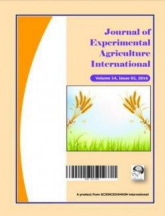

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# Influence of Harvesting Stage on Seed Quality Aspects of Pumpkin (*Cucurbita pepo L.*)

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author WJT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JOO and NKR managed the analyses of the study. Author JOO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Statement of the Problem:** The availability of certified seeds to rural farmers is very important for sustainable agriculture in developing countries. Farmers usually harvest the fruits of pumpkin for food then use the extracted seed at the time of planting. Yet the fruit at harvest may not coincide with the seed quality. This causes the mixture of mature and immature seeds at harvest hence causing poor seed quality.
**Aim:** This study was done to examine the influence of harvesting stage-Days After Anthesis-(DAA) and different sites of the North-rift of Kenya, on seed quality aspects of pumpkin.
**Methodology:** Two types of pumpkin seeds, from round fruit and oval fruit, were planted in the rainfall season of 2015. Four harvesting stages (30 DAA, 40 DAA, 50 DAA and 60 DAA) were done on the two pumpkin types at three sites (AIC Cheptebo, Kaiboi Technical Training Institute-KTTI and University of Eldoret-UOE). The seeds were then extracted and seed testing was done on germination and seed vigor.
**Results:** The seeds from fruits harvested at 60 DAA showed higher seed weight, germination and vigor compared to those from fruits harvested at an earlier stage of 30 DAA. Seed weight,

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germination and vigor for the two pumpkin types were not significantly different at P≤0.05. In spite of the different harvesting stages and pumpkin types, the best seed qualities were observed for seeds harvested from AIC Cheptebo compared to seeds from KTTI and UOE.
**Conclusion:** This showed that seed physiological maturity occurs at 60 DAA and that higher temperature (30ºC) which was at AIC Cheptebo favored better physiological maturity evaluation in the pumpkin. Therefore, pumpkin is a warm-season crop.

*Keywords: Harvest; germination; site; physiological maturity.*

## 1. INTRODUCTION

Pumpkin crop is very useful in curbing food insecurity because its fruits, leaves and seeds are edible and contain a high level of protein, oil, vitamin and minerals [1]. Hull-less seeds are usually used in pharmaceutical products [2].

Pumpkin fruit is rich in carbohydrate, protein and antioxidant activities. These antioxidants are required to boost the human body immunity against cancer and other deadly human diseases [3]. Pumpkin also enhances biodiversity which is important in crop improvement and adaptation to the changing environmental conditions, thus the sustainability of farming systems [4]. It is extensively grown throughout the tropical and sub-tropical countries and thus considered a high value crop [5].

Even though pumpkin is highly nutritive, medicinal and has the potential of food biodiversity, its seed certification has not been looked into, hence low seed quality. Farmers usually obtain on-farm seed after harvesting pumpkins, from their neighbors or at the market place where seeds are sold. The farmers usually harvest the pumpkin fruit at complete plant senescence, purposely for food and then use the seeds that are extracted from the fruit in planting for the following season, thus causing a long term poor pumpkin seed quality [6]. According to [7], production of high quality seed in Cucurbits depends on the fruit age at harvest.

Harvesting of seeds at optimum stage of physiological maturity helps to obtain better quality seed. Harvesting stage influences the quality of seed in relation to germination, vigor, viability and also storability [8]. 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 vigor increases with increase in relative humidity, temperature and seed moisture after the seed attains physiological maturity. Seeds harvested at physiological maturity are well developed, matured and possess maximum viability and vigor [9]. Seeds that are harvested early have been found to be immature and poorly developed, therefore, have poor storage compared to seeds harvested at physiological maturity [10]. Higher vigor seeds were obtained from fruits harvested at 60 or 70 DAA, and storage of such fruits before seed extraction could not improve their performance [11].

Farmers find pumpkins ready for harvest when the stalk becomes cracked and corky. The rind should also be hard for thumb or finger nail entry. The rind color should also turn from being glossy to being dull. The maximum production of pumpkins is best at temperatures between 20ºC to 35ºC and can take about 13 to 25 weeks for it to mature depending on variety and climate [12]. The main aim of this study was to examine the influence of harvesting stage-Days After Anthesis-(DAA) and different sites of the North-rift of Kenya, on seed quality aspects of pumpkin.

## 2. MATERIALS AND METHODS

### 2.1 Site Description

The experiment was done at three agro-ecological zones represented by Cheptebo AIC 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.

### 2.2 Planting Materials

Two local pumpkin (*Cucurbita pepo* L.) seeds from round shaped fruit characterized by bulged seeds and oval-shaped fruit characterized by relatively flattened seeds (Fig. 1) obtained from the farmers were planted during the rainfall season (from May to August 2015) at the experimental farm of University of Eldoret (Uasin-Gishu county), Cheptebo AIC farm (Elgeyo Marakwet county) and Kaiboi Technical Training institute farm in Nandi county at an intra-row and inter-row spacing of 4 m × 6 m.

To obtain vigorous seedlings and high yield, Diammonium phosphate fertilizer was used at planting. This field was regularly maintained by three weedings. Approximately 150 pistillate flowers were tagged at anthesis, and 150 fruits which developed from them were manually harvested by simple rupture of stalk at 30, 40, 50 and 60 Days After Anthesis (DAA).

## 2.3 Experimental Design

The experimental sowing design in the field was Randomized Complete Block Design laid in split plot, in which the whole plot was variety and the sub-plot was harvesting stage; done on two types of seeds, four fruit ages (30, 40, 50 and 60 DAA) blocked by three sites (Cheptebo, U.O.E, Kaiboi T.T.I). Completely Randomized Design was then used in the laboratory for seed testing.

## 2.4 Data Collection and Analysis

Pumpkin seeds from the harvested fruits of each age (30, 40, 50 and 60 DAA) were extracted, then they were thoroughly washed with running tap water and then sun-dried at ambient air (22-32°C) for a period of one week until constant weight. A thousand seed weight from each site and harvesting stage was then collected. Four subsamples of 25 seeds from each lot were placed on sand moistened with distilled water. The sowed pumpkin seeds were then incubated at 25°C. The first count was done at four days from sowing and the last count eight days after sowing [13]. The results were expressed as a percentage of normal seedlings and then calculated to the nearest whole number. Seed vigor was recorded by the rate of seedling growth, which was done by measuring the seedling height from the fourth day from sowing to the tenth day from sowing so as to show the rate of growth. The electrical conductivity of the seed leachate was determined according to [13] 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 $25^0$C. After 24 hours, the electrical conductivity of the seed leachates was determined using a conductivity meter. The conductivity 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 conductivity meter and recorded as $\mu sg^{-1}$.


**Fig. 1. Fruit morphology of two types of pumpkin; (A) Round fruited and (B) Oval fruited (C) Seeds of the Round fruit and (D) Seeds of Oval fruit harvested at 60DAA**

## 2.5 Data Statistical Analysis

The data obtained for each parameter was subjected to ANOVA using General Linear Model; PROC GLM procedure for [14]. The treatment means for each test was compared by protected Fischers 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.

**Model**: $Y_{ijk} = \mu + V_i + S_j + VS_{ij} + H_k + VH_{ik} + \Sigma_{ijkl}$

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
$VS_{ij}$ is the effect due to the interaction of $i^{th}$ Variety and $j^{th}$ Site
$H_k$ is the effect due to Harvesting stage k
$VH_{ik}$ is the effect due to the interaction of $i^{th}$ Variety and $k^{th}$ Harvesting stage
$\sum_{ijkl}$ is the random error effect

## 3. RESULTS

Soil pH for the three sites was done, whereby UOE had (4.96), Kaiboi (5.4) and Cheptebo (6.9). The temperature and precipitation for the planting season of April to August was also recorded. AIC Cheptebo recorded the highest temperature level of 30ºC but lowest precipitation of 107.5mm compared to Eldoret and Kaiboi which had lower temperature and higher precipitation (Table 1).

### 3.1 Effect of Different Harvesting Stages and Sites 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 AIC 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. The two local types of pumpkin per harvesting stage showed no significant difference (Table 2).

### 3.2 Effect of Different Harvesting Stages and Sites on Seed Germination

The different harvesting stages of pumpkin fruits from different sites showed a significant difference ($P<0.05$). Harvesting at 60 DAA from AIC Cheptebo showed the highest germination percentage of 90.9% for the round type and 87.6% for the oval type and was significantly different from 50 DAA, 40 DAA and 30 DAA. The three sites; AIC Cheptebo, KTTI and UOE showed a significant difference at $P \leq 0.05$, whereby, seeds from AIC Cheptebo gave the highest germination percentage. The two types of pumpkin showed no significant difference (Fig. 2).

**Table 1. Weather conditions for Kaiboi, Eldoret and AIC Cheptebo, as of the year 2015**

| Site | Temperature ($^0$C) (April-August) | Rainfall (MM) (April-August) | SOIL pH |
|---|---|---|---|
| Kaiboi | 18 | 600 | 5.4 |
| Eldoret | 23.7 | 280.8 | 4.96 |
| Cheptebo | 30 | 107.5 | 6.9 |

**Table 2. A Thousand seeds weight in grams (g) for seeds harvested at 30, 40, 50 and 60 DAA from UOE, KTTI and AIC Cheptebo**

| Site | Type | HARVEST (DAA) | | | |
|---|---|---|---|---|---|
| | | 30 | 40 | 50 | 60 |
| UOE | Round | 4.03±0.22d | 6.30±0.47c | 9.05±0.89b | 25.95±1.02a |
| | Oval | 2.45±0.44d | 5.65±0.31c | 8.35±0.52b | 21.43±3.39a |
| KTTI | Round | 6.55±1.80d | 10.50±1.02c | 15.80±0.37b | 28.58±1.58a |
| | Oval | 4.15±0.66d | 7.43±0.36c | 10.85±0.48b | 24.20±2.22a |
| AIC CHEPTEBO | Round | 7.83±0.52d | 12.40±2.16c | 27.00±0.62b | 39.45±2.28a |
| | Oval | 5.93±0.28d | 8.85±1.37c | 22.80±2.55b | 29.03±1.11a |
| | **MEAN** | 5.15 | 8.52 | 15.64 | 28.10 |
| | **SD** | 1.98 | 2.62 | 7.29 | 6.10 |

*DAA; Days after Anthesis, Means with different letters in the row are significantly different at P≤0.05 using protected Fischers test Least Significant Difference (LSD); ±SD n=4*

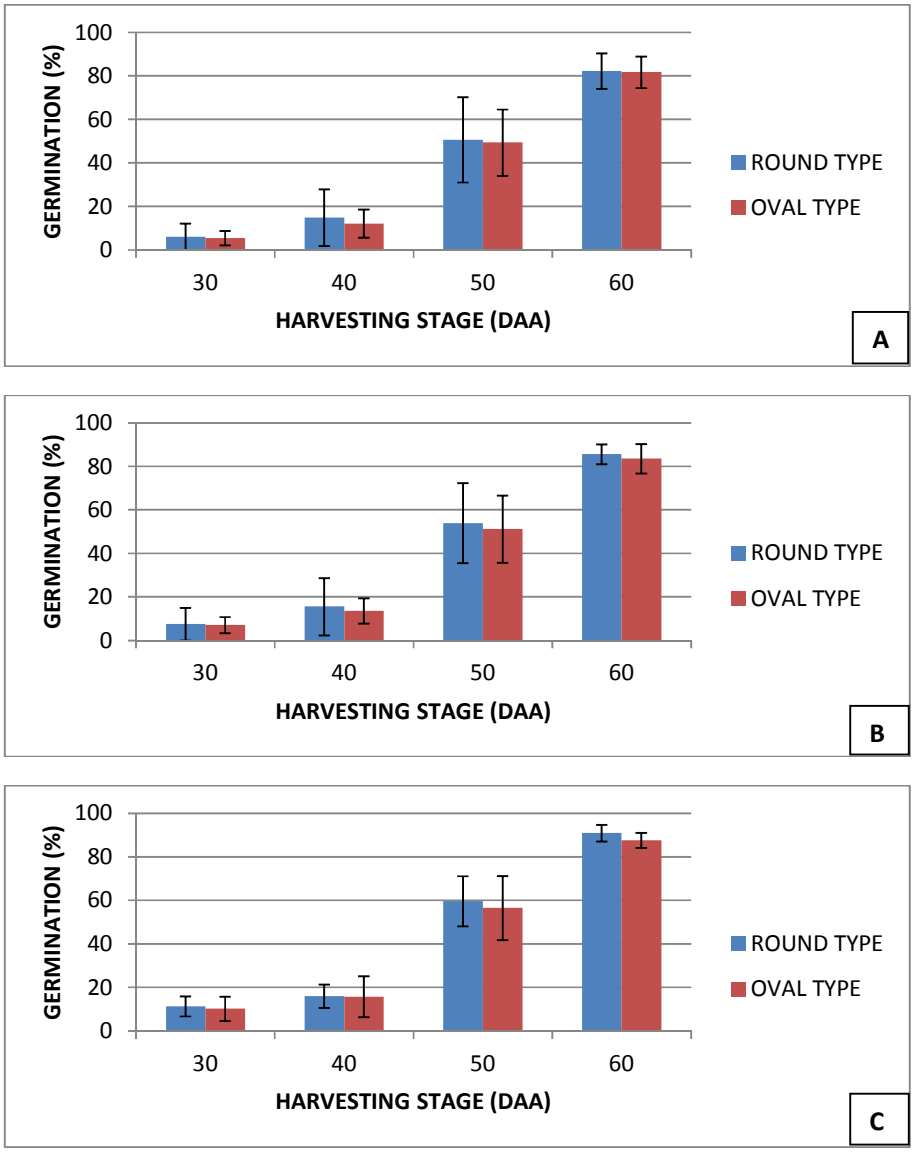


**Fig. 2. Germination percentage of pumpkin seeds harvested from UOE (A), KTTI (B) and AIC Cheptebo (C) at 30, 40, 50 and 60 DAA. Error bars represent the Standard Deviation (SD) n=15**

### 3.3 Effect of Harvesting Stages and Sites on Pumpkin Seed Vigour (Seedling Growth Rate)

The seedling growth rate at different harvesting stages of pumpkin fruits was significant at (P≤0.05). Harvesting at 60 DAA resulted in the highest seedling growth rate which was significantly different from 50 DAA, 40 DAA and 30 DAA. Pumpkin fruits harvested from UOE, KTTI and AIC Cheptebo had a significant effect on seedling growth rate at (P≤0.05). Pumpkin fruits from AIC Cheptebo produced seeds that had the highest seedling growth rate. The two local types of pumpkin showed no significant difference (Figs. 3 and 4).

### 3.4 Effect of Harvesting Stages and Sites on Pumpkin Seed Vigor (Electrical Conductivity)

The electrical conductivity at different harvesting stages of pumpkin fruits was significant at (P≤0.05). Harvesting at 60 DAA resulted in the lowest level of electrical conductivity which was significantly different from 40 DAA and 30 DAA

but was not significantly different from 50 DAA. The two local types of pumpkin showed no significant difference. Pumpkin fruits harvested from UOE, KTTI and AIC Cheptebo had a significant effect on seed vigor at (P≤0.05). Pumpkin fruits from AIC Cheptebo produced seeds that had the highest seed vigor (low electrical conductivity) (Table 3).

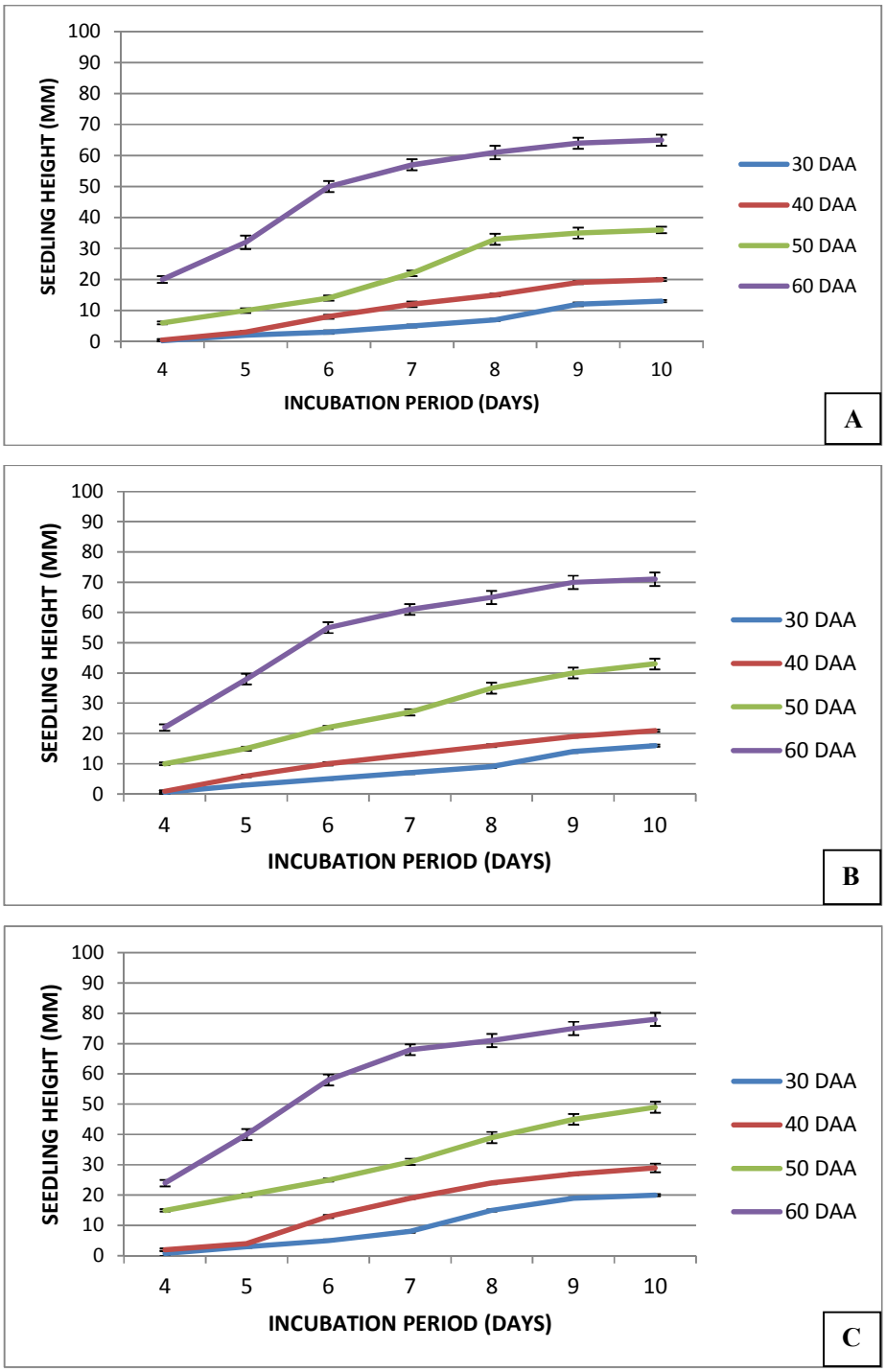


**Fig. 3. Round type of pumpkin harvested from UOE (A), KTTI (B) and AIC Cheptebo (C) at 30, 40, 50 and 60 DAA. Error bars represent Standard Deviation (SD) n=5**

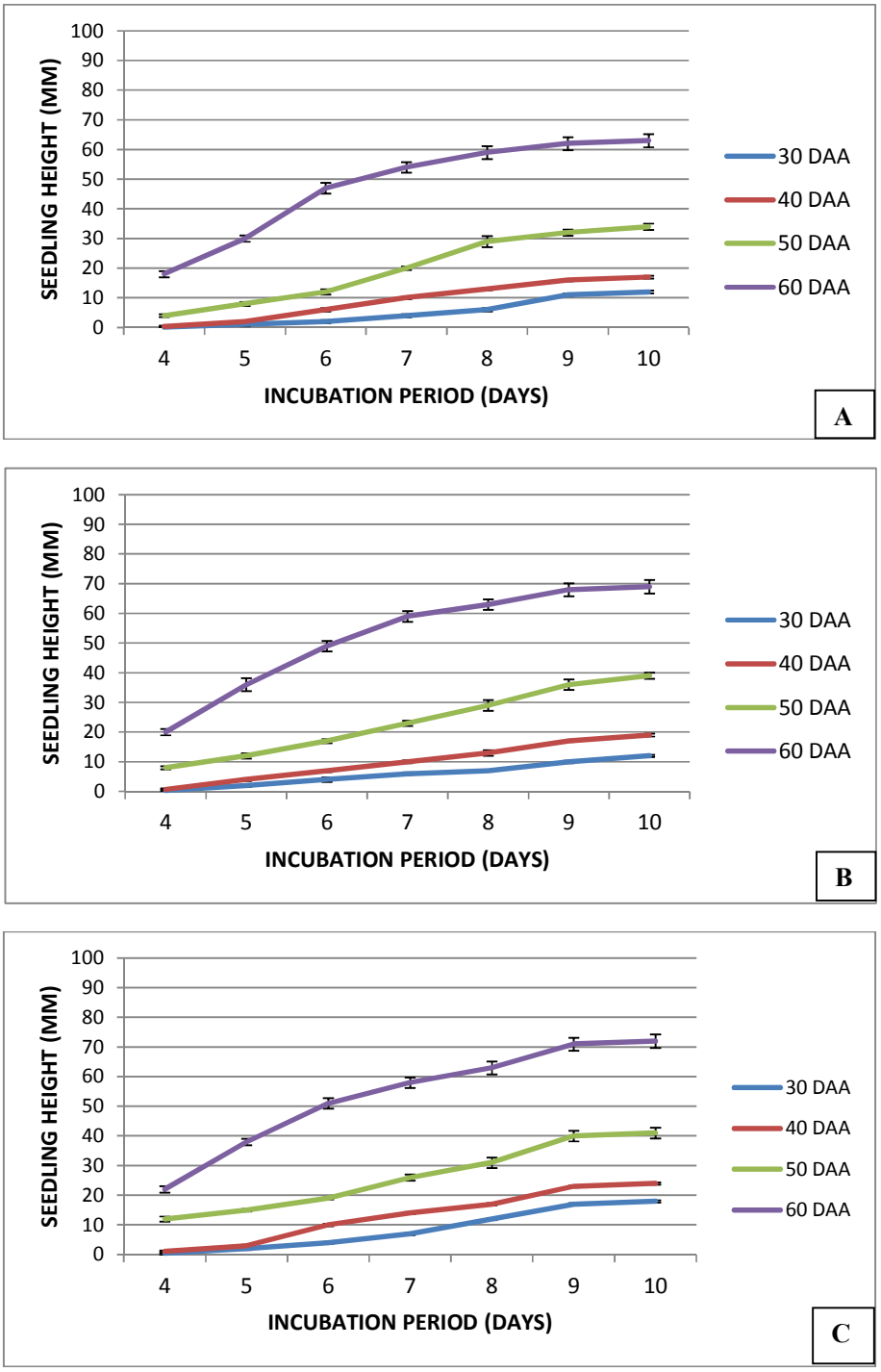


**Fig. 4. Oval type of pumpkin harvested from UOE (A), KTTI (B) and AIC Cheptebo (C) at 30, 40, 50 and 60 DAA. Error bars represent Standard Deviation (SD) n=5**

## 4. DISCUSSION

The seed quality response to physiological maturity was carried out in pumpkin with respect to pumpkin type and location of the experiment. The results showed that the effect of the different harvesting stages at different sites had effect on seed germination and seedling growth rate.

**Table 3. Electrical Conductivity (μsg$^{-1}$) for harvesting stages of 30, 40, 50 and 60 DAA from UOE, KTTI and AIC Cheptebo**

| Site | Type | Harvest (DAA) | | | |
|---|---|---|---|---|---|
| | | **30** | **40** | **50** | **60** |
| UOE | Round | 61.3±5.24a | 59.3±4.7ab | 48.0±2.31bc | 46.3±2.33c |
| | Oval | 65.3±3.29a | 62.0±4.51a | 51.3±0.88b | 49.7±0.88b |
| KTTI | Round | 58.7±4.91a | 56.7±4.37ab | 46.0±2.31bc | 44.3±2.33c |
| | Oval | 62.3±2.9a | 59.7±4.84a | 49.0±0.58b | 47.3±1.2b |
| AIC CHEPTEBO | Round | 56.3±5.24a | 55.7±3.85a | 43.7±2.03b | 42.7±2.6b |
| | Oval | 60.7±2.96a | 57.7±4.84a | 46.0±0.58b | 45.3±1.2b |
| | **Mean** | 60.8 | 58.5 | 47.3 | 45.9 |
| | **SD** | 6.80 | 6.94 | 3.48 | 3.57 |

*DAA; Days after Anthesis.*
*Means with different letters in the row are significantly different at P≤0.05 using protected Fischers test Least Significant Difference (LSD); ±SD n=4.*

There was a significant increase in the dry weight of pumpkin seeds from 30 to 60 DAA. The dry mass was lesser in the early stages of fruit growth (30 DAA) while in the late and final stage of 60 DAA, the fruits had a higher dry matter accumulation. The seed dry mass is considered as one of the important criteria for measuring seed maturity. These findings agree with [15], who reported that seed reaches physiological maturity when it reaches maximum dry weight. 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 60DAA compared to those extracted from fruits harvested at 30DAA.

The seedling growth rate was slow at 30 DAA, compared to the seedling growth rate at 60 DAA which was higher and faster. According to [16], these results indicate that initially, seeds had lower physiological quality, thus releasing high amounts of leachate due to the low structure and selectivity of the membranes hence a lower rate of seedling growth. 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 and high seed vigor.

The reduction in seed germination and vigor varied due to the different growing sites because of the changes in temperature and rainfall conditions during vegetative, reproduction and harvesting periods. Seed quality was modified by the environment during seed development and maturation. Adverse weather conditions have a strong relation with seed quality aspects. In mustard seeds for example, there is accumulation of soluble sugars through starch synthase activity and Late Embryogenesis Abundance (LEA) proteins when approaching maturity. The solutes, enzymes and proteins contribute to seed development, followed by storage longevity and desiccation tolerance [17].

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. 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 [18].

## 5. CONCLUSION

Fruits that are harvested at maturity provide seeds with higher viability, vigor and weight than those harvested at an earlier stage. Pumpkin fruits that are grown at a relatively warm region grow faster and give seeds with high weight, viability and vigor. Following the positive interaction of fruit age at harvest and the environment in which the fruits were grown, the pumpkin fruits should be harvested at 60DAA and planted in warmer areas, like AIC Cheptebo in Elgeyo-Marakwet county. The variations of seed germinability and vigor in this study can be closely associated with fruit size, fruit color, seed color and seed dry weight. Fruit size, fruit color, seed color and seed dry weight can be used as indicators of fruit maturity in both types of pumpkin (round and oval type).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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