pulp. The process of fermentation improves seed quality but depends on the condition it takes for it to be efficient (Nerson, 2002, 2007).

The best germination and vigour were obtained from seeds fermented under anaerobic, dark and relatively low temperature conditions of 26^0C (Woo and Song, 2010). Fermenting the seeds for too long caused them to germinate, hence no chances for storage because they die immediately when dried after germination.

5.6 Effect of seed weight on seed quality

Germination percentage and vigour were significant at $P \leq 0.05$ for heavier seeds which were harvested at 60 DAA, from Cheptebo, compared to those seeds harvested at an earlier stage of 30 DAA from UOE. These results agree with those of Rezapour *et al*. (2013), who realized that an increased seed size caused an increase in germination percentage, root and shoot length of naked oat. The lowest amount of seedling length was detected by small seeds. The germination percentage of large seeds is higher than that of medium and small seeds. Germination parameters are significantly related to seed weight and the large seeds germinate earlier than small seeds.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Fruits harvested at 60 DAA provided seeds with the highest germination percentage and vigour than those harvested at 30 DAA.

ii. Seeds harvested from Round shaped fruit and those harvested from Oval shaped fruit, and fermented for four days, showed higher germinability than those fermented for two days period. This shows that fermentation does not only facilitate seed extraction but also improves seed viability.

iii. Fruits harvested from Cheptebo (Elgeyo-Marakwet) site produced seeds with higher seed dry weight, hence higher germinability compared to seeds harvested from fruits taken from Kaiboi (Nandi) and UOE (Uasin-Gishu).

iv. Pumpkin seeds reach physiological maturity at the growth stage of 60 DAA.

v. Storage of pumpkin seeds at cooler room temperatures of 22^0 C and 27.7^0 C of Kaiboi and UOE respectively is better compared to Cheptebo at 38^0C.

6.2 Recommendations

i. Pumpkin fruits should be harvested at 60 DAA from Elgeyo-Marakwet (Cheptebo).

ii. Although fruits are harvested from 60 DAA, a period of 30 days of storage before germination is required to improve maturity in order to reach a maximum germination potential.

iii. The variations of seed viability and seed vigour were closely linked to seed colour, seed dry weight.

iv. Seed colour, fruit colour, rind texture, seed dry weight, seed germination percentage and vigour can be used as indicators for pumpkin (*Cucurbita pepo L.*) maturity.

v. Further selection and breeding to come up with certified seeds of pumpkin should be done.

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APPENDICES

Appendix I: Highest air temperature and precipitation during the planting season at Kaiboi, UOE and Cheptebo as of the year 2015.

SITE	TEMPERATURE (^{0}C) **(APRIL-AUGUST)**	RAINFALL (MM) **(APRIL-AUGUST)**
KAIBOI	18	600
UOE	23.7	280.8
CHEPTEBO	30	107.5

Appendix II: ANOVA table of Germination percentage of pumpkin seeds harvested at 30, 40, 50 and 60 DAA.

R-Square	Coeff Var	Root MSE	Gp Mean
0.910260	24.80677	10.01022	40.35278

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Type	1	255.0250	255.0250	2.55	0.1115
Harvest	3	351419.1417	117139.7139	1169.01	<.0001
Site	2	1990.2722	995.1361	9.93	<.0001
Type*Harvest	3	35.7639	11.9213	0.12	0.9489
Type*Site	2	6.8167	3.4083	0.03	0.9666

Appendix III: ANOVA table of seedling height of pumpkins harvested at 30, 40, 50 and 60 DAA.

R-Square	Coeff Var	Root MSE	Seedlingheight Mean
0.971862	14.50455	3.638155	25.08286

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Type	1	2020.5808	2020.5808	152.66	<.0001
Harvest	3	301619.0206	100539.6735	7595.83	<.0001
Site	2	4791.8715	2395.9357	181.01	<.0001
Days	6	66797.1478	11132.8580	841.09	<.0001
Harvest*Type	3	272.5404	90.8468	6.86	0.0001
Site*Type	2	289.1167	144.5584	10.92	<.0001

Appendix IV: ANOVA table of seed vigour (Electrical Conductivity) of pumpkin seeds harvested at 30, 40, 50 and 60 DAA.

R-Square	Coeff Var	Root MSE	EC Mean
0.686016	9.752917	5.182592	53.13889

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Harvest	3	3106.055556	1035.351852	38.55	<.0001
Site	2	234.777778	117.388889	4.37	0.0169
Type	1	174.222222	174.222222	6.49	0.0135
Harvest*Type	3	5.222222	1.740741	0.06	0.9783
Site*Type	2	0.777778	0.388889	0.01	0.9856

Appendix V: ANOVA table of a Thousand Seed Weight (TSW) of pumpkin seeds harvested at 30, 40, 50 and 60 DAA.

R-Square	Coeff Var	Root MSE	Seedweight Mean
0.932947	19.43766	2.790317	14.35521

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Site	2	1262.221458	631.110729	81.06	<.0001
Harvest	3	7425.310312	2475.103437	317.90	<.0001
Type	1	298.567604	298.567604	38.35	<.0001
Site*Type	2	40.206458	20.103229	2.58	0.0816
Harvest*Type	3	73.378646	24.459549	3.14	0.0295

Appendix VI: ANOVA table of Germination percentage of pumpkin seeds fermented for 0, 2 and 4 days.

R-Square	Coeff Var	Root MSE	Gp Mean
0.818412	19.03019	10.79259	56.71296

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Fd	2	87197.45370	43598.72685	374.30	<.0001
Type	1	1980.16667	1980.16667	17.00	<.0001
Site	2	20012.23148	10006.11574	85.90	<.0001
Type*Site	2	4.52778	2.26389	0.02	0.9808

Appendix VII: ANOVA table of seedling height of pumpkin seeds fermented for 0, 2 and 4 days.

R-Square	Coeff Var	Root MSE	Seedlnghgt Mean
0.805749	19.73579	1.372368	6.953704

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Fd	2	944.0645370	472.0322685	250.63	<.0001
Type	1	26.4600000	26.4600000	14.05	0.0002
Site	2	652.9156481	326.4578241	173.33	<.0001
Type*Site	2	1.5108333	0.7554167	0.40	0.6701

Appendix VIII: ANOVA table of Germination percentage of pumpkin seeds stored at UOE, Kaiboi and Cheptebo; at room temperature, for a period of three months.

R-Square	Coeff Var	Root MSE	Gp Mean
0.247016	31.96214	20.97959	65.63889

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Storage condition	2	29100.33333	14550.16667	33.06	<.0001
Type	1	1166.68519	1166.68519	2.65	0.1050
Storage condition*Type	2	54.70370	27.35185	0.06	0.9398

Appendix IX: ANOVA table of seedling height of pumpkin seeds stored at UOE, Kaiboi and Cheptebo; at room temperature, for a period of three months.

R-Square	Coeff Var	Root MSE	Seedlingheight Mean
0.974348	8.216461	2.415640	29.40000

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
Storage condition	2	8123.22857	4061.61429	696.04	<.0001
Type	1	840.00000	840.00000	143.95	<.0001
Days	6	34648.33333	5774.72222	989.62	<.0001
Storage condition*Type	2	273.68571	136.84286	23.45	<.0001