UNIVERSITY OF CAPE COAST

EFFECT OF HERMETIC STORAGE ON THE QUALITY AND SHELF LIFE OF *'OBATAMPA'* MAIZE (*Zea mays L.*) VARIETY

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidates Signature……Date ……

Name : MAMOUTOU DIARRA

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

Control of the storage pest Larger Grain Borer (LGB) (*Prostephanus truncates)* and aflatoxin contamination by the mould *Aspergillus spp.* are the major challenges to maize storage in Africa. In this study, the effect of hermetic storage on LGB and *Aspergillus spp.* during maize storage was evaluated. A 2 x 3 factorial experiment of two storage atmospheres (hermetic and non-hermetic) and infestation levels (LGB, *Aspergillus spp.* and uninfected control) were evaluated during the storage of '*Obatampa*' maize. LGB and *Aspergillus spp.* were each introduced into 1.5 kg of the maize grains in hermetic and non-hermetic bags and stored alongside uninfected grains of same weight. Oxygen depletion in the hermetic bags was significant (p = 0.012) while temperature in both hermetic and non-hermetic bags remained fairly constant at 26.99 ºC and 27.4 ºC, respectively. Relative humidity, moisture content, grain damage and weight loss percentages were significantly different in the various bags ($p < 0.001$). There was 100 % LGB mortality in the hermetic storage after 52 days. *Aspergillus flavus* contamination in the non-hermetic bags was highly significant compared to the hermetic bags (p = 0.002). The aflatoxin group B_2 was found in both storage systems. While the aflatoxin group G_1 was not detected, the G_2 group was only detected at the concentration 0.1 ppb in the hermetic storage. The double layer hermetic SuperGrainbag better preserved the quality and shelf life of the maize grains and maintained seed viability ($p < 0.001$) much longer than the non-hermetic polypropylene bag.

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DEDICATION

To my loving and supportive wife, Mrs. Fanta Berthe DIARRA and children;

Korotoumou, Ousmane and Aminata; nephews Gaoussou and Yacouba.

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LIST OF ACRONYMS

ACDI/VOCA	Agricultural Cooperative Development International and Volunteers in Overseas Cooperative Assistance
***AfB*$_1$ and *AfB*$_2$**	*Aspergillus flavus* toxin B_1 and B_2
***AfG*$_1$ and *AfG*$_2$**	*Aspergillus flavus* toxins G_1 and G_2
AGRA	Alliance for a Green Revolution in Africa
APHIS	African Post Harvest Losses Information System
BFAP	Bureau for Food and Agricultural Policy
CA	Controlled Amosphere
CGIAR	Consultative Group for International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Centre
CO_2	Carbon Dioxide
CRD	Completely Randomized Design
EMC	Equilibrium Moisture Content
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agricultural Organization Statistics
FFAs	Free Fatty Acids
G-HF	Gas-Hermetic Fumigation
HPLC	High Pressure Line Chromatograph
ICRISAT	International Centre Research for Semi-Arid Tropic
IITA	International Institute of Tropical Agriculture
ISTA	International Seed Testing Association
KNUST	Kwame Nkrumah University of Sciences and Technologies

LGB	Larger Grain Borer
LSD	Least Significant Difference
MA	Modified Atmosphere
MC_{wb}	Moisture Content wet basis
MoFA	Ministry of Food and Agriculture
NRI	Natural Research Institute
O_2	Oxygen
O-HS	Organic-Hermetic Storage
Pg	Pico gram
PICS	Purdue Improved Crop Storage
PPB	Part per billion
PPRSD	Plant Protection Regulatory Services Directorate
RH	Relative Humidity
SGB	SuperGrainbag
SSA	Sub-Sahara of Africa
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
USSR	United Socialist Soviet Republic
Viz	Namely
$\mu l\ L^{-1}$	Microliter per liter
$\mu g kg^{-1}$	Microgram per kilogram
%	Percent

CHAPTER ONE

INTRODUCTION

Background to the Study

Maize is a member of the grass family; Poaceae (Gramineae) (Piperno & Flannery, 2001). It is further organized in the genus *Zea*, a group of annual and perennial grasses native to Mexico and Central America (Buckler & Stevens, 2005). Maize is currently the world's third most important cereal after wheat and rice (Belfield & Brown, 2008). It is, however, the most important cereal in most African countries including Sub-Sahara Africa (Du Plessis, 2003). It also serves as a staple food for some 200 million people in developing countries, especially in Sub-Sahara Africa (SSA) (Hussein, Metwally, Farghaly, & Bahawirth, 2011). Maize is used for three main purposes; food for mankind, raw material in brewing industries and for animal feed. According to Onimisi, Omage, Dafwang and Bawa, G. S. (2009), seventy percent (70 %) of maize produced worldwide is also used for livestock feed and as staple food for more than 1.2 billion people in SSA and Latin America. In Sub-Saharan Africa, maize is consumed by 50 % of the population and is the preferred food for one-third of all malnourished children and 900 million poor people worldwide. By 2025, maize will be the developing world's largest crop and between now and 2050, the demand for maize in the developing world is expected to double. By 2050, global maize consumption is expected to increase from 32 to 52 kilograms per person per year (http://maize.org/why-maize/ Accessed on April 9, 2015).

Despite the importance and place of maize in Africa, its production is subject to high post-harvest losses due to poor handling and inadequate

storage techniques. Losses after harvest of both quantity (weight losses) and quality (bio deterioration) are brought about by insect pests, mould growth, fungi, rodents and sometimes, birds which deprive farmers of the full benefits of their labour (Boxall, 2001). Traditionally, maize grain is stored by African farmers for consumption and sold later depending on the quantity produced per household. The stored maize is usually destroyed by several pests which eventually leads to deterioration in quality forcing farmers to sell at reduced prices and below the production cost. The value of storage protection to a market-oriented grower is a function of price seasonality, value loss prevention, and their opportunity costs of capital (Jones, Alexander, & Lowenberg-DeBoer, 2011).

The maize weevil or *Sitophilus zeamais* and *Prostephanus truncatus* or Larger Grain Borer (LGB) are the major primary pests of maize during the drying and storage periods. They cause significant storage losses for African maize producers, expressly in tropical and sub-tropical regions (Anankware, Obeng-Ofori, Afreh-Nuamah, Oluwole, & Ansah, 2013). Maize weevils and LGB can be extremely destructive to stored maize (Bbosa, Brumm, Bern, & Rosentrater, 2014). Furthermore, inadequate storage protection allows the entry of water and facilitates easy access by insects and rodents, while in large-scale bag storage chemical browning reactions may lead to grain discoloration called ‘stack-burn’. Maize kernels damaged by insects may be contaminated with dangerous levels of aflatoxins apart from the actual nutrient losses. The feeding activities of these insects also lead to the loss of seed viability hence, low yields resulting in hunger and poverty (Anankware & Bonu-Ire, 2013).

Fungi are the second significant cause of deterioration and loss of maize next to insects. Fungi could cause about 50 % to 80 % of damage on farmers' maize during storage if conditions are favorable for their development (Khosravi, Mansouri, Bahonar, & Shokri, 2007). Fungi infection of maize grain before and after harvest remains a major problem of food safety in most parts of Africa. Problems associated with this infection include loss of germination, mustiness, mouldy smell (Sauer, 1992) and aflatoxins contamination (McAlpin, Wicklow, & Horn, 2002). Aflatoxin B_1 is one of the most potent naturally occurring animal carcinogens and found in all cereal grains and other oil seeds. All animal species appear to be susceptible to aflatoxins and susceptibility varies from species to species. Aflatoxins were identified as the cause of epidemic liver cancer (hepatoma) in rainbow trout. It was found that 4 μgkg^{-1} of diet fed for 16 months causes liver cancer. The control of maize storage is therefore very important to boost maize availability. Various approaches have been used over many decades to control maize post-harvest losses. Among other methods, the integrated approach involving the use of the narrow crib with appropriate pesticides or fumigants is very popular (Affognon, Mutungi, Sanginga, & Borgemeister, 2015).

Major storage techniques utilized by small-holder producers in Western Africa vary greatly, but include on field, open storage, jute bags, polyethylene or polypropylene bags, raised platforms, conical structures with thatched roofs, clay structures, and giant woven baskets (Addo, Birkinshaw, & Hodges, 2002). Farmers may also store bags in their personal rooms, on cobs above fireplaces, or simply heaped on floors (Ofosu, Compton, Magrath, Acquaye, & Ayertey, 1995; Hell, Cardwell, Sétamou, & Poehling, 2000).

These are generally considered 'traditional' storage methods, while improved covered structures or 'cribs' may be termed 'semi-modern', and formal silos and warehouses termed 'modern' storage systems (Sekumade & Akinleye, 2009). Though shelling of grain and insecticide application is officially encouraged by many Ministries of Agriculture, storage of maize on cobs (husked and de-husked) is almost universal.

In recent years, there has been a rising interest in hermetic storage systems as alternative methods for grain preservation against insects that devour the stored grain. These methods are attractive to farmers as they stop survival of the insects without the use of chemicals, and have less destructive impact on the environment and human health. A quantity of these technologies apply flexible plastic liners or bags that have low air permeability properties, which enable them to secure modified atmosphere involving low oxygen and high carbon dioxide concentrations around the grains (Affognon *et al.*, 2015). To avoid or reduce losses during those periods, scientists have developed methods or technologies which nowadays give more satisfaction in matters of cereal storage in general and maize grain in particular. One of these methods and technologies is hermetic storage. Hermetic storage isolates the storage ecosystem from the external environment while respiration within the storage ecosystem causes O_2 reduction and CO_2 accumulation leading to suffocation and dehydration of weevils (Navarro, Donahaye, & Fishman, 1994).

In line with this, the present study compares the effect of hermetic and non-hermetic storage on the quality and shelf life of '*Obatampa*' maize variety grain to determine which of the two is the most effective to preserve grain quality and seed viability of maize.

Problem Statement

World population has been predicted to reach 9.1 billion by 2050 and this will require a 70 % increase in food production (www.fao.org. Accessed on April 15, 2015). Almost all of this growth will occur in less developed countries including Africa. However, Africa is suffering from 20-30 % post-harvest losses valued at 4 billion dollars annually (George, 2011).

Maize (*Zea mays L.*) is a main food crop for many households in Sub-Sahara Africa. As a subsistence crop, it accounts for 40 % of total energy intake in Eastern and Southern Africa (Mutungi, Ng'ang'a, & Affognon, 2015). For countless farmers, however, proper storage of maize grains still a main challenge and a number of biotic and abiotic factors eventually cause storage losses that manifest as loss of the quantities available as food, loss of quality, and loss of economic value. A main biotic factor that causes post-harvest losses in maize is mould and insect pest infection. Infection of maize with moulds can occur at various stages of the crop cycle including cultivation, harvesting, drying, storage, transportation and marketing. When the infection has taken place, it does not only reduce quality but also may prime contamination with toxic metabolites which might accumulate and become a significant safety concern if the fungi are mycotoxigenic, and the conditions of storage favor *Aspergillus flavus* toxins production (http://theagriknot.info/hermetic-storage-bags-aflatoxins/. Accessed on December 27, 2015). Traditional storage practices in developing countries cannot guarantee protection against major storage pests of staple food crops like maize. Post-harvest maize grain losses in Sub-Sahara Africa has reached the highest levels in recent history with the accidental introduction of the

storage pest *Prostephanus truncatus* or Larger Grain Borer (LGB) into Eastern and Western Africa in the late 1970s and early 1980s (Jones *et al*., 2011).

According to the Food and Agriculture Organization (FAO), 25 % of the world's food crops are significantly contaminated with mycotoxins (Boutrif & Canet, 1998). Because maize is dietary staples for the majority of the poor communities in Sub-Sahara Africa (SSA), mycotoxin poisoning is common in this region. Thus, there is a direct link between socioeconomic status and exposure to mycotoxins in SSA's countries, with poor families experiencing significantly higher exposure (Wagacha & Muthomi, 2008). According to Alliance for a Green Revolution in Africa [AGRA], (2013), maize post-harvest losses in Ghana and Mali are estimated to be about 9-15 % and 20-30 %, respectively. In 2010, Mali lost 280,716-421,074 tons in maize post-harvest processing with cost estimated about $ 21,833,928- $ 32,735,891 (Yusuf & He, 2013).

Kamanula *et al*. (2010), recorded that after harvesting, most small-holder farmers do not test the initial moisture content before grain storage, no fumigation is performed and they lack storage management skills. This results in high post-harvest losses during storage of about 30 % of the grains in Sub-Sahara Africa, attributable mainly to pests (Admire & Tinashe, 2014). The use of pesticides to control pests and diseases, however, leaves chemical residues that pose health risks to consumers and the environment. Safe storage of maize at the farm level is critical, as it directly impacts on poverty mitigation, food and income security and prosperity for the small-holder farmers. The deficiency of suitable storage structures for maize grain and the nonexistence of storage management technologies is critical in SSA. That often force the

small-holders to sell their produce instantly after harvest when prices are low to avoid post-harvest losses from storage pests and pathogens and cannot use their harvest as collateral to access credit. Finally, their food security is disrupted. Hence, food security and safe storage at the farmer level go hand in hand. As well as providing food security for times of insufficiency, effective grain storage is an inflation-proof savings bank; grain can be cashed as needed or used directly as a medium of exchange (i.e. in payment for work such as field clearance and weeding). Safer and more environmentally friendly storage methods are therefore being sought and one of such proper methods is the hermetic storage method.

To sustainably achieve the goals of food security, food availability also needs to be increased over declines in the post-harvest process at farm, retail and consumer levels. Hermetic storage is a non-residue organic technology which preserves the health of consumers and also protects the yield against pest attacks for the protection of stored grain.

Justification

Maize (*Zea mays L.)* is the third most important cereal grain worldwide after wheat and rice (Hodges & Farrell, 2004). It is mentioned as the cereal of the future for its nutritional value and utilization of its products and by-products (Lee, 1999). The demand for maize has been projected to increase by 50 % from 558 million metric tons in 1995 to over 800 million metric tons in 2020 (Moreno-Martinez *et al*., 2011). Maize production in Mali has for the last two decades recorded the fastest growth of any of the rain-fed coarse grains. Its production has increased from about 200,000 tons in 1991 to close to 700,000 tons in 2009, thanks to agronomic research and rural development

projects as well as increasing maize price levels (Diallo, 2011). Currently, maize represents 15 % of the total cereal production (Fofana, Abdoulaye, Coulibaly, Sanogo, & Langyintuo, 2010).

Food and Agriculture Organization predicts that about 1.3 billion tons of food are globally wasted or lost per year (Gustavsson, Cederberg, Sonesson, Van Otterdijk, & Meybeck, 2011). Reduction in these losses would increase the amount of food available for human consumption and enhance global food security, a growing concern with rising food prices due to growing consumer demand (Mundial, 2008). During post-harvest operations, there may be losses of both maize grain quantity and quality. Qualitative post-harvest loses can lead to a loss in market opportunity and nutritional value; under certain situations, these may pose a serious health hazard if linked to the consumption of aflatoxin contaminated grain. The major grain and seed losses during maize storage in Sub-Sahara African countries is due, in part, to high ambient relative humidity, and the fact that storage ecosystems and the stored maize equilibrate with ambient moisture and temperature. According to Williams (2011), a survey of local African markets shown that 40 % of the commodities found there exceeded allowable aflatoxin levels (in excess of the international standard of 10-20 ppb) and that an estimated 4.5 billion people in developing countries are at risk of uncontrolled or poorly controlled exposure to aflatoxins. More recently, recurrent acute aflatoxicosis in Kenya in 2004 and 2005 caused more than 150 human deaths and were linked to inadequately stored, homegrown maize infected by *Aspergillus spp* (Barry, Robert, & Mostrom, 2007). According to Campbell, Arthur and Mullen (2004), the current estimates of the cost of grain loss due to insect and microorganism

damage of grain stored in developing countries each year vacillated from $ 500 million to $1 billion.

To maintain high quality maize during storage, maize should be protected from the weather (including relative humidity and temperature), growth of microorganisms, and insects (Oyekale, Daniel, Ajala, & Sanni, 2012). Storage losses can be reduced by introducing improved storage methods. Several farmers in Sub-Sahara Africa used to safeguard their post-production product with fumigants and contact insecticides which generate health risks in the food. Constraints due to the adverse effects of pesticide residues in food and the environment resulted in the hassle of strict limitations on pesticide registration by regulatory agencies. Consumer demand for chemical free and insect contamination free products increased the attention to the application of non-residue organic technologies to replace fumigants for the protection of stored grain. A more recent but increasingly popular form of hermetic storage system is the triple layer bag. This system utilizes a thin, transparent and low permeability co-extruded multi-layer plastic as a liner to a conventional jute or polypropylene bag. Nowadays the SuperGrainbag (SGB) is an appropriate type for use by the small farmer to store maize on the farm. The significant work being done to reduce aflatoxin levels in the field is mentioned, as well as its probable implications on post-harvest storage (Villers, 2014). To date, there is no reported work about the effects of hermetic storage on contamination by *Aspergillus flavus* groups, which produce toxins responsible for maize poisoning during storage.

Different authors worked on causes and effect of post-harvest losses under different methods. Anankware *et al.* (2013) found that the grain damage

and weight loss were significantly (p <0.001) higher in the non-hermetic polypropylene and jute bags than the hermetic bags. Another study showed that the triple-layer hermetic bag preserved seed quality and viability much longer than the conventional jute and polypropylene bags (Anankware & Bonu-Ire, 2013).Therefore, the authors did not report the weight loss and the relative humidity evolution during and at the end of storage. They also were not specific about the critical atmospheric conditions responsible for the death of insects. However, knowledge of specific atmosphere mix is important for the design of effective Control Atmosphere or Modified Atmosphere storage without time lag for insect mortality. Correlation between insect damage and aflatoxin contamination was positive in the studies conducted by Bowen & Mack, 1991; Lynch & Wilson, 1991; Lynch, Dicko, Some, & Ouedraogo, 1991; Gorman & Kang, 1991), but the authors did not assess the difference between hermetic and non-hermetic in this correlation.

This study therefore aims to elucidate the functionality of the double layer hermetic bag storage system and evaluate its effectiveness in protecting maize grains against moist air and major pests responsible for storage losses.

Objectives of the Study

Main objective

The aim was to study the effect of hermetic storage on the quality and shelf life of *"OBATAMPA"* maize variety.

Specific Objectives

1. To examine the effects of hermetic storage on the changes in the store atmospheric conditions (viz. O_2 concentration, temperature and relative humidity),

2. To determine the effect of hermetic storage on the activity and mortality of insect Larger Grain Borer (LGB),
3. To determine the effect of hermetic storage on the total aflatoxins concentration and *Aspergillus flavus* groups contamination,
4. To examine the effect of hermetic storage on the quality of maize grains (moisture content, weight loss, percentage grain damage and germination potential).

Hypotheses

Null Hypothesis

1. There is no significant difference between hermetic and non-hermetic storage methods for their effect on the changes in the atmospheric composition of maize storage environment.
2. There is no significant difference between hermetic and non-hermetic storage methods for their effect on the quality of maize during storage.
3. There is no significant difference between hermetic and non-hermetic storage methods for their effect on the activity and mortality of LGB of maize during storage.
4. There is no significant difference between hermetic and non-hermetic storage methods for their effect on the *Aspergillus flavus* groups contamination of maize during storage.

Alternative Hypothesis

1. There is a significant difference between hermetic and non-hermetic storage methods for their effect on the changes in the physical environment conditions of maize grain during the storage.

2. There is a significant difference between hermetic and non-hermetic storage methods for their effect on the quality of maize during storage.
3. There is a significant difference between hermetic and non-hermetic storage methods for their effect on the activity and mortality of LGB of maize grain during the storage.
4. There is a significant difference between hermetic and non-hermetic storage methods for their effect on *Aspergillus flavus* groups contamination of maize grain during the storage.

Significance of the Study

The results from this study could be used by farmers to protect their maize grains during storage against the principal storage pests. The results from this study could also be used for the optimal design of controlled atmospheres in hermetic storage systems.

Delimitation

This study took place in the laboratory of the University of Cape Coast Research Farm. Cape Coast lies on latitude 05-06 ºN and longitude 01-15 ºS at an altitude of 1.1 m above sea level. The annual temperature is 30 ºC–34 ºC during the day and 22 ºC–24 ºC during the night and a relative humidity of 75–79 %.

Limitations

In this study the main methodological weakness was the inability of the measuring instrument (SCY-2A Oxygen Analyzer) to read carbon dioxide concentration accurately. Knowledge of specific atmosphere mix is important for the design of effective controlled atmospheric/modified atmospheric storage without time lag for insect mortality.

Definition of Terms

1. Temperature is the state describing how hot or cold the storage room during the experiment.
2. Relative humidity is the amount of water vapor in the air expressed in percentage (%).
3. Moisture content is the weight of water in the commodity expressed in percentage (%).
4. *Aspergillus flavus* contamination is the invasion and quality destruction of the commodity by the toxins produce by *Aspergillus spp*.
5. Insect mortality is the state of insect and not living because of the environmental conditions.
6. Seed viability is the ability for a seed to germinate if germination conditions are satisfied.
7. Hermetic storage is the storage system in which there is no air exchange between the internal atmosphere of the commodity container and its external atmosphere.
8. Non-hermetic storage system in which there is air exchange between the internal atmosphere of the commodity container and its external atmosphere.

Organisation of the Study

The background of the research, problem statement with the objectives of the study were described in Chapter 1. Chapter 2 presents the review of research related to the problem being investigated. The methodologies as well as the materials used to carry out the results are described in Chapter 3. The mathematical background relating to the determine some specific parameters

(moisture content, percentage of weight loss, grain damage and germination potential) of maize grain are included in this chapter. Chapter 4 gives the general results and their related discussion. Finally, chapter 5 states conclusions and recommendations derived from the complete study.

CHAPTER TWO

LITERATURE REVIEW

Maize Crop and Origin

Maize (*Zea mays L.*) or corn is a monoic annual plant belonging to the grass family of Gramineae (Poaceae), with the cells having 2n chromosomes (Mejía, 2003). Worldwide, the cereals wheat, rice and maize are produced in greater quantities than any other crop. Of these crops, maize has the highest average yield per hectare and it is third after wheat and rice in the area harvested and total production. It has the basic structure of the grass family with conspicuous nodes and internodes on the stem. The leaves grow on opposite sides; one leaf per node. Maize is botanically unique among cereal crops. It is monoecious (separate male and female inflorescences on the same plant) and produces grains on lateral rather than terminal branches. Maize is a cross-pollinating (allogamous) species; hence, the natural population is usually heterogeneous. It is grown widely throughout the world in a range of agro ecological environments (www.fao.org. Accessed on December 10, 2015). About 50 species exist and consist of different colors, textures and grain shapes and sizes. White, yellow and red are the most common types. The white and yellow varieties are preferred by most people depending on the region (Yakubu, Bern, Coast, & Bailey, 2009).

The centre of origin of maize is the Mesoamerican region, probably in the Mexican highlands, from where it spread rapidly. Archaeological records and phylogenetic analysis propose that domestication began at least 6,000 years ago (Piperno & Flannery, 2001). Maize spread around the world after European discovery of the Americas in the 15th century, predominantly in

temperate zones (Farnham, Benson, Pearce, White, & Johnson, 2003). Maize is only known as a cultivated crop and its exact genealogy remains uncertain. *Zea mays ssp*. parviglumis is hypothesized to be the progenitor of cultivated maize. This hypothesis is supported by the close genetic compatibility and relationship between the two sub-species. Various hypotheses have been proposed on the origin/domestication of maize, conversely, it is generally accepted that the word has its origin in Araguaco and the name was brought back to the old world by Christopher Columbus who heard it for the first time in the Caribbean islands (Doebley, 2004).

Arrival of Maize in Africa

After the opening of the Atlantic basin to trade and cultural exchange, maize arrived in Africa after 1500 as part of the massive global ecological and demographic transformation. The importation of maize seeds to various parts of Africa generally went unremarked, though it certainly was not unremarkable. The first reference to maize's introduction to Africa may be that of an anonymous Portuguese pilot in 1540, who described its already well established cultivation on the Cape Verde Islands: "At the beginning of August they begin to sow grain. It is like chick pea, and grows all over these islands and along the West African coast, and is the chief food of the people" (McCann, 2001).

Maize Growth and Production

Maize is a versatile crop grown over a range of agro climatic zones. In fact, the suitability of maize to varied environments is matchless by any other crop. It is grown from 58 ºN to 40 ºS, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per

year Shaw (1988). Currently, the area under this crop is nearly 162 million hectares, out of which, nearly 100 million hectares is covered by 125 developing countries with global production reaching a mark of 845 million tons with global productivity of 5.21 tons/ha, 67 % coming from low and lower middle income countries (Pratap & Kumar, 2014). Maize is grown throughout the world, although there are large differences in yields. It is documented that in 2012, the total world production of maize was 875,226,630.27 tons (Peña-Rosas, Garcia-Casal, Pachón, Mclean, & Arabi, 2014). According to Smale, Byerlee, and Jayne (2011), maize currently covers 25 million hectares in Sub-Saharan Africa, largely in small-holder systems that produced 38 million tons in 2005-8, primarily for food. From 2005-8 (Table 1), maize represented an average of 27 % of cereal area, 34 % of cereal production and 8 % of the value of all primary crop production. The potential for expanding maize production in Sub-Sahara Africa is huge. Even after excluding protected and forested areas, an estimated 88 million hectares of land that has not yet been cultivated with maize is suited to the crop. Worldwide, this amount is equivalent to four times the area now planted to maize and over half of the additional land area that is suitable for maize (Deininger & Byerlee, 2011). The Food and Agriculture Organization of the United Nations' indices of agricultural production include produces that are considered edible and contain nutrients, and show the relative level of the total volume of agricultural production for each year in comparison with the base period 1999-2001 (www.fao.org/3/a-i3621e/i3621e02.pdf. Accessed on February 4, 2016).

In Sub-Sahara Africa, the maize yield is lower and various research programs planned to boost the yield per hectare. Although the use of improved maize can be a catalyst for increasing farmers use of other inputs and especially fertilizer, such broad based change has only occurred in some parts of SSA. The majority of farmers do not adopt the additional production practices needed to sustain the improvement. For all of Sub-Sahara Africa, about 40 % of fertilizer is used on maize, implying that the average dose is only about 17 $kgha^{-1}$ of nutrients compared to the developing country average of 100 and the industrialized country average of 270 $kgha^{-1}$ on the same crop (Morris, Kelly, Kopicki, & Byerlee, 2007; Heisey & Norton, 2007). Maize is a heavy consumer of fertilizer, leading fertilizer demand in industrialized countries among major cereals, and the second most heavily fertilized crop on a global scale, after potatoes (Heisey & Norton, 2007).

Table 1: Area, Production, Yield Consumption in Regions of Sub-Saharan *Africa, 1961-2008*

	West Africa	Central Africa	East Africa	South Africa	SSA
Maize area (million ha, 2005-2008)	7.75	2.31	7.79	6.77	24.84
Maize production (million tons, 2005-2008)	12.86	2.42	11.62	7.62	38.21
Maize yield (2005-2008)	1.66	1.05	1.49	1.09	1.39
Growth in maize area (% Year, 1961-2008)	3.09	1.92	1.84	1.30	2.03
Growth in maize production (% Year, 1961-2008)	4.08	2.90	3.02	1.30	2.99
Growth in maize yield (% Year, 1961-2008)	1.71	0.98	1.18	0.00	0.95
Average Kg/cap/year (2003-2005)	24.40	24.90	26.90	81.80	39.60
Average % of calories/cap/year (2003-2005)	8.60	12.40	19.30	36.10	19.10

Source: (FAOSTAT).

Economic Importance and Use of Maize

Over the past three decades, economists have described maize research and development in Sub-Sahara Africa as an 'emerging maize revolution' Byerlee and Eicher (1997), a 'stop and go revolution' Howard and Mungoma (1997), a 'delayed green revolution' Smale (1995), an 'obscured revolution' Gilbert *et al*. (1994), and a 'failure' (Kydd, 1989). According to International Institute of Tropical Agriculture [IITA] (2009), maize is the most important cereal crop in Sub-Sahara Africa and with rice and wheat, one of the three most important cereal crops in the world. It is a high yielding crop, easy to process, readily digested, and cheaper than other cereals. Maize is also a useful crop; growing across a range of agro ecological zones. Every part of the plant has economic value: the grain, leaves, stalk, tassel, and cob can all be used to produce a large variety of food and non-food products. In developed countries, maize is largely used as livestock feed and as a raw material for industrial products, while in emerging countries, it is mainly used for human consumption. In Sub-Sahara Africa, maize is a staple food for an estimated 50 % of the population. Maize is an important source of carbohydrate, protein, iron, vitamin B, and minerals. Africans consume maize as a starchy base in a wide variety of porridges, pastes, grits, and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled; playing an important role in filling the hunger gap after the dry season. A significant part of maize production is being used to generate ethanol fuel (ethyl alcohol), the same type of alcohol found in alcoholic beverages. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. Maize is the primary feedstuff used to produce ethanol. Strong demand for ethanol production has resulted in

increased maize prices and has provided incentives to increase maize acreage (Afiff, Wilkenson, Carriquiry, Jumbe, & Searchinger, 2013). It is estimated that nearly 40 % has been used in recent years to make ethanol for fuel. Of this, 27 % becomes ethanol and 12 % is the distillers' dry grain residue that goes to animal feed, making the total animal feed use at 50 % (Wallington *et al*., 2012). Exports accounted for 13 % and 4 % are used to make high glucose corn syrup. Part of the remaining 7 % is used to make corn oil, cornstarch, corn syrups, and other industrial applications, while some is used to make whiskey and other alcoholic beverages (www.infonet-biovision.org. Accessed on January 2, 2016).

Maize Consumption

Worldwide consumption of maize is more than 116 million tons, with Africa consuming 30 % and Sub-Sahara Africa 21 % (Baributsa, Lowenberg-DeBoer, Murdock, & Moussa, 2010). Estimated maize consumption in grams per person per day in countries where maize is considered an important food source is above 50 g. It is clear that maize is a staple in the African region where the consumption ranges from 52 to 328 g person^{-1} day^{-1} and the region of the Americas where the highest consumption was 267 g $^{-1}$ person^{-1} day^{-1} in Mexico (Ranum, Peña-Rosas, & Garcia-Casal, 2014). World production of maize has shown a slight but steady increase over the years, but human consumption of the grain has remained steady. It is thought that the majority of the increase in production has corresponded to an increase in the use of maize for animal feed. However, maize is still a staple food for many people, especially in Africa. Maize has food, feed, and industrial uses. It is a major component of livestock feed. The amount of maize used for feed depends on

the crop's supply and price. It also depends on the amount of supplemental ingredients used in feed rations, and the supplies and prices of competing ingredients (Peña-Rosas *et al*., 2014). In high income countries, an estimated 70 % of maize is destined for feed, only 3 % is consumed directly by humans, and the remainder is used for biofuels, industrial products and seed. In Sub-Sahara Africa outside of South Africa, 77 % of maize is used as food and only 12 % serves as feed (Smale, Byerlee, & Jayne, 2013). The two types of white maize (dent and flint) are largely associated with different food products (Food and Agriculture Organization [FAO] & International Maize and Wheat Improvement Centre [CIMMYT], 1997). Maize has accounted for 22 to 25 % of starchy staple consumption in Africa from 1980, representing the largest single source of calories, followed closely by cassava. The worth of maize as a staple varies across the continent. The highest amounts of maize consumed are found in Southern Africa at 85 kg/capita/year as compared to 27 in East Africa and 25 in West and Central Africa (Smale *et al*., 2013). In some countries in Sub-Sahara Africa, maize is important enough in farm production, incomes and diets that yield gains could have impacts on producer and consumer welfare similar to those that occurred with improved rice in Southeast Asia (Larson, Otsuka, Kajisa, Estudillo, & Diagne, 2010).

Maize is processed and prepared in different forms depending on the country. Ground maize is prepared into porridge in Eastern and Southern Africa, while maize flour is prepared into porridge in West Africa. Ground maize is also fried or baked in many countries (www.iita.org/maize. Accessed on October 12, 2015). In all parts of Africa, green (fresh) maize is boiled or roasted on its cob and served as a snack. Maize ground and maize flour

(cornmeal) in the form of 'banku', 'ugali' and porridge etc. are a staple food around the world. Again, popcorn is a common snack, while corn flakes, hominy, grits, and canjica are common breakfast foods. It includes an average of 30 to 50 % of the daily caloric intake of people in most southern African countries (FAO, 2011). Maize is also consumed as a vegetable, in addition to being used for livestock and dog feed, plus fish bait (Yakubu, 2009).

Structure of Maize Grain

External Structure

Maize grain is not a seed, but a single-seed fruit (Fig. 1). Its fruit-wall and seed-coast are fused into a single layer. The grain is monocotyledonous and endospermic protected by the pericarp. There is a small tube near the top of the grain. A very slight, whitish patch on one side of the grain, marks the embryo. The micropyle is situates at the base of the grain (http://www.enmuangplanting. blogspot.com/2012/04/germination-of-maize-grains.html. Accesses on March 11, 2016).

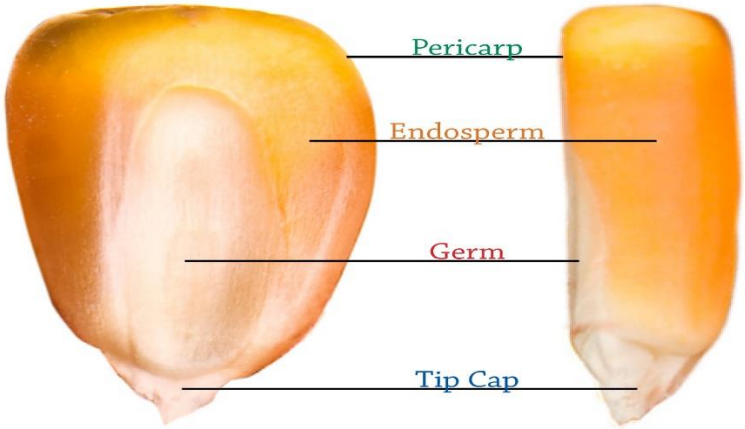

Figure 1: Primary components of maize grain.

Source : (http://igrow.org/agronomy/corn/heat-stress-on-late-grain-filling-in-corn/ . Accessed on March 18, 2016).

The Internal Structure

The outermost layer is formed by the fusion of fruit-wall and seed-coast (Fig. 2). The endosperm constitutes the upper one-third to the three-quarters of the grain. The endosperm has thin outer aleurone layer and an inner part containing starch grain. The aleurone layer consists to proteins and fats. A shield-shaped single cotyledon is known as the scutellum. It is separated from the endosperm by the epithelial layer. The embryo is embedded in the scutellum. Its plumule is covert by a sheath called coleoptile and the radicle by the coleorhiza (http://www.enmuangplanting.blogspot.com/2012/04/germination-of-maize-grains.html. Accessed on March 11, 2016).

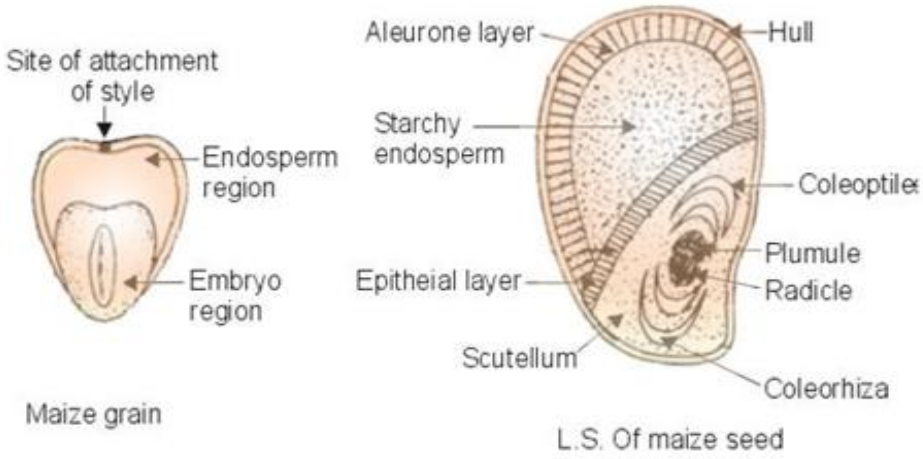

Figure 2: Internal structure of maize grain.

Source : http://www.enmuangplanting.blogspot.com/2012/04/germination-of-maize-grains.html. Accessed on March 11, 2016).

Production Constraints of Maize

The maize plant is quite hardy and adaptable to harsh conditions. In addition, it is a highly diverse crop, offering ample scope for genetically enhancing its tolerance to constraining factors. This notwithstanding,

production has been affected by certain factors which have led to decreased yields and high post-harvest losses. These limiting factors comprise both biological and physical factors. Pertinent physical variables are temperature and precipitation together with other traditional inputs such as labour, seed, fertilizer and irrigation (Bureau for Food and Agricultural Policy [BFAP], 2007). Maize does not tolerate drought well and the grain can rot during storage in tropical climates. In addition, periodic drought caused by irregular rainfall distribution has made farming a very risky endeavor for millions of small scale farmers who rely on rainfall to water their crops. In Sub-Sahara Africa, periodic drought caused by irregular rainfall distribution reduces maize yields by an average of 15 % each year. This is equivalent to at least US $200 million in foregone grain and adversely affects the lives of about 300 million people (http://www.iita.org. Accessed on November 4, 2015).

Biological factors limiting maize production are diseases and pests. A range of diseases plagues maize growing areas in Sub-Sahara Africa. These include downy mildew, rust, leaf blight, stalk and ear rots, leaf spot, and maize streak virus. Insect pests, including stem and ear borers, armyworms, cutworms, grain moths, beetles, weevils, grain borers, rootworms, and white grubs are also a great threat to the survival of maize in Africa. They can cause 20-40 % losses during cultivation and 30-90 % losses post-harvest and during storage (IITA, 2009). Weeds including the parasitic witch weed (Striga) are major pests in Sub-Sahara Africa and cause estimated cereal grain loses up to US $7 billion per annum. This adversely affects the lives of about 300 million people (www.iita.org. Accessed on November 1, 2015).

Maize Moisture Content at Harvesting

Maize can be stored for a considerable period in unprocessed form without undergoing deterioration. Its shelf life greatly depends on the prevailing ambient temperature and relative humidity, and other factors like the inherent moisture, pests and diseases. Thus, recommended post-harvest handling and managing operations involve the manipulation of the above factors in order to obtain high quality maize grains. Quality control starts with harvesting. Harvesting is the single deliberate action to separate the cob from its grown medium. The optimum time of harvesting maize is when the stalks have dried and moisture of grain as about 20-17 %. (www.teca.fao.org. Accessed on December 30, 2015). The crop is harvested nearest to optimum moisture contents and placed at the bottom or back of storage structures. Maize with higher than desirable moisture levels may be more of a problem at feed-out during the warm months and is best to put on the top or front of the silo for winter feeding. Very wet maize may be disposed to aerobic instability (heating) upon removal from the silo.

Drying of Maize

Drying of agriculture products is an imperative unit operation under post-harvest phase. It refers to removal of moisture from grains and other products to a predetermined level. It is a thermos-physical and physico-chemical operation by which the excess moisture from a product is removed. The main purpose of drying is to prevent germination, the growth of bacteria and fungi and retard considerably, the development of mites and insects. For safe storage, the maize must be dried since the moisture content at harvest is generally higher than the desirable moisture content for storage. Drying makes

the commodity suitable for safe storage and protects them against attacks of insects, moulds and other micro-organism during storage.

Maize is usually harvested with moisture content in the range of 18-26 % and the full cobs or the shelled grains are further dried in the sun; if possible the maize should not be put directly on the ground to avoid contaminating the grain or cobs with soil or dirt. Polythene sheeting or sheets made out of nylon sacks are useful for drying. Grain must be turned often to ensure homogenous drying. After harvesting, the greatest enemy of grain is moisture, wet grains attract insects and mould. Therefore, the grain must be dried as soon as possible after harvesting. Drying is the systematic reduction of crop moisture down to safe levels for storage, usually 12 % to 15.5 % moisture content.

In many tropical and sub-tropical regions, sun drying remains the favored method of grain drying, mostly for economic reasons. Traditional sun drying has changed little over the centuries. The grain is spread on mats or paved ground in layers of 5 to 15 cm thickness and is exposed to the ambient conditions. The grain is stirred intermittently, usually is covered at night, and dries adequately in 2 to 4 days. However, sun drying is an unreliable process because it is weather dependent. Also, the solar radiation changes with the season and the time of day, and the flux density is low. The sun drying of grain is affected by the solar radiation, the ambient air temperature, the ambient relative humidity, the wind velocity, the soil temperature, the grain layer thickness, and the grain type (http://mykonspekts.ru/1-85826.html. Accessed on December 15, 2015). There is no definitive method for the drying of maize since each method depends on a number of factors, such as the level of maize production, the intentional use of the grain, the capital and expertise

available, fuel availability and the local weather after harvest. Maize must be adequately dried before subsequent storage to avert germination of the grain, the growth of micro-organisms and insect infestation. Most drying processes, either of cob maize or shelled grain, take place at or near the point of maize production. During the drying process, moisture which evaporates from the wet grain is rapidly absorbed into the drying air until an equilibrium state is reached where no further moisture is lost. The final grain moisture content is termed the "equilibrium moisture content" for specific ambient conditions. The rate at which drying takes place depends upon the moisture content of the grain and the flow rate, temperature and humidity of the drying air.

Chemical Composition of Maize

Maize is a multiuse grain. It can be used directly as a human food, but provides even greater nutritional values when used as an ingredient in the food processing industry and the animal feeding industry (Ullah, Ali, & Farooqi, 2010). Typical proximate compositions of the main parts of the maize kernel (yellow dent corn). Chemically, dried maize kernel contains about 10.4 % moisture, 6.8 % to 12 % protein, 4 % lipid, 1.2 % ash, 2.0 % fiber and 72 % to 74 % carbohydrate Kulp (2000) as shown (Table 2). It also contains macro and micronutrients such as calcium, phosphorus, iron, sodium, potassium, zinc, copper, magnesium, and manganese, with 7 mg/100 g, 210 mg/100 g, 2.7 mg/100 g, 35 mg/100 g, 287 mg/100 g, 2.2 mg/100 g, 0.3 mg/100 g, 127 mg/100 g, and 0.45 mg/100 g each, respectively in dry matter basis (db.). Maize also contains important vitamins such as thiamine 0.38 mg/100 g, riboflavin 0.20 mg/100 g and niacin 3.63 mg/100 g, pantothenic acid 0.42

mg/100 g and folate 19 µg/100 g. These will vary due to variety, hybrid, growing seasons, and soil conditions (Nuss & Tanumihardjo, 2010).

Table 2: ***Proximate Chemical Composition of main Parts of Maize Kernel (% db.)***

Chemical Composition	Pericarp	Endosperm	Germ
Protein	3.70	8.00	18.40
Fat	1.00	0.80	33.20
Crude Fiber	86.70	2.70	8.80
Ash	0.80	0.30	10.50
Starch	7.30	87.60	8.30
Sugar	0.34	0.62	10.80

Source: (Nuss & Tanumihardjo, 2010*).*

Quality of Maize Grain

Grain quality is an ill-defined term because its meaning is interpreted differently by various end-users. For the livestock producer, the nutritive value of grain is important. For the cereal manufacturer, some physical grain property such as the breakage susceptibility may be of significance. And to the seed producer, only the seed viability is of interest. Regardless of the particular grain quality criterion, the post-harvest operations to which a grain sample is subjected determine its value. The economic and nutritional value of the maize kernel is mainly due to its high starch content that represents approximately 75 % of the mature seed weight. However, the protein complement (*ca*. 10 % of the mature seed weight) mainly found in the form of zeins which is a protein of the prolamine group occurring in maize and is used in the manufacture of plastics, coatings, adhesives, etc.) and oil (*ca*. 4.6 %) are essentials for human and animal nutrition (Motto, Hartings, Fracassetti, & Consonni, 2012).

Maize grain quality is increasingly important as more grain is processed into other specialty end uses. Its quality is determined by growing conditions, harvest practices, drying operations and storage conditions. Except for growing conditions, these quality factors are generally under the control of the grower. Harvesting grain at too high moisture content can result in severe kernel damage during threshing and drying. Conversely, allowing maize to dry in the field for too long can lead to reduced yield and quality as stalk or ear rot diseases and insect feeding damage increase (Aguirrezábal, Martre, Pereyra-Irujo, Mercedes-Echarte, Izquierdo, 2014). Broken kernels and fines can create problems during grain storage, with lower quality for many end uses. The grains with a large number of stress cracks are more likely to be broken, produce smaller grits during dry milling, absorb water too rapidly during wet milling, and are more vulnerable to insect and mould damage during storage. The wetter the grain, the lower the temperature must be to maintain a better kernel quality and density (www.pioneer.com/home/harvest/corn/grain-quality. Accessed on December 10, 2015). It has been found with maize in Ghana that for every 1 percent damage above 5 % (damage being grains with insect holes), the value decreases by 1 percent (Golop, Boxal, & Gallat, 2009).

Maize Storage

Storage is the art of keeping the quality of agricultural materials and preventing them from deterioration for specific period of time, beyond their normal shelf life. Different crops are harvested and stored by various means depending on the end utilization. On the other hand, it is an interim and a repeated phase during transit of agricultural products from producer to processor and its products from the consumer. Agricultural products need to

be stored from one harvest to next thus, demanding additional carry over as safeguard against a following crop of low yield or poor quality, against speculation in price and market demand or against shortage and famine.

The objective of grain storage is to maintain the quality of the grain during the storage period, either short-term (i.e., 2-6 weeks) or long-term (i.e., over 4-8 weeks). The quality factors to be preserved depend on the requirements of the end user of the grain. To keep grain in good condition, it should be stored at a relatively low moisture content and cool temperature in order to prevent the development of moulds and insects. The practice of crib storage of ear maize and bag storage of grains still is exercised on smaller farms in many developing countries, but bulk storage is rapidly replacing both methods worldwide (Consultative Group for International Agricultural Research [CGIAR], 1999).

Crib Storage of Maize

The use of wire bin cribs (Fig. 3) for the storage of ear maize is rarely practiced. Ear maize can be safely stored with natural ventilation at 20 to 25 % moisture (wet basis) in temperate climates if excessive foreign matter (i.e., husks and silks) is absent. In warm and humid regions, cribs must permit fumigation to control insect infestation. According to Hall (1957), proper ventilation can generally remove 3 to 5 % excess moisture; therefore, high moisture (>25 % wet basis) ear maize can be stored with mechanical ventilation. An airflow rate of 5.6 to 11.1 $m^3min^{-1}ton^{-1}$ is recommended for the safe storage of high moisture ear maize.

Figure 3: Unshelled maize harvest, stored in three wire bins.

Source : www.123rf.com/photo_3756049. Accessed on January 2, 2016.

Bag Storage of Maize

Bag storage of grains is suitable for small-scale systems in some regions of the world (Lee, Tan, & Seet, 1977). Bag storage has the advantage that the grain can be moved easily, and segregated in individual farmers' lots. Bags may be piled under any shelter and can be handled without special equipment. Bag storage can become overly expensive in locations in which labor costs are high. Bags typically made of woven jute, hemp, local grass, or cotton offer no protection against moisture, insects, and rodents. Polypropylene bags are mechanically stronger and are rodent proof but are expensive and susceptible to deterioration by ultraviolet radiation. Jute bags can be stacked up to a height of 6 m in warehouses, polypropylene bags only to 3 m because of slipping (CGIAR, 1999).

Maize Post-Harvest Losses and Damage

Post-harvest loss can be defined as the degradation in both quantity and quality of a food production from harvest to consumption. Quality losses

embrace those that affect the nutrient or caloric composition, the acceptability, and the edibility of a given product. These losses are generally more common in developed countries (Kader, 2002). Quantity losses refer to those that result in the loss of the amount of a product. Loss of quantity is more common in developing countries (Kitinoja & Gorny, 1999).

Damage is a subjective term referring to the evidence of deterioration, such as infested grains, which may result in a loss and its importance will depend upon the financial status of the consumer. The term "Weight loss" designates the disappearance of food and should be directly measurable in quantitative, qualitative or economic terms. Quantitative is exhibited by reduction in weight (or volume) and qualitative loss is often measured by reference to locally accepted quality standards. Economic loss is the reduction in the monetary value of grain as a result of physical loss or downgrading because of a loss quality (Natural Research Institute [NRI], 2000). Losses occur between harvest and the moment of human consumption. They include on farm losses, such as when grain is threshed, winnowed and dried, as well as losses along the chain during transportation, storage and processing. In Africa the post-harvest level varied from one country to other as show on Fig. 4. The losses incurred at each step vary depending upon the organization and technologies used in the food supply chain. For example, in less developed countries where the supply chain is less mechanized, larger losses are incurred during drying, storage, processing and in transportation (Fig. 5 and 6).

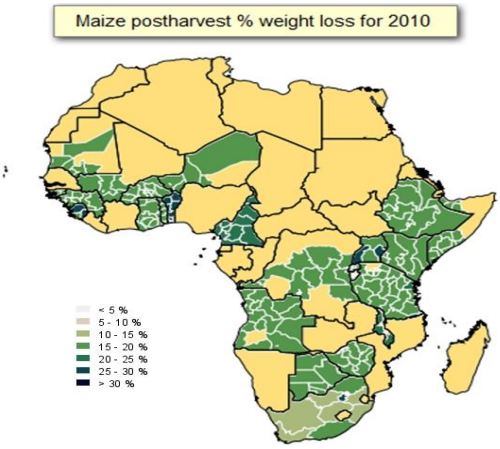

Figure 4: Post-harvest losses map for Africa

Source : (http//www.aphis.net/?from=overview. Accessed on December 31, 20115).

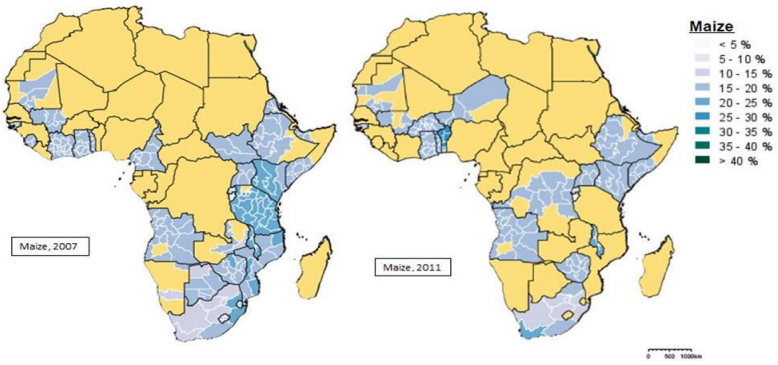

Figure 5: Estimated % cumulative post-harvest weight loss of maize in year 2007 followed by year 2011 by countries. Source: (http://www.aphlis.net. Accessed on December 31, 2015).

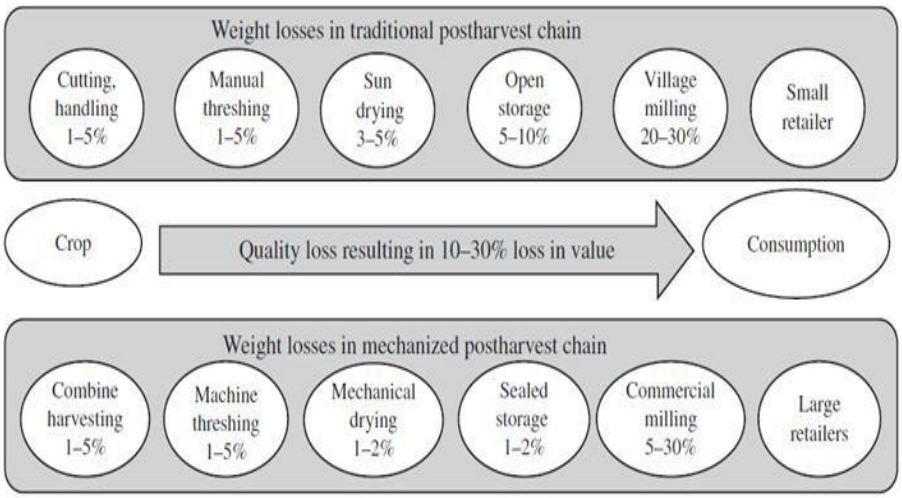

Figure 6: Traditional vs mechanized post-harvest chain (Hodges et al., 2011).

Respiration and Dry Matter Loss

During grain storage, insects, moulds, mites are living organisms and they breathe; during the respiration process, oxygen is consumed and carbon dioxide, water and heat are produced (Bern, Yakubu, Brumm, & Rosentrater, 2013). The carbon dioxide, moisture, and heat produced through respiration of the grain causes an increase in temperature and dry matter loss of the stored grain. Carbon dioxide has been used by many researchers as one way of quantifying the deterioration of maize grain over time (Muir, Waterer, & Sinha, 1985). The carbon dioxide, moisture, and heat produced through respiration Suleiman, Rosentrater, and Bern (2013), of the grain causes an increase in temperature and dry matter loss of the stored grain (Lee, 1999). According to Reed, Doyungan, Ioerger, and Getchell (2007), three different levels of moisture content (low 15.0 %, medium 16.6 % and high 18.0 %) show a gradual increase in moisture content of 15.1 ± 0.01 %, 16.6 ± 0.04 %, and 18.2 ± 0.03 %, for low, medium, and high moisture content maize, respectively. The respiration activity of stored grain is also considerably influenced by the condition, or soundness of the product.

Moulds and Fungi

The major effect of storage fungi on grain are decrease in germination, discoloration, heating and mustiness, dry matter loss, mycotoxin production, and nutritional changes. The importance of these effects, however, depends on the grain's final use. Depending on severity, infestation by fungi can affect grain quality and completely destroy the usefulness of grain. When conditions are right for growth, storage fungi invade the seed embryos preferentially and

sometimes almost exclusively. The storage fungi kill usually the embryos before any discoloration is evident. When germ discoloration is obvious, the seeds are not likely to germinate. Both field and storage fungi can discolor seeds, and when invasion discolors the germ or endosperm, the grain is classified as damaged. Damaged kernels lower the grade of grain in market channels, which can result in considerable financial loss (Christensen & Sauer, 1982).

Mould and fungal species can develop on grains, in the field as well as in storage contamination of maize grain. There are considered as one of the most serious safety problems in the tropical countries and throughout the world (Kaaya & Kyamuhangire, 2006). Toxigenic fungi invading maize are divided into two distinct groups, field fungi and storage fungi (Barney, Price, Sedlacek, & Siddiqui, 1995). Field fungi invade maize and produce toxins before harvest or before the grains are threshed, and can develop under high relative humidity of over 80 %, with moisture content of 22 % to 33 % and wide range of temperature (10 ± 35 °C) Montross. E, Montross. M, & Bakker-Arkema (1999), as shown in (Table 3). These usually die out in storage, but some can live under storage conditions Sanchis, Vinas, Jimenez, Calvo, & Hernandez (1982), cause significant damage reducing the yield and quality, especially in warm humid climates (Moturi, 2008). Conversely, storage fungi invade grain primarily during storage and require moisture content in equilibrium with relative humidity of 70 % to 90 %. In both circumstances, fungi originated from the field. Storage moulds replace field moulds that invade contaminate the maize before harvest (Reed *et al.*, 2007).

Table 3: ***Optimum Conditions for Growth of Common Storage Moulds on Cereal and Grain at 20 °C***

Species	Relative Humidity (%)	Moisture content (%)
Aspergillus Halophilieus	68	Dec-14
Aspergillus Restritus	70	13-15
Aspergillus Glaucus	73	13-15
Aspergillus Candidus,	80	14-16
Aspergillus Flavus	82	15-18
Penicillium spp	80-90	15-18

Source: (Montross *et al*., 1999).

Moulds growing on maize grains present a great threat, especially through production of secondary metabolites (mycotoxins) (Weinberg *et al*., 2008). Mycotoxins are a chronic problem for maize grown in warm, humid, tropical, and sub-tropical regions (Kaaya & Kyamuhangire, 2006). Moulds and fungal infections can result in mycotoxin contamination in all stage from growing, harvesting, storage to processing (Chulze, 2010). The most important mycotoxins that frequently occur in cereal grains are aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone (Pitt, 2000). The two most common and toxic mycotoxin compounds faced on maize in tropical and subtropical regions are aflatoxins and fumonisins (Krska *et al*., 2008).

Insects

One of the major organisms that are responsible for the decline in quantity, quality and germination potential of maize seeds in storage are insect pests. Insect pest infestation accounts for about 20-50 % of all food crop losses (Anankware *et al*., 2013). Effective and adequate storage method of maize grains is therefore a major research thrust for enhanced maize productivity in order to reduce the huge economic loss. Likewise, Boxall (1998) described that a common strategy in many African countries is to sell maize grains

immediately after harvest to avoid losses to insect pests. Insects eat and ruin a lot of grain because they grow inside the grain kernels, some insects are not found in grain until they have done a lot of damages. Insects' damages can be grouped into direct damages and indirect damages. During the direct damages, a) some insects consume germ, some prefer endosperm and the other eat away both. This results in loss of weight, loss or conversion of nutriments, loss of germination potential, loss in gradation and consequently fall in the available market volume. b) The contamination may be with the dead bodies, cast skin, excreta, obnoxious odour and webbings. c) Structure and containers may also be damaged by causing tunneling in wooden parts resulting in the weakening of the structure/container. During the indirect damages, a) it may create heating and migration of moisture. b) It may create distribution of parasites to man. Certain tape worms use stored grain insects as intermediate hosts. c) It causes customer's resistance/repulsion which may lower the prestige (Sahay & Singh, 2001). Maize may be infested with insects such as maize weevils (*Sitophilus zeamais*) before harvest. Without proper management, losses of maize stored by subsistent farmers can be 100 % of the crop. According to African post-harvest losses information system (APHIS), the countries of Sub-Sahara Africa lost in the year 2013 about 17.8 % of the total maize production. In traditional storage systems, losses are usually well contained at about 5 % (Tyler & Boxall, 1984). Insect activity, and the damage which results from this activity are strictly related to temperature and moisture in the stored grain. For example, warm, moist grain provide conducive conditions so that a large number of insects can grow. More insects will produce more heat and water, and they create the right conditions for the growth of moulds.

The most important primary pest insects in Maize in Sub-Sahara Africa are the *Prostephanus truncatus* or Larger Grain Borer (LGB) and the *Sitophilus zeamais* or maize weevil indicated by red color on Fig. 7 (hppt//www.infonet-biovision.org. Accessed on January 2, 2016).

Figure 7: Geographical distribution of the larger grain borer in Africa (Orange marked). Source: hppt//www.infonet-biovision.org. Accessed on January 2, 2016.

Prostephanus truncatus (Fig 8A) is aboriginal to Central America, tropical South America, and the extreme south of the USA as a major, but localized, pest of farm-stored maize. It was first found in West Africa in Togo in 1984 and it has since spread to Benin, Burkina Faso, Ghana, Niger and Nigeria. A separate outbreak occurred in Guinea Conakry. It could potentially invade all maize and cassava growing areas of tropical and sub-tropical Africa, and it is the only recent example of a serious storage pest invading on a regional or continental scale. Adults frequently initiate their attack on stored maize cobs with intact sheaths by boring into the base of the maize cob cores, although they eventually gain access to the grain via the apex of the cob by crawling between the sheathing leaves (Hodges & Meik, 1984). Adult's insect (2-3 mm long, reddish brown in colour with a slim cylindrical shape) bore into

the maize grains, making neat round holes, and as they tunnel from grain to grain they generate large quantities of maize dust. Adult females lay eggs (ovoid in shape, 0.6 mm in length, 0.2 mm in diameter, laid loosely in grains, white when first laid, and turn rose to brown before hatching) in chambers bored at right angles to the main tunnels. Larvae hatch from the eggs after about three days at 27 °C and seem to thrive on the dust produced by boring adults (www.cabi.org/isc/datasheet/44524. Accessed on November 4, 2015). Development of the larva through to the adult stage at the optimum conditions of 32 °C and 80 % relative humidity takes 27 days on a diet of maize grain. Humidity within the range 50-80 % relative humidity does not greatly affect the development period or mortality; at 32 °C, a drop in relative humidity from 80 to 50 % (giving maize with an equilibrium moisture content of about 10.5 %) extended the mean development period by just 6 days and increased the mean mortality by only 13.3 % (Hodges & Meik, 1984). The life cycle of the female is longer (61 days) than the life cycle of the male (45 days) (www.keys.lucidcentral.org. Accessed on October 13, 2015).

Sitophilus zeamais adult (Fig. 8B) is 3 to 3.5 mm long. The eggs hatch in a few days into a soft, white, legless, fleshly grub which feeds on the interior of the grain kernel. The grub changes to a naked white pupa and later emerges as an adult beetle. A minimum of thirty days is required for passing through the egg, larval and pupa stage. Weevils were shown to carry significant *Aspergillus flavus* contamination more than others pest insects responsible on maize damages. The life cycle is 28 days (www.kznhealth.gov.za/environ/vector/maizeweevil.htm. Accessed November 4, 2015).

Sitophilus zeamais are able to increase in number by as much as 100 times in each generation of about five weeks under optimum conditions (NRI, 2000).

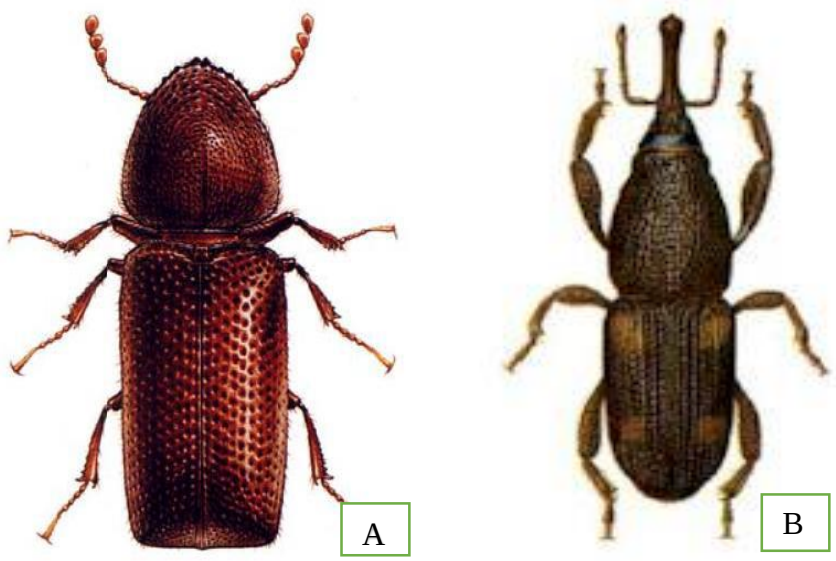

Figure 8: Adults of *Prostephanus truncatus* (A) and *Sitophilus zeamais* (B). Source : http://www.kznhealth.gov.za. Accessed on December 10, 2015.

Farmers and traders of maize respond to storage insect pests by taking protective or avoidance measures. Synthetic insecticides: Actellic Super (Pirimiphos-methyl (1.6 %) Permethrin (0.3 %) and Sofagrain (Pirimiphos-methyl (1.5 %) Deltamethrin (0.5 %) are most commonly used for grain protection. The effectiveness of these insecticides is limited. Even with appropriate application, long term storage may still result in considerable grain damage and dry weight losses. It was related that a weight loss of 7 % and a depression of market value by 27 % following six month storage with Sofagrain (Njoroge *et al*., 2014).

Rodents

Rodents are the most prolific mammals found throughout the word. This group includes rats, mice, bandicoots, hamsters, voles, gerbils, squirrels,

porcupines, jerboas and birch mice, dormice and bamboo rats (Pathak & Kumar, 2000). According to NRI (2000), it seems likely that the house mouse was once a wild species somewhere on the borders of Persia and the USSR, and gradually spread from there with the practice of agriculture some thousand years ago. The most important species are *Ratus ratus* and *ratus norvegicus*. These pests do not only damage the standing crops but also largely the stored grains. Rodents have three major impacts and are major pests in grain stores, causing both direct and indirect effects (Leirs, 1992). The first is the substantial damage they can cause at any stage of the growing crop. The second is the losses they cause post-harvest to stored grain (Fig. 9B) and vegetables. The third, and often overlooked, impact is on the health of smallholder farmers, rodents are carriers of at least 20 severely debilitating human diseases (Meerburg, Singleton, & Kijlstra, 2009).

According to Mdangi *et al*. (2013) more severe rodent damage, weight loss and contamination occurs in open storage structures and improved storage structures can reduce rodent damage to stored maize. The most common causes of damage by rodents in maize storage are (1) eating of the germ of seeds, which reduces the nutritive content and causes germination failure when the seeds are used for planting, and (2) contamination of the grain with faeces (Fig. 9A), hair and urine, which results in lower market values and potential disease transmission (Ministry of Agriculture and Food Security [MoAFS], 1984).

On average, rodents need to consume about 10 % of their weight body per day, but consumption will vary with the size and species of rodent, and with the prevailing climatic conditions. Adult Ratus norvegicus eat about 28 g

of dry food a day. A population of 100 adults would therefore consume just over 1 ton of dry food a year and the saving in grain resulting from rat control measures was sufficient to provide food for 900,000 people (NRI, 2000).

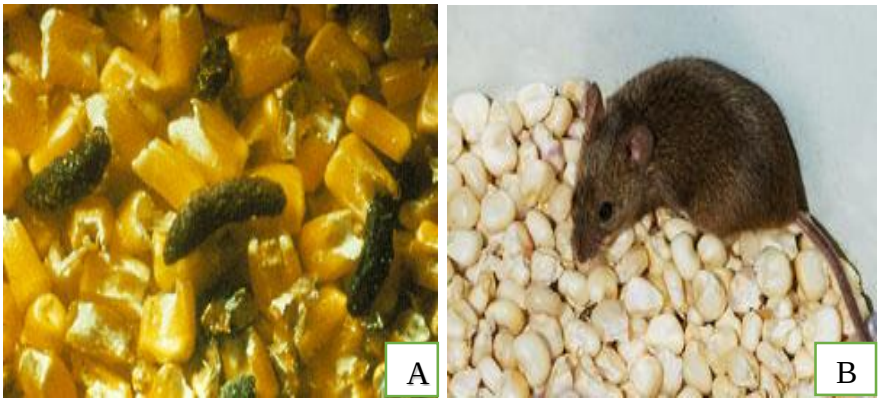

Figure 9: Stored maize grains contaminated by rodent droppings (A) and small rat on maize grain (B).

Source : http://www.dreamstime.com/photos-images/pet-mouse-rodent-animal.html. Accessed March 10, 2016.

Physical Factors Affecting Maize Grain Storage

The three principal physical factors in grain storage are: temperature, moisture content and relative humidity. All three factors have an important effect on storage pests. They are also closely correlated to each other. Temperature and moisture content of the cereal grains are the two key features affecting the resulting quality of the grain, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grain, that because of the permanently exchange between this two factors (Gonzales, Armstrong, & Maghirang, 2009). The optimum temperature range for mould growth is 25-30 °C, and temperatures above 15 °C are ideal for insect growth and reproduction. Insect metabolic activity in dry commodities (below 15 % moisture content) can result in heating up to 42 °C (Mills, 1989).

Temperature

Temperature has an important quantitative effect upon insect development. At low temperature, development of individuals is very slow, mortality is relatively high, and the activity of individual insect is low, as the result the population growth is also low. As temperature increases, the rate of development of individuals increases, activity increases and, as a result the rate of population growth becomes high. All species have an optimum temperature at which population growth is at its maximum. As the temperature increases above the optimum, rate of development and activity of the individual increase, but mortality rises rapidly and rate of population growth falls. There are two temperatures which are important. One is the outside temperature of the air; the other is the temperature of the air and the grain in the storage place. It is easy to store grain in areas where the air temperature is low or never gets too hot. In very cold weather, insects and moulds do not grow very quickly, or at all. In warm places, the grain is warm when it is put into storage. Then, as the outside temperature go up, the temperature in stored grain is likely to get even higher. When the temperature in the grain goes up, certain activities start happening like insects growing and breeding, the mould spore multiplication, as well as insects, and grains all live and breathe faster, causing heat, water, and carbon dioxide to increase in stored grain. Keeping storage containers protected from the hot sun is therefore important. (NRI, 2000).

Relative Humidity

Relative humidity can be termed as the amount of water vapor that is contained in the air, expressed as a proportion of the amount of water vapor

required to saturate the air at the same temperature (Lawrence, 2005). It can be measured using a wet and dry bulb thermometer, which is either whirled around in the air or has air blown over it. The dry bulb measures the temperature of the air, whereas the wet bulb, which has a damp wick around it, is cooled by evaporation of water from the wick to the air and therefore shows as a lower temperature. The extent to which the wet bulb temperature is lowered will depend upon how much evaporation there is from the wick the more evaporation, the lower the temperature. The amount of evaporation that occurs depends upon the relative humidity of the air. If the relative humidity of air is low, i.e. there is not much water in the air, then the evaporation rate will be high and consequently the wet bulb temperature will be low. If the relative humidity is high, the air is close to saturation and little evaporation can take place, so little cooling will occur and the wet bulb temperature will be close to the dry bulb temperature (NRI, 2000). The relative humidity can also be expressed as the ratio of the actual water vapor pressure to the equilibrium vapor pressure over a plane of water (often called the "saturation" vapor pressure) (Suleiman *et al*., 2013).

Moisture Content

The moisture content of grains and other agricultural products play an important role in maintaining the desirable quality of the product. Changes in moisture content of agricultural product occur during their harvesting, processing and marketing. The change in moisture content during successive post-harvest stages is dependent upon the initial moisture content of product and atmospheric conditions. Biological and biochemical activities occur only when moisture is present. Hence, for safe storage of grain, both the moisture

content of the grain and that of the surrounding air should be reduced and monitored (Jayas & White, 2003). Maize grains, like other stored commodities, are hygroscopic materials (i.e. they absorb and release water). They consist of a constant amount of dry matter but water content will vary (Devereau, Myhara, & Anderson, 2008). Moisture content plays a weighty role in the storage of grain; when grain has more moisture, it heats up and can have mould spoilage (Brewbaker, 2003). Living organisms, such as moulds and insects, and thermal heat produced by respiration of the grain itself will enhance water vapor, which in turn will lead to further deterioration of the grain (Freer, Siebenmorgen, Couvillion, & Loewer, 1990). Insect pests of stored grain are adapted to dry conditions: free (liquid) water is usually not available and the moisture content of their food is low (compared with the living plant tissues attacked by pre-harvest pest). The moisture content as well as relative humidity of commodities influence the development, survival and behaviour of storage pests. As a general expression, the higher the moisture content, the more susceptible the maize grain is to mould and insect deterioration. Grain moisture content can be expressed as a percentage of moisture, based on wet weight or dry matter. Wet basis moisture content is generally used (Agricultural Cooperative Development International and Volunteers in Overseas Cooperative Assistance [ACDI/VOCA], 2003).

Interaction of Moisture Content, Temperature and Relative Humidity

The moisture content is the weight of water contained in a commodity, expressed as the percentage of the total weight of the commodity (i.e. dry grain plus water), while the relative humidity is the amount of water vapor contained in the air, expressed as a proportion of the amount of water vapor

contained in the air when it is saturated at the same temperature. Temperature and moisture content act together upon insect pests. Different combinations of the two factors produce conditions that differentially favor species according to their interacting temperature and moisture content optima. In the presence of stored commodity such as grain, a rise in moisture content or temperature causes the relative humidity to rise and vice versa. This is because the grain is hygroscopic and acts as a reservoir of water: when the moisture content or temperature rises, more water is released from the grain into the air and the relative humidity rises, and when the moisture content or temperature falls, water is absorbed into the grain from the air and the relative humidity falls. When they are not changing, the moisture content of the stored products and the relative humidity of the air around them exist in an equilibrium or balanced state which depends upon their temperature. Various commodities have equilibrium moisture

Drying to this moisture content or below will ensure that the commodity will be safe from mould growth. Different stored products have different corresponding equilibrium moisture contents ranging from 65-85 % relative humidity (Table 4). For example, at temperature of 25 °C, the safe moisture content for maize is 14 %. The moisture content of commodities and relative humidity thus influence the development, survival and behavior of storage pests. Moisture content affects the amount of water ingested by insects ant the relative humidity affect the rate at insects lose water to the atmosphere (NRI, 2000).

The growth of moulds in a stored product tends to cause a rise in temperature and moisture content, and therefore relative humidity, due to

mould's respiration and insulating properties of stored commodities. Growth of one particular mould species therefore leads to conditions that are suitable for growth of another, and damage rapidly increase as a series of mould develop. Poor storage conditions therefore risk the growth of not one but many mould species.

Table 4: ***Moisture Contents of Various Grains and Seeds in Equilibrium with Different Relative Humidities at 25-30 ºC***

Relative humidity	Maize and Sorghum	Soybeans	Groundnuts
65%	12.50-13.50	12.50	6.20
70%	13.50-14.50	13.00	7.00
75%	14.50-15.50	14.00	7.90
80%	15.50-16.50	16.00	9.00
85%	18.00-18.50	18.00	10.50

Source : (NRI, 2000).

Equilibrium Moisture Content

Any hygroscopic material (including grain) has its own characteristic balance (or equilibrium) between the moisture it contains and the water vapor in the air with which it is in contact. When maize grains containing certain amounts of moisture are exposed to air, moisture moves from the grain to the air, or *vice versa*, until there is a balance between the moisture in the grain and in the air. This is known as the Equilibrium Moisture Content (E.M.C).

The moisture content of a commodity and its temperature determine the equilibrium relative humidity of the air around the grains. It is by reducing the moisture content of a commodity, thereby reducing its equilibrium relative humidity that drying techniques prevent spoilage of foodstuffs. The moisture content in equilibrium with the relative humidity of 70 % is referred as the

safe moisture content for storage. The equilibrium moisture content playing a decisive role in drying and storage of grains varies slightly with temperature and for most cereals, it will drop by approximately 0.5 % for every 10 °C temperature rise at the same percentage relative humidity of the air (CGIAR, 1999). In grain storage, the air space between kernels in a storage structure of maize will have the humidity indicated at the corresponding moisture and temperature (Table 5). For example, 15 % maize at 60 ºC will generate a relative humidity in the air space between kernels of 70 %, but when cooled to 45 ºC will have a relative humidity of 65 %. Mould growth is inhibited during storage when the environment is retained at a relative humidity level of 65 % or lower.

Table 5: ***Maize Equilibrium Moisture Content***

		Relative Humidity (%)						
		60	65	70	75	80	85	90
Temperature (°C)	21.11	13.40	14.20	15.00	16.00	17.00	18.20	19.80
	23.88	13.10	13.90	14.80	15.70	16.70	17.90	19.40
	26.66	12.90	13.70	14.50	15.40	16.40	17.60	19.10
	29.44	12.60	13.40	14.30	15.20	16.20	17.30	18.80
	32.22	12.40	13.20	14.00	14.90	15.90	17.00	18.50
	35.00	12.20	13.00	13.80	14.70	15.60	16.80	18.20
	37.77	12.00	12.80	13.60	14.50	15.40	16.50	17.90

Source **:** (https://www.uaex.edu/publications/pdf/FSA-1074.pd. Accessed on January 27, 2016).

Interactions Between Temperature and Relative Humidity

Several studies have been conducted to examine the relationship between temperature and relative humidity in grain storage in the tropics, and results have revealed a direct relationship between them, that is, as temperature increases, grain will lose moisture to the surrounding air, thus increasing the relative humidity (Devereau, Myhara, & Anderson, 2002). It has been perceived that in most cereal grains, every 10 °C rise in temperature

causes an increase of about 3 % in relative humidity (ACDI/VOCA, 2003). Shah, Rehman, Kausar, and Hussain (2002), explained that changing temperature and relative humidity not only promotes moulds growth, but also causes considerable nutrient losses of grain. For the case of nutrients reported by Rehman, Habit, and Zafar (2002), after six months of maize storage at 45 °C and 12 % relative humidity, result showed significant decreases in protein soluble sugars, up to 20.4 %. Moreover, according to Samuel, Saburi, Usango, Ikotun, and Isong (2011), even after drying, maize grain harvested in tropical countries retained a certain amount of moisture, and when exposed to air, exchanges of moisture between the maize grains and surrounding occur until the equilibrium is reached. Beside this, fluctuation of temperature and relative humidity in tropical countries accelerates rapid proliferation of moulds and insects, which facilitate further damage of grain (Yakubu, 2009).

Aflatoxin Contamination of Maize

One of the major food safety hazards associated with maize is from the mycotoxins that are produced by many species of fungi which contaminate maize during pre and post-harvest periods. Currently the primary mycotoxin fungi of concern in the Sub-Saharan Africa' maize value chain are aflatoxins which are toxic metabolites produced by fungal species during their growth under favorable conditions of temperature and moisture. A large number of pathogenic fungi, bacteria, viruses and insects infecting and infesting maize grain cause combined worldwide annual losses of 9.4 % (Varga, Tóth, & Téren, 2005). Fungi affect the quality of grain; as a result, there will be increase in fatty acid, reduction in germination, increase its mustiness, production of toxins and finally leading to spoilage of grain in many ways.

Fungi are the second important cause of deterioration and loss of maize next to insects. Fungi could cause about 50 % to 80 % of damage on farmers' maize during storage if conditions are favorable for their development (Khosravi *et al*., 2007).

Aflatoxins are a group of structurally related toxic secondary metabolites produced mainly by certain strains of *Aspergillus flav*us and *Aspergillus parasiticus. Aspergillus flavus* particularly, is a common contaminate in agriculture (Bhatnagar, Cotty, & Cleveland, 2001; Bennet and Klich, 2003). Aflatoxins were discovered in the early 1960s and family of toxic compounds Wild and Turner (2002) and the name aflatoxin is derived from (***A****spergillus* ***fla****vus* **toxin**) and was derived from a toxin producing fungus which caused a disease referred to as 'Turkey X disease' in England in 1960 which resulted in the death of 100,000 young turkeys (Bullerman, 1999). The most important aflatoxin producing species are *Aspergillus flavus* and *Aspergillus parasiticus*. The main cereals affected are maize, sorghum, rice and wheat and other crops like groundnuts and cassava.

Grains (cereals and oilseeds) and nuts in general are subject to mould attack at pre-harvest and post-harvest times. Among moulds that can attack these foods, *Aspergillus flavus*, and *Aspergillus parasiticus* are important because they can produce aflatoxins that are considered a potent natural toxin (Wild & Gong, 2010). Aflatoxin can be produced principally by different *Aspergillus* species (Fig. 10), but *Emiricella* and *Petromyces* have been reported as aflatoxin producers (Frisvad, Skouboe, & Samson, 2005). Aflatoxin contamination has been reported for grains such as maize, soya,

wheat, rice, and cottonseed, and nuts such as peanuts, cashew nuts by producing spores (Gürses, 2006).

Figure 10: Maize cob infected by *Aspergillus flavus* (http//www.aspergillusproject11.wordpress.com. Accessed on December 31, 2015).

Contamination can occur any time from pre-harvest to storage. Pre-harvest infection is substantial in the semi-arid tropics, especially when end of season drought occurs. Poor post-harvest conditions in warm moist area, and bad harvesting and storage practices lead to fast development of the fungi and higher levels of toxins (http://www.icrisat.org/aflatoxin/food_security.asp. Accessed on December 10, 2015).

In West Africa the main vulnerable crops are maize (*Zea mays*), groundnut (*Arachid hypogaea*), and tree nuts (Cardwell & Henry, 2004). Maize has been studied most intensively with respect to infection by primary inoculum in soil (Horn, 2007). Aflatoxin contamination of maize occurs all-inclusive (Payne & Widstrom, 1992). The occurrence of *Aspergillus flavus* in field maize was reported in 1920 (Taubenhaus, 1992). Aflatoxins can be produced in pre-harvest as well as during maize storage (Marsh & Payne,

1984; Hell, Cardwell, & Poehling, 2003). Contamination of maize by *Aspergillus flavus* are complex and include colonization of silks as well as wounding of kernels by insects (Marsh & Payne, 1984; Brown, Cotty, Cleveland, & Widstrom, 1993). In nature, *Aspergillus flavus* can directly infect maize kernels under drought stress and high temperature (32 to 36 °C) known to compromise the hot's physiological defense systems as well as cause cracks in the seed (Payne, Thompson, Lillehoj, Zuber, & Adkins, 1988). Colonized waste maize kernels and cobs that overwinter following harvest also serve as important sources of maize infection due to wind dispersed conidia (Olanya, Hoyos, Tiffany, & McGee, 1997; Jaime-Garcia & Cotty, 2004).

Other food products, derived from areal parts of plants (maize, cottonseed, tree nut) have a pattern typical for Aspergillus flavus. As a result, over 90 % of the contaminated maize samples only contain AfB_1 and AfB_2 (Moss, 1989). Aflatoxins contamination having been observed in several foodstuffs, the contamination of maize, peanuts, and oilseeds can be considered, in terms of diet exposure, the most important worldwide (Ding, Li, Bai, & Zhou, 2012).

In post-harvest, factors such as temperature, availability of water, oxygen, and carbon dioxide, insect and rodent's infestation, incidence of broken grains or nuts, the cleaning of the product, toxigenic fungal load, microbial competition, antifungal compound presence, and substrate composition are important too. Transport, waiting time for drying, drying system (temperature and drying rate), and storage conditions can affect these factors during the post-harvest period (Dorner, 2008). African communities

and populations are exposed to aflatoxins before birth and throughout their lives with serious impact on their health (Williams *et al.*, 2004). It was revealed that aflatoxins can be found in significant fractions of different population. Studies carried out in Africa indicated that approximately 12 to 37 % of African population has measurable amounts of aflatoxin in the blood serum (Bullerman, 1999). The daily intake of aflatoxins was estimated to be 2.7 ng kg^{-1} body weight day^{-1} for US citizen, it was up to 220 ng kg^{-1} body weight day^{-1} for African (Smith, Lewis, Anderson, & Solomons, 1994). In 1981, the maximum limit for aflatoxin in food (aflatoxin B) or the sum of aflatoxins B_1, B_2, G_1 and G_2 varied from zero to 50 parts per billion (ppb) (Jewers, 1987), and the contamination level limit in food for human consumption is 20.

Aflatoxin levels beyond certain concentrations lead to various health problems in humans and animals. Kumar, Reddy, Waliyar (2000) list the general levels permitted and the consequences of ingestion beyond those levels as in Table 6.

Table 6: Aflatoxin Ingestion Limits and Health Effects

Aflatoxin level (parts per billion)	Limitation/consequences
20	Highest level allowed for human
50	Highest level allowed for animal
100	Slowed growth of young ones
200-400	Slowed growth for adults
Beyond 400	Liver damage and cancer

Source: (Kumar *et al.*, 2000).

Infection is most common after the kernels have been damaged by insects, birds, mites, hail, early frost, heat and drought stress, windstorms, and other unfavorable weather. The presence of *Aspergillus flavus* or *Aspergillus*

parasiticus in a given feed sample does not mean that aflatoxins are present, but the presence of the toxigenic fungi does increase the risk for aflatoxin production. Aflatoxins persist under the majority of environmental conditions, and aflatoxins are not destroyed during feed manufacturing processes. Pelletizing feeds may eliminate fungi presence in the stock, but will not reduce or eliminate aflatoxin present in any of the ingredients. Food processing and baking does not destroy aflatoxins. Aflatoxins are not destroyed during alcohol production, and on a dry matter basis, aflatoxins are concentrated in stillage and distillers soluble (Barry *et al.,* 2007).

Several technologies have been tested in Africa to reduce mycotoxin risk. Field management practices that increase yields may also prevent aflatoxin. They comprise use of resistant varieties, timely planting, fertilizer application, weed control, insect control and avoiding drought and nutritional stress. Other options are used to control the toxin causing fungi *Aspergillus flavus* contamination in the field. Among them are toxigenic fungi and timely harvest. Post-harvest interventions that reduce mycotoxins are rapid and proper drying, sorting, cleaning, drying, post-harvest insect control, smoking and the use of botanicals or synthetic pesticides as storage protectant. Another approach is to reduce the frequent consumption of 'high risk' foods (especially maize and groundnut) by consuming a more varied diet, and diversifying into less risky staples like sorghum and millet (Hell *et al*., 2010).

Chemical Structures of Aflatoxins

A potent hepatotoxic and hepatocarcinogenic mycotoxin produced by the *Aspergillus flavus* group of fungi. Aflatoxin B_1 is the most hepatotoxic and hepatocarcinogenic of the aflatoxins and occurs as a contaminant in a variety

of foods(www.pubchem.ncbi.nlm.nih.gov/compound/aflatoxin Accessed on June, 21 2016). The chemical structures of some aflatoxins are shown in Fig. 11.

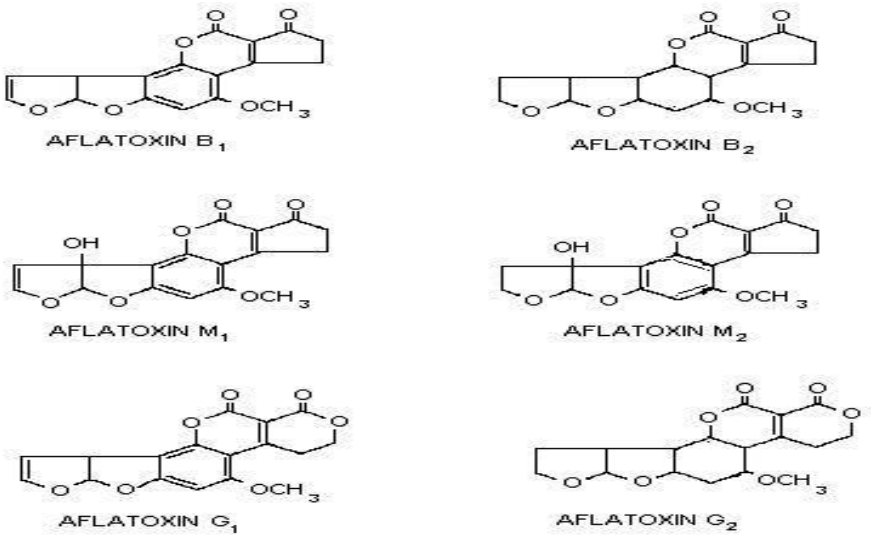

Figure 11: *Chemical structure of aflatoxins* (Cole & Cox, 1981).

Properties of Aflatoxins

Hell (1997) stated that four major groups of aflatoxins are identified: B_1, B_2, G_1 and G_2 as shown in the Table 7. These abbreviations are indicative of the colors these exhibit/fluorescence under the ultraviolet light (385 nm); thus B is for blue and G is for yellow-green. Among these toxins, the Aflatoxin B_1 (AfB_1) being the most abundant toxic metabolite in the group (Bullerman 1999). They do not seem to have physiological functions for the fungus they are now recognized as potential carcinogens, teratogens, mutagens, immune-suppressants and have eostrogenic effects in humans (Kang'ethe, 2011).

Table 7*: The Properties of Aflatoxins*

Property	*Aspergillus flavus*			
	B1	B2	G1	G2
Chemical formular	$C_{17}H_{12}O_8$	$C_{17}H_{14}O_8$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$
Molecular weight	312	314	328	330
Melting point (°C)	268-269 D)[1]	287-289 (D)	244-249 (D)	230
Flourescence	425 nm	425 nm	450 nm	425 nm

D[1] = Decomposition, Source: Cole and Cox (1981).

Life Cycle of *Aspergillus flavus*

The fungi of *Aspergillus* section *Flavi* are one of the most abundant and widely distributed soil borne moulds and can be found anywhere on earth (Yu, Cleveland, Nierman, & Bennett, 2005). *Aspergillus flavus* is a saprophytic fungus that is capable of surviving on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insects and animal carcasses, outdoor and indoor air environments, stored grains, and even on live humans and animals (Klich, 1998).

The life cycle in agricultural fields can be divided into two stages as shown on Fig.12. The first is the colonization of plant debris in soil and the second is the invasion of seeds and grain in actively growing crop plants (Horn, 2007). Soil serves as a reservoir for primary inoculum of *Aspergillus flavus* and *Aspergillus parasiticus* (Horn, Greene, & Dorner, 1995; Payne, 1998). *Aspergillus parasiticus* appears to be more adapted to a soil environment, being prominent in peanuts, whereas *Aspergillus flavus* seems

adapted to the aerial and foliar environment, being dominant in maize, cotton seed, and tree nuts (Diener *et al.*, 1987).

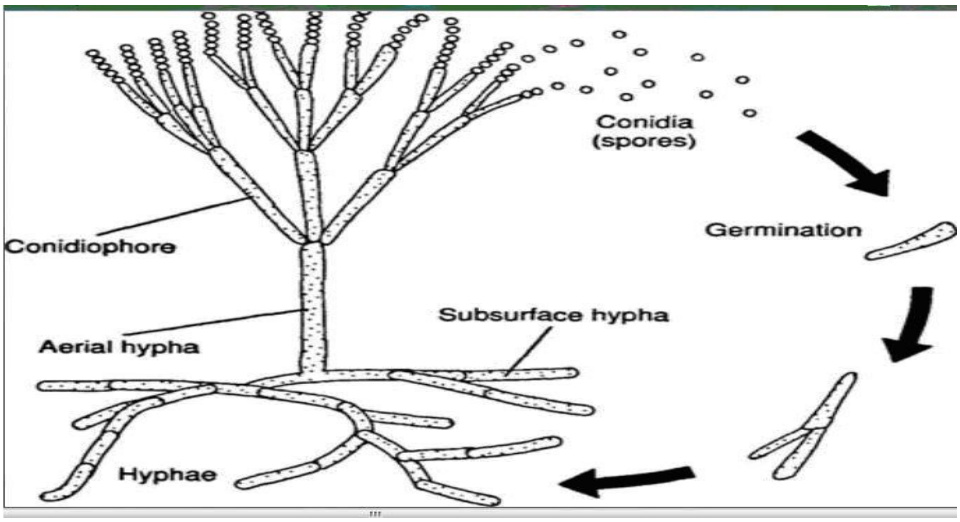

Figure 12: Life cycle of *Aspergillus flavus.*

Source : (www.aspergillusproject11.wordpress.com. Accessed on December, 2015).

Under adverse conditions such as dry and poor nutrition, the mycelium congregates to form resistant structures called sclerotia (Yu *et al.*, 2005). Sclerotia are pigmented, compacted aggregates of hyphae, which resist unfavorable environmental conditions and are capable of remaining dormant for long periods (Wicklow *et al.*, 1983; Cotty, 1988; Rollins and Dickman, 1998). The fungus overwinters either as mycelium in plant debris and litter on the soil, on insects or as sclerotia in the soil (Diener *et al.*, 1987). When the growth conditions are favorable the sclerotia either germinates to produce additional hyphae or they produce conidia (asexual spores), which can be further spread in the soil and air (Bennett, Phaik-Mooi, Kruger, & Keyes, 1986; Cotty, 1988). The fungus mostly exists in the form of mycelium or asexual conidia spores.

Aflatoxin contamination (Fig. 13) can be divided into two distinct phases with the infection of the developing crop in the first phase and increase

in contamination after maturation in the second phase (Bhatnagar, Cotty, & Cleveland, 2001). Both phases contribute to many contamination events, weather influences the two phases of contamination differently (Cotty and Jaime-Garcia, 2007). During the first phase of contamination infections by *Aspergillus flavus* and *Aspergillus parasiticus* of susceptible crops are promoted due to wounding of developing crops by birds, mammals, insects, mechanically or drought stress and elevated temperatures (Dowd, 1998; Payne, 1998; Guo, Sobolev, Holbrook, & Lynch, 2002). Its capability to attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground, demonstrates that this fungus has evolved a battery of mechanisms to breach the host's resistance (Yu *et al*., 2005). Conidia of plant, insect, and human derived strains of *Aspergillus flavus* rapidly colonize leaves, kernels, and insects injured during inoculation but do not affect uninjured plant or insect material (Leger, Screen, & Shams-Pirzadeh, 2000).

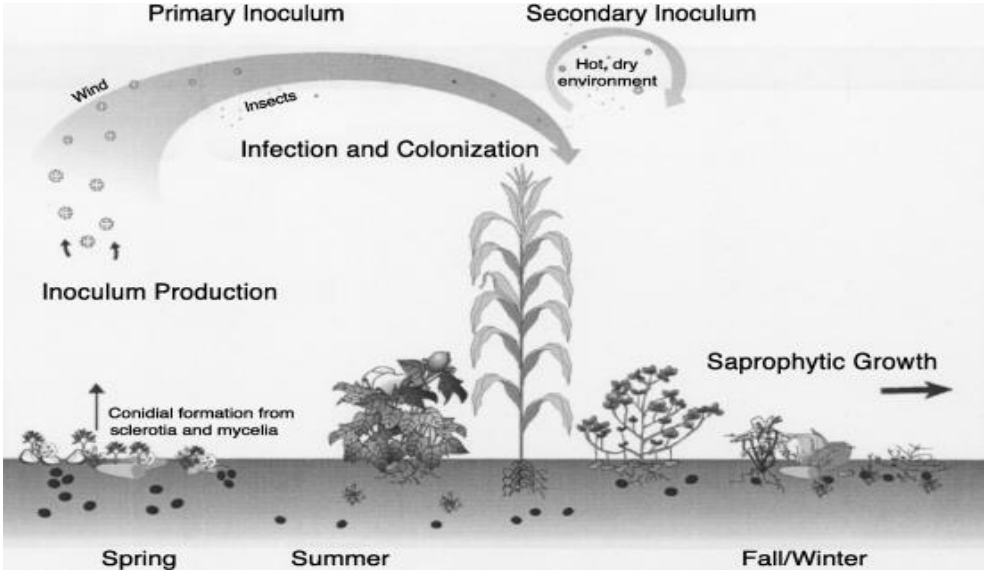

Figure 13: Diagram of the preharvest infection of maize by *Aspergillus flavus.* Source: (Scheidegger & Payne, 2003).

Influence of Insect Infestation on Aflatoxin Contamination

Maize grain is subject to numerous fungi infestation during the growth and storage period. Fungal growth can lead to reduction in the quantity and weight of grain, deterioration in the quality of produce and in food value, and the development of mycotoxins (Lubulwa & Davis, 1994). *Aspergillus flavus* produces the most potent natural toxin and its contamination of food is a serious health hazard to humans and animals (Diener *et al.*, 1987; Cardwell & Miller, 1996). Insect damage and aflatoxin contamination are positively correlated (Bowen & Mack, 1991; Lynch &Wilson, 1991; Lynch *et al.*, 1991; Gorman & Kang, 1991). These insects consumed *Aspergillus flavus* spores, deprived of detrimental effect to themselves (Wicklow, 1988). Feeding by insects breaks the pericarp, rendering grain more vulnerable to invasion by storage fungi, including *Aspergillus flavus* (Tuite, Koh-Knox, Stroshine, Cantone, & Bauman, 1985; Barry *et al.*, 1992). *Aspergillus* spores have been isolated from the bodies of maize weevil (McMillian, 1987; McMillian, Widstrom, Wilson, & Evans, 1990). Hell *et al*., (2000), reported that in 1993, no aflatoxin was detected in maize that was free of insect damage. In the same year, in maize with more than 70 % of cobs damaged by insects 30.3 % were aflatoxin positive, with a mean aflatoxin contamination of 77.8 ppb (parts per billion or µg/kg).

There is little information relating beetles and borers, to an increased risk of fungal infection and subsequent aflatoxin development in Sub-Sahara African countries. A survey conducted in Kenya investigated microorganisms present on stem and cob-borers, 7 % of the larvae were infected with *Aspergillus* fungi (Odindo, Otieno, Oloo, Kilori, & Odhiambo, 1989). In

Benin the cob-borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) was found to be significantly correlated to *Aspergillus flavus* infection and aflatoxin contamination in the field (Sétamou, Cardwell, Schulthess, & Hell, 1998).

Principal Storage Systems of Maize

According to Bern *et al.* (2013), drying maize to 14 % moisture or less is necessary to avert growth of fungi. Storage in a secure container can prevent losses from rats and birds. Maize may be infested with insects such as maize weevils before harvest. Without proper management, losses of maize stored by subsistent farmers can be 100 % of the crop. The objective of grain storage is to maintain the quality of the grain during the storage period either short-term (2-6 weeks) or long-term (4-8 weeks). The quality features to be preserved depend on the requirements of the end user of the grain. To keep grain in good condition, it should be stored at relatively low moisture content and cool temperature in order to prevent the development of moulds and insects. To be able to achieve these objectives, the store must satisfy the following parameters as far as possible:

a. The grain must be kept dry;

b. The grain should be kept at a uniform temperature;

c. The grain should be protected from insect attack and

d. Rodents and birds should be excluded (Mrema, Gumbe, Chepete, & Agullo, 2011).

Non-Hermetic Storage

Non-hermetic storage allows the air exchange between the commodity on storage and the external environment. The atmospheric gases (O_2 and CO_2) concentration are not modified or controlled and allow microorganism proliferation causing damage to the storage commodity. Maize storage and pest control method are adept in traditional clay granaries by farmers from Sub-Sahara African region. They also use pesticides to control pests and diseases during maize storage period with satisfactory results using this method**.** Despite these vast use and benefits of chemicals, they can cause and involve some health and environmental hazards. Examples of problems associated with them are diseases like cancer, kidney, and acute poisoning or cause environmental problems like ozone depleting, effect on terrestrial and aquatic animals, contamination on environmental media (air, water, food, land). All these are making clear to everyone that "chemical safety a national challenge" is not an empty phrase (Abdel, Azhari, Ahmed, Elhindi, & Ali, 2006).

The practice of crib storage of ear maize, the metal silos, granaries, baskets or earthen pots and bag storage of grains still is implemented on smaller farms in many developing countries, but bulk storage is rapidly replacing both methods worldwide. In order to reduce the losses incurred after harvesting, farmers take measures such as sufficiently drying maize before storage, using storage structures which are moisture proof and are adequately aired. Farmers also store their grains in the living houses, which are perceived to be secure as grain losses through theft are minimized. In addition to the use of traditional storage structures, farmers use other integrated storage strategies

aimed at reducing post-harvest losses based on traditional knowledge. These include the use of herbs like the Mexican marigold and hot pepper in storage, selling grain soon after harvest and cleaning or dusting the storage structure with pesticide thoroughly before depositing the maize or acquire the new maize storage technologies (Bett & Nguyo, 2007).

Modified Atmosphere Storage

Modified atmosphere (MA) is proposed as the general term, including all cases in which the composition of atmospheric gases or their partial pressures in the treatment enclosure have been modified to create conditions favorable for the control of storage insects. A type of (MA) that can be applied for the safeguard of grain is hermetic storage, also termed "sealed storage" or "airtight storage" or "sacrificial sealed storage". This method takes advantage of adequately sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the modified atmosphere by reducing oxygen (O_2) and increasing carbon dioxide (CO_2) concentrations through respiratory metabolism. Respiration of the living organisms in storage (insects, fungi, and grain) consumes oxygen (O_2), reducing it from near 21 % in air to 1 to 2 %, while production of carbon dioxide (CO_2) rises from an ambient 0.035 % to near 20 % (White & Jayas, 2003). This environment kills insect and mite pests and prevents aerobic fungi from growing (Weinberg *et al*., 2008). Elevated CO_2 and depleted O_2 levels will generally maintain stored grain quality for long periods of time (White & Jayas 1993).

Effective low-cost non-chemical grain protection technologies have the potential for tremendous impact in SSA. Hermetic grain storage has been used since ancient times for grain preservation (De Lima, 1990). Storage of maize

under airtight conditions was shown to effectively control *Sitophilus zeamais* under laboratory trials (Yakubu *et al*., 2009). According to Hell *et al*. (2010) there is an enormous reduction in grain weight losses from 18 % in ordinary polypropylene bag to 0.3 % when maize was stored in hermetic bags for six months, while Ognakossan, Tounou, Lamboni, Hell (2013) have reported a weight loss of 6 % in Benin following five month storage. The effectiveness of hermetic storage depends primarily on the hermetic seal, the commodity stored, agro-climatic conditions, type and prevalence of insect pests, and mechanical strength of the barrier material. Prostephanus truncatus is quite versatile and is known to attack storage facilities and structures (Borgemeister, Holst, & Hodges, 2003).

Hermetic storage isolates the storage ecosystem from the external environment while respiration within the storage ecosystem causes O_2 reduction and CO_2 accumulation, leading to suffocation and dehydration of weevils (Navarro *et al*., 1994). This means that as a result of respiration effects, there generally develops a very low oxygen and high carbon dioxide. The low penetrability envelope maintains a constant moisture environment. Pioneering modern hermetic storage, has resulted in the broad use of safe, pesticide-free hermetic storage suitable for many commodities and seeds, particularly in hot, humid climate (Navarro, Donahaye, Caliboso, &. Sabio, 1989). These hermetic storage systems are used primarily in Africa, Asia, South and Central America for a growing variety of both high and medium value commodities. According to Navarro, (2006b), these methods create a low oxygen modified atmosphere which normally results in 100 % insect mortality (maize weevils) of all life stages in a few days to two weeks as well

as preventing mould development, protecting quality and preventing losses in the commodity. However, some hermetic storage systems are discredited because *Prostephanus truncatus* is able to burrow through from inside or outside breaking the hermetic seal, hence allowing both *Sitophilus zeamais* and *Prostephanus truncatus* to multiply freely (Hell *et al*., 2010; Popoola, 2012; Ognakossan *et al*., 2013).

According to Villers *et al*. (2006), hermetic storage prevents development of cancer causing mycotoxins such as aflatoxins and ochratoxin A (OTA). It is also used to prevent rodent penetration during storage, and prevent the growth of moulds as well as deterioration of the commodity by protecting it from the high outside relative humidity levels that prevail in hot humid climates.

In some applications, such as for rice bran, brown rice, peanuts, and cocoa beans the quality loss due to increase of Free Fatty Acids (FFAs) is prevented through a low oxygen environment (De Bruin & Murali, 2006). The principal reasons for using hermetic storage for grains is to prevent further insect development, by creating a low oxygen, high CO_2 atmosphere lethal to insects already present inside the container. It is also used to prevent rodent penetration during storage, and prevent the growth of moulds as well as deterioration of the commodity by protecting it from the high outside relative humidity levels that prevail in hot humid climates (Villers *et al*., 2006). In the case of seeds, preserving seed viability and vigor is the dominant consideration.

Hermetic storage of seeds show large differences over conventional unrefrigerated bagged storage in retention of germination and vigor when

stored in hot humid climates (De Bruin, 2005). It is used for medium to long term storage of conventional grain bags. Bulk products such as maize, beans, sorghum, rice, wheat, barley, as well as seeds can be stored in silos. According to Bern *et al*. (2013), the product must be clean and dry (below 14 % moisture for maize) before being placed in the silo to prevent fungal spoilage. If clean maize at 14 % moisture or below is placed in a silo and managed properly, losses during one year or more of storage will be near zero. If the silo is filled with maize and hermetically sealed, maize weevils and other insects will be kept under control.

According to Baributsa (2011), the Purdue Improved Crop Storage (PICS) technology was developed for storage of cowpeas in West and Central Africa using plastic bags to accomplish hermetic storage of cowpeas and other grains. It is composed of three layers. A first bag is filled completely, but with a 20 to 30 cm neck, which is tied securely. Then, this bag is surrounded by a second bag with the same thickness. The second bag's neck is also tied securely. Finally, these two bags are placed in a third woven nylon or polypropylene bag used for its strength. With the third bag tied securely, the container can be handled without bursting the inner bags, and is readily accepted by grain handlers who are accustomed to handling cowpea in this type of woven bag. Nowadays, the plastic barrel is used to safeguard the maize grain against damage caused by rodents. The rat problem was solved by placing the bag containing maize inside a plastic barrel (Dowell, 2011).

Controlled Atmosphere

Controlled atmosphere (CA) storage involves changing the composition of the gas in the store so that it is different to that of atmospheric air or most if not all the storage period. In other words, CA includes placing the commodity in a very well-sealed store, allowing the products of grain respiration to increase CO_2 levels, thereby overturning insect development (NRI, 2000). These set concentrations are maintained for the time necessary to control the storage pests. Navarro *et al*. (2012b) reported that a widely used source for production of such atmospheric gas compositions is tanker delivered liquefied CO_2 or N_2, when the target controlled atmospheric gas composition is < 1% O_2 or high CO_2 concentration. For large scale application of N_2 or CO_2, vaporizers are essential. These vaporizers consist of a suitably designed receptacle with a heating medium (electricity, steam, diesel fuel, or propane), a super-heated coil with hot water jacket, and forced or natural draught.

According to Jay (1971), N_2 and CO_2 have been used as agents for controlled atmospheric storage for many years. Carbon dioxide has been considered to be more efficient than N_2 due to the concentrations necessary for control and the level of gas tightness of the structure being used. A CO_2 concentration of about 60 % can provide 95 % control insect pests of most stored products at 27 °C, while N_2 use requires interstitial O_2 levels to be reduced to 1 % or less. Mann, Jayas, Muir, &. White (1999) revealed that CO_2 generated from dry ice and circulated with a vacuum pump at a concentration of 51 % caused 100 % mortality of *Cystobacter ferrugenius* after 10 days at 20 °C. Carbon dioxide can also be added to bulk stored products as compressed

gas. White and Jayas (1991) also demonstrated that by circulating CO_2 released from compressed cylinders, high mortality of several stored product arthropod pests could be achieved within 14 days. Banks and Annis (1980) establish that bin sealing was crucial to maintain efficacy especially when commodity temperature fell below 20 °C, and that utilizing pressure testing techniques which is a useful means of determining a bin's seal. A recent development has been reported by Clamp and Moore (2000), in which N_2 supplied as a bulk liquid under pressure was used to treat 1,800 tons' bins. Nitrogen also can be easily generated using molecular membrane generators and these are capable of purging vertical grain storages of 120 tons' capacity within 3 hours (Timlick, Dickie, & McKinnon, 2002; Navarro, 1978) stated that insects can tolerate low levels of oxygen for prolonged periods and using N_2 to replace O_2 must result in O_2 being below 2 %, preferably 1 % for rapid death.

Fumigation

According to NRI (2000), fumigation is a method of killing insect pest in storage products using poison gas and does not include the use of insecticidal sprays, vapors, and smokes. Fumigation is a very specific operation in which a gas is held in an airtight enclosure for a set period of time. This technique when applied correctly can kill all insects, mites and rodents within the gastight enclosure. Fumigant gases are able to pass into the bag, and even into the grain itself to kill insects developing inside. Therefore, it provides no lasting protection as soon as grain is no longer in the gas-tight enclosure may become re-infested.

The reason why fumigation cannot be used as the only means of pest control is that it needs to be linked to a wider pest management strategy that limits the opportunity for re-infestation. Fumigation is a very expedient pest control technique as grain can be treated without undue disturbance. Grain can be fumigated wherever it is stored provided that it can be sealed to give sufficient gas tightness. According to Golop *et al*. (2009), the gas is lethal even in low concentrations to humans and livestock. Gas is released from the tablets within 20 minutes in some climates. But, leakage of gas is extremely dangerous for the farmer's family and animals and does not kill insects in the grain. To protect themselves against fumigation gas effects, farmers may be able to take their produce to a fumigation centre where it can be fumigated under controlled conditions by trained personnel who can guarantee that the grain will be free of insects after treatment.

The three most common gases that can be used as a fumigant are: phosphine, methyl bromide and carbon dioxide. For fumigation to be successful, recommended concentration of gas and the length of time must be followed and are interchangeable. For example, if fumigation time is short, high fumigant concentration must be used, while if fumigation time is long, low concentration of fumigant must be used (Lilford, Fulford, Schlipalius, & Ridley, 2009).

Hermetic Seed Storage

Storage of seed grain requires conditions that will not only maintain peak viability but will avoid also all possibility of germination while in storage. High moisture content and low oxygen may decrease viability and

therefore should be avoided for seed storage. At the same time, to avoid any danger of germination or fungal and insect damage while in storage, seed should be dried 1-2 % more for human consumption. Additionally, it is important to keep the temperature of the seed as low as possible for good conservation of the viability (www.fao.org/docrep/015/i2433e/i2433e10.pdf. Accessed on January 2, 2016). The vast common of seed utilized by small scale farmers in Sub-Sahara African countries, especially cereals, are produced and stored on farm. Major problems such as mould and insect damage can be avoided and higher seed quality retained through embracing more effective storage strategies such as hermetic storage technologies.

Hermetic storage is a technology that enables farmers to store their own seed for long periods without loss due to insects and without using any insecticides. The technology consists of enclosing seed in airtight containers that prevent or reduce gas exchange. Insect aerobic respiration depletes O_2 and increases CO_2. Insect feeding ceases, and therefore insects begin dying (Murdock, Margam, Baoua, Balfe, & Shade, 2012). Additionally, hermetic storage can impede the growth of fungi as these organisms also need oxygen to proliferate (Quezada *et al*., 2006). This technique can maintain seed quality for up to one year of storage and both locks in and locks out moisture hence, adequate drying of seed prior to storage must be a challenge. Seed must be dry prior to storage, approximately 12-14 % moisture and high moisture contents in hermetically stored grain such as maize can lead to loss in germination and viability hence dryness must be ensured (Weinberg *et al*., 2008). Hermetic storage can also keep seed dry in the event of flooding. Hermetic storage

works by allowing the insect to naturally respire and exhaust oxygen level in an airtight environment to the point where they cannot survive.

Three types of hermetic seed storage containers are promoted for use by small-holder farmers. These include locally available containers, Purdue Improved Crop Storage (PICS) triple layer sacks Baributsa, Baoua, Lowenberg-DeBoer, Abdoulaye, & Murdock (2012), and GrainPro Super Bag (Villers, Navarro, & De Bruin, 2008). GrainPro Super Bags are sold as a single polyethylene liner with a proprietary formula, for which farmers must generally purchase the necessary woven sack for reinforcement. Unlike local woven bags which simply "organize" grain without providing protection against insects, hermetic bags provide full protection against insects without the need for any additional treatment. The most common locally available containers include simple water bottles and recycled vegetable oil containers. The 5 and 20 liters' vegetable oil containers are quite popular in villages throughout Africa and are typically used to store water and local beverages and can provide a hermetic seal. Purdue Improved Crop Storage and GrainPro sacks come in 50 and 100 kg sizes (Baributsa *et al*., 2012).

Viability of Maize Seed

Viability of seed is defined as the ability of the seed to develop into a young plant under favorable growing conditions and is expressed in percentage. The viability of a sample of grain is of interest principally if the grain is to be used for seed, hence viability is too stringent a measure for the quality of grain. Seed grain usually is marketed with a minimum viability (CGIAR, 1999). Seed maize for International Seed Testing Association has a

viability of at least 75 % (International Seed Testing Association [ISTA], 2001).

According to Navarro, Donahaye, Caliboso, &. Sabio (1998), seeds below their critical moisture content are not significantly affected at high CO_2 or low O_2 atmospheres but increasing grain moisture contents, CO_2 rich atmospheres could reduce the physiological quality of grain by interfering with the enzymatic activity of glutamine decarboxylase. They reported that, to preserve a good germination potential, low O_2 atmospheres is preferred if expected temperatures are significantly above 30 °C than the use of CO_2-free. Viability of maize stored under hermetic (148 days' storage) and non-hermetic (120 days' storage) conditions did not indicate significant changes between the initial and final samples (Navarro & Caliboso, 1996).

Challenge in Maize Storage

The main objective of storing grain in a proper way, is to maintain throughout the whole storage period, the biological, chemical, and physical characteristics that the grain possessed immediately after harvest. One of the most acute physiological factors that determines a successful grain storage process is its moisture content. A high moisture content leads to storage problems because it creates a favorable condition for mould and insect growth, an increased grain respiration rate and reduction of the viability during storage. One of the products of respiration is heat, and reduction of temperature of the harvested crop can help reduce the rate of respiration and thus increase the storage life of the product. Also, lowering the temperature reduces insect and mould activities, and subsequently the rate of spoilage is

reduced. Intense insect and mould growth are recognized as the major problems preventing successful grain storage in the tropics (CGIAR, 1999).

Storage of harvested maize in West Africa is generally done by farmers. This takes place at the farm gate or the village level. Many challenges are encountered during storage. These are mainly due to the lack of good storage knowledge or practices, among others. Farmers have to grapple with losses in quantity and quality by way of moisture content, insect infestation and rodent attack. Improper storage, such as open air storage in granaries leads to rapid insect growth and damaged stored maize resulting in post-harvest storage losses (Caddick, 2007). Studies have shown that the combined effect of maize weevils and moulds is capable of causing up to 100 % damage to stored maize (Demissie *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

Plant Materials

Obatampa variety of maize free from pesticides was provided by farmers after harvest (August, 2015) at Brimso (Cape Coast). *Obatampa* is an improved variety of the white type. The main reason for choosing this variety for the study was that *Obatampa* is cultivated in many West African countries like Burkina, Ghana, Mali and Nigeria. Research indicates that this variety is promoted as part of the Quality Protein Maize (QPM) being high yielding, very nutritious and mature earlier than other varieties. The untreated maize acquired at 28.29 % moisture content was cleaned to remove broken grains and foreign materials at the School of Agriculture Teaching and Research Farm, University of Cape Coast. The whole maize was dried to 13.8 % moisture content (wb) at Alhaji Musa Farm in Abura Dunkwa village.

Experimental Storage Bags

The hermetic SuperGrainbag (manufactured by GrainPro, INC) shown in Fig. 1D was used. The bags were obtained from AGRIMAT Ltd. in Legon (Accra). The polyethylene material (100 µm thick) was cut into pieces and sewn into smaller bags (45 cm x 25 cm) and sealed with an electric sealing machine after filling with the grain. This was then placed inside a second bag made of woven polypropylene to give additional protection and strength.

The non-hermetic polypropylene bag, consisting of flexible, plastic fabric was used as the control check. The bag allows ventilation of the product and the fabric is also able to absorb moisture from the product and does not offer adequate protection of the commodity against moisture, insects, and

rodents. Despite these shortcomings, it is still an ideal packaging solution for many different products as maize, cocoa, potatoes etc. The bags were cut and sewn into 45 cm length and 25 cm width dimensions. After filling with the weighed quantities (1.5 units) of maize, bags were stored in wire-meshed shelves to protect them against rodents and rats attack (Fig. 14).

Figure 14: Storage of filled bags in wooden shelves.

Culture of Insects

A parent stock of *Prostephanus truncatus* was obtained from the Plant Protection Regulatory Services Directorate of the Ministry of Food and Agriculture (PPRSD/MOFA) at Pokuase in Accra. They were cultured in a glass bottle covered with a nylon mesh to allow ventilation. Whole and untreated maize grain were used to feed them. The grains were sterilized in a refrigerator for 24 hr. and dried in an oven at 40 °C for six hours (Bonu-Ire, 2001). After two weeks of oviposition, the parents were removed using an aspirator (in order to get the uniform generation) and killed by freezing. This ensured the emergence of same age progeny for use in establishing the main culture with subsequent re-culturing every two weeks. By this, insects of the

same cohort were always available for the experiment. This method was used by Anankware *et al*. (2013) on maize storage.

Culture of *Aspergillus flavus*

A quantity of 600 g of maize grain was mixed with 100 ml of distilled water and shaken by hand. The preparation was maintained at ambient temperature for 10 days in a storage room. The average storage room temperature and relative humidity were 27.3 ºC and 71 %, respectively. Aflatoxin produced cannot be seen with the naked eye. However, suspect materials tend to rot, get moldy or discolored and this coloration is the main characteristic of *Aspergillus flavus* (Hong-Lian & Tsung-Che, 1975; Abbas, Shier, & Weaver, 2004). The blue and green fluorescence coloration were visible after 7 days which means that the culture contained *Aspergillus flavus*.

Experimental Design

A 2x3 factorial experiment, with two levels of store atmosphere (hermetic and non-hermetic), three levels of infestation (uninfected, *Aspergillus flavus* and insect infestation) at three replications each (1.5 kg maize per replicate) were used (Table 7). For the insect infected treatments, each bag was seeded with twenty (20) *Prostephanus truncates* while for the *Aspergillus flavus* infested treatment, a 100 g of grain previously cultured with the mould was mixed with 1400 g uninfected maize. Hermetic bags were tightly sealed to create hermetic conditions while the non-hermetic bags were just tight enough to prevent spillage but allowed the exchange of gases.

During storage, the temperature and relative humidity (internal and external), grain moisture content, oxygen concentration in the bags, insect mortality, aflatoxins contamination, weight loss, percentage grain damaged

and seed viability were monitored. The initial moisture content, germination rate and aflatoxin concentration were assessed at the beginning of the experiment. Data monitoring of the temperature, the relative humidity and the oxygen concentration was done every two days. Every two weeks for three months, destructive sampling was done to assess the insect mortality/activity (dead and/or life), the moisture content and the grain damage as well as the weight loss. Because of the impossibility of reusing an opened hermetic bag for the subsequent sampling, additional bags were provided for this treatment. The aflatoxin concentration and the germination potential were determined monthly. The storage duration was three months.

Table 8: Experimental Design

Factor	Factor levels	
*Store atmosphere	1- Hermetic bag	2- Non-hermetic bag
*Infestation level	1- Insect (LGB) 2- Uninfected maize 3- Aspergillus flavus	1- Insect (LGB) 2- Uninfected maize 3- Aspergillus flavus

Measurement of Temperature and Relative Humidity (Inside and Outside)

An infra-red thermometer (Victor 303B, China) (Fig. 15A) was used to measure the temperature inside the bags. The measuring range of this instrument is between -20 and 350 ℃ with ± 1.5 ℃ accuracy. The working range is 30 cm between the instrument and the target. The contact of the infrared and the target allows the temperature inside the bags to be read in 6 s. The relative humidity was measured using electronic RH-44550 Pen device (China) (Fig. 15B). This instrument can be used to measure the relative

humidity (20 to 90 % with ± 5 % accuracy) and the temperature (-10 to 50 ºC with ± 1 ºC accuracy). The device is introduced into the bag already opened and the data (temperature and relative humidity inside and outside) is read in 80 s.

Figure 15: Infra-Red Thermometer Victor 303B (A) and the RH-44550 Pen (B) used to measure temperature and relative humidity.

Determination of the Moisture Content

About 50 g of maize was put into a crucible and weighed as W_1. The weighed sample was immediately transferred to an oven and dried at 105 °C for 24 h allowed to cool for 30 min and placed in a desiccator for moisture equilibration. The final weight was noted as W_2. The process was repeated for all the samples. The moisture contents (wb) were then calculated for all the samples, using the formula as shown in Equation 1.

$$MC_{(wb)} = \frac{W1-W2}{W1} x\ 100 \quad (1)$$

Determination of Oxygen Concentration

The SCY-2A (GrainPro) oxygen analyzer was used (Fig. 16). This instrument measures O_2 content in mixed gases. Its range is 0-25 % with an

accuracy of ± 1.0 for oxygen and works at relative humidity < 90 %. Butterfly needles were connected to the hermetic SuperGrainbag from the oxygen analyzer and sealed with Epoxy glue. The butterfly needle consists of a sharp tiny needle and a narrow tube with a caped head to prevent air exchange when closed. The needle was pushed into each bag and firmly held in place with Epoxy glue. The GrainPro oxygen analyzer was earlier calibrated in nitrogen gas to 21 % and used to measure the oxygen concentration in the hermetic bags. This was done immediately after the set up and repeated every two days. This method was used by Anankware *et al*. (2013) to check the seed viability and oxygen depletion rate of hermetically stored maize infested by major insect pests.

Figure 16: Recording oxygen concentration using GrainPro oxygen Analyzer.

Determination of Insect Mortality

Sampling was done every two weeks up to the third month of storage. At each sampling stage, the contents of each hermetic and non-hermetic bag (Fig. 1E) was sieved using a set of USA standard sieves with mesh Nº 1.00 and 2.00 mm. Dead and life insects (Fig.17) were collected separately,

counted and their percentages calculated (Compton, Floyd, Magrath, Addo, Gbedevi, Agbo, & Penni, 1998).

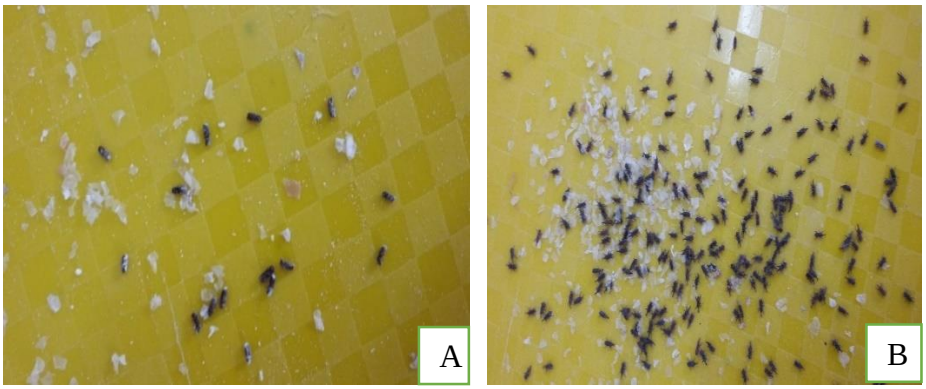

Figure 17: Dead bodies of LGB in a hermetic bag (A) and life insects (LGB and *Sitophilus zeamais*) in a non-hermetic bag (B).

Determination of *Aspergillus flavus* Concentrations

The aflatoxin analysis took place in the Mycotoxins Lab in Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The concentration of aflatoxin was determined using the Aflatest method at the beginning of the storage and every month during the three-month period with the use of the protocol below (Iqbal, Bhatti, Asi, Bhatti, & Sheikh, 2011).

Extraction and Clean-Up

Maize samples were ground to uniform consistency using the Preethi Trio Blender made in India (500 W, MG 182/100 class 'F'). A mixture of the ground sample (25 g) was mixed with 5 g of sodium chloride and 125 ml of methanol 70 % blended at high speed for 2 min using a waring blender (400 W, model HGBTWS3, USA) and filtered through a fluted filter paper. The extract (15 ml) was diluted with 30 ml of deionized water and filtered through a 1.0 µm microfiber filter.

Quantification of *Aspergillus flavus* Groups and their Concentrations

A Cecil Adept Binary Pump High Pressure Line Chromatograph (UK) (Fig. 18) coupled with Shimadzu 10AxL fluorescence detector (Japan) (Excitation: 360 nm, Emission: 435) with Waters Novapak C_{18} Column (150 x 4.60 mm, 5 µm) was the device used to detect the presence and quantify the abundance of *Aspergillus flavus* toxins. The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1 mL/min with column temperature maintained at 40 °C. To 1 liter of the mobile phase were added 119 mg of potassium bromide and 350 µL of 4 mL nitric acid (required for post column electrochemical derivatization with Kobra Cell, R-Biopharm Rhone).

Calibration curve of aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ngg^{-1}) were prepared from Supelco aflatoxin standard of 2.6 ng μL^{-1} in methanol. Limit of Detection and Limit of Quantification for total aflatoxin was established at 0.5 ng g^{-1} and 1ng g^{-1}, respectively. The injection volume was 1 mL. The system run for 22 min and by simple reading from the screen it is possible to see the amount of different mycotoxins (G_1, G_2, B_1 and B_2) as well as the total aflatoxin in the sample (Fig. 19).

Figure 18: A Cecil Adept Binary Pump High Pressure Line Chromatograph (HPLC) for aflatoxin analysis.

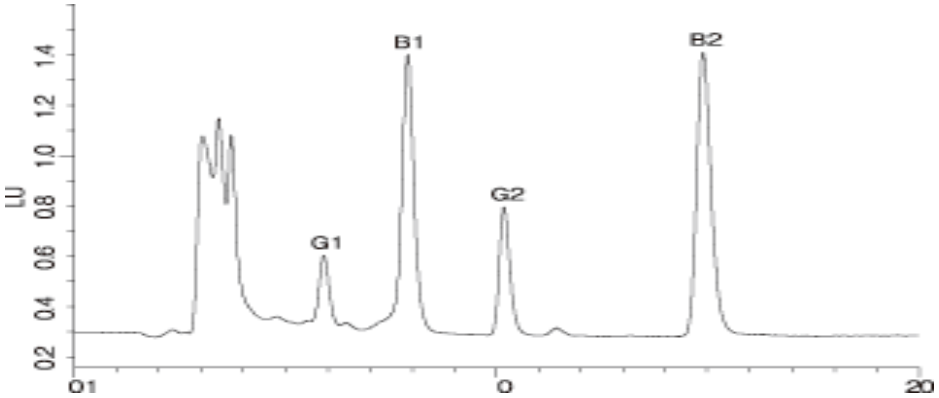

Figure 19: Chromatograms obtained by HPLC-Cecil Adept Binary Pump in maize grain.

Determination of Weight Loss

The weight of standard volume method (SVM) which provides an estimate of loss was used. The content from each bag was first collected, weighed (W_1) and sieved to remove power, dust, foreign matter and free living insects every two weeks. The sieved sample was weighed as W_2. The percentage of weight loss was estimated using Equation 2.

$$\text{Percentage Grain Weight Loss} = \frac{W1-W2}{W1} x\ 100 \qquad (2)$$

Where, W_1 is the weight of baseline sample (initial weight), W_2 is subsequent sample weight at different storage intervals (Ngatia & Kimondo, 2011).

Determination of the Percentage Damage

From each bag, a container was filled, the total number of grain was counted as well as the number of damaged grains (Fig. 20) and expressed as a percentage. (Adams & Schulten, 1976). The percentage of damaged grains was calculated using Equation 3.

$$\text{Percentage grain Damage} = \frac{Number\ of\ damaged\ grains}{Total\ number\ of\ grains\ counted} x\ 100) \qquad (3).$$

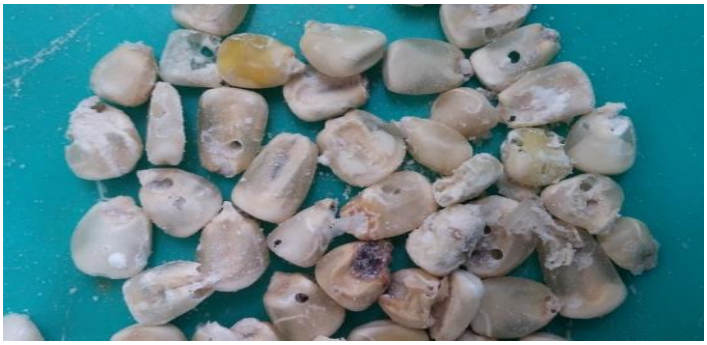

Figure 20: Maize grains from non-hermetic bag.

Determination of Seed Viability

The seed viability test was conducted in the laboratory using a box containing sterilized soil. Fifteen (15) seeds were randomly taken from the various bags and cultured in plastic boxes containing soil and irrigated using distilled water. The test was monitored for seven days and observed for emergence. On the seventh day, the germinated seeds from each were counted (Fig. 21A and 21B). This was repeated every month. The seed viability or germination potential was calculated using Equation 4.

$$\text{Percentage of germination} = \frac{Ngx100}{Tn} \quad (4)$$

Where Ng = Number of germinated seeds and Tn = Total number of seeds in the sample or initial number of seeds in sample (Anankware & Bonu-Ire, 2013).

Figure 21: Seed viability test. The first three lines (A) are from hermetic bags and the last three (B) from the non-hermetic bags.

Statistical Analysis

The effects of hermetic storage on the quality of maize grains were investigated using analysis of variance (ANOVA) with GenStat Discovery Edition (4th Edition) software. A confidence level of 95 % was used for all analysis. Mean differences in the treatments were tested for significance using Fisher's Least Significant Difference (LSD) at ($p < 0.05$) and the significant differences were observed. The Duncan Multiple Range Test (DMRT) was used to separate the means. The software SigmaPlot 12.0 was used to plot the different graphs.

Chapter Summary

All methods were described succinctly mentioning those used by previous authors and duly referencing them. The experimental design used was clearly explained as well as the statistical analysis. Also the materials used in this study were described and illustrated by pictures. The inability to read the carbon dioxide concentration limits the complete data required for the design of Controlled Atmosphere or Modified Atmosphere storage without time lag of the insect mortality.

CHAPTER FOUR

RESULTS AND DISCUSSION

Temperature Changes

Temperature generally increased significant at $p < 0.001$ with storage time in all the treatments (Table 1A) from the initial mean value of 23.8 ^{o}C to a maximum of 29.6 ^{o}C and the changes are show on Fig. 22. The hermetic bags recorded lower temperatures (26.99 ^{o}C) than the non-hermetic bags (27.05 ^{o}C) and the average outside temperature was 28.7 ^{o}C. The room storage reached its highest temperature (29.9 ^{o}C) on day 54 while the lowest (26.2 ^{o}C) was recorded on day 18 (Fig. 1C). The mean temperature between hermetic and non-hermetic atmospheres were not significant ($p = 0.09$) as shown on Table 1A. Insect infected bags recorded significantly higher temperatures (27.2 ^{o}C) than both aflatoxins infected (26.9 ^{o}C) and the uninfected control bags (26.9 ^{o}C) in the non-hermetic storage, and 27.2 %, 26.94 % and 26.8 % respectively in the hermetic storage (Table 1B).

The increase in temperature can be due to the heat released during the respiratory and the metabolic processes of maize grain and weevils as reported by Bern *et al*. (2013). As the grain is a living entity, it also respires and releases heat. The higher insect population and activity in the non-hermetic bags may also explain the higher temperature recorded than the hermetic bags. Higher temperatures accelerate insect metabolism.

Similar results were recorded by Sawant, Patil, Kalse, & Thakor (2012). Their research revealed increased temperature in a hermetic silo from 29.30 ℃ to 42.90 ℃ during the storage of wheat grain for eight months. Temperatures recorded inside the hermetic bags were 23.81-28.97 ^{o}C and the

same trend was recorded by Bbosa *et al*. (2014) (21.6-34.7 ºC) and Foster, Kaler and Whistler (1955) (21.1-26.7 ºC) for maize grain storage during 120 days. Njoroge *et al.* (2014), recorded that the temperature in the hermetic bags remained lower than the non-hermetic bags during the last month of maize grains storage. This lowering in temperature in hermetic bags than the non-hermetic may reflect the moisture content and the relative humidity during the storage period.

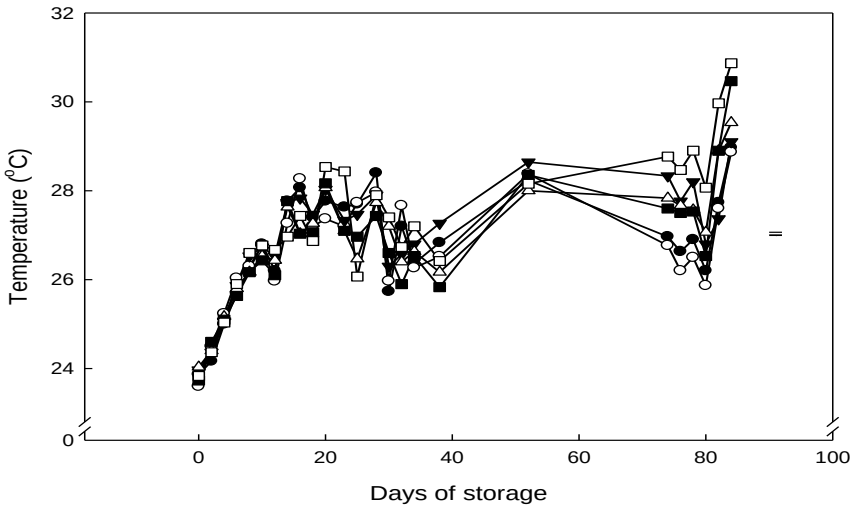

Figure 22: Temperature changes during the storage of maize grains**.** (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table1A).

Relative Humidity

Relative humidity during hermetic storage declined from the initial mean of 82.44 % to 64.11 %, while in non-hermetic storage, it increased from 80.56 % to 84.11 % after an initial transient fall (Fig. 23) and the outside relative humidity was 80.50 %. The lower relative humidity from the storage room was 72 % on day 26 and reached its higher percentage (91 %) on first day of the storage (Fig. 2C). The treatment comparisons from ANOVA (Table

2A) showed significantly different values ($p < 0.001$) between the non-hermetic and the hermetic storage bags. The mean values were 75.17 % (aflatoxin and insect infected bags) and 73.75 % (uninfected bags) in hermetic storage and 78 % (aflatoxin infected bags), 78.83 % (control bags) and 81 % (insect infected bags) in non-hermetic storage as show in Table 2B. The relative humidity in the hermetic bags was fairly constant between day 14 and day 28 and reached a maximum of 83.78 %, but it decreased sharply thereafter.

The relative humidity affects the rate at which insect and other pests lose water to the atmosphere. Low relative humidity increases moisture loss from the biotic organisms including the grains. The non-hermetic structure allows exchange of air with the outside environment which can also cause the entry of prevailing moist air into the bags. High relative humidity in the non-hermetic bags could also be due to the release of more water from the grain into the air which accelerate the multiplication of major micro-organisms responsible for damage of the grain during the storage. On the other hand, the hermetic bags are airtight and do not permit moisture exchange with the environment. The lower relative humidities recorded in the hermetic bags suggests that there was lower respiratory moisture loss from the biotic contents.

Villers *et al*. (2006) related that humidity requirements for rapid mould growth of aerobic microflora are within the range of 65 % to 85 % in hermetic storage structures. In a study by Sawant *et al*. (2012), lower relative humidity (16.1 %) was observed in a hermetic silo than a non-hermetic silo during wheat storage. Vales, Ranga, Sudini, Patil, & Murdock (2014) reported that

the relative humidity inside Purdue Improved Crop Storage bags was more stable over time than in gunny bags (jute bags) on pigeon pea storage for eight months respectively 11 to 21 % and 35 to 58 %. Similarly, Njoroge *et al.* (2014) recorded that relative humidity remained relatively steady at about 65 % in Purdue Improved Crop Storage bags, whereas notable fluctuations occurred in non-hermetic polyethylene bags during maize grains storage. It is apparent that hermetic storage might prevent deterioration of stored maize by protecting it from high external relative humidity levels that prevail in hot humid climates because relative humidity in the hermetic bags did not increase like that of the non-hermetic bags' environment. Temperature and relative humidity in the non-hermetic bags were higher than the hermetic bags which could considerably affect the deterioration of grains. For wet grains (equilibrium relative humidity higher than 67 %) the CO_2 increases from 15 to 25 % (Bartosik, 2012). From the results recorded, it may be deduced that hermetic storage protects grains against the storage pests better than non-hermetic storage.

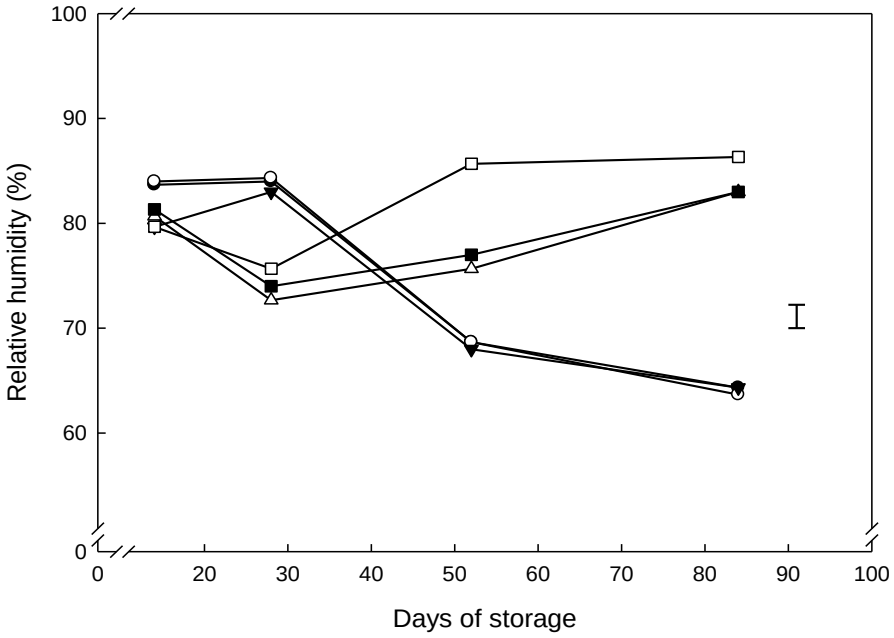

Figure 23: Relative humidity changes during the storage of maize grains. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 2A).

Moisture Content

Significantly higher moisture levels were recorded in the non-hermetic bags (15.1 %) compared to the hermetic bags (13.8 %). Maize moisture in the hermetic bags decreased from the initial average of 13.7 % to 13.2 % and conversely, it increased from the average of 13.7 % to 16.2 % in the non-hermetic storage as shown Fig. 24. Pairwise comparison showed no difference in the moisture levels between the insect infected bags (14.6 %) and the aflatoxin infected bags (14.6 %) but both were significantly higher than the uninfected control bags (14.2 %). There was a highly significant difference ($p < 0.001$) between the hermetic and the non-hermetic bag in moisture content level that for insect and aflatoxin infected bags and uninfected bags (Table 3A). The mean moisture content was 13.86 % in aflatoxin infected bags, 13.70

% in uninfected bags and 13.87 % in insect infected bags (hermetic storage) and 15.28 % in aflatoxin infected bags, 14.68 % in control bags and 15.32 % in insect infected bags (non-hermetic storage) (Table 3B).

The determination of moisture content is highly imperative as this determines the keeping quality of grain. The differences observed in this study might be explained by bag permeability, insect density and micro-organism activity in the various bags. In the hermetic bags, the transient increase in moisture during the first 14 days was probably due to the initial respiration of maize grains and weevils. The subsequent decline could also be as a result of the maize grains establishing equilibrium moisture content and reduction of respiration and biotic activities due to the low oxygen concentration and the low respiration. The results of this study show that the hermetic SuperGrainbag can create the suitable moisture content for stored maize grain. The storage temperature and relative humidity were 26 ºC and 64 %, respectively. The combination of these values equilibrated the storage moisture content to 13.70 % according to Table 5. In the non-hermetic bags, the temperature and relative humidity were 27.05 ºC and 80.5 %, respectively, which produced a storage moisture content of 16.2 % (Table 5). Moisture content affects the amount of water ingested by insects. At high moisture contents, increasing competition from fungi and other micro-organisms rapidly reduces the survival of many storage pests which may be replaced by fungi species. The low permeability of the hermetic structure also maintains safe constant moisture levels in previously dried commodities regardless of ambient humidity.

The same trend as observed in the current study was also reported by Anankware *et al.* (2013); Jonfia-Essien, Navarro, and Villers (2010) and Weinberg *et al.* (2008) on maize grain storage. Njoroge *et al*. (2014) recorded a lower moisture content in the hermetic bag (13.34 %) than the non-hermetic (13.55 %) after six months' storage period of maize. When maize was stored in the double layer hermetic bag, moisture content remained practically the same (about 13 %) during the whole storage period, showing that exchanges between hermetic bags and the external environment were limited. However, this also means that no further drying is possible within this structure, so that grains have to be well dried at 12-14 % prior to storage. On the contrary, grain moisture in non-hermetic polypropylene bags followed the trend of the room relative humidity due to the permeability of these bags. This confirms that in hermetic bags, initial moisture content remains largely unchanged during storage. The increase of moisture content in the non-hermetic bag could influence aflatoxin contamination in these bags and Beti, Philips,and Smalley *(*1995) recorded an increase of moisture content from 15 to 20 % after 30 days in maize infested with *Sitophilus zeamais* and significantly more aflatoxin B_1 was founded in *Sitophilus zeamais* infested maize, than in control maize that had been inoculated with *Aspergillus flavus.* With a moisture content of 15.1 and 13.8 % respectively in non-hermetic and hermetic bags, the germination potential will be affected because more the moisture content is increasing more the seed viability will decrease (Weinberg *et al*., 2008).

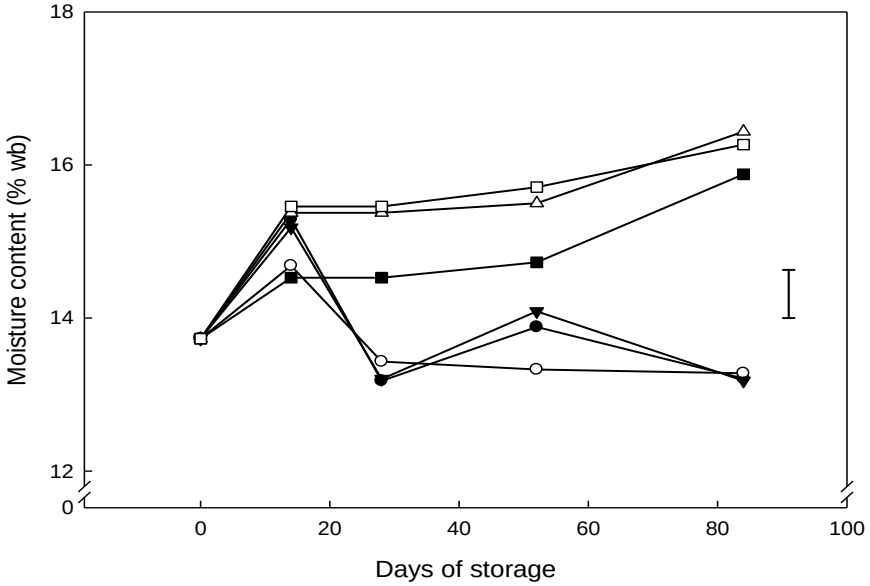

Figure 24: Moisture content of maize grain during storage. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 3A).

Oxygen Concentration

The initial atmospheric oxygen concentration in the hermetic bags was measured as 20.77 %. After 60 days of storage, oxygen concentration declined significantly to the minimum level of 6.3 % in the uninfected maize bags, 6.4 % in the aflatoxin infected bags and 6.3 % in the insect infected bags. At these values the concentrations remained fairly constant. Mean oxygen levels in the uninfected maize bags and those infected with aflatoxin or insects were 12.87 %, 12.70 % and 12.85 % respectively (Table 4B). ANOVA show that there were significantly different at (p = 0.012) as shown on Table 4A. During the study, the oxygen level was considerably dropping as shown the Fig.25.

Grains, insects, fungi and other microorganisms respire, consuming grain constituents and O_2 from the environment, and releasing to the interstitial environment CO_2, water and heat. Grain type, moisture content,

temperature, storage time and O_2 and CO_2 concentrations affect the respiration rate. A fundamental nature of stored agricultural produce is that, the tissues are alive and are therefore engaged in the process of metabolism, especially respiration. The hermetic bags are waterproof and have a certain degree of gas-tightness which does not allow the exchange of gases with the immediate environment. Thus during hermetic storage, there is a gradual depletion of available oxygen in the enclosed atmosphere with concomitant build-up of carbon dioxide concentration. The presence of other biotic organisms such as insects and moulds contribute to the changes in the atmospheric gas composition, generating a low oxygen modified atmosphere. Hence as noted in the results, oxygen depletion was higher in the insect and aflatoxin infected bags than the uninfected control.

Low O_2 levels cause spiracles to open resulting in insect death from water loss. In some cases, CO_2 can acidify the hemolymph leading to membrane failure in some tissues (Nicolas & Sillans, 1989). Bailey and Banks (1980) noted that oxygen concentration depletion retarded insect development, impaired metamorphosis and altered fecundity without necessarily having to kill them. Atmospheres with 60 % CO_2 and 8 % O_2 are very effective at killing internal seed-feeding insects, while low O_2 atmospheres are more rapid in killing external-feeding insects (Banks & Annis, 1990). High CO_2 levels, even with 20 % O_2, rapidly kill insects because of CO_2 toxicity (Annis, 1987).

The results obtained in the current study are similar to that observed by Njoroge *et al*. (2014) where oxygen concentration depleted to 7.82 % in the Purdue Improved Crop Storage bags and carbon dioxide increased to 13.93 % in six months of maize storage. In another study of maize grains storage,

Bbosa *et al.* (2014) found that oxygen declined from 21 % to between 3 % and 10 % by 120 days and increased to 6.7 % from the 120-120 days. The fluctuations seen were observed for both laboratory and field experiments, and it was attributed to a residual insect population that remained behind over an extended period of time before a steady-state was attained (Banks & Fields, 1995; Navarro *et al.*, 1994). For peanut stored at 8 % moisture content in hermetic SGBIIZ (GrainPro Inc.), oxygen was depleted after 44 days (Navarro *et al.*, 2011). Jonfia-Essien, Navarro and Dator (2008) also observed an oxygen depletion in GrainPro Cocoons to 0 % during cocoa bean storage.

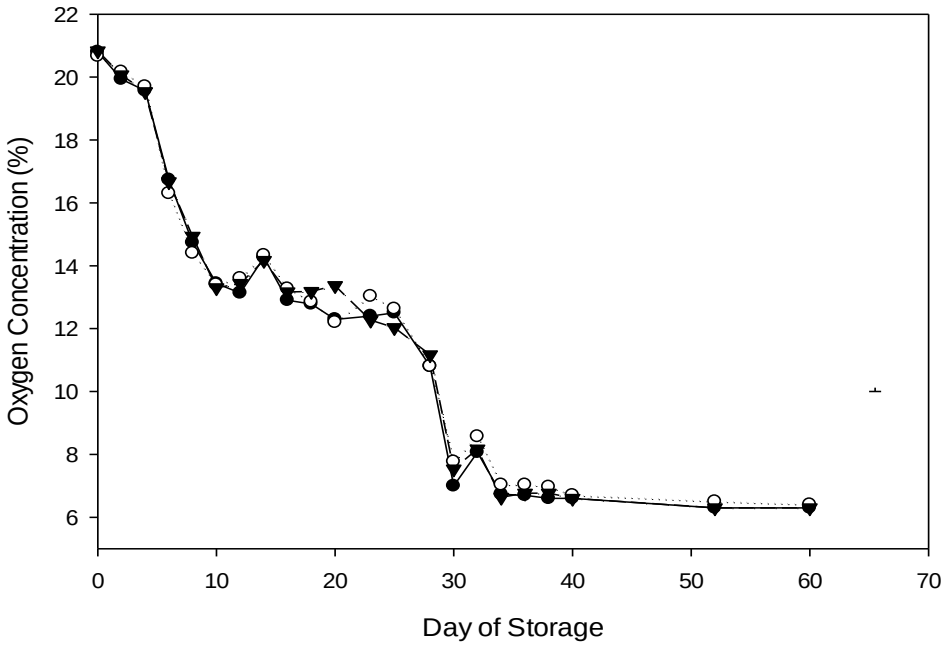

Figure 25: Variation of oxygen concentration during the storage of maize grains. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags and LSD $_{(0.05)}$ bars shown are for the interaction effects (ANOVA Table 4A).

Insect Mortality

The Analysis of Variance (Table 5A) and the mean table (Table 5B) showed a highly significant difference between the interaction day and treatments about the insect dead and alive ($p < 0.001$). The insect mortality

was increasing during the whole storage period (0 to 100 %) in the hermetic bags with a strong increase between 14th and 28th days in the hermetic bags where 86.96 % of the 20 larger grain borers introduced died while the mortality in the non-hermetic was increasing slowly at the same period (21.29 %) and at the end of the experiment 79.81 % of the larger grain borer introduced alive was recorded (Fig. 26). On day 28 (second opened), 100 % of the insects seeded in the double layer hermetic bag died (rep. 3) when the non-hermetic counted 111 Larger Grain Borer (LGB) insects and 87 *Sitophilus zeamais* not introduced in the bags at the beginning of the experiment. The number of *Sitophilus* was increasing in the non-hermetic bags and 198 was counted in the bag (rep. 3) 0n day 52, it was only 7 on day 28. *Sitophilus zeamais* multiplication was faster than that larger grain borer and was also higher in non-hermetic infected bags than the uninfected one and the aflatoxin bags had more *Sitophilus zeamais* than control one respectively 6 and 3 for rep 3 on day 52. At least 50 % of insect introduced died when the oxygen concentration was 12 %. In general, 59.3 % in the hermetic bags and 30.7 % in the non-hermetic insect mortality was recorded during the storage period. There was more maize flour due to insect damages in non-hermetic than the hermetic storage as shown the fig. 2E.

This observation indicates that the double-layer hermetic bag successfully prevented insect proliferation from within the bag and inward infestation from the storage environment. LGB and *Sitophilus zeamais* invade maize while the crop is still in the field or before storage (Golob & Hanks, 1990). The population increase in the non-hermetic bags was because of the favorable maize moisture and temperature Sone (2000) and the complete

mortality in hermetically airtight bags was because of oxygen depletion and CO_2 enrichment (Anankware *et al*., 2013; Anankware & Bonu-Ire, 2013; Fleurat, 1990; Navarro *et al*., 1990; Villers, Navarro, & De Bruin, 2010; Yakubu, Bern, Coats, & Bailey, 2011). The mortality observed in the non-hermetic bags is due to the natural mortality because LGBs seem to have a natural mortality rate, which is dependent on natural mortality factors, irrespective of the treatment combination. The lowest oxygen concentration reached was 6.3 % in this current experiment. This oxygen concentration was well above that needed to completely eliminate live insects. The hermetic storage system of SuperGrainbag, nonetheless, suppressed destructive insect multiplication. Insect multiplication in non-hermetic bags, on the contrary, was ferocious and resulted in severe grains damage. Besides the high LGB population, a heavy *Sitophilus zeamais* infestation occurred in all the non-hermetic bags even those not infested by insects. At three months of storage, *Sitophilus zeamais* also outnumbered LGB in the non-hermetic bags. Compared to LGB, the intrinsic rate of increase of *Sitophilus zeamais* in stores tends to be higher and it may be that the bags had larger numbers of grain borers.

The same trend was recorded by Bbosa *et al.* (2014) where zero weevils were observed in the hermetic bag and 214 in the non-hermetic after 21 days of maize grain storage. Edoh, Tounou, Lamboni, & Hell (2013) recorded 73.04 % of LGB die in hermetic bags and only 19.41 % in the non-hermetic, and high insect mortality rates were observed in the hermetic bags compared with the non-hermetic with respectively 7 and 820 live LGB and 412 live *Sitophilus zeamais* according to Anankware *et al*. (2013). As with all

aerobic organisms, development and survival of insects is strongly correlated with oxygen concentration, so that in hermetic storage, all insect development ceases Donahaye and Navarro (2000) and insects perish if levels fall below 2-3 %; Moreno-Martinez, Jiménez, and Vázquez, 2000; Bailey and Banks (1980) stated that hermetic condition delayed insect development, impaired metamorphosis and altered fecundity. Many others, Makundi *et al*. (2010); Giga and Canhao (1993); Meikle *et al*., (1998); and Vowotor, Meikle, Ayertey, Borgemeister and Markham (1998) have as well reported that presence of Sitophilus zeamais negatively influences development of LGB. Vowotor *et al.* (1998), for instance, observed that later instars of *Sitophilus zeamais* larvae are able to kill *Prostephanus truncatus* larvae. This can also be the reason for the lower *Prostephanus truncates* number in the non-hermetic bags compared to the *Sitophilus Zeamais* number. From the results of this experiment, hermetic bags prevent insect's proliferation more than the non-hermetic bags thereby maintaining the quality of maize grain.

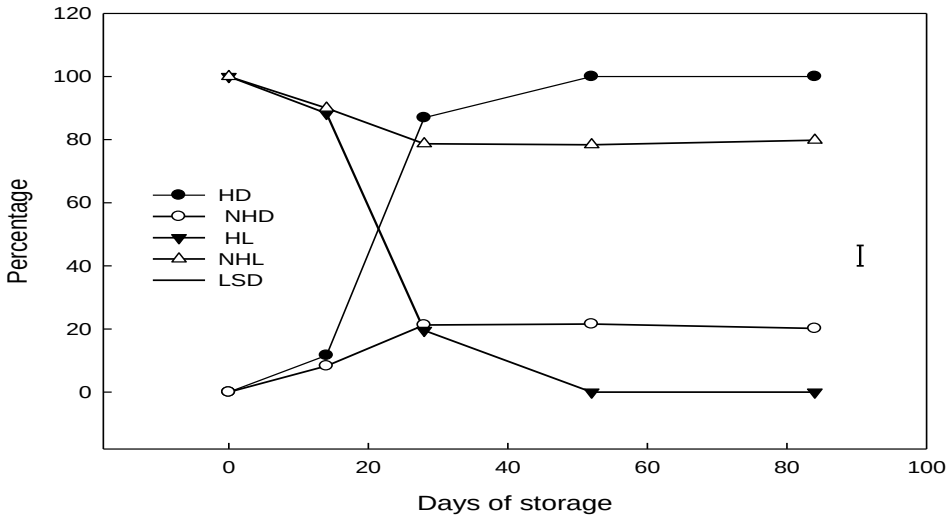

Figure 26: Percentage insect mortality and alive during the storage. (●) Insect mortality in hermetic bags, (▼) Insect alive in hermetic bags, (○) Insect

mortality in non-hermetic bags, (Δ) Insect alive in non-hermetic bags and $LSD_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 5A).

Aspergillus flavus Groups and their Concentrations

The analysis of variance shows the significant difference ($p < 0.001$) between the hermetic and the non-hermetic on the toxins concentration one hand and between the different types of *Aspergillus flavus* groups in other hand (Table 6A). All *Aspergillus* fungi detected from maize grain were found to be *Aspergillus flavus*, the *Aspergillus flavus* toxin B_1 (AfB_1) was significantly abundant than the three others as shown in Fig. 27. The AfB_1 was 1ppb in the hermetic and 42.6 ppb in the non-hermetic bags, while AfB_2 was 0.4 and 15.4 ppb, respectively in hermetic and non-hermetic bags. When AfG_1 and AfG_2 were not detected in the hermetic bags, there 4 and 1.5 ppb, respectively in the non-hermetic bags (Table 6B). It is well known that growth of Aspergillus ssp. and subsequent production of aflatoxins in maize is depend on a number of factors such as temperature, relative humidity, insect injury, handling during harvest and storage (Hell *et al*., 2003).

Many authors assessed aflatoxin contamination in stored maize, but they did not check out the diversity of *Aspergillus flavus* group under hermetic condition. It can be concluded that the double layer hermetic reduces the growth of *Aspergillus flavus* B_1 and B_2 and inhibits this of the G_1 and G_2. The results revealed that mycotoxigenic fungi can develop in maize stored in hermetic plastic bags.

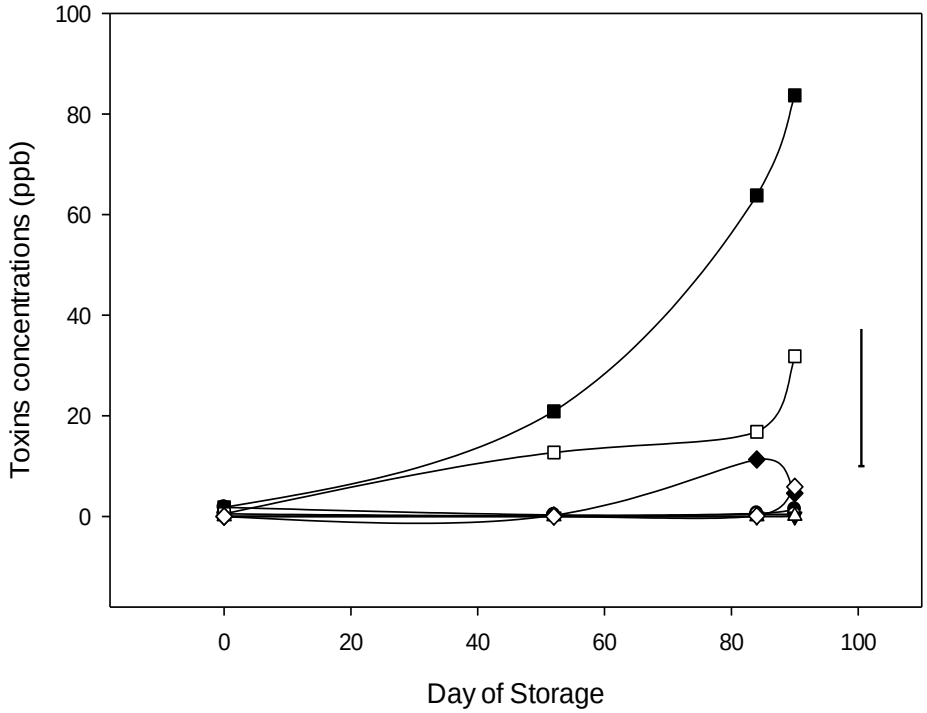

Figure 27: Aspergillus flavus toxins group and their concentration during the storage. (●) AfB_1 hermetic bags, (○) AfB_2 hermetic bags, (▼) AfG_1 hermetic bags, (Δ) AfG_2 hermetic bags, (■) AfB_1 non-hermetic bags, □ AfB_2 non-hermetic bags, (♦) AfG_1 non-hermetic bags, (◊) AfG_2 and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 6A).

The aflatoxin concentration in the hermetic bags (1.4 ppb) was lower significantly than the non-hermetic bags (63.6 ppb) (Table 7B) and the ANOVA shows a significant difference between the hermetic and the non-hermetic bags (p =0.002) as indicated on Table 7A. The aflatoxin level sharply increased in the non-hermetic bags from week 9 to week 12 (33.9 ppb to 126 ppb), while it slowly increased in the hermetic bags from 0.3 to 1.9 ppb in the same period (Fig. 28).

The effect of these storage systems on mould proliferation, and the possibility of maize being contaminated with aflatoxin during storage are a subject of interest because, incidentally, the low air permeability liners also have moisture barrier properties.

So far, many researchers did not compare the aflatoxins contamination during maize storage in hermetic and non-hermetic conditions. But different researchers report different observations with respect to mould growth. Richard-Molard (1988) has reported that a fungi-static effect is induced when oxygen concentration drops to 1 % or below. On a differing note, Castellari, Valle, Mutti, Cardoso, and Bartosik *(*2010) reported that fungi could develop on maize stored in hermetic plastic bags thus creating the risk of contamination with mycotoxins. Navarro. H., Navarro. S and Finkelman (2011) recorded a level below the threshold limit (<0.3 ppb) in hermetic conditions during peanut storage in 3 months. In this study, the samples moisture content in the hermetic bags was 13.41 % while that in the non-hermetic storage was 14.99 %. In maize, it was determined that a storage moisture content of 13 % is sufficiently low to prevent fungal development and mycotoxin production (Castellari *et al.,* 2010). The lowest oxygen level (6.3 %) reached in the present study was not sufficiently low to guarantee inhibition of fungal growth. Nevertheless, at grain moisture content of 13 % or below, growth of moulds would be relatively minimal (Diener *et al.,* 1987; Quezada *et al*., 2006). The increase of aflatoxin concentration in the non-hermetic conditions was recorded by FAO (1992) where maize samples analyzed 20 days after harvest had levels of 130 µg aflatoxin of total maize. The same samples analyzed 60 days later showed rapid increase, up to 1,680 ppb. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Mali shows that conventional storage for more than 2 months causes rapid growth of aflatoxins (Waliyar *et al*., 2002). Sanders, Davis, and Diener (1968), reported that little aflatoxin was found on inoculated peanuts

maintained in an atmosphere of 60 % CO_2, 20 % O_2, and 20 % N_2, as compared with 206 pg. of aflatoxin per g of peanuts stored in normal air at 25 °C and 90 % relative humidity. The limit aflatoxin below 5 % increase per month in the PICS bag compared to the traditional polypropylene bag which experienced a 92 % increase per month but no increase per month in the Grain Pro Bulk Bag (USAID & Bill and Melinda Gates, 2015). The aflatoxin content was low and not significantly different between gunny and PICS bags, respectively 1.10 and 0.68 ppb after 8 months of storage of pigeon pea (Vales *et al*., 2014).

In this study, *Aspergillus* was introduced but the contamination could also occur in the field. However, insects in stored products found in the field before harvest generally require previous damage to the husk, or ears that protrude from the sheath (some hybrids), in order to gain entry. They may carry *Aspergillus flavus* spores into the ear and inoculate kernels (www.micotoxinas.com.br. Accessed on February 6, 2016). The increasing damage of insect in the non-hermetic bag and the rapid growth of aflatoxin can lead to the insects bringing *Aspergillus flavus* in the storage. Even the maize weevil, for example, is considered a poor vector of *Aspergillus flavus* in the field (LaPrade & Manwiller, 1977). The main role of maize weevil in *Aspergillus flavus* infection and subsequent aflatoxins contamination occurs during storage. Large populations of weevils can change conditions within bagged or bulk grain to encourage the growth of storage fungi (Christensen & Kaufmann, 1969). Insects can disseminate spores of *Aspergillus flavus* in the field and stored products (McMillian, 1987). However, Sinha and Sinha (1991, 1992) recorded that, the incidence of fungi of the *Aspergillus flavus* group and

aflatoxin contamination was higher in insect damaged maize than in insect free samples.

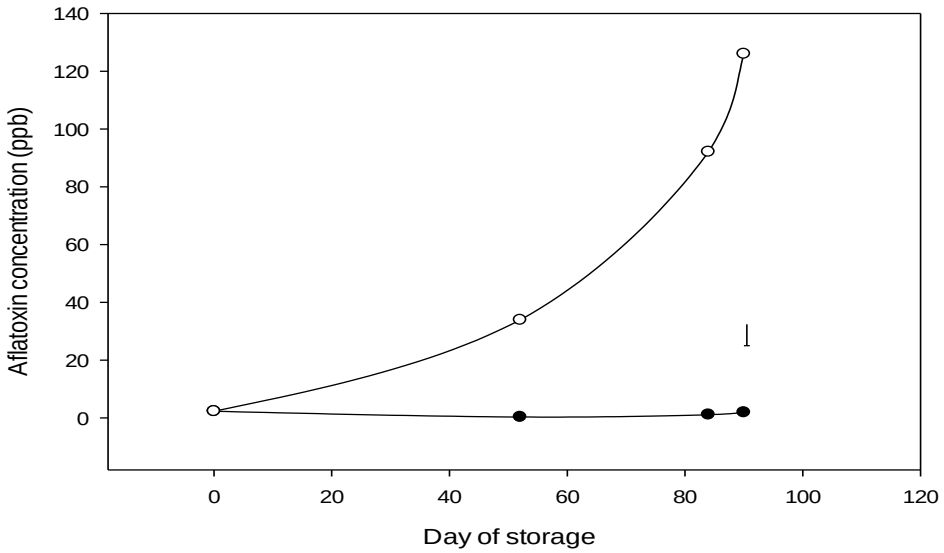

Figure 28: Total aflatoxins concentration during the storage. (●) Aflatoxin level in hermetic bags, (○) Aflatoxin level in non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 7A).

Weight Loss and Grain Damage

The analysis of variance for weight loss and grain damage (Tables 8A and 9A) showed highly significant differences between the hermetic and the non-hermetic storage at ($p < 0.001$). The means for weight loss in the hermetic storage were 0.29 % in aflatoxin infected bags, 0.43 % in control bags and 0.48 % in insect infected bags, while in the non-hermetic storage there were 6.29 % in aflatoxin infected, 8.98 % in uninfected and 9.09 % in insect infected bags as shown in Tables 8B. The results showed that there was no significant change in grain weight from the inception of the hermetic storage (mean weight loss, 0.4 %), irrespective of whether or not the grain was infected. There was only a little grain damage in the hermetic storage, 2.3 % for infested aflatoxin and insect bag, 2.17 % for uninfected bag in hermetic

storage and 7.78 %, 8.39 % and 16.29 % for aflatoxin, control and insect bags respectively in non-hermetic storage (Table 9B). This is a sharp contrast to the non-hermetic storage which sustained significant grain weight loss and damage of 16.5 % and 26.4 %, respectively, after just 52 days of storage (Fig. 29 and 30). Significantly higher grain weight loss and damage were incurred in the insect infected bags (4.8 % and 9.3 %, respectively) compared to the aflatoxin infected bags (3.5 % and 5.1 %, respectively) and this occurred predominantly in the non-hermetic storage ($p < 0.001$). Both grain weight loss and percentage damage in the uninfected control lied between the infected treatments.

The trend observed in the study herein, was similarly recorded by some authors or researchers. Bbosa *et al*. (2014) noted that there was a significant difference between hermetic and non-hermetic treatments of maize grain during storage for 120 days while Njoroge *et al*. (2014) also found 2.0 % and 3.1 %, respectively, as the damage in hermetic bags: and 0.5 % and 1.8 % respectively as weight loss in non-hermetic bags during the one-month storage. Grain losses were significantly lower in hermetic bag compared with woven bag, and their results show the number (mean ± SE, n = 3) of holes caused by *Prostephanus truncatus* to be 9.33 to 109, respectively, during maize storage for 120 days (Edoh *et al*., 2013). The triple layer hermetic bags recorded the least mean weight loss of 2.94 % while the polypropylene bags recorded higher mean values of 23.65 % (Anankware *et al*., 2013). The weight loss of 0.4 % and 8.2 %, respectively in hermetic and non-hermetic recorded in this experiment is similar to that founded by Weinberg *et al*. (2008), where

there were 0.2 and 9.56 %, respectively in hermetic and polypropylene bag after three months' storage period.

Losses observed in the bags are linked to the infestation level. The storage of maize in hermetic conditions minimized grain weight losses to less than 6 % for infestation with *Prostephanus truncatus* or mixed insect population Edoh *et al.* (2013); Navarro *et al*. (1994); and Navarro, (2012) recorded that, an ingress rate of 0.05 % O_2 day^{-1} is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018 % over a one-year storage period. The greater losses recorded in the non-hermetic bags could be due to the fact that the polypropylene bags allow atmospheric air exchange between the grain and the external environment and therefore encourages the growth of insects and aflatoxins that cause damage. This is lacking in the hermetic bag due to its air tightness, hence the inability of the insects to survive, reproduce and cause damage and weight loss (Murdock, Seck, Ntoukam, Kitch, & Shade, 2003; Navarro & Donahaye, 2005). Grain damage and weight loss have double effects on overall losses to maize farmers and traders. Loss of grain weight, on the one hand, is a direct loss of food and saleable weight. Grain damage, on the other hand, culminates in the loss of market opportunity as a result of downgraded quality and therefore lower price. It could also affect the germination potential of seed grains meant for reproduction.

Compton *et al*. (1998) demonstrated in an exploratory study in Ghana that a strong quasi-linear negative relationship exists between grain damage and price. Maize with grain damage of 5-6 % or below did not attract discounted price. However, when grain damage exceeded this level, every 1 %

increase in grain damage attracted a discounted price of 0.6 -1.0 %. Furthermore, extensive damage renders grain unfits for human food and therefore makes the produce completely unsaleable. With or without prior *Prostephanus truncatus* infestation, the double layer hermetic bags kept grain damage and weight loss below 2.29 % and 0.42 % respectively; such grains would retail close to the highest price.

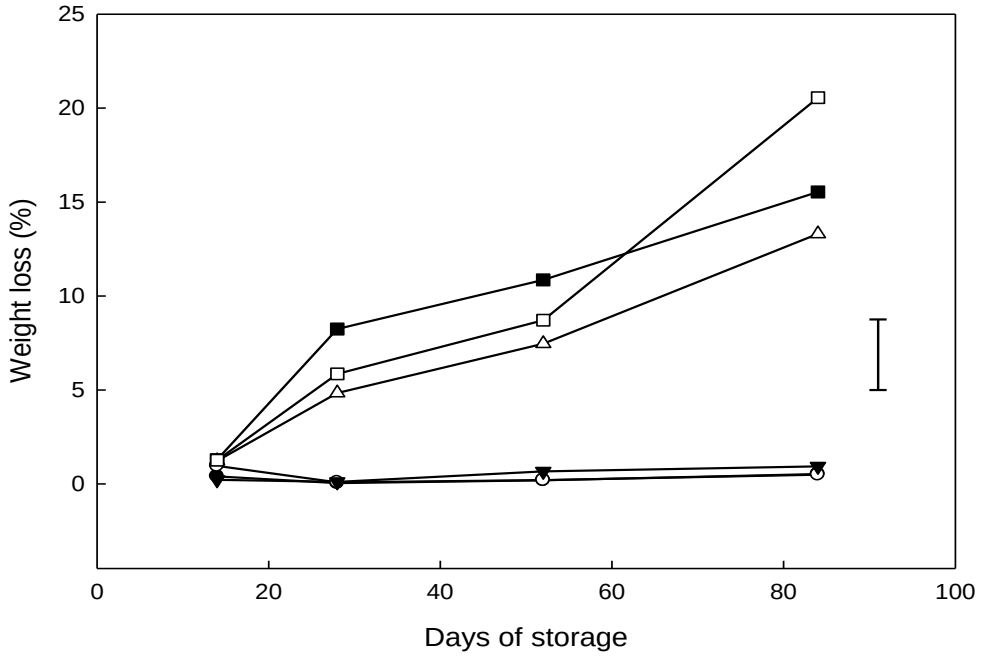

Figure 29: Percentage weight loss of maize grain during storage. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 8A).

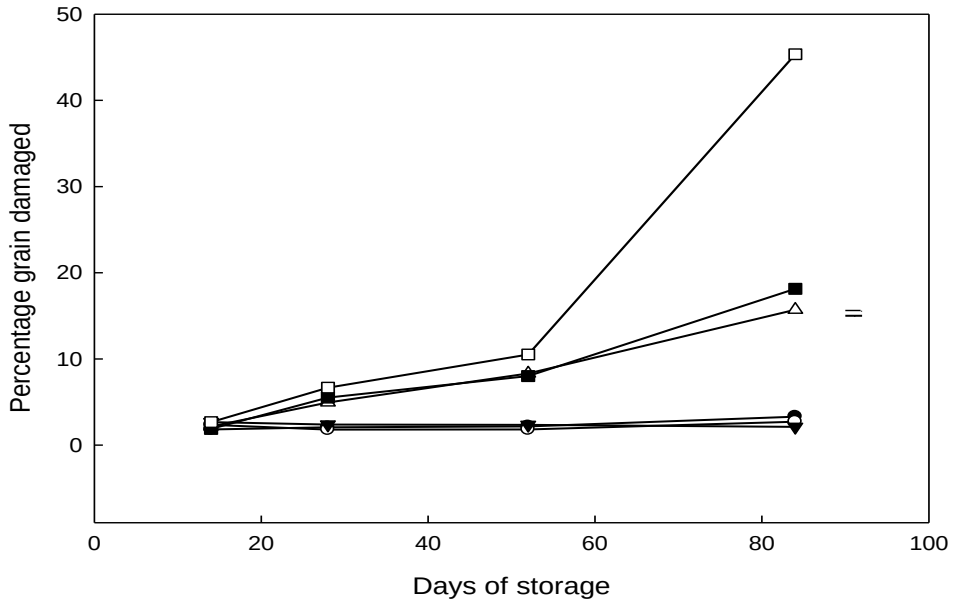

Figure 30: Percentage damaged maize grain during the storage. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 9A).

Seed Viability

The ANOVA (Table 10A) found that the potential germination was significantly higher ($p < 0.001$) in the hermetically stored maize than that stored in the non-hermetic bags and Fig. 31 shows the trend. The grand mean values being 75.5 % and 60.9 %, respectively. Also the seed viability was significantly higher in the uninfected control (79.17 %) compared to the insect infected (74.3 %) and the aflatoxin infected (72.92 %) in hermetic storage but was lower in non-hermetic storage maize with 65.28 % for control bags, 65.97 % for aflatoxin infected and 51.39 % for insect infected bags (Table 10B). The initial germination percentage was on average 83.33 % and dropped to 68.83 % and to 38.89 % after three months of storage respectively in the hermetic and non-hermetic storage.

There was a difference in maize seed viability between the two storage systems (non-hermetic storage dropped to 60.9 % and in the hermetic storage 75.5 %) after 11 weeks. This was due respectively due to the extensive insect and mould damage and the effect of higher mortality of insects and reduction multiplication of mould. The potential germination decreased in both hermetic and non-hermetic bags. As expected, loss of viability was lower in hermetic bags than in the free polypropylene bags especially from the beginning to 52th day of storage. At the end of the three months, germination rates reached 74.30 % and 51.39 % in hermetic bags infested and non-hermetic bags infested by Grain Larger Borer, respectively. This could only mean that the damage inflicted was not severe because of the low moisture content and low relative humidity, and may be because of the expected low activity of insects and as a result preserve the viability of the seed under hermetic storage. Also, increasing grain moisture contents, O_2 poor atmospheres could reduce the physiological quality of grain by interfering with the enzymatic activity of glutamine-decarboxylase. The lower viability of seed in the non-hermetic storage can lead to insect metabolic activities which will increase relative humidity, providing favorable conditions for the growth of *Aspergillus flavus*, leading to reduced seed germination (Sauer & Burroughs, 1980; Mills, 1983). The germination rate of 75.46 % recorded by grains from the double-layer hermetic bags fall within the acceptable range given by the International Seed Testing Association (ISTA, 2001). Maintaining grain germination and vigor is an important consideration especially because farmers often use some of the stored produce as seed for the subsequent season (De Bruin, 2005). The drop of viability in non-hermetic bags could be due to extensive insect and mould

damage, the trend in the SGB could be due to the effect of higher mortality of insects and reduction multiplication of mould.

Similar results were obtained from studies on maize and coffee quality and viability after hermetic storage (Moreno, Benavides, & Ramirez, 1988; De Briun & Murali, 2006). Njoroge *et al*. (2014) reported that the germinability was lower in non-hermetic bags (12.7 %) than the hermetic one (71 %). In their study, Anankware *et al*. (2013) recorded a mean germination of 78.52 % in the triple layer bags and 54.66 % in the polypropylene bags. The dropping of the seed viability from 83.88 % to 68.83 % with a moisture content of 13.8 % is similar to Weinberg *et al*. (2008), where in hermetic storage condition of maize grain, the germination potential dropped from 84.3 % to 58.3 % with 14 % moisture content after 75 days of storage. It can be concluded that the double layer hermetic bag preserves seed viability far better than conventional storage bags and so can be used for effective seed storage even in the presence of *Prostephanus truncatus* (De Briun, 2005).

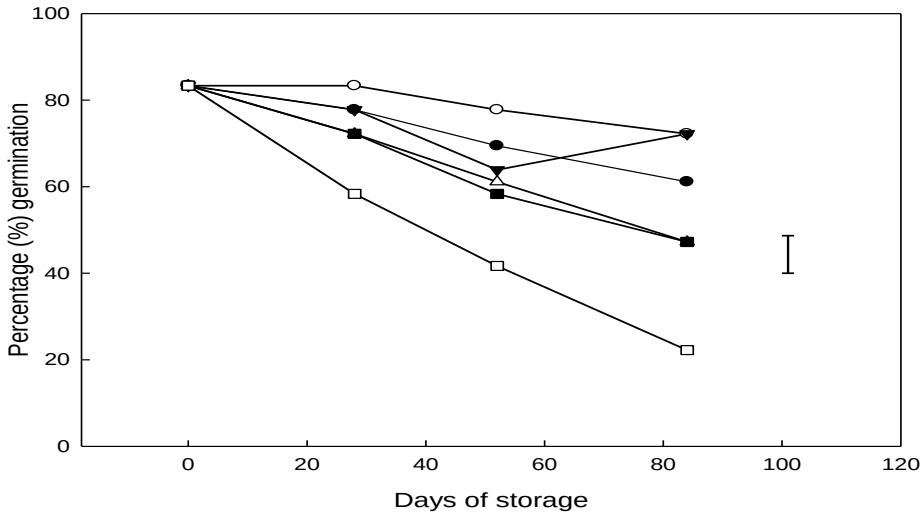

Figure 31: The potential germination of maize grain during storage. (●) Aflatoxin infected hermetic bags, (▼) Insect infected hermetic bags, (○) Uninfected hermetic bags, (Δ) Aflatoxin infected non-hermetic bags, □ Insect infected non-hermetic bags, (■) Uninfected non-hermetic bags and LSD $_{(0.05)}$ bar shown is for the interaction effects (ANOVA Table 10A).

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study investigated the effect of hermetic storage on the quality and shelf life of *"OBATAMPA"* maize variety grain and identified different groups of *Aspergillus flavus* toxins and the total aflatoxins concentration. To achieve the objectives, hypothetical assumptions were made that there is no significant difference between hermetic and non-hermetic storage on the quality and shelf life of maize grain, as well as on the growth of LGB and different *Aspergillus flavus.* groups during the storage period.

The results of this experiment revealed that there is no difference between hermetic and non-hermetic on temperature changes. Oxygen depletion was significantly higher in the insect infested grains and uninfected grains. Hermetic storage significantly reduced the relative humidity, moisture content, insect activity (dead and alive), weight loss, grain damage, *Aspergillus flavus* contamination compared to non-hermetic storage. Also, seed viability was better improved in the hermetic storage.

Conclusions

The conclusion drawn from the experimental findings is that, storage systems of maize influence the quality and shelf life. From the results of this comparative study, the double-layer hermetic bag facilitates the decrease of oxygen concentration in the storage atmosphere with its corollary effect in minimizing insect populations and lowering grain damage. It also created the

equilibrium moisture content, minimized weight loss significantly and inhibited *Aspergillus flavus* proliferation in the maize during the post-harvest storage.

The double-layer hermetic is capable of keeping aflatoxin levels below the International Standard (10-20 ppb) and promises the storage grain against the insect LGB better than the non-hermetic bag. In addition, the double-layer hermetic bag conserved grain quality and seed viability fairly well; almost 100 % better than the conventional storage bags. Optimum atmospheric conditions for Controlled Atmosphere/Modified Atmosphere systems design were found to be 28 ºC, 68 % RH, 13.5 % (MC/wb) and below 6.3 % O_2.

Recommendations

In light of the results of this study, the following recommendations are made with some suggestions for further study.

1. Maize farmers must be educated on the benefits of hermetic bag storage in order to adopt the technology to reduce maize grain post-harvest losses and contamination by *Aspergillus flavus*.
2. Further studies on the effect of hermetic storage of maize on the nutritional value should be conducted.
3. The changes in concentration of CO_2 should be determined in further research for the purpose of developing an appropriate modified atmosphere for long term storage.
4. Maize seed dealers or seed Companies are advised to use the hermetic techniques of storing maize seed for a better storage and germination potential.

5. The media should also educate the public on the health hazards of aflatoxins.

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APPENDICES

APPENDIX A

Analysis of variance tables of effect of hermetic storage on quality and shelf life of maize grain.

Table 1A: *ANOVA for the Effect of Hermetic Storage on Temperature in the Storage Atmosphere* (Fig.22)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	24	744.566	31.0236	237.63	<0.001
Atmosphere	1	0.3701	0.3701	2.84	0.093
Organism	2	11.2949	5.6474	43.26	<0.001
Day.Atmosphere	24	66.0634	2.7526	21.08	<0.001
Day.Organism	48	29.1401	0.6071	4.65	<0.001
Atm.Organism	2	0.2539	0.127	0.97	0.379
Day.Atm.Organism	48	13.557	0.2824	2.16	<0.001
Residual	372	48.5656	0.1306		
Total	521	913.811			

Table 2A: ***ANOVA for the effect of hermetic Storage on Relative Humidity during Maize Grains Storage (Fig. 23)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	749.708	249.903	135.29	<0.001
Atmosphere	1	425.347	425.347	230.26	<0.001
Organism	2	18.083	9.042	4.89	0.012
Day. Atmosphere	3	2355.708	785.236	425.09	<0.001
Day.Organism	6	106.583	17.764	9.62	<0.001
Atm.Organism	2	95.528	47.764	25.86	<0.001
Day.Atm.Organism	6	36.25	6.042	3.27	0.009
Residual	48	88.667	1.847		
Total	71	3875.875			

Table 3A: ***ANOVA for the Effect of Hermetic Storage on Moisture Content during Maize Grains Storage (Fig. 24)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	19.1278	4.7819	31.69	<0.001
Atmosphere	1	37.2349	37.2349	246.76	<0.001
Organism	2	3.2394	1.6197	10.73	<0.001
Day.Atm	4	28.6112	7.1528	47.4	<0.001
Day.Organism	8	1.9404	0.2426	1.61	0.142
Atm.Organism	2	1.0044	0.5022	3.33	0.043
Day.Atm.Organism	8	0.7837	0.098	0.65	0.733
Residual	60	9.0539	0.1509		
Total	89	100.996			

Table 4A: ***ANOVA for the Effect of Hermetic Storage on Oxygen Depletion during Maize Grains Storage (Fig. 25)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	18	3096.35	172.02	1508.45	<0.001
Organism	2	1.0403	0.5201	4.56	0.012
Day.Organism	36	7.4594	0.2072	1.82	0.009
Residual	123	14.0266	0.114		
Total	179	3118.88			

Table 5A: ***ANOVA for the Effect of Hermetic Storage on Insect Mortality during Maize Grains Storage (Fig. 26)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	30.45	7.61	0.49	0.74
Atmosphere	1	10515.12	10515.12	681.81	<0.001
Status	1	10.06	10.06	0.65	0.424
Day.Atmosphere	4	40984.77	10246.19	664.38	<0.001
Day.Status	4	23.93	5.98	0.39	0.816
Status.Treatements	1	29866.14	29866.14	1936.56	<0.001
Day.Status.Trts	4	19381.03	4845.26	314.17	<0.001
Residual	39	601.47	15.42		
Total	58	100455.9			

Table 6A: ***ANOVA Table for the Effect of Hermetic Storage on mean of Aflatoxins Group Growth (Fig. 27)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	3504.2	1168.1	4.17	0.009
Atmosphere	1	5794.6	5794.6	20.7	<0.001
Type	3	6674.9	2225	7.95	<0.001
Day.Atmosphere	3	3512.4	1170.8	4.18	0.009
Day. Type	9	3800	422.2	1.51	0.164
Atmosphere. Type	3	6029.4	2009.8	7.18	<0.001
Day.Atmosphere. Type	9	3847	427.4	1.53	0.158
Residual	64	17914.7	279.9		
Total	95	51077.1			

Table 7A: ***ANOVA Table for the Effect of Hermetic Storage on mean of Aspergillus Contamination on Maize Stored for Three months' period (Fig. 28)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	14026	4675	2.91	0.067
Atmosphere	1	23193	23193	14.42	0.002
Day.Atm	3	14043	4681	2.91	0.067
Residual	16	25734	1608		
Total	23	76996			

Table 8A: ***ANOVA for the Effect of Hermetic Storage on Maize Grains Weight Loss during Storage (Fig. 29)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	559.786	186.595	1315.42	<0.001
Atmosphere	1	1111.69	1111.69	7836.97	<0.001
Organism	2	24.9719	12.4859	88.02	<0.001
Day. Atmosphere	3	528.546	176.182	1242.01	<0.001
Day.Organism	6	40.0955	6.6826	47.11	<0.001
Atm.Organism	2	18.7456	9.3728	66.07	<0.001
Day.Atm.Organism	6	36.5671	6.0945	42.96	<0.001
Residual	48	6.8089	0.1419		
Total	71	2327.21			

Table 9A: ***ANOVA for the Effect of Hermetic Storage on Maize Grains Damage during Storage (Fig.30)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	1628.92	542.973	104.25	<0.001
Atmosphere	1	1308.22	1308.22	251.18	<0.001
Organism	2	278.59	139.295	26.74	<0.001
Day. Atmosphere	3	1485.76	495.254	95.09	<0.001
Day.Organism	6	494.696	82.449	15.83	<0.001
Atm.Organism	2	263.211	131.605	25.27	<0.001
Day.Atm.Organism	6	609.376	101.563	19.5	<0.001
Residual	48	249.998	5.208		

Total	71	6318.77			

Table 10A: ***ANOVA table for the Effect of Hermetic Storage on mean Viability of Maize stored for Three months' period (Fig. 31)***

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	9113.73	3037.91	108.61	<0.001
Atmosphere	1	3828.12	3828.12	136.86	<0.001
Organism	2	1113.04	556.52	19.9	<0.001
Day.Atm	3	2024.5	674.83	24.13	<0.001
Day.Organism	6	453.32	75.55	2.7	0.024
Atm.Organism	2	769.68	384.84	13.76	<0.001
Day.Atm.Organism	6	565.2	94.2	3.37	0.007
Residual	48	1342.59	27.97		
Total	71	19210.18			

APPENDIX B

Means tables of the effect of hermetic storage on the quality and shelf life of maize grain. Least Significant Difference (LSD) comparison.

Table 1B: *Effect of Hermetic Storage on Temperature Changes during Maize Grains Storage*

Atmosphere	Aflatoxin	Control	Insect
Hermetic	26.947a	26.862a	27.172a
Non-Hermetic	26.946a	26.916a	27.279a
LSD	0.107		

Note: The values with same letter are not different.

Table 2B: *Effect of Hermetic Storage on Relative Humidity during Maize Grains Storage*

Atmosphere	Aflatoxin	Control	Insect
Hermetic	75.17b	73.75b	75.18b
Non-Hermetic	78a	78.83a	81.83a
LSD	1.116		

Note: The values with same letter are not different.

Table 3B: *Effect of Hermetic Storage on Maize Grains Moisture Content during Storage*

Atmosphere	Aflatoxin	Control	Insect
Hermetic	13.859b	13.69b	13.877b
Non-Hermetic	15.284a	14.678a	15.324a
LSD	0.283		

Note: The values with same letter are not different.

Table 4B: E*ffect of Hermetic Storage on Oxygen Depletion during Maize Storage*

Atmosphere	Aflatoxin	Control	Insect
Hermetic	12.708b	12.878a	12.858a
LSD	0.122		

Note: The values with same letter are not different.

Table 5B: *Effect of Hermetic Storage on Insect Mortality during Maize Grains Storage*

Atmosphere	Percentage
Hermetic	59.3a
Non- hermetic	30.7b
LSD	5.43

Note: The values with same letter are not different.

Table 6B: ***Effect of Hermetic Storage on Aflatoxins groups growth***

Atmosphere	AfB_1	AfB_2	AfG_1	AfG_2
Hermetic	1b	0.4b	0b	0b
Non-Hermetic	42.6a	15.4a	4a	1.5a
Lsd	13.65			

Note: The values with same letter are not different.

Table 7B: ***Effect of Hermetic Storage on Maize Grains Contamination by Aspergillus flavus***

Atmosphere	Level (ppb)
Hermetic	1.4b
Non-Hermetic	63.6a
LSD	34.71

Note: The values with same letter are not different.

Table 8B: ***Effect of Hermetic Storage on Maize Grans Weight Loss during Storage***

Atmosphere	Aflatoxin	Control	Insect
Hermetic	0.291b	0.434b	0.48b
Non-Hermetic	6.29a	8.982a	9.091a
LSD	0.309		

Note: The values with same letter are not different.

Table 9B: ***Effect of Hermetic Storage on Maize Grain Damage Percentage during the Storage***

Atmosphere	Aflatoxin	Control	Insect
Hermetic	2.34b	2.17b	2.38b
Non-Hermetic	7.78a	8.39a	16.29a
LSD	1.873		

Note: The values with same letter are not different.

Table 10B: ***Effect of Hermetic Storage on Maize Seed Viability during Storage***

Atmosphere	Aflatoxin	Control	Insect
Hermetic	72.92a	79.17a	74.3a

Non-Hermetic	65.97b	65.28b	51.39b
LSD	4.341		

Note: The values with same letter are not different.

APPENDIX C

Storage room temperature and relative humidity changes during the storage period.

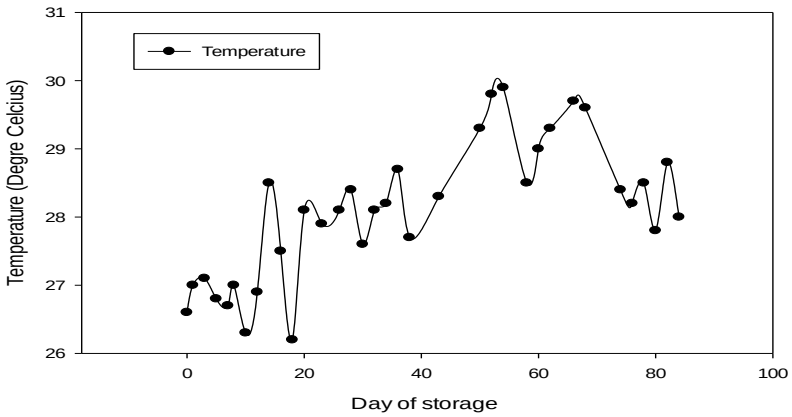

Figure 1C: Outside temperature changes during three months' storage of maize grains.

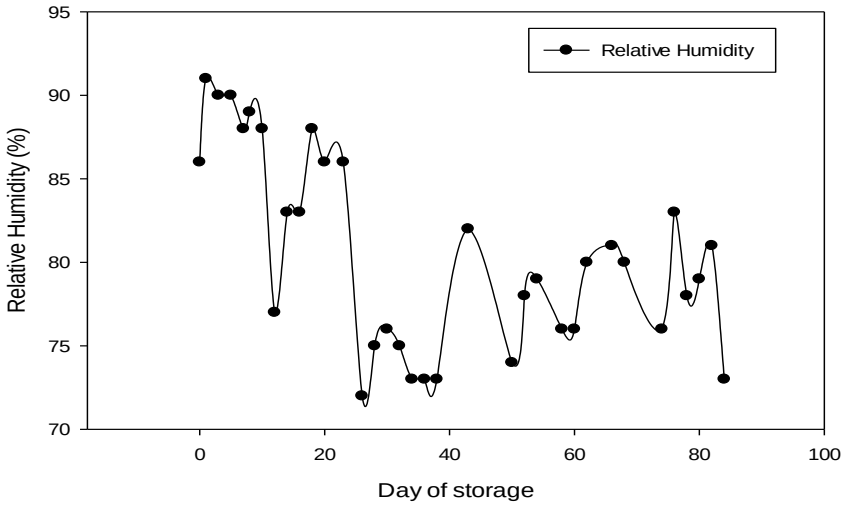

Figure 2C: Outside relative humidity changes during three months' storage of maize grains.

APPENDIX D

Hermetic storage material.

Figure 1D: The SuperGrainbag material.

Appendix D: Comparison of insect effect on stored maize grains in hermetic and non-hermetic storage.

Figure 1E: Maize grains from hermetic (A) and non-hermetic (B) bags.