EFFECTS OF HERMETIC BAG STORAGE ON INSECT PEST DAMAGE, MOULD INFECTION AND AFLATOXIN CONTAMINATION ON MAIZE GRAIN IN MAKUENI COUNTY, KENYA

JEREMIAH KINUTHIA NG'ANG'A

MASTER OF SCIENCE

(Food Science and Technology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

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Effects of hermetic bag storage on insect pest damage, mould infection and aflatoxin contamination on maize grain in Makueni County, Kenya

Jeremiah Kinuthia Ng'ang'a

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature…….Date….....

Jeremiah Kinuthia Ng'ang'a

This thesis has been submitted for examination with our approval as university supervisors:

Signature ……. Date……

Dr. Samuel Imathiu
JKUAT, Kenya

Signature ……. Date……

Dr. Christopher Mutungi

EU, Kenya

Signature ……. Date……

Dr. Hippolyte Affognon

ICRISAT, Mali

DEDICATION

To my beloved parents Edward and Ruth, brothers and sisters

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ACRONYMS

ANOVA	Analysis Of Variance
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
EU	Egerton University
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
HCC	Hepatocellular Carcinoma
HDPE	High Density Polyethylene
HS	Hermetic Storage
ICIPE	International Center of Insect Physiology and Ecology
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
IRRI	International Rice Research Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LGB	Larger Grain Borer
m.c.	Moisture content
PICS	Purdue Improved Crop Storage
PP	Polypropylene
SDA	Sabourauds Dextrose Agar
SSA	Sub-Saharan Africa
µg/kg	Microgram per kilogram

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ABSTRACT

For centuries, maize producers in sub-Saharan Africa have been plagued by post-harvest losses from insect infestation and mould infections, with small-scale producers representing the most vulnerable populations. Participatory on-farm trials were conducted to assess the effectiveness of triple layer hermetic (PICS™) bags for storage of maize in small-scale farmers' stores in rural villages in Makueni County, Kenya. PICS bags are three-layer hermetic bag-system that forms a barrier against the influx of oxygen and the escape of carbon dioxide. Shelled maize grain was purchased from the participating farmers, filled into jute, woven polypropylene or PICS bags, and kept in the farmers' own stores for 35 weeks. Temperature and relative humidity in the trial site and in all the bags, as well as oxygen and carbon dioxide levels in the PICS bags were also monitored. Grain moisture, live insect population, grain damage and weight loss, total mould count and mould incidence levels were examined at onset and after every 7 weeks while total aflatoxin contamination was examined at onset and after 14, 28 and 35 weeks. Initial moisture content of maize varied from farmer to farmer and ranged between 12.4 – 15.0%.

Oxygen and carbon dioxide compositions demonstrated that PICS bags are capable of sustaining good air-barrier properties. Generally, moisture content of maize stored in PICS bags was significantly higher ($P < 0.05$) than moisture content of maize stored in woven polypropylene and jute bags in the last 14 weeks of storage. Maize stored in hermetic bags remained free from insect infestation and the weight loss due to insect damage was below 1%. In contrast maize stored in woven polypropylene and jute bags permitted profuse build-up of insect populations and grain damage reached 77.6% and 82.3% corresponding to 41.2% and 48.5% weight loss respectively. Total mould count and aflatoxin contamination of maize stored at an initial moisture content < 13% and 13% ≤ m.c. ≤ 14% increased significantly in woven polypropylene and jute bags but not in PICS bags. After 35 weeks, total aflatoxin of maize stored in the woven polypropylene and jute bags at initial moisture content < 13% and 13% ≤ m.c. ≤14% increased 5 - 8 folds. Total mould and aflatoxin contamination of maize stored at initial

moisture content > 14% increased in the three types of bags. These findings demonstrate that PICS bags are effective in controlling losses caused by storage pests and can prevent mould infection and aflatoxin contamination on maize in rural farmers' stores if grain moisture is < 14%.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize (*Zea mays* L.) is one of the most important grain staples in Sub-Saharan Africa (SSA). In Kenya, maize and maize products are a major source of food for over 85% of the population. The crop accounts for nearly 20% of plant-based food supply (Abebe *et al*., 2009), and is a major source of calories and income for many households (Zia-Ur-Rehman, 2006). The per capita daily consumption of maize meals in Kenya is estimated at 0.4 Kg (Siboe & Muriuki, 1995). In Kenya, the bulk of production is carried out by small-scale farmers who cultivate less than 5 ha of the crop annually due to resource constraints. However, biotic and abiotic factors, especially after harvesting contribute to further losses in quantity, quality (safety and nutritional value) and economic value of the grain available for consumption or trade (World Bank, 2010). The main biotic causes of postharvest losses in maize are insect pest and mould infection.

Insect pest causes enormous amounts of losses to the harvested and stored grain because control of these pests is still a challenge to many small-scale farmers, particularly in poorly managed stores. The destructive effects are aggravated by the lack of knowledge and appropriate, affordable and effective grain care technologies (Moreno-Martinez *et al*., 2000; Baributsa *et al*., 2014). Consequently, food and income security of many rural farmers, become tremendously diminished when stored volumes, quality, food value, and marketability of the grain is lost to insect feeding, damage and contamination (Affognon *et al*., 2015). The main insects that attack stored maize are larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (De Groote *et al*., 2013). Others include *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner, [1813]), and *Rhyzopertha dominica* (Fabricius) (Ortega, 1987).

In addition to insect pest, maize becomes infected at any stage of production including cultivation, harvesting, drying, storage, transportation and marketing by a variety of moulds, such as *Fusarium*, *Aspergillus* and *Penicillium* spp. (Quezada *et al*., 2006; Blandino *et al*., 2009; Chulze, 2010). The infection not only reduces quality of the maize through discoloration and reduction of nutritional value (Ehrlich, 2007), but also culminates in deposition of toxic metabolites when the colonizing fungi are mycotoxigenic, and the conditions favor production of the toxins (Bennett & Klich, 2003; Wagacha & Muthomi, 2008).

Dried maize may be infected by three main aflatoxigenic species of the genus *Aspergillus*, namely, *A. flavus, A. parasiticus* and *A. nomius* (Peraica *et al*., 1999; Guo, 2000). Aflatoxin contamination of maize is almost exclusively by *A. flavus*, which produces aflatoxin B1 and B2 (Mutungi *et al*., 2008). Typically, *A. flavus* grows optimally at 25°C with a minimum water activity of 0.75 (Parry, 1990; Oladiran & Iwu, 1993), but the optimal conditions for subsequent production of aflatoxin include moisture content above 14%, temperature of 28 – 30°C and water activity of 0.83 – 0.97 (Oladiran & Iwu, 1993). The oxygen - carbon dioxide ratio, physical integrity of the grain, initial level of *A. flavus* infection, presence of competing moulds, pest activity, and genetic properties of the grain have also been reported to determine the degree of contamination and subsequent aflatoxin contamination (Dierner *et al*., 1987).

Contamination of maize and other food commodities with aflatoxins is of public health concern because of the ability of aflatoxins to cause human and animal diseases (CDC, 2004; *Gong et al*., 2004). Aflatoxin has been implicated with acute and chronic aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of the immune system, aggravation of kwashiorkor and impaired childhood growth (Hall & Wild, 1994). Aflatoxin contamination has been shown to be more prevalent in the tropical and subtropical regions due to the warm and humid conditions (Choudhary & Sinha, 1993; Cotty, 1994). Aflatoxigenic fungi may infect the maize crop before harvest and remain associated with the kernel through harvesting and storage (Cotty, 1990). Thus

contamination is likely to continue in the postharvest stage if the produce is not handled or stored properly to minimize the growth of the toxigenic fungal spp. (Wilson & Abramson, 1992). Outbreaks of acute human aflatoxicosis occur frequently especially with respect to maize in Kenya, and are well documented (Ngindu *et al*., 1982; CDC, 2004; Azziz-Baumgarter *et al*., 2005; Lewis *et al*., 2005).

Chemical-free hermetic storage technologies that have less destructive impact to environment and human health may offer safe and cost-effective protection of stored grains against insect pest (Murdock *et al*., 2012) and mould infection and aflatoxin contamination (Williams *et al*., 2014). One such technology is the Purdue Improved Crop Storage (PICS®) triple-layer hermetic storage bags which applies a two-layer envelope made of 80µm thick high density polyethylene (HDPE) liners inserted in an outer woven polypropylene sack. The HDPE liners have low permeability to air, and are thus able to secure a modified low oxygen and high carbon dioxide atmosphere generated by respiration of the grain, insects and other life-forms enclosed when the bag is sealed, thus stopping damage of the stored produce by insect pests and moulds (Murdock *et al*., 2012). A major health and safety concern in hermetically stored maize is the possibility of proliferation of aflatoxigenic moulds and potential aflatoxin contamination during multi-month storage because of possibility of moisture build-up in impermeable enclosures. Some findings have been reported, for instance Richard-Molard (1988) demonstrated that under hermetic storage, fungistatic effect is induced when oxygen concentration drops to 1% or below. Contrary to these findings Castellari *et al*. (2010) reported that mycotoxigenic fungi can develop in maize stored in hermetic plastic bags and a potential risk of contamination with aflatoxins and /or fumonisins in the grain may occur. This study was therefore conducted to compare the performance of the PICS, woven polypropylene (PP) and jute bags in controlling insect pest damage, mould proliferation and aflatoxin contamination of maize during long-term storage under farmers' storage conditions in Makueni County, Kenya.

1.2 Problem statement

In Sub-Saharan Africa, maize producers are often plagued by postharvest losses due to insect infestation and mould infections, with small-scale producers representing the most vulnerable populations. Annual total postharvest loss has been reported to be $US 4 billion out of $US 27 billion worth of grains produced (FAO, 2004) leading to food insecurity in many countries including Kenya.

Insect pest for instance, causes enormous amounts of losses to the harvested and stored grain because control of these pests is still a challenge to many small-scale farmers. The *P. truncatus* is the most damaging pest for farm-stored maize causing over 30% dry weight loss after only 3 - 6 months of storage (Lamboni & Hell, 2009; Mutambuki & Ngatia, 2012). The maize weevil (*S. zeamais*) can cause 10 - 20% weight loss after 3 - 6 months of storage, and up to 80% loss may occur if untreated maize is stored in traditional structures (Mutiro *et al*., 1992; Boxall, 2002). To avoid the losses caused by these insects, farmers opt to sell their maize shortly after harvest and this translate to loss of incomes by farmers. Other farmers apply synthetic insecticides as storage protectants but adequate protection is often not achieved (Meikle *et al*., 2002; Obeng-Ofori, 2011). Consequently, food and income security of many rural farmers, become tremendously diminished when stored volumes, quality, food value, and marketability of the grain is lost to insect feeding, damage and contamination.

In addition, maize becomes infected by a variety of moulds, such as *Fusarium*, *Aspergillus* and *Penicillium* spp. (Chulze, 2010). The infection not only reduces quality of the maize through discoloration and reduction of nutritional value but also culminates in deposition of toxic metabolites when the colonizing fungi are mycotoxigenic (Wagacha & Muthomi, 2008). Globally, aflatoxin is a public health concern because of the ability of aflatoxins to cause human and animal diseases (CDC, 2004; Gong *et al*., 2004). Aflatoxin has been implicated with acute and chronic aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of the immune system, aggravation of

kwashiorkor and impaired childhood growth (Hall & Wild, 1994). In Kenya, outbreaks of acute human aflatoxicosis occur frequently especially with respect to maize, the dietary staple to over 85% of the population, and are well documented (Ngindu *et al*., 1982; Azziz-Baumgarter *et al*., 2005; Lewis *et al*., 2005).

1.3 Justification of the study

Maize is the most important cereal crop in Kenya without which most of the communities in the country would be rendered food insecure. In Kenya, the crop is a major source of calories and income for many households (Zia-Ur-Rehman, 2006). However, maize grains are often contaminated by spoilage and mycotoxin producing microorganisms that may impact negatively on human and animal health. In addition, infestation by both insects and rodents create pathways for microbial contamination, spoilage and thus food loss. Today's consumers demand for chemical-free food due to increased attention to health hazards posed by contaminants calls for noble methods of postharvest commodity storage. In this regard there is need to develop more efficient and effective technologically sound approaches for ensuring food safety and security. One such approach is the use of hermetic technologies such as triple layer hermetic bagging (PICS®) to store and preserve the harvested produce. In Kenya, PICS® bags were shown to successfully control *S. zeamais* and *P. truncatus* in artificially infested maize stored under laboratory conditions (Njoroge *et al*., 2014). Baoua *et al*. (2014) reported similar results using naturally infested maize in West Africa. Recently, Williams *et al*. (2014) reported that PICS® bags can prevent spoilage by moulds and aflatoxins accumulation. This study was therefore to compare the performance of the PICS®, PP and jute bags in controlling shelled maize against insect pest damage, mould proliferation and aflatoxin contamination in farm stores of individual rural small-scale farmers in Makueni County, Kenya.

1.4 Objectives

1.4.1 Main objective

To study the effect of hermetic bag storage on insect pest damage, mould infection and aflatoxin contamination in stored maize grain in Makueni County, Kenya.

1.4.2 Specific objectives

1. To establish storage practices of maize farmers in the study site.
2. To determine the efficacy of hermetic Purdue Improved Crop Storage (PICS®) bags in the reduction of postharvest losses of maize grain caused by insect pests under farmer storage conditions.
3. To determine the effect of PICS® bag storage on mould infection and mould incidence levels in stored maize grain.
4. To determine the effect of PICS® bag on aflatoxin contamination on stored maize grain.

1.5 Hypotheses

1. Ho: storage practices among maize farmers do not differ
2. Ho: storage of maize in PICS® bags does not significantly reduce postharvest losses caused by insects under farmers' storage conditions.
3. Ho: Storage of maize grains in PICS® bags has no significant effect on mould infection and mould incidence levels.
4. Ho: Storage of maize grains in PICS® bag has no significant effect on aflatoxin accumulation.

CHAPTER TWO

LITERATURE REVIEW

2.1 The maize plant, origin and distribution

Maize (*Zea mays* L. ssp. *mays*) or corn (**Figure 2.1**) is a monoicannual plant belonging to the maideas tribe and the grass family of *gramineae* (*Poaceae*), with cells having 2n chromosomes (Mejía, 2008).

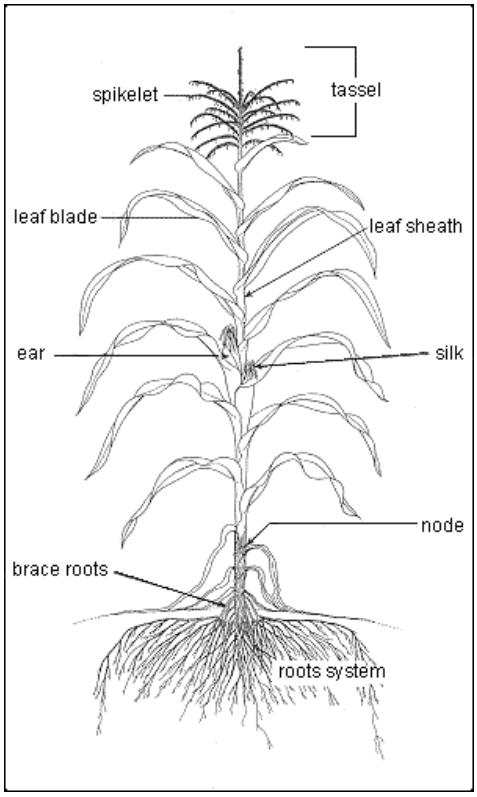


Figure 2.1: The maize plant (IITA, 2007)

Maize is a tall, annual grass with overlapping sheaths and broad conspicuously distichous blades, as well as staminate spikelets in long spike-like racemes that form

large spreading terminal panicles (tassels). It also has pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, on a thickened, almost woody axis (cob) (Mejía, 2008). The ear is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads. The pollen is produced in the staminate inflorescence and the gametes are produced in the pistillate inflorescence (Mejía, 2008).

Maize is one of the oldest human-cultivated crops. The center of origin is believed to be the Mesoamerica region, at least 7000 years ago when it was grown as a wild grass called *teosinte* in the Mexican highlands (Mangelsdorf, 1974). It spread around the globe after European discovery of the Americas in the 15th century (Watson & Dallwitz, 1992). It has tremendous variability in kernel color, texture, composition and appearance. The crop was probably introduced to tropical Africa at more than one point and at different times (Watson & Dallwitz, 1992). It was widely grown along the coast from the River Gambia to Sâo Tomé, around the mouth of the River Congo, and possibly in Ethiopia, in the sixteenth century (Mangelsdorf, 1974). There is reference to it in all these places, in Zanzibar, and around the mouth of the River Ruvuma in the seventeenth century; and it was not only mentioned but described as an important foodstuff and a major provision for slave ships between Liberia and the Niger Delta during the same century. Until the present century, it was neither a major export nor a mainstay of the diet in most of Eastern and Central tropical Africa, the bulk of the areas where it is now of major importance (Mangelsdorf, 1974). Currently is grown globally with major producers being USA, China, Brazil, Argentina, Mexico, South Africa, Romania and India (FAO, 2006).

2.2 Soil and climatic requirements for maize production

Maize grows on a wide range of soil types, provided they are well aerated and well drained to allow for the maintenance of sufficient oxygen for good root growth and activity, and enough water-holding capacity to provide adequate water throughout the growing season (Hoeft, 1992). Successful maize cultivation is achieved on soils which

are of light to medium texture but their organic status and fertility should be high and capable of providing the nutrients needed by the crop. The crop can be successfully cultivated on moderately acidic soils of a pH 5 – 8, but best growth has been reported in the range of a pH 5.6 – 7.5 (Hoeft, 1992). It does not grow well in saline soils. The crop requires a temperature of 9 - 30°C from planting to emergence (Du Plessis, 2003). From emergence to silking, leaf number increases with temperature and photoperiod. Maximum rate of grain filling occurs between 20 - 30°C. The longer the grain filling period, the higher the grain yield provided no freezing temperature is experienced (Du Plessis, 2003). Generally, it takes 3 - 5 months from emergence to maturity, and this often depends on the type of cultivar.

2.3 Maize production and utilization

It is estimated that in 2012, the total world production of maize was 875,226,630 tons, (FAO, 2012) with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production, respectively. In Kenya, maize production by regions is shown in **Table 2.1**. This indicates that there was increase in maize production in subsequent year with Rift Valley province being the largest producer in Kenya.

Maize cereal is popular with consumers because it is high yielding, easy to process, readily digested, cheaper than other cereals and grows across a wide range of agro-ecological zones. It comprises an average of 30 to 50% of the daily caloric intake of people in most Eastern African countries (FAO, 2001), and is a major staple food where per capita human consumption exceed industrial uses (Aquino *et al*., 2000; IITA, 2007).

Maize and maize flour (cornmeal), in the form of *oje, nshima, ugali, mealie pap, atole*, etc are a staple food around the world (FAO, 2001). Popcorn is a common snack, while corn flakes, hominy, grits, and canjica are common breakfast foods derived from maize. Maize is a significant source of starch, and a feedstock for the production of corn oil,

high fructose corn syrup, grain alcohol, and biofuels (FAO, 2001). It is also consumed as a vegetable; in addition to being used for livestock feed.

Table 2.1: Maize production in Kenya in metric tonnes by regions, 2009 - 2010

Region	**2009**	**2010**
Central	137,700	146,511
North Eastern	5,629	5,581
Nyanza	448,276	388,635
Western	397,815	508,757
Coast	51,105	46,410
Eastern	409,203	329,419
Rift Valley	1,037,997	1,358,127
Total	**2,481,725**	**2,783,375**

Source: Ministry of Agriculture, Crop Development Division, 2010.

2.4 Nutritional importance of maize cereal

The maize kernel (**Figure 2.2**) has nutritional properties that are comparable to other cereals such as rice and wheat (Mejía, 2008). **Table 2.2** shows the nutritional composition of maize compared with wheat and rice. It consists of carbohydrates, protein and small quantities of fat, vitamins, dietary fibres and minerals such as iron and phosphorus (Iken *et al.*, 2002).

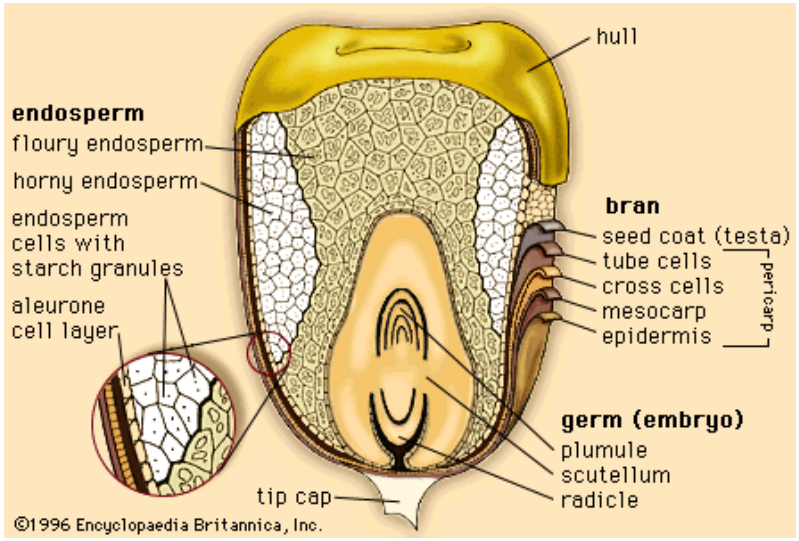


Figure 2.2: Maize kernel: outer layer and internal structure (Britannica, 1996)

Table 2.2: Nutritional composition comparison per 100 g maize, wheat and rice grain

Contents	Maize ground meal	Wheat flour	Rice polished grain
Calories	362	359	360
Carbohydrates	74.5g	74.1g	78.9g
Water	12g	12g	13g
Protein	9g	12g	6.8g
Fat	3.4g	1.3g	0.7g
Ash	1.1g	0.65g	0.6g
Starch fiber	1g	0.5g	0.2g
Phosphorous	178mg	191mg	140mg
Calcium	6mg	24mg	6mg
Niacin	1.9mg	2.0mg	1.5mg
Iron	1.8mg	1.3mg	0.8mg
Thiamine	0.30mg	0.26mg	0.12mg
Riboflavin	0.08mg	0.07mg	0.03mg

Source: Mejía (2008).

2.5 Maize storage and potential problems

Grain storage and preservation takes many forms depending on the quantity and type of grain to be stored, the purpose of storage, and the location of the store. Grains and grain storage systems are classified as either bag or bulk storage (IRRI, 2008). Maize grain storage moisture content and potential problems during storage are shown in **Table 2.3**. Maize grain is hygroscopic and its moisture content easily equilibrates with the

surrounding air, in open-air storage. This, in addition to high relative humidity and temperature in the tropics, promotes the rapid infestation and multiplication of insects, moulds as well as rodents and birds attack in open-air storage (IRRI, 2008).

Table 2.3: Safe maize grain storage moisture content requirements

Required moisture content for safe storage	Storage period	Potential problems
14 – 18%	2 to 3 weeks	Molds infection, discoloration
12 - 13%	8 to 12 months	Insect damage
9% or less	More than 1 year	Loss of viability

Source: IRRI (2008).

Primary causes of stored grain spoilage include incomplete drying resulting in wet pockets, temperature variations between storage bin and the outside, and the associated moisture condensation within the bin. In addition, inadequate observation and management during storage, improper storage bin preparation and insufficient cooling of grain after drying could result to further spoilage (IRRI, 2008).

2.6 Common maize storage methods used by small-scale farmers in Africa

In Africa, the poor status of small scale farmers leads them to select storage methods which are cheap to construct regardless of their inadequacy, consequently, most of the grain losses occur during storage (Obetta & Daniel, 2007). This necessitates improvement of the storage technologies. Factors that usually affect the farmers' choice of the storage methods include the cost of building and running the storage method,

availability of the materials and expertise for building the storage facility, climatic conditions of the area and the types of pest problems in the area (FAO, 1985).

2.6.1 Sack storage

Storage sacks are made of different materials such as sisal natural fibers and synthetic fibers, and they can store up to 100 kg of grain each (Lindblad & Druben, 1976). To prevent the storage sacks from absorbing moisture from the floor, the sacks need to be stacked on platforms raised off the floor, with space between them to allow air to flow under the sacks and between them. This cools the stored cereal from the heat that results from respiration of the grain. Regular inspection of crops stored in sacks is necessary to keep the grains safe from attacks by pests (De Groote, 2013). The weakness of sack storage is that they do not last long (FAO, 1994). New storage sacks are likely to be needed after every harvest, which makes this storage method expensive for small-scale farmers. Sack storage methods require that the storage sacks be treated with pesticides prior to storage to reduce chances of infestation (FAO, 1994). The advantage of sack storage is that it provides the farmer with ease of access to the stored crops because the farmer can choose to store the grain filled sacks at any convenient place in the home. However, they can be easily damaged by rodents, which would expose the stored maize to rodents' infestations.

2.6.2 Storage cribs

These storage structures can last for more than a year and the amount of crops that they can store depend on the size of the crib (UNIFEM, 1995). The main advantage of storage cribs is that maize stored in them continues to dry through ventilation due to the manner in which the cribs are built. However, the rate at which maize dries in a crib depends upon the force at which air currents pass through the maize cobs, and this is influenced by the width of a crib (FAO, 1994). Placing the longer side of the crib in line with the orientation of the prevailing wind has been found to be helpful in allowing as

much air current as possible to be blown into the stored maize cobs (FAO, 1994). Thus, purposeful designing and positioning of cribs may be helpful in maize drying. However, it has also been noted that it takes 8 – 10 days to bring maize in a crib to the right moisture content during the dry season, and 80 days during the wet season (FAO, 1985). Generally, maize with 30% moisture content at harvest can take about six weeks to be appropriately dried in a crib (Shepherd, 2010).

Storage cribs can be metallic or non-metallic (**Figure 2.3**). Walls and floors of non-metallic cribs are made using wood and mud, while roofs are thatched. Rodents can easily make holes through them, while moisture can also penetrate into the cribs and cause moisture content problems in the stored grain. As opposed to non-metallic cribs, metallic ones are made using materials which rodents cannot make holes through such as iron or aluminum sheets, and they can be rodent proofed by fitting into them structures that prevent rodents from getting into the cribs.

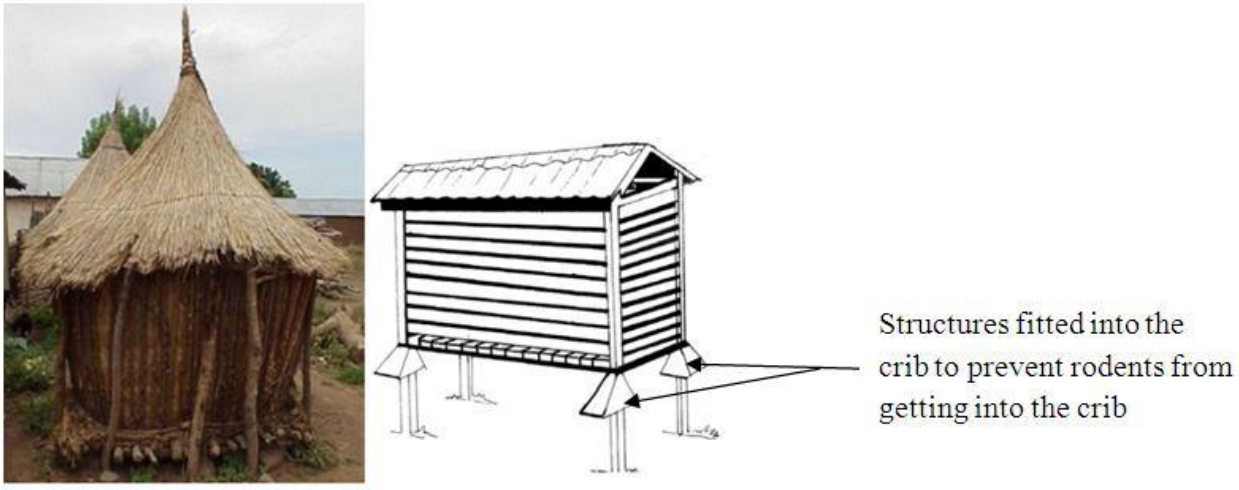


Figure2.3: Storage cribs for maize storage (IITA, 2009)

2.6.3 Concrete silos

Concrete silos (**Figure 2.4**) are structures made using concrete bricks and they can store up to five tonnes of grain depending on the size of the silo (Villers *et al*., 2006). They include cement-stave silos which have the capacity to store up to 10 tonnes of grain and Thai-Ferro cement silos which can store four to six tonnes of grain. Although they are durable, concrete silos are expensive because the materials required and the cost of constructing them is high (Villers *et al*., 2006). Thus, poor small-scale farmers may not be able to cope with the cost of making concrete silos regardless of the benefits. Furthermore, concrete silos have constraints such as allowing build-up of heat leading to condensation and moisture problems, and ultimately to infestations by insect pests and moulds infections (Villers *et al*., 2006).

Figure 2.4: Concrete silo for maize storage (Shepherd, 2010)

2.6.4 Mud block silos

Mud block silos (**Figure 2.5**) are structures made using compacted soil (Lindblad & Druben, 1976). Compact soil is naturally a poor conductor of heat and has high thermal inertia (Darlington, 2007) because heat does not flow through it easily. Thus, when exposed to heat a brick silo would build up heat on the surface while the inner parts of the bricks remain cool. This helps to keep crops stored in the brick silo cool, leading to longer storage (UNIFEM, 1995). However, due to the nature of the material used to make the mud brick silos, moisture can easily get into the storage facilities and cause moisture problems and damage to the storage facilities. The ease with which mud block silos break renders them expensive since new ones will have to be built after every breakage (Coulter & Schneider, 2004). Rodents can also easily make holes through the mud bricks and attack stored grain.

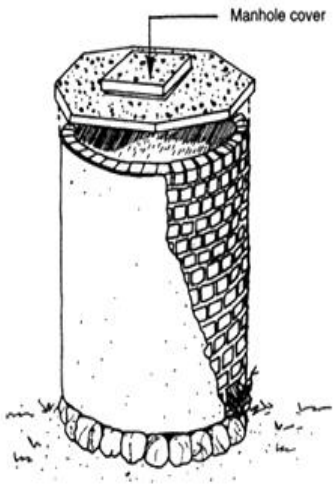


Figure 2.5: Mud block silo (Shepherd, 2010)

2.6.5 Underground storage pits

Underground storage pits can offer protection to stored crops for up to two years if the walls of the pits are lined with bricks or concrete to hinder moisture from getting in

(Ikisan, 2000). The amount of maize that an underground storage pit can store depends on the size of the storage pit. It has been noted that while this method can protect stored crops from rodents and insects, moisture and air from other parts of the soil may move into the storage pit, and crops stored in such pits may be attacked by mites (UNIFEM, 1995). Grain stored in an underground storage pit cannot be accessed easily because the entrance to the pit is covered with soil to prevent air and moisture from getting into it (Ikisan, 2000). Therefore, underground storage pits cannot be used for storage of grain intended for daily consumption.

2.6.6 Suspension of crops on a tree or above the fireplace

Hanging unthreshed crops such as maize cobs on a bunch in a tree or drying above the fire-place (**Figure 2.6**) is used for storage of small quantities of maize (Lemma, 2006). This method allows drying of maize to continue through exposure to the sun (UNIFEM, 1995). However, this storage method has been criticized for exposing the crop to rain, wind, rodents, birds and insects, which may lead to infestations, infections and moisture problems. Hanging above the fire allows continuous drying of maize. However, this method is usually associated with food safety issues such as accumulation of smoke on maize grain making it unpalatable.

Figure 2.6: Drying of unshelled maize above the fire-place (UNIFEM, 1995)

2.7 Improved storage technologies

2.7.1 Admixing grain with synthetic pesticides

In Sub-Saharan Africa, the main commercially available grain protectant recommended for storage against insect pest control is a dilute dust containing 1.6% pirimiphos-methyl + 0.3% permethrin (Actellic Super dust (ASD)), although recently several other similar mixtures have entered the market. Actellic super has been adopted by small-scale farmers for grain storage in Kenya as well as other African countries. In Tanzania, it was the most common control method of treating maize before storage having been reported by more than 93% of farmers in both high rainfall and low rainfall zones (Mutambuki & Ngatia, 2012). Unfortunately, since the distribution of these pesticides was privatized in Africa in the 1990s, there have been widespread reports from farmers of inefficiency. The limited effectiveness of synthetic insecticides used by the farmers could be explained by a number of reasons, among them possible adulteration of the insecticides by vendors, improper application practices such as delayed treatment (Golob &Hanks, 1990), incorrect dosage and patchy use by the farmers (Mutambuki & Ngatia, 2012), or the progressive loss of insecticidal potency of the active ingredients (Denloye *et al*., 2008). In order to reduce losses farmers' and other agricultural stakeholders' have been demanding for alternative grain protectants.

2.7.2 Metal silos

Metal silo is a cylindrical metal container constructed from galvanized iron sheet. Metal silos have been used widely in Central America for on-farm grain storage (Farrell & Schulten, 2002). This storage structure can be fabricated in different sizes, 100 kg – 3000 kg holding capacity by trained local artisans. The metal silo works on the hermetic technology concept, where the lack of air inside the container suffocates and kills insect pests and discourages growth and multiplication of moulds (Tadele *et al*., 2011). The impact of metal silo technology in Africa, Asia and Latin America includes, improving

food security, empowering smallholder farmers, enhancing income opportunities and job creation, and safeguarding the agro-ecosystems. It has also been promoted in various countries in Africa including Kenya, Malawi and Swaziland by various NGOs and FAO as one way of improving food security (FAO, 2001). Adoption of this technology by small-scale farmers is not well documented. In Kenya, for instance, Kimenju and De Groote (2010) reported that metal silos were not economically feasible if the capacity is smaller than 500 kg. Thus, even though they demonstrated large impact on the welfare and food security of users, high initial cost became a disincentive to adoption especially for individual small-scale farmers who store less than five 90 kg bags, and whose opportunity cost of capital is high (Gitonga *et al*., 2013). On the other hand, the larger more cost effective silos would require communal ownership which is unpopular because many small-scale farmers still prefer to store their own produce, so as to retain greater control and flexibility in its marketing and use in the household (World Bank, 2010).

2.7.3 Fumigation

Fumigants are effective against storage pests because as gases they can reach the pests in the most remote hiding place (Winks, 1986). The range of safe fumigant chemicals that can be used is now restricted to phosphine and carbon dioxide due to food safety and health related issues (Friendship *et al*., 1986).

Phosphine fumigation is undertaken using tablets and pellets as shown in **Table 2.4**. These tablets and pellets release phosphine gas when they come into contact with humid air (Friendship *et al*., 1986). Phosphine is toxic to all insects. When insects are exposed to fumigation in a sealed environment all stages of development from the eggs, larvae, pupae to adults are killed (Winks, 1986). Phosphine does not impair the grain nor leave residues that could be hazardous to the consumer when correctly applied and the grain aerated (Friendship *et al*., 1986).

Table 2.4: Phosphine fumigation minimum exposure times at 60% relative humidity

Temperature (^{o}C)	Tablets (days)	Pellets (days)
Under 5	No fumigation	No fumigation
5 - 10	10	8
11 - 15	5	4
16 - 25	4	3
Over 25	3	3

Source: Friendship *et al*. (1986).

Fumigation must be carried out by highly trained personnel. Care must be taken when using phosphine gas as it is very toxic to humans. In addition, fumigation must take place in an enclosure that can be tightly sealed. Once the exposure time is ended, the grain must be aerated and the bin checked for residual phosphine gas before entry.

Insects need oxygen for respiration. With carbon dioxide fumigation, much of the oxygen in the storage bin is replaced by carbon dioxide that suffocates, dehydrates and also produces toxic chemicals in the insects' fluid (Navarro *et al*., 2012). To be effective, elevated carbon dioxide levels must be maintained until all insects die. The required exposure time depends on the percentage of carbon dioxide and the temperature of the grain (Navarro *et al*., 2012).

2.7.4 Triple-layer hermetic storage bags

Purdue Improved Crop Storage (PICS®) triple-layer hermetic storage bags which applies a two-layer envelope made of 80µm thick high density polyethylene (HDPE) liners inserted in an outer woven polypropylene sack (**Figure 2.7**) has seen emergent interest in hermetic storage systems as alternative grain preservation methods that are more attractive to small-scale farmers (Baributsa *et al*., 2014). The HDPE liners have low permeability to air, and are thus able to secure a modified low oxygen and high carbon dioxide atmosphere generated by respiration of the grain, insects and other life-forms

enclosed when the bag is sealed, thus stopping damage of the stored produce by insect pests and moulds (Murdock *et al*., 2012; Williams *et al*., 2014).

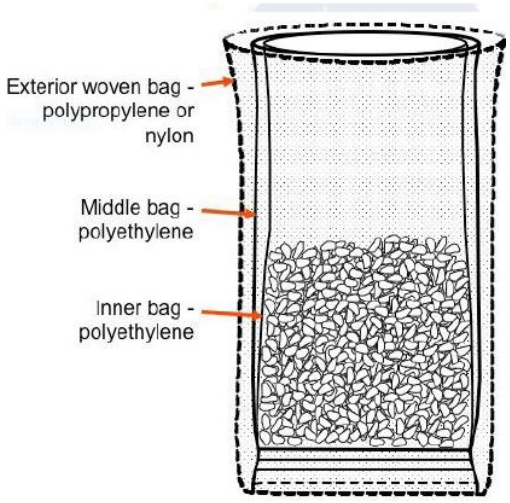


Figure 2.7: Schematic representation of PICS bag (Murdock *et al*., 2003).

Although the two HDPE liners are not perfectly oxygen impermeable and leak oxygen extremely slowly, they nevertheless greatly hinder diffusion of oxygen from the exterior air into the interior of the bag. However, as long as insects present in the grain are actively feeding, growing and developing and are using oxygen faster than it is leaking in from the outside, the oxygen content of the airspace within the bag will necessarily fall (Murdock *et al*., 2012). PICS® bags were originally developed for preservation of cowpeas against cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in West Africa (Murdock *et al*., 2003). **Figure 2.8** demonstrates how to use PICS bags.

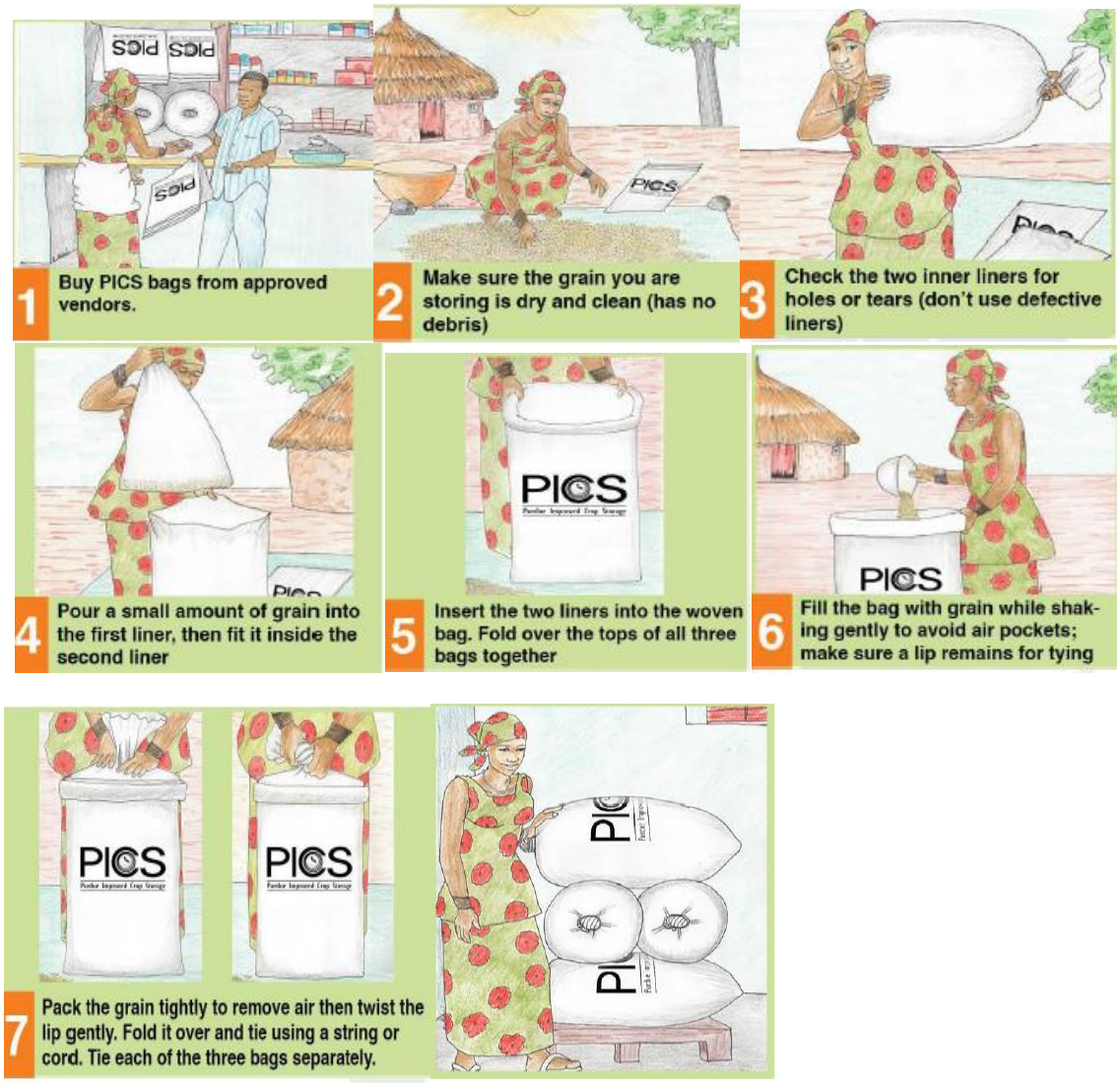


Figure 2.8: How to use PICS bags (Murdock *et al*., 2003).

Previous research has seen PICS bags investigated in its effectiveness in storing other products. (Edoh-Ognakossan *et al*., 2013; Baoua *et al*., 2014; Mutungi *et al*., 2014; Njoroge *et al*. 2014; Vales *et al*., 2014). In addition, to insect pest control, hermetic condition induces fungistatic effect when oxygen concentration drops to 1% or below (Richard-Molard, 1988). Thus, stored products could be free from mould infection and aflatoxin accumulation during storage if initial moisture content is safe for long term storage (Williams *et al*., 2014).

2.8 Maize grain damaging pests

The most economically destructive maize storage insects in Sub-Saharan Africa are the larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (De Groote *et al*., 2013). Others include *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner, [1813]), and *Rhyzopertha dominica* (Fabricius) (Ortega, 1987). The *P. truncatus* is the most damaging pest for farm-stored maize, and in endemic situations, extensive grain damage results in over 30% dry weight loss after only 3 - 6 months of storage (Lamboni & Hell, 2009; Mutambuki & Ngatia, 2012). Such weight loss can be accompanied by heavy grain damage, which could render the grain totally unfit for human consumption (Njoroge *et al*., 2014). Lager grain borer infestations were first reported in Tanzania (1981), Kenya (1983), Burundi (1984),Togo (1984), Benin (1984), Guinea (1987), Ghana (1989), Burkina Faso (1991), Nigeria (1992),Malawi (1992), Rwanda (1993), Niger (1996), Zambia (1996), Uganda (1997), Namibia (1998), South Africa (1999), Mozambique (1999), and Senegal (2007) (Cugala *et al*., 2007), indicating that they are spreading and threatening the productivity of the maize storage in Africa. Mutambuki & Ngatia (2012) noted that *P. truncatus* outbreaks could be sporadic for various biological reasons.

Figure 2.1 show the maize weevil and weevil infested maize. The maize weevil (*S. zeamais*) can cause 10 - 20% weight losses after 3 - 6 months of storage, and up to 80% loss may occur if untreated maize is stored in traditional structures (Mutiro *et al*., 1992; Boxall, 2002). In addition, insects physically transfer conidia adhering to their bodies to plant parts in feeding and this would lead to mould infection and aflatoxin contamination (Diener *et al*., 1987). Thus, due to inefficient storage technologies millions of tonnes of maize may be lost due to insect pests in the world each year.

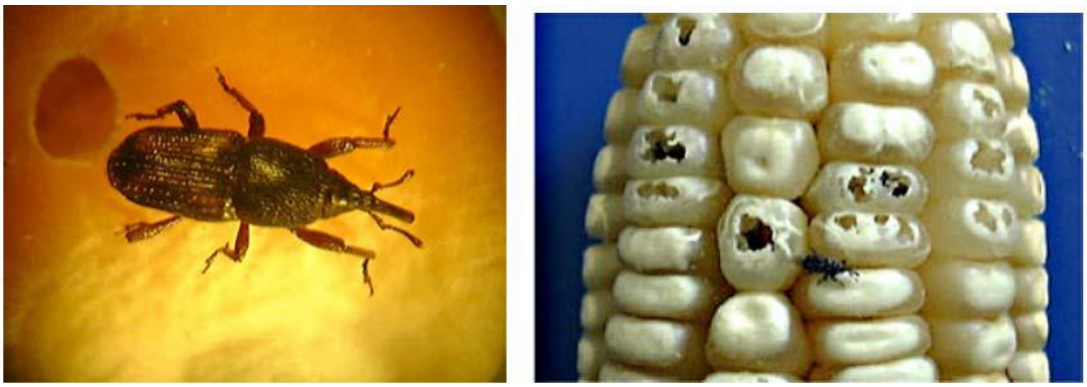


Figure 2.9: The maize weevil and weevil infested maize on the cob (Savidan, 2002)

2.9 Mould infection and aflatoxin contamination on maize

Maize grains are subject to infection by a variety of toxigenic fungi, particularly from the genera *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium*. Toxigenic *Alternaria* and *Fusarium* species are often classified as field fungi because they infect the grain while in the field, while *Aspergillus* and *Penicillium* species are considered as storage fungi because they grow and infect grain during storage if conditions are favourable for their growth (Logrieco *et al*., 2003).

Figure 2.10 shows maize cob infected by field fungi**.** Field fungi populations such as *Fusarium* generally decrease with duration of storage period as moisture content declines (Fandohan *et al*., 2005).

Figure 2.10: Maize cob infected by field fungi (Fandohan *et al*., 2005)

In the field during the dry hot seasons, *A. flavus* spore population increases on crop debris and on senescent or dormant plant tissues leading to high levels of mould propagules in the air (Wilson and Payne, 1994). Heavy inoculum of *A. flavus* may thus be introduced to the crop as air-borne propagules throughout the period of crop growth and maturation. Insect activity facilitates infection of pre-harvest maize by enhancing dissemination of inoculum within ears, and creating a favourable habitat for *A. flavus* through injury associated feeding (McMillan, 1983; Payne, 1992). After introduction of inoculum, the environment to which the standing crop is exposed determines the extent of infection.

Aflatoxins are toxic fungal metabolites that accumulate in stored produce when storage conditions favor proliferation of aflatoxigenic fungi (Krishnamurthy and Shashikala, 2006). There are 18 different types of toxins in the aflatoxin group identified. Among these, the major members are aflatoxins B1, B2, G1, G2, M1 and M2 (Wrather, 2008). Aflatoxin B1 is produced most abundantly and is the most toxic followed by G1, B2 and G2. Aflatoxins B1, B2, G1 and G2 are classified as Group I human carcinogens whereas M1 is classified as Group 2B probable human carcinogen (Krishnamurthy and Shashikala, 2006). The four major aflatoxins: B1, B2, G1 and G2 are named according

to the fluorescent colours when thin layer chromatographic preparations are viewed under long wavelength ultraviolet light (365nm). Aflatoxin B1 and B2 show bright blue fluorescence while G1 and G2 fluoresce green. Natural formation of aflatoxins is more prevalent in the tropical and subtropical regions due to the warm humid conditions (Cotty, 1994). High levels of aflatoxins in agricultural commodities occur during the wet season after spells of severe drought (Philips et al., 1994).

Aflatoxin contamination of maize is almost exclusively by *A. flavus*, which produces aflatoxin B1 and B2 (Mutungi *et al*., 2008). Fungi, including *A. flavus* are generally incapable of growing in water activity below 0.70. The optimum water activity for growth of *A. flavus* group ranges between 0.91 - 0.99. In mature maize kernels, *A. flavus* does not exhibit extensive growth below water activity of 0.85 (Wilson & Abramson, 1992). Spore formation and sporulation however do occur at water activity of between 0.81 - 0.83, respectively. At slightly higher water activity levels of 0.87, *A. flavus* grows and produces aflatoxins. Very high water activity however, induces heavy colonization by competing mycoflora such as *A. oryzae, A. niger* and *Rhizopus nigricans* that can significantly reduce growth of *A. flavus* (Sauer & Burroughs, 1986).

The development of aflatoxigenic and other storage fungi in stored maize is principally a function of inoculum availability, moisture, and temperature of the grain (Wilson & Abramson, 1992). Maize grains are hygroscopic and depending on the moisture gradient and on whether grains are adsorbing or desorbing, kernels may equilibrate to a moisture level that permits development of *A. flavus* and subsequent production of aflatoxins. Aflatoxigenic moulds can grow and elaborate toxins in localized spots of high moisture. Even when grain is stored at safe initial moisture content of 13%, the xerotolerant moulds such as the *A. glaucus* group continue to exhibit slow growth and predominance at 13 - 15% moisture. At 15% moisture, the less xerotolerant fungi such as *A. ochraceous, A. versicolor,* and *A. flavus* begin to grow. Although the growth ability of *A. flavus* is greatly inhibited at this moisture level, the rapid growth of the xerotolerant fungi creates localized wet spots enhancing conditions that favour growth of *A. flavus*

and subsequent production of aflatoxin (Wilson & Abramson, 1992). In addition, the oxygen - carbon dioxide ratio, physical integrity of the grain, initial level of *A. flavus* infection, presence of other moulds, pest activity, and genetic properties of the grain also determine the degree of contamination (Dierner *et al*., 1987). Moreover, immature, broken, undersized, discoloured kernels are most likely to be contaminated with aflatoxin compared to mature sound kernels (Beaver, 1991; Chiou *et al*., 1994; Rucker *et al.*, 1994). Drought stress has also been shown to increase the risk of field contamination of maize with aflatoxins (Sanders *et al*., 1981).

Occurrence of aflatoxin poisoning in Kenya was first reported in 1960 (Asplin & Carnaghan, 1961) following an outbreak in which 14 000 ducklings were reported to have died in one farm after being fed on a toxic groundnut meal imported into the country from Uganda. However, the presence of aflatoxin in food is often overlooked due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, wars, political and economic instability (MERCK, 2006). In Kenya, the largest mycotoxin poisoning epidemic in the last decade occurred in 2004, with concentrations of aflatoxin B1 in maize being as high as 4,400 ppb, which is 220 times greater than the Kenyan regulatory limit of 20 ppb. (CDC, 2004; Lewis *et al*., 2005). The aflatoxin poisoning was associated with eating home-grown maize that was stored under damp conditions (Lewis *et al*., 2005). Acute aflatoxin poisoning has continued to occur every year since 2004 in Eastern and Central regions of Kenya (Ngindu *et al*., 1982; CDC, 2004; Lewis *et al*., 2005). The outbreak covered more than seven districts and resulted in 317 case-patients and 125 deaths. Of great concern is that there seems to be an annual outbreak of aflatoxicosis in Eastern region of Kenya.

2.10 Effects of aflatoxins on humans and animal health

The effects of aflatoxins on animal health have been observed in many species for over forty years (Patten, 1981) beginning with the documentation of Turkey X disease in 1960 in United Kingdom which was attributed to a groundnut containing feed which was heavily infested with *A. flavus* (Blount, 1961). The primary target of aflatoxins is the hepatic system. Acute effects include hemorrhagic necrosis of the liver and bile duct proliferation while chronic effects include hepatocellular carcinoma.

In humans, acute exposure can result in aflatoxicosis, which manifests as severe, acute hepatotoxicity with a case fatality rate of approximately 25% (Patten, 1981). Early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low-grade fever. Acute high level exposure can progress to potentially lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death. In addition, suppression of immunity, growth retardation, and increased susceptibility to infectious disease due to aflatoxin exposure is well-documented (Patten, 1981).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Trial site and experimental conditions

On-farm storage trials were conducted with individual farmers in Kibwezi (02° 23'S, 37° 57'E; 1036 m), Machinery (02° 54'S, 37° 28'E; 1004 m) and Makindu sub-Counties (02° 18'S, 37° 50'E; 1019 m) in Makueni County, Eastern Kenya. The trial site was selected for being a hot-spot for insect-induced storage losses and aflatoxin outbreaks in Kenya (Lewis *et al*., 2005; Mutambuki & Ngatia, 2012). The region is generally semi-arid and experiences a bimodal rainfall pattern in which rains fall in March - April and November - December. Annual rainfall ranges between 200 - 700 mm, and day-time temperature ranges between 20 - 30°C.

3.2 Experimental design

Factorial design was applied in this study. Three villages in each sub-County were randomly selected. A total of 33 farmers (3 - 4 farmers in each village) who had a harvest of at least five (5) 90 kg bags of maize, and who also expected to store part of it were recruited for the trial using a combinations of both random and purposive sampling techniques. Trials were conducted over a 35-week storage period beginning end May 2014 to early February 2015, and covered the typical maize storage cycle which spans 8 - 9 months starting shortly after the short rains harvest season.

3.3 Storage practices of farmers

A rapid appraisal using semi-structured questionnaire was conducted to gather information on maize production, storage problems, strategies used to cope with storage

problems and storage practices of the farmers. (Details of the questionnaire are inserted in Appendix I).

3.4 Materials

One bag of 90 kg of shelled maize grain which had not been treated with insecticide or mixed with indigenous grain admixtures (wood ashes, animal dung, and botanical protectants) was purchased from each of the participating farmer. Each farmer also provided storage structure in the homestead. Jute and PP bags of 50 kg capacity were purchased from a grain dealer in Nyamakima market in Nairobi, Kenya. The PICS™ bags (50 kg capacity) were supplied by Lela Agro Industries Limited (Kano, Nigeria).

3.5 Bagging, storage and sampling

Each 90 kg bag of maize grain was sieved through a 2 mm aperture sieve to remove any insects, dirt and other debris, and subdivided into three equal portions by weight (30 kg per bag). The three portions were randomly filled into either a triple-layer hermetic PICS™ bag, PP or jute bag each of 50 kg capacity. An EL-USB-2 data logger (Lascar electronics Inc., Pennsylvania, USA), programmed to record data every one hour, was placed in each of the storage bag to record the temperature, relative humidity and dew point conditions during storage. The bags were then sealed by firmly twisting the open end, and fastening with sisal twine, and placed on wooden planks in the farmer's store. To record the temperature, relative humidity and dew point conditions of the local environment, another EL-USB-2 data logger was placed at an open strategic place in the compound of at least one farmer in each village.

Sampling was done during trial set-up (baseline data) and subsequently at seven-week intervals. Before opening the PICS bags, oxygen and carbon dioxide levels were measured using a portable Mocon Pac Check Model 325 oxygen/carbon dioxide analyzer (MOCON Inc., Minneapolis, USA) fitted with a 20-gauge hypodermic needle

for sampling inside the bag. To take gas composition measurements, the inner HDPE liner was punctured with the analyzer needle at the top, middle and bottom. Needle holes were then immediately sealed with plastic adhesive tape after taking the readings. Subsequent measurements were performed from the same spot by lifting and replacing the tape. To obtain samples for examination of other parameters, the bags were opened and a composite sample of 1000 g of maize from each storage bag was drawn from five random points by pushing a two-inch diameter hollow tube sampler from the top of the bag. The 1000 g sample from each storage bag was thoroughly mixed and divided into two equal portions of about 500 g. One sub-sample was used to determine moisture content. In the remaining sub-sample through coning and quartering method three sub-samples of about 125 g were randomly separated. One sub-sample was used in determination of total mould counts and mould incidence levels, another for live insect count and another for insect damage weight loss. Thereafter, the sub-samples were combined and milled using a laboratory scale Knife Mill Cup KM 400 MRC Lab (MRC International, Westminster, UK). A portion of milled sample (100 g) was stored at -15°C awaiting aflatoxin analysis.

3.6 Determination of live adult insect counts

Sub-samples (125 g) were first stored in a refrigerator maintained at 2°C for 3 hours to immobilize crawling insects. The damaged grains were further split open to remove any insects lodged within the grain. Insect counts were reported as the number of live adult *P. truncatus* or *S. zeamais* or *T. castaneum* per 125 g of grain.

3.7 Determination of insect damage and weight loss

Sub-samples (125 g) were sieved through a 2 mm mesh kitchen sieve, and the dust-free grains were sorted into insect damaged and undamaged grains. Weight of undamaged grains (*Wu*), weight of insect damaged grains (*Wd*), number of undamaged grains (*Nu*), and number of insect damaged grains (*Nd*) were determined. Percent damage was

calculated as [Nd/ (Nd +Nu)] x 100. Percentage weight loss was calculated by the count and weigh method using the expression: % weight loss = [(Wu × Nd) - (Wd × Nu)]/Wu (Nu + Nd) × 100 (Boxall, 1986).

3.8 Moisture content determination

A Dickey-John mini GAC® plus moisture tester (DICKEY-john Corporation, Illinois, USA) calibrated on the basis of U.S. federal standard grain calibration was used. About 400 g whole grain samples were filled into the tester cup, levelled off and the moisture content read directly and recorded.

3.9 Mould count, isolation and identification

Total mould count was determined using dilution plating (Pitt & Hocking, 1997) on Sabourand Dextrose Agar (SDA) amended with 20 mg chloramphenicol (SDA-C) (Appendix II). Maize grains (10 g) were added in 90 mL of sterilized peptone water in 200 mL conical flask and mixed thoroughly by shaking for one minute. Then, one milliliter of the suspension was drawn and added into 9 mL sterile peptone water and serially diluted to a dilution of 10^{-4}. Duplicate of 0.1 mL aliquots of $10^{-1,}$ 10^{-2}, 10^{-3} and 10^{-4} were spread-plated on SDA-C Agar and incubated at 25°C for 3 days. The number of colonies in plates bearing 10 – 100 were enumerated and reported as number of colony forming units per gram (cfu/g).

Mould incidence levels, isolation and identification was done using direct plating technique for internal infestation (Pitt & Hocking, 1997) on Czapek-dox Agar (Appendix II). One hundred maize kernels were randomly scooped from each sample. The kernels were surface-sterilized for 2 minutes in NaOCl (2%) and rinsed twice with sterile distilled water. The kernels were then planted on Czapek-dox Agar plates (10 kernels per plate). The plates were incubated at 25°C for 5 days, and the number of kernels showing growth of fungal species in each Petri dish counted. Fungal colonies

were then isolated and sub-cultured on Czapek-dox Agar for 5 days and identified based on cultural and morphological characteristics as described by Watanabe (1994). The percentage of grains infected by each fungi species was calculated to determine their incidence on maize grains.

3.10 Determination of aflatoxin contamination

Ridascreen® Enzyme-Linked Immunosorbent Assay kit for total Aflatoxin (R-Biopharm AG, Darmstadt, Germany) was used as described by manufacturer. Milled maize samples (2 g) were weighed into a 50 mL screw cup centrifuge tube and mixed with 10 mL of methanol/ distilled water (70/30 v/v). The mixture was agitated gently on a vortex mixer at room temperature (20 - 25°C) for 10 min, centrifuged at 3000×*g* and the supernatant recovered. The supernatant was diluted appropriately while ensuring that the final extract contained 10% v/v methanol. Aliquots (50 µL) of the dilute extract and equal volumes of the calibrated aflatoxin standards (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, and 4.05 ppb) were added in separate duplicate wells of anti-aflatoxin antibody coated microtitre plate. In to each well, 50 µL of enzyme conjugate was added followed by another 50 µL of antibody solution and mixed gently by tapping the plate manually. The plate was covered with aluminum foil and incubated for 30 min at room temperature (20 - 25°C) in a dark cabinet after which the liquid in the plate wells was poured off and the wells filled with 250 µL washing buffer (10 mM phosphate buffer, pH 7.4 containing 0.05% Tween 20). The washing procedure was repeated twice and the wells semi-dried by tapping the plate gently on adsorbent paper. A hundred (100) µL of substrate/ chromogen solution was added to each well, and after mixing gently the plate was incubated for 15 min at room temperature in a dark cabinet following which 100 µL of stop solution (1 mol/L sulfuric acid) was added. Absorbance of liquid in each well was measured at 450 nm using a UT-6100 auto microplate reader (MRC International, UK) within 20 minutes of adding the stop solution. Aflatoxin concentrations (µg/kg) of samples were done in triplicates and determined from a calibration curve prepared from

the known standards. Limit of detection of Ridascreen® Enzyme-Linked Immunosorbent Assay kits for total aflatoxin was 0.005 µg/kg.

3.11 Statistical analysis

To stabilize variances insect count, mould count and aflatoxin data (x) were log transformed $Y = \log_e(x + 1)$ whereas percentage data (P) (grain damage, weight loss, moisture content and mould incidence levels data) were arcsine $Y = \sin^{-1}\sqrt{P}$), transformed, where Y is the result of transformation. The transformed data were then subjected to analysis of variance (ANOVA) using Stata SE version 12 (StataCorp LP, Texas, USA). Further due to inherent limitations of ANOVA in describing difference in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was also applied to test effects of treatment and storage duration, and the interaction effects. When the coefficient of the interaction term was significant ($P< 0.05$), it was concluded that there was a significant difference between treatments over the storage period. One-way ANOVA was performed where treatment outcomes at a specific point in storage time needed to be compared. Means were separated using Bonferroni adjustment at 95% confidence level.

CHAPTER FOUR

RESULTS

4.1 Farmers storage practices

Figure 4.1 summarizes key characteristics of the farmers involved in the storage experiment with respect to maize production, storage structures and storage challenges. Analysis of questionnaire data revealed that the average maize production of participating farmers varied widely. About half of the farmers (46.7%) harvested 10 – 20 bags of 90 kg. Another 30% harvested more than 20 bags while 23.3% of the farmers harvested less than 10 bags. The quantity of grain reserved for household consumption and other household uses varied as well. Two thirds of the farmers (63.3%) stored between 6 – 10 bags, 20% stored less than 5 bags while 16.7% stored more than 10 bags. Slightly more than half of the farmers (53.3%) harvested and stored traditional maize varieties (*Kinyanya*) whereas 36.7% of the farmers had pure improved varieties. A small proportion (10%) harvested and stored both traditional and improved varieties.

Most of the farmers (90%) stored their maize as shelled grain while 10% stored as dehusked cobs as well as shelled grain. About three quarters of farmers (73.3%) stored their maize grain mainly for household use for a period exceeding 7 months. Only a small proportion (26.7%) of the farmers stored their maize for less than 6 months. The majority of farmers (66.7%) who stored shelled maize packed the grain in woven polypropylene bags and placed the bags in granaries (*ikumbi*). A third of the farmers (33.3%), however, preferred to store the bags in designated rooms in the living house. The granaries were mainly raised structures constructed using wooden slats or sisal stems with either grass thatch (traditional granaries, 42.1%) (**Figure 4.2**) or iron sheet roofing (**Figure 4.3**) (improved cribs, 57.9%). Some of the granaries particularly the improved cribs were fitted with rat guards (5.3%) but many (94.7%) were not, and the farmers used commercial baits or kept domestic cats for rodent control. The special

rooms used for maize storage by the farmers were mainly brick wall rooms with concrete floor but farmers habitually installed raised wooden platforms on which bags were laid. Most farmers (83.3%) stored maize grain together with other commodities including cowpeas, pigeon peas and green grams in the same storage structure.

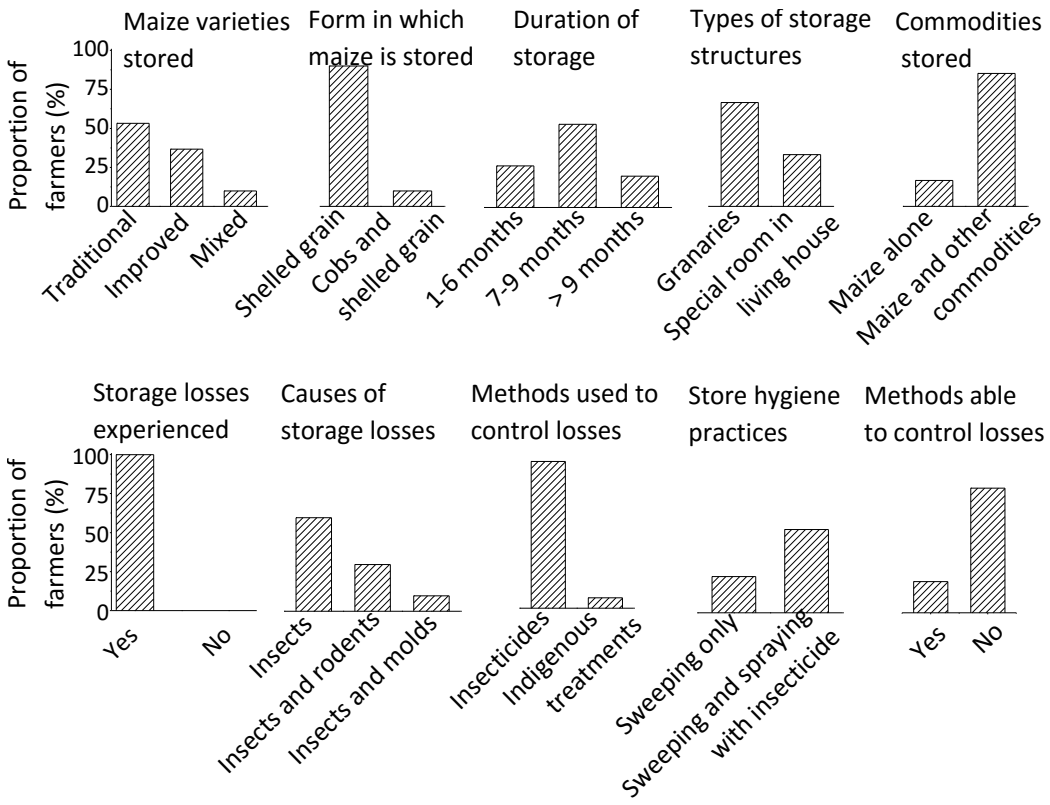


Figure 4.1: Maize storage practices and experiences of farmers with on-farm storage losses in the trial site (n = 30)

All participating farmers reported to experience losses during storage. Close to 60% of the farmers attributed this loss to insect infestation, while 30% of the farmers attributed it to both insects and rodents, and only 10% of farmers attributed the losses to mould infections. In farmers own estimation, the losses due insect amounted to 100 - 200 kg (about 1 – 2 bags; average losses 16.4%) for farmers who stored 6 – 10 bags for home

use. A higher loss of more than 200 kg reported by farmers who stored more than 10 bags particularly to sell at peak market prices (average losses 24.8%) while for farmers who stored less than 5 bags, the losses were less than 100 kg, average losses of 6.7%. Almost all farmers (93.3%) applied insecticides mainly Actellic Super® dust ((Pirimiphos methyl (1.6 g / 100 g) + permethrin (0.3g /100 g)) to control storage pests and losses whereas a few (6.7%) used indigenous methods such as admixing with wood ashes.

Figure 4.2: Sisal stalk grass roofed granaries for maize storage

Figure 4.3: Sisal stalk iron roofed granaries for maize storage

Among the farmers who participated in the storage trial, 60% were aware of aflatoxin poisoning in the area. However, only a small proportion (10%) of these farmers attributed mould infection to storage losses. The small proportion (10%) of the farmers who reported loss due mould infections experienced mouldy grains either during harvesting, drying or storage, and used it to feed chicken and livestock or disposed of together with other household wastes. Farmers who participated in this trial were also aware of good storage hygiene. All the farmers (100%) removed the old stock and cleaned their stores before introducing new harvest.

4.2 Effect of type of storage bag on live insects count

In this study only *S. zeamais* and *T. castaneum* were observed. **Figure 4.4** shows the populations of surviving adults of the two pests in the three types of bags. At the start of the experiment, some of the maize acquired from farmers had already been infested to different levels, but the visible emergent infestations were relatively low. The average number of live *S. zeamais* adults was 4 - 5 insects per kg whereas no live adults of *T. castaneum* were present or counted at the beginning of the experiment.

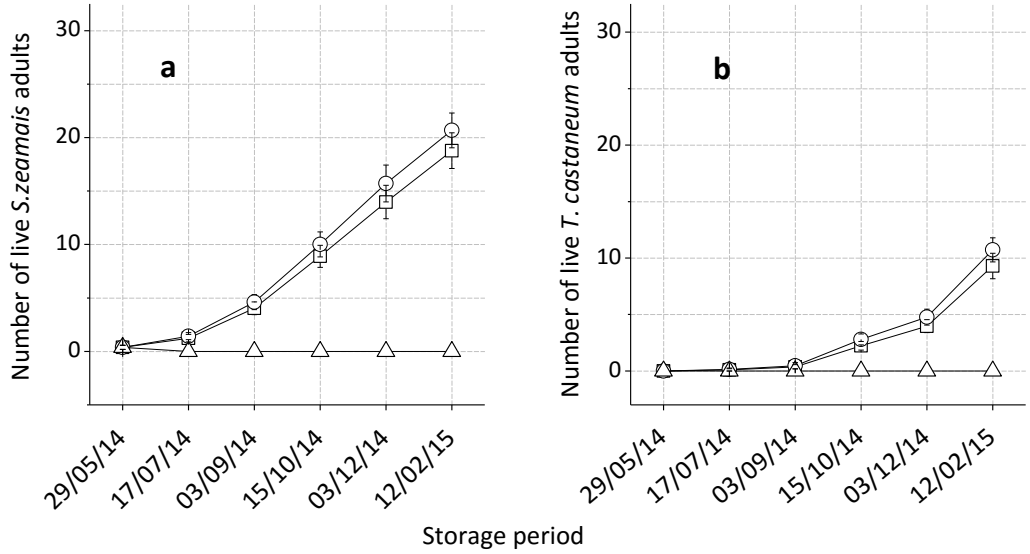


Figure 4.4: Populations (number per 125 g) of live adult *S. zeamais* (a) and live adult *T.castaneum* (b) in PP (□), Jute (○) and PICS bags (Δ). Plotted data are means ± standard errors (n=30)

Interaction effect between type of storage bag and storage duration was significant for both *S. Zeamais* (F = 25.98; df = 10, 522; $P < 0.001$) and *T. castaneum* (F=25.72; df = 10, 522; $P < 0.001$).

On all sampling occasions except the baseline sampling, no live insects were detected in the PICS bags. On the contrary, proliferation of insects in PP and jute bags continued, and the populations of live adult *S. zeamais* remained higher than those of *T. castaneum*. Significant numbers of *T. castaneum* became evident starting from the 14th week of storage, and a drastic increase occurred in the 28th - 35th week interval, when the ratio of live adult *S. zeamais* to *T. castaneum* approximated 2:1. The populations of these pests in PP and jute bags, however, did not differ significantly (**Figure 4.4**).

4.3 Effect of type of storage bag on grain damage and weight loss

Grain damage and grain weight loss in the three types of storage bags are presented in **Table 4.1**. At the start of the experiment, the maize had a low level of grain damage of

3.6 ± 1.3%. No further damage was observed in PICS bags during the 35 weeks of storage (**Table 4.1**). In contrast, grain damage in PP and jute bags increased steadily as demonstrated in **Figure 4.5**, and reached 77.5 ± 5.6% and 82.3 ± 5.1%, respectively at the end of the storage trial. The interaction effect of type of storage bag and storage period on grain damage was highly significant (F = 21.21; df = 10, 522; P < 0.001). Notably, beginning from the 7th storage week, significantly higher damage was determined in the PP and jute bags as compared to the PICS bags, and the trend continued throughout the storage period (F = 26.05; df = 2, 87; P < 0.001). Moreover, although grain damage measured in jute bags was consistently higher than that measured in the PP bags throughout the entire storage period, the weight loss in the two bags did not differ significantly.

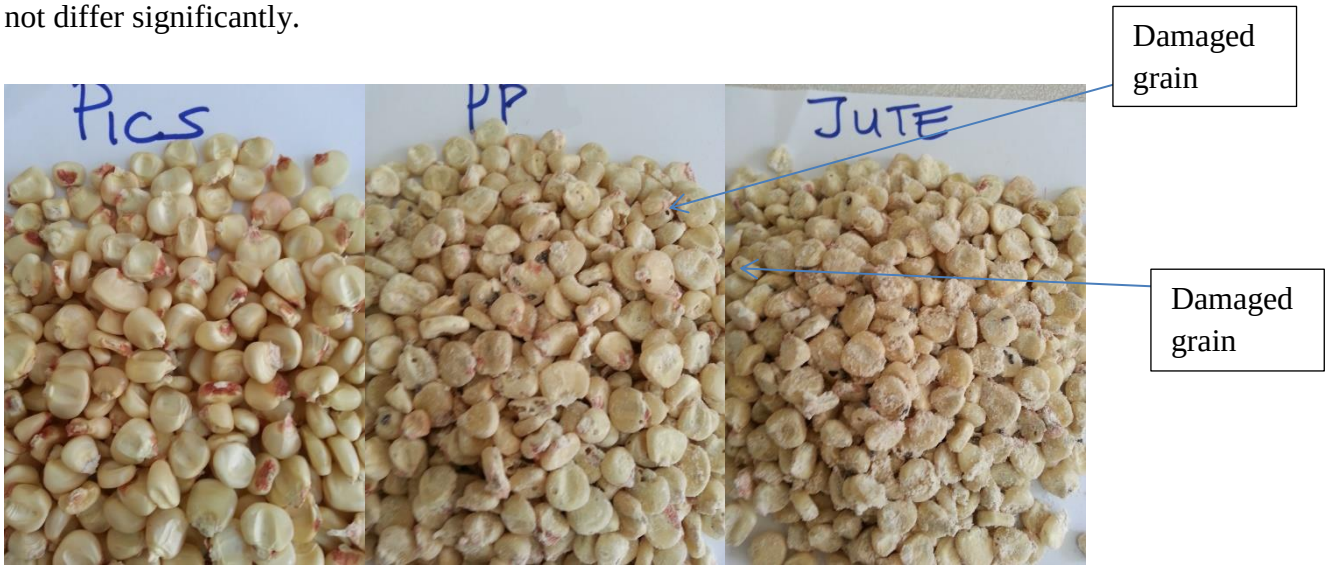


Figure 4.5: Comparison of samples damage from the three bag types during 28th week's storage (PICS, PP and Jute bags)

Initial weight loss of maize grain at the start of experiment was 0.7 ± 0.3%. No further losses were observed in the PICS bag during storage. However, weight losses of maize stored in PP and jute bags exceeded 5% in 14 weeks, and increased steadily to 41.2 ±

3.3% and 48.5 ± 3.4%, respectively in the 35th week. The interaction effect between type of bag and storage duration was significant (F = 33.70; df = 10, 522; P < 0.001). Notably, beginning from the 14th storage week, significantly higher weight loss was observed in the PP and jute bags as compared to the PICS bags, and the trend continued throughout the storage period (F = 18.41; df = 2, 87; P < 0.001). Similar to insect damage, there was no significant difference between the weight loss of maize stored in PP bags and that stored in jute bags (**Table 4.1**) although weight losses measured in the jute bags were consistently higher during the entire period of storage.

Table 4.1: Percentage grain damage and weight loss of maize grain stored in PP, Jute and PICS bags for 35 weeks (n=30)

Bag type	Storage duration (weeks)*					
	0	7	14	21	28	35
Grain damage (%)						
PICS	3.6±1.3a	3.5±1.3a	3.5±1.2a	3.7±1.2a	3.7±1.2a	4.0±1.2a
PP	3.6±1.3a	9.2±2.4b	25.8±3.9b	50.1±5.5b	56.9±5.6b	77.6±5.6b
Jute	3.6±1.3a	10.0±2.5b	30.1±4.3b	53.7±5.7b	61.4±5.8b	82.3±5.1b
Weight loss (%)						
PICS	0.7±0.2a	0.7±0.2a	0.8±0.3a	1.2±0.4a	0.8±0.3a	0.8±0.2a
PP	0.7±0.2a	1.8±0.5b	6.3±0.9b	17.7±1.9b	24.8±2.8b	41.2±3.3b
Jute	0.7±0.2a	2.1±0.5b	7.5±1.1b	20.1±2.4b	27.6±3.0b	48.5±3.4b

*Data are means ± standard errors. Entries in the same column followed by same letter are not significantly different (P > 0.05). Means were separated using Bonferroni adjustment.

4.4 Effect of type of storage bag on grain moisture content

The initial moisture content (m.c.) of maize varied from farmer to farmer. Three m.c. levels were identified as follows: m.c. <13%, 13% ≤ m.c. ≤14% and m.c. > 14%. Data was clustered into these initial m.c. levels for purpose of analysis and interpretation. **Figure 4.6** shows the progression of grain m.c. in PICS, PP and jute bags over the 35 weeks of storage. For m.c. < 13%, (n = 7) maize, the average m.c. was 12.7 ± 0.1% (range: 12.4 - 12.9%) at start of experiment. Maize stored in PICS bags retained this m.c. throughout (F = 0.95; df = 5, 36; P = 0.463). Contrastingly, m.c. of maize stored in PP and jute bags started to decline from the 14th week, and reached levels that were significantly lower than in PICS bags from the 28th weeks of storage onwards (F = 16.91; df = 2, 18; P < 0.001). Throughout the entire storage period, m.c. of maize stored in PP and jute bags were not significantly different (**Figure 4.6**) and the lowest m.c. levels reached for two bags were 11.1 ± 0.2% and 10.9 ± 0.2%, respectively.

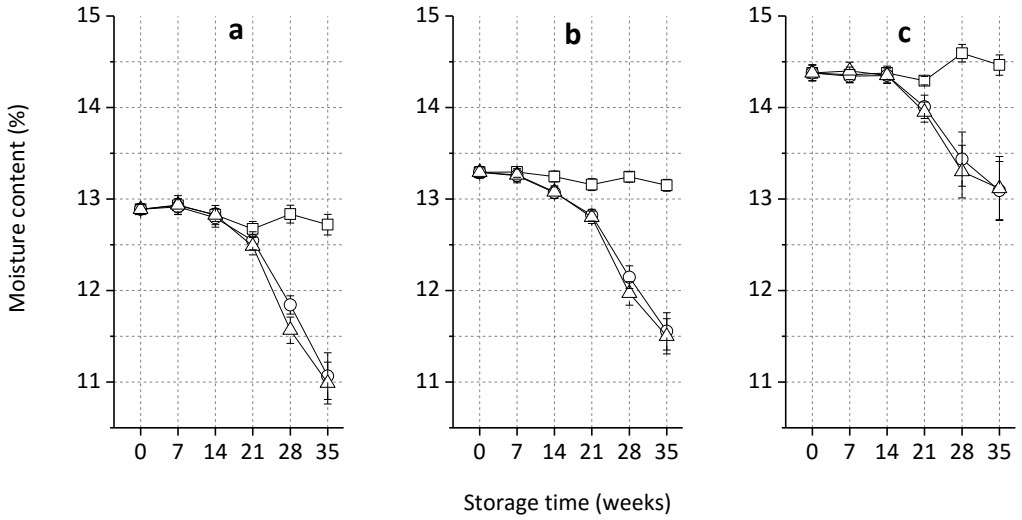


Figure 4.6: Moisture content of maize stored in PICS (□), PP (○) and jute bags (Δ) at initial moisture contents of (a) m.c. < 13%, (b) 13% ≤ m.c. ≤ 14%, and (c) m.c. > 14% for 35 weeks.

For maize stored at initial 13% ≤ m.c. ≤14% (n = 13), the average m.c. at start of experiment was 13.3 ± 0.1% (range: 13.0 – 13.8%). Maize stored in PICS bags generally retained its m.c. throughout the storage period (F = 0.58; df = 5, 72; P = 0.712) at about 13.3 ± 0.1%. On the contrast, m.c. of maize stored in PP and jute bags started to decline from the 7th week, and reached levels that were significantly lower than in PICS bags from the 21st weeks of storage onwards (F = 9.16; df = 2, 36; P < 0.001). Likewise the m.c. of maize packed in PP and jute bags did not differ significantly throughout the entire storage period and the lowest m.c. levels attained were 11.7 ± 0.2% and 11.5 ± 0.2%, respectively.

In the storage trials containing initial m.c. > 14% (n = 7), the average m.c. at start of experiment was 14.4 ± 0.1% (range: 14.2 – 15.0%). As with other m.c. levels, maize stored in PICS bags generally retained its m.c. throughout the storage period (F = 0.86; df = 5, 36; P = 0.517) whereas m.c. of maize stored in PP and jute bags continued to decline reaching levels that were significantly lower than in PICS bags (13.1 ± 0.3% and 13.3 ± 0.2% respectively) in the 35th week (F = 3.72; df = 2, 18; P < 0.045). There was

also no significant difference in the m.c. of maize stored in PP and jute bags throughout the entire storage period. ANCOVA tests revealed that interaction effect between type of bag and storage period was significant for the three m.c. levels (m.c. <13%: F=7.57; df = 10, 108; P< 0.001, 13% ≤ m.c. ≤14%: F = 9.61, df = 10, 216, P < 0.001; m.c. > 14%: F=2.37; df = 10, 108; P< 0.014).

4.5 Effect of PICS bags on gas composition

Figure 4.7 shows one of the storage trials in the farmers store as well as oxygen and carbon dioxide levels being measured using a portable Mocon Pac Check Model 325 oxygen/carbon dioxide analyzer in the PICS bags. **Figure 4.8** shows the mean oxygen and carbon dioxide concentrations in the PICS bags containing maize at three levels of m.c. From the atmospheric oxygen and carbon dioxide concentrations, that is, 21% and 0.03% respectively, the oxygen levels in PIC bags containing maize stored with an initial m.c < 13%, dropped to 4.7 ± 0.7% in the first 7 weeks whereas carbon dioxide increased to 11.2 ± 1.5%. During the rest of storage period, oxygen concentration increased to 10.6 ± 0.5% while carbon dioxide averaged at 8.7 ± 0.8% at 35 weeks of storage.

(a)


(b)

Figure 4.7: (a) storage trial in farmer's store, (b) measurement of oxygen and carbon dioxide levels using a portable Mocon Pac Check Model 325 oxygen/carbon dioxide analyzer in PICS bag

Similar trends were observed in PICS bags containing initial 13% ≤ m.c. ≤ 14% maize, where oxygen levels dropped to 5.2 ± 0.2% in the first 7 weeks of storage while carbon dioxide increased to 11.0 ± 0.6%. During the rest of storage period, oxygen concentration increased and averaged 10.6 ± 0.4% while carbon dioxide stabilized at 11.1 ± 0.7% at 35 weeks of storage.

For maize with an initial m.c > 14%, oxygen levels dropped to 4.1 ± 0.6% while carbon dioxide increased to 10.2 ± 0.3% in the first 7 weeks of storage. During subsequent weeks of storage oxygen concentration increased to 9.8 ± 0.4% while carbon dioxide stabilized to 12.9 ± 0.8% at 35 weeks of storage. ANCOVA results showed significant differences in oxygen (F=2.59; df = 10, 144; P< 0.007) and carbon dioxide (F=2.22; df = 10, 144; P< 0.019) progression patterns at three levels of moisture content.

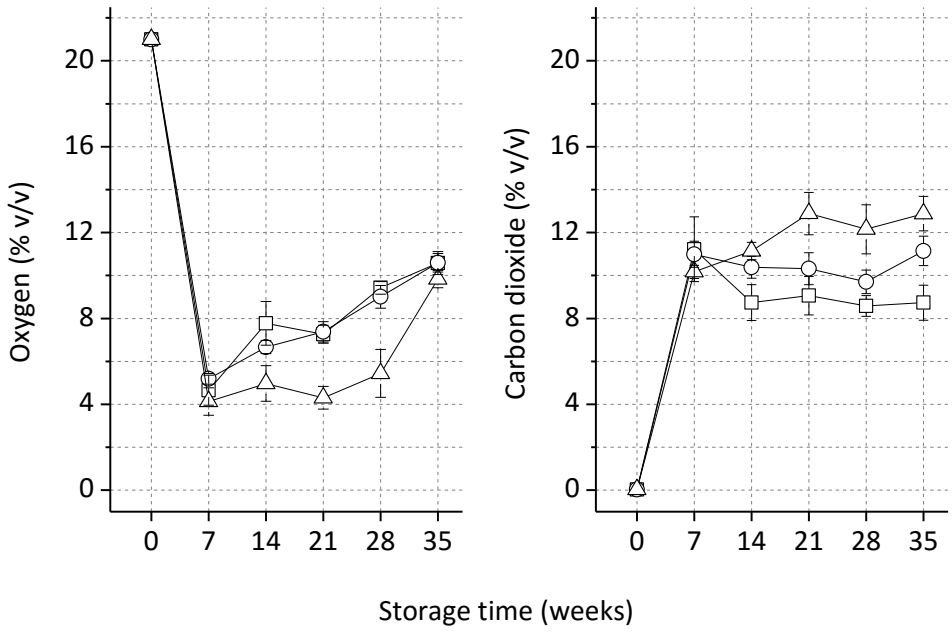


Figure 4.8: Oxygen and carbon dioxide levels in PICS bags containing maize grain stored with initial moisture contents of m.c. < 13% (□); 13% ≤ m.c. ≤ 14%, (○), and m.c. > 14% (Δ) for 35 weeks.

4.6 Temperature, relative humidity and dew point condition in storage bags

Figure 4.9 shows mean temperature, relative humidity and dew point conditions prevailing in the trial sites and the storage bags over the 35 weeks of storage. The mean atmospheric temperature, relative humidity and dew point were 23.9 ± 3.2°C, 59.9 ± 11.1%, and 15.9 ± 2.2°C, respectively. These patterns were characterized by wide ranges between 17.2 – 35.2°C (temperature), 24.4 - 91.5% (relative humidity) and 7.9 - 20.7°C (dew point). In the storage bags, temperature varied with varying environmental temperature. For maize stored at initial m.c. < 13%, average temperature in the PICS bags was 26.1 ± 0.4°C. On the other hand, temperature prevailing in PP and jute bags averaged 29.9 ± 0.2°C and 29.6 ± 0.5°C, respectively. These temperature conditions

were similar to those prevailing in bags containing maize stored at initial 13% ≤ m.c. ≤ 14%, which averaged 25.4 ± 0.5°C in PICS bags, 29.1 ± 0.4°C in PP bags and 29.0 ± 0.3°C in jute bags. Regarding maize stored at initial m.c. > 14%, temperature in the PICS bags averaged 26.1 ± 0.3°C whereas the mean temperatures were 29.7 ± 0.4°C and 30.1 ± 0.5°C in PP and jute bags, respectively. Generally, temperature conditions in PICS bags remained lower than in PP or jute bags (**Figure 4.9**).

Relative humidity in the storage bags varied considerably (**Figure 4.9**). In the PICS bags fairly constant relative humidity levels were maintained. In the PICS bags packed with maize at initial m.c. < 13% relative humidity increased from 58.7 – 63.8% (mean 61.7 ±1.8%) whereas the relative humidity in the PICS bags containing maize with an initial moisture of 13% ≤ m.c. ≤ 14% and m.c. > 14% increased from 62.9 – 68.3% (mean 66.0 ±1.9%) and 71.5 – 80.5%, (mean 76.4 ± 1.9%), respectively. In the three storage moisture categories, relative humidity in PICS bags was higher compared to the relative humidity in PP or Jute bags (**Figure 4.9**).

With regard to dew point, the dew point temperatures in bags containing maize stored at initial m.c. < 13% in PICS bags averaged 18.1 ± 0.3°C (range: 14.5 – 22.5°C), PP bags 18.8 ± 0.3°C (range: 14.5 – 22.6°C) and jute bags 18.7 ± 0.4°C (range: 15.1 – 22.7°C). Similarly, the mean dew point temperatures in bags containing maize stored at initial 13% ≤ m.c. ≤14% in PICS bags was 18.7 ± 0.3°C (range: 14.6 – 23.0°C), PP bags 19.0 ± 0.3°C (range: 15.3 – 24.9°C) and jute bags 18.7 ± 0.2°C (range: 14.9 – 24.0°C). A significant difference among the bags occurred in the maize stored at an initial m.c. > 14% where dew point temperature exceeded and remained about 25°C in the PICS bags beginning from the 20^{th} – 21^{st} weeks of storage onwards (**Figure 4.9**).

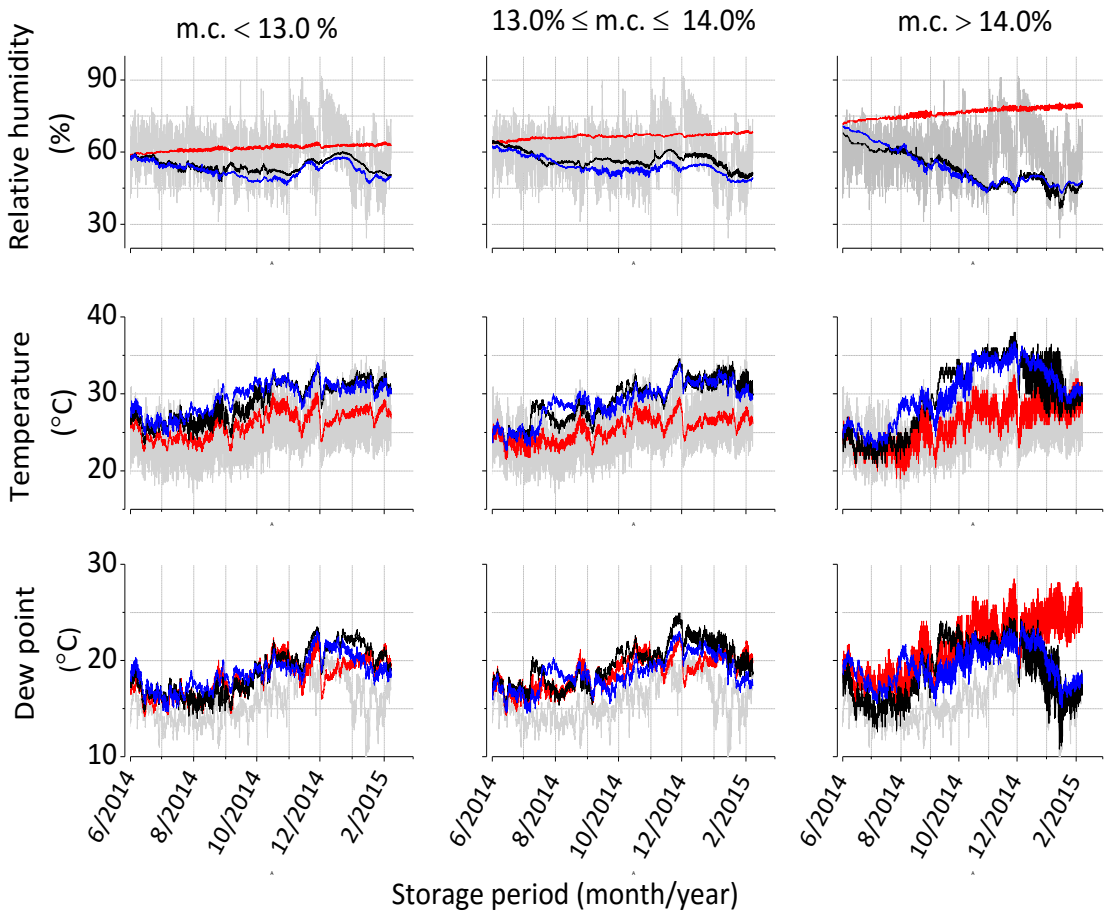


Figure 4.9: Relative humidity, temperature and dew point conditions in the trial site (grey), and relative humidity, temperature and dew point conditions prevailing in PICS (red), PP (black) and jute bags (blue) filled with maize having moisture contents of m.c. < 13%; 13% ≤ m.c. ≤ 14% and m.c. > 14%.

4.7 Effect of storage bag on mould infection

Results of total mould count on maize stored in PICS, PP and jute bags at different storage moisture levels are presented in **Table 4.2**. At onset mould infection was three times higher in maize at initial m.c. >14% than the maize at initial m.c. < 13%. Infection

levels did not change significantly in the maize stored in PICS bags at m.c. < 13%: F=0.06; df= 5, 36; P= 0.997 and 13% ≤ m.c. ≤ 14%: F=0.13; df= 5, 72; P= 0.985 throughout 35 weeks of storage. In contrast, mould count in PP and jute bags increased up to six-fold reaching levels that were significantly higher than in PICS bags (m.c. < 13%: F=4.51; df= 2, 18; P= 0.025) and (13% ≤ m.c. ≤ 14%: F=10.32; df= 2, 36; P= 0.003) at the end of storage. For maize grain containing initial m.c. > 14%, the total mould counts were not significantly different in various storage bags (F=1.97; df= 2, 18; P= 0.169).

Table 4.2: Total mould count (×10^3 cfu/g) of maize grain stored in PP, Jute and PICS bags for 35 weeks.

Bag type	Storage duration (weeks)					
	0	7	14	21	28	35
m.c. < 13%						
PICS	19.4a	19.0a	19.2a	20.4a	22.1a	21.6a
PP	19.4a	45.1a	59.8b	74.1b	91.4b	126.3b
Jute	19.4a	62.2a	70.7b	93.2b	99.2b	115.6b
13% ≤ m.c. ≤ 14%						
PICS	31.6a	34.3a	30.6a	29.1a	31.8a	25.8a
PP	31.6a	46.2a	66.7a	77.2b	111.3b	119.6b
Jute	31.6a	57.1a	68.7a	99.5b	121.2b	154.0b
m.c > 14%						
PICS	59.8a	65.9a	47.6a	67.9a	105.7a	160.3a
PP	59.8a	88.7a	111.4b	162.1b	178.5b	201.4a
Jute	59.8a	89.8a	122.9b	132.7b	198.5b	215.7a

Data are means values (m.c. < 13% n=7, 13% ≤ m.c. ≤ 14% n=13, m.c. > 14% n=7). Entries in the same column followed by same letter are not significantly different (P > 0.05). Means were separated using Bonferroni adjustment.

Moulds of the genera *Aspergillus, Fusarium* and *Pencillium* were isolated at higher frequencies. **Figure 4.10** shows the plates containing maize kernels after 5 days of incubation. Purified colonies after sub-culturing are shown on **Figure 4.11**. **Figure 4.12** shows microscopic view of the mould of genus *Aspergillus, Fusarium* and *Penicillium* as observed in the microscope.

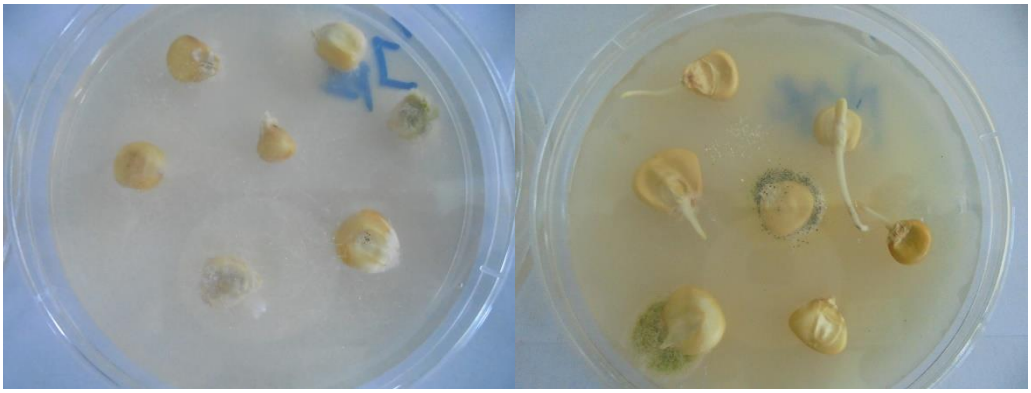


Figure 4.10: Fungi growing on maize kernels after 5 days incubation in Czapek-dox Agar

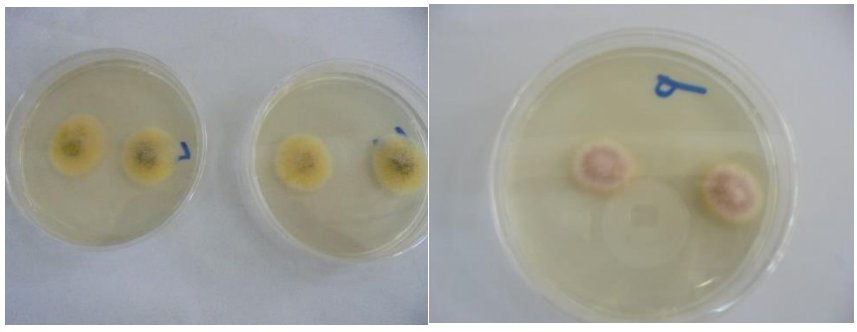


Figure 4.11: Purified colonies of fungi after sub-culturing in Czapek-dox Agar

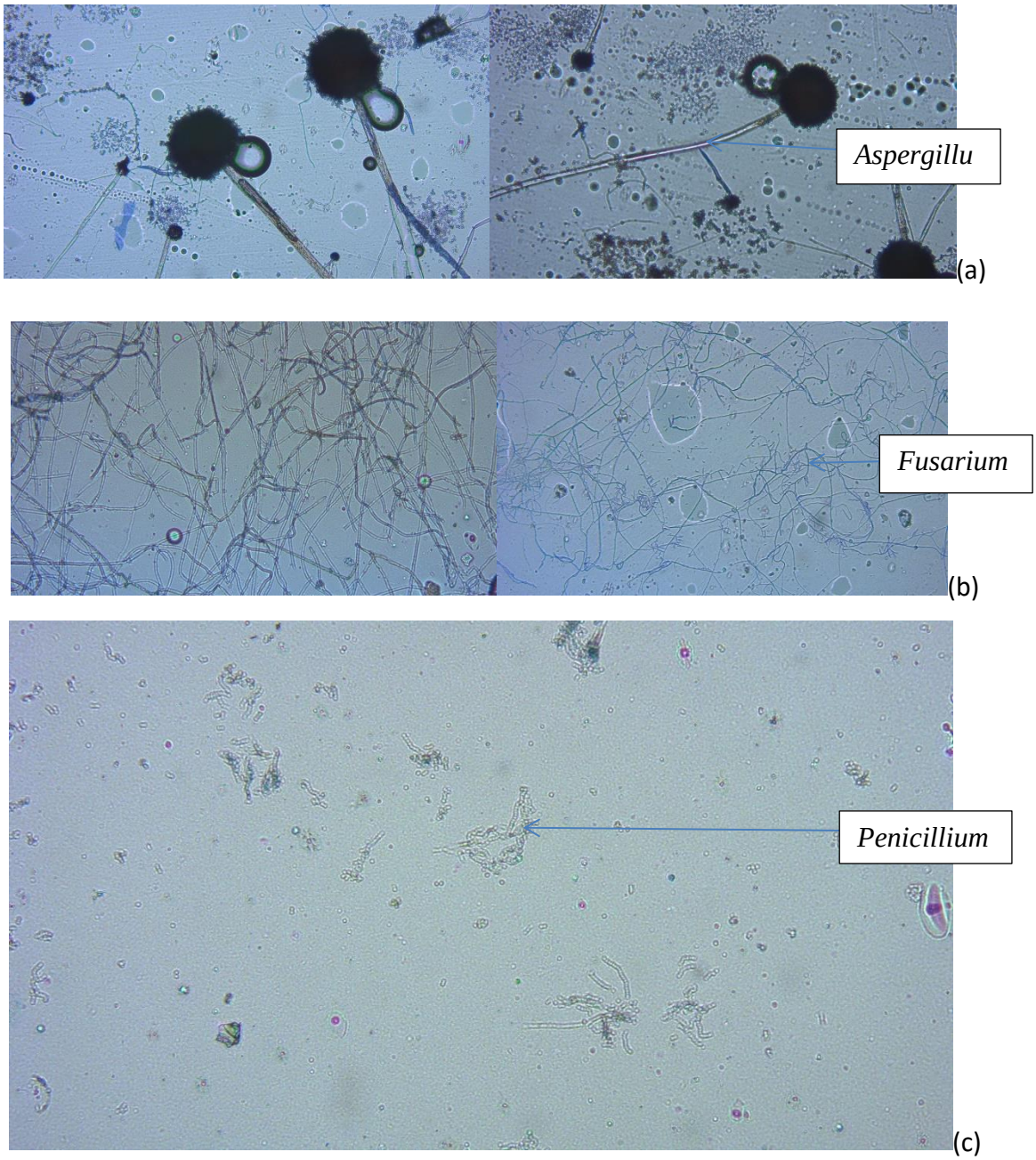


Figure 4.12: Mould of genus (a) *Aspergillus,* (b) *Fusarium* and (c) *Penicillium* as observed in the microscope.

Figure 4.13 shows the mould incidence levels in different storage bags containing maize with initial moisture of 13% ≤ m.c. ≤ 14%. Interaction effect between type of bag and storage duration was significant for *Aspergillus* (F=2.31; df= 10, 162; P= 0.014) and *Pencillium* (F=3.30; df= 10, 162; P< 0.001).

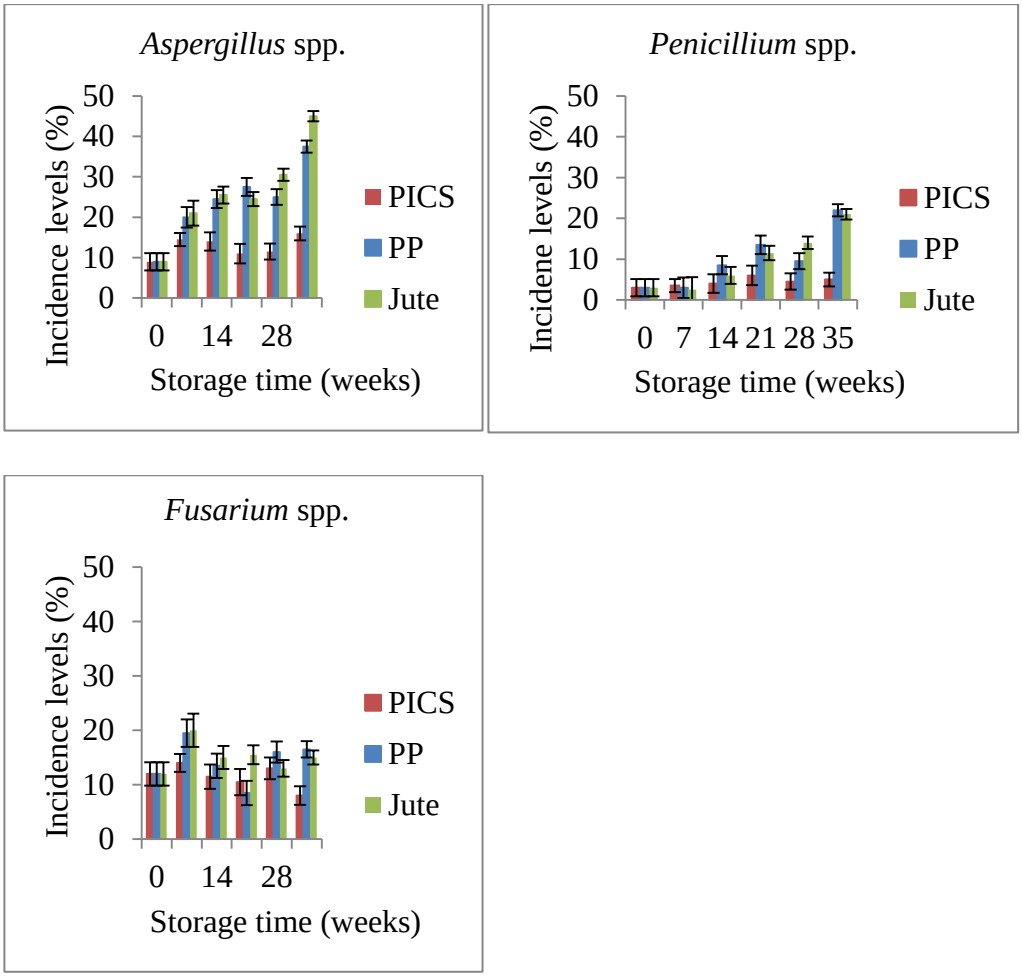


Figure 4.13: The percentage incidence levels of mould that were frequently isolated on maize grain (13% ≤ m.c. ≤ 14%) stored in PP, Jute and PICS bags for 35 weeks (n = 10)

In the PICS bags, incidences of *Aspergillus* (9 - 16%) and *Penicillium* (3 - 6%) did not change significantly with storage time (F=0.60; df= 5, 54; P= 0.699; F=0.48; df= 5, 54; P= 0.790), respectively. In the PP and jute bags, however, the incidence levels increased

up to five-fold (*Aspergillus*) and seven-fold (*Penicillium*), and reached significantly higher levels than in PICS bags at the end of storage duration (F=11.12; df= 2, 27; P= 0.003; F=21.37; df= 2, 27; P< 0.001), respectively.

For *Fusarium* incidence levels, there was no significant interaction effect between type of bag and storage duration (F=1.36; df= 10, 162; P= 0.202), but the main effects, that is, type of bag and storage duration were significant (P= 0.007 and P= 0.004 respectively).

4.8 Effect of type of storage bag on aflatoxin contamination

In this study the results of samples analyzed varied considerably as shown in two of the Ridascreen® Enzyme-Linked Immunosorbent Assay kits used (**Figure 4.14**). The results of aflatoxin contamination on three levels of initial m.c. are presented in **Table 4.3**. For maize stored at an initial m.c. < 13%, interaction effect between the storage duration and storage bag was not significant (F=0.54; df= 6, 72; P= 0.799). The aflatoxin contamination of the maize stored in PICS bags did not change significantly over the storage period (F=0.24; df= 3, 24; P= 0.868). Significant increase in contamination level, however, occurred in the maize stored in PP (F=2.95; df= 3, 24; P= 0.050) and jute bags (F=3.20; df= 3, 24; P= 0.042). For the maize stored at initial m.c. of 13%≤ m.c. ≤14% a significant interaction effect between type of bag and storage duration was observed (F=2.47; df= 6, 144; P= 0.026). As with maize stored at an initial m.c. <13%, no significant change in aflatoxin contamination was noticed in the PICS bags throughout the 35 weeks of storage (F=0.24; df= 3, 48; P=0.865). In contrast, aflatoxin concentration increased in PP and jute bags and reached levels that were significantly higher than in PICS bags from the 14th week onwards. In addition, aflatoxin contamination levels in PP and jute bags did not differ significantly (**Table 4.3**).

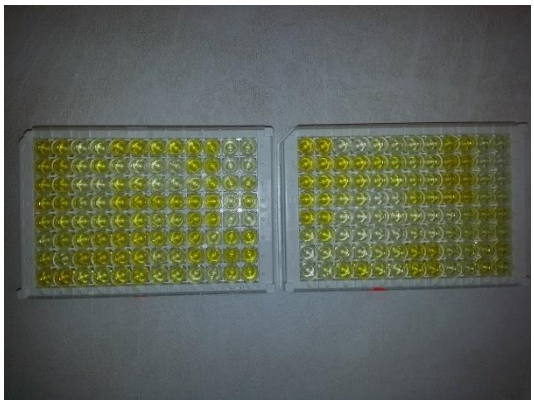

(a)

(b)

Figure 4.14: (a) Ridascreen® ELISA kit during analysis (b) Ridascreen® ELISA kits after analysis

In maize stored at initial m.c. > 14%, interaction effect between the type of bag and storage duration was not significant (F=0.14; df= 6, 72; P=0.991). Further analysis of the main effects showed that storage duration was significant (P< 0.001) but the type of storage bag was not (P = 0.525). Thus, aflatoxin contamination increased significantly with storage time (PICS: F=4.60; df= 3, 25; P=0.011; PP: F=4.91; df= 3, 24; P=0.008; jute: F=3.52; df= 3, 24; P=0.030) but did not significantly differ with type of storage bag (F=0.48; df= 2, 81; P=0.621).

Table 4.3: Total aflatoxin concentration (µg/kg) of maize grain stored in PP, jute and PICS bags for 35 weeks.

Bag type	Storage duration (weeks)			
	0	14	28	35
m.c. < 13%				
PICS	62.6±13.2a	50.7±14.5a	51.7±12.7a	53.3±15.7a
PP	62.6±13.2a	158.5±57.9b	182.8±65.1b	306.8±116.3b
Jute	62.6±13.2a	221.5±73.8b	253.5±71.8b	393.8±132.4b
13% ≤ m.c. ≤ 14%				
PICS	64.7±17.5a	66.9±18.4a	48.9±15.2a	59.1±14.3a
PP	64.7±17.5a	143.5±37.3b	201.8±51.8b	414.9±134.4b
Jute	64.7±17.5a	167.2±39.5b	211.9±49.8b	492.7±141.9b
m.c. > 14%				
PICS	107.2±39.3a	89.6±29.4a	254.6±94.7a	630.9±158.6a
PP	107.2±39.3a	159.7±34.6a	354.5±117.4a	864.4±208.6a
Jute	107.2±39.3a	177.5±49.6a	407.9±127.8a	823.5±198.5a

Data are means ± standard errors (m.c. < 13% n=7, 13% ≤ m.c. ≤ 14% n=13, m.c. > 14% n=7). Entries in the same column followed by same letter are not significantly different ($P > 0.05$). Means were separated using Bonferroni adjustment.

Overall, there was a significant correlation between aflatoxin contaminations and total mould count (r = 0.677; $P = 0.001$), incidence of *Aspergillus* spp. (r = 0.640; $P = 0.001$), and incidence of *Penicillium* spp. (r = 0.298; $P = 0.002$). Also a significant correlation was found between total mould count and incidences of *Aspergillus* spp. (r = 0.802; $P = 0.001$) and *Penicillium* spp. (r = 0.339; $P = 0.001$).

CHAPTER FIVE

DISCUSSION

In many rural villages, small-scale farmers store varying quantities of grain for subsistence use under varying traditional storage conditions for different periods. Traditional storage methods that these rural farmers adopt at farm level are usually adapted to their environments and the types of crops they traditionally expect to store. However, the advent of factors such as new crop varieties some of which are more susceptible to infestation by insects than traditional ones (Amason *et al*., 1997), and the spread of exotic storage pests such as *Prostephanus truncatus* could introduce a disruption of erstwhile effective storage practices.

All the participant farmers in this study experienced storage losses due to insect attacks. Whereas the farmers applied insecticides and other traditional treatments to control the pests, only less than 25% succeeded to mitigate the losses. They thus perceived these control methods as ineffective. Indigenous treatments such as admixing with wood ashes, have low efficacy because there exist no standard application guidelines (Stathers *et al*., 2008), and their use is only practical when preserving small quantities of grain in the short-term. The limited effectiveness of synthetic insecticides used by the farmers could be explained by a number of reasons among them possible adulteration of the insecticides by vendors, improper application practices such delayed treatment (Golob & Hanks, 1990), incorrect dosage and patchy use by the farmers (Mutambuki & Ngatia, 2012), or the progressive loss of insecticidal potency of the active ingredients (Denloye *et al*., 2008). This limited efficacy of common insecticides on stored maize was also reported by other authors. For instance, Meikle *et al*. (2002) reported a weight loss of 7% and a depreciation of the market value of 27% in maize stored for six-months with Sofagrain™ (Pirimiphos-methyl (1.5%) + Deltamethrin (0.5%)) in Ghana. Similar observations were also reported for maize stored with Actellic Super® dust in West-

(Biliwa & Richter, 1990) and East Africa (Stathers *et al*., 2008; Mutambuki & Ngatia, 2012).

During storage of grains, frequent monitoring is important in order to to detect grain spoilage and mycotoxigenic fungi development in good time. Recently, hermetic bags have been promoted as effective chemical free storage alternatives for grains among small-scale farmers (Baoua *et al*., 2013; Baoua *et al*., 2014; Baributsa *et al*., 2014). As knowledge of the effects of the hermetic bags on the quality of stored produce is still limited especially under farmer storage conditions, this study investigated the effect of hermetic bag storage on aflatoxin contamination of maize with a group of rural farmers in an aflatoxin endemic region where environmental factors and storage practices, structures and duration were previously linked to high aflatoxin contaminations (Hell *et al*., 1997). Hell *et al*. (1997) stated that the influence of storage duration and storing maize in different storage structures, depending on the agroecological conditions could be the major factors associated with high aflatoxin contamination in stored maize.

In this study, the modified environment created by sealing PICS bags with maize effectively suppressed insect survival thereby stopping grain damage and losses. Oxygen depletion and carbon dioxide enrichment of intergranular atmosphere form the basis for suppression of insect infestations in hermetic storage via a number of mechanisms. The lowest oxygen level for multiplication of insect pests is 2 - 3% (Moreno-Martinez *et al*., 2000; Vachanth *et al*., 2010), although some studies also indicate that insects may adapt to low oxygen tensions and evolve into forms that resist sub-normal oxygen levels of about 1% (Annis, 1986; Donahaye, 1990). A low oxygen level of about 2 - 3% has been found to interfere with feeding of larval forms of insects which could become extremely slow or even cease, causing death (Moreno-Martinez *et al*., 2000; Murdock *et al*., 2012). In addition, Bailey and Banks (1980) indicated that oxygen depletion retarded development, impaired metamorphosis and altered fecundity of insects without necessarily having to kill them.

Extremely low oxygen levels were not attained in this study. However, according to Banks and Annis (1990), the simultaneous exposure of insects to low oxygen and high carbon dioxide could contribute to insect inactivity or mortality in a synergistic way. Nicolas & Sillians (1989) reported that at lower humidity, more water loss from permanently open spiracles caused by stimulation with high carbon dioxide would lead to desiccation then to death. Carbon dioxide also dissolves body fluids to form carbonic acid which could decrease haemolymph pH (Lea & Ashley, 1978) and NADPH (Friedlander *et al*., 1984), influencing activities at cell membranes or inhibiting various enzymes systems. On the other hand, accumulated carbon dioxide induces diapause in some insects without necessarily causing mortality. Recently, Murdock *et al*. (2012) concluded that insects enclosed in limiting oxygen conditions died of desiccation because they are unable to generate the water they need to maintain vital life processes, which they do by oxidizing energy-rich substrates in their diets.

This study has demonstrated significant grain damage and weight loss in maize stored in PP and jute bags compared to that which was stored in PICS bags. The high levels of grain damage and weight loss in PP and jute bags may be attributed to high rate of grain respiration and insect pest multiplication as a result of presence of conducive environment particularly high oxygen concentrations within the bags. On the other hand, multiplication of insect pests was discouraged in PICs bags by the fact that the environment within the bags was modified (high carbon dioxide and low oxygen concentrations) reducing the extent of the damage and losses. In this study the damage and losses were primarily a consequence of *S. zeamais* infestation. Birkinshaw *et al*. (2002) and Hodges (2002) have reported that, *Sitophilus* spp. are widespread and in most seasons and years, a high risk of their attack exists whereas *P. truncatus* outbreaks could be sporadic for various biological reasons. Although losses by *S. zeamais* are regarded to be low, other researchers have reported devastating losses caused by *S. zeamais* in farm stores. For instance, Sori & Ayana (2012) reported grain damage and weight losses averaging 54 - 80% and 41 - 74% respectively, on maize stored for 6 months in farmers stores in Jimma zone, Ethiopia.

In a separate study, Nukenine *et al*. (2002) reported that *S. zeamais* caused upto 80% losses in traditional storage systems in Cameroon after 6 - 8 months of storage. Recently, Baoua *et al*. (2014) in storage trials with maize grain using PICS and woven polypropylene bags in Benin, Burkina Faso and Ghana under natural infestation conditions reported grain damage of 6.7 - 53.9% corresponding to weight loss of 1.1 - 21.5% in maize stored in PP bags where densities of *S. zeamais* were the dominant species after 6.5 months. During the storage trials, Baoua *et al*. (2014) utilized local storage spaces provided by the participants, however, despite the diversity of species and variability of pest density from one site to another the quality of grain stored in PICS bags was protected. In addition, Jay (1983) clarified that environmental factors such as temperature and relative humidity play an important role in stored product insect pests' proliferation. The optimal conditions for reproduction and growth of *S. zeamais* and *T. castaneum* are 60 - 70% relative humidity and 25 - 30°C (Madrid *et al*., 1990; Schwartz & Burkholder, 1991), and these conditions prevailed in our trial sites which might explain the extensive damage and losses caused by *S. zeamais* on the maize stored in PP and jute bags. As expected, *T. castaneum* appeared in the PP and jute bags later when *S. zeamais* had caused sufficient damage to the whole grains. Further to weight loss which represents direct loss of edible and sellable mass, grain damage causes quality loss often associated with low food value and palatability. Such grain is also of low market value. Thus storage of maize in PICS bags would also abate quality and market value losses. In an exploratory study in Ghana, Compton *et al*. (1998) demonstrated strong quasi-linear negative relationship between grain damage and price. Whereas grain damage of 5 - 6% or below did not attract discounted price, maize with damage in excess of 5 - 6%, was discounted to 0.6 - 1% for every 1% increase in grain damage. Furthermore, extensive damage renders grain unfit for human food and is occasionally unsafe as it is highly susceptible to mold infection and mycotoxin contamination.

Grain moisture is an important factor that needs to be controlled when storing maize in hermetic containers. From farmer to farmer, the initial moisture content of maize varied, because many farmers do not have a standard objective method of determining grain

moisture before or during storage but instead rely on subjective knowledge such as the rattling sound of grains. In the three broad grain moisture content categories established for purposes of this study, 26% of farmers had maize with moisture content above 14%. Nonetheless, maize stored in PICS bags neither gained nor lost moisture content during the entire storage period of 35 weeks. On the other hand, moisture of grain stored in PP and jute bags declined over time because of the low barrier properties of the bags which allows equilibration with the surrounding ambient conditions. Traditionally, such properties are preferred as the grain would continue to dry during storage although excessive drying could translate to economic loss due to loss of sellable weight (Compton *et al*., 1998). In this study, experiments were set up immediately after short rainy season, thereafter, ambient relative humidity continued to drop, as result maize stored in PP and jute bags lost moisture due to evaporation. Similar observations were also made elsewhere (Baoua *et al*., 2014; Williams *et al*., 2014). However, in other instances, where ambient relative humidity is much higher, maize may gain moisture when stored in PP and jute bags. In such cases, storing the grain in PICS bags can prevent moisture migration and hence preserve the quality of the stored grain (Njoroge *et al*., 2014). Other factors that can cause moisture gain in stored maize are high insect activity and heavy fungal growth especially on insect damaged grains as was reported by Compton *et al*. (1998) and Njoroge *et al*. (2014).

With regard to oxygen and carbon dioxide levels in the PICS bags, extreme low oxygen levels were not attained unlike in some other studies (Murdock *et al*., 2012; Baoua *et al*., 2013; Williams *et al*., 2014). It has been argued that low oxygen and high carbon dioxide levels in hermetic storage systems could control mould proliferation (Richard-Molard, 1988; Williams *et al*., 2014). The drop in oxygen and rise in carbon dioxide observed when maize was stored in PICS bags was as the result of aerobic metabolism of life forms enclosed together with the maize (Murdock *et al*., 2012) and could be influenced by elements of the storage system such as insect populations, moisture content of grain, fungal inocula, quality of the grain and gas-tightness (Moreno-Martinez

et al., 2000). Thus, oxygen depletion and carbon dioxide build-up may be slow in grains that are well dried, and free from insects and moulds.

However, oxygen and carbon dioxide levels of about 4 – 5% and 10 – 11% respectively were evident during the first 7 weeks of storage in the three levels of moisture content, reaching concentrations of 5.4 - 9.4% and 8.5 - 12.2%, respectively, in 28 weeks (9.8 - 10.6% and 8.7 - 12.8%, respectively, in 35 weeks). These results compare closely with those of Baoua *et al*. (2014) who reported oxygen and carbon dioxide concentrations of 6.1 - 12.4% and 3.1 - 7.7%, respectively, in PICS bags packed with naturally infested maize stored at 10.3 – 13.5% moisture content for 6.5 months in storage trials involving traders, marketing cooperatives, private seed companies and private food processors. Williams *et al*. (2014) reported lack of significant oxygen depletion in maize stored at 12% moisture content but a depletion of up to 0 – 1% in maize conditioned at moisture content of 15, 18 and 21% during one month of storage in PICS bags indicating the role of grain moisture. Similarly, Murdock *et al*. (2012), for instance, observed a rapid drop in oxygen levels to about 1 – 2% with a concomitant rise in carbon dioxide to 9% within 24 hours of closing PICS bags filled with highly infested cowpeas. Seemingly, however, as observed in our results, the modified gas conditions in the bags could be lost overtime. A similar observation was reported by Baoua *et al*. (2012a) where oxygen levels dropped to a range 2 – 3% within 12 days before gradually rising to 12 – 15%, while carbon dioxide rose to 5% before gradually decreasing again. It is reasoned that during protracted storage, oxidative metabolism is severely attenuated, and as oxygen consumption drops, the concentration of oxygen around individual grains tends to increase as air proceeds to leak slowly through the partially impermeable HDPE liners following concentration gradient (Baoua *et al*., 2012b).

It was expected that packing maize in PICS bags would alter the course of mould proliferation by creating a modified micro-environment of storage. All in all, high mould counts were determined in all maize samples at the onset of the storage trials. This observation might be related to an interaction between the ubiquitous nature of fungi

associated with maize and agro-climatic conditions of the trial site. The fungi usually form sclerotia that allow for saprophytic survival for extended periods in the soil, maize residue and maize cobs (Wagacha & Muthomi, 2008), while high temperatures and drier conditions in semi-arid areas predispose maize to mould infections at pre-harvest stage in the field and post-harvest stage during storage (Okoth *et al*., 2012). Moreover, maize grains that are internally infected with fungi, when left to germinate, could give rise to plants that were internally infected with the same fungi (Mycock *et al*., 1992). The present trials demonstrated that maize stored in PICS bags with m.c. < 14% can be successfully kept without further mould infection during typical storage periods experienced in most rural households. Mould infection on maize stored in PP and jute bags, nevertheless, increased with increasing duration of storage irrespective of the initial storage moisture level of the grain. Magan & Lacey (1988) observed that mycoflora development in stored cereals is influenced by environmental factors, especially temperature, water activity and gas atmosphere. In the present study, maize stored in PICS bags with m.c. ≤ 14% did not show an increase in mould infection although it is unlikely that the oxygen/ carbon dioxide environment achieved in the PICS bags could inhibit mould development (Richard-Molard, 1988). Early works by Magan & Lacey, (1984) reported that decreasing oxygen to < 0.14% is required before mould growth can be substantially reduced and increasing carbon dioxide to > 50% is required for inhibition of mycelial growth. Other studies also reported the effect of modified atmospheres in controlling fungal growth and mycotoxin production in stored products (Dixon & Kell, 1989; Ellis *et al*., 1993). Studies on modified atmospheres with different carbon dioxide levels balanced with oxygen and nitrogen showed that *A. flavus* grew on wheat and rye with up to 75% carbon dioxide (Suhr & Nielsen, 2005). On maize, Giorni *et al*. (2008) indicted that treatment with 25% carbon dioxide reduced *A. flavus* development, but at least 50% carbon dioxide was necessary to reduce aflatoxin synthesis.

Notably, in maize grain with an initial 13% ≤ m.c. ≤ 14%, potentially toxigenic fungal isolates of the genera *Aspergillus*, *Fusarium* and *Pencillium* which have implications on

safety and quality of stored maize grain were frequently isolated. The genus *Aspergillus* was most frequently isolated. According to Abbas (2005), toxigenic *Aspergillus* and *Pencillium* spp. are often classified as storage fungi that can survive and grow on a variety of substrates and under a wide range of environmental conditions. The two mould spp. increased during storage in PP and jute bags but not in PICS bags. Infection by the genus *Fusarium* decreased during storage. Fandohan *et al*. (2005) noted that genus *Fusarium* generally decreases with duration of storage as moisture content and water activity of the grain declines. Previously, Bii *et al*. (2012) found that moulds belonging to the genus *Aspergillus* were most frequently isolated (35.8%) in two locations in Eastern Kenya. Other genera including *Fusarium, penicillium* and *Rhizopus* were isolated at frequencies of 15.5%, 9.2%, and 5.3 %, respectively. In a similar study, Muthomi *et al*. (2012) reported high incidence levels of *Aspergillus* species isolated from soil samples, whole maize grain and maize products in the Eastern region of Kenya. The pervasive nature of *Aspergillus* spp. and their high ability to colonize diverse substrates (Muthomi *et al*., 2009) may be reason for high occurrence in the maize samples.

In order to minimise mould proliferation, moisture content of maize to be packed in PICS bags should not exceed 14%. For long term storage, moisture content of 13 - 13.5% is recommended (KEBS, 2014) to avoid mould growth. However, a better indicator of the likelihood for moulds to colonize stored products is water activity which, in addition to moisture content, is related to temperature (Mahmoud *et al*., 1992). Water activity (a_W) is a measure of the fraction of water content which is free and therefore available for fungal growth (Reichmuth, 2008), and is equivalent to equilibrium relative humidity expressed as a fraction. The growth limit for most fungi during storage of durable products is a_W of 0.65 - 0.70 (Reichmuth, 2008). For maize at 26°C, the average temperature recorded in the PICS bags, a_W of 0.7 corresponds to moisture content of 14% (ASAE, 1995), although slight variations may occur depending on variety. Relative humidity in PICS bags packed with maize at moisture content of 14% or less did not exceed 70%. The relative humidity measured in the bags may be

regarded as the equilibrium relative humidity or a_W of the enclosed maize. Accordingly, mould counts on maize in these bags did not increase as a_W did not exceed 0.7. However, for the PICS bags packed with maize at an initial m.c. > 14% the relative humidity exceeded 70% (71.5 - 80.5%) representing $a_W > 0.7$. This explains the steady increase in mould infection. Lacey & Magan (1991) reported that commodities stored at relative humidity > 75% and m.c. > 15% are susceptible to fungal attack within normal storage time. Moreover, studies have shown that the less xerotolerant fungi such as *A. ochraceous* and *A. versicolor* also begin to grow at moisture of 14% thus increasing mould infection (Wilson & Abramson, 1992).

A main observation made during the course of this trial was high insect population and grain damage by insects of the maize stored in the PP and jute bags (Ng'ang'a *et al*., 2016). Insects' role in mould infection of stored maize was reviewed extensively; they are able to physically disseminate conidia in stored grain lots during movement and feeding, and also deposit them via defecation (Barry, 1987; Diener *et al*., 1987). Furthermore, damage inflicted by feeding insects, and the heat and moisture generated could enhance mould growth (Wright, 1992). These reasons related to profuse insect activity probably explain the increase in total mould count on maize stored in PP and jute bags even when m.c. was within the limit for safe storage, that is, below 14%. Moreno-Martinez *et al*. (2000) also reported low *A. chevalieli* invasion on maize stored in hermetic containers as compared to maize stored in non-hermetic ones, and attributed the difference to high insect activity in the non-hermetic containers.

Similar to mould infection, initial aflatoxin contamination of maize used in this study was high, suggesting field or pre-storage contamination. In maize agro-ecological zones characterized by dry hot seasons such as in the present study area, spore populations of *A. flavus* increase on crop debris leading to high levels of mould propagules in the air (Wilson & Payne, 1994). Thus, heavy *A. flavus* innocula may have been introduced to the crop during growth and maturation or during pre-storage handling. Drought stress and delayed harvesting also increase the risk of field contamination with aflatoxins

(Wagacha & Muthomi, 2008). However, these findings demonstrate that PICS bags can prevent further aflatoxin accumulation on maize during post-harvest storage provided the maize is dried below 14% moisture. Das *et al*. (2012) noted that *A. flavus* is a mesophilic fungus which grows optimally at a temperature of 30°C and relative humidity above 80%. Lacey & Magan (1991) stated that the minimum a_W for germination and growth of *A. flavus* is 0.78 which corresponds to moisture content of 16% at 27 °C or 15.5% at 32°C. Other researchers, (Fernandez-Pinto *et al*., 1991) observed that minimal aflatoxin production by *A. flavus* occurred at a_W of 0.85 when temperature is about 20°C, while maximum toxin production required a_W of 0.95 and temperature of 35°C. Likewise, Faraj *et al*. (1991) reported maximal colony growth of *A. flavus* and aflatoxin production at 30°C and 0.98 a_W, suggesting that a combination of fairly warm and humid conditions is necessary. Eventually, in the PICS bags, aflatoxin accumulation was observed when moisture of stored maize exceeded 14% in which relative humidity and temperature in the bags averaged 76% (71.5 - 80.5%) and 26°C, respectively. According to Sumner and Lee, (2012) development of the aflatoxin-producing moulds usually stops when moisture of the maize is below 12 – 13.5% and a_W is below 0.70. While aflatoxin accumulation was not observed in maize stored in PICS bags at moisture content of 14% or below, the same did not happen for the maize stored in PP and jute bags probably because of the influence of insect infestation in these bags (Ng'ang'a *et al*., 2016). Wilson & Abramson (1992) indicated that pest activity may increase the extent of aflatoxin contamination as insects break the physical integrity of grains, and could create localised spots of high moisture and temperature in the grain lot. Other earlier studies also associated insect damaged maize with increased risk of aflatoxin contamination (Diener *et al*., 1987; McMillian, 1987; Sinha & Sinha, 1991). These results concur with the findings of Baoua *et al*. (2014) in West Africa, who reported lower levels of aflatoxin in 10 - 13.5% moisture content maize stored in PICS bags as compared to PP bags.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Storage losses due to insect pest infestations, fungal infection and aflatoxin contamination are a serious problem that threatens the food security, nutrition and livelihood of rural farmers who rely on traditional storage systems. Because many infestations in endemic areas begin on the farm, prophylactic treatment using insecticides is almost never achieved. Moreover, in settings where adherence to best practices in the use of insecticides is poor, farmers who choose to protect their grain with insecticide may have to apply the insecticide more than once in order to achieve longer-term storage, which has cost, environmental and consumer health implications. Findings of this study showed that farmers experience losses during storage and control methods currently practiced are not effective. However, PICS bags storage is capable of halting destructive losses due to insect pest even for produce that may enter storage with some level of pre-storage infestation arising from field infestation or improperly cleaned storage structures.

In addition, the continued occurrence of acute aflatoxin poisoning in parts of the country and the numerous reports of contamination of human foods with aflatoxins demonstrate the great extent to which unsuspecting consumers are exposed to the toxic compounds in their diets. In Kenya, consumers of maize and products of maize origin in rural and urban areas alike are especially vulnerable to the acute and/ or long-term effects of aflatoxins, which act synergistically with primary infectious agents and malnutrition. In this study findings demonstrated that PICS bags are capable of storing maize grain with moisture content <14% with minimum risk of fungal growth and aflatoxin contamination during multi-month of storage. However, grain should be clean, damage free either by insect or mechanical and insect free as possible to limit the growth of

toxigenic fungi. Since the PICS technology does not require use of chemicals, it is cheap, and would allow the high level of control and flexibility that subsistence farmer's desire in the use and handling of their grain.

6.2 Recommendations

1. Farmers training on proper use of PICS bags
2. Future work should look at whether imperfections on HDPE lines such as those caused by repeated use and twisting could provide additional leverage for common maize weevils to bore through hence affecting performance of the bags.
3. A limitation with the PICS bag as demonstrated in this study which might require pre-storage precautions relates to the ability to sustain a constant relative humidity while storing grain with high moisture content. Since many farmers do not have a standard objective method of determining grain moisture before or during storage but instead rely on subjective knowledge such as the rattling sound of grains, this constraint could affect quality of grain stored. Therefore, frequent monitoring during storage is recommended.
4. Further research is needed to establish the effect on nutritional composition of stored produce such as maize in PICS bags during multi-month of postharvest storage.

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APPENDICES

Appendix I: Sample questionnaire used

Questionnaire No:
01.Interviewers' Name
02. Date:
03. County:
04. Village:
GPS coordinates S_____ 0 _____', E_____ 0 _____', Alt _____M

Questionnaire for insect pest damage, mould infection and aflatoxin contamination in maize grain during storage in Makueni County, Kenya

1.0 Maize agronomic and harvesting condition

1.1How did you prepare your farm for new cropping? 1. Human labour /_____2.Animals/_____/

3. Both/_____/ 4. Other specify/_____/

1.2 What did you do to left over stock/residuals of previous harvest? 1. Burned/_____/

2. Buried/_____/ 3.Both/_____/ 4. Other specify/_____/

1.3 What time of the year did you introduce new crop? 1. Aug/_____/ 2. Sept/_____/

3. Oct/_____/ 4. Nov/_____/ 5. Other specify/_____/

1.4 How can you rate the amount of rainfall experienced during sowing/planting?

1. Very low/_____/ 2. Low/_____/ 3. Medium/_____/ 4. High/____/ 5.Very high/_____/

1.5 How can you rate the amount of rainfall experienced during weeding?

1. Very low/_____/ 2. Low/_____/ 3. Medium/_____/ 4. High/____/ 5.Very high/_____/

1.6 How can you rate the amount of rainfall experienced during grain filling?

1. Very low/_____/ 2. Low/_____/ 3. Medium/_____/ 4. High/____/ 5.Very high/_____/

1.7 How can you rate the amount of rainfall experienced during harvesting?

1. Very low/_____/ 2. Low/_____/ 3. Medium/_____/ 4. High/____/ 5.Very high/_____/

1.8 How do you usually know your maize is ready for harvesting? 1. Calendar (month calculation)/_____ / 2. When ears dry up and cob fall/_____/ 3. Other specify/_____ /

1.9 Where did you dry your maize after harvesting? 1. Field/_____/ 2.Homestead/_____/

3. Both/_____/ 4. Other specify/_____ /

1.10 Did you experience/notice mouldy cobs or grain during harvesting? 1. Yes/____/0.No/____/

1.11 If Yes, what did you do to mouldy cobs or grain during harvesting?

1. Mixed with other grain/___/ 2. Burned/___ / 3.Buried/_____/ 4. Other specify/_____ /

1.12 Any other constraint/peculiar observation during maize production and harvesting? State please_____ _ /

1.13 Approximately, how long did your maize take to dry up and be ready for storage?

1. One week/_____/ 2.Two week/_____/ 3. Three week/____/ 4. Other specify/_____/

2.0 Storage practices

2.1 In which form do you store your maize? 1. As cobs /_____/2. As grain /_____/

3. Both /_____/ 4. Other specify/_____/

2.2 What storage structure do you use to store your maize cobs/grain?

1. Crib (local name)/ _____/
2. Bags (specify the bag) /_____/
3. On the floor in room /_____/
4. Others (please list) /_____/

2.3 Where is your crib located? 1. Field/_____ / 2.Within the homestead/_____/3. Both /_____/

2.4 After you put your maize in bags, in which place do you store them?

1. Room (living) /_____/
2. Bedroom /_____ /
3. Kitchen separate from the main house/_____ /
4. Special store room within the main house/_____/
5. Cribs (give the name) /_____/
6. Other (specify)/ _____

2.5 For how many month(s) do you store maize before the stock is exhausted? /_____/

2.6 What quantity of grain can your storage structure hold? /_____/ (kg)

2.7 Do you store other products together with maize?

A. In cribs 1. Yes/_____/ 0. No/_____/

If Yes above, what else do you store together with maize?

/_____

B. In room 1. Yes/_____/ 0. No_____/

If Yes above, what else do you store together with maize?

/_____

C. In other containers 1.Yes/_____/ 0.No/_____/

If Yes above, what else do you store together with maize?

/_____

3.0 Storage problems

3.1 Do you have storage problems/ challenges? 1. Yes/____/0. No/____/

3.2 In your opinion, which storage problem is the most important? (Please range per order of importance: **1, 2, 3, 4, 5**, **6. 1** = Very important /, **2** = Important /, **3** = Moderately important/, **4** = Of little importance/ **5** = Not important, **6=** Others

Storage problem	**Order of importance** (**1, 2, 3, 4, 5, 6**)
Drying (moisture content)	
Insects	
Mould	
Rodents (Rats and mice)	
Birds	
Other (specify)	

3.3 Have you ever experienced loss of maize during storage? 1. YES /_____ /0. NO/ _____/

If Yes, what was the cause of the loss?

1. Infestation by insects /_____/
2. Infestation by moulds /_____/
3. Infestation by rodents /_____/
4. Other (specify) /_____

3.4 Approximately, how many kg of the maize grain do you lose due to storage problems? /_____/ (kg)

3.5 How many kg of your losses on maize grain will you attribute to:

1. Insects/_____/ 2. Mould/_____/ 3. Others, specify/_____

3.6 (i) When do you observe this problem of insect on maize grain during storage?

1. At the beginning of storage/_____/

2. After a few weeks after the beginning of storage / _____/

3. At the end of storage /_____/

4. Other specify/_____

(ii) When do you observe this problem of mould on maize grain during storage?

1. At the beginning of storage/_____/

2. After a few weeks after the beginning of storage / _____/

3. At the end of storage /_____/

4. Other specify/_____

4.0 Strategies to cope with storage problems

4.1 Are there any activities/ methods you use for controlling the storage problem?

1. YES /_____/ 0. NO /_____/

4.2 If Yes, what do you do to solve storage problem on maize grain?

Against insects	Control method
A. Use of insecticide? 1.Yes___/ 0. No____	**If yes, give name:**
B. Other (specify)	
Against mould growth	
A. Any chemical? 1.Yes___/0. No____	**If yes, give name:**
B. Other(specify)	

4.3 Do you think the control you implement has reduced insect/mould (aflatoxin poisoning) problems? 1. Yes/_____/ 0. No/_____/

4.4 Do you clean the storehouse (cribs, room or other structures) before a new storage?

1. Yes/_____/ 0. No/_____/

4.5 Do you remove old grains before introduction of new stock? 1.Yes/___/ 0. No/____/

4.6 What do you do to clean the store (cribs, room or other structure) before storage? (Narrative)_____

_____

_____

_____

4.7 Have you received any training on protection of stored products? 1.Yes/___/ 0.No/____/

If Yes, who provided the training? 1. ICIPE staff/____/ 2. Government officers/_____/

3. Farmer to farmer/_____/ 4.Any other organization specify/_____

4.8 What specific training/level training received? State /_____

5.0 Production, consumption and sale of maize

5.1 Please give information needed in the table below

Description	**A. Maize growing season- low rainy season**
Acreage cropped	
Quantity harvested (kg)	
Quantity reserved for home consumption (kg)	
Quantity reserved for home consumption (kg), how long does it last (months)	
Quantity sold Immediately (kg)	
Remainder if any? When is it sold?	

Appendix II: Composition of mycological media

1. Sabouraud's Dextrose agar, modified with antibiotic

Enzymatic digest of casein 5 g

Enzymatic digest of animal tissue 5 g

Dextrose 40 g

Agar 15 g in 1000 mL distilled water

pH 5.6 ± 0.2 at 25°C modified with 20 mg chloramphenicol

2. Czapek-dox Agar

Sucrose 30 g

Sodium nitrate 2 g

Dipotassium phosphate 1 g

Magnesium sulphate 0.5 g

Potassium chloride 0.5 g

Ferrous sulphate 0.01 g

Agar 15 g in 1000 mL distilled water

pH 7.3±0.2 at 25°C