

**EVALUATION OF HERMETIC TECHNOLOGIES IN THE CONTROL OF
INSECT INFESTATION, MOLD PROLIFERATION AND MYCOTOXIN
CONTAMINATION OF STORED MAIZE IN KENYA**

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FACULTY OF AGRICULTURE

2019

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

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ACRONYMS AND ABBREVIATION

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
A-Z	Agro Z Bag
A-Z⁺	Agro Z Impregnated with pesticides
CDC	Center for Disease Control and Prevention
CIMMYT	International Center for Maize and Wheat Improvement
EC	European Commission
FAO	Food and Agriculture Organization
FAW	Fall Army Worm
FDA	Food and Drug Authority
GAP	Good Agricultural Practices
GDP	Gross Domestic Produce
GENSTAT	General Statistics
ppb	Parts per billion
ppm	Parts per million
HACCP	Hazard Analysis Critical Control Points
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
IFPRI	International Food Policy Research Institute
KARLO	Kenya Agricultural Research and Livestock Organization
KEBS	Kenya Bureau of Standards
LGB	Lesser Grain Borer
LSD	Least Significant Difference
MLND	Maize Lethal Necrosis Disease

MOFA	Ministry of Food and Agriculture
MW	Maize Weevil
MS	Metal Silo
Ng/g	nanogram per gram
PCA	Principal Components Analysis
PDA	Potato Dextrose Agar
PICS	Purdue Improved Crop Storage
PP	Poly Propylene
PS	Plastic Silo
RCBD	completely randomized block design
RH	Relative Humidity
SGB	Super Grain IV-R™ bag
SSA	Southern Saharan Africa
SWP	Standard woven Polypropylene.
SWP+	Standard woven Polypropylene with insecticide.
USAID	United States Agency for International Development
USD	United States Dollars
USDA	United States Development Agency
WHO	World Health Organization

GENERAL ABSTRACT

In Africa, maize is one of the most important food crops, yet it is also susceptible to insects infestation, microbial attack and mycotoxin contamination. These cause significant economic losses and deleterious health effects to humans and animals. Strategies such as storage of maize in hermetic bags are known to be effective in reducing post-harvest contamination by fungi and mycotoxin production. This study was conducted with the aim to investigate the effectiveness of using hermetic technologies in the control of insect infestation, fungal proliferation, and mycotoxin contamination as well as preserve the nutritional quality and seed viability in a safe and environmentally friendly system. Maize used for this study was collected from the farmers in Nakuru County and data on maize production practices recorded using a semi-structured questionnaire. Three factors were used in the design of this study: contaminated grain with molds vs. clean grain; two levels of grain moisture levels; and ten storage methods, of which eight were hermetic. The overall study design was a 2 x 2 x 10 completely randomized block design (RCBD) with 3 replications. The first factor was artificial infestation with fungi (fusarium and aspergillus strains). All technologies were tested with artificially inoculated molds and the other set was not be inoculated. The second factor is grain moisture. All the treatments were subjected to two grain moisture levels, low (12-13%) or high (14-15%) determined using standard methods. The final factor was the storage technologies; metal silos (MS); plastic silos (PS); Super Grain IV-R™ bag (SGB); AGRO-Z (A-Z); AGRO-Z⁺ (A-Z⁺) impregnated with insecticides PICS bag; Elite bag; ZeroFly and two controls; the standard woven polypropylene (PP) bags one with grain treated with 0.05% Actellic Super (PP⁺) and one without insecticide. Each technology was analyzed for gas composition. Grain samples of approximately 1kg were collected from each storage technology at 0, 4 and 8 months and analyzed for moisture levels, insect infestation, fungal proliferation, mycotoxin contamination, seed viability and grain composition during the

experiment. During artificial inoculation, the fungal inoculums (fusarium and aspergillus strains) were placed in the middle of the grain in perforated bags that allowed contact with the other grains. *Sitophilus zeamais* and *Postephanus truncatus* types of storage insects were counted and recorded as live and dead from 1000 sampled kernels. Natural infestation relied on existing insect infestation only. Isolation of mycotoxin producing fungi was done employing the serial dilution and spread plate technique on Potato Dextrose Agar; and total aflatoxin and fumonisin in maize kernels was analyzed by Vicam kits.

Maize from the farmers was found to contain less than 10 ppb aflatoxin with an average of 1.63 ppb under moisture content that varied from 13-16.47 %. Hermetic technologies were able to modify gas composition, increasing CO₂ by 12.98 % and dropping O₂ by 11.77%. After 8 months of maize storage, there was 21% insects infestation in hermetic technologies and 53 % in PP bags and the least amount 17% in PP⁺ bags. Mycotoxin fungi and aflatoxin contamination were higher in PP bags above the local and international standards and the metal and plastic silos with un-inoculated grains complied with the European Commission (EC 4 ppb) limit but grain inoculated was above this limit. The hermetic bags were effective in the control of aflatoxin contamination below the limit of 10 ppb set by KEBS except for the grain inoculated in PP bags that were above 20 ppb (FDA max limit), KEBS and EC limits. The percentage seed viability reduced to less than 5% in PP bags, 45% in hermetic storages and 55% in PP⁺ bags. There was no significant difference in nutritional composition of maize in all the storage technologies at P>0.001. The success of the hermetic technologies in preserving the quality of maize is synergized with proper drying of maize before storage and good handling practices of both the maize and the technologies.

CHAPTER 1: INTRODUCTION

1.1 Background information

Maize (*Zea mays* L.) can conveniently be classified as the most important cereal crop owing to its nutritional value and utilization of its by-products (Shiferaw *et al.*, 2011). Maize is staple food to almost half the population of people in sub-Saharan Africa alone (CIMMYT, 2010) and for more than 90% Kenyans (Anankware *et al.*, 2013). It accounts for 40% of the total dietary intake in East and Southern Africa (Doss *et al.*, 2003).

Maize production in Kenya has been constrained due to factors such as high rates of disease prevalence like the Maize Lethal Necrosis Disease (MLND), human and animal population influx, changes in weather patterns, high post-harvest losses, high cost of value addition production and the new emerging pests such as the Fall Army Worm (FAW) (USDA, 2013; (Wangai *et al.*, 2012) Maize has many uses; as poultry feed, livestock feed and in brewing industry to replace sorghum (Ranum, Peña-Rosas and Garcia-Casal, 2014).

Mould contamination of maize grain in the tropical countries is one of the most important food safety risk (Schulthess, Cardwell and Gounou, 2002); (Kimanya *et al.*, 2008); (Gong *et al.*, 2004); (Kaaya and Kyamuhangire, 2006); (Strosnider *et al.*, 2006a); (Campbell and Arbogast, 2004). After insects, storage fungi rank highly in causing reduction of maize quality and quantity (Ominski *et al.*, 1994). In favorable conditions, fungi could cause up to 80 % of damage on maize during storage period (Tsedaley *et al.*, 2016). Economical loss of stored grain caused due to mycotoxins and insect pests is estimated at US \$ 500 million to 1 billion annually (Campbell and Arbogast, 2004). Conventional storage methods which result in up to 30% loss as a result of insect pests and mycotoxins, force the smallholder farmers to sell off their grain soon after harvest at the time when prices are still low, only to purchase it back later at a costly price, hence being trapped in a vicious cycle of poverty (Tefera *et al.*,

2011). On-farm grain losses result in food insecurity and negatively affect the farmers' livelihood income (Gitonga *et al.*, 2013; Ndegwa *et al.*, 2016; Mutambuki *et al.*, 2012).

A number of moulds which include, *fusarium*, *aspergillus* and *penicillium* are involved in post-harvest losses (Quezada *et al.*, 2006); (Blandino *et al.*, 2009); (Chulze, 2010). Among over 400 mycotoxins known, aflatoxins are the most important mycotoxins (Reddy *et al.*, 2018) (Al-Ruwaili *et al.*, 2018). The maize is susceptible to aflatoxigenic fungi from the time of harvest through storage duration (Cotty and Jaime-Garcia, 2007). If not handled properly or stored efficiently to minimize growth and multiplication of these fungi, the grain damage is likely to proceed through the post-harvest stage (Abramson *et al.*, 1992). The Kenya quality standard specification requires a safe maximum limit of total aflatoxins for imported maize at 10 ppb (NCPB, 2017). Mycotoxin contamination is escalated with long storage time when conditions are favorable for mold growth (Kaaya and Kyamuhangire, 2006). The infection may thereby reduce the nutritional value and result in discoloration of the grain (Ehrlich *et al.*, 2007). When the colonizing fungi are mycotoxigenic, then the infection also results in the spread of toxic metabolites (Klich, 2009); (Wagacha and Muthomi, 2008).

Fungal growth, especially *Aspergillus flavus* and *Fusarium spp* in stored maize, is mainly facilitated by hot, and humid conditions, hence deterioration of maize. This poses a major risk through production of mycotoxins which implies that animal and human health risk will be greatest in the absence of control system for mycotoxin. Further, the existence of mycotoxins in food may be escalated in the presence of high insect infestation which spread mould spores. (Hubert *et al.*, 2018; Nayak and Darglish, 2018).

Hermetic bags have been known to preserve the quality of grain and aesthetic appearance by reducing mould growth (Moussa *et al.*, 2014); (Murashiki *et al.*, 2018). Hermetic technology works synergistically to promote conditions of low oxygen and high carbon dioxide levels

produced by aerobic metabolism of insects, micro-organisms and grain respiration. Aerobic metabolism uses up oxygen and produce carbon dioxide to levels that are lethal to insects and moulds in the grain mass (Weinberg *et al.*, 2008); (Yakubu *et al.*, 2011). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues, aflatoxin and insect contamination (Weinberg *et al.*, 2008).

Improved storage technologies at both household and national levels which reduce losses by preventing insect pest attack are an important component of food security. Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal/plastic silo provides affordable and more effective storage alternative for farmers, especially the vulnerable women, that would markedly contribute to food security (Gitonga *et al.*, 2013); (Obeng-Ofori, 2011a; Ndegwa *et al.*, 2016) (Mutambuki *et al.*, 2012).

This study, therefore analyzed the multiple effect of hermetic storage in the control of mycotoxin contamination as well as insect infestation in safe and environmentally friendly system.

1.2 Statement of the problem

The main biotic cause for post-harvest losses in maize is mould infection. This has resulted into outbreaks of aflatoxicosis which are endemic across Africa. Lack of public awareness of the mycotoxin existence coupled with; poor regulatory policies, dumping and introduction of contaminated food into the food chain during chronic shortage, drought, increases the exposure to the consumers (MERCK, 2006). Maize infested with insects and moulds losses its palatability and is discolored due to the excretion and the by-products from the moulds and insect respectively. Insects burrow through the grains, leaving holes and large quantities of dust (Holst, Meikle and Markham, 2000).

Most of the maize produced in Africa is either used for producer 's own consumption or sold in the local market. The maize becomes infected at any stage of production including cultivation, harvesting, drying, storage, transportation and at the market place (Wagacha and Muthomi, 2008). Due to prevailing high temperatures and higher humidity, maize produced in Eastern Kenya is prone to aflatoxin and fumonisin contamination. Aflatoxin occurrence together with outbreaks in fumonisins can present unacceptable levels of toxins in the same grain samples (Martinez, 2000; Ono, *et al*, 2001). A study conducted by Bii *et al.* (2012) in Eastern province of Kenya on 86 stored maize samples, found mean fumonisin contamination in maize samples ranging from 0.912 mg/kg in Kitui to 1.17 mg/kg in Makueni.

There have been many studies about the effectiveness against post-harvest pests in hermetic technologies in Sub-Saharan Africa but little information exists on the mould and mycotoxin infestation with regards to hermetic technologies. Hermetic bags are now widely used in West and Central Africa for the storage of pulses such as cowpeas (Moussa *et al.*, 2014). Research on effectiveness of hermetic bags has been extended to other crops such as maize (Ognakossan *et al.*, 2013a); (Murdock and Baoua, 2014). However, adoption of hermetic storage technologies in Kenya is still very slow.

There is need for a comparative assessment of available hermetic storage technologies for the control of mycotoxin and insect pest infestation. A great number of farmers store their grain in, woven polypropylene bags with no barrier to air yet there is evidence that this method facilitates fungal contamination and aflatoxin development (Udoh, Cardwell and Ikotun, 2000b; Hell *et al.*, 2000).

1.3 Justification

Maize is central to household food security for most Kenyans and Africans in general. It has also consistently been consumed widely as a staple food in most sub-Saharan countries.

However it also provides nourishment to the fungi which produce toxic metabolites such as aflatoxin (Wagacha and Muthomi, 2008). Food security is threatened as a result of reduced nutritional quality, and agricultural production due to the quality and safety issues resulting from fungal attack and mycotoxin contamination. Despite long time of study, maize has continuously been contaminated with mycotoxin producing fungi posing serious problems in Kenya; hence, aflatoxicosis outbreaks in 2004 that caused several hundred Kenyans to become severely ill, and 125 died, of acute aflatoxicosis: a disease of liver failure associated with consuming extremely high levels of aflatoxin in food (Lewis *et al.*, 2005); (Strosnider *et al.*, 2006a); (Shephard, 2008a); (Probst, Bandyopadhyay and Cotty, 2014). Moreover, during the 2004 - 2006 outbreak, more than 2.3 million bags of maize were burnt due to aflatoxin contamination in Kenya (Atser, 2010).

Infection of maize with aflatoxin is a public health concern due to its potential to cause animal and human health complications and diseases (CDC, 2004; Gong *et al.*, 2004). Over 5 billion of people in developing countries in the world especially in the tropics and sub tropics are exposed to aflatoxin contaminated foods due to the humid and warm conditions (Shephard, 2008b; Strosnider *et al.*, 2006b); (Weinberg *et al.*, 2008). This has been known to cause or increase kwashiorkor in African children, high prevalence of liver cirrhosis, and reduced rate of childhood growth (Strosnider *et al.*, 2006a); Gong *et al.*, 2002; (Gong *et al.*, 2003); (Gong *et al.*, 2004); (Jolly *et al.*, 2006) Jiang *et al.*, 2008; Khlangwiset *et al.*, 2011).

Several methods including chemical, biological and cultural methods have been explored but none is efficient and cost effective especially in the control of larger grain borer (De Groote *et al.*, 2013). Insects have also developed resistance to pesticides resulting in their resurgence (Wangui, 2016). Insect pests have been reported to provide ideal environment for mould growth through respiration, feeding activities and waste excretion. Insects also reduce the

nutrient content of the maize as they tend to feed mainly on the germ which is known for high oil levels, they shed off their wings, legs and some die which cause discoloration and musty smell in the grain.

This study therefore was aimed at determining the mycotoxigenic fungi in maize and assessing the effect of hermetic storage technologies on fungal population, and aflatoxin and fumonisin levels in maize.

1.4 Objectives

1.4.1 General Objective

To evaluate how hermetic storage technologies can be leveraged in the prevention of mould growth and insect infestation in stored maize grain.

1.4.2 Specific objectives

1. To determine the effect of hermetic storage technologies on insect infestation at different moisture levels in maize grain.
2. To determine the effect of hermetic storage technologies on mould growth at different moisture levels in maize grain.
3. To determine the effect of hermetic storage technologies on maize grain mycotoxin contamination at different moisture levels.
4. To evaluate the nutritional quality of the maize grain stored under hermetic and conventional storage.

1.5 Hypothesis

- i. There will not be a difference in the insect infestation between hermetic technologies and polypropylene bags.

- ii. The mould growth will not increase with increased moisture content of stored grain in hermetic technologies.
- iii. Mycotoxin contamination will not increase with increased moisture content of stored grain in both hermetic technologies and polypropylene bags.
- iv. There is no difference in maize grain nutritional composition between hermetic and conventional storage.

Thesis Layout

The study was categorized into four sub-studies and includes elaborated chapters that are presented to develop a Thesis as follows;

Chapter 1: General Introduction that articulates the study background, challenges that deserved this study, objectives and study design.

Chapter 2: Literature review that elucidates the work done previously on the topic of interest.

Chapter 3: Evaluation of Hermetic Technology in the Control of Insect infestation in Stored Maize Grains.

Chapter 4: Effect of Hermetic Technologies in Controlling Mould Proliferation of Stored Maize.

Chapter 5: Mycotoxin Contamination Control in Maize Grains Stored Using Hermetic Technologies.

Chapter 6: Evaluation of the nutritional quality of the grain stored under hermetic and convectional storage.

Chapter 7: General conclusion and recommendations

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of maize production trends

The FAO (1996) declaration states that “food security exists when all people at all times, have physical and economic access to sufficient, safe and nutritious food to meet dietary needs and food preferences for an active and healthy life”. The need for extended storage periods to cater for household food security has increased owing to occurrence of frequent droughts in many parts of Africa; necessitating use of effective and environmentally-benign alternative methods to extend the storage period (Chigoverah and Mvumi, 2016) (Chigoverah and Mvumi, 2018). The production of maize in Kenya declined from 3.1 to 2.6 million tons in 2011 and 2012, respectively (Karemu, Ndung'u and Githua, 2013); it increased to 3.5 million tons in 2013 but declined to 3.19 MT in 2017 (KEBS 2018). Among the factors that contributed to the decline in maize production include unpredictable rainfall pattern, conversion of land under maize into sugarcane-producing farms following the construction of new sugar factories in western Kenya, and the Maize Lethal Necrosis disease (MLN) (MoA, 2013). The majority of maize producers are small- to medium-scale farmers. Since maize production is seasonal, storage of the harvested grain for prolonged periods becomes necessary. However, small-scale farmers still experience high postharvest losses due to application of ineffective storage methods (Likhayo *et al.*, 2016). Postharvest losses in developing countries are high due to, among other factors, poor handling practices and inadequate and ineffective storage structures (World Bank 2011; (Affognon *et al.*, 2015). At the micro level, factors such as drought, diseases and pests, price fluctuation, nutrient deficiencies, and poor storage facilities have been attributed to the low levels of maize production in Africa (Sesay *et al.*, 2017). The reuse of contaminated and perforated bags among small scale farmers predisposes the stored grain to insect infestation (Abass *et al.*, 2014). The on-farm storage losses of maize in Kenya are estimated at 30 % (Mutambuki *et*

al., 2012); (Karemu, Ndung'u and Githua, 2013)). (Zorya *et al.*, 2011) estimated maize grain loss at 17.5% for East and Southern Africa. The total postharvest losses of maize in Africa vary from 14 to 36% (Tefera *et al.*, 2011) whereas developing countries have subsidized farm inputs such as fertilizer to increase productivity, small-scale farmers lack effective storage facilities to store the surplus. The high grain losses during on-farm storage therefore deny the farmers the opportunity to attain food security and increased income (Likhayo *et al.*, 2016).

Maize has many uses including poultry feed, livestock feed and in brewing industry to replace sorghum (Ranum, Peña-Rosas and Garcia-Casal, 2014). Maize nutritional value is comparable to that of rice, wheat and other cereals (Gijón-Hernandez *et al.*, 2008). It consists of the major nutrients required by the body. Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g (Nuss and Tanumihardjo, 2010) and minerals such as iron and phosphorus with the exception of essential amino acids-tryptophan and lysine (Iken, Amusa and Obatolu, 2002). Notwithstanding the maize production constraints, maize is central to household food security for most Kenyans and Africans in general where each person is estimated to consume at least 98 kg per year on average (Kilonzo *et al.*, 2014). Approximately, in excess of 90% of the households in the countryside depend on maize, therefore dominating all countrywide food security considerations (Ouma and De Groote, 2011).

In Kenya, maize is grown in the high potential areas of the rift valley; in the medium potential areas in central province and western province; in the marginal areas of Eastern province and South Nyanza; in the arid areas of North Eastern and in the coastal lowlands of Kenya (FAO, 2014). With the average annual production of maize estimated at 2.7 million tons and average annual consumption at 3.4 million tons, a fluctuating trend in maize production over the years threaten household food security and income sources (ROK, 2015).

Maize production per capita, which had risen to over 150 kg per capita in the mid-1970s, has dropped steadily since then to a current all-time low of 70 kg per capita (Ouma, De Groote and Owuor, 2006). This is substantially less than the estimated consumption needs of 103 kg per capita, which necessitates regular imports of large quantities of maize (De Groote *et al.*, 2005). In 2008 maize production stood at 2.4 million metric tones (26 million bags) against a national requirement of 3.1 million tons (34 million bags) (Suleiman and Kurt, 2015). In 2013, Kenya produced a total of 38.9 million bags of maize, which was a deficit of 2.0 % compared to 39.7 million bags in 2012 (KEBS, 2014). With the country's population projected to be 43.1 million by the year 2020, the demand for maize is likely to be 5 million metric tons (Rosentrater and Suleiman, 2015). This means based on the prevailing maize production rates that the maize deficit will be around of 1.2 million metric tons in 2020 (Kang'ethe, 2011).

Table 2.1: Nutritional composition of maize in comparison with the most common cereal crops

Contents	Maize ground	Wheat flour	Rice polished grain
Per 100g	meal		
Calories (kCal)	362	359	360
Carbohydrates (g)	74.5	74.1	78.9
Water (g)	12	12	13
Protein (g)	9	12	6.8
Fat (g)	3.4	1.3	0.7
Ash (g)	11	0.6	0.6
Starch fibre (g)	1	0.5	0.2
Phosphorous (mg)	178	191	140
Calcium (mg)	6	24	6
Niacin (mg)	1.9	2.0	1.5
Iron (mg)	1.8	1.3	0.8
Thiamine (mg)	0.3	0.2	0.26
Riboflavin (mg)	0.08	0.07	0.03

Source; (Meijia, 2008)

All over the world, maize is primarily consumed as the main source of starch (Meijia, 2008). Food producers in developed countries have advanced technologies that add value to the maize with the aim to produce more convenient maize products such as cereals and instant mixes. Currently maize and its products contribute 50% of the total agricultural revenue in the world, however maize production has been constrained in Sub-Saharan Africa leading to losses during postharvest storage of up to 40% (Kang'ethe, 2011). Maize also makes a large contribution to the economies of developed and developing countries (Suwa *et al.*, 2010). It

represents 3% of Kenya's gross domestic product (GDP), 12% of the agricultural Gross Domestic Product (GDP) and 21 % of the total value of primary agricultural commodities (De Groot *et al.*, 2005).

The most important concern in maize production is the inadequate mechanism of quality preservation and measurable losses of maize during postharvest storage. These losses are caused by both biotic and abiotic factors which if not managed, maize will be condemned unsafe for both human and animal consumption. The main post harvest losses which include; insect infestation, mould infection, mycotoxin contamination and reduced nutritional quality have been investigated to have been caused by inadequate drying of grains to safe moisture levels of <13 %.

Fungal growth, especially *aspergillus flavus* and *fusarium sp* in stored maize, is mainly facilitated by hot, and humid conditions, hence deterioration of maize. This poses a major risk through production of mycotoxins which implies that animal and human health risk will be greatest in the absence of control system for mycotoxin. Further, the existence of mycotoxins in food may be escalated in the presence of high insect infestation which spread mould spores. (Buerstmayr *et al.*, 1999).

Hermetic technology works synergistically to promote conditions of low oxygen and high carbon dioxide levels produced by aerobic metabolism of insects, micro-organisms and grain respiration. Aerobic metabolism uses up oxygen and produce carbon dioxide to levels that are lethal to insects and moulds in the grain mass (Navarro *et al.*, 2007; Yakubu *et al.*, 2011). Hermetic bags are now widely used in West and Central Africa for the storage of pulses such as cowpeas (Moussa *et al.*, 2014). Research on effectiveness of hermetic bags has been extended to other crops such as maize (Ognakossan *et al.*, 2013a); (Murdock and Baoua, 2014). However, adoption of hermetic storage technologies in Kenya is still very slow. To

move forward in mitigating post-harvest losses in maize from mould infection and mycotoxin contamination, it will demand the detection and eradication of the limitations to the usage of a particular technology. Consequently, safe on-farm storage of maize is vital in improving food and income security for the smallholder farmers (Maria, 2011). The effect of modified atmospheres could significantly control fungal contamination in stored grains and reduce the incidence for insect infestation. Elevated concentrations of CO₂ of >75% inside the hermetic bags are essential in prevention of growth of mycotoxigenic fungi in relatively dried maize.

Most parts of Africa still practice rudimentary storage methods that expose the maize to insect infestation, mould and mycotoxin contamination (Olakojo and Akinlosotu, 2004). The losses threaten Africa's food security and may lead to deteriorated health in both humans and animals. Studies by FAO and University of Nairobi Project have indicated that most farmers lack knowledge on food safety issues around mycotoxins, proper harvest and post-harvest practices to manage mycotoxin production (Kang'ethe, 2011). A number of studies have suggested and proved the effectiveness of reduction and eradication of moulds and mycotoxins in maize;

2.1.1 Challenges on farm and postharvest losses in maize

Most African homesteads rely on maize as food but it is threatened by a series of production constraints that hamper not only the livelihoods of the farming population but also meeting of the government objectives for agricultural sector transformation (Ndegwa *et al.*, 2016). These production constraints include a combination of abiotic and biotic stresses (Kang'ethe, 2011) (CIMMYT, 2010). Abiotic factors like drought, extreme temperatures, land degradation, high soil aluminum (soil acidity), flooding and salinity limit maize production (Mugo, Bergvinson and Hoisington, 2001). Biotic factors such as storage fungi contribute to loss of more than 50% of maize grain in the tropics and ranks second after insects as the major cause of deterioration and loss of maize (Fandohan *et al.*, 2005). The cost of grain loss due to micro-

organisms and damage by insect pests of stored grain in developing countries is estimated at US \$500 million - 1 billion of foreign exchange annually (Campbell and Arbogast, 2004). To prevent mould growth and insect infestation, it is recommended to dry maize grain to safe moisture of 10 - 13% (Hell *et al.*, 2008). Many African communities still use traditional storage methods which are ineffective in reducing the high moisture levels of grain after harvest leading to fungal contamination (Fandohan *et al.*, 2005).

Food security is threatened, reduced nutritional quality, and agricultural production due to the quality and safety issues resulting from fungal attack and mycotoxin contamination (Kang'ethe *et al.*, 2017a). Despite long time of study, maize has continuously been contaminated with mycotoxin producing fungi posing serious problems in Kenya; hence, aflatoxicosis outbreaks in 2004 that caused several hundred Kenyans to become severely ill, and 125 died, of acute aflatoxicosis (Probst, Bandyopadhyay and Cotty, 2014). Moreover, during the 2004 to 2006 outbreak, more than 2.3 million bags of maize were burnt due to aflatoxin contamination in Kenya (Atser, 2010).

The fact that maize production is seasonal, it is necessary to store the harvested maize for extended periods. However, smallholder farmers still experience high postharvest losses due to application of ineffective storage methods. Postharvest losses in developing countries are high due to, among other factors, insect pests, poor handling practices, poor market structures, bad road infrastructure and ineffective storage systems (World Bank, 2011; Affognon *et al* 2015). The reuse of contaminated and perforated bags among small-scale farmers predisposes the stored grain to insect infestation (Abass *et al.*, 2014). Whereas on-farm storage losses of maize in Kenya are estimated at 30% (Mutambuki *et al.*, 2011; Karemu *et al.*, 2013; Paddy *et al.*, 2016; Ndegwa *et al.*, 2016), the total postharvest losses of maize in Africa range from 14 to 36% (Tefera, 2012). Subsidized farm inputs such as

fertilizer have resulted in increased productivity; however, smallholder farmers lack effective storage facilities to store the surplus. The high grain losses incurred on-farm therefore deny farmers the opportunity to attain food security and livelihood income. Adequate levels of improved storage technologies at household and national levels which reduce losses by maintaining stored grain free from insect pest attack and mold contamination are important component of food security.

2.2 Methods of postharvest loss reduction

2.2.1 Cultural control and store management

The storage of husked and un-husked or shelled and unshelled maize is not uncommon among small-holder farmers in Africa. Storage of maize on the cob with the husk intact provides protection to grain against insect pest infestation and aflatoxicogenic fungi (Hell *et al.*, 2008). Traditional storage structures used by farmers for on the farm storage include containers made of plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet. Essentially the stores are constructed to prevent insect and rodent attack and to prevent moisture from getting into the grains. Maize is subjected to several kinds of treatments prior to storage. Traditionally, stored maize is protected against damage by mixing with ash from cooking fire, sand or leaves from certain plant (Hayma, 2003). Cobs may be exposed to smoke and heat from kitchen fire or, when outside the house from a fire underneath the main structure to facilitate drying and disinfect the maize from destructive biotic agents such as insects, mites, and fungi (Udoh, Cardwell and Ikotun, 2000a). Good agricultural practices and HACCP, proper storage and transportation facilities, breeding improved varieties and diet diversification are some of the ways solution to mycotoxin prevalence will reduce.

2.3 Overview of mycotoxins in cereal grains

Mycotoxins e.g. aflatoxin are poisonous secondary metabolites produced by fungi. Estimates indicate that approximately 25% of the world's food crops are contaminated with aflatoxins, but the magnitude of contamination is greater in sub-Saharan Africa (Wagacha and Muthomi, 2008; Hell *et al.*, 2000b; Cardwell *et al.*, 2000; (Atehnkeng *et al.*, 2008). One of the critical concerns of inappropriate drying and storage methods coupled with warm, humid environment found in the tropics facilitates rapid increase in mycotoxin concentrations levels (Bankole *et al.*, 2006). Apart from high grain moisture levels, insects, temperature and relative humidity fluctuations in the tropics have been attributed to accelerated and rapid multiplication of moulds and aflatoxin contamination (Yakubu, 2009). Insect metabolic activities results in increased relative humidity, providing favorable conditions for growth of *Aspergillus flavus* leading to reduced seed germination (Hell *et al.*, 2010).

2.3.1 Prevalence of aflatoxin in maize

Aflatoxin contamination of maize is unavoidable due to the varied factors in pre-harvest, harvesting, and post-harvest stages of maize. Aflatoxin contamination in maize can occur in the field before harvest, during harvesting, or after harvest, and can be affected by many factors (Beuchat, 2017). Pre-harvest factors that influence aflatoxin contamination are; maize cultivars, soil type, species of fungi, climate, weather conditions, agricultural practices, water activity and maturity of maize; the optimum harvest time and timely drying of maize etc. (Cotty and Jaime-Garcia, 2007). Aflatoxin producing fungi have very few nutritional, environmental and reproductive requirements thus their ability to survive and multiply (Wagacha and Muthomi, 2008). Food availability as well as income of maize farmers and traders suffers setbacks due to extensive losses in and marketability due insect damage (Likhayo *et al.*, 2016). In addition to direct damage, several studies have proven that insect activities result in qualitative losses such as aflatoxin contamination, discoloration, obnoxious

smell and bitter taste (Ng'ang'a, 2016; Mehmood *et al.*, 2018). Aflatoxin is a major food security threat especially in Africa; aflatoxin producing fungi have very few nutritional, environmental and reproductive requirements thus their ability to survive and multiply (Wagacha *et al.*, 2008). The major aflatoxin producing species in grains/cereals or crops are *Aspergillus flavus* and *A. parasiticus*. The main cereals affected by aflatoxins are maize, groundnuts, sorghum, rice, wheat and cassava (Khlanguiset, Shephard and Wu, 2011). Makueni County, a semi-arid area located in Eastern Kenya, is trending high in aflatoxin prevalence owing to the drastic climate changes it is known for. This comment from AGRA 2016 calls for interventions that will be sustainable in Maize production in this area and Kenya in general; erratic rainfall and weather patterns coupled with maize diseases have greatly affected the produce in Makueni as a result, maize yields have significantly dropped and therefore affecting farmers' income.

In 2004, aflatoxicosis outbreaks caused several hundred Kenyans to become severely ill, and 125 died of acute aflatoxicosis: a disease of liver failure associated with consuming extremely high levels of aflatoxin in food (Strosnider *et al.*, 2006a); (Shephard, 2008a); (Probst, Bandyopadhyay and Cotty, 2014). Moreover, during the 2004 to 2006 outbreak, more than 2.3 million bags of maize were incinerated due to aflatoxin contamination in Kenya (Atser, 2010). According to the studies made, FDA has set a tolerable maximum level of aflatoxins in human food as 20 ppb. Kenya Bureau of Standards (KEBS) has its aflatoxin maximum limit set at <10ppb (Table 5.1).

It has been estimated that over 5 billion of people in developing countries in the world especially in the tropics and sub tropics are at risk of chronic exposure to aflatoxins through contaminated foods due to the warm conditions (Shephard, 2008b; Strosnider *et al.*, 2006b); Donahaye and Navarro (2000). This has been known to cause or increase kwashiorkor in

African children, high prevalence of liver cirrhosis, and reduced rate of childhood growth (Strosnider *et al.*, 2006; Gong *et al.*, 2002; Gong *et al.*, 2003; Gong *et al.*, 2004; Jiang *et al.*, 2008; Khlangwiset *et al.*, 2011).

Aflatoxins are toxic metabolites produced by fungal species during their growth under favorable conditions of temperature and moisture. The major aflatoxin producing species are *Aspergillus flavus* and *Aspergillus parasiticus* (Nayak and Daglish, 2018; Hubert *et al.*, 2018). The main cereals affected are maize, sorghum, rice and wheat and other crops like groundnuts and cassava. *Aspergillus* species generate four significant aflatoxins: B1, B2, G1 and G2. "B" and "G" refers to the blue and green fluorescent colors produced under UV light on thin chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Khlangwiset, Shephard and Wu, 2011). Aflatoxin B1 is the most potent carcinogenic compared with the other aflatoxins, and it has been classified as a Group 1 carcinogen (Ainiza, Jinap and Sanny, 2015). The hierarchy of toxicity are in the order of B1>G1> B2>G2 (Suleiman *et al.*, 2013). In milk, aflatoxin appears as aflatoxin M1, which is its metabolite (Akande *et al.*, 2006). While these toxins do not seem to have physiological functions for the fungus they are now recognized as potential carcinogens, teratogens, mutagens, immune-suppressants and have oestrogenic effects in humans. . The various toxic repercussions and good thermal stability make the existence of aflatoxins on food and feeds potentially hazardous to the health of both humans and animals (Waliyar *et al.*, 2014); (Mauro *et al.*, 2018). Aflatoxins in humans or animals are characterized as food or feed related, non-contagious, non-transferrable, non-infectious, and non-traceable to microorganisms other than fungi. The Kenyan tragedy speaks volume of the magnitude of aflatoxin contamination in Africa (Atser, 2010). In Uganda, (Kaaya and Kyamuhangire, 2006) reported of higher levels of aflatoxins in the moist regions of the country than in the dry regions.

Aflatoxin levels of about 30 times higher than the legal limits (10 ppb) have been reported in peanut butter given to school children in Eastern Cape, South Africa (MERCK, 2006).

Two areas in Eastern Kenya considered high prevalence of aflatoxins (from Mbeere to Embu and in Makueni) and one low-risk region in South western Kenya (Homa Bay, Rongo and Kisii; with maximum levels of 3,479 ppb in Makueni, 3,442 ppb in Kisii, 255 ppb in Mbeere and only 21ppb in Embu (IFPRI, 2010).

The World Health Organization (WHO) categorizes aflatoxin as class 1 carcinogens (Martinez *et al.*, 2011) and it is associated with stunting in children, immune suppression, micronutrient deficiencies, and higher prevalence of cancers in sub-Saharan Africa, East Asia, and China (Smith Shephard 2003; (Strosnider *et al.*, 2006b; Alim *et al.*, 2016) . It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Strosnider *et al.*, 2006a); (Shephard, 2008a). Aflatoxin exposure has been implicated as a causal or aggravating factor in kwashiorkor in African children and higher prevalence of hepatocellular cancer in Africa and also in acute and chronic aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of the immune system and impaired childhood growth (Gong *et al.*, 2004); (Strosnider *et al.*, 2006a); (Khlanguiswet, Shephard and Wu, 2011).

2.3.2 Prevalence of fumonisin in maize

Fusarium moniliforme occurs worldwide on corn intended for human and animal consumption (Wagacha and Muthomi, 2008). A closely related species *Fusarium proliferatum* also occurs frequently on corn; yellow dent corn, white dent corn, white and yellow popcorn and sweet corn may be contaminated (Kimanya *et al.*, 2008). Both organisms are capable of producing a group of toxins known as fumonisins, of which fumonisin B₁ (FB₁), fumonisin B₂ (FB₂) and fumonisin B₃ (FB₃) are most common. Fumonisin may be

found in sound whole kernel corn at levels at or below 1.0 µg/g (Kimanya *et al.*, 2014). By contrast animal disease problems begin to occur at fumonisin levels above 5.0 to 10.0 µg/g. Corn-based food products that have the most frequent and highest fumonisin levels, besides whole kernels, are corn meal and corn grits. Popcorn, sweet corn and hominy corn have been found contaminated with sporadic, low levels (0.01 to 0.08 µg/g) of fumonisins. *Fusarium* grain mold is often, but not always, characterized by white streaks under the cap of the kernel. Infected kernels usually are scattered across the ear; however, colonized kernels do not always show evidence of the mold but not all colonized kernels will have fumonisin. Colonized kernels with no visible symptoms of the mold may contain fumonisin (Santiago, Cao and Butrón, 2015). Occurrence of *Fusarium* spp. in Kenya also threatens the productivity of maize (Maina *et al.*, 2009). *Fusarium verticillioides* that causes ear rot in maize is a major contributor to maize yield decline (Maina *et al.*, 2009) in Kenya. In addition to lowering maize quality, *Fusarium* species on maize produce trichothecene, zearalenone and fumonisin toxins which cause severe devastating effects to human and animal health (Logrieco *et al.*, 2002).

2.3.3 Control measures of mycotoxin contamination in maize grain

2.3.3.1 Biological control

Biological control methods have been explored as an alternative to decontamination of aflatoxin. Biocontrol is one of the solutions that are effective in the soil, where aflatoxin contamination begins and carries through the value chain through storage and consumption (Cotty, and Mellon, 2006). The beneficial native atoxigenic strains of the fungus multiply and dominate over the bad aflatoxin producing strains in the soil, making the positive effects on crops last for several seasons. Numerous organisms according to Yan *et al.*, (2008) have been tested for biological control of aflatoxin including bacteria, yeasts, toxigenic strains of causal organisms. According to Lopez-Garcia *et al.*, (1999) the efficacy of biological control

methods usually depends on specific compounds produced by the selected organism. For instance, *aspergillus flavus* are known to degrade aflatoxins, probably through fungal peroxidases (Lopez-Garcia *et al.*, 1999). Researches by the International Institute for Tropical Agriculture (IITA) discovered a less toxigenic strain of *aspergillus flavus* which grows on grain stored under humid conditions which can displace virulent strains capable of causing considerable amount of toxins (IITA, 2003). Also, field application of non-toxigenic strains of *aspergillus flavus* and *A. parasiticus* can drastically minimize postharvest aflatoxin contamination by 95.9% (Dorner and Cole, 2002). Atoxigenic strains of *A. flavus* from Nigeria have been combined as a bio-control product and registered as AflaSafe that is hugely reducing aflatoxin levels in maize and groundnut in Africa. Stored products had 2408 ppb in an untreated sample while AflaSafe treated samples had 105 ppb which represent a 96% reduction in aflatoxin levels. Fungal strains of *Trichoderma* spp according Benitez *et al.*, (2004) have demonstrated to be pathogenic fungi through mechanisms such as competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, myco-parasitism, bio-fertilization and stimulation of plant defense mechanisms (Alakonya and Monda, 2001). Although corn hybrids are reported to vary in susceptibility to *Fusarium* species, susceptibility to grain mold and fumonisin contamination are not characteristics listed in the seed catalogs.

2.3.3.2 Planting resistant maize varieties

Maize genotypes with aflatoxin resistance have been identified in West and Central Africa, (Brown *et al.*, 2001) and their sources of resistance are being used in a breeding programme to develop aflatoxin resistant, high-yielding cultivars adapted to tropical Africa (Menkir 2008). Tropical maize germ plasm with resistance to Aflatoxin has been registered and these are among the varieties that have been distributed to National programmes for the development of locally adopted hybrid (Menkir *et al.*, 2008). In Diourbel (Senegal), peanuts

treated with AflaSafe had aflatoxin level of 1.9 ng/g while control had 29.7 ng/g giving a reduction in aflatoxin level of 93%. Due to good performance of atoxigenic strains, peanut producers in Senegal and Gambia are willing to adopt competitive exclusion technology for aflatoxin control in peanuts (Alakonya and Monda, 2001).

2.3.3.3 Chemical use

Some mycotoxins such as aflatoxins B1 and B2 can also be destroyed chemically with calcium hydroxide, monoethylamine (Hell *et al.*, 2008). Fungicides such as intraconazole and amphotericin B have also proven to be effective against *aspergillus* spp (Ni and Streett, 2005). Chemical compounds tested on feeds such as propionic acids, sodium propionate, benzoic acid and ammonia were found to be effective against anti-fungal compounds followed by urea and citric acids (Gowda *et al.*, 2004). Aflatoxins reduced from 93 ppb to 9 ppb during a study by (Méndez-Albores *et al.*, 2004) to establish the fate of aflatoxins B1 and B2 employing nixtamalization process of tortilla production. The process involve separating the hull from the kernel and the hull subjected to alkaline heating and later mixing the treated hull with the untreated endoplasm-germ fraction (Ramírez-Jiménez *et al.*, 2019).

2.3.3.4 Hermetic storage technology

Hermetic bags provide modified atmospheric conditions that suppress the growth of moulds and insect activities; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects and mycotoxin contamination in stored maize (Williams *et al.*, 2014). However, grain with high moisture content stored over long months in hermetic bags is likely to produce and increase the moisture levels leading to mould production hence mycotoxin infection. Some findings reported that under hermetic storage, fungal static effect is included when oxygen concentration drops to 1% or below (Tubbs *et al.*, 2016); Yeole *et al.*, 2018). It has also been identified that mycotoxigenic fungi can be

produced in maize of 13-25.1% moisture content stored in hermetic systems with the considerable risk of contamination with aflatoxin and fumonisins (Castellari *et al.*, 2010). Also, studies on PICS bags have shown that maize stored at moisture content of 10-13.5% have minimal levels of aflatoxin (Nganga *et al.*, 2016). In as much as the trend is changing, the previous storage pest management initiative was aimed at the use of synthetic pesticides (Mvumi and Stathers, 2003; Collins, 2006). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues, aflatoxin and insect contamination (Weinberg *et al.*, 2008). Hermetic bags provide modified atmospheric conditions that suppress the growth of moulds; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects and mycotoxin contamination in stored maize (Williams *et al.*, 2014).

Report on “Missing Food” in 2011 by World Bank indicate that there is a substantial lack of adoption of modified grain storage systems in Africa (Villane *et al.*, 2012). There are many factors that contribute to low adoption including lack of information on the existing storage systems and their effectiveness. Hence, of late more importance has been specified by CIMMYT on hermetic storage. Outside Africa the effectiveness of hermetic storage at both small and commercial scales has been well researched and documented (Quezada *et al.*, 2006).

Metal silo research has gained more prominence in recent years in regard to hermetic storage as an option for grain storage method in Africa. (Tefera *et al.*, 2011; Murdock *et al.*, 2012; Baoua *et al.*, 2013; de Groote *et al.*, 2013; Guenha *et al.*, 2014). Improved storage technologies at both household and national levels which reduce losses by preventing insect pest attack are important component of food security. Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal/plastic silo provides affordable

and more effective storage alternative for farmers, especially the vulnerable women, that would markedly contribute to food security (Gitonga *et al.*, 2013); (Obeng-Ofori, 2011a); (Ndegwa *et al.*, 2016) (Mutambuki *et al.*, 2012).

To move forward in mitigating post-harvest losses in maize from mycotoxin contamination and insect infestation, it will demand the detection and eradication of the limitations to the usage of a particular technology. Consequently, safe on-farm storage of maize is vital in improving food and income security for the smallholder farmers (Maria, 2011). Elevated concentrations of carbon dioxide of >75% inside the hermetic bags are essential in prevention of growth of mycotoxigenic fungi in relatively dried maize.

2.3.3.5 Education and extension services to maize farmers

The problem posed to the health and economy by mycotoxins is not known to a larger percentage of the populace including even the educated ones. It is therefore necessary that the national agency in each country responsible for food safety, should embrace the task of creating awareness in the populace about the need to consume pathogen-free or good quality food. Private non-governmental organizations could also be roped in to spread information especially to the most remote villages. There should be regular programmes on radio and television on mycotoxin hazards and discussion on the issue should also feature regularly in daily newspapers and magazines. Appropriately, the cradle of the education should be the farmers or producers, whom the extension staff of the Ministry of Food and Agriculture (MOFA), should educate on the need to adopt Good Agricultural Practices (GAP) to produce food free of hazards. Hazard Analysis Critical Control Point (HACCP), a food safety control system based on a systematic identification and assessment of hazards in food and the identification of their control is useful in this situation. In an ideal HACCP-based system mycotoxin would be minimized at every phase of production, harvesting, processing and distribution (Kang'ethe, 2011).

2.3.3.6 Mechanical methods use to manage mycotoxin contamination

Rapid drying of agricultural products to low moisture content is often emphasized, because all scenarios leading to mycotoxin contamination relate to non-maintenance of stored products at safe moisture content. Drying maize to 15.5% moisture content or lower within 24 to 48 hours of harvest will reduce the risk of fungus growth and consequent aflatoxin production. Hamilton, 2000 found that if shelled grain was immediately sundried the likelihood of contamination of maize grain was reduced significantly. In Africa, most farmers sun-dry their harvests which often require longer durations for the product to attain 'safe' moisture level especially in times of cloudy weather. The grains are spread out on polyethylene sheets spread on the floor, and the stirring or turning is done manually till the product is dry. Due to the high rainfall at the time of harvest, farmers take some steps such as stacking the products to shield it from rain, drying grains over the fire and mixing of moist and dry grains (Frimpong, 2016). Since sun drying may be a difficult task due to the high rainfall at the time of harvest (Opit *et al.*, 2014), a lot of work has been done on the design of solar and mechanical dryers for use by farmers in the tropics (Pachpor and Lad, 2018). Mechanical dryers could be set up in strategic locations, which farmers can utilize if sun drying is proven difficult (Singh, 2015). However, these dryers are not in use by farmers because large capital investment is involved (Baral and Hoffmann, 2018).

2.4 Gaps in knowledge

Hermetic storage containers utilization is still low in SSA because of the knowledge gaps about their performance yet high in the conventional storage methods of using insecticides. There are many studies about the effectiveness against post-harvest pests in hermetic technologies in SSA but little information exist on the mould and mycotoxin infestation with regard to hermetic technologies. In addition, studies have been done only for the short term hermetic storage and have not compared the performance of hermetic technologies with the

conventional synthetic pesticides and their effectiveness against mould proliferation and insect infestation (Baoua *et al.*, 2013; de Groote *et al.*, 2013; Ognakossan *et al.*, 2013; Baoua *et al.*, 2014). In as much as the trend is changing, the previous storage pest management initiatives were aimed at the use of synthetic pesticides (Mvumi and Stathers, 2003; Collins, 2006). Studies by FAO and University of Nairobi Project have indicated that most farmers lack knowledge on food safety issues around mycotoxins, proper harvest and post-harvest practices to manage mycotoxin production (Kang'ethe, 2011).

2.5 Storage technologies for maize storage

Metal silo was a 90kg standard design cylindrical, galvanized metal sheet (gauge number 24), with a centered filling inlet and a lateral outlet; fabricated by the local tinsmith (Bravo 2009) and sealed hermetically with rubber cork (Tefera *et al.* 2011; Likhayo *et al.*, 2016). Super grain bag is a bag made of tougher than the traditional polyethylene (PE) inner liner, 78 mm thick (Villers *et al.* 2008, Garcia-Lara *et al.* 2013). The users have to buy the polypropylene woven bags separately from the hermetic inner liners (Likhayo *et al.*, 2016). Super Grain IV-R comes in storage capacities of 25kg, 50kg, 90kg and 100kg with the thickness tolerance of ± 0.002 . Agro-Z bag is a multi-layer hermetic storage bag developed by A to Z Textile Mills Ltd. through its R&D wing, the Africa Technical Research Centre (ATRC). Agro-Z Bag and it comes in standard size of 80 cm x 130 cm which can store 100 kg of maize and Agro-Z bag is composed of two distinct bags; One polypropylene (PP) outer bag and a multi-layer inner liner (H Coffi *et al.*, 2016). Agro Z+ bag is a treated hermetic storage bag specifically designed to control insect borers such as LGB. Similar to non-impregnated bag, AgroZ® Bag Plus is composed of two distinct bags with the central layer incorporated with a repellent insecticide (alpha-cypermethrin) sandwiched between two barrier layers which should prevent the migration of the insecticide either to inner surface or to the grain within the bag

or to the outside surface of the bag (Mwaijande, 2017). PICS bags were developed by Purdue University and is a double layer hermetic storage bag which has an outer woven polypropylene bag and two inner bags - 80 microns thick each - of high-density polyethylene (HDPE) bags (Murdock *et al.*, 2003). Initially these bags were of 50 kg capacities which were later increased to 100 kg capacity on farmers demand in west and central Africa (Baoua *et al* 2013). Elite grain storage bags are also developed by Purdue University of 50kg capacity bag comes with a multi-layered liner and is mainly used to store legumes while its 109kg bag comes with two liners and is meant to store grains. Standard propylene bags dusted with Actellic Super is effective in controlling both insect species for up to four months; Actellic super is a cocktail of 1.6% Pirimiphos-methyl and 0.3% Permethrin (Sekyembe *et al.*, Undated). Zerofly® storage bag is manufactured by Vestergaard (vestergaard.com), a Swiss-based global company in Switzerland (Christopher J. Smith, 2016). The bag is made with pyrethroid incorporated into woven polypropylene yarns.

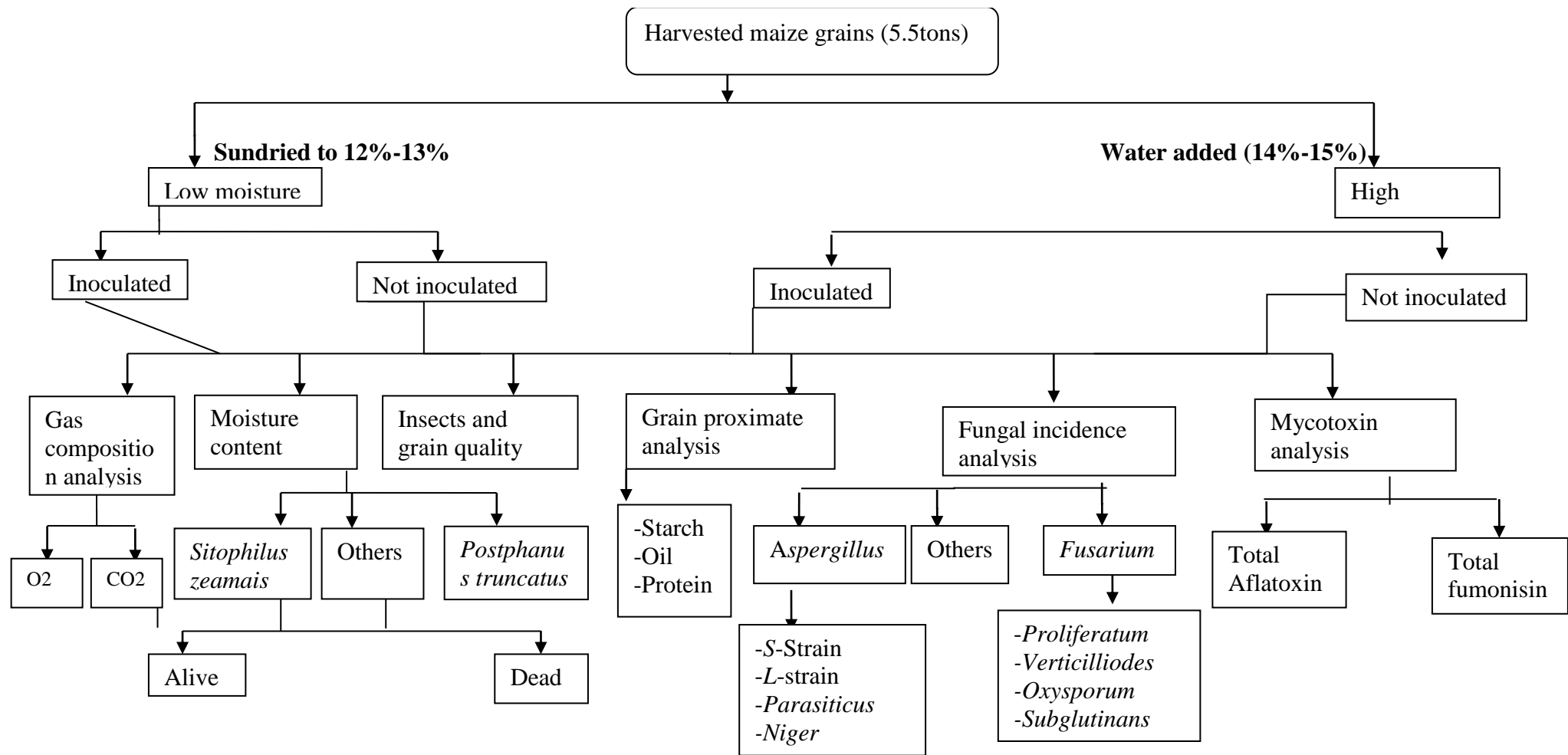


Figure 2.1: Experimental design of the study

Source: Self

CHAPTER THREE: EVALUATION OF HERMETIC TECHNOLOGIES IN THE CONTROL OF INSECT INFESTATION OF STORED MAIZE GRAINS IN KENYA.

3.0 ABSTRACT

Grain losses due to insects infestation during on-farm storage increases food insecurity which results in huge economic losses. The losses also negatively affect farmers' livelihoods, and increases exposure to mycotoxins that negatively affect human and animal health. Several studies have reported the effectiveness of hermetic technologies against post-harvest insects in Africa but provide limited evidence on the comparative effectiveness of over eight storage technologies against two controls and under varying moisture levels. This study evaluated the effectiveness of selected hermetic technologies in the control of insect infestation in maize grains during eight months period. Maize used for this study was collected from the farmers in Nakuru County at harvest and two factors were investigated: two levels of grain moisture levels and ten storage methods, of which eight were hermetic as mentioned in the main abstract. The overall study design was a 2 x 10 completely randomized block design (RCBD) with 3 replications. Each technology was analyzed for gas composition and grain analyzed for moisture content and insect infestation at 0, 4 and 8 months. Hermetic technologies were superior over farmer practice in all the factors tested in varying degree in reducing insect infestation. A-Z and A-Z+ bags were the most optimum technologies in managing both *Sitophilus zeamais* and *Prostephanus truncatus* insects infestation both at four and eight months of storage compared with the two controls which registered an increase of 33.4% of *P.truncatus*. There was an increase in moisture values by 1% among the hermetic technologies and a reduction by 4.3% in the control bags. The hermetic technologies were able to modify gas composition, increasing CO₂ by 12.98% and dropping O₂ by 11.77%. The study indicated that hermetic bags are superior at (P<0.05) in the management of both *Sitophilus zeamais* and *Prostephanus truncatus* insect infestation compared with the silos and

the conventional storage practices. The use of hermetic bags is an economically reasonable option for farmers notwithstanding the importance of adequate grain drying.

3.1 Introduction

Maize (*Zea mays* L.) can conveniently be classified as the most important cereal crop in the world owing to its nutritional value and utilization of its by-products (Chilaka *et al.*, 2012). If the grains are not adequately stored, the losses due to insect infestation results in increased cases of malnutrition and reduced revenue especially among the rural farmers leading to abject poverty (Obeng-Ofori, 2011a). Damages caused by insect pests represent a huge setback in the world 's effort to achieve food security globally. According to (Ileleji, Maier and Woloshuk, 2007) and (Nukenine, 2010) an estimated 1 % to 5 % of stored grain in developed countries and 20 % to 50 % of stored grain in developing countries are lost due to insect damage. Cracked or broken grains provide an entry point for infestation by insects and moulds during storage (Mbata, Ivey and Shapiro-Ilan, 2018; Papanikolaou *et al.*, 2018). Variation in temperature and humidity has been identified to support the metamorphosis of *P. Truncatus* (Papanikolaou *et al.*, 2018). They lay eggs which hatch in about three days at 27°C day temperature and the dust provide the nourishment to the larvae. Larva development to adult stage takes place within 27 days and is facilitated by ideal conditions of 32°C, 80% relative humidity and grain moisture content of 13 % (Chigoverah and Mvumi, 2018). Maize weevil, *Sitophilus zeamais*, is one of the broad-based pests of stored cereals, especially maize (Demissie, Tefera and Tadesse, 2008). It damages stored maize and cob maize prior to harvest. It may also infest other cereals if the moisture content is moderate or high (Arena *et al.*, 2017). Eggs are laid at temperatures between 15 and 35 °C (with an optimum around 25 °C) and at grain moisture contents over 10% (Demissie, Tefera and Tadesse, 2008). Subsequent infestations in stores result from the transfer of infested grain into store or from

the pest flying into storage facilities, probably attracted by the odor of the stored grain. Dry weight loss from *S. zeamais* infestation alone is about 5% by weight after six months of storage. The 5% dry weight loss translates into 22% of total grains displaying damage (Holst, Meikle and Markham, 2000). As a start, it should always be recognized that an intact grain is an essential item for successful storing.

Several methods including chemical, biological and cultural methods have been explored but none is efficient and cost effective especially in the control of larger grain borer (De Groote *et al.*, 2013). Improved storage technologies at both household and national levels which reduce losses by preventing insect infestation are important components of food security. Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal and plastic silos provide affordable and more effective storage alternative for farmers, that would markedly contribute to food security (Ndegwa *et al.*, 2016). The data generated from this study will facilitate sustainable adoption of the hermetic technologies among smallholder farmers in Sub Saharan Africa. This study suggests the most efficient storage options for the small holder farmers considering the robustness and cost of the hermetic storage that will have been identified as effective and less expensive.

3.2 Materials and methods

The trial was conducted at Kiboko Research Centre shared by the International Maize and Wheat Improvement Centre (CIMMYT) and Kenya Agriculture Research and Livestock Organisation (KALRO) located in (Makueni County), 170km from Nairobi in a semi-arid region in Eastern Kenya. The trial site was selected for being a hotspot for aflatoxin outbreaks in Kenya. Kiboko provided the ideal environment (high temperatures and humid conditions) which facilitates insect infestation. The study was conducted under the conditions Kenyan farmers subject the maize grains after harvesting. Maize grain used for this trial was analysed

to have very minimal insect infestation. It is of importance to establish these varying environmental conditions in order to identify the limitations for a particular storage technology and therefore suitability for the 8 months storage period in the areas where they are most needed. Maize used for this study was collected from the farmers in Neissuit and Kigogo villages in Gilgil sub-county, Nakuru County (Figure.1) and data on maize production practices recorded using a semi-structured questionnaire. Two factors used in the design of this study included low (12-13%) and high (14-15%) grain moisture levels; and ten storage technologies. The hermetic storage technologies evaluated in the study were metal and plastic silos, while the hermetic bags were: Super Grain IV-RTM, AGRO-Z with pesticides and AGRO-Z without pesticides, PICS, Elite, and ZeroFly. The two controls were two farmer practices: the standard woven polypropylene bags, one with grain treated with insecticide and one without insecticide treatment. Each storage technology was filled with 90kg of maize grain and grouped into two sets, high and low moisture in three replicates each. The experimental design was a 2 x 2 x 10 randomized complete block design (RCBD). The duration of the experiment was 8 months with non-destructive sampling at baseline and every 120 days. Each grain sample was divided in two for insect pest and grain quality analysis.

3.2.1 Region where maize was collected

Nakuru County situated in the Rift Valley region is located between longitude 35° 28' and 35° 36' East and Latitude 0° 13' and 1° 10' south and covers an area of 7,495.1 m². The county borders: Kericho and Bomet to the west, Baringo and Laikipia to the north, Nyandarau to the east, Narok to the southwest and Kajiado and Kiambu to the south.

Nakuru county is classified under three broad climatic zones (II, III and IV) as influenced by altitude. Zone II covers high altitude between 1980 and 2700m above sea level that receives at least 1000mm rainfall per annum. Zone III are the mid-altitude between 900-1800m above

sea level where rainfall ranges between 950-1500mm per annum. Zone IV occurs within similar altitude as Zone III, however with not more than 1000mm rainfall per annum. The temperatures range from 29.3°C during the hottest months of December, January, February to as low as 12°C during the coldest months in June and July (Office of the Governor Nakuru County, 2013). Nakuru County inhabitants depend mainly on their small holder farming as their source of livelihood; maize being the main dietary staple crop. The decision to conduct the study in Nakuru was on the basis of the previous reports of low prevalence of Aflatoxin in the region (IFPRI, 2010).

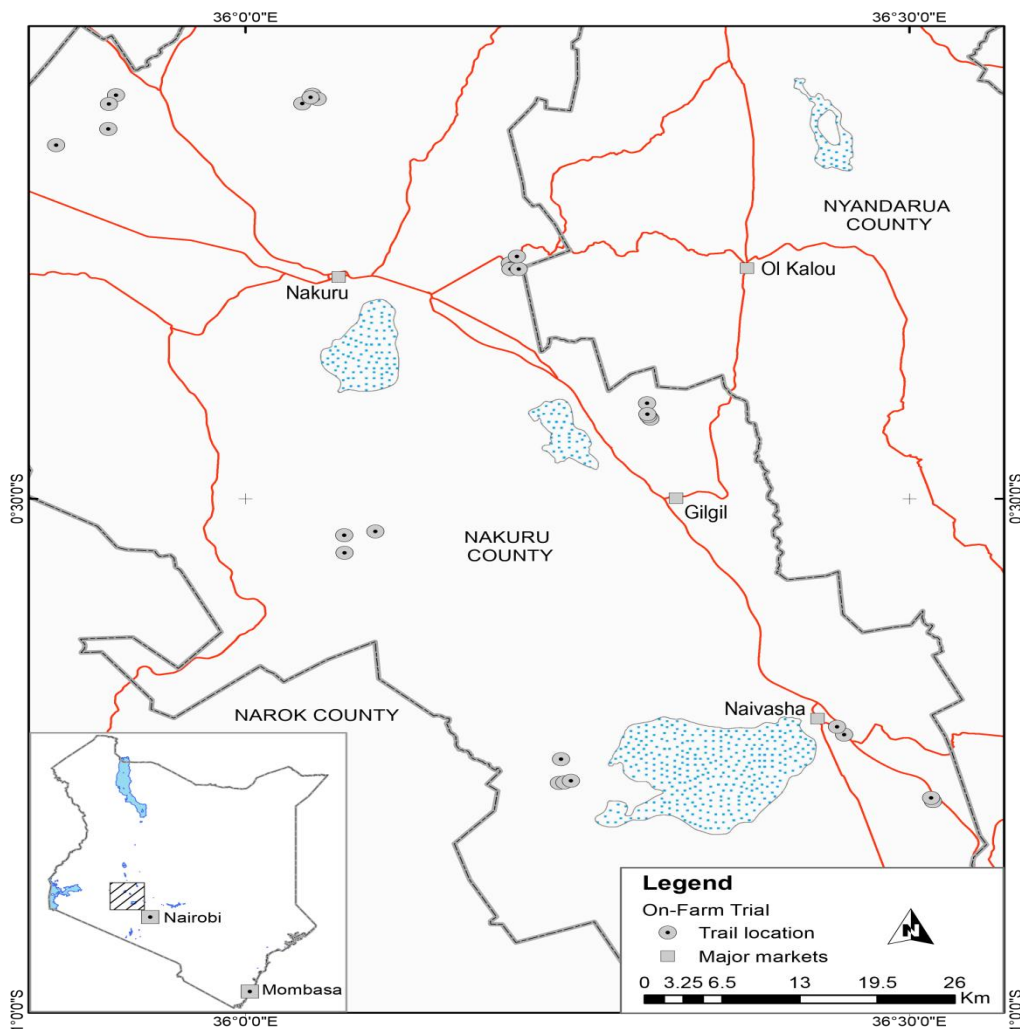


Figure 3.1: Map of the study regions in Nakuru County

3.2.2 Study Site

The trial was conducted at CIMMYT/KARLO Kiboko Research Centre (Makueni county), 170km from Nairobi located in Eastern Kenya with GPS coordinates 1° 48' 0" South, 37° 37' 0" East (Figure. 3.2). The trial site was selected for being a trouble spot for insect storage losses and Aflatoxin outbreaks in Kenya (Lewis *et al.*, 2005); (Mutambuki *et al.*, 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. in a semi-arid region in Eastern Kenya.

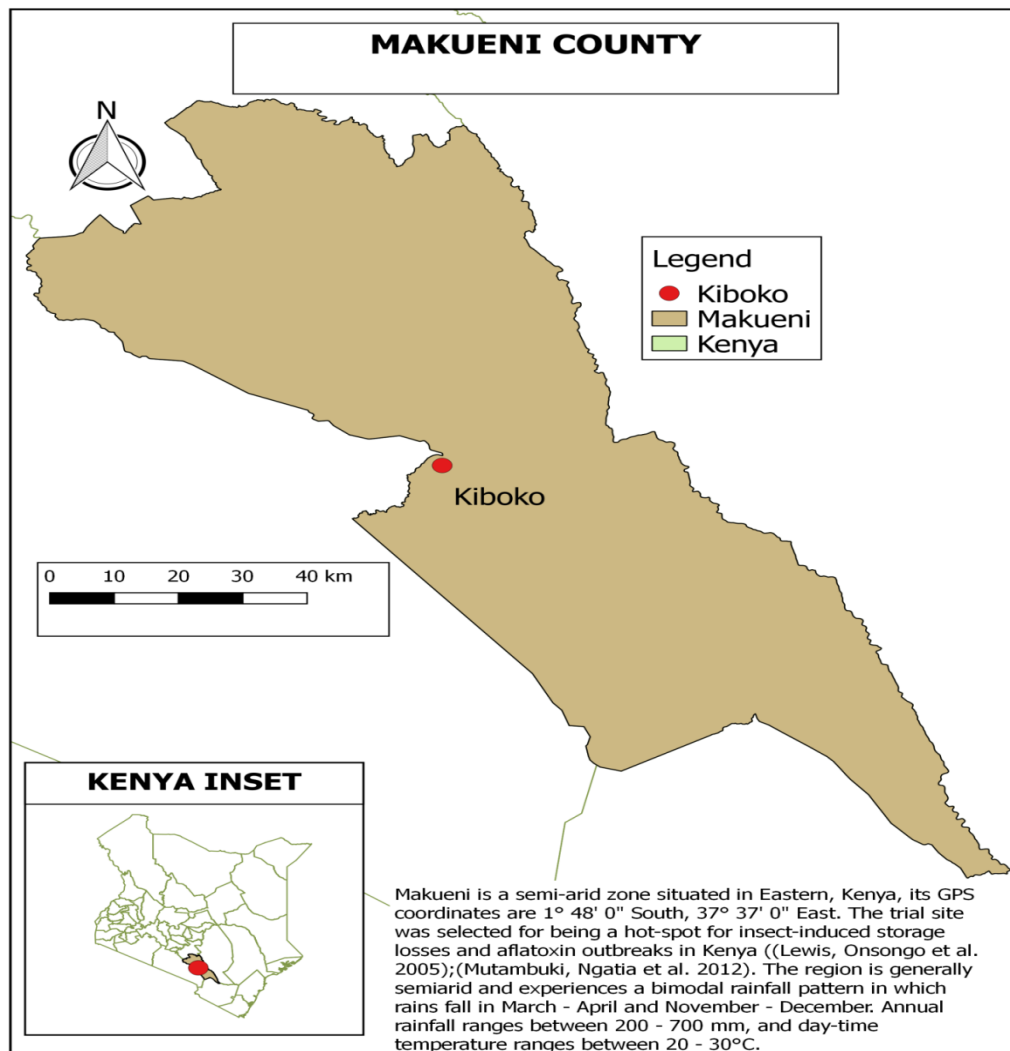


Figure 3.2: Map showing the study regions in Makueni County

3.2.3 Sample collection and preparation

About 5.5 tons of maize grain used for this study was of maize varieties H614 and H618 hybrid. The untreated grain was cleaned by sieving to remove chaff, broken and rotten kernels. At the onset of the experiment, the grain was mixed and conditioned at the appropriate moisture content before transferring in the respective study technologies.

About a Kilogram of maize grains was required for the analysis. Sampling was done from five different points, about 1 inch from the walls of the storage technology using a grain sampling spear. Sampling was done carefully not to puncture the linings of the bags and the spear cleaned with cotton wool dampened with 75% ethanol before sampling the next storage technology to avoid cross contamination. The sampled grain was transferred into a zip-lock plastic bag and sealed carefully to exclude air.

3.2.4 Grain moisture

The high moisture content (14-15%) was achieved by subjecting the grain to high relative humidity and tests were carried out progressively to determine the required moisture contents. The grain spread on plastic sheet was sprayed with potable water for 1.5 to 2 days required. The quantity of water sprayed on the grains was calculated from the formula below:

Quantity of water required(g) = weight of grain $\times \frac{mcf - mc}{100 - mc}$ Where: mcf is the final moisture content; and mc the initial moisture content (Kiburi *et al.*, 2014). In case of moisture levels above 13%, the grain was sun-dried for 3.5 hours to achieve the moisture range of 12-13%. Moisture content of the maize grains was determined by Dickey John digital multi Grain moisture tester M3G™ model (Dickey John Corporation, Minneapolis, USA).

3.2.5 Gas composition analysis

The air in the bags was pressed out to reduce the available oxygen and tightly tied with rubber bands; and the silos were sealed with both masking tapes and rubber bands shortly after placing burning candles inside the silos to get rid of the oxygen inside the silos. Before opening the storage technologies for the grain sampling, oxygen and carbon dioxide levels were measured. The gas composition was measured from each bag/silo using a portable Mocon Pac Check Model 325 oxygen carbon dioxide analyzer (MOCAN Inc, Minneapolis, USA), fitted with 20-gauge hypodermic needle. The metal and plastic silos were appropriately made to allow taking of the gas composition measurements. The metal silos are designed to have a small hole fitted with an elastic rubber cork, 5 cm from the neck of the metal silo (Kimani, 2016) while for the plastic silo, a red hot needle was used to puncture the upper section of the container.

3.2.6 Assessment of insects infestation

One kilogram of maize grain was analyzed for dead and live insects before bagging and at every 120 days. This was done to investigate whether the storage technologies are able to prevent entry of insects/encourage insects' activities. The number of live and dead insects, both adult *S. zeamais* and *P. truncatus* were counted and recorded (Boxall, 1986) method.

3.3 Statistical analysis

Variances of insect count, (x) was stabilized by log transformation $Y=\log(x+1)$ whereas percentage data (P) was arcsine $Y=\sin^{-1}\sqrt{P}$, transformed, where Y is the result of transformation. The transformed data was then be subjected to analysis of variance (ANOVA) using Stata SE version 12 (Stata Corp LP, Texas, USA). Further due to inherent limitations of ANOVA in describing differences in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression were

applied to test effects of treatment and storage duration, and the interaction effects. Means were separated using Bonferroni adjustment at 95% confidence level (Ognakossan *et al.*, 2013a).

3.4 Results

3.4.1 Gas composition

The gas composition over the eight months of storage in all the storage technologies varied. At the beginning of the trial, the concentrations of both oxygen and carbon dioxide were measured immediately after closing the storage technologies and the respective means were 15.55 % and 1.54 %.

3.4.1.1 Oxygen composition

At 4 months, the oxygen concentration reduced drastically to 4.3 % and the storage technology with minimum oxygen composition was SGB (1.7 %) and maximum value in Agro-Z+ (8.78 %). At 8 months, the oxygen concentrations slightly dropped to 4.1 % with minimum value in Zerofly (1.15 %) and maximum of 6.86 % in Plastic silo (Table 3.2).

Table 3. 1: Percentage oxygen levels in different hermetic storage systems over eight months of maize grain storage

Moisture	Low			High		
	Oxygen					
Technology	0 month	4 months	8 months	0 month	4 months	8 months
Metal silo	15.89 ± 0.20	4.70 ± 0.20	5.91 ± 0.90	15.79 ± 0.22	2.69 ± 0.59	1.66 ± 0.74
Plastic silo	18.20 ± 0.09	5.93 ± 0.59	6.88 ± 0.10	17.38 ± 0.34	4.82 ± 0.20	2.19 ± 0.75
Agro Z	16.91 ± 1.64	6.82 ± 0.39	6.37 ± .77	14.84 ± 0.72	5.08 ± 0.73	2.96 ± 0.47
Agro Z imp	17.01 ± 1.62	6.92 ± 0.60	6.54 ± 0.51	15.90 ± 0.94	2.89 ± 0.63	2.90 ± 0.48
Elite	15.31 ± 1.32	3.72 ± 0.81	5.49 ± 0.67	13.59 ± 1.04	2.52 ± 0.81	1.63 ± 0.45
PICS	15.45 ± 1.92	3.25 ± 0.81	3.86 ± 0.75	14.58 ± 1.41	3.64 ± 0.05	2.56 ± 0.82
Super grain	15.22 ± 1.86	5.52 ± 0.27	6.63 ± 0.79	16.15 ± 1.17	1.95 ± 0.53	3.80 ± 1.11
Zerofly	16.78 ± 1.69	5.03 ± 0.65	6.36 ± 0.17	16.14 ± 0.76	3.21 ± 0.16	1.56 ± 0.06

3.4.1.2 Carbon dioxide Composition

The carbon dioxide concentration at four months increased to a mean value of 14.72 % from 1.54 % with minimum composition measured in Elite bag (8.11 %) and maximum in Agro-Z bag (21.62 %). On the other hand, the carbon dioxide mean value at eight months was 14.52 % and the minimum measured in PICS bag (10.74 %) and maximum in Zerofly (21.95 %). Generally, the carbon dioxide increases stabilized at 14.6 %. ANCOVA results indicated that the interaction between storage technologies and duration of the storage was significant for carbon dioxide at $P=0.021$ at 0 month, $P<0.01$ at 4 months, and $P=0.028$ at eight months however, at 8 months there was no significant interaction ($P, 0.797$) but significant at 0 and 4 months ($P, 0.048$ and 0.011 respectively) (Table 3.3).

Table 3. 2: Percentage carbon dioxide levels in different hermetic storage systems over eight months of maize grain storage.

Moisture	Low			High		
	Carbon dioxide					
Treatment	0 month	4 months	8 months	0 month	4 months	8 months
Metal silo	2.80 ± 0.16	14.54 ± 1.15	13.58 ± 0.77	2.91 ± 0.18	17.04 ± 0.95	17.59 ± 0.85
Plastic silo	1.99 ± 0.05	12.32 ± 1.29	11.97 ± 1.07	2.59 ± 0.02	13.59 ± 1.81	16.57 ± 0.93
Agro Z	0.23 ± 0.04	15.32 ± 1.80	12.52 ± 1.09	1.54 ± 0.06	21.91 ± 2.34	19.03 ± 1.08
Agro Z imp	0.49 ± 0.02	15.44 ± 1.77	13.09 ± 1.67	1.05 ± 0.04	21.31 ± 1.74	16.37 ± 1.09
Elite	1.12 ± 0.06	11.13 ± 1.37	9.92 ± 0.30	1.47 ± 0.06	17.74 ± 1.86	12.43 ± 0.51
PICS	0.47 ± 0.01	9.50 ± 0.20	10.98 ± 1.51	1.54 ± 0.83	9.11 ± 0.03	14.34 ± 1.22
Super grain	1.52 ± 0.06	14.95 ± 1.61	12.24 ± 0.94	3.38 ± 0.69	16.81 ± 1.93	15.34 ± 1.41
Zerofly	0.25 ± 0.08	15.57 ± 1.22	8.29 ± 0.04	1.15 ± 0.44	17.04 ± 1.17	21.00 ± 1.15

3.4.2 Grain moisture levels

Initial moisture content of maize grain was found to be between 9.8 to 17 % and most of it having higher than 13 % moisture content indicating that farmers choose not to adequately dry the grain to the required storage moisture levels. This exposes the maize grain to insect infestation and fungal contamination. Moisture content of maize grains stored in hermetic bags did not significantly change over the month's storage period ($P>0.05$) however, grain moisture was significantly lost in both the silos and woven storage bags. At the time of the trial the area temperatures were well over 30°C and the maize grain stored in woven bags lost a considerable portion of moisture (10-22 %) due to evaporation. After conditioning, the dry grain moisture levels ranged between 12-13 % and 14-15 % shortly before bagging. For moisture content, results indicated that there was a significant ($P<0.001$) interaction of treatment and storage period. At four and eight months of storage, moisture content significantly ($P<0.05$) increased in both the grain with low and high moisture content in hermetic storage technologies by 6.7 % except for woven bags where there was a drop by 2.3 % (Figure.3.3). The overall moisture content in the woven bags was between 10.75% and 12.45% from the baseline range of 12 % to 15 %. This moisture drop was possibly due to the permeability of the bags and the insects' activities.

On the other hand, the moisture levels of the grain remained stable in the hermetic technologies but drastically dropped in the woven bags (Figure 3.3).

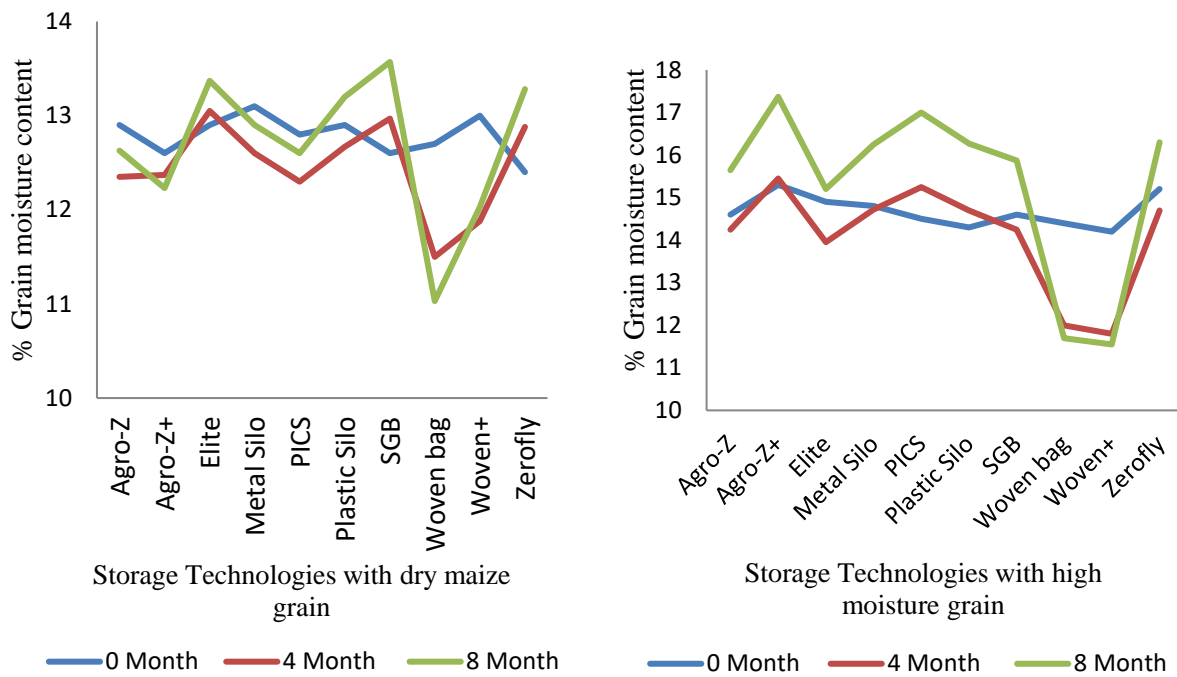


Figure 3.3. Percentage moisture levels of maize grains in the 10 storage technologies at the time of bagging and every 120 days.

3.4.3 Insect infestation

The number of dead insects was linked with the type of storage at $P < 0.05$. Grains stored in hermetic bags had less infestation by insects than the farmer practice storage technologies. At high relative humidity, insect infestation was significantly high in grains with moisture content greater than 13% and lower in the dry grains regardless of the mode of inoculation. There was a positive correlation between the total insects infestation and the type of storage technology (treatment), at $P = 0.109$. Mode of inoculation and Relative Humidity (RH) did not have significant effect on the insect infestation in the eight months storage period (Table. 3.3)

Table 3.3: Efficacy of hermetic storage, inoculation and Relative Humidity (RH) on insect infestation in maize grain stored for 4 months.

Insects	Factors	P- Value	Corrected p-value	Significance
	Inoculation	0.196	0.169	n.s.
	RH	0.492	0.048	n.s.
	Treatment	0.109	0.26	Sig

There is a significant difference at a corrected p-value greater than the p-value. Not significant is denoted by (ns) and significant (Sig).

3.4.3.1 Infestation of maize grain with *Prostephanus truncatus*

At the onset of the experiment there was no record of *P. truncatus* in the grain, however, infestation was significantly high in the non-inoculated and lower in the inoculated grains at ($P < 0.001$) during the fourth months of storage in the woven bags. Moreover, at eight months infestation was higher in the grains inoculated with the fusarium and aspergillus fungi (Table 3.4). Both the woven bags with pesticides and hermetic technologies were able to prevent the invasion of *P. truncatus* to a certain measure with the exception of woven bags where there was an increase in the infestation by 33.4% of the live insects (Table 3.4). The ability of the technology to desiccate the insects varied across the hermetic technologies; the plastic silo was heavily infested but also was able to desiccate the insects, yet the ratio of dead to live insects in Zerofly hermetic bag was 1:1 at four months and increased to 1:3.33 at eight months. This indicates that conditions within the bag were not terminally injurious to the *P. truncatus* to cause death and therefore this bag may not be substantially effective to store grains for more than eight months.

Table 3.4: Population of adult *P. truncatus* in maize grain stored analyzed at 0, 4 and 8 months of storage comparing.

*P. truncatus	0 Month		4 Month				8 Month			
			Low moisture		High moisture		Low moisture		High moisture	
Technologies	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Metal silo	0.0 ^a	0.0 ^a	0.00 ^a	3.00 ^{ab}	0.00 ^a	0.00 ^a	0.00 ^a	2.00 ^{ab}	0.00 ^a	0.00 ^a
Plastic silo	0.0 ^a	0.0 ^a	3.00 ^b	4.00 ^b	0.00 ^a	1.00 ^{ab}	2.00 ^b	2.67 ^{ab}	0.83 ^a	0.67 ^a
PICS	0.0 ^a	0.0 ^a	0.00 ^a	2.00 ^{ab}	0.00 ^a	0.50 ^a	0.00 ^a	9.33 ^c	0.00 ^a	1.33 ^a
Super grain	0.0 ^a	0.0 ^a	1.00 ^{ab}	2.00 ^{ab}	0.00 ^a	0.50 ^a	0.67 ^a	1.66 ^{ab}	0.00 ^a	1.66 ^a
Agro-Z	0.0 ^a	0.0 ^a	0.00 ^a	0.50 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.33 ^a	0.00 ^a	0.00 ^a
Agro-Z	0.0 ^a	0.0 ^a	0.00 ^a	0.50 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.33 ^a	0.00 ^a	0.00 ^a
Elite	0.0 ^a	0.0 ^a	1.00 ^{ab}	2.00 ^{ab}	0.00 ^a	0.00 ^a	0.67 ^a	1.33 ^{ab}	0.00 ^a	0.00 ^a
Zerofly	0.0 ^a	0.0 ^a	1.00 ^{ab}	0.50 ^a	0.00 ^a	0.50 ^a	1.00 ^a	0.00 ^a	0.00 ^a	0.33 ^a
SWP	0.0 ^a	0.0 ^a	27.50 ^c	10.50 ^c	10.50 ^b	2.50 ^b	41.34 ^c	24.10 ^d	9.34 ^b	3.34 ^b
SWP	0.0 ^a	0.0 ^a	0.00 ^a	0.00 ^a	0.00 ^a	1.00 ^{ab}	0.00 ^a	0.00 ^a	0.00 ^a	0.33 ^a

*Means with the same letters within columns are not significantly different at LSD 5 %.

* Numbers per 1000g sample

3.4.3.2 Infestation of maize grain with *Sitophilus zeamais*

Sitophilus zeamais infestation decreased with increasing months of storage in most of the hermetic technologies. Except for the Agro-Z and plastic silo where the infestation increased as storage time increased. The reduction was more than 50% and also with the dead insects (Table 3.5). Among the hermetic technologies, Agro-Z bags were more efficient in managing the infestation of *S. zeamais* and plastic silos were not as effective in controlling the infestation compared with the hermetic bags and metal silos. The standard woven bags were comparatively effective in managing the insect infestation both at four and eight months of storage. Generally, the abundance of these insects varied across the storage technologies but lower in hermetic bags.

Table 3.5: Population of adult *S. zeamais* in maize grain analyzed at 0, 4 and 8 months of storage.

*S. zeamais	0 Month		4 Month storage				8 Month storage			
	Alive	Dead	Low moisture		High moisture		Low moisture		High moisture	
Technology	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Metal silo	0.125 ^a	0.75 ^a	4.5 ^b	23.0 ^e	0.0 ^a	16.0 ^{abc}	0.0 ^a	23.0 ^b	3.2 ^b	9.2 ^{ab}
Plastic silo	0.125 ^a	0.75 ^a	6.75 ^{bc}	12.25 ^c	12.75 ^c	42.0 ^d	2.5 ^a	17.75 ^{ab}	0.0 ^a	62.5 ^d
PICS	0.125 ^a	0.75 ^a	3.0 ^b	19.5 ^{cd}	1.5 ^a	25.25 ^{bc}	1.5 ^a	8.75 ^a	1.0 ^a	24.75 ^c
Super grain	0.125 ^a	0.75 ^a	13.5 ^c	23.0 ^e	1.0 ^a	16.0 ^{ab}	0.0 ^a	6.25 ^a	1.0 ^a	14.25 ^b
Agro-Z4	0.125 ^a	0.75 ^a	0.0 ^a	3.75 ^b	0.0 ^a	17.75 ^{abc}	1.75 ^a	7.0 ^a	1.25 ^a	5.25 ^a
Agro-Z+	0.125 ^a	0.75 ^a	0.0 ^a	0.5 ^a	0.25 ^a	2.25 ^a	0.5 ^a	8.75 ^a	0.5 ^a	14.25 ^b
Elite	0.125 ^a	0.75 ^a	0.25 ^a	13.75 ^c	1.5 ^a	10.0 ^{ab}	2.0 ^a	11.25 ^{ab}	0.75 ^a	3.5 ^a
Zerofly	0.125 ^a	0.75 ^a	0.0 ^a	15.25 ^{cd}	3.5 ^b	20.25 ^{bc}	0.0 ^a	16.0 ^{ab}	8.5 ^b	5.75 ^a
PP-	0.125 ^a	0.75 ^a	30.0 ^d	22.25.0 ^{de}	31.5 ^d	8.25 ^a	169.25 ^b	45.5 ^c	155.5 ^c	104.25 ^e
PP+	0.125 ^a	0.75 ^a	0.0 ^a	16.25 ^{cd}	0.25 ^a	7.5 ^a	0.0 ^a	20.25 ^b	0.0 ^a	7.75 ^a

*Means with the same letters within columns are not significantly different at LSD 5%. Baseline means of dead and alive *S.zeamais* were 0.75 and 0.125 respectively.

* numbers per 1kg sample.

3.5 Discussion

Hermetic storage technologies restrict gaseous exchange and act as a barrier hence reduced contamination. The failure for oxygen to drop further is attributed to the factors such as the extent of gas exchange restriction in a particular technology, the insect population, the extent of fungal proliferation, the quality and moisture levels of the grain at the time of storage (Quezada *et al.*, 2006). He also suggested that due to the low oxygen demand within the storage system, the grains essentially facilitate an environment where oxygen develops regardless of how airtight the storage technology. The variations indicate that there was a slight difference in the progression of both oxygen and carbon dioxide profiles in all the hermetic technologies as storage period increased. The oxygen depletion and carbon dioxide accumulation was evidently due to the activities of the insect infestation therefore environment modification was as a result of respiration and metabolism by the insects, fungi and the maize grain as reported by (Ognakossan *et al.*, 2013b).

In effect, hermetic bags seem to retain the grain moisture far better than the woven bags in the same environmental conditions. This evidence has been identified in a study conducted by (Baoua *et al.*, 2014) where moisture content in PICS bags was retained in maize grain stored under varying environmental conditions. The ability for the hermetic bags to retain grain moisture is attributed to the airtight/watertight double or triple inner HDPE lining inside the hermetic bags when tied tightly. The woven propylene bags do not have any certainty for restraining the movement of air and moisture and thereby open to ambient conditions creating equilibrium with the environment over time. The hermetic system creates a barrier from the external relative humidity preventing the moisture fluctuations evident in hot and humid places. This is one of the properties that make hermetic systems superior in the prevention of grain deterioration during storage unlike in the woven bags. However, the moisture content in

grain will remain relatively constant throughout the storage period and if high, the grain quality will deteriorate emphasizing the need to sufficiently dry the grain prior to storage (Quezada *et al.*, 2006). The moisture levels did not influence the insect infestation during the eight months storage period as this may be due to the design of the bag that is permeable and therefore allowed for an equilibrium between the relative humidity in the bag and that of the atmosphere. Insect infestation was higher under conventional storage systems with *S. zeamais* being the dominant species and *P. truncatus* with the least infestation. Similar results were reported in Benin, a trial conducted for 6.5 months (Baoua *et al.*, 2014). The prevalence of *P. truncatus* was primarily due to its ability to adapt in moisture conditions as low as of 9% (Obeng-Ofori, 2011b). The variations in relative humidity and temperatures are also known to support the growth and reproduction of this insect; temperatures of 32°C and relative humidity of over 80% have been particularly identified with larva to adult development (Zorya *et al.*, 2011).

There was a positive correlation between inoculation and insect infestation where insect infestation was higher in the maize that was not inoculated. This is because maize free from aflatoxin and fumonisin may still be nutritionally rich and its palatability desired by the insects. This is also agreeable with the findings that mycotoxins development increases with the insects activities in the grain (Garbaba *et al.*; Munkvold, 2003a). Insect infestation could have significant impact on the mycotoxin contamination of maize. Insects act as vectors by carrying spores of mycotoxin producing fungi from plant surfaces to the interior of the stalk or kernels or create infection wounds through their feeding habits (Munkvold, 2003b). Insects attack in storage could also be devastating because their level of damage influences the extent of mycotoxin production in the store. Hermetic bags have also been known to preserve the quality of grain, appearance and aroma by reducing mold growth and insect infestation (Moussa *et al.*, 2014). The actellic powder was effective as a pesticide in the prevention of

the infestation as agreed by (Udoh, Cardwell and Ikotun, 2000a). Hermetic technology works synergistically to promote conditions of limited oxygen and high carbon dioxide levels produced by aerobic metabolism of insects, micro-organisms and grain respiration; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects in stored maize (Williams, Baributsa and Woloshuk, 2014). Aerobic metabolism uses up oxygen and produces carbon dioxide to levels that are lethal to insects in the grain mass (Yakubu *et al.*, 2011). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues and insect contamination (Ortiz *et al.*, 2010; Weinberg *et al.*, 2008).

3.6 Conclusion

Application of hermetic techniques to store maize grains offers far more benefits to substantially control storage insect infestation leading to increased value, maligns the use of insecticides and increase food security globally. If well handled, undamaged hermetic bags are extremely effective in managing insect infestation for over eight months of storage however; super-grain bags and the plastic silos would not be recommended for longer periods of maize storage.

CHAPTER FOUR: EFFECT OF HERMETIC TECHNOLOGIES ON MOULD GROWTH OF STORED MAIZE

4.0 ABSTRACT

This study compared hermetic technologies with farmer practice in their effectiveness against mould growth. The study also analyzed the synergistic effect of hermetic storage to control mould proliferation in a safe and environmentally friendly system. Three factors were investigated i.e. natural or artificial fungal inoculation with *Fusarium* and *Aspergillus*; low (12-13%) or high (14-15%) grain moisture levels; and ten storage technologies. The hermetic storage technologies under study were metal and plastic silos, as well as various hermetic bags. The two controls were two farmer practices; the standard woven polypropylene bags (PP), one with grain treated with insecticide and one without insecticide. The duration of the experiment was 8 months with non-destructive sampling at baseline and every 120 days afterwards. The mycotoxin producing fungal strains identified in the maize grains were *Aspergillus* (*L*-strain, *S*-strain, *Parasiticus*, and *Niger*), and *Fusarium* (*Proliferatum*, *Verticilliodes*, *Oxysporum*, *Subglutinans*) as well as other fungi predominantly; *Trichoderma* and *Penicillium*. The *L*-strain, *Parasiticus*, and *S*-strain were the most predominant *Aspergillus* strains in all the storage technologies with the highest values found in PP bags and the least registered was in the PICS bags. The *Fusarium* strains most predominant included *Proliferatum* and *Verticilliodes*, higher in PP bags and lower in Agro-Z and PICS bags. Hermetic bags greatly reduced the fungal proliferation in maize than the silos and PP bags. The technologies had a significant effect ($p < 0.001$) on the mould proliferation at both 4 and 8 months of storage. There was an increase of mycotoxin fungi in the hermetic technologies by 33% at four months and 42% at eight months compared with the baseline. The findings can enable farmers make informed decision on the ideal storage options for the small holder farmers considering the robustness and cost of the hermetic storage that will

have been identified as effective and less expensive. In retrospect, the adoption of hermetic storage options will reduce the prevalence of ill health related to consuming infected maize; increase the market potential of the maize grains and therefore improving livelihoods.

4.1. Introduction

Globally but mostly in the tropical countries, mould contamination and fungi that produce mycotoxin contamination of maize grain is one of the most important food safety risk (Schulthess, Cardwell and Gounou, 2002; Kimanya *et al.*, 2008) (Gong *et al.*, 2004); (Kaaya and Kyamuhangire, 2006); (Strosnider *et al.*, 2006a); (Campbell and Arbogast, 2004). After insects, storage fungi rank highly in causing reduction of maize quality and quantity (Ominski *et al.*, 1994). In favorable conditions, fungi could cause up to 80 % of damage on maize during storage period. Economical loss of stored grain caused due to mycotoxins and insect pests is estimated at US \$ 500 million to 1 billion annually (Campbell and Arbogast, 2004). Conventional storage methods which result in up to 30% loss as a result of insect pests and mycotoxins, force the smallholder farmers to sell off their grain soon after harvest at the time when prices are still low, only to purchase it back later at a costly price, hence being trapped in a vicious cycle of poverty (Tefera *et al.*, 2011). On-farm grain losses result in food insecurity and negatively affect the farmers' livelihood income (Mutambuki *et al.*, 2012; Gitonga *et al.*, 2013; Ndegwa *et al.*, 2016)

The maize becomes infected at any stage of production including cultivation, harvesting, drying, storage, transportation and at the market place (Wagacha and Muthomi, 2008). A number of moulds which include, *Fusarium*, *Aspergillus* and *Penicillium* are involved in post-harvest losses (Quezada *et al.*, 2006; Blandino *et al.*, 2009; Chulze, 2010). Among over 400 mycotoxins known, aflatoxins are the most important mycotoxins (Reddy *et al.*, 2010). The maize is susceptible to aflatoxigenic fungi from the time of harvest through storage

duration (Cotty and Jaime-Garcia, 2007). If not handled properly or stored inefficiently to minimize growth and multiplication of these fungi, the grain damage is likely to proceed through the post-harvest stage (Fandohan *et al.*, 2005). The Kenya quality standard specification requires a safe maximum limit of total aflatoxins for imported maize as 10 ppb (NCPB, 2017). Mycotoxin contamination is escalated with long storage time (Kaaya and Kyamuhangire, 2006). The infection will thereby reduce the nutritional value and result in discoloration of the grain (Ehrlich *et al.*, 2007). When the colonizing fungi are mycotoxigenic, then the infection also results in the spread of toxic metabolites (Klich, 2009; Wagacha and Muthomi, 2008).

There have been many studies about the effectiveness against post-harvest pests in hermetic technologies in SSA, but little information exists on the mould and mycotoxin infestation with regards to hermetic technologies. Studies have also been conducted only for a short term hermetic storage and have not compared the performance of hermetic technologies with the conventional synthetic pesticides and their effectiveness against mould proliferation and insect infestation (Baoua *et al.*, 2013; de Groote *et al.*, 2013; Ognakossan *et al.*, 2013; Baoua *et al.*, 2014).

There is need for a comparative assessment of available hermetic storage technologies for the control of mould development. A great number of farmers store their grain in, woven polypropylene bags with no barrier to air yet there is evidence that this method facilitates fungal contamination and aflatoxin development (Udoh *et al.*, 2000b; Hell *et al.*, 2000). The information generated from this study will aim at sustainable adoption of the hermetic technologies among smallholder farmers in Sub - Saharan Africa.

4.2. Materials and methods

The hermetic technologies that were assessed for this trial included Super Grain IV-RTM, AGRO-Z with pesticides, A-Z without pesticides, PICS bags, metal, plastic silos, and the two controls (Polypropylene bags with actellic powder and the other without).

4.2.1 Experimental design and empirical framework

Grain samples of approximately 1kg were collected at 0, 4, 8 and 12 months after storage using the spear sampler and analyzed for aflatoxin and fumonisin levels and fungal population. The experimental design was a 2 x 2 x 10 randomized complete block design (RCBD) with 3 replications. Maize used for this study was collected from the farmers in Neissuit and Kigogo villages in Gilgil sub-county, Nakuru County (Figure.1) and data on maize production practices recorded using a simple questionnaire.

4.2.2 Sample collection and preparation

The procedure was similar with sample collection and preparation for the insets analysis in the chapter three, section 3.2.3.

4.2.3 Maize inoculation

The grain used in this study was not disinfected, purchased from farmers in Nakuru County and Naivasha sub-county. For artificial inoculation, portions of 500g maize grains were contaminated with fungal inoculums; *Fusarium*, (*F. versitilliodes*, *F. proliferatum*) and *Aspergillus* (*A. parasiticus* and *A. flavus* of *S* and *L* strains). The aliquots were placed in the middle of the maize grain in the storage technologies in perforated bags that allowed contact with the rest of the grains.

4.3 Methodology

Gas composition was measured and recorded before sampling the maize grain.

4.3.1 Isolation and enumeration of mycotoxin producing fungi from maize grains

A sample of 500 g of maize grain was mixed and ground in the laboratory using a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two equal sub-samples for microbial and mycotoxin analysis. Isolation of mycotoxin producing fungi was carried out using the serial dilution and spread plate technique on Potato Dextrose Agar (PDA) amended with 50 mg penicillin, 50 mg tetracycline and 50 mgs antibiotics (Muthomi, 2001). One gram of each ground maize sample was suspended in 9 ml of sterile distilled water to form a stock solution, vortexed for 30 seconds and serially diluted with sterile distilled water to 10^{-2} of the original concentration. A hundred micro liter of each suspension was spread onto potato dextrose agar amended with antibiotics. The plated cultures were incubated for 5 to 7 days at 25°C. The isolation procedure was carried out in three replicates for each sample. The population of each fungal species was expressed as described in the formulae below:

$$\text{Number of fungi/g sample} = \frac{\text{Number of colonies of a fungal species}}{\text{Amount plated} \times \text{Dilution factor}}$$

4.3.2 Isolation and identification of fungus

The morphological identification of fungus was done by scraping mycelia plugs advancing from margins of the aliquot with flamed scalpel. The plugs were mounted on slides for microscopic examination using distilled water. The prepared slides were examined under a compound microscope and identification of the isolates was done based on color, morphology of mycelial, conidia and sporulating structures as described by Agrios, 2005 and, Peres *et al.*, 2018.

For identification of *Aspergillus spp.*, isolates were isolated on PDA amended with antibiotics and sub-cultured on to 5/2 agar, that is, 5% V8 juice and 2% agar at pH 5.2 (Agbetiameh *et al.*, 2018). The cultures on 5/2 agar were incubated at 31°C for 5 days. Isolates that produced small numerous dark sclerotia on 5/2 were identified as *A. flavus S*-strain, while those with

yellow to bright green colonies without sclerotia were identified as *A. flavus* L-strain. Isolates that had dark green colonies on 5/2 and produced rough conidia were considered *A. Parasiticus* (Atehnkeng *et al.*, 2008). Colonies that were black on the top side, while the bottom side remained pale were identified as *A. Niger*. *Aspergillus spp.* were distinguished based on colony color, shape, elevation, pigmentation, texture and pattern of growth (Klich, 2009).

4.3.3 Morphological identification of mycotoxin producing fungi

Aspergillus spp. were examined and identified under microscope with the use of modified Riddell slides (Riddell, 1950; Titilayo *et al.*, 2017). Slide cultures of *Aspergillus spp.* were made by placing 5/2 blocks on a microscopic slide raised with a V – shaped glass rod in a sterile Petri plate covered with a dump sterile paper towel at the bottom. *Aspergillus* spores were carefully transferred from their isolates to the four edges of the agar block using a sterile inoculating needle. A clean cover slip was placed on the surface of each agar block and the plate partially sealed with parafilm TM. Cultures of *Aspergillus spp.* were incubated at 31°C for 5 days. Slides for light microscopy were prepared by removing the agar block and then adding a drop of sterile distilled water on the slide and cover slip added to cover the growth on the slide. The prepared slides were used for identification and taking images of morphological characteristics of the commonly isolated *Aspergillus spp.* All prepared slides were examined under a Light microscope (1000x) and the corresponding images taken with the inbuilt camera (LEICA ICC 50, Leica Microsystems, Wetzler, Germany) fitted to a microscope. Microscopic characteristics used in identification of *Aspergillus spp.* were conidial heads, serration, conidia size, shape and raggedness as described by Klich, 2009 and Pitt and Hocking, 2017.

4.4 Statistical analysis

Data was collected every four months and analyzed using GENSTAT Stata SE version 12 software. The mould count, was log transformed while the mould incidence, was square root transformed. The transformed data was first analyzed using one-way repeated ANOVA to compare grain moisture, the percentage of oxygen and carbon dioxide. Significant differences in the mould incidence levels were tested using two-way ANOVA. Fischer's LSD was used to separate statistically different means. Pearson correlation test was used to assess relationship among *Fusarium* incidence, and *Aspergillus* incidence (Hell *et al.*, 2014).

4.5 Results

4.5.1 Diversity of mycotoxin producing fungi in maize samples

There were nine commonly isolated mycotoxin producing fungi in the maize samples and in both hermetic and Polypropylene (PP) bags at harvest and in the eight months storage period. Notwithstanding the levels of moisture in the grain, the storage technologies had a significant effect at ($p < 0.001$) on the mould development. A high relative humidity resulted into higher mould incidences at $p < 0.05$. The major genera of mycotoxin producing fungi isolated were *Aspergillus spp* (Figure 4.1) and (Figure 4.4) *Fusarium spp*. In grains with high moisture levels the predominant species in descending order included; *A. Parasiticus* (4.87×10^3 CFU/g), *F. Proliferatum* (4.6×10^3 CFU/g), *F. Vericilliodes* (4.59×10^3 CFU/g), *F. Oxysporum* and *F. Subglutinans* (4.4×10^3 CFU/g), *A. L-strain* (4.24×10^3 CFU/g), and *A. Niger* (3.8×10^3 CFU/g) (Table 4.1). At low moisture levels, the most prevalent fungal species in the descending order included; *F. Proliferatum* (4.96×10^3 CFU/g), *F. veticilliodes* (4.9×10^3 CFU/g), *A. Parasiticus* (4.72×10^3 CFU/g), *A. L-strain* (4.58×10^3 CFU/g), *A. S-strain* (4.57×10^3 CFU/g), *F. Oxysporum* (4.4×10^3 CFU/g), *F. Subglutinans* (4.3×10^3 CFU/g), and *A. Niger* (3.33×10^3 CFU/g).

4.5.2 Morphological characteristics of *Aspergillus* fungal strains

The most common members of *Aspergillus* section Flavi isolated from maize grain samples were; *A. flavus* (*S* and *L*-strains) and *A. Parasiticus* (Figure 4.1). *Aspergillus Niger* was also commonly isolated from maize grain samples. Colonies of *A. flavus L*-strain were yellow to bright green with no sclerotia while *A. flavus S*-strain produced numerous small and dark sclerotia. *Aspergillus Parasiticus* produced dark green colonies with rough conidia which were more compact than spores of *A. flavus L*-strain. Colonies of *A. Niger* were initially white but soon turned black on the top side, while the bottom side remained pale yellow (Figure 4.1).

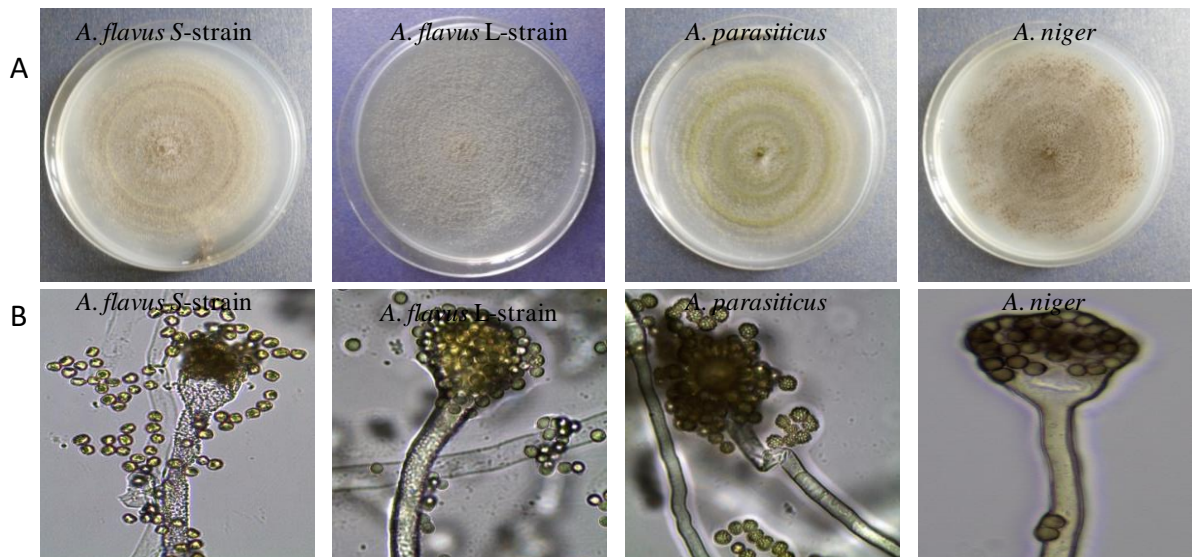


Figure 4. 1: Cultures of *Aspergillus* spp. on 5/2 agar (A) and spores and conidial heads of *Aspergillus* spp. (B) isolated from maize grains.

4.5.3 Hermetic storage effectiveness over farmer practice in the control of *aspergillus* development under low and high relative humidity.

It was observed that the moisture content of maize stored in PP bags decreased with time due to the low moisture barrier properties of the bags considering that the trial proceeded during the dry weather season. It was also observed that, hermetic technologies were superior to

farmer practice in reducing *Aspergillus* development; however, the plastic silo was not as effective in managing the *Aspergillus* growth. Among hermetic technologies, there were no significant differences ($P>0.05$) in performance between metal silos and hermetic bags for *Aspergillus* development regardless of the mode of inoculation. Table 4.2 and 4.3 indicate the total mould counts in maize stored in the woven bags, silos and hermetic bags in the two moisture levels. At the onset, there was a 3-fold increase in the mould infection in the maize with moisture content above 13% than in the maize grains of less than 13% moisture content. During the entire eight months of storage, fungal infection did not change significantly in maize stored in hermetic bags at initial moisture content of $<13\%$ at $p> 0.05$. However, there was a significant increase in mould infection in the silos and woven bags at ($p< 0.001$) increasing by 7-folds at the eighth month of storage higher than in hermetic bags (m.c. $<13\%$: $P< 0.001$). Interaction effect between type of bag and storage duration was significant for *Aspergillus spp.* ($P<0.001$). In the hermetic bags, occurrence of *Aspergillus spp.* did not change significantly with storage time ($F \frac{1}{4} 0.60$). In the silos and woven bags, however, incidence levels increased up to five-fold (*Aspergillus spp.*) and reached significantly higher incidence levels than in hermetic bags at the end of storage period ($P < 0.001$). Further analysis of the main effects showed that both storage duration ($P < 0.004$) and the type of storage bag ($P < 0.007$) were significant.

4.5.4 Efficacy of gas composition within the storages on mould growth and development

Among the hermetic technologies assessed, Agro-Z storage bags prevented the accumulation of *Aspergillus* fungi with a mean value of 2.96×10^3 CFU/g compared with the 4.9×10^3 CFU/g observed in the metal silo. The PP bags registered the highest fungal growth of 5.04×10^3 CFU/g in PP bags with pesticides and 5.39×10^3 CFU/g in PP bags without pesticides.

4.5.5 Effect of time on *Aspergillus* fungal strains in different RH

Generally, it was observed that as storage time progressed on, the *Aspergillus spp.* in grains with high moisture levels increased. *Aspergillus L*-strain, *A-* strain and *A. Parasiticus* were predominantly isolated with *A. Niger* being the least predominant in the maize samples over the eight months of storage in all the storage technologies (Table 4.1).

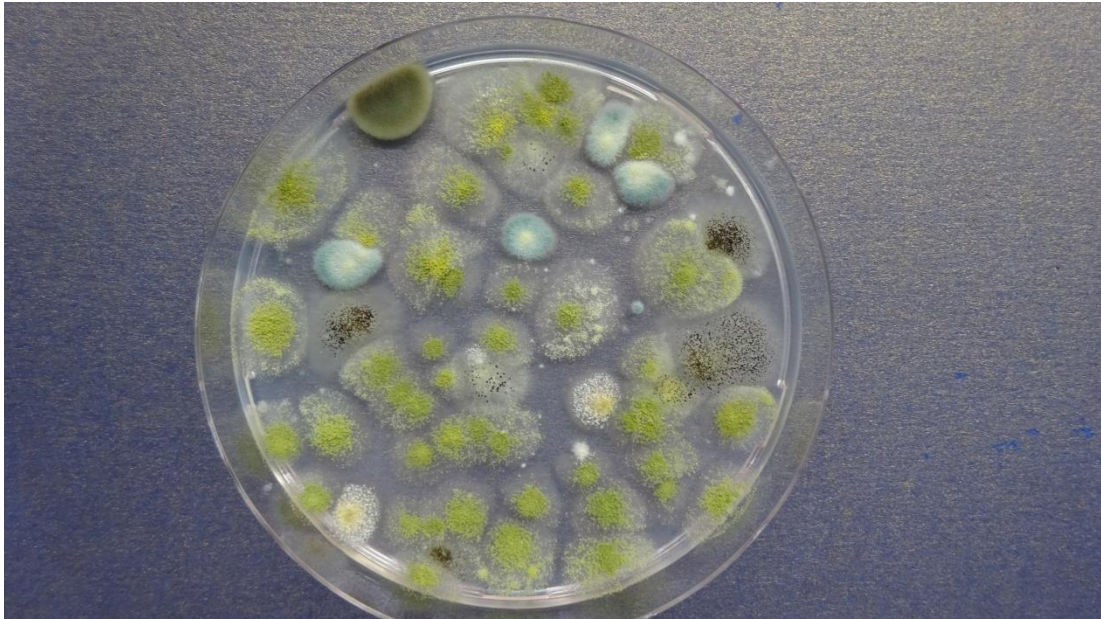


Figure 4.2: Colony forming strains with high population of *Aspergillus* strains in one of the inoculated maize samples with high M.C in one of the hermetic technologies after eight months of storage

4.5.5.1 Diversity of *Aspergillus* strains in maize grain

As indicated in (Table 4.1) there was no significant difference in the *Aspergillus L*-strain population in both grains with low moisture and in high moisture content at ($p>0.05$). However, the technology that managed to control the fungi from multiplying was Agro-Z+ at the minimum mean value of $(3.21\pm 2.49 \times 10^3 \text{ CFU/g})$ and the maximum value was in the polypropylene bag with a maximum value of $(5.55\pm 0.43 \times 10^3 \text{ CFU/g})$.

Aspergillus S-strain was not significantly different between the grains stored at high or low moisture levels with a mean value of $(4.57 \times 10^3 \text{ CFU/g})$ but the technology with more fungal

proliferation was the PP+ bags with pesticides (4.9×10^3 CFU/g) followed by PP- bag at 4.84×10^3 CFU/g (Table 4.2). The hermetic technologies registered low population of the fungi, values ranging from 4.3×10^3 CFU/g to 4.6×10^3 CFU/g.

AGROZ technology proved most effective in inhibiting growth of *A. niger* where no mould incidences were detected. The interaction between hermetic storage and relative humidity was significant at $p < 0.05$ in mould incidences in *A. niger*.

A. parasiticus was one of the major species of mycotoxin producing fungi isolated especially predominant in grains with high moisture levels at a mean value of (4.9×10^3 CFU/g) and lower in the grains stored at low moisture content (4.7×10^3 CFU/g). Agro-Z bag had the least colony forming units but not so different from the other hermetic technologies at (4.6×10^3 CFU/g). Both the PP- and PP+ had higher and closer values of (5.0×10^3 CFU/g) and (4.94×10^3 CFU/g) respectively (Table 4.1).

Table 4.1: Aspergillus growth CFU/g) as influenced by different hermetic technologies and relative humidity

Treatment*	<i>L</i> -strain		<i>S</i> -strain		<i>Aspergillus niger</i>		<i>A .parasiticus</i>	
	Low RH	High RH	Low RH	High RH	Low RH	High RH	Low RH	High RH
Metal silo	4.98±0.48 ^b	4.91±0.28 ^{bc}	ND	4.30±0.00 ^a	ND	1.23±0.45 ^c	4.30±0.00 ^b	4.84±0.09 ^b
Plastic silo	4.96±0.56 ^b	4.24±1.90 ^{ab}	4.48±0.27 ^{bc}	4.58±0.15 ^{ab}	ND	4.90±0.00 ^{ab}	4.80±0.46 ^a	4.84±0.58 ^b
Agro-Z	4.53±0.29 ^b	4.40±0.17 ^b	4.60±0.00 ^{ab}	4.60±0.00 ^a	ND	ND	4.60±0.43 ^{ab}	ND
Agro-Z+	2.96±2.48 ^a	3.43±2.63 ^a	4.30±0.00 ^a	4.40±0.17 ^a	4.30±0.00 ^a	4.30±0.00 ^b	4.90±0.00 ^a	4.70±0.17 ^b
PICS	4.67±0.38 ^b	4.36±0.13 ^b	4.60±0.00 ^{ab}	4.38±0.15 ^a	4.60±0.00 ^a	ND	ND	4.63±0.35 ^b
PP-	5.39±0.43 ^c	5.55±0.43 ^c	4.75±0.16 ^c	4.84±0.47 ^b	5.09±0.43 ^b	5.32±0.22 ^a	4.78±0.44 ^a	5.34±0.79 ^a
PP+	5.04±0.43 ^b	3.29±2.55 ^a	4.60±0.43 ^{ab}	4.90±0.85 ^b	4.95±0.56 ^{ab}	3.99±2.28 ^b	4.78±0.00 ^a	4.98±0.55 ^{ab}

Values with different superscripts, lowercase along a column and superscript across a row, for a microorganism are statistically different at p<0.05.

4.5.6 Time effect on the proliferation of fusarium spp in maize grain stored in hermetic and farmer storage systems.

At month, it was observed that *A. parasiticus* was highest with 4.85 CFU/g in grains stored under high moisture conditions. This was followed by *AL* strain in grains under low moisture levels at 4.8 CFU/g, and then *AL* strain in grains under high moisture at 4.73 CFU/g. *A. parasiticus* was the least in grains stored under low moisture conditions at 4.30 CFU/g.

At month four, the *Aspergillus* population in stored grains significantly increased. Under low moisture levels, *AL* strain was the highest at 5.14 CFU/g, followed by *A. parasiticus* at 4.70 CFU/g and the lowest was *AS* strain at 4.53 CFU/g. At month four, the *Aspergillus* population in stored grains under high moisture levels had *A. parasiticus* gaining the highest level of 4.97 CFU/g, followed by *AL* stain and *A. parasiticus* with 4.7 CFU/g and *A. niger* as the lowest with 4.28 CFU/g. At eight months in grains stored under low moisture conditions, it was discovered that *A. niger* at 5.04 CFU/g, *A. parasiticus* at 4.79 CFU/g were the highest in content followed by *AS* strain at 4.61% and *AL* strain at 4.25 CFU/g.

At month four, *AL*. strain was found highest at 5.22 CFU/g in inoculated grains, followed by *A. parasiticus* at 4.96 CFU/g in inoculated grains while the lowest content was *A. niger* at 4.30 CFU/g in inoculated grains. The rest of the strains were average with approximately 4.5 CFU/g. At month eight, high content of *A. niger* was detected in inoculated grains at 5.26 CFU/g, followed by *A. parasiticus* in inoculated grains at 4.899 CFU/g. *AL*. strain was the lowest at 3.26 CFU/g in non-inoculated grains. The rest of the strains were more or less the same with an average level of 4.5 CFU/g (Figure 4.3). At eight months in grains stored under high moisture conditions, we found *A.niger* to be highest with 5.06 CFU/g, followed by *A. parasiticus* at 4.82CFU/g, and *AL*. strain was the lowest at 3.72 CFU/g. At month zero, *AL*

strain was found highest at 4.86 CFU/g in the non inoculated grains, followed by *A. parasiticus* in inoculated grains at 4.78 CFU/g.

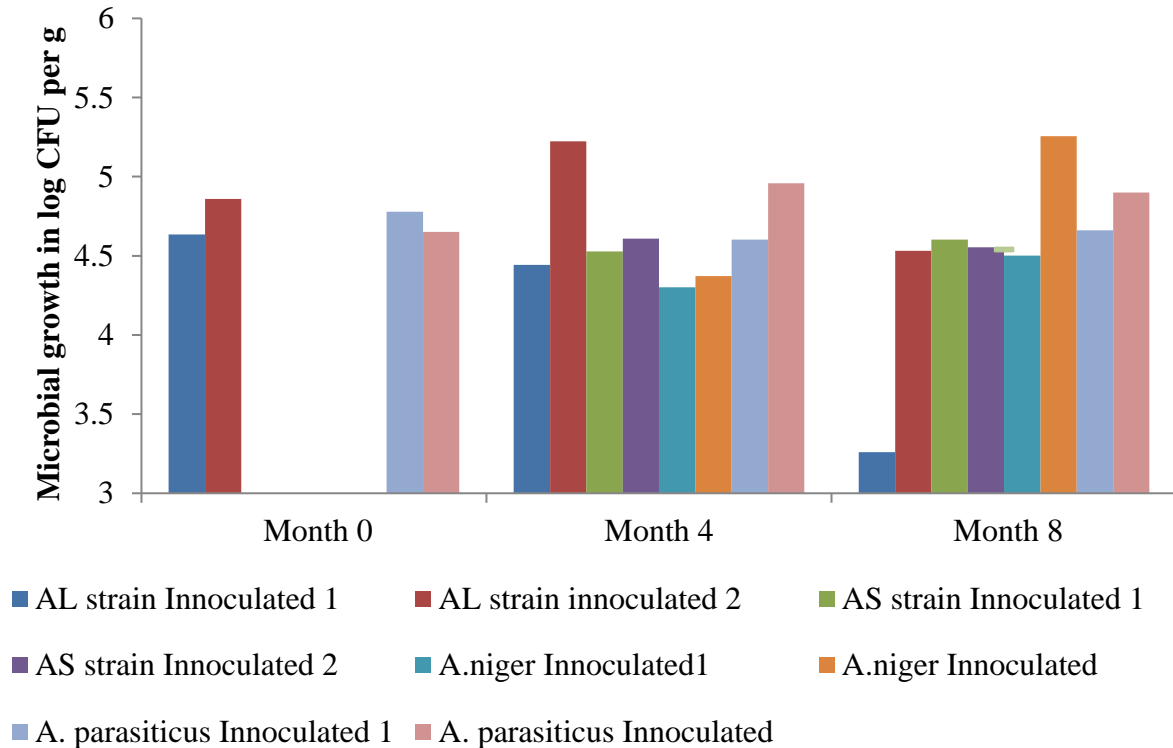


Figure 4.3: Aspergillus infection in the grains both naturally contaminated and artificially contaminated grains. 1Low M.C maize grains, 2 High M.C maize grains

4.5.7. Morphological characteristics of Fusarium fungal strains

Fusarium proliferatum produced white aerial mycelium that grew rapidly and was tinged with purple colour. *Fusarium verticillioides* produced mycelia with white pigmentation. Sporodochia of *F. verticillioides* was dark in colour. *Fusarium oxysporum* produced floccose mycelia that were abundant and white to pale violet and the under surface was pale purple. *Fusarium subglutinans* produced aerial mycelia that grew rapidly and was white in colour while sporodochia was cream in colour. Microscopically, *Fusarium proliferatum* produced curved apical end that were slender, thin walled and relatively straight club shaped microconidia (Figure 4.4).

Microconidia of *F. proliferatum* were club shaped, non-septate and with a flattened base. *Fusarium oxysporum* produced non-septate kidney shaped microconidia and slightly curved 3-septate macroconidia. *Fusarium verticillioides* produced club shaped, non-septate microconidia that were in long chains and aggregates (Figure 4.4). *Fusarium subglutinans* produced oval non-septate microconidia on false heads on the aerial mycelium.

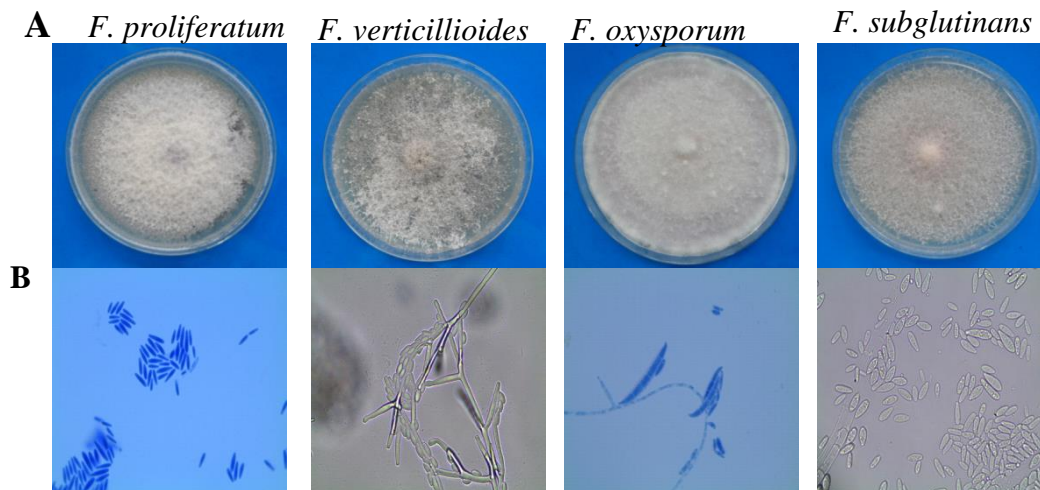


Figure 4.4: Cultures of major *Fusarium* spp. on potato dextrose agar (A) micro- and macro-conidia of major *Fusarium* spp. (B) isolated from maize grains

4.5.8 Hermetic effectiveness over farmer practice in the control of *Fusarium* development in maize grain.

In non-inoculated grain, fungal populations were varied but included mycotoxin-producing *Fusarium* spp. in the harvested maize grains indicating that the grain was naturally contaminated and acted as a good reservoir for these fungi. There was no significant interaction effect between type of bag and storage duration ($P > 0.202$). Fungal population increased with higher moisture in non-inoculated grain by six - folds. In Table 4.5 the fungal population was significantly high in the grains at $p < 0.01$ that were inoculated with the mycotoxin producing fungi in all the technologies.

There was no significant difference $P > 0.05$ between the *Fusarium* infection in all storage technologies except for the AGROZ bags with the lowest *Fusarium* contamination (3.11×10^3 CFU/g) and in the PICS bag 4.2×10^3 CFU/g. Among the hermetic technologies, Agro-Z bag

did not effectively manage the fungal infection in the grain with the overall mean value of 4.5×10^3 CFU/g (Table 4.2). This observation could be linked to the fact that the bags are permeable to allow enough oxygen for the fungi to grow and multiply.

Table 4.2 indicates that *F. verticillioides* was more predominant in grains at low moisture level (4.9×10^3 CFU/g) than in grains conditioned at high moisture levels (4.59×10^3 CFU/g). However, the technologies that had the most contamination were PP- (5.4×10^3 CFU/g), PICS (4.32×10^3 CFU/g), and metal silo (5.3×10^3 CFU /g). The least infected was the grain in Agro-Z+ at 5.3×10^3 CFU/g (Table 4.2)

There was no statistical difference ($p > 0.05$) between the number of colonies of *F. oxysporum* for both the grain with low and high moisture content. In some technologies, *F. Oxysporum* was not detected such as Agro-Z, PP- and PP+ in grain with low moisture content; Agro-Z+ and PICS bags in grain with high moisture content (Table 4.2).

F. subglutinans were not detected in Agro-Z, and in PP- bags in the both the grains at low and high moisture levels. The fungi was also not detected in grain with low moisture stored in Metal and plastic silo but detected at higher levels at (4.6×10^3 CFU/g) and (5.51×10^3 CFU/g) respectively. At high moisture level, the fungi were not detected in PICS bag but was detected in the grain stored at low MC (5.51×10^3 CFU/g). Generally, the *F. subglutinans* fungi were not common in all the storage technologies regardless of the moisture levels as indicated in (Table 4.2)

Table 4.2: *Fusarium proliferatum* growth (CFU/g) as influenced by different hermetic technologies and relative humidity.

Treatment	<i>F. proliferatum</i>		<i>F. verticillioides</i>		<i>F. oxysporum</i>		<i>F. subglutinans</i>	
	Low RH	High RH	Low RH	High RH	Low RH	High RH	Low RH	High RH
Metal silo	4.92±0.44 ^{abc}	4.86±0.44 ^a	5.30±0.00 ^a	ND	4.30±0.00 ^a	4.70±0.46 ^a	ND	4.60±0.00 ^a
Plastic silo	4.61±0.33 ^c	4.74±0.32 ^a	4.63±0.50 ^b	4.46±0.20 ^{bc}	4.30±0.00 ^a	4.42±0.24 ^a	ND	4.30±0.00 ^a
Agro-Z	5.09±0.35 ^{ab}	5.02±0.35 ^a	4.30±0.00 ^c	5.58±0.00 ^a	ND	4.30±0.00 ^a	ND	ND
Agro-Z+	4.87±0.38 ^{abc}	4.80±0.35 ^a	4.72±0.60 ^b	4.36±0.13 ^{bc}	4.30±0.00 ^a	ND	4.30±0.00 ^a	4.60±0.00 ^a
PICS	5.24±0.58 ^a	1.43±2.48 ^b	5.48±0.20 ^a	4.30±0.00 ^c	4.50±0.17 ^a	ND	4.30±0.00 ^a	ND
PP-	5.15±0.31 ^a	5.01±0.35 ^a	ND	5.40±0.70 ^a	ND	4.30±0.00 ^a	ND	ND
PP+	4.69±0.55 ^{bc}	4.66±0.32 ^a	4.54±0.34 ^b	4.50±0.17 ^b	ND	4.30±0.00 ^a	4.30±0.00 ^a	4.30±0.00 ^a

Values with different superscripts, lowercase along a column and superscript across a row, for a microorganism are statistically different at $p < 0.05$.

4.5.9 Other fungal strains in maize grain stored in hermetic technologies and farmer conventional storage systems

Other fungi were identified to include more of *Penicillium spp.* and *Trichoderma*. The other strains were unknown and therefore grouped together. This category had the most contamination compared to the common mycotoxin producing fungi. Overall, the grain with high moisture content was more infected (5.51×10^3 CFU/g) than the maize conditioned to <13 % moisture content (5.28×10^3 CFU/g). Comparison between individual storage technologies did not show any significant difference in the mean population of the other fungal infection at $p < 0.05$ (Table 4.3)

Table 4.3: Other fungal strains in maize samples under different relative humidity during the 8 months of storage.

Technology	Other fungi	
	Low RH	High RH
Metal silo	5.20±0.60 ^a	5.65±0.62 ^a
Plastic silo	5.26±0.62 ^a	5.59±0.61 ^a
Agro-Z	5.12±0.75 ^a	5.36±0.56 ^a
Agro-Z+	5.53±0.51 ^a	5.50±0.59 ^a
PICS	5.55±0.48 ^a	5.36±0.66 ^a
Woven	5.30±0.65 ^a	5.52±0.60 ^a
Woven+	5.09±0.53 ^a	5.60±0.47 ^a
Average	5.28±0.60 ^B	5.51±0.58 ^A

Values with different superscripts, lowercase along a column and superscript across a row, for a microorganism are statistically different at $p < 0.05$.

4.5.10 Time effect on the proliferation of *Fusarium* spp. in maize grain stored in hermetic and farmer storage systems.

At month zero, the non-inoculated grains had a high concentration of *F. proliferatum* at 5.12 % of the total population of the identified fungi. Among the hermetic treatments, PICS bag had the least population of the *F. proliferatum* (3.81×10^3 CFU/g) and SGB bag registered high population of the fungi (5.04×10^3 CFU/g) higher than the farmer practice treatment with pesticides at (4.67×10^3 CFU/g) and about the same as the growth in PP- bag at (5.07×10^3 CFU/g). The mean values significantly differed between grains at low moisture (4.96×10^3 CFU/g) and grains at high moisture (4.6×10^3 CFU/g).

At month 4, the level of fungal strain infection varied greatly. The inoculated grains with mycotoxin fungi were found to contain the highest levels of other fungi at 5.37%. The non-inoculated grains had a 5.01% rate of infection with other fungal strains. At month eight, a variation of infection of the different strains was observed. The inoculated grains with mycotoxin producing fungi and the non-inoculated grains had a high close range of infection by other fungal strains between 5.69 % and 5.84%. A 5.45 % of the non-inoculated grains were infected with *F. verticilloides*. The non-inoculated grains had the lowest infection levels with *F. subglutinans* and *F. oxysporum* at 4.30% which was the same level in non-inoculated grains with mycotoxin producing fungi infected with *F. proliferatum*. The rest of the strains were found between the average parameters (Figure 4.3).

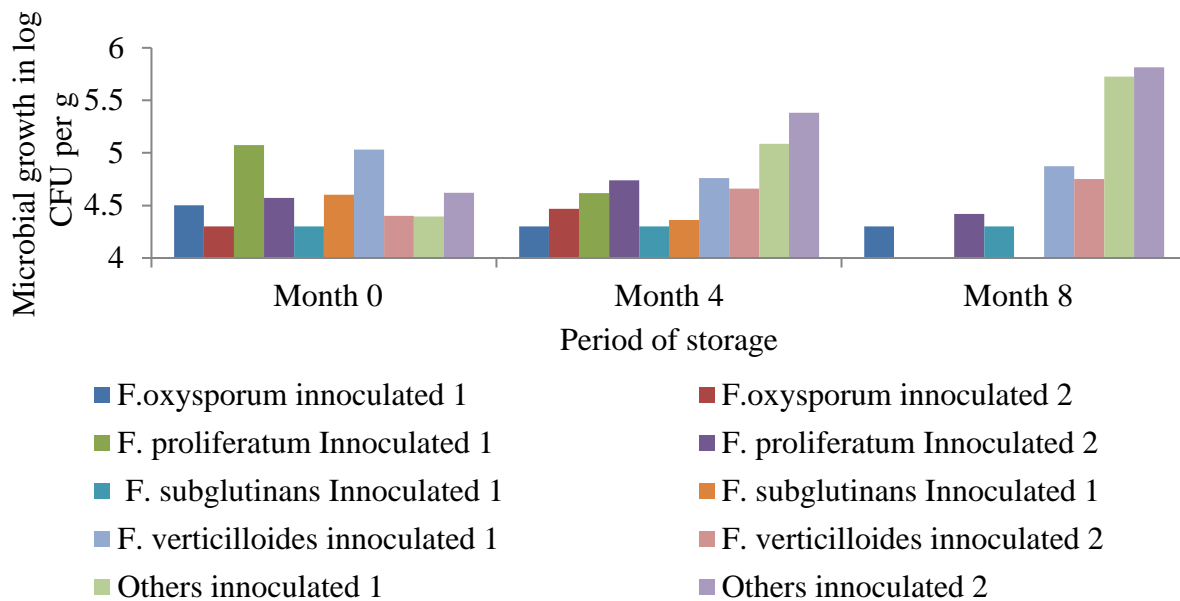


Figure 4.5: Fusarium fungal strains in maize grains under different MC. 1- Not inoculated, 2- Inoculated maize grains with mycotoxin producing fungi.

4. 6 Discussion

It was expected that packing maize in hermetic bags would alter the course of mould proliferation by creating a modified storage microenvironment. 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. Similarly (Baoua *et al.*, 2014) who conducted storage trials involving traders, marketing cooperatives, private seed companies, and private food processors reported on average 24 % m.c. loss of maize stored in woven bags as compared to maize stored in PICS bags for 6.5 months. During a two months laboratory trial, (Williams *et al.*, 2014) observed moisture loss on maize stored in woven bags as compared to maize stored in PICS bags and attributed this to dry environment of the room in which they were stored. It is important to note that there are other factors that may result in the accumulation of moisture in the grain stored for many months; such are, high insect infestation and the breakdown of organic matter by the fungal activities into carbon dioxide, heat and water as reported by (Njoroge *et al.*, 2017).

In this study, the hermetic technologies with high maize moisture were not able to manage the proliferation of the toxigenic moulds. At high moisture level PICS bags were more effective with a mean CFU/g of 2.9×10^3 compared with the others in descending order, metal silo, plastic silo, Agro-Z⁺ and Agro-Z at 3.7×10^3 , 4.5×10^3 , 4.6×10^3 and 4.9×10^3 respectively. However, at low moisture level PICS and Agro-Z⁺ bags were the least effective in controlling the mould growth at mean values of 4.8×10^3 CFU/g and 4.5×10^3 CFU/g respectively compared with metal and plastic silos at 3.6×10^3 CFU/g and 3.4×10^3 CFU/g respectively Agro-Z rating high in managing proliferation of moulds. It is likely that the moisture gain in hermetic storages was as a result of high fungal population, hence further facilitating the multiplication. The heat and water together with resident oxygen facilitated mould infection. Generally, the technology that managed favourably the mould growth was PICS with the least number of colony forming units at 3.1×10^3 compared with Agro-Z, Agro-Z⁺, plastic silo and metal silo at 4.1×10^3 , 4.0×10^3 , 3.9×10^3 and 3.6×10^3 CFU/g respectively. The mould population was low in PP without pesticides at 1.3×10^3 CFU/g compared with 3.4×10^3 CFU/g in the bags with pesticides however, maize dusted with pesticides had the least population of moulds in the maize grains with high moisture content at 3.4×10^3 CFU/g compared with 4.9×10^3 CFU/g in polypropylene bags without pesticides. Previously, (Krnjaja *et al.*, 2013) found that moulds belonging to the genus *Aspergillus* were most frequently isolated (35.8%) in Kenya. In a similar study, (Muthomi, 2001, Thathana *et al.*, 2017) 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 (Wagacha and Muthomi, 2008; Stasiewicz *et al.*, 2017) may be reason for high occurrence in the maize samples even at low moisture levels.

The fungi usually form sclerotia that allow saprophytic survival for extended periods in the soil, maize residue and maize cobs (Wagacha and 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.*, 2017; Kang'ethe *et al.*, 2017b). At the eighth month (52%) of the moulds increased significantly not regarding the type of storage technologies. This finding is leveled with the observation that mycotoxin contamination is escalated with long storage time (Kaaya and Kyamuhangire, 2006). Mould infection on maize stored in woven bags, nevertheless, increased with increasing storage duration irrespective of the initial storage moisture. (Beuchat, 2017) observed that mycoflora development in stored cereals is influenced by environmental factors, especially temperature, water-activity and gas atmosphere as was also noted by (Mannaa and Kim, 2018). Fungistatic effect is induced when oxygen concentration drops to 1% or below as investigated by (Beuchat, 2017) who 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 (Addae-Mensah, 2014). Some findings reported that under hermetic storage, fungal static effect is included when oxygen concentration drops to 1% or below (Storm, 2009). It has been argued that low oxygen and high carbon dioxide levels in hermetic storage systems could control mould proliferation (Williams *et al.*, 2014; Schmidt *et al.*, 2018). The drop in oxygen and rise in carbon dioxide observed when maize was stored in hermetic technologies was as the result of aerobic metabolism of life forms enclosed together with the maize (Murdock and Baoua, 2014) and could be influenced by elements of the storage system such as insect populations, moisture content of grain, fungal inoculums, quality of the grain, and gas-tightness of the

hermetic package (Quezada *et al.*, 2006). Thus, oxygen depletion and carbon dioxide build-up may be slow in grains that are well dried, and free from insects and moulds.

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 and Nielsen, 2005). On maize, (Giorni *et al.*, 2008) indicated that treatment with 25% carbon dioxide reduced *A. flavus* development, but at least 50% carbon dioxide was necessary to reduce aflatoxin synthesis. In order to minimize mould proliferation, m.c. of maize to be packed in hermetic bags should not exceed 14%. For long term storage, m.c. of 13% - 13.5% is recommended by KEBS to avoid mould growth (Mutungi *et al.*, 2016). However, a better indicator of the likelihood for moulds to colonize stored products is water activity which, in addition to m.c., is related to temperature (Beuchat, 2017). Water activity (aw) is a measure of the fraction of water content which is free and therefore available for fungal growth (Abass *et al.*, 2018; Abass *et al.*, 2014), and is equivalent to equilibrium relative humidity expressed as a fraction. The growth limit for most fungi during storage of durable products is aw of 0.65e (Amante *et al.*, 2017). For maize at 26°C, the average temperature recorded in the PICS bags, at water activity of 0.7 corresponds to moisture content of 14% (Lane *et al.*, 2018), although slight variations may occur depending on variety. This explains the steady increase in mould infection (Suleiman *et al.*, 2018; Manandhar *et al.*, 2018) reported that cereals of m.c. > 15% are susceptible to fungal attack within normal storage time. Moreover, studies have shown that the less xero tolerant fungi such as *A. ochraceous* and *A. versicolor* also begin to grow at moisture of 14% thus increasing mould infection (Gupta *et al.*, 2017). These reasons related to profuse insect activity probably explain the increase in total mould count on maize stored in woven bags even when m.c. was within the limit for safe storage, that is, below 14%. (Moreno-Martinez *et al.*, 2000; Quezada *et al.*, 2006) also reported low *Aspergillus* 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, of maize quantified 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 (Strosnider *et al.*, 2006b).

Hermetic bags provide modified atmospheric conditions that suppress the growth of moulds; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects and mycotoxin contamination in stored maize (Williams *et al.*, 2014). It has also been identified that mycotoxigenic fungi can be produced in maize of 13-25.1% moisture content stored in hermetic systems with the considerable risk of contamination with aflatoxin and fumonisins (Castellari *et al.*, 2010).

4. 7 Conclusion

In this study maize stored in hermetic bags with high moisture content 14-15% did not show an increase in mould infection although it is unlikely that the oxygen / carbon dioxide environment achieved in the hermetic bags could inhibit mould development. Among the hermetic bags, Agro-Z and PICS bags were more effective in managing the mould growth while the metal silos were superior over plastic silos. Hermetic technologies work best when the maize grain is dried to 13 % or less to avoid possibilities of mould growth.

CHAPTER 5: EFFECT OF HERMETIC STORAGE TECHNOLOGIES ON MAIZE GRAIN MYCOTOXIN CONTAMINATION AT DIFFERENT MOISTURE LEVELS

5.0 ABSTRACT

Grain losses due to mycotoxin contamination during on-farm storage increases food insecurity which results in economic losses, negatively affect farmers' livelihoods, and negatively affect human and animal health. Storing dry maize in hermetic systems can sufficiently reduce post-harvest losses as a result of mycotoxin contamination. There is limited and inadequate proof on the success of more than five hermetic technologies against mycotoxin contamination. Three factors were investigated i.e. natural or artificial fungal inoculation with *Fusarium* and *Aspergillus*; low (12-13%) or high (14-15%) grain moisture levels; and ten storage technologies. This study evaluated the effectiveness of PICS, SGB, A-Z, A-Z+, Elite, Zerofly, metal silo, and plastic silo hermetic technologies comparing with two controls (standard woven with and without pesticides) in the control of mycotoxin contamination in stored maize grain. Three samples of maize grains were taken from each storage technology, ground and analysed for aflatoxin and fumonisins using VICAM kit. Findings show that hermetic technologies were superior to farmer practices in reducing mycotoxin accumulation. There was no significant differences ($P>0.05$) in performance among hermetic bags in the management of mycotoxin contamination. Mycotoxin levels increased with higher moisture even in non-inoculated grain. Aflatoxin and fumonisin levels at eight months were significantly higher than at four months of storage. There was no significant increase of mycotoxins ($P>0.05$) from the baseline to four months although contamination was slightly higher in the inoculated maize grain with high moisture across all hermetic storage technologies indicating that hermetic technologies will not prevent mycotoxin contamination in maize grain with high moisture. Aflatoxin and fumonisin were significantly higher at 1.69 ppb and 0.25 ppm respectively in non-inoculated grains at high

moisture indicating the need to adequately dry grain before storage in hermetic conditions. This trend was observed collectively in all the storage technologies where aflatoxin and fumonisin were higher than the baseline values by 2.03 ppb and 0.311 ppm respectively. In inoculated grains at high moisture, there was an increase in aflatoxin in both hermetic treatments and the control by 5.7 ppb and 12.14 ppb respectively at the fourth month. The study indicated that hermetic bags are superior in the management of mycotoxin contamination compared to the silos; the conventional storage being by far inferior. In retrospect this finding adequately provides facts that PICS, AgroZ, AgroZ⁺ bags and metal silos as the most appropriate and sustainable technologies to use for the maize grain storage for upto eight months.

5.1 Introduction

Aflatoxins and fumonisins are the two commonest and highly toxic mycotoxins encountered in maize in the tropical and sub-tropical regions of the world (Krska *et al.*, 2008). Food security encompasses availability and the ability for people to afford, access, safe food. Over the years, Kenya has lost maize grain contaminated with aflatoxin with over 830 tons of maize was recalled from the market in (2016) due to aflatoxin contamination exceeding 10 ppb. This led to a reduction in revenue, malnutrition especially in children, and ill health in the wider mass whose staple food is maize (Kang'ethe *et al.*, 2017a).

Fumonisin are mycotoxins produced by *Fusarium verticillioides* and *Fusarium proliferatum* and are categorized as B1, B2 and B3 and are usually found to be greater than 1 ppm in the corn samples tested. However, the FDA/USDA advises less than 4ppm in corn meant for human consumption and less than 50ppm for cattle feed (Table 5.1). Fumonisin are not always produced where the fungi have colonized the kernels, but many factors contribute to the subsequent mycotoxin contamination including host susceptibility, and environmental

conditions (Wagacha *et al.*, 2008). All these factors together determine the incidence and severity of mould contamination on the grain. The conditions that favor Fumonisin production are not well known (Bellerman and Wei-Yun, 1998). The magnitude of the effect of mycotoxin exposure is facilitated by the level and exposure period, as well as health, age and the species of the animal. Improved storage technologies at both household and national levels which reduce losses by preventing mycotoxin contamination are important components of food security (Vales *et al.*, 2014).

Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal and plastic silos provide affordable and more effective storage alternative for farmers, that would markedly contribute to food security (Ndegwa *et al.*, 2016). Hermetic storage containers utilization is still low in SSA because of the knowledge gaps about their performance in comparison with the conventional storage methods of using insecticides. There have been numerous studies on how to mitigate the aflatoxin contamination in maize grains and they have not been entirely successful considering some limitations. Alleviation initiatives to manage aflatoxin and fumonisin should be aimed at prevention of plant infection during pre-harvest and grain contamination during post-harvest activities. Current good agricultural practices may help to reduce the possibilities of fungal infection during pre-harvest (Munkvold, 2003) and postharvest practices such as proper drying, biological control use of mycotoxin adsorbents and chemoreceptors are some of the mitigation initiatives to reduce or prevent the absorption of the toxins and exposure to humans and animals (Kensler *et al.*, 2013; Miller *et al.*, 2014).

The aim of this study was to analyze the synergy effect of hermetic storage to manage mycotoxin contamination in safe and environmentally friendly systems. The data generated from this study will facilitate sustainable adoption of the hermetic technologies among smallholder farmers in Sub Saharan Africa. This study suggests the most efficient storage

options for the small holder farmers considering the robustness and cost of the hermetic storage that will have been identified as effective and less expensive. The study also evaluated the use of improved storage technology in preservation of quantity and quality of grain stored for more than four months under a simulated farmers practice conditions.

Table 5.1: Mycotoxin limit in maize

Mycotoxin	Food stuff	Maximum level(ug/kg)		
		EC	FDA	KEBS
Aflatoxin	All cereal products	4 ppb	20 ppb	10 ppb
	Maize to be subjected to sorting or other physical treatment before human consumption	4 ppm	4 ppm	
	Maize meant for cattle feed		50 ppm	

5.2 Materials and methods

5.2.1 Sample collection and preparation

The trial was conducted at Kiboko Research Centre Organisation located in Makueni County, 170km from Nairobi in a semi-arid region in Eastern Kenya. The trial site was selected for being a hotspot for aflatoxin outbreaks in Kenya. The grain was prepared as explained in chapter 3 section 3.2. Maize grain used for this trial was analysed to have very minimal mycotoxin contamination. Maize was purchased from the maize farmers at the time of harvest, mixed, divided into low moisture (12%-13%) and high moisture content (14%-15%). The maize grain was transferred into the 10 different storage technologies and one half of the

low/high moisture grain was inoculated and the other was not inoculated with *Aspergillus* and *Fusarium* fungal strains.

About 100 grams of maize samples was ground from 1 kg of maize grain sampled and 25g required for both aflatoxin and fumonisin analyses.

5.2.2 Determination of levels of aflatoxin

Detection and quantification of aflatoxin levels in maize grains will be performed using VICAM (Milford, MA, USA) protocol (Vicam, 2013; Herrman *et al.*, 2014). Five grams of each ground maize sample will be placed in an extraction tube and 30 mL of Agua premix added. The mixture will be vortexed for 5 min and filtered through a 24 cm fluted filter paper (VICAM, Watertown, USA). A hundred micro litre of the Afla-V diluent was transferred to a strip test vial and 100 μ L of the sample extract added and vortexed for two minutes. A hundred micro litre of the mixture will be transferred to the Afla-V strip test at a flow rate of one drop per second vertically into the circular opening (Vicam, 2013). The strip tests will be allowed to develop for five minutes on a flat surface. Afla-V strip tests will be inserted into the Vertue reader (VICAM, Watertown, USA) for quantification of total aflatoxin in parts per billion (ppb) (Vicam, 2013; Herrman *et al.*, 2014).

5.2.3 Determination of fumonisin levels in maize grains

The levels of fumonisin in maize was determined using the VICAM method described by VICAM, (2012) and Atukwase *et al.*, 2009 with modification. Five grams from each finely ground maize grain sample was placed in an extraction tube and 10 ml of methanol/water (70:30) added. The mixture was vortexed for 5 min and filtered through a 24 cm fluted filter paper (VICAM, Watertown, USA). A hundred micro litre of Fumo-V Diluent was transferred to the strip test vial and 100 μ L of the sample extract added and vortexed for two minutes. A hundred micro litre of the mixture was transferred to the Fumo-V strip tests at a flow rate of

one drop per second vertically into the circular opening. The strip tests were allowed to develop for five minutes on a flat surface. Fumo-V strip tests were inserted into the Vertue reader (VICAM, Watertown, USA) for quantification of fumonisin in parts per million, ppm (Atukwase *et al.*, 2009).

5.3 Statistical analysis

The data collected for the four and eight months storage periods was analyzed using GENSTAT software and variances of the fumonisin and aflatoxin (x) results were evened out through log transformation [$Y = \log(x+1)$] while arcsine $Y = \sin^{-1}P$ was used to transform the percentage moisture data Y representing the results of transformation. The transformed data was 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 applied to test effects of treatment and storage duration, and the interaction effects. One-way ANOVA was performed to compare treatment outcomes at a specific point in storage time. Means were separated using Bonferroni adjustment at 95% confidence level (Hell *et al.*, 2014).

5.4 Results

5.4.1 Aflatoxin levels in maize at harvest

The initial mean aflatoxin levels in the maize was 1.7 ppb, values ranging between 0.7 ppb to 3.9 ppb and mean moisture content was found to be 14.94% ranging between 13% to 17% (Figure 5.2). Over 90% of the samples had higher than 13% moisture content indicating that farmers do not adequately dry the grain to the required storage moisture levels. This exposes the maize grain to fungal growth and therefore mycotoxin contamination.

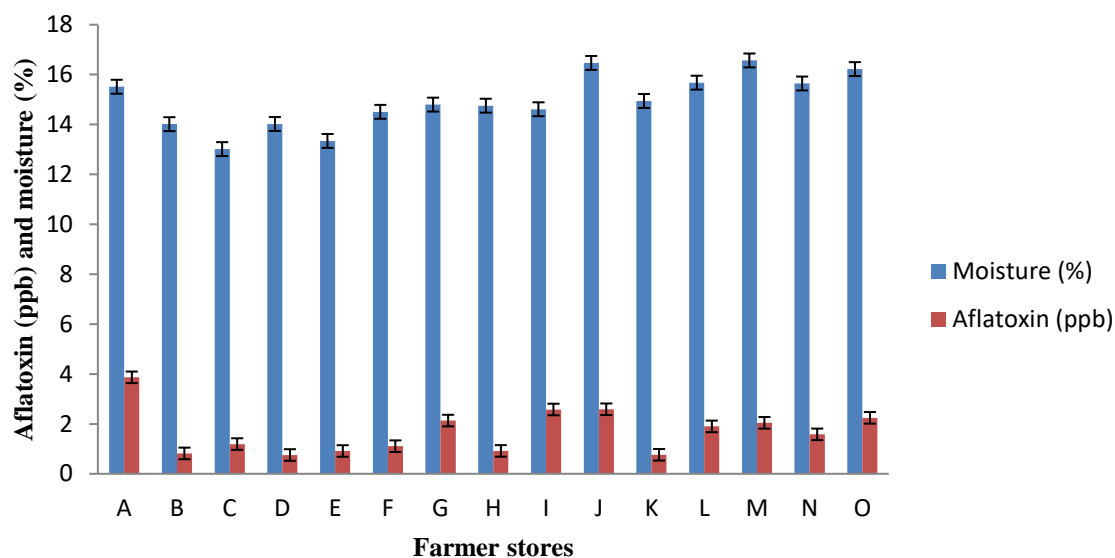


Figure 5.1: Relationship between moisture levels and aflatoxin contamination in maize used for the study. A – O represent the maize samples from different farmers’ stores.

5.4.2 Effectiveness of hermetic storage on aflatoxin contamination in maize grains subjected to different moisture levels and mould inoculation

Maize stored in polypropylene bags was 33.4% more contaminated with aflatoxin compared to samples stored in hermetic bags. By the end of eight months’ storage, aflatoxin levels varied greatly from undetected to as high as 20.5 ppb in polypropylene bags and were not significantly high in hermetic bags at $p > 0.05$.

The aflatoxin accumulation was lower in the dry maize grains and higher in grains with high moisture regardless of the mode of inoculation but still higher in the inoculated grains and (Figure 5.2) in all the respective treatments. At four months the difference between aflatoxin contamination in the inoculated maize grain with low M.C and that in grain not inoculated conditioned at low M.C was high (2.88 ppb). However, there was a slight difference at month eight (0.46 ppb) between the aflatoxin contamination in the inoculated maize grain with high moisture and that in the maize grain not inoculated at high M.C (Figure 5.2).

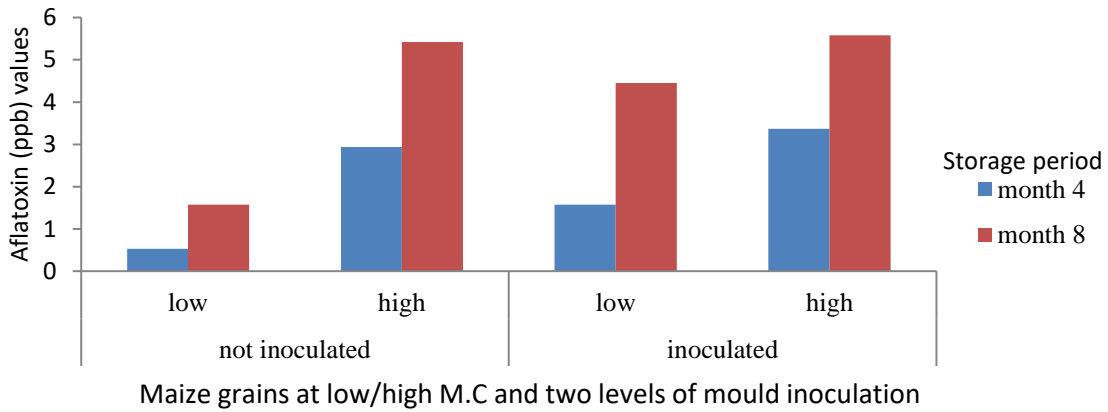


Figure 5.2: Efficacy of technologies, relative humidity (low and high) and inoculation on aflatoxin contamination.

PICS, Agro-Z+, and Agro-Z in that order had the minimum aflatoxin accumulation in the eight months storage period (1.71 ppb, 3.01 ppb, 3.35 ppb respectively), however maize grains in zerofly, super grain and elite bags accumulated more aflatoxins (3.68 ppb, 3.71ppb and 4.36 ppb respectively). The silos were not as effective in managing the aflatoxin accumulation with means of 4.8 ppb in metal silos and 4.9 ppb in plastic silos. On the other hand, grains dusted with actellic powder had lower levels of aflatoxin with a mean of 5.7 ppb compared with the grains in PP bags without pesticides (9.3 ppb) containing up to 20.5 ppb in inoculated grains with high moisture (Table 5.2).

Table 5.2: Aflatoxin levels in maize grain during four and after eight months of storage in hermetic technologies and in woven bags

Technologies	Initial aflatoxin level	Low moisture	High moisture	Low moisture	High moisture
		Inoculated		Not inoculated	
Metal silo 4	1.7 ^a	1.90 ^{ab}	4.81 ^{bc}	0.85 ^a	0.90 ^a
Metal silo 8		4.85 ^c	4.75 ^{bc}	1.45 ^{ab}	7.20 ^e
Plastic silo 4	1.7 ^a	6.45 ^d	5.79 ^{bcd}	0.95 ^a	2.74 ^{bc}
Plastic silo 8		8.65 ^d	6.35 ^a	0.90 ^a	2.95 ^{bc}
PICS 4	1.7 ^a	0.11 ^a	1.96 ^a	0.01 ^a	0.40 ^a
PICS 8		0.00 ^a	5.10 ^c	1.65 ^{ab}	1.05 ^a
Super grain	1.7 ^a	0.71 ^a	2.58 ^a	0.29 ^a	1.64 ^a
Super grain		4.70 ^c	3.10 ^a	0.33 ^a	1.35 ^a
Agro-Z 4	1.7 ^a	0.00 ^a	1.20 ^a	0.01 ^a	0.70 ^a
Agro-Z 8		1.60 ^{ab}	2.50 ^a	2.95 ^b	0.00 ^a
Agro-Z+ 4	1.7 ^a	0.00 ^a	1.09 ^a	0.00 ^a	0.65 ^a
Agro-Z+ 8		2.00 ^b	5.60 ^{cd}	2.35 ^b	0.00 ^a
Elite 4	1.7 ^a	0.10 ^a	2.97 ^{ab}	1.15 ^{ab}	2.21 ^b
Elite 8		8.65 ^d	4.10 ^b	0.15 ^a	6.85 ^a
Zero fly 4	1.7 ^a	1.95 ^{ab}	3.45 ^b	0.23 ^a	0.20 ^a
Zero fly 8		2.85 ^{abc}	3.75 ^b	6.80 ^d	3.39 ^d
Standard woven 4	1.7 ^a	3.83 ^c	15.38 ^{ef}	0.94 ^a	5.0 ^{cd}
Standard woven 8		8.05 ^d	20.45 ^f	3.20 ^c	5.20 ^{cd}
Standard woven+ 4	1.7 ^a	0.83 ^a	6.15 ^{cd}	0.69 ^a	3.29 ^c
Standard woven+ 8		1.80 ^{ab}	10.00 ^e	2.10 ^e	6.15 ^d

5.4.3 Efficacy of hermetic storage and moisture levels on aflatoxin contamination in maize grains

Aflatoxin contamination increased with relative humidity in both hermetic and farmer practice storages at $P < 0.001$. The treatment type had a significant effect ($p < 0.001$) on the level of aflatoxin contamination. The effect of relative humidity on aflatoxin contamination was significant with the mean values of 3.07 ppb in the grains with high moisture content, 1.69 ppb in the just harvested grains and 1.05 ppb in the dry grains. Generally, aflatoxin levels increased from the initial levels by 19.5 % at month four and by 76.4 % at month eight. The grains with low moisture content only had an increase of 27.6 % and the levels almost doubled at 51.8 % in grains with high moisture (Figure. 5.3).

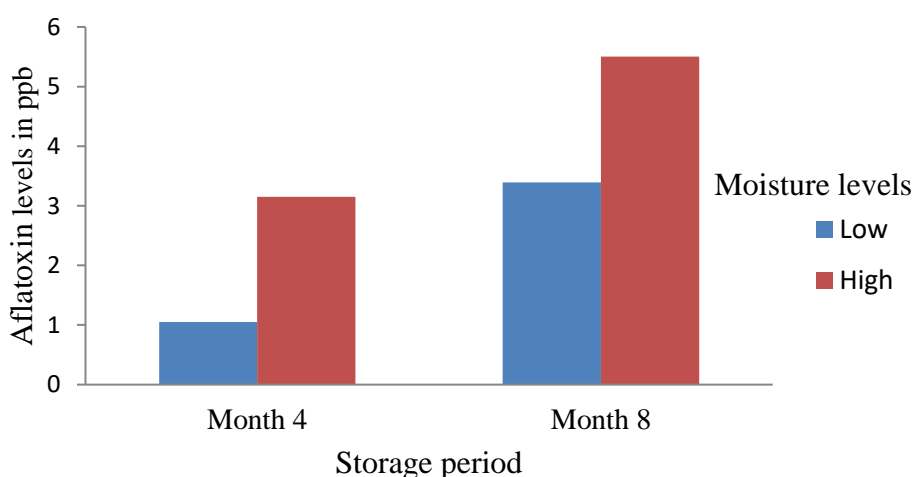


Figure 5.3: Moisture content (low and high) effect on aflatoxin contamination in maize grain at four months and eight months' storage periods.

The dry grains in metal and plastic silos were contaminated with aflatoxin to levels of 3.3 ppb and 6.6 ppb respectively and at high moisture levels, both silos had mean aflatoxin levels of 5.25 ppb. The hermetic bags registered lower aflatoxin contamination with the lowest mean observed in PICS bags at 0.08 ppb, 1.67 ppb in Agro-Z and 1.42 ppb in Agro-Z+ after four months of storage; the highest mean value (3.92 ppb) was observed in Elite bag, Zerofly

(3.33 ppb) and SGB (2.44 ppb). By the end of 8 months storage, aflatoxin had increased by 57.7% and the highest mean observed in Agro-Z+ with 5.8 ppb and the lowest in Agro-Z with mean of 2.7 ppb (Figure. 5.4).

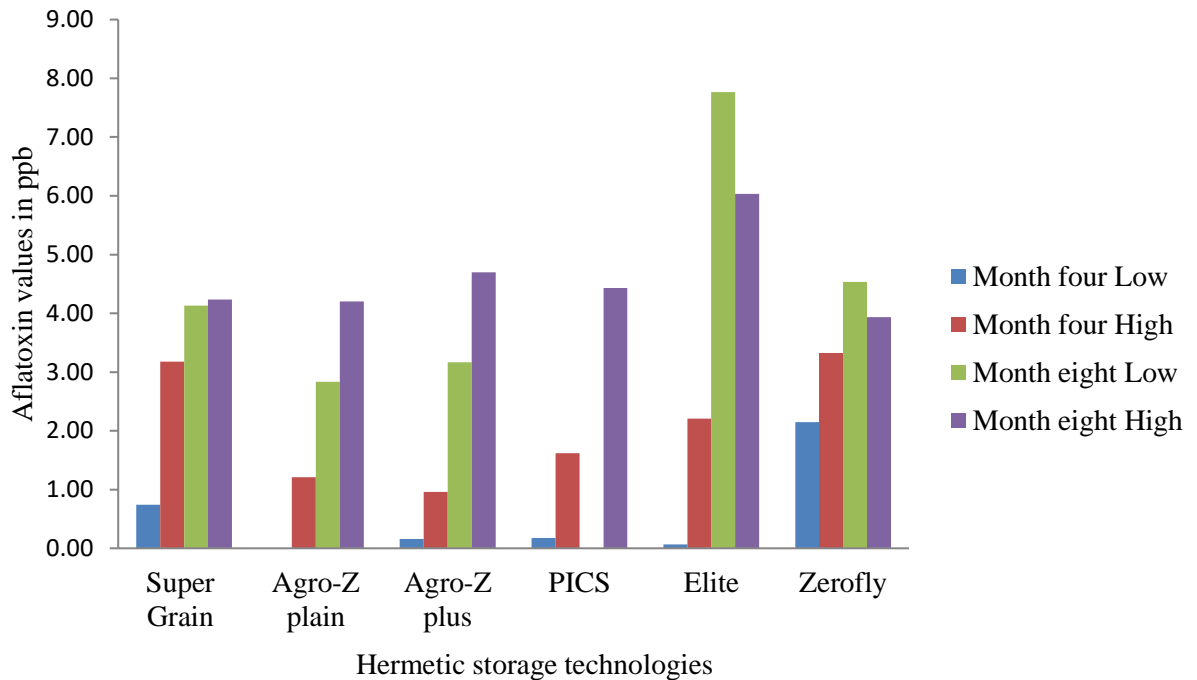


Figure 5.4: Effect of hermetic storage technologies against Aflatoxin contamination in maize grains subjected under low and high moisture levels at four and eight months of storage

5.4.4. Efficacy of hermetic technologies and level of inoculation on Aflatoxin contamination in maize

There was a general increase in aflatoxin contamination in the grains inoculated with the fungal strains. At four months, aflatoxin in grains without inoculums was at an average of 1.7 ppb and increased to 3.9 ppb in the eight months of storage. The contamination was even higher in grains inoculated with the fungi; the highest level of contamination (5.02 ppb) observed at eight months increasing from 2.5 ppb at four months. In general, the mean values of aflatoxin contamination assessed in all the technologies collectively were 2.59 ppb in inoculated grains and 1.65 ppb non-inoculated grains (Figure.5.5).

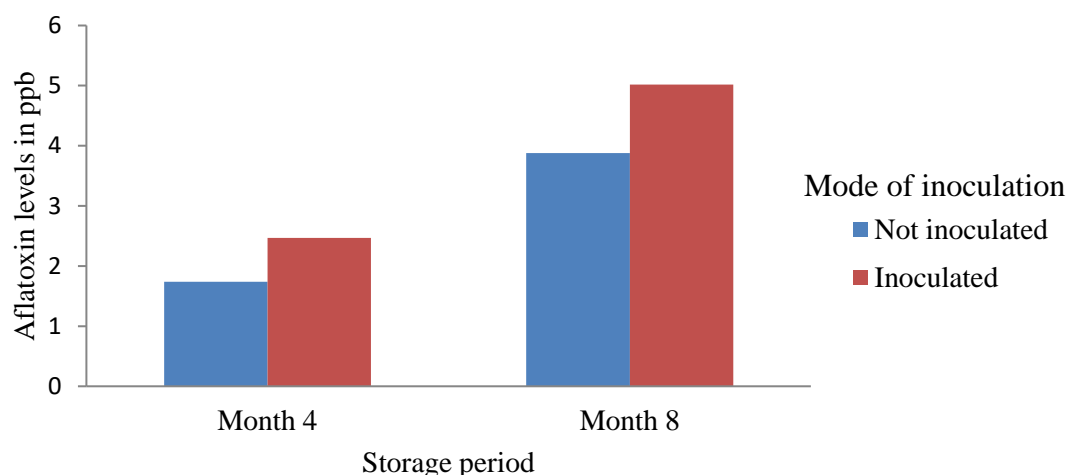


Figure 5. 5: Aflatoxin contamination in maize inoculated and not inoculated by mycotoxin producing fungi.

5.4.5. Effect of technologies, relative humidity and mode of inoculation on Fumonisin levels in maize grains

There were no significant differences ($P>0.05$) in the level of fumonisin across all the storage technologies. However, there was a correlation between moisture level and fumonisin contamination in both inoculated and non-inoculated technologies with inoculated technologies having a grand mean of 0.32 ppm while the non-inoculated technologies had a grand mean of 0.28 ppm. However, there was no significant difference ($p>0.05$) observed between treatment and the level of fumonisin contamination (Table 5.3).

Table 5.3: Effect of moisture, inoculation and different storage technologies on fumonisin contamination of maize grains.

Technologies	Inoculated*		Not inoculated*	
	Low moisture	High moisture	Low moisture	High moisture
Metal silo4	0.647 ^a	0.527 ^a	0.257 ^a	0.457 ^a
Metal silo8	0.33 ^a	0.3767 ^a	0.2967 ^a	0.3933 ^a
Plastic silo4	0.377 ^a	0.243 ^a	0.177 ^a	0.507 ^a
Plastic silo8	0.32 ^a	0.1967 ^a	0.1433 ^a	0.3667 ^a
PICS4	0.1567 ^a	0.373 ^a	0.107 ^a	0.157 ^a
PICS8	0.17 ^a	0.1967 ^a	0.3967 ^a	0.1733 ^a
Super grain4	0.24 ^a	0.373 ^a	0.423 ^a	0.107 ^a
Super grain8	0.3633 ^a	0.2233 ^a	0.21 ^a	0.1933 ^a
Agro-Z4	0.263 ^a	0.21 ^a	0.05 ^a	0.293 ^a
Agro-Z8	0.33 ^a	0.4367 ^a	0.1833 ^a	0.2567 ^a
Agro-Z+4	0.32 ^a	0.48 ^a	0.603 ^a	0.257 ^a
Agro-Z+8	0.18 ^a	0.4533 ^a	0.0833 ^a	0.2933 ^a
Elite4	0.303 ^a	0.647 ^a	0.227 ^a	0.343 ^a
Elite8	0.3733 ^a	0.4033 ^a	0.0133 ^a	0.2033 ^a
Zerofly4	0.07 ^a	0.213 ^a	0.073 ^a	0.44 ^a
Zerofly8	0.44 ^a	0.2867 ^a	0.1933 ^a	0.3967 ^a
Standard woven4	0.137 ^a	0.107 ^a	0.197 ^a	0.3433 ^a
Standard woven8	0.6 ^a	0.3033 ^a	0.3733 ^a	0.1367 ^a
Standard woven+4	0.2367 ^a	0.137 ^a	0.123 ^a	0.237 ^a
Standard woven+8	0.6 ^a	0.2267 ^a	0.2033 ^a	0.45 ^a

*Means within columns and rows followed by the same lower case letters are not significantly different at P<0.05.

There was no correlation between the aflatoxin and the fumonisin at P>0.05 (Table 5.4).

Table 5.4: Interaction between aflatoxin/fumonisin and RH, inoculation and treatments

Mycotoxin	Factors	P- Value	Corrected p-value
Aflatoxin			
	Relative Humidity	<.001	1.711
	Treatment	<.001	0.904
	Inoculation	<.001	1.032
Fumonisin			
	Relative Humidity	0.555	0.059
	Treatment	0.092	0.169
	Inoculation	0.413	0.069

5.5 Discussion

The grain moisture reduced with storage time, the greatest reduction noticed at 4 months storage time, and up to (20 %) reduction in woven bags. This is in agreement with (Vales *et al.*, 2014) whose trial on pigeon pea seed registered a reduction of up to 7.7 % moisture by eight months storage period in PICS bags. They also indicated the high possibility of fungal development and therefore mycotoxin contamination in seeds with higher moisture content due to the high relative humidity resident in these hermetic bags over a long storage time.

The high Aflatoxin levels in polypropylene bags could be attributed to retention of high moisture and heat (Nyukuri, 2007; Wagacha *et al.*, 2013) which favor fungal growth and aflatoxins contamination. A study by Domenico *et al.* (2016) reported the mean levels of total aflatoxins of 85 and 85.4 µg/kg in maize stored in hermetic and conventional bags, respectively. Overall, 90 % and 100 % of maize samples stored in hermetic bags in this study

met the Kenyan regulatory threshold of ≤ 10 ppb and FDA standard of ≤ 20 ppb for total aflatoxins. Hermetic bags effectively reduced aflatoxin levels by 55.3% after four months of storage which could be attributed to low O₂ content $< 3\%$ and elevated CO₂ levels in hermetic bags which hinder the growth of fungal and production of aflatoxins (Moreno-Martinez *et al.*, 2000). Hockings (2003) reported that carbon dioxide enrichment hinders Aflatoxin formation in the substrate. Studies by Bartosik *et al.* (2008) reported that the ability of *A. flavus* to produce aflatoxins in groundnuts was significantly reduced with the raise in CO₂ and decline in O₂ concentrations. This implies that the storage of maize in hermetic bags provided conditions that were unfavorable for fungal growth and aflatoxins contamination.

Aflatoxin was found higher in grains inoculated with the toxin producing fungi. This observation is in agreement with Cotty (2007), who described water activity as one of the conditions that encourage aflatoxins contamination. In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues, aflatoxins and insect contamination (Weinberg *et al.*, 2008).

The levels of fumonisin in all the maize samples obtained at harvest were less than 4 ppm. In a similar study, Bii *et al.* (2012) reported that the mean fumonisin content in maize samples from Makueni and Kitui Districts was 1.2 $\mu\text{g/g}$ and 0.9 $\mu\text{g/g}$, respectively. High levels of fumonism in woven bags could be attributed to large open spaces that allow for free flow of air. This is normally attributed to accumulation of heat and moisture which results in proliferation of fungal growth and hence mycotoxin contamination (James and Zikankuba, 2018, Quezada *et al.*, 2006 and Samapundo *et al.*, 2007) reported that the ability of *F. verticillioides* and *F. proliferatum* to produce fumonisin in maize stored in sealed bags was inhibited by high CO₂ concentration of 30 %. This modified atmosphere within the hermetic

bags as a result of oxidative metabolism by fungi, insect pest and the stored grain led to diminished O₂ and high CO₂ levels. The fumonisin levels in all the treatments were within the 4 ppm FDA maximum regulatory limits in maize grains meant for human consumption. Maize grain samples stored in polypropylene bags were 40 % more contaminated than samples stored in hermetic bags. About 93 % and 100 % of the maize stored for eight months in hermetic bags in this study met the European Commission and the US Food and Drug Administration threshold for total Fumonisin of ≤ 2 ppm and ≤ 4 ppm, respectively.

Hermetic technology works synergistically to promote conditions of limited oxygen and high carbon dioxide levels produced by aerobic metabolism of micro-organisms and grain respiration; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of mycotoxin contamination in stored maize (Williams *et al.*, 2014). Also, studies on PICS bags have shown that maize stored at moisture content of 10-13.5% have minimal levels of aflatoxin (Nganga *et al.*, 2016).

5.6 Conclusion

Eight months' storage was sufficient to indicate that hermetic bags were far superior in controlling aflatoxins and fumonisin contamination; however, maize with high moisture content was susceptible to aflatoxins contamination. Further studies may be useful in understanding the effect of hermetic storage in controlling mycotoxins for longer period of storage considering the same conditions.

CHAPTER 6: ASSESSMENT OF HERMETIC TECHNOLOGIES IN MANAGING NUTRIENT LOSS OF STORED MAIZE GRAINS

6.0 ABSTRACT

The World Health Organization recommends that an adult consumes 2000 Kcal/day and children to consume 3000 Kcal/day for normal functioning of the body. Maize contains 40% of the total calories required for an adult and 18% of the calories required by children. Both the aesthetic and nutritional quality of maize contributes to a wholesome and palatable meal. Strategies such as storage of maize in hermetic bags have been developed to reduce post-harvest fungal and mycotoxin contamination of maize. This study is, therefore to assess the synergy effect of hermetic storage to ascertain their effectiveness in conserving the grain quality and nutritional components in the grains in a safe and environmentally friendly system. All the treatments/technologies were subjected to two grain moisture levels, low (12-13 %) or high (14-15 %) and two levels of inoculation. Moisture content of the maize grains was determined by the standard methods. The technologies evaluated included, eight hermetic storage technologies and two controls with conventional, non-hermetic storage; the positive control (SWP⁺), and the other without insecticide, the negative control (SWP⁻). Grain samples of approximately 1kg were collected from each storage technology at 0, 4 and 8 months and analyzed for grain composition during the experiment. Insect infestation was evaluated by sampling 1000 kernels separating them into grains, insects and dust by sieving across a set of 4.7 mm and 1.0 mm aperture screens. The number of live and dead insects, both weevils and LGB counted and recorded.

It was found to contain the average nutritional values of fats as 4-5%, protein 8-9%, starch 71-75% and moisture ranged from 12-15%. Three factors were used in the design of this study: 1) artificial infestation of grain with mycotoxin producing fungi vs. natural infestation;

2) two levels of grain moisture levels; 3) Ten storage methods, of which eight hermetic. The overall experimental design is a 2 x 2 x 10 completely randomized block design (RCBD) with 3 replications. The findings indicated that maize nutritional and aesthetic values were affected by the mould proliferation, insect infestation and moisture levels in the maize grains over the twelve months of storage. Maize used for this study was collected from the farmers in Neissuit and Kigogo villages in Gilgil sub-county, Nakuru County and data on maize production practices recorded using a simple questionnaire.

The generated data from this study will facilitate sustainable adoption of the hermetic technologies among smallholder farmers in Sub Saharan Africa if these technologies can maintain the nutritional quality of the maize grains as is suggested in this study.

6.1 Introduction

Maize (*Zea mays* L.) can conveniently be classified as the most important cereal crop owing to its nutritional value and utilization of its by-products (Lee, 1999, Ranum *et al.*, 2014, Ekpa *et al.*, 2018). Maize is staple food to almost half the population of people in sub-Sahara Africa alone (CIMMYT, 2010) and for more than 90% Kenyans (Anankware *et al.*, 2013). It accounts for 40% of the total dietary intake in East and Southern Africa (Doss *et al.*, 2003). Maize has many uses; as poultry feed, livestock feed and in brewing industry to replace sorghum (Ranum *et al.*, 2014). Maize nutritional value is compared to that of rice, wheat and other cereals (Gijón-Hernandez *et al.*, 2008). It consists of the ideal nutrients required by the body i.e., Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g, (Nuss and Tanumihardjo, 2010) and minerals such as iron and phosphorus with the exception of essential amino acids- tryptophan and lysine (Iken *et al.*, 2002). Maize also provides nourishment to the fungi which produce toxic metabolites such as aflatoxin (Wagacha and Muthomi, 2008).

However, maize production in Kenya has been constrained due to factors such as high rates of disease prevalence like the Maize Lethal Necrosis Disease (MLND), human and animal population influx, changes in weather patterns, high post-harvest losses, high cost of value addition production and the new emerging pests such as the Fall Army Worm (FAW) (USDA, 2013; Wangai *et al.*, 2012). The environmental factors and variation in rainfall distribution are believed to be the most substantial abiotic factors that constrain maize production in Eastern Kenya (Omoyo *et al.*, 2015). Food security is threatened, reduced nutritional quality, and agricultural production due to the quality and safety issues resulting from fungal attack and mycotoxin contamination (Lewis *et al.*, 2005; Strosnider *et al.*, 2006).

Insect pests have been reported to provide ideal environment for mould growth through respiration, feeding activities and waste excretion. Insects also reduce the nutrient content of the maize as they tend to feed mainly on the germ which is known for high oil levels, they shed off their wings, legs and some die which cause discoloration and musty smell in the grain (Papanikolaou *et al.*, 2018; Hubert *et al.*, 2018). In favorable conditions, fungi could cause up to 80 % of damage on maize during storage period. If not handled and / stored properly to minimize growth and multiplication of these fungi, the grain damage is likely to proceed through the post-harvest stage (Abramson *et al.*, 1992, Kaaya and Kyamuhangire, 2006). The infection will thereby reduce the nutritional value and result in discoloration of the grain (Ehrlich *et al.*, 2007).

Conventional storage methods which result in up to 30 % loss as a result of insect pests and mycotoxins, force the smallholder farmers to sell off their grain soon after harvest at the time when prices are still low, only to purchase it back later at a costly price, hence being trapped in a vicious cycle of poverty (Tefera *et al.*, 2011). On-farm grain losses result in food

insecurity and negatively affect the farmers' livelihood income (Gitonga *et al.*, 2013; Ndegwa *et al.*, 2016; Mutambuki *et al.*, 2012).

Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal/plastic silo provides affordable and more effective storage alternative for farmers, especially the vulnerable women, that would markedly contribute to food security (Gitonga *et al.*, 2013; Obeng-Ofori, 2011b; Ndegwa *et al.*, 2016; Mutambuki *et al.*, 2012). There have been many studies about the effectiveness against post-harvest pests in hermetic technologies in SSA but little information exists on the mould and insects effect on the nutritional quality of the grain with regards to hermetic technologies. Hermetic bags are now widely used in West and Central Africa for the storage of pulses such as cowpeas (Moussa *et al.*, 2014). Research on effectiveness of hermetic bags has been extended to other crops such as maize (Ognakossan *et al.*, 2013b); (Murdock and Baoua, 2014). However, adoption of hermetic storage technologies in Kenya is still very slow. A great number of farmers store their grain in woven polypropylene bags with no barrier to air yet there is evidence that this method facilitates fungal contamination (Udoh *et al.*, 2000b, Hell *et al.*, 2000). The information generated from this study will aim at sustainable adoption of the hermetic technologies among smallholder farmers in Sub - Saharan Africa.

Hermetic bags have been known to preserve the quality of grain, appearance and aroma by reducing mould growth (Moussa *et al.*, 2014). Hermetic technology works synergistically to promote conditions of low oxygen and high carbon dioxide levels produced by aerobic metabolism of insects, micro-organisms and grain respiration. Aerobic metabolism uses up oxygen and produce carbon dioxide to levels that are lethal to insects and moulds in the grain mass (Navarro *et al.*, 2007; Yakubu *et al.*, 2011). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality

products that are free from chemical residues and insect contamination (Weinberg *et al.*, 2008).

Hermetic bags provide modified atmospheric conditions that suppress the growth of moulds and insect activities; a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects in stored maize (Williams *et al.*, 2014). However, grain stored over long months in hermetic bags is likely to produce and increase the moisture levels leading to mould production hence fungal infection. Some findings reported that under hermetic storage, fungal static effect is included when oxygen concentration drops to 1% or below (Murashiki *et al.*, 2018). Also, studies on PICS bags have shown that maize stored at moisture content of 10-13.5% have minimal levels of fungal infection (Nganga *et al.*, 2016).

To move forward in mitigating post-harvest losses in maize from mycotoxin contamination and insect infestation, it will demand the detection and eradication of the limitations to the usage of a particular technology. Consequently, safe on-farm storage of maize is vital in improving food and income security for the smallholder farmers (Maria, 2011). The effect of modified atmospheres could significantly control fungal contamination in stored grains and reduce the incidence for insect infestation. Elevated concentrations of CO₂ of >75% inside the hermetic bags are essential in prevention of growth of mycotoxigenic fungi in relatively dried maize. Hence, of late more importance has been specified by CIMMYT on hermetic storage. Report on “Missing Food” in 2011 by World Bank indicates that there is a substantial lack of adoption of modified grain storage systems in Africa (Villane *et al.*, 2012). There are many factors that contribute to low adoption including lack of information on the existing storage systems and their effectiveness. Outside Africa the effectiveness of hermetic storage at both small and commercial scales has been well researched and documented. (Quezada *et al.*, 2006). Metal silo research has gained more prominence in recent years in

regard to hermetic storage as an option for grain storage method in Africa (Tefera *et al.*, 2011; Murdock *et al.*, 2012; Baoua *et al.*, 2013; de Groote *et al.*, 2013; Guenha *et al.*, 2014). Hermetic storage containers utilization is still low in SSA because of the knowledge gaps about their performance *Visa* via the conventional storage methods of using insecticides. In addition, studies have been done only for the short term hermetic storage and have not compared the performance of hermetic technologies with the conventional synthetic pesticides and their effectiveness against mould proliferation and insect infestation (Baoua *et al.*, 2013; de Groote *et al.*, 2013; Ognakossan *et al.*, 2013; Baoua *et al.*, 2014). In as much as the trend is changing, the previous storage pest management initiatives were aimed at the use of synthetic pesticides (Mvumi and Stathers, 2003; Collins, 2006). The storage of husked and unhusked or shelled and unshelled maize is not uncommon among small-holder farmers in Africa. Storage of maize on the cob with the husk intact provides protection to grain against insect pest infestation and mould infection (Hell *et al.*, 2008). Traditional storage structures used by farmers for on the farm storage include containers made of plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet. Essentially the stores are constructed to prevent insect and rodent attack and to prevent moisture from getting into the grains. Maize is subjected to several kinds of treatments prior to storage. Traditionally, stored maize is protected against damage by mixing with ash from cooking fire, sand or leaves from certain plant (Hayma, 2003). Cobs may be exposed to smoke and heat from kitchen fire or, when outside the house from a fire underneath the main structure to facilitate drying and disinfect the maize from destructive biotic agents such as insects, mites, and fungi (Udoh *et al.*, 2000).

This study is aimed at evaluating the effect of hermetic storage technologies in preserving grain quality and nutritional value of the maize grains with regard to the moisture levels, mode of inoculation and insect infestation in the twelve months of storage.

6.2. Study design

Purposive sampling of maize was done from five cardinal points in the storage technologies using a sampling spear. A representative sample from the collected sample was taken to the University of Nairobi – Kabete Nutrition laboratory for proximate analyses. Three factors were used in the design of this study: 1) artificial infestation of grain with 1 Kg mycotoxins vs. natural infestation; *Aspergillus parasiticus* and *Aspergillus flavus* of S and L-strains and inoculums of Fumonisin; *Fusarium verticilloides* and *Fusarium proliferatum* 2) two levels of grain moisture levels [low (12-13%) and high (14-15%)]; 3) Ten storage technologies. The overall experimental design was a 2 x 2 x 10 completely randomized block design (RCBD) with 3 replications. Sampling was done at 0, 4, 8 and 12 months durations. The grain composition and quality was compared against the moisture levels, the mode of inoculation and insect infestation considering the stages/frequency of sampling.

6.3. Data collection

6.3.1 Sample collection and preparation (as explained previously)

1. Moisture analysis
2. Grain composition analysis
3. Proximate analysis
4. Germination assessment

6.3.2 Grain Composition Analysis

One hundred and twenty maize samples of about 100g ground kernels was analyzed using AOAC analytical method.

6.3.2.1 Oil analysis (ether extract) (AOAC, 2009)

A sample of 15 g of maize was crashed in a mortar of which 2 g of fine flour was weighed and placed in a Soxhlet thimble. Ether was heated at 105°C in an oven for one hour and volatilized, then condensed and allowed to pass through the ground maize carrying ether soluble materials along. This process was repeated over and over until no more extractable material remained in sample (Thiex., 2009).

6.3.2.2 Determination of crude protein- macro unit (AOAC 2009)

The Nitrogen or protein and other organic compounds were transformed into sulphate by acid digestion with boiling concentrated Sulfuric acid and a catalyst. When digestion was complete the acidic sample solution was cooled, diluted with water and neutralized with strong Sodium Hydroxide. The ammonia was released and distilled into a boric acid solution. The boric acid solution was titrated with standardized hydrochloric acid from where the amount of nitrogen was determined. This was multiplied by 6.25 (conversion factor) to correct to protein quantity (Thiex., 2009).

6.3.2.3 Starch analysis

Starch was analyzed using the Southgate (AOAC 2009) method where samples 30% of ethyl alcohol was used to extract followed by hydrolyzing sugars in the maize samples. Dubiss Phenol Sulfuric acid was used to estimate the amount of sugar in the alcohol extract and the sugar in the acid hydrolysate was estimated using the anthrone method and the value multiplied by a factor (0.9%) to obtain the percentage value of starch (Hall.,2015).

Three reading of each parameter were taken and average recorded.

6.4 Statistical analysis

Data was collected every four months and was analyzed using GENSTAT software. The proximate and germination data was analyzed using one way repeated measure ANOVA to make a comparison of grain moisture/level of inoculation with starch, protein and oil values (Ognakossan *et al.*, 2014).

6.5 Results and interpretation

6.5.1 The effect of hermetic technologies on moisture levels in maize grains stored at high moisture levels for a period of four months.

The moisture levels at four months were generally high in all the technologies with a grand mean of (14.8 %) except for The highest moisture level was observed in the grain inoculated in Elite bags with a mean average of (15.8 %) and the lowest in the grain that was not inoculated in A-Z bag at (13.7 %). Generally, grain inoculated did maintain a significantly high moisture level, of (15 %) and above compared with the maize grain which was not inoculated registering slightly less than 14% moisture content. In these technologies, we observed that the grains contained moisture levels with a mean grade of (12.70 %). The moisture levels were within our minimum base value range from (12 %) to (13 %) but we need to point out the fact that the inoculated grains contained more moisture with the maximum being (13.51 %) in relation to the grains which had (12.52 %) as the highest moisture level. Plastic silo grains contained the least amount of moisture at a level of (11.59 %) (Figure 6.1). The moisture content was significantly low with a mean level of (11.7 %) which is lower than our minimum base value of (12 %) in all the technologies used. The inoculated grains in the woven bags retained a significantly higher moisture level of (12.3 %) which was within the conditioned range as opposed to the non-inoculated grains in the woven pesticide bags which had the lowest value of (11.4 %) moisture retention. The moisture

content in the dry maize grain stored in woven bags was the same value as the maize originally conditioned at high moisture with an average mean of (11.7 %) less than the base value. It was observed that the non-inoculated grains that were stored in woven bags had no significant difference with the inoculated maize at (11.82 %) and (11.37 %) moisture content respectively. The moisture content was significantly high above (15 %) in all the technologies used. We observed that the inoculated grains had higher moisture content than the grains that were not inoculated. The moisture levels under these hermetic technologies had a grand mean of (13.04 %) which was merely higher than our base level. We observed that both inoculated and non-inoculated grains had a moisture level slightly above (12 %). The variation was minimal with the highest being non-inoculated grains in metal silo with (14 %) and the lowest being Agro-Z+ bag with 12 % moisture content. In PP bags, we observed moisture levels had a mean level of (11.48 %) across the technologies used. The inoculated pesticide dusted maize had a moisture level within our base value range of (12 %) to (13 %) which is also the lowest level in this experiment. The inoculated grains generally had higher moisture levels. Also, the non-inoculated maize stored in PP bags recorded the lowest moisture level of (10.8 %). We observed a significant variation in the moisture content with a grand mean of (11.69 %) which was slightly below our base value. However, we observed that both inoculated and non-inoculated grains, the insecticide dusted grains had higher moisture levels recording a maximum high of (12.52 %). Whereas the grains stored in woven bags had low moisture levels with a minimum of (10.8 %) (Figure 6.1).

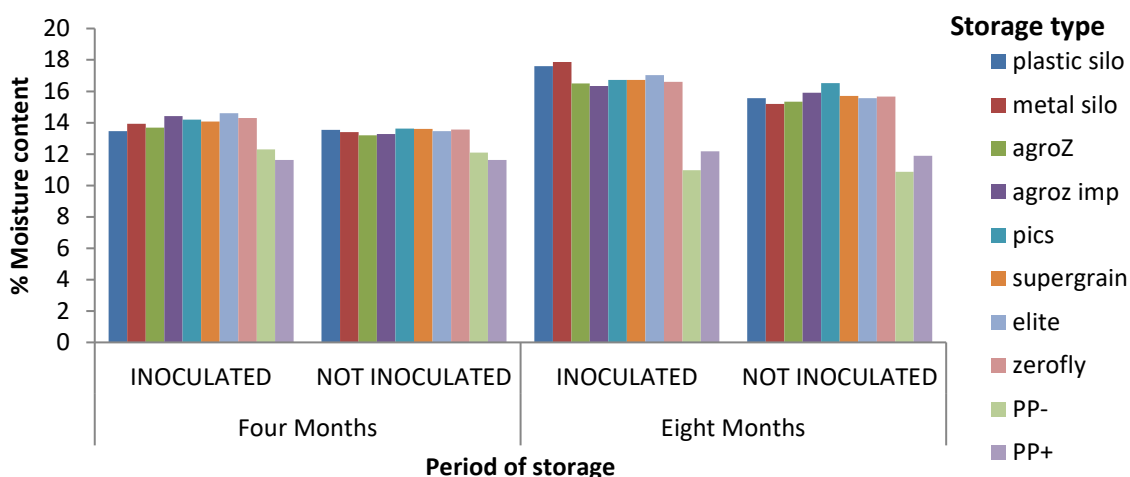


Figure 6.1: Effects of hermetic technologies on moisture content

6.5.2 The effect of hermetic technologies on oil content in maize grains.

Generally, oil content in all the technologies was lower than the base value with a grand mean of (4.8 %) at four months. It was observed that only Agro-Z bag had retained oil content higher than the base value of (4.9 %) (Figure 6.2). Generally, the oil levels in these grains were a bit higher than our base value as we got a grand mean of (4.99 %) with these technologies. There wasn't much variation in the oil content levels between the inoculated and the non-inoculated grains except for the non-inoculated grains in Agro-Z+ bag which had the highest level of oil at (5.28 %) and the inoculated grains in Elite bag where the oil levels were lowest at (4.78 %). The oil levels did not significantly differ at eight months of storage in all the hermetic storage technologies with a mean value of (4.7 %). The level of inoculation did not affect the oil content, $p > 0.001$. The technology that preserved oil the most was identified as PICS bags with a mean of (4.9 %). However, the oil level in SGB bag varied significantly with (5.9 %) mean value in inoculated grains and (4.7 %) in the grains not inoculated. The oil content in the maize grains stored in hermetic bags varied greatly with a mean value of (4.93 %) which is slightly higher than the base value of (4.9 %). We observed that the non-inoculated grains in plastic silo, Agro-Z+, metal silo, Elite, and zerofly

bags were higher with a maximum of (5.26 %) than the other technologies. From our observation, the inoculated maize stored generally had oil content less than the base value.

In PP bags, the oil levels significantly differed at four months of storage in the grains with a mean value of (4.8 %). The non-inoculated pesticide dusted grains in the woven bags retained the highest oil content level of (5 %) which is slightly higher than our base value of (4.9 %). We observed that the non-inoculated grains had more oil content than the inoculated grains in the PP bag technology which had a minimum level of (4.7 %) oil content. The oil content in dry maize grains had a mean value (5.1 %) which was higher than the base value of (4.9 %). The non-inoculated grains in PP bags had the least amount of oil with 4.97% (Figure 6.2). We observed a grand mean of (4.5 %) of oil content across the technologies used, which was significantly lower than our base value. The non-inoculated pesticide dusted maize grains stored in woven bags had an oil content of (4.9 %) which is just at our base value level. The inoculated grains stored in woven bags had significantly low oil content with a minimum of (4.2 %) (Figure. 6.2). The oil content generally was below the base value at a grand mean of (4.72 %). It was observed that the pesticide dusted grains stored in woven bags had a higher level of oil content with a maximum of approximately (5.02 %) in the non-inoculated grains. We also observed that the grains stored in PP bags recorded the least oil content level of (4.52 %) in the inoculated grains (Figure 6.2).

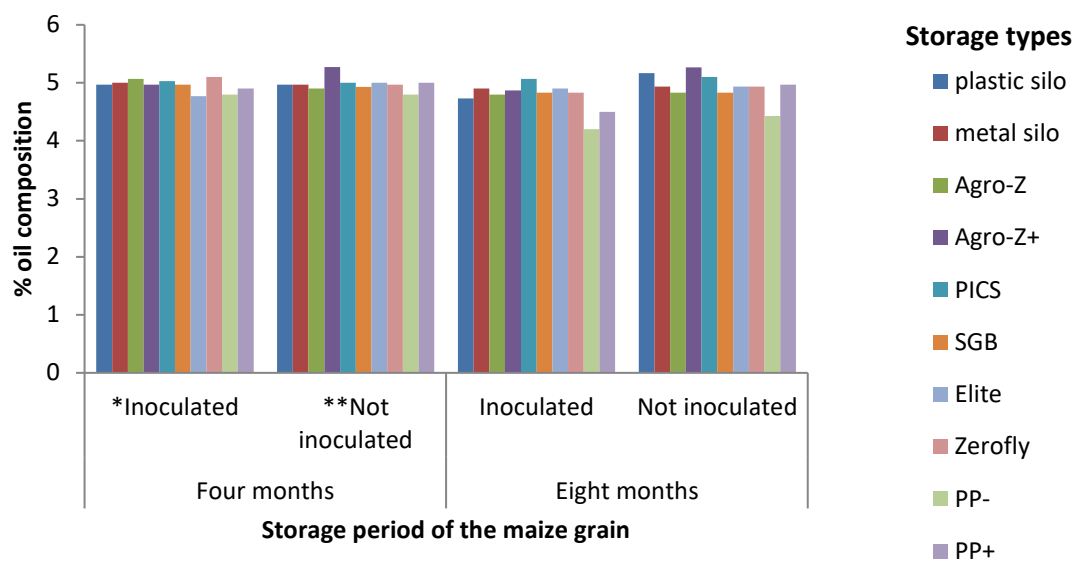


Figure 6.2: Effect of hermetic technologies on maize grain oil content

*Maize inoculated with mycotoxin producing fungi

6.5.3 The effect of storage technologies on Protein content in maize grains

At 4 months we observed a grand mean of (8.58 %) protein content which was slightly higher than the base value of (8.5 %). At four months, there was no significant variation in the protein levels in all these hermetic technologies with a grand mean of (8.45 %) which is lower than our base level required. We noticed that only the inoculated grains in Agro-Z had protein levels of (8.5 %) and the non-inoculated grains in Bag 3 had the highest protein content levels of about (9.8 %). Besides PICS and SGB bags, the inoculated grains in all the other technologies retained a higher level of protein (Figure 6.3). The grains that were not inoculated in plastic silo, Agro-Z+, and Zerofly bags had protein content less than the base value while the rest of the technologies retained slightly higher protein content.

At 4 months of storage, the protein content in maize grains stored in PP bag technologies at low moisture levels was approximately average with a grand mean value of (8.5 %) just in line with our base value. From our results we observed that the protein content in inoculated

** Maize not inoculated with mycotoxin producing fungi

maize grains stored in woven bags was significantly high to a maximum level of (8.8 %). The non-inoculated pesticide dusted grains stored in woven bags had the least protein content of (8.3 %) (Figure 6.3). It was observed that the protein levels in woven bags were slightly above the base value at a mean value of (8.6 %). The inoculated grains stored in woven bags had the highest protein content at (8.8 %) while the non-inoculated grains stored in woven bags had the lowest levels of protein at (8.2 %). There was minimal difference in the protein levels of pesticide dusted grains stored in woven bags. The oil levels did not significantly differ at eight months of storage in all the hermetic storage technologies with a mean value of (4.7 %). The level of inoculation did not affect the oil content, $p>0.001$. The technology that preserved oil the most was identified as PICS with a mean of (4.9 %). However, the oil level in SGB varied significantly with (5.9 %) mean value in inoculated grains and (4.7 %) in the grains not inoculated. The oil content in the maize grains stored in hermetic bags varied greatly with a mean value of (4.9 %) which is slightly higher than the base value of (4.9 %). We observed that the non-inoculated grain in plastic silo, Agro-Z bag, metal silo, Elite bag, and zerofly bags were higher with a maximum of (5.26 %) than the other technologies. From our observation, the inoculated maize stored generally had an oil content less than the base value. At 8 months of storage, the protein content in maize grains stored in hermetic technologies at high moisture levels significantly varied with a grand mean value of (8.7 %). From our results we observed that the protein content in maize grains stored was within the acceptable range of not less than (8.5 %). However, we observed that the grains that were not inoculated retained more protein as opposed to the inoculated grains. The protein content was at a mean level of (8.6 %) slightly higher than our base value. It was observed that the protein levels in the inoculated grains did not show much variation as PICS bag and plastic silo recorded a maximum high of (9.2 %). It was also observed that the non-inoculated grains retained a higher protein level with a maximum of (9.7 %) and a minimum of (8.25 %)

(Figure 6.3). Protein levels were higher than base value with a grand mean level of (8.7 %). All the grains stored in PP bags recorded high protein content with a maximum height of (9.2 %) and a minimum of (8.8 %). However, the pesticide dusted grains both inoculated and non-inoculated had a low protein content of approximately (8.4 %). The protein levels were considerably higher than the base value at a grand mean of (8.7 %) with these technologies. However, the grains in the woven bags, both inoculated and non-inoculated had higher level of protein content with a maximum high of (9.2 %). The pesticide dusted grains had the lowest protein content with a minimum low of 8.4% (Figure 6.3).

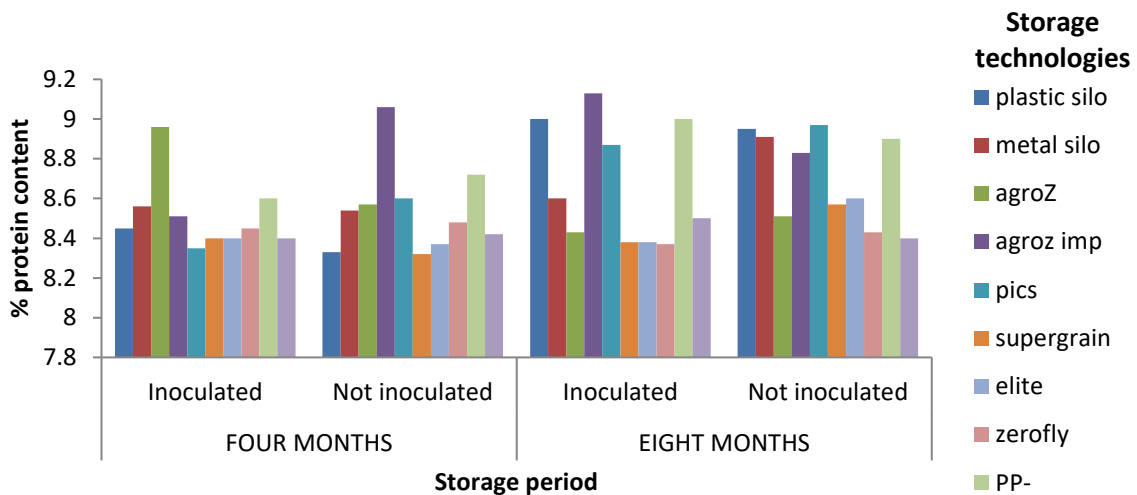


Figure 6.3: Effect of hermetic technologies on protein content in maize grain

6.5.4 The effect of hermetic technologies on Starch content in maize grains.

Generally, the starch levels in the hermetic technologies were slightly less than our base line value with a grand mean value of (71.3 %). We observed that all grains in plastic silo and metal silo retained starch levels higher than the base value reaching a maximum of (72.5 %). The non-inoculated grain in Agro-Z bag and the inoculated grain in PICS bag also had a starch content of (71.6 %) which was slightly higher than our base value. The rest of the technologies had starch content less than our base value (Figure 6.4). Starch content in the maize grains varied greatly within the different hermetic technologies even though the grand

mean level was (70.8 %) across the graph. We found the starch levels to be lower than the base value of (71.4 %). Even with a mean grade of 71.7 %, the starch content in the maize grains varied greatly within the different hermetic technologies. It was observed that the inoculated grains under metal silo, Agro-Z, Agro-Z+, SGB, Elite, and Zerofly technologies retained significantly lower starch level below the 71.4 % base value. It's only plastic silo and PICS bag that retained starch levels above base value. The grain that was not inoculated under the Plastic silo, metal silo, Agro-Z, and Agro-Z+ hermetic technologies retained a higher level of starch above the baseline value. There was no significant difference ($P>0.05$) between the mean grade and the baseline value (Figure 6.4). In this experiment, the mean level of starch content across the hermetic technologies was (17.7 %). We observed quite a variation in the starch content levels in both the inoculated the non-inoculated grains despite the fact that they were generally below the base value. The inoculated grains had more starch levels recording a high of (71.2 %) and the non-inoculated grains recording a low of (69.2 %) starch content.

The starch levels were significantly low ($P<0.05$) at four months of storage in the grains with these technologies with a mean value of 70.83 %. The inoculated grains in the woven bags retained the highest oil content levels of 71.25 % which is still lower than our base value of 71.4 %. We observed that the non-inoculated pesticide dusted grains stored in woven bags had less starch content of 70.25 %. In general, the non-inoculated grains were seen to retain the least amounts of starch in this technology. It was observed that the starch levels were considerably low with a mean level 70.44 % which is lower than the base value of 71.4 %. The non-inoculated grains stored in woven bags had 71 % starch content and the non-inoculated pesticide dusted grains were found to contain the least amount of starch at 70 %. The starch levels were generally lower than the base level with a grand mean of 70.80 %. However, the inoculated grains had higher starch content above 71.2 %. It was observed that

the non-inoculated pesticide dusted grains had a higher starch level 70.52 % and the non-inoculated grains stored in woven bags had the lowest level of starch which was 70 %. The starch content was at a mean level of 70.29 % which was lower than the base value. The pesticide dusted inoculated grains had the highest level of starch content with 70.82 %. The inoculated grains stored in woven bags had the lowest starch content level of 69.85 %. The non-inoculated grains had the same starch content level of 70.7% (Figure.6.4)

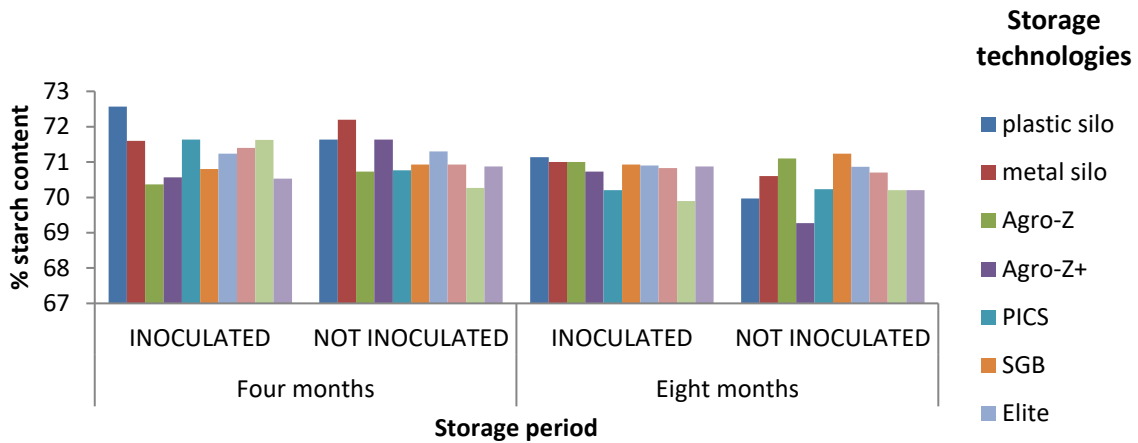


Figure 6.4: Effect of hermetic technologies on Starch content in maize grain.

6.5.5 The effect of storage technologies on germination of grains stored for a period of four months.

It was observed that the hermetic technologies had diverse effects on the maize grain's ability to germinate at the end of the storage period of four months. We observed the germination rate was lower than our base value of 89 % and that we had a grand mean germination rate of 22.3 %. To be specific, we observed that the germination rate sequentially was; non inoculated grains stored at low moisture levels - 32.87 %, inoculated grains at low moisture levels – 27.3 %, non-inoculated grains at high moisture levels – 17.4 %, and inoculated at high moisture levels – 11.5 %.

The non-inoculated under low moisture conditions generally germinated most, followed by the inoculated grains under low moisture conditions, followed by the non-inoculated grains under high moisture conditions, and finally the inoculated grains under high moisture conditions (Figure 6.5). The grains that were subjected to the PICS bag, Plastic silo and Metal silo germinated the most with a peak of 40 % germination rate. Inoculated grains subjected to Plastic silo under high moisture conditions germinated the least with 2 %. The pesticide dusted grains stored in woven bags had a higher germination rate with the inoculated grains

stored under low moisture conditions having a 34 % success level and a minimum of inoculated grains under high moisture conditions whose rate was 25 %. The non-inoculated grains stored in the woven bag under low moisture had a yield of 30 % and inoculated grains under low moisture had the least germination rate of 17 %.

In general, the germination rate dwindled sequentially starting with the non-inoculated grains under low moisture conditions, then came the inoculated grains under low moisture conditions, followed by the non-inoculated grains under high moisture levels and finally, the inoculated grains under high moisture conditions. We observed that non-inoculated grains with PICS bag, under low moisture conditions had the least failure rate of 10 % and the inoculated grains with Plastic silo under high moisture conditions had the highest germination failure rate of 48 %.

The grains stored in woven bags had a higher failure rate with the inoculated grains stored under low moisture conditions having 33 % un-germinated grain and a minimum of non-inoculated grains under low moisture conditions whose rate was 20 %. The pesticide dusted inoculated grains stored in the woven bag under high moisture had a 25 % germination failure rate and inoculated grains under low moisture had the least germination rate of 16 %.

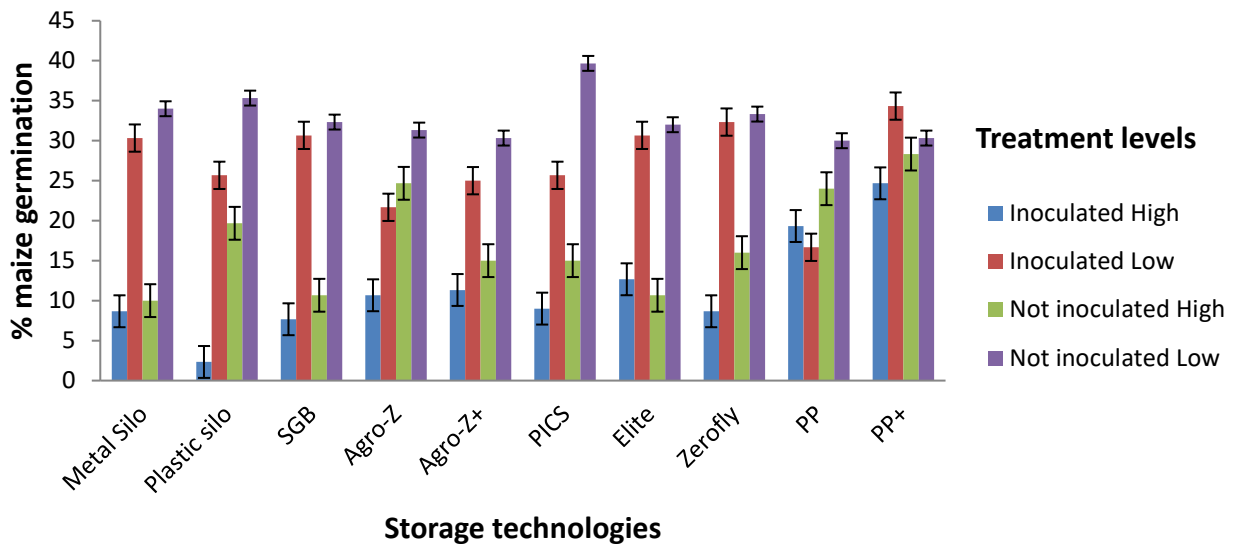


Figure 6.5: Effect of storage technologies and storage duration of four months on germination of maize grain

6.5.6 The effect of storage technologies on germination of grains stored for a period of eight months

After eight months of storage under the hermetic technologies, the maize grain's ability to germinate was almost insignificant as we observed an 82.8% grand mean un-germination rate which is so far from the desired 89 % germination rate. The specifics revealed a sequence of failure ranging from the inoculated grains stored at high moisture levels (97.5 %), to non-inoculated grains at high moisture levels (94.8 %), to inoculated grains at low moisture levels (75.5 %), and finally, non-inoculated grains at low moisture levels (63.6 %).

We generally observed a very high failure rate of germination especially in the inoculated grains stored at high moisture levels in the hermetic technologies with a peak of 100 %. The non-inoculated grains in PICS bag under low moisture conditions were seen to have the lowest germination failure rate of 40.7 %.

In the PP bags, grain stored in plain woven bags had an extremely high germination failure rate 98.3 % to 100 %. The pesticide dusted inoculated grains stored in the woven bag under high moisture had a 76 % germination failure rate whereas inoculated grains under low moisture had the least germination failure rate of 46.3 %.

Even though the germination rate across the technologies was generally low after eight months of storage, it was observed that the non-inoculated grains had fairly high germination rates with grains in PICS bag stored in low moisture conditions having the highest germination rate of 59.3 % and metal silo with 17 % success (Figure 6.6). The non-inoculated grains stored in high moisture conditions had the lowest germination rate with only PICS bag and plastic silo having 10 % and 5 % germination rate.

The pesticide dusted grains stored in woven bags had a higher germination rate with inoculated grains stored under low moisture conditions having the highest germination rate of 53 % and the inoculated grains stored under high moisture conditions having a 23 % as the minimum rate. Grains stored in plain woven bags had almost no germination with only the inoculated grains stored at low moisture levels giving a 1.7 % success rate in germination.

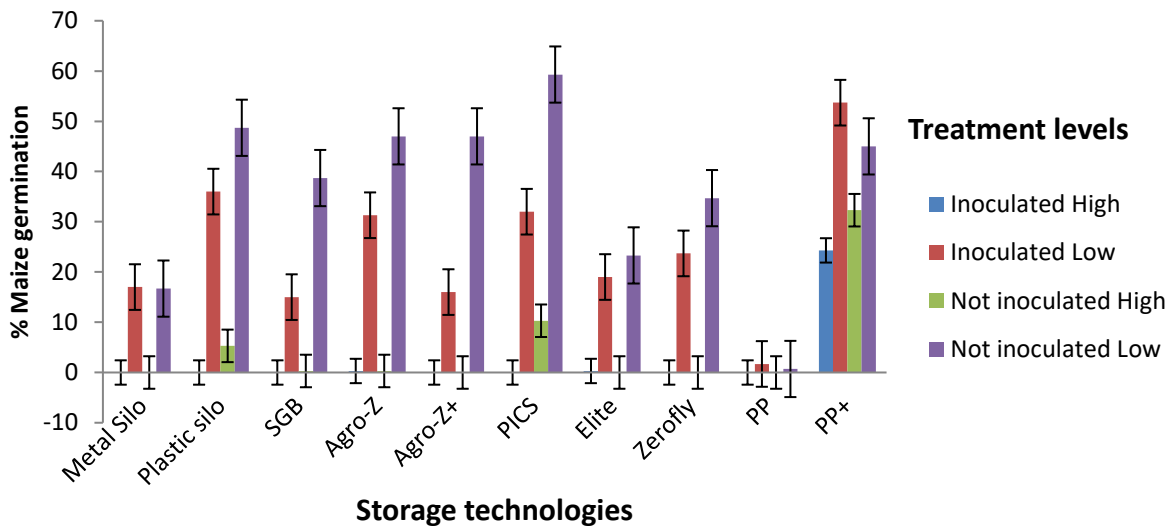


Figure 6.6: Effect of storage technologies and storage duration of four months on germination of maize grain

To sum it up, the pesticide dusted grains in the woven bag technology and grains stored in the PICS bag technology had the highest germination rate across the technologies used in this study for a period of 240 days.

6.6 Discussion

The storage technologies in this study were subjected to the same conditions to simulate the storage environment of the Kenyan farmers where the maize grain is stored after harvesting and aggregation. At play, the storage conditions such as humidity and temperatures were not controlled. Such variations are important to be able to grapple with the varying conditions under which grains are stored by the Kenyan farmers and this study enabled us to effectively judge how these technologies will perform in these areas.

The oxygen depletion and carbon dioxide accumulation were likely due to the fungal infection and insect infestation (Walker *et al.*, 2018; Bhandari *et al.*, 2017; Di Domenico *et al.*, 2015). These organisms together with biological activities in maize use up oxygen and produce carbon dioxide therefore modifying the storage environment as reported by (Ognakossan *et al.*, 2013a).

Initial moisture levels of the grain ranged from (9.8 %) to (17 %) indicating that farmers do not have a standard measurement of extent of drying before storage. In the earlier months, hermetic bags retained grain moisture far better than the woven bags in the same environmental conditions. This evidence has been identified in a study conducted by (Baoua *et al.*, 2014) where moisture content in PICS bags was retained in maize grain stored under varying environmental conditions. However, in this study the moisture levels in hermetic bags increased by 1 % as storage time progressed.

The maize used for this study had minimum infestation of the cosmopolitan *Sitophilus zeamais* at a mean of 0.6 and *Postephanus truncatus* at zero before transferring into the test storage technologies. The level of infestation in these technologies varied where the hermetic bags were far more superior in suppressing the emergence insects under all subjected

treatments. However, the level of moisture in the grain modulated the extent of infestation where more infestation was identified in grains with high moisture levels.

The moisture build up in hermetic technologies coupled with the warm storage conditions could have contributed to the high fungal population, hence further facilitating the multiplication. It is likely that the heat and water together with resident oxygen facilitated mould infection in all the storage technologies. (Krnjaja *et al.*, 2013) found that moulds belonging to the genus *Aspergillus* were most frequently isolated (35.8%) in Kenya. In a similar study, (Muthomi, 2001, Thathana *et al.*, 2017) 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 (Wagacha and Muthomi, 2008; Stasiewicz *et al.*, 2017) may be reason for high occurrence in the maize samples even at low moisture levels.

However the hermetic technologies were able to manage the nutritional loss to a certain extent compared with the conventional storage methods as was noted by (Murashiki *et al.*, 2018). It is also worth mentioning that grains with high moisture stored in hermetic technologies were discolored more than grains stored in conventional storages as was also observed in a hermetic storage study by (Walker *et al.*, 2018).

It was also observed that the oil, protein and moisture contents increased during the storage period. This is likely due to the high infestation of insects especially in the eighth month of storage as was also noted by (Tripathi, 2018; Chattha and others (2016) reported losses in lipids (2.4%), starch (64.8%) but recorded an increase in protein content (11.78%) of maize infested with *Callosobruchus maculatus* by 26%, and contaminated by fungi by 25% when stored in room stores in bulk for three months. In contrast, the maize did not reduce in protein levels in all the storage technologies. This is concisely in agreement with the findings by

(Mehmood *et al.*, 2018) who found that only oil, fibre, ash, starch and moisture levels of the maize grain had increased due to infestation of maize flour by *Tribolium casteneum* in three months of storage. (MENDES and others (2017) also investigated the effect of insects infestation on beans, wheat and maize nutritional quality/quantity and found that protein quality was not altered by the infestation instead an increase was observed. Interestingly, the there was a reduction in Amino acids in beans than in corn and wheat. As was expected, it was evident that the standard woven bags were not effective in preserving the nutritional content of the grains as backed up by (Walker *et al.*, 2018). This could probably be that insects lodging in the grains were also ground and analyzed collectively hence the increase in oil values especially in heavily infested grains. It is also true that maize is susceptible to moulds which use up the starch, protein and oils as their source of food to grow and multiply (Lane *et al.*, 2018). This explains the reduction of starch in the maize heavily infected by moulds (Chattha *et al.*, 2016).

6.7 Conclusion

The data collected is essential in providing the local communities with the ideal storage option that will preserve the nutritional quality of grains stored up to twelve months. High moisture grains inoculated with moulds were more depleted than grains that were not inoculated despite the moisture levels. The polypropylene bags dusted with the pesticides were fairly effective in reducing the nutritional loss. Hermetic technologies are only effective and reasonably economical options for dry maize grain.

CHAPTER SEVEN: GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.0 CONCLUSION

This work supports the promotion of both hermetic storage technologies and improved drying practices. Bags with double liners and those impregnated with pesticides were better at managing the insect infestation more than the single liners and certainly superior over the standard woven bags. The use of actellic powder as pesticide was helpful in the control of *P. truncatus* but did not particularly prevent *S. Zeamais* infestation especially at eight months of storage probably due to the reduced lethal action. The hermetic technologies are not yet satisfactorily effective due to the likely miss handling if not used as instructed. Although the mortality rate was high in plastic silo, it also allowed for infestation of both insects and did not preserve the aesthetic quality of the grain with high moisture. Research may be needed to assess its efficacy in insect management and grain quality preservation using >90kg grain quantities. The manufacturers might need to make adjustments to consider the users capabilities to maximize the potential of hermetic technologies. The idea of zip locking might not only be convenient but also effective in ensuring that the existing insects are deprived of the oxygen. Hermetic storage technologies can be an effective solution to reliably manage insect infestation during on-farm storage, thereby reducing food loss and potential human /animal exposure to mycotoxins. However, if farmers do not adequately dry grain, /apply appropriate postharvest practices to avoid fungal infection, even hermetic storage technologies may not be effective in the control of insect infestation.

From our findings, it was evidently noted that maize nutritional value is affected by high storage moisture levels, mould infection and insect infestation over a longer period of storage. However the hermetic technologies were able to manage the nutritional loss to a certain extent compared with the conventional storage methods. High moisture grains

inoculated with moulds were more discolored than grains that were not inoculated despite the moisture levels. The PP bags dusted with the pesticides were fairly effective in reducing the nutritional loss caused by insects. If the pesticide residues are not harmful to humans, then grains dusted with actellic powder in standard woven bags is equally sustainable. Hermetic storage technologies can be an effective solution to manage mycotoxin contamination during on-farm storage, thereby reducing potential human and animal exposure to mycotoxins. Hermetic storage technologies restrict gaseous exchange and act as a barrier hence reduced contamination. However, if farmers do not adequately dry grain, even hermetic storage technologies may not be effective in the control of mycotoxins contamination. It is therefore recommended that grains be stored at the safe storage moisture in the hermetic technologies.

This work supports the promotion of both hermetic storage technologies and improved drying practices. Adoption of hermetic technologies especially the bags will improve the marketability of the farmers' maize therefore improving their economic status. Maintaining mycotoxin free maize and its products along the production process will not only increase the economy's Gross Domestic Product but also reduce the exposure of both humans and animals to the various health concerns as a result of consuming these toxins.

Hermetic storage technologies can be an effective solution to reduce mould proliferation during on-farm storage, thereby reducing potential human and animal exposure to mycotoxins. However, if farmers do not adequately dry grain, even hermetic storage technologies may not be effective in the control of mould and mycotoxin contamination, and contamination will be even greater underconventional storage systems. This work supports the promotion of both hermetic storage technologies and improved drying practices.

7.1 RECOMMENDATION

The grains with high moisture stored in hermetic technologies were discolored more than grains stored in conventional storages. It is therefore recommended that grains be stored at the safe storage moisture in the hermetic technologies.

We recommend that other studies should consider carefully sorting out the maize grains harboring insects / pupae before grinding as a way of only assessing the grain composition.

It will be helpful if the maize is stored in these hermetic storage technologies immediately after harvesting and drying. There is still need to improve the technologies to facilitate proper sealing and use of materials that will not be easily damaged during the storage period. Bare ground should be avoided as a drying platform to avoid contamination from the fungi resident in soil.

There is a need for more public sensitization mostly in the rural farmer groups about the dangers of mycotoxins and the most effective ways of eradicating the contamination. This requires a holistic intervention mechanism where stake holders are involved, right from the farmers, Research Institutions, Social workers, input suppliers, package manufacturers, millers and value addition processors, retailers, consumers and most importantly the good will of the government. Strategies by the government to restructure food safety policies including revamping extension services to carry the right message to farmers will be some of the plausible initiatives to manage the aflatoxin crisis. The farming systems need to be upgraded to match those used in developed countries, however there is an urgent need to restructure our culture of over reliance on maize as the sole food and diversify diets to other crops that had been removed from the menus.

It is strongly recommended to conduct the same study with the participation of farmers in effort to evaluate how easily these technologies can fit into the diverse and complex activities

farmers engage in. this will give an overview of how user friendly these technologies are in reality and the realistic shelf life of these technologies.

A subsidized pricing program can be effective to encourage the use of these technologies.

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Appendix

Appendix 1: Assessment of gas composition in the technologies set up for the experiment



Appendix 2: Figure Maize infested by insects at 8 months of storage in PP bags



Appendix 3: Insect infestations at four month (A), at eight months (B) and insects infestation assessment



Appendix 4: Serial dilution in the University laboratory



Appendix 5: Grain germination set up



Appendix 6: Maize purchase guide

Data sheet for maize purchase

Date	6/12/16	Location	LIPTAEB	GPS readings	
County	MURURI	Sub-location	LIPTAEB	Elevation (meters)	2342
District	ULUHL	Village	NESSAT	Latitude in decimal degrees S	
Division	KARUMU	Age (years)	45	Longitude in decimal degrees N	
Farmer's name	OPPE WANGA	Education (years)	11	Waypoint number	
Mobile phone No.	070 52228	Gender	F	Main occupation	FARMER
Secondary occupation	FARMER	What is the size of your farm	2 Acres	How much maize did you harvest?	20 bags
Aspect of the grain	Good	Date of harvest	28/12/16	How much of your harvested grain were able to be store in the silo (s) % and in hermetic bag (s) %	100%

S 00°44.933'
E 038° 20.078'

Data sheet for maize purchase

Date		Location		GPS readings	
County		Sub-location		Elevation (meters)	
District		Village		Latitude in decimal degrees S	
Division		Age (years)		Longitude in decimal degrees N	
Farmer's name		Education (years)		Waypoint number	
Mobile phone No.		Gender		Main occupation	
Secondary occupation		What is the size of your farm		How much maize did you harvest?	
Aspect of the grain		Date of harvest		How much of your harvested grain were able to be store in the silo (s) % and in hermetic bag (s) %	

bags	Moisture measures			Aflatoxin reading			Variety	Observation
Number Code	1	2	3	1	2	3		

1	636	16.5	16.7	16.4				
2	637	16.6	16.7	16.6				
3	638	16.8	16.5	16.6	1.95	2.03	1.99	
4	639	16.5	16.5	16.6				
5								

16

bags	Moisture measures			Aflatoxin reading			Variety	Observation
Number Code	1	2	3	1	2	3		

1	640	13.6	13.2	13.2	0.00	0.00	0.00	
2	740	15.3	14.8	14.6	0.00	0.00	0.00	
3	840	14.8	15.0	14.4	1.46	1.38	1.46	
4	940	14.7	15.0	14.8	2.62	2.67	2.66	
5	1040	14.2	14.2	14.6	0.00	0.00	0.00	

2