EFFECTIVENESS OF TRIPLE-LAYER HERMETIC BAGS AGAINST AFLATOXINS IN STORED MAIZE

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DECLARATION

This is to certify that, with the exception of the references to other work which have been cited and duly acknowledged, this thesis is the result of research undertaken by me towards award of Master of Philosophy degree in Post-harvest Science and Technology, Department of Crop Science, University of Ghana. This thesis has neither in whole nor in part been presented for a degree elsewhere.

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DEDICATION

To the Lord, God Almighty.

My parents Yaw Tawiah and Yaa Boahemaa, and all my siblings for their invaluable supports.

To Mrs. Grace Bamfo and sons Richard Bamfo and David Bamfo for their unique hospitality.

To my wife Belinda Ibrahim

ABSTRACT

Aflatoxin contamination of agricultural commodities is gaining public prominence in Africa where it is pervasive due to favourable environmental conditions and high consumption of maize. Aflatoxins are toxic secondary fungal metabolites that contaminate dietary staples such as maize and groundnut resulting in adverse effect on food security, health and trade. This study evaluated the effectiveness of triple-layer hermetic bag to control aflatoxin in stored maize. A factorial experiment was conducted involving 2.5 kg of Obatanpa maize variety at moisture content between 12%-13%. Two storage bags (triple-layer hermetic bag and polypropylene interwoven bag) and two insect pests (*Sitophillus zeamais* and *Prostephanus truncatus*) were used in the study. Oxygen depletion and carbon dioxide elevation, temperature, relative humidity and dew point in the different storage bags and the storage environment were measured during the six months storage period. Different levels of temperature (16 ^{o}C, 30 ^{o}C and 38 ^{o}C) were also monitored to ascertain their effect on the performance of the triple-layer bag against aflatoxins in stored maize. The study showed that most farmers introduced to the hermetic technology by NGOs adopted and used it to preserve various agricultural commodities from insect pests. The vulnerability of the high-density polyethylene (HDP) bags to leakages and tearing and the cost of replacing a torn HDP bag were predominant concerns expressed by farmers during the survey in the Techiman Municipality. Field studies revealed that extreme fluctuation in temperature in the field negated the effectiveness of the air-tight condition created in the triple-layer hermetic bag to control aflatoxigenic fungi. There was significant difference ($p<0.05$) in moisture content of

maize grains in the different storage bags with respect to insect infestation and changes in season from dry to rainy season in the storage crib. The mean aflatoxin level of maize infested with insect pests in the conventional bag was significantly higher ($p<0.05$) than insect-infested grains in the triple-layer hermetic bag. The triple-layer hermetic bag preserved the germination capacity of the seed maize much longer than polypropylene interwoven bag. There were significant differences ($p<0.05$) in the concentration of aflatoxin at the different temperature levels in the different storage bag technologies. The polypropylene bag had aflatoxin level (38.8 µg/kg) near the initial aflatoxin content of 38.2 µg/kg after three months of storing at a constant warm temperature (38 ^{o}C). The result of the research showed that, irrespective of the type of storage bag used for storing maize, the most critical factor to control aflatoxin is storing grains at save moisture content (<11%) and keeping them in storage structures that ensure environmental conditions that are relatively stable.

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LIST OF ABBREVIATION

AFB_1: Aflatoxin B1

AFB_2: Aflatoxin B2

AFG_1: Aflatoxin G1

AFG_2: Aflatoxin G2

ANOVA: Analysis of Variance

APHILIS: African Postharvest Losses Information System

AUC: African Union Commission

CAC: Codex Alimentarius Commission

CAST: Council for Agriculture Science and Technology

CRD: Completely Randomised Design

CRSP: Collaborative Research Support Program

CYMMT: International Maize and Wheat Improvement Center

EC: European Commission

EU: European Union

F: Fumonisins

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Food and Agriculture Organisation Statistics

FARA: Forum for Agriculture Research in Africa

FDA: Food and Drug Administration

G-HF: Gas-Hermetic Fumigation

GTZ: German Agency for Technical Cooperation

HACCP: Hazard Analysis Critical Control Point

HCC: Hepato Cellular Carcinoma

HDPE: High Density Polyethylene

HIV/AIDS: Human Immuno Virus/Acquire Immune Deficiency Syndrome

HPLC: High Performance Liquid Chromatography

HS: Hermetic Storage

IARC: International Agency for Research on Cancer

IITA: International Institute for Tropical Agriculture

ISO: International Organization for Standardisation

JECFA: Joint FAO/WHO Expert Committee on Food Additives

LGB: Larger Grain Borer

MAS: Modified Atmosphere Storage

MERCK: Medical Research Council

MOFA: Ministry of Food and Agriculture

OT: Ochratoxins

PACA: Partnership for Aflatoxin Control in Africa

PDA: Potato Dextrose Agar

PICS: Purdue Improve Hermetic Bag

TLHB: Triple Layer Hermetic Bag

US: United States

USAID: United States Agency for International Development

V-HF: Vacuum Hermetic Fumigation

ZEN: Zearalenone

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.) is referred to as the cereal of the future for its nutritional value and utilization of its by-products (Lee, 1999). It is one of the commonest strategic crops in Africa and the developing world in general. Due to its importance, maize is a major staple and cash crop for smallholder farmers. In sub-Sahara Africa alone, maize is a staple food for an estimated 50% of the population (CIMMYT and IITA, 2010). It is the most important cereal in Ghana and a staple for over 90% of the population (Anankware *et al.,* 2013). According to FAO (2002) the global attention is continuously focused on maize because it is one of the most important dietary staple foods in the world.

Global maize production increased from 200 million tonnes to 600 million tonnes from 1963 to 2003 (FAOSTAT, 2006). The area planted to maize in West Africa alone increased from 3.2 million in 1961 to 8.9 million in 2005 (IITA, 2009). The average maize yield in Ghana is estimated to be 1.7 metric tonnes/hectare (MOFA, 2009). Proper grain storage thus occupies a pivotal stage in the economy of Ghana and most sub-Saharan countries in relation to availability and stability of prices as well as in the attainment of household food security among poor resourced farmers (Wambugu *et al.*, 2009).

One of the most serious food safety problems throughout the world, especially in the tropical countries is the contamination of maize grain with mould and fungi (Kaaya and Kyamuhangire, 2006). It has been reported by Fandohan *et al*. (2003), that storage fungi

contributes to loss of more than 50% of maize grain in tropical countries, and ranks second after insects as the major cause of deterioration and loss of maize. Campbell *et al*. (2004) estimated the cost of grain loss due to insect pests and micro-organisms damage of grain stored in developing countries at of US $500 million to US $1 billion of foreign exchange annually.

According to Hell *et al*. (2008) the general recommendation is to dry maize to safe moisture of 10% to 13% to minimise insects and mould damages. Investigations by Olakojo and Akinlosotu (2004) and Thamaga-Chitja *et al*. (2001) revealed that, many African communities still depend on traditional storage methods which are not effective in reducing the high moisture levels of grain after harvest leading to fungal contamination.

One of the critical concerns of inappropriate drying and storage methods is mycotoxin concentrations which increase rapidly in the warm, humid environment found in tropical regions (Bankole *et al*., 2006). Mycotoxin (e.g aflatoxin) literally means poison (toxin) produced from secondary metabolites of fungi. Today, the problem of aflatoxins and other mycotoxins in grains and foodstuff is one of the most serious and chronic one confronting agriculture and the livestock and poultry industries all over the world. 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).

Apart from grains moisture contents; insects, fluctuations of temperature and relative humidity in the tropics have been attributed to acceleration and rapid multiplication of mold and aflatoxin contamination (Bowen and Mack 1991; Lynch and Wilson 1991; Yakubu, 2009). Insect metabolic activities results in increased relative humidity, providing favourable conditions for growth of *A. flavus* leading to reduce seed germination (Hell *et al.,* 2010).

The World Health Organization (WHO) categorizes aflatoxin as class1 carcinogens, as they are highly poisonous, toxic substances (Martinez *et al.,* 2011). Aflatoxin contamination has been associated with stunting in children, immune suppression, micronutrient deficiencies, and higher prevalence of cancers in sub-Saharan Africa, East Asia, and China (Moturi, 2008; Hell, 2010 and Smith *et al.,* 2012).

1.1 Problems and Justification

Maize remains the most widely consumed staple food in Ghana (Anankware *et al.,* 2013) and most sub-Saharan countries. Unfortunately, maize is also one of the richest substrates for aflatoxin production (Wagacha and Muthomi, 2008). The majority of maize produce in most African countries is either used for producer's own consumption or sold in the local market. This implies that the human health impact will be greatest if there is no control mechanism of aflatoxins. In 2004, several hundred Kenyans became 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.,* 2006). Aflatoxin exposure has been implicated as a causal or aggravating factor in kwashiorkor

in African children and higher prevalence of hepatocellular cancer in Africa (Ramjee *et al.,* 1992; Strosnider *et al.,* 2006).

Furthermore, because aflatoxin contamination is generally not appropriately controlled and regulated in developing countries, there have been frequent rejections from importers with stringent aflatoxins regulations such as the European Union, a major trading partner of Africa. Between 2007 and 2012; the EU alone has issued 346 notifications to African countries (PACA, 2012). A study by Kpodo (1996) pointed out that all maize sampled from silos and warehouses in Ghana contain aflatoxin level ranging from 20 -335 µg/Kg while fermented dough from major processing sites contains levels of 0.7 to 313 µg/Kg which far exceeded the acceptable levels of 15 µg/kg by the Ghana Standard Authority.

Hell *et al.* (2000b) reported that, the uses of pesticide to control mycotoxins by farmers in Africa are not well practiced and deaths due to pesticides application have been reported. The consumers today expect the food products that are pesticide free or with lower levels of residues (Conyers and Bell, 2007). There is therefore the need for an effective and efficient, non-pesticide post-harvest aflatoxins management technology.

Donahaye and Navarro (2000) reported the use of modified atmospheres storage (MAS) as a non-toxic and environmentally benign alternative to fumigation for the control of insects in stored products. Hermetic technology is a form of MAS that works by creating an airtight seal in which oxygen levels are drastically reduced in a relatively short period through insect, fungal and grain respiration. Purdue Improved Crop Storage (PICS) bags

have been disseminated to millions of farmers in West and Central Africa to store grain hermetically (Moussa *et al.*, 2014).

The Purdue Improved Crop Storage (PICS) hermetic bag system has proven effective in storing a variety of crops including cowpeas, maize, peanuts, sorghum, wheat, and common beans against insect pests (Murdock *et al.*, 2012 ; Anankware *et al*., 2013; Baoua *et al*., 2014; Mutungi *et al.*, 2014; Njoroge *et al*., 2014; Vales *et al.*, 2014). The PICS bag is a triple bagging hermetic technology consisting of two liners made out of high-density polyethylene (HDPE) and an outer woven layer of polypropylene that provides protection during handling. Together, these bags establish MAS by creating an oxygen-depleted environment which is lethal to insect growth and development (Murdock *et al*., 2012). The ability of the PICS technology to generate MAS system has raised the question of its capacity to manage aflatogenic fungi. An investigation in U.S by William *et al.* (2014) proved that PICS were effective in preventing aflatoxin contamination in maize stored in the laboratory.

In the Philippines, Elepano and Navarro (2008) reported that, the aflatoxins in maize grains stored in single-layer GrainPro® (an alternative to triple-layer hermetic bags) cocoons hermetic bags did not increase with storage time.

The current study was undertaken to establish the effectiveness of the triple-layer hermetic bag against aflatoxin in maize stored under the field condition. An assessment of social acceptability, benefits and challenges farmers have encountered upon using the PICS bag was made.

The research also contributed to the filling of knowledge gap by assessing the influence of insect pests in aflatoxin contamination, since there are limited information regarding beetle pests to an increase risk of fungal infection and subsequent aflatoxin development (Hell *et al.*, 2000).

1.2 Objectives

The main objective of the research was:

To determine the effectiveness of the triple-layer hermetic storage bag technology against aflatoxins in stored maize.

The specific objectives of the study were:

1. To assess the social acceptability, benefits and challenges of the triple-layer hermetic bags

2. To determine the aflatoxins level of insect-free maize and insect infested maize in the triple layer hermetic bags

3. Assess the influence of temperature on the control of aflatoxins in the triple-layer hermetic bags

4. Assess the percentage viability of maize under hermetic storage conditions

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MAIZE PRODUCTION AND CONSUMPTION

Maize (*Zea mays* L) is the third largest planted crop after wheat and rice and is considered the most crucial and strategic food security cereal crop in Africa and the developing world in general (FARA, 2009). It is cultivated throughout the world with disparities in yield. It is estimated that in 2012, the total world production of maize was 875,226,630 tonnes (FAO, 2012a) with the United States (US), China, and Brazil harvesting 31%, 24%, and 8% of the total production of maize respectively.

Table 2. 1: Major maize production countries in the world in 2012

Country	Maize production in 2011 (million MT/year)
United States of America	274
China	208
Brazil	71
Mexico	22
Argentina	21
India	21
Ukraine	21
Indonesia	19
France	16
Canada	12
South Africa	12

Source: (FAO, 2012b)

Maize production in sub-Saharan Africa tripled from the early 1960s to late 1990s because of nearly two-fold increase in area under cultivation and a >40% increase in productivity. It is estimated that by 2050 the demand for maize will double, and by 2025

maize would globally become the crop with the highest production (FARA, 2009). In 2006, the Abuja Summit on Food Security identified maize, among other crops, as a strategic commodity for food production and poverty reduction. The summit calls for the promotion of maize production across the continent (AUC, 2006). Across the regions in Africa, the West Africa sub-region has raised its maize production levels (350% for product, 64% for productivity and 170% for area) with Nigeria playing a leading role where there were 385% increase for production, 46% for productivity and 231% for area of production (FAOSTAT, 2003).

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 (Ojo, 2003).These factors among others has created the situation whereby demand sometimes outstrip supply on the continent (Akande, 1994). Maize continues to receive priority research attention (Morris *et al.*, 1999) because of the strategic role it plays in addressing food security (Gyasi, 2001; Alderman and Hingis 1992; Boateng *et al.*, 1990). Several improved maize varieties have been developed and extended to farmers to increase production in Ghana. Among them are the following improved varieties: Dobidi, Aburotia, Okomasa, Abeleehe, Obatanpa, Mamaba, and Dorke.

In developed countries, maize is mainly used as livestock feed and raw material for industrial products whiles in low income countries, it is largely used for human consumption (IITA, 2001). According to FAO (2005), maize consumption in Africa ranges from 8 kg/year per person in Eastern and Southern Africa to 105 kg/year per

person in West Africa. Maize provides about 30% of the food calories to more than 4.5 billion people in 94 countries (von Braun *et al*., 2010). This encompasses 900 million poor consumers for whom maize is a preferred staple, 120-140 million poor farmers and about one-third of all malnourished children.

Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g, (Nuss and Tanumihardjo, 2010) as compared to rice and wheat, but has lower protein content. Like many African countries maize is staple food in Ghana. Varieties of cuisines are prepared from maize in various parts of Ghana and among different ethnic groups notably Akans, Gas, Ewes, and Dagombas. Maize may be cooked, roasted, milled to prepare various food items including banku, kenkey, akple, and tuo zaafi. The relatively high starch and minerals present in maize have been extracted in making confectionaries and noodles. Maize is also a key and significant component of poultry feed and to some extent the livestock feed sector as well as a substitute for the brewing industry (Ranum *et al*., 2014).

2.2 MAIZE STORAGE

Storage of maize in most parts of the world is to prevent deterioration of grain quality and quantity to insure farmer's household against chronic food insecurity. In sub-Saharan Africa maize is stored in myriad of structures depending on farmer's social status and the prevailing environmental conditions (Hayma, 2003). In West Africa many smallholder farmers use different traditional structures including raised platforms, conical structures with thatch roofs, clay structures, jute bags, polyethylene or polypropylene bags, and giant woven baskets (Motte *et al.*, 1995; Addo *et al.*, 2002).

In the East and Southern part of Africa, subsistence farmers store their farm output in small bags with cow dung ash, wood and open-air or roofed crib, raised platform, and roofed iron drums enclosed with mud, or may hung cobs over a fireplace (Wambugu *et al.*, 2009; Kankolongo *et al.*, 2009). The poor aeration polypropylene may encourage fungal growth and aflatoxin production if grains are not dried to safe moisture level (Udoh *et al*., 2000; Hell *et al*., 2000b).

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 aflatogenic fungi (Mora and Lacey, 1997; Hell *et al.*, 2008).

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 (Hodges *et al*., 1983; Udoh *et al.*, 2000).

The use of pesticides is a common storage practice in Africa. Udoh *et al.* (2000) reported that 24.2% of farmers in the Northern Guinea Savanna of Nigeria use actellic whilst a significant proportion of 22.6% still use non-recommended insecticides such as aldrex to store produce for consumption due to unavailability of appropriate chemicals. Phosphine

fumigation is also practiced widely in the tropics, as it is relatively cheap, easy to apply, and effective in controlling insect pests in grain (Bond, 1984).

2.2.1 Crib

The crib is one of the improved covered structures that have proven to be excellent for drying and storing of maize. It is constructed by using wood with rat guard attached to the base of the post, wire mesh and corrugated iron sheet or by using thinly split bamboo sticks (Udoh *et al*., 2000). In many sub-Saharan countries, the cribs are round or rectangular in shape, and are built approximately ½ meter off the ground (David, 1998). In humid wetter areas, it is more appropriate to put crops in crib type storage container (David, 1998).

According to Nyanteng and Asuming-Bempong (2003) the government of Ghana had established modern storage facilities in major maize producing regions in Ghana as part of its buffer stock policy aimed at reducing post-harvest losses and to reduce high price of maize in the post-harvest season. Lack of access to these modern storage structures and the continues preference for traditional storage structures as the crib culminated in the failure of the buffer stock policy in key maize producing areas in Ghana such as Techiman, Nkoranza and Kintampo all in the Brong Ahafo Region (Armah and Asante, 2006). The crib storage unit is easy and cheap to make, but the storage losses due to insects and rodents are often as high as 40% (Taylor-Davis, 2005)

Plate 2. 1: Bamboo crib

Plate 2. 2: A wooden crib

2.3 MYCOTOXINS

Food safety, according to Hell *et al.* (2008) occurs when microbial contaminants and chemical toxicants are present below tolerance levels in foods. Mycotoxins are toxic secondary metabolites produced by fungi such as *Aspergillus* and contaminate various agricultural commodities either before harvest or under postharvest conditions (FAO, 1991). When mycotoxins are ingested, inhaled or absorbed through the skin, it lowers

performance, causes sickness or death in human or animals (APHLIS, 2012). Some mycotoxins pass through the food chain to become associated with foods which have not been moulded.

According to Hussein and Brasel (2001), it has been well-established that some moulds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungi species. Usually, more than one mycotoxin is found on a contaminated substrate. Besides not all moulds are toxigenic and not all secondary metabolites of fungi are toxigenic (Hussein and Brasel, 2001).

Mycotoxins are diverse in their structure, their biosynthesis and their toxicity and fungi producing them have diverse ecology, ranging from obligates plant pathogens and endophytes, to saprotrophs active in the field and postharvest spoilage fungi active at stored commodities (Moss, 1996). Even though mycotoxin contamination depends on the fungi isolates, environmental and genetic backgrounds of the maize crops are also crucial to successful contamination (Picot *et al*., 2010; Warfield and Gilchrist, 1999). These toxins account for millions of dollars annually in loss worldwide in human health, animal health and, condemned agricultural commodities (Shane, 1994; Vasanthi and Bhat, 1998).

Prominent mycotoxins that are of greatest public health and agro-economic significance are aflatoxins (AF), ochratoxins (OT), fumonisins (F), zearaleonone (ZEN), trichothecenes, ergot alkaloids and tremogenic toxins. Among these mycotoxins, the two

commonest and highly toxic mycotoxins compound encountered on maize in the tropical and sub-tropical regions of the world are aflatoxins and fumonisins (Krska *et al.*, 2008).

2.3.1 The Genus *Aspergillus*

The genus *Aspergillus*, is member of the phylum Ascomycota, including a wide number of mycotoxin producing species. Three main species of fungi*; Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* are noted to produce mycotoxins. *Aspergillus* species may occur in different agricultural products such as cereals destined for human and animal consumption.

2.3.2 Aflatoxins

The most important mycotoxins that are frequently present in cereals particularly stored maize in store is aflatoxins. Aflatoxin contamination is unavoidable and unpredictable, which makes it a unique challenge to food safety (Park and Stollof, 1989; FAO, 1997). Aflatoxin-producing fungi have very few nutritional, environmental and, reproductive requirements, thus their ability to survive and well developed. *Aspergillus* species generate four significant aflatoxins: B_1, B_2, G_1 and G_2. "B'' and "G'' refers to the blue and green fluorescent colours produced under UV light on thin chromatography plates, whiles the subscript numbers 1 and 2 indicates major and minor compounds, respectively (Wu *et al*., 2011).

Aflatoxin B_1 is the most potent carcinogenic compared with the other aflatoxins, and it has been classified as a Group 1 carcinogen (IARC, 1993; Steyn, 1995). The hierarchy of

toxicity are in the order of B_1>G_1> B_2>G_2 (Suleiman *et al.*, 2013). In milk, aflatoxin appears as aflatoxin M_1, which is its metabolites (Akande *et al.*, 2006).

2.3.3 Chemical structures of aflatoxins

Aflatoxins differ in their chemical structures, which have been shown in Figure 2.1

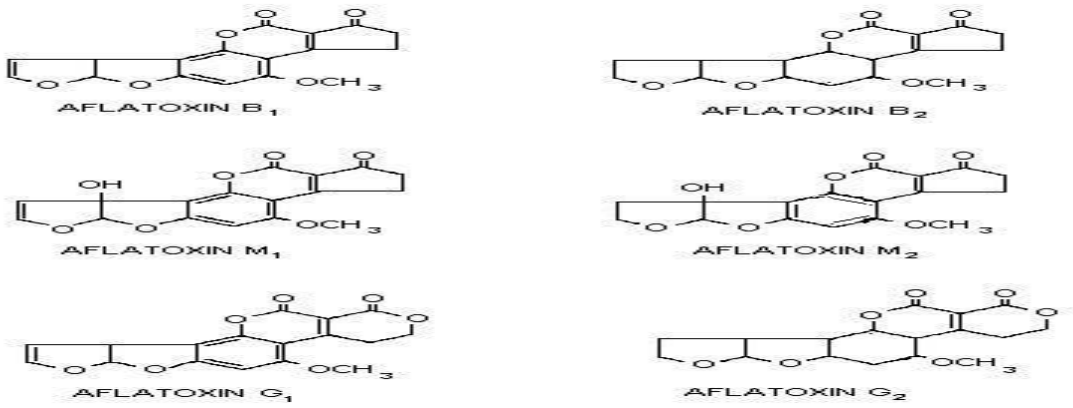

Figure 2. 1 : The chemical structures of some aflatoxins (Cole and Cox, 1981)

2.4 PRE-HARVEST FACTORS INFLUENCING AFLATOXIN CONTAMINATION

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. 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. (Dorner *et al.*, 1989; Cotty and Jaime-Garcia, 2007).

2.4.1 Soil type

Soil type, conditions and the availability of viable spores, are important factors (Horn, 2003) to generate aflatoxin production. Maize grows in different types of soil such as light sandy soils and heavy soils. Light sandy soil benefits from the rapid proliferation of *Aspergillus flavus* particularly under dry conditions in the later growth period. Conversely, heavier soil can reduced the level of aflatoxins contamination in maize grown because of its characteristic good water holding capacity (CAC, 2004; Torres *et al*., 2014).

2.4.2 Maize cultivar

Cultivar of maize planted has a tremendous influence on the level of aflatoxin contamination. Hoenisch and Davis (1994) reported that, maize kernel of thicker pericarp provide high level of resistance towards mycotoxin contamination fungi than kernels with soft pericarp. Hybrid with tight husk coverage according to Warfield and Davis (1996) tend to be less susceptible to toxigenic fungi. Maize genotypes with aflatoxin resistance have been identified in West and Central Africa (Brown *et aI.*, 2001).

2.4.3 Environmental conditions

Environmental conditions that favour *Aspergillus* infection in the field include high soil and air temperature, drought stress, nitrogen stress and any other condition that aid the dispersal of conidia during silking (Diener *et al*., 1987). Several researches have proved high temperature and drought to be major factors influencing aflatoxin contamination and fungal growth (Cole *et al.*, 1984; Sanders *et al.*, 1993; Widstrom, 1996; Tubayika and Damann 2001; Crauford *et al*., 2006; Kaaya and Kyamuhangire, 2006). *A. flavus* grows

well in temperature range of 19–35 °C (Northolt and van Egmond, 1981) with 28 °C being optimum for aflatoxin production (Scott *et al*., 1970; Sanchis and Magan, 2004).

End of season drought stress and elevated soil temperature are more conducive for the promotion of aflatoxin contamination (Rachaputi *et al*., 2002; Bankole *et al*., 2006). According to Payne and Hagler (1983), drought stress induces a great increase in proline in plants, which can enhance aflatoxin production. Lewis *et al*. (2005) pointed out that, in tropical countries, drought and semi-arid to arid conditions are linked to contamination and the poor subsist on frequently contaminated staples. Lewis *et al*. (2005) highlighted that in such regions, shifts in weather patterns may lead to acute aflatoxicoses and deaths.

Humidity also has significant influence on aflatoxin contamination both on and off the field. Under high humidity, an initially dry seed develops water content conducive for contamination. According to Cotty and Jaime-Garcia (2007) substrate moisture content and temperature dictates the extent of contamination. Alborch *et al*. (2011) reported that temperature and water activity influence not only the rate of fungal spoilage, but also the production of mycotoxins.

The combine activities of temperature, humidity and precipitation are known to have a remarkable effect on toxigenic moulds and on their interaction with the plant hosts. Generally, conditions that are injurious to the host plant (e.g. drought stress, pest-induced stress and poor nutrient status) encourage the fungi to develop more than under favourable plant conditions with the expectation of greater production of mycotoxins (FAO, 2008; Magnani *et al.*, 2007). In Vietnam, Le Van *et al.* (1995) reported that,

aflatoxin contamination was higher during raining season and increased with time. Aflatoxin contamination is very rare in latitude above 45 ºC but quite prevalent between latitude 26 ºC -35 ºC, relative humidity of 70% and Temperature of >32 ºC (Manabe and Tsuruta, 1978; Klich, 2002; CAST, 2003; Lagrieco and Visconti, 2004 and APHLS, 2012)

2.4.4 Agronomic practices associated with aflatoxin contamination

Aflatoxins are produced by fungal action during production, harvest storage and processing. It is worthy to note that, once crop becomes infected under field conditions, fungal growth continues with increasing vigour at the postharvest and storage conditions. Tillage practices, crop rotation, fertilizer application, weed control, late season rainfall, irrigation, wind and pest vectors all can affect the source and level of fungal inoculum maintaining the disease cycle in maize (Diener *et aI*., 1987). In sub-Sahara Africa, crops are cultivated under rain fed conditions with low level of fertilizer and little or no pesticide application. These situations according to Hell *et al.* (2008) promote *Aspergillus flavus* infection of fertility stressed plants.

Farming system also has great influence on mycotoxin production on the field. When maize is intercropped with cowpea there is high probability of aflatoxin contamination (Hell, 1997). Unlike mono-cropping, crop rotation and proper management of crop residues reduce the incidence of aflatoxin production (Hell *et al*., 2008). Poor agronomic practices such as poor weed control, high crop density, poor spacing increases crop vulnerability to fungi infection and subsequent aflatoxin contamination. The time of planting and harvesting crops also affect the degree of aflatoxin contamination. Lynch *et*

al. (1991) reported that peanut kernel from delayed harvest had significantly more aflatoxin than those harvested on time. Delayed harvest increases mould incidence, insect damage and aflatoxin levels (Kaaya *et al.*, 2005).

2.5 POST HARVEST AFLATOXIN CONTAMINATION

Considerable number of biotic and abiotic factors can determine fungal infection and growth as well as aflatoxin production after harvest. Timely drying and maintaining grain at safe moisture level pivotal to achieving effective control of aflatoxin contamination of maize. This is because they restrict the devastating activities of particularly the biotic agents responsible for aflatoxin contamination (Torres *et al*., 2014). Postharvest factors such as temperature, availability of water, oxygen and carbon dioxide, insects and rodent infestation, incidence of broken grains, the cleanness of product, toxigenic fungal load, microbial competition, antifungal compound presence and substrate composition are crucial factors that influence mold strains capacity to produce aflatoxins in maize (Hell *et al*., 2008). Besides, transport, waiting time for drying, drying system (temperature and drying rate), and storage conditions can affect these factors during post-harvest period (Dorner, 2008; Diener *et al*., 1987; Molyneaux *et al*., 2007).

Temperature and moisture content of maize are two crucial parameters that affect significantly the quality of grains, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grains (Gonzales *et al*., 2009). Yakubu (2009) explained that fluctuations in temperature and relative humidity in tropical countries accelerate rapid multiplication and proliferation of moulds and insects which undermine grain quality.

Similarly, White and Sinha (1980) reported that, the survival and multiplication of moulds and insects in grain greatly depend on the temperature and moisture content of the grain. Mould growth on grain is highly favoured when relative humidity of the grain surface layer is more than 70%. The humidity at the grain layer according to APHLS, (2012) is determined by the grain moisture content for cereals the corresponding moisture content in equilibrium with 70% relative humidity is about 14%. APHLS (2012) also, emphasized that, mould growth apart from resulting in mycotoxin production, causes heating and caking of grains and subsequent discolouration due to either the production of pigments or browning reaction occurring at elevated temperatures.

Proper storage of grains also has significant impact on the quality of grain. The type of storage and the type of storage structure have enormous influence on the levels of aflatoxin. Poor aeration in the storage structure and dirty floors may promote fungal growth on wet maize kernels. The storage form (cobs and shelled grain) of maize influences contamination by toxigenic fungi (Hell *et al.*, 2008). In India, maize with good husk cover had lower aflatoxin levels than those without husk (Bilgrami *et al*., 1991). The same conclusion was drawn by McMillian *et al*. (1987), after aflatoxin levels in tight husk cover varieties were found to be lower than loose husk cover varieties.

Shelling maize by beating cobs in bags with a stick bruises the kernels and predisposes the maize to fungal infections which consequently lead to aflatoxin contamination (Fandohan *et al*., 2006). The practice of storing maize with other commodities after harvest also has the potential of enhancing aflatoxin contamination. Wholesome and aflatoxin-free maize grains stored with aflatoxin infected cowpea (Gill *et al*., 1983;

Umerchuruba, 1985) would result in production of aflatoxin due to cross contamination (Seenappa *et al*., 1983; Koehler *et al*., 1985). Also cross contamination was reported between maize and sorghum (McMillian *et al.,* 1983) but no such incidence was reported when an aflatoxin prone commodity; groundnut (Mehan *et al*., 1991) was stored together with maize in Benin (Hell *et al*., 2000b).

2.6 PREVALENCE AND MAGNITUDE OF AFLATOXIN IN AFRICA

It is generally accepted that, no region of the world can escape the threat of mycotoxins (Lawlor and Lynch, 2005). But the magnitude of the problem is enormous in Africa where maize is the people's staple and there is availability of suitable conditions for fungi growth and development. Unfortunately, the presence of mycotoxins in food is overlooked in Africa due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into human food chain during chronic shortage, drought, wars, political and economic instability (MERCK, 2006).

Several hundred of Kenyans became severely ill, and 125 died, due to acute aflatoxicosis: a disease of liver failure associated with consuming extremely high levels of aflatoxin in food in 2004 (Lewis *et al*., 2005; Strosnider *et al.*, 2006). The Kenyan tragedy speaks volume of the magnitude of aflatoxin contamination in Africa. 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).

In 2003, Gong *et al*. (2003) reported of high contamination of aflatoxin in West Africa. According to James *et al*. (2007), aflatoxin levels measured in maize sold to the public in West Africa were higher and ranged from 0.4 ng/g to 490 ng/g in Ghana, 0.7 ng/g to 110 ng/g in Togo, and 0.2 ng/g to 120 ng/g in Benin. The chronic incidence of aflatoxin in diet is also evident from the presence of aflatoxin M_1 in human breast milk in Ghana, Kenya, Nigeria, Sierra Leone and Sudan and in the umbilical cord blood samples from Ghana, Kenya, Nigeria and Sierra Leone (Bhat and Vasanthi, 2003). In Nigeria 33% of maize sampled from the different agro-ecological zones of the country were found to be aflatoxin contaminated. The Southern Guinea Savanna appears to be the agro-ecological zone with the highest contamination of aflatoxins (Udoh, 2000). This zone according to Hell *et al*. (2008) has a bimodal rainfall pattern with the first crop being harvested at the beginning of the rainy season which makes drying crop difficult. The second crop often does not get enough rain and high insect pressure increasing the likelihood of aflatoxin contamination.

All the maize samples collected by Kpodo (1996), from silos and warehouses in Ghana contained aflatoxins at levels ranging from 20 µg/kg to 355 µg/kg, while fermented maize dough collected from major processing sites contained aflatoxin levels of 0.7 µg/kg to 313 µg/kg. Yameogo and Kassamba (1999) reported that seeds of groundnuts from Burkina Faso inoculated with *A. flavus* excreted all the four major aflatoxins, which peaked at 170 ppb after 6 days.

2.7 IMPACT OF MYCOTOXIN

Mycotoxins according to WHO (2006) and Wu (2006) has attracted worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade. Mycotoxicoses are diseases caused by consumption of mycotoxins. The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (WHO, 1999). The magnitude of aflatoxicoses in Africa is enormous, because maize is a prominent staple diet on the continent. Atanda *et al*. (2011) reported that, a common feature of all outbreaks has been the involvement of staple foods such as maize, wheat or pearl millet, following unseasonable rains or drought either in the growing season or at harvest.

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 (Fellinger, 2006; Barug *et al*., 2003). Aflatoxins in humans or animals are characterised as food or feed related, non-contagious, non-transferrable, non-infectious, and non-traceable to micro-organisms other than fungi

2.7.1 Impact of aflatoxins on human health

The disease, aflatoxicoses, like all toxicological syndromes, can be categorized as acute and chronic. Acute toxicity generally has a rapid onset and an obvious toxic response, while chronic toxicity is characterised by low-dose exposure over a long period resulting in cancer and other irreversible effects (James, 2005).

Acute toxicity is caused by ingestion of large amount of aflatoxins from heavily contaminated food. This causes decreased liver function and leads to blood clotting mechanism, jaundice, a decrease in serum proteins that are synthesized by the liver, edema, abdominal pain, vomiting and death of affected person. This was the case in Kenya in 2004 where 125 deaths was reported due to consumption of maize contaminated with aflatoxins with *Aspergillus flavus* implicated as the causal agent of the outbreak (CAC, 2005; Probst *et al*., 2010).

Chronic exposure to aflatoxins according to William *et al*. (2004) is associated with impaired immunity, malnutrition and liver cancer. Aflatoxins have also been implicated in Kwashiorkor, a disease usually considered a form of protein and energy malnutrition and also in Reyes syndrome which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral edema (Blunden *et al*., 1991).

MERCK (2006) reports of epidemiological studies of human population exposed to diet contaminated with aflatoxins and revealed the association between the incidence of liver cancer in Africa and elsewhere. Out of all the diseases resulting from aflatoxin consumption, the primary among them is hepatocellular carcinoma (HCC or liver cancer). This disease is the third-leading cause of cancer death globally according to (WHO, 2008) with 83% of these deaths occurring in East Asia and sub-Saharan Africa. Turner *et al.* (2000) reported that, aflatoxin consumption raise the risk of liver cancer in people who are infected with hepatitis B and C, by more than ten-fold compared to either exposure alone.

According to Gong *et al*. (2003) preliminary evidence proved that, there may be an interaction between chronic mycotoxin exposure and malnutrition, immune-suppression and impaired growth in diseases such as malaria and HIV/AIDS. Ingestion of 2-6 mg/day of aflatoxin for a month can cause acute hepatitis and death (Pattern, 1981)

2.7.2 Impact of aflatoxin on animal health

The deleterious consequences of aflatoxins on animal health and production has been identified largely in farm animals such as poultry, pigs and ruminant as result of consumption of high levels of cereals and oilseeds in diets (Smith *et al*., 1994; Charoenpornsook and Kavisarasai, 2006).

Akande *et al*. (2006) reported that mycotoxins have negative effects on animal feed intake, animal performance, reproductive rate, growth efficiency, immunological defense, the central nervous system, hemorrhagic, as well as causing damage to the livers and kidney. Ruminants such as cattle, sheep and goats are generally more tolerant to the adverse effects of mycotoxins. This is because the rumen microbiota is capable of degrading mycotoxins (Wogan, 1966; Heinferich *et al*., 1986). Nevertheless, Hussein and Brasel (2001), highlighted that, the production of milk, beef, or wool and reproduction can be altered when ruminant consume mycotoxin-contaminated feed for extended period of time.

Aside affecting animal performance, large chunk of aflatoxins become concentrated in animal products such as milk, meat, and eggs which pose serious health threat to humans. Example of such aflatoxin is aflatoxin M_1 which appeared in milk of animals following

high and extensive consumption of aflatoxin-contaminated feed by farm animals (Akande *et al*., 2006).

Clinical signs such as reduced feed consumption, drastic drop in milk production, weight loss, liver damage and reduced immune system functions and rumen metabolism were reported in cattle (Bodine and Mertens, 1983; Lawloy and Lynch, 2001). Unlike ruminant that are generally tolerant to aflatoxins, pigs are the most sensitive with poultry being intermediate (Ratcliff, 2002). In poultry, ducks are most sensitive to aflatoxins, followed by turkey, broilers and layers. It is worthwhile to note that aflatoxins were initially isolated and implicated as the cause of death of more than 100,000 turkey poults (Turkey X disease) following the consumption of a mould-contaminated peanut meal (Asao *et al*., 1963). The following symptoms according to Cortyl (2008) have been observed following the contamination with aflatoxin in poultry: fatty liver, kidney disorder, legs and bones problems, pigmentation problems (carcass, egg yolk), reduced hatchability, smaller eggs and reduced egg shelled.

2.7.3 Impact of aflatoxin on agriculture and food security

Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (FAO, 1996). Aflatoxin problems have the potential to affect each of the pillars of food security (availability, access, utilization, and stability). This is because contamination of the major staple food can directly reduce availability of food. Producers of affected food crops may also earn less because of product rejection, reduced market value or inability to gain access to high-value international trade market. Lower

farm income in turn limits ability to purchase food for family, which translates into reduced access to food. According to PACA (2012) over 4.5 billion people in developing countries are at the risk of chronic aflatoxin exposure. This therefore means that, the profound effort of the international community to achieve greater food security and improve health would be undermined, particularly in sub-Saharan Africa where aflatoxin contamination is widespread. Miller (1996) reported that 40% of productivity is lost in developing countries due to diseases such as HIV/AIDS, malaria, jaundice that is exacerbated by aflatoxicoses. Such condition increases food insecurity by affecting farmer's ability to produce food, because productive and skilled members of the household have become ill or died, making household unable to cultivate land, and buy food, because farmers family can no longer continue working, hence there is no income or income is diverted to care for the sick.

2.7.4 Impact of aflatoxin on trade and economy

According to Zain (2011) there are multiple criteria for assessing the economic impact of mycotoxins on humans and on animals. Considerations include loss of human and animal life, health care and veterinary care costs, loss of livestock production, loss of forage crops and feeds, regulatory costs, and research cost focusing on relieving the impact and severity of the mycotoxin problem. The economic impact of aflatoxin contamination depends on the contribution that the susceptible commodity makes to a country's consumption and income. Aflatoxin-producing fungi also cause direct economic losses by spoiling grain. Commodities contaminated with aflatoxins have a lower market value and often are consumed locally, since they cannot be exported (Hell *et al*., 2008).

According to Food and Agriculture Organisation of the United Nations (FAO) estimates, the direct cost of mycotoxin contamination of corn and peanut in Southeast Asia (Thailand, Indonesia, and Philippines) amounted to several hundred million dollars annually, with most of the losses accounted for by corn (Bhat and Vasanthi, 1999).

Levels of mycotoxins acceptable in foods in developed countries have been lowered, which has resulted in lower export earnings by Africa and other developing countries that cannot comply with stricter regulations. Otsuki *et al.* (2001) pointed out that the potential for disruption to developing countries food exports resulting from regulation actions in high income market such as the European Union (EU) and the United States (US) is underscored by the fact that significant number, nearly 70% of developing African and Middle East countries food exports are destined for high income market.

This tighter phytosanitary standard set by the European Union (EU) in the early 1980s caused a decline in India's peanut export to the EU by more US $30 million dollars a year (Bhat and Vasanthi, 1999). These stringent legislations to regulate aflatoxin in developed countries can result in forgone trade revenues arising from increased cost of meeting standards including cost of testing, rejection of shipments and eventual loss of admissibility into foreign markets.

Dohlman (2003) reported that an average of US $466 million is spent on efforts to prevent or reduce aflatoxin contamination through regulation, enforcement, testing and other quality control efforts. Overall costs for mycotoxin management and monitoring in the United States are estimated at between US $0.5 million to >$1.5 billion for aflatoxin

in maize and peanuts, fumonisin in maize, and deoxynivalenol in wheat (CAST, 2003). Is worthy to emphasize that, trade disputes from mycotoxin contamination arise because it is considered as unavoidable risk because of the several factors that influence the levels of contamination (Lopez-Garcia, 2010).

2.8 MANAGEMENT OF AFLATOXIN

Considering the magnitude of aflatoxin contamination on food security, health and trade, several countries around the world are taking pragmatic steps to address aflatoxin and all other mycotoxins contaminations. Wagacha and Muthomi (2008), emphasized that, factors fundamental to countries ability to protect their population from mycotoxins include the political will to address the problem and the capability to test food contamination, which determines whether requirement can be enforced.

Evidence presented by Finley *et al*. (1992), states that the problem of aflatoxin cannot be solved by mixing healthy grains or feeding it to animals, as the toxin will be accumulated in their body and later consumed by people in the form of milk, meat, or egg. Prevention of mycotoxins through proper measures prior to harvest is an effective way to control aflatoxins. This is because once contamination starts, it is difficult to manage it. There are myriad of measures that can be employed to manage aflatoxin contamination. Cultural controls, biological controls, chemical controls are among the various management practices that have the potential to prevent or limit aflatoxin contamination.

2.8.1 Cultural control methods

Cultural practices designed to reduce mycotoxin contamination of crops have their roots in plant disease epidemiology. The general strategy is to alter the conditions under which the crop is grown so that infection by the offending fungus or fungi is avoided (Munkvold, 2003). Some of these good agronomic practices that have shown profound effect on mycotoxins contamination of crops in the field are:

2.8.1.1 Planting resistant varieties (Breeding for resistance varieties)

Host resistance according to Wagacha and Muthomi (2008), represents the most promising long term strategies in controlling mycotoxin contamination menace in Africa. In spite of the enormous steps by scientist to develop resistance varieties, only few, commercial cultivars have adequate levels of resistance to mycotoxin-contaminating fungi (Munkvold, 2003). Some high yielding yellow maize varieties with good resistance to *Aspergillus spp* have been developed. This includes A0901-25 that have grain yield of 7115 kg/h and a low aflatoxin level (Munkvold, 2003). Two maize lines that are resistance to mycotoxin-producing fungi have been identified by scientist in U.S Department of Agriculture (Hamiton, 2000). Maize genotypes with aflatoxin resistance have been identified in West and Central Africa too, (Brown *et aI.,* 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 *et aI.,* 2008). Tropical maize germplasm with resistance to aflatoxin have been registered and these are among the varieties that have been distributed to National programmes for the development of locally adopted hybrid (Menkir *et aI.,* 2008).

2.8.1.2 Timely planting and harvesting

Pre-harvest sowing can have profound impact on later contamination by mycotoxins. Magan and Aldred (2007) reported that late sowing times in Europe were found to have four times higher fumonisins than earlier sowing. Likewise sowing dates, timing of harvesting could also have enormous influence on the level of aflatoxin contamination. Early harvesting reduces fungal infection of crops in the field before harvest and consequent contamination of harvested produce (Wagacha and Muthomi, 2008).

Delayed harvest increase mould incidence, insect infestation and consequent aflatoxin contamination levels (Kaaya *et al.*, 2005). Aflatoxin contamination according to Kaaya *et al*. (2005) increased in 4-fold and more than 7-fold when maize harvest was delayed by 3 and 4 weeks respectively, after maturity. According to Amyot (1983) African farmers have good knowledge in appropriate time to harvest their produce but, unpredictable weather, labour constraints, cash problems, threat of thieves, rodents and other animals may compel them to harvest at inappropriate time.

2.8.1.3 Insect management

Insect infestation could have significant impact on the mycotoxin contamination of maize. It is worthwhile to know that, the level of insect damage influences the extent of mycotoxins contamination. 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, 2003). Insects attack in storage could also be devastating because their level of damage influences the extent of

mycotoxin production in store. Therefore proper management of insects both on and off the field is crucial in reducing mycotoxin contamination.

2.8.1.4 Proper irrigation and fertilizer application

Soil fertility and drought stress has been implicated to be contributing factors in pre-harvest aflatoxin contamination of maize. A higher rate of nitrogen fertilization consistently resulted in reduced aflatoxin levels. Jones and Duncan (1981) reported that a higher rate of nitrogen fertilization consistently resulted in reduced aflatoxin levels. Plants receiving 145.7 kg/ha N had aflatoxins ranging from 19 to 2000 ng/g, while those receiving only 11.2 kg/ha N ranged from 64 to 4875 ng/g aflatoxins. High moisture and high relative humidity are essential for spore germination and fungal proliferation. According to Lopez-Garcia *et al*. (1999), studies have shown that drought stress followed by high-moisture conditions is ideal for *Fusarium moniliforme* proliferation and fumonisin production.

2.8.1.5 Proper drying

Apart from critical factors such as timeliness in harvesting and clean-up after harvest, drying is a crucial factor in preventing aflatoxin contamination of maize during storage. This is because dried grains keep longer, are rarely attacked by insects and usually do not support mould growth, since free water required for the development and proliferation of fungi is not available (Hell *et al.,* 2008). Aflatoxin contamination can increase by ten-fold in three days if maize is not dried properly (Tamboo-ek, 1989). Drying harvested maize to 15.5% moisture content or lower within 24- 48 hours would reduce the risk of fungal multiplication and subsequent aflatoxin production (Hamiton, 2000). According to

Munkvold (2003), lower moisture content by artificial drying of maize is a valuable tool for arresting fungal development and mycotoxin production because it reduces both toxigenic and non-toxigenic moulds physiological activities.

Wagacha and Muthomi (2008) highlighted that, during storage, transportation and marketing, maintaining low moisture levels by avoiding leaking roofs, and condensation arising from insufficient ventilation is critical to avoid aflatoxin accumulation. Therefore, a common recommendation according to Hell *et al.* (2008) is that harvested maize should be dried as quickly as possible to safe moisture content of 10-13 % and cooled before storage to avoid aflatoxin contamination.

2.8.1.6 Other cultural methods

Cultural practices such as crop rotation, tillage, appropriate plant density, management of crop residues, weed control have positive effects on infection and subsequent aflatoxin accumulation (Champiel *et al.*, 2004; Jones *et al.*, 1981; Munkvold, 2003). A study conducted in Mexico demonstrated that a combination of cultural practices (early planting, reduced plant population and irrigation, hybrid selection, and insect control) reduced aflatoxin concentrations down to 0-6 ng/g, compare to 63-167 ng/g in late planted, non-irrigated, maize at higher plant density without insect control (Del-Bosque, 1996).

2.8.2 Physical control methods

In post-harvest, critical factors such as temperature, relative humidity, moisture content of the grain, oxygen and carbon dioxide, insects and rodents infestation, incidence of

broken grains, the cleanness of the product, toxigenic fungal load, microbial competition and anti-fungal factors are crucial and worthy of consideration in terms of managing aflatoxin. Fandohan *et al.* (2005) reported that physical post-harvest measures such as sorting, winnowing, washing, crushing combined with dehulling of maize grains were effective in achieving significant mycotoxins removal.

According to Hell *et al*. (2008), the first step to reduce aflatoxin is to sort cobs that are damaged, insect infested, have incomplete husk cover, or mouldy grains from the rest of the grains. This is because sorting out of physically damaged and infected grains from the intact commodities can result in 40-80% reduction in aflatoxin levels (Park, 2002; Fandohan *et al*., 2005; Afolabi *et al*., 2006). Grains are sorted based on their physical properties such as colour, size and density. Aflatoxin contamination has been reported to been related to smaller grains and nuts in commodities (Dorner, *et al.*, 1989; Whitaker *et al*., 2005; Shatzi and Pan, 1996).

An experiment by Piadade *et al.* (2002) proved that, when a sieve of 4.5 mm of round holes was used to sieve maize samples, the larger grain fraction has lower levels of aflatoxin (84.8 µg/kg) than smaller grains (204.0 µg/kg). Also, sorting grains by density helps to segregate poor quality grains from good quality grain. This is because grains and nuts contaminated by fungus and infested by insects have lower density than sound ones (Kebak *et al*., 2006). Huff (1980) obtained 60% reduction of aflatoxin levels when buoyant maize in water was removed. Ensuring sanitation measures such as removal and destruction of debris from previous harvest and cleaning stores before loading new harvest can reduce aflatoxin contamination (Hell *et al.,* 2000b).

Storage form (cobs or shelled) and proper storage structure are known to have considerable influence on the degree of aflatoxin contamination. According to Mora and Lacey (1997) aflatoxigenic fungi were highly present in maize shelled immediately after harvest than maize left on the cobs through drying. Storage temperature is the most critical factor in managing potential mycotoxin problems in dried grain (Munkvold, 2003). Storage structures that allow proper ventilation, and gives protection to grains against insects, rodents and rainstorms can highly minimise aflatoxin contamination during storage.

2.8.3 Biological control methods

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, atoxigenic 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 minimise post-harvest aflatoxin contamination by 95.9% (Dorner and Cole, 2002).

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, fungistatis, antibiosis, rhizosphere modification, mycoparasitism, biofertilization and stimulation of plant defense mechanisms. 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 samples whiles AflaSafe treated samples had 105 ppb which represent a 96% reduction in aflatoxin levels. 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).

2.8.4 Chemical control methods

Generally, using any appropriate and recommended pesticides during the production phase of a crop to control pathogenic fungi have the potential of reducing aflatoxin development. Also, because insect damage and aflatoxin contamination are positively correlated (Bowen and Mack, 1991; Lynch and Wilson, 1991; Lynch *et al*. 1991; Gorman and Kang, 1991), any chemical measure taken to reduce insects would directly affect the

levels of aflatoxin accumulation. But for any chemical control measure to be acceptable, it must be efficient, safe, and cost effective while safeguarding the nutritional quality of the crop. A number of chemicals have specifically been identified to decontaminate mycotoxins in maize. Ammoniation has been considered to be one of the effective methods of detoxifying animal feed rich in aflatoxins (Lopez-Garcia *et al.,* 1999). Ammonia is known to degrade 95-98% of aflatoxin B_1 present in maize (Hell *et al.,* 2008). Ammoniation, unfortunately may not be effective against all mycotoxin and the treatment is limited to animal feed. Some mycotoxins 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 the best anti-fungal compounds followed by urea and citric acids (Gowda *et al.,* 2004).

2.8.5 Legislations

Wagacha and Muthomi (2008) reported that, factors fundamental to any country's ability to protect its population from mycotoxicoses includes the political will to address mycotoxin exposure and the capability to test food contamination, which determines whether requirement can be enforced. One third of European Union agricultural trade regulations are mycotoxin related. Trade and economic development efforts are hurt when commodities are rejected because they do not meet safety standards. Hence it would benefit developing countries whose economic backbone is agriculture to legislate regulation to control mycotoxins.

Mycotoxin regulations have been established in about 100 countries out of which only 15 are African countries (Fellinger, 2006; Barug *et al*., 2003; Van Egmond, 2002). Thirteen countries are known to have no specific regulations, and no data are available for about 50 countries, many of which are in Africa (Van Egmond, 1999). Human food are allowed 4-30 ppb aflatoxin, depending on the country involved (FDA, 2004; Henry *et al.*, 1999). In the U.S, 20 µg/Kg is the maximum limit for human consumption (Wu, 2006; FAO, 1996) while a quite significantly lower levels of 2 µg/kg total aflatoxin in food for human consumption are the maximum acceptable limits in the E.U, the strictest in standard worldwide (EC, 2006 ; Wu, 2006). In Ghana, the Ghana Standard Authority has set a maximum aflatoxin limit of 15 µg/kg in maize meant for human consumption.

Table 2.2: Maximum amount of aflatoxin allowed in foodstuffs in different countries

Country	Aflatoxin level (µg/kg)
Australia/New Zealand	15
Brazil	30
Canada	15
China	20
EU	2
India	30
Japan	10
Malaysia	35
South Africa	15
United Kingdom	10
United State	20

Source: (Liu *et al.,* 2006).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), the scientific body that develops advisory international standards on food additives and contaminants for the

CODEX Alimentarius Commission has set an aflatoxin standard of 15 ppb (Dohlman, 2003).

2.8.6 Surveillance and awareness creation

There are ample evidences to support that, considerable numbers of people living in sub-Sahara Africa are daily consuming aflatoxin contaminated maize. Apart from the 125 deaths recorded in Kenya in 2004 (Lewis *et al.*, 2005; Strosnider *et al.*, 2006), several cases including the presence of aflatoxin M_1 in human breast milk in Ghana, Kenya, Nigeria, Sudan are among others that have been reported (Bhat and Vasanthi, 2003). Unfortunately, due to ignorant, illiteracy, and socio-economic factors majority of the people in Africa continues to be exposed to aflatoxin contamination.

According to James (2005) and WHO (2006) surveillance and awareness creation could be an effective tool to limit the consumption of aflatoxin-rich food in Africa. Wagacha and Muthomi (2008) advised that, African countries should strengthen nationwide surveillance, increase food and feed inspections to ensure that food and animal feed are harvested correctly, dried completely and stored properly to reduce aflatoxins problems in African countries. In Ghana national agencies such as Ghana Standard Authority, Food and Drug Authority, have pivotal roles to create awareness and enforce existing regulation to limit consumption of aflatoxins by the populace. The Ministry of Food and Agriculture also should ensure that farmers are trained on good agricultural practices to produce grains which are safe for consumption. Awareness, sensitization and control measures designed to address aflatoxin contamination according to Ogunlela and Mukhtar (2009) should focus more on women because of the pivotal role they play in

decisions on pre-harvest and postharvest production including storage and marketing practices.

2.8.7 Dietary change, dietary variation and dietary intervention

People in developed countries according to Hell *et al.* (2008) experienced a low risk of mycotoxin contamination primarily due to their diverse diet that contains food from a range of climatic zones in which crops are produced with varying risks of mycotoxin exposure. Similar intervention has been adopted by parts of China, where individuals have changed their diet from maize to rice to reduce their risk of aflatoxin exposure (Yu, 1995). The levels of mycotoxin contamination in produce prior to consumption may be reduced by food processing methods such as wet and milling, grain cleaning, canning (autoclaving), roasting, baking, frying, alkali cooking (nixtamalization), and extrusion cooking (Hell *et al*, 2008).

2.8.8 Integrated mycotoxin management system

Mycotoxins are not considered as single group of toxicants on the basis of their mechanism of action because they are chemically diverse. For the same reason it would be impossible to develop or employ one of the various control measures to address mycotoxin contamination of any agricultural commodity. In addition, the heterogonous nature of mycotoxins distribution results in complications in the sampling and analysis due to ‘hot spot’. Taken all these factors into account, one can conclude that designing a programme for mycotoxin control is not a simple issue (Park, 1993; Park and Liang, 1993).

An integrated mycotoxin management system is considered as one possible approach to managing mycotoxin. The concept behind integrated management system according to Lopez-Garcia and Park (1998) is similar to the 'hurdle' effect, where each phase of production, i.e. pre-harvest and post-harvest processing, the risks are minimized. In summary it involves the adherence to good agricultural practices, proper control of biotic and abiotic factors that enhance aflatoxin contamination and the adoption of Hazard Analysis and Critical Control Point (HACCP) approach during the processing and distribution of food and feed to the final consumer.

2.8.9 Modified atmospheric storage (MAS)

The used of modified or controlled atmosphere in grain storage date back in antiquity. According to Donahaye and Navarro (2000) the use of MAS serves as a non-toxic and environmentally friendly alternative to fumigation to maintain product quality. MAS systems increase carbon dioxide or decrease oxygen atmospheres or a combination of both (Bell, 2000; Donahaye and Navarro, 2000).

Many storage fungi are capable of growth in low pressures of oxygen and reduction of available oxygen is often not enough to inhibit mycotoxigenic fungi in most especially high moisture grain. Elevated levels of carbon dioxide are severely inhibitory to mold growth (Hocking, 1989). Generally, decreasing oxygen to <0.14% is required before growth of aflatoxigenic fungi is substantially reduced. Also increasing carbon dioxide to >50% is required for inhibition of mycelia growth (Magan and Lacey, 1984).

A systematic investigation by Diener and Davis (1977) regarding how these gases affects aflatoxin production in maize showed that: when O_2 was decreased from 21% to 15% there was no effect on aflatoxin accumulation but a significant inhibition occurred when O_2 concentration was decreased to <5%. Moreover aflatoxin production was reduced by 25% when the CO_2 was elevated to 20% although it had no visible effect on growth and sporulation. The tolerance of low oxygen and high carbon dioxide is also influenced by the interactions with the grain type and water availability. The drier the grain, the more effective the modified atmosphere treatment would be (Magan and Aldred, 2007).

2.8.9.1 Hermetic storage

Hermetic storage system (HS) which simple means 'airtight' has been grain storage method practiced centuries ago in the form of sealed clay drums stored underground in Persia and Egypt (Murdoch and Baoua, 2014). It has evolved as a significant alternative to other methods of storage that maintains the wholesomeness of agricultural commodities by protecting them from insect and mould. Hermetic storage is a type of modified atmosphere that have been applied to maintain the quality and quantity of several agriculture commodities including corn, cocoa beans, coffee, rice, pulse and seeds (Navarro *et al.*, 1995;Varnava and Mouskos, 1997; Navarro, 2006; Sabio *et al.*, 2006).

Hermetic Storage is based on the principle of generating an oxygen-depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of living organisms in the ecological system of a sealed storage (Obeng-Ofori, 2011). The effectiveness of hermetic storage depends primarily on veracity of the hermetic seal, the commodity

stored, agro-climatic conditions, type and prevalence of insect pests, and mechanical strength of the barrier material (Njoroge *et al.,* 2014).

2.8.9.2 Forms of hermetic storage

The principal reasons for using hermetic storage for grains preservation is to prevent further insect, and microflora development by creating a low oxygen, high carbon dioxide atmosphere lethal to insects already present inside the container. The oxygen depleted atmosphere thus generated prevents development of cancer causing mycotoxins and maintains the moisture level of the commodity regardless of ambient humidity. According to Villers *et al*. (2006) hermetic storage takes three distinct forms. These are:

a) Organic-Hermetic storage- often referred to as "Hermetic Storage" relies on the metabolic activity and respiration of insects, microflora and the commodity itself to generate a modified, non-life sustaining low oxygen atmosphere.

b) Vacuum-Hermetic Fumigation (V-HF)- uses a vacuum pump to rapidly create a very low pressure atmosphere for accelerated disinfestation of non-crushable commodities through asphyxiation.

c) Gas-Hermetic Fumigation (G-HF)- uses an external gas source (usually CO_2) for crushable commodities, such as dried fruit, prior to shipment. These methods create a low oxygen modified atmosphere which normally results in 100% insect mortality of all life stages in a few days to two weeks as well as preventing mould development, protecting quality and preventing losses in the commodity. It also prevents development of cancer causing mycotoxins such as aflatoxins and ochratoxin A (OTA).

2.8.9.3 Types of hermetic storage

The existence of three different forms of hermetic storage systems have to do with meeting the postharvest storage and transportation needs. Several types of hermetic storage structures have been designed to reflect on the principles underlying modified atmospheric storage. These include the Transliner, GrainPro bumper, Mega cocoons, SuperGrain bags and Purdue Improved Crop Storage (PICS) bags and outside Storage bags shown in Plate 2.3-2.6.

Most hermetic storage technologies (SuperGrain and PICS bags) in the hands of farmers in the developing countries are made from flexible food grade plastic to facilitate transportation and distribution. Villers *et al.* (2006) reported that the low permeability of these types of hermetic structures maintains constant moisture levels in previously dried commodities regardless of ambient exterior humidity.

Plate 2. 3: TranSafeliner™ being installed in a shipping container

Plate 2. 4: One tonne SuperGrainbag-HC™ storing paddy with woven inside protective polypropylene bag

Plate 2. 5: 1050 Tonne Mega Cocoon™

Plate 2. 6: Hermetically sealed SuperGrainbag™

Source: Jonfia-Essien *et al.*, 2010

2.8.9.4 The PICS bag Technology

The triple-layer hermetic PICS bags were developed under the Bean/Cowpea CRSP project in the late 1980s with funding from USAID (Murdock *et al.,* 2003). As an organic-hermetic storage, PICS bag works by creating an airtight seal in which oxygen levels are dramatically decreased in a relatively short time through insect, fungal and seed respiration (Quezada *et al.,* 2006). This technology was originally created for West and Central African cowpea farmers under the name "Purdue Improved Cowpea Storage" (PICS) bags and served as protection against extremely destructive cowpea seed beetles, which prevented long-term storage to capture price increases later in the marketing season.

The Purdue Improved Crop Storage (PICS) hermetic bag system is now known to have proven effective in storing a variety of crops including cowpeas, maize, peanuts, sorghum, wheat, and common beans against insect pests (Murdock *et al.,* 2012; Anankware *et al.,* 2013; Baoua *et al.,* 2014; Mutungi *et al.,* 2014; Njoroge *et al.,* 2014; Vales *et al*., 2014). PICS bags have been disseminated to millions of farmers in West and Central Africa, with close to 50% of the cowpea not sold at harvest being stored in these simple containers (Moussa *et al.,* 2014; Ibro *et al*., 2014). To prevent moulding and rotting in tropical and subtropical conditions, maize should be dried at save moisture content and impurities removed before storing in hermetic conditions (Weinberg *et al.,* 2008).

2.8.9.5 Mode of action of PICS bags

Purdue Improved Cowpea Storage (PICS) bags consist of three plastic bags: two 80-mm high-density polyethylene (HDPE) bags, one surrounded by the second; both are enclosed by a third bag made of woven polypropylene. The polyethylene inner liners have finite oxygen permeability but it is nevertheless sufficiently low that it greatly hinders oxygen leakage into the bag interior from the surrounding air. The woven outer bag is of the type commonly used for grain storage throughout West Africa. Grains are put into the inner HDPE bag and tied shut with twine or string. The second (middle) bag, enveloping the first, is then tied shut in the same way such that it completely surrounds the inner bag. The outer woven bag is then tied shut completely surrounding the inner two bags. Together, these bags create a low-oxygen environment (Murdock *et al*., 2012). When oxygen level becomes sufficiently low, pests in the bag stop feeding, become inactive, and eventually die of asphyxiation (Moreno-Martinez *et al.*, 2000) or desiccation (Murdock *et al*., 2012).

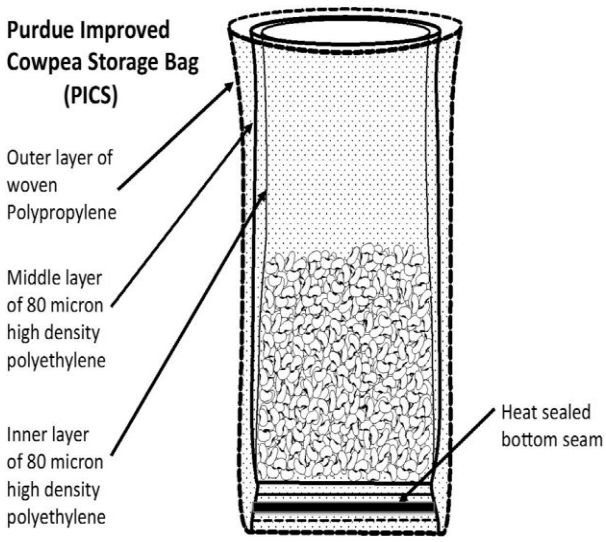

Figure 2. 2 Schemetic representation of the PICS bag

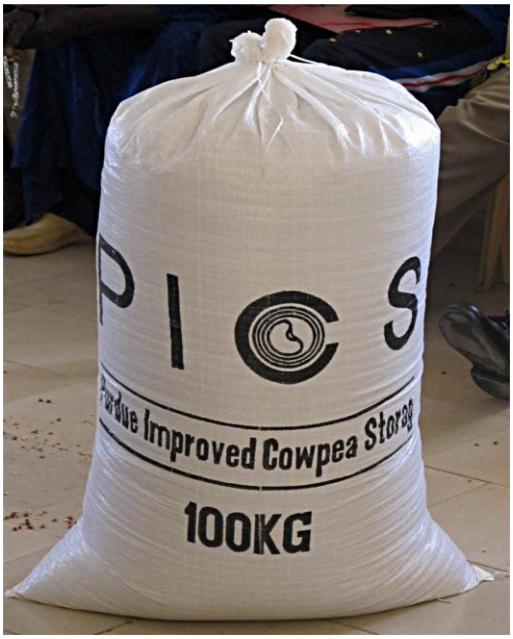

Plate 2. 7: PICS Bag with grains

Source: Murdoch and Baoua, 2014

2.9 PRESERVATION OF SEED VIABILITY BY HERMETIC STORAGE SYSTEM

Maintaining grain germination and vigour is of great significance to most farmers in the developing world because majority of these farmers often use some of the seed of stored produce as seed for subsequent season (De Bruin, 2005). An effective seed preservation technology ensures most seed maintain good qualities such as sprouting energy and viability.

Hermetic storage of seeds modifies the atmospheric composition surrounding them by depleting the oxygen through respiration of insects, moulds and seeds creating an insecticidal, fungistatic, or fungicidal atmosphere (Navarro, 2006). Relative humidity, high storage moisture content and high storage temperature are some factors that negatively correlate with loss of seed viability (Moreno *et al.*, 1988; Guberac *et al.*, 2003; Weinberg *et al*., 2008).

Generally, the lower the moisture content of seed and the lower the temperature the longer the seed can be stored (Copeland and McDonald, 1985). An investigation by Moreno *et al*. (1988) revealed that, maize stored at moisture contents between 15.3% and 17.7% were not invaded by fungus, when stored under hermetic conditions and maintained a higher viability than seed not stored hermetically. Similar results were obtained from studies on maize and coffee quality and viability after hermetic storage (Banks, 1981; Moreno *et al*., 1988; De Bruin, 2006; Anankware and Bornu-Ire, 2013).

2.10 INSECT

Damages caused by insect pests represent a huge setback in the world's effort to achieve food security globally. According to Ileleji *et al*. (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. The most economically destructive storage insect pests of maize in sub-Saharan Africa are maize weevil (*Sitophilus zeamais*) and the Larger Grain Borer (*Prostephanus truncatus*) (Meikle, 2002).

2.10.1 Recognition and identification of *Prostephanus truncatus*

The Larger grain Borer (LGB), *Prostephanus truncatus* (Horn) belongs to the family Bostrichidae which are mainly wood boring beetles. Nansen *et al*. (2004) suggested that bostrichids in general live on felled timber or dead wood, and that *P. truncatus* was considered a wood-boring species that has become adapted to stored commodities. Tubers and roots serve as the natural host of LGB.

The larvae are white, fleshy and sparsely covered with hairs. They are parallel-sided, i.e. they do not taper. The legs are short and the head capsule is small relative to the size of the body. The adult has the typical cylindrical bostrichid shape. The declivity is flattened and steep and has many small tubercles over its surface. The limits of the declivity, apically and laterally, are marked by a carina. The body is 3-4.5 mm long with the thorax bearing two rows of teeth. The head is turn underneath the thorax so that it cannot be seen from above.

Haines, (1991) used the following features to distinguish adult *P. truncatus*;

- Typical bostrichid shape, body cylindrical and dark brown in colour
- Head ventral to the prothorax
- Rows of teeth on the anterior part of pronotum
- Antennae 10-segemented with a loose three-segmented club
- Slender funicles clothed with long hairs
- Posterior flattened and steeply inclined elytra
- Five segmented tarsis

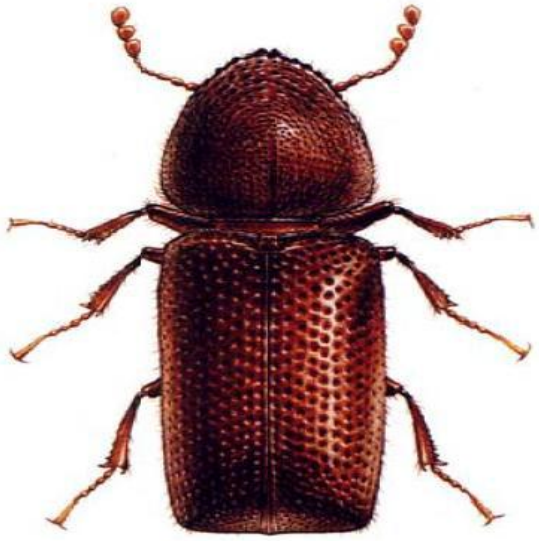

Plate 2. 8: Adult *P. truncatus* (Courtesy of Hodges R.J)

2.10.1.1 Life History and Behaviour

The life cycle of *P. truncatus* has been investigated at a range of temperatures and humilities (Shires, 1980; Bell and Watters, 1982; Hodges and Meik, 1984). Adults frequently initiate their attack on stored maize cobs with intact sheaths by boring into the

base of the maize cob cores, and eventually gain access to the grain via the apex of the cob by crawling between the sheathing leaves (Hodges and Meik, 1984).

Adults bore into the maize grains, making neat round holes, and as they tunnel from grain to grain, generating large quantities of maize dust. Adult females lay eggs in chambers bored at right angles to the main tunnels. Eggs are laid in batches of 20 and covered with finely chewed maize dust. Oviposition begins 5-10 days after adult emergence, reaching a peak at 15-20 days (Bell and Watters, 1982). Larvae hatch from the eggs after about three days at 27 °C and seem to thrive on the dust produced by boring adults. Development of the larva through to the adult stage at the optimum conditions of 32 °C and 80% RH takes 27 days on a diet of maize grain. Optimum conditions for development on maize are 32 ^{o}C and 70-80% relative humidity, and under these conditions, the life cycle can be completed in 24-25 days (Hodges, 1986). Details of flight performance and factors affecting flight and distribution behaviour have been investigated in the laboratory (Fadamiro and Wyatt, 1996; Fadamiro, 1997). A field study in Honduras showed flight activity of *P. truncatus* following a daily bimodal pattern with a major peak at 06.00-08.00 h and a minor peak at 18.00-20.00 h (Novillo, 1991). A similar pattern was observed by Tigar *et al.* (1993) in Central Mexico and Birkinshaw *et al.* (2004) in Ghana, but in both these cases the major peak was associated with dusk.

2.10.1.2 Geographical distribution of *Prostephanus truncatus*

P. truncatus is originally indigenous to Central America, tropical South America, (Hodges, 1986) but has spread remarkably to African countries. Its distribution is deemed phenomenal when compared to other storage pests, in that it is very discrete and

delimited (Markham *et al.*, 1991). Its success has also been attributed to its ability to tolerate maize of very low moisture content of 9% (Obeng-Ofori, 2008)

2.10.1.3 Incidence of *Prostephanus truncatus* in Africa

P. truncatus was accidentally introduced into Africa in the early 1980s (Dustan and Mangazini, 1981). Prior to 1981 there were no records of this pest in Africa (Bonu-Ire, 2001). However, in 1981 it was identified as a new pest causing severe damage to farm stored maize in the hot dry Tabora region of Tanzania (Gilman, 1984). It subsequently spread within Tanzania, into Southern Kenya (Kega and Wurui, 1983), Burundi (Gilman, 1984), Rwanda (GTZ, unpublished) and Malawi (GTZ, unpublished).

In West Africa, a serious outbreak of the pest was found in 1984 in Togo (Harnisch and Krall, 1984), and later in Ghana (Dick *et al.*, 1989), Benin (Krall and Favi, 1986), Guinea Conakry (Kalivogui and Muck, 1991), Burkina Faso (Bosque-Perez *et al.*, 1991) and Niger (Adda *et al.*, 1996). The pest has now spread into 17 countries in West, East and Southern Africa (Nansen and Miekle, 2002; Cugula *et al.*, 2007). In Ghana the pest was first reported in the Volta Region of the country, which shares a common boundary with Togo, where the pest was reported first in West Africa (Harnisch and Krall, 1984).

2.10.1.4 Economic importance of *Prostephanus truncatus*

The introduction of this pest in Africa has influenced the economy of several countries, especially those depending on exportation of maize. Many countries now refuse to import maize from areas infested with the larger grain borer (Boeye *et al.*, 1990). In the early days after the arrival of *P. truncatus* in East Africa, countries with the pest found their

maize exports banned. For example in 1987-88, it is estimated that Tanzania lost US $634,000 in export earnings. This situation improved following efforts to upgrade phytosanitary procedures in the region but such procedures, involving fumigation, have their own continuing costs (Boxall, 2002). Infestations in maize may start on the mature crop in the field, i.e. when moisture content is at or below 18%. Weight losses of up to 40% have been recorded in Nicaragua from maize cobs stored on the farm for 6 months (Giles and Leon, 1975). In Tanzania, up to 34% losses have been observed after 3 months storage on the farm, with an average loss of 8.7% (Hodges *et al.*, 1983). Adults of *P. truncatus* bore in maize grains and produce large quantities of dust, in which their larvae seem to feed and pupate. Apart from maize, there have been reports of damages to wide range of produce such as hard winter wheat, short grain rice, butter beans, and cocoa beans. Hodges *et al*. (1985) reported 70% losses in dried cassava roots after four months of storage due to this species.

2.10.2 Recognition and identification of maize weevil (*Sitophilus zeamais*)

Maize weevil, *Sitophilus zeamais* (Motschulsky), is one of the cosmopolitan pests of stored cereals, especially maize (Throne, 1994). It damages stored maize and of cob maize prior to harvest. It may also infest other cereals if the moisture content is moderate or high. *S. zeamais* occurs throughout the warmer, more humid regions of the world, especially where maize is grown (Longstaff, 1981).

The developmental stages (eggs, larvae and pupae) are all found within tunnels and chambers bored in the grain and are thus not normally seen. The larvae are apodous. It is usually blacker than *S. oryzae*, with fine microsculpture and is shinier. Its scutellum has

lateral elevations further apart than their longitudinal length which is about half as long as the scutellum. Males have median lobe of aedeagus with two longitudinal grooves dorsally, except in the apical quarter, and is thus sinuous in cross section. Females with lateral lobes of the Y-shaped sclerite pointed and their separation is greater than for *S. oryzae*. The antennae have eight segments and are often carried in an extended position when the insect is walking. It has four pale reddish-brown or orange-brown oval markings on the elytra, but these are often indistinct.

2.10.2.1 Biology and Ecology

S. zeamais is 5 mm in length and a very active flier. Infestation usually starts in the field and later continues in the store. Adult females chew grains creating a small hole in which they lay eggs throughout most of their adult lives, although 50% may be laid in the first 4-5 weeks; each female may lay up to 150 eggs. The eggs are laid individually in small cavities chewed into cereal grains by the female; each cavity is sealed, thus protecting the egg, by a waxy secretion (usually referred to as an 'egg-plug') produced by the female. The incubation period of the egg is about 6 days at 25 °C (Howe, 1952).

Eggs are laid at temperatures between 15 and 35 °C (with an optimum around 25 °C and at grain moisture contents over 10%. However, rates of oviposition are very low below 20 °C or above 32 °C, and below about 12% moisture content (Birch, 1944). Larvae tunnel in grains and are responsible for most of the damage. There are four larval instars all of which remain within the grain. Immediately after hatching, the first instar feeds by burrowing through the tissues of the grain. At the end of the fourth instar the larva uses a

mixture of frass and larval secretion to close off the end of the burrow, to form a pupal cell.

Pupation takes place inside the grain and adults chew their way out through the outer layer of the grain leaving a large, characteristic emergence hole. Total development periods range from about 35 days under optimal conditions to over 110 days in unfavourable conditions (Birch, 1944; Howe, 1952). Adults live for 5-6 months depending on the temperature and humidity of grains (Mound, 1989). *Sitophilus zeamais* is capable of inhabiting reserved breeding grounds near the threshing floors that are normally full of plant residues, where the population builds up in before moving to granaries. Even though *S. zeamais* is a serious primary pest of stored maize, it is capable of developing on all cereals, dried cassava and other processed food products.

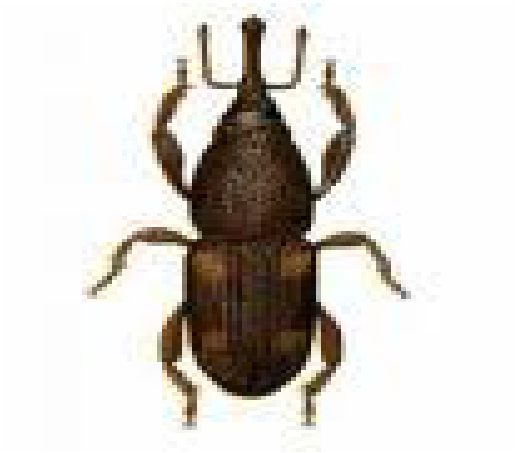

Plate 2. 9: Adult *S. zeamais* courtesy of U.S Department of Agriculture

2.10.2.2 Economic importance of *Sitophilus zeamais*

S. zeamais is very important pests of cereals with the ability to cause damage to mature crop on the field when the moisture content of the grain has fallen to 18-20%. Subsequent

infestations in store result from the transfer of infested grain into store or from the pest flying into storage facilities, probably attracted by the odour of the stored grain. Dry weight loss from *S. zeamais* infestation alone averaged about 5% by weight after six months of storage. The 5% dry weight loss translates into 22% of total grains displaying damage (Holst *et al.,* 2000).

2.10.3 Insect role in aflatoxin contamination

Food availability as well as income of maize farmers and traders usually suffers setbacks due to extensive losses in physical weight, food value and marketability due to insect damage. In addition to direct damage to stored grains, several studies have proven that insect activities result in qualitative loss including aflatoxins contamination, discolouration, and obnoxious smell. Sinha and Sinha (1992) reported that *A. flavus* infection in insect-damaged grain was 87% whiles insect-free samples was 25%. Hence insect damage of maize is a good predictor of aflatoxin contamination and can serve as early warning.

Insects that feed on maize ear in the field predispose kernels to fungal infection through physical damage while storage insect pests open the kernel to fungal invasion (Avantaggio *et al.,* 2002). Insect-damaged grains provide an opportunity for fungus to circumvent the natural protection of integument and establish infection sites in vulnerable interior (St. Leger *et al.,* 2000). Insects can disseminate spores of *A. flavus* in the field and stored products (McMillian, 1987). They act as vectors by transporting fungal spores on their bodies, contaminating grains as they move about (Lynch and Wilson, 1991). Insects carry spores from the plant surfaces to the interior of the stock or kernel or create

infection wounds due to feeding of the larvae on the stalk or kernels (Munkvold and Hellminch, 2000).

A survey of insects collected on the Southern United States showed that, some were infected with *A. flavus,* both internally and externally. Similarly, in India, Pande and Mehrotra (1988) sampled wheat grains for *Sitophilus oryzeae* and found out that *A. flavus* was the more frequent found species in their alimentary canals, followed by *A. candidus, A. sojae, A. fumigatus, Penecellium rugulosum* and *Cladosporium cladosporioides.* In South Africa, Flett and Van Rensburg (1992) showed that *Busseola fusca* infestation significantly increased the incidence of *F. verticillioides* infected maize cobs, irrespective of whether the cobs are artificially inoculated with the fungus or not. A recent study in Benin, reported that cob/stem infection by *F. verticillioides* positively correlated with infestation of *Eldana saccharina*, *Cryptophlebia leucotreta*, *Mussidia nigrivenella* and *Sesamia calamistis* (Schulthess *et al.*, 2002).

These findings prove the possibility that, insects can transmit fungus spores from infected grains to healthy grains and vice-versa.

Insects metabolic activities results in increase relative humidity, providing favourable conditions for growth of *A. flavus* which results in aflatoxin contamination and reduction in seed germination capacity (Sauer and Burroughs, 1980; Mills, 1983).

According to Beti *et al*. (1995) moisture content increased from 15% to 20% after 30 days in maize infested with *S. zeamais* and significant aflataoxin B_1 was found in *S. zeamais* infested grain that had been inoculated with *A. flavus.*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey

A field survey was carried out in Techiman Township, Forikrom, Tanoso and Fiaso in the Techiman Municipality in the Brong Ahafo Region of Ghana. The survey was used to assess the social acceptability, opportunities and challenges farmers faced upon the usage of the triple-layer hermetic bags. A total of one hundred and seven (107) questionnaires were administered to farmers who have benefited from the intervention. Questions regarding the level of acceptance of the technology by farmers, benefits such as insect pests control, household food security, increment in income and the short falls of the technology were addressed in the questionnaire.

3.2 Treatment and experimental design

An obatanpa maize variety cultivated on farmer's field in Techiman, Brong Ahafo was brought to the Entomology Laboratory of Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences (CBAS), University of Ghana (UG), Legon, for the study.

A factorial treatment combination of two (2) insects (*Sitophillus zeamais* and *Prostephanus. truncatus*) and two (2) bagging technologies (triple-layer hermetic bag and polypropylene interwoven bag) were used for the experiment in a completely randomized design.

A five kilogram triple-layer bag (150 um thick and measure 34 cm× 62 cm in width and length, respectively) supplied by Bioplastic Company Limited Accra was used for the study. Polypropylene bag of the same dimension as the hermetic bag was also bought from the open market to serve as the control for the study.

3.2.1 Culturing of insects

An unsexed adults *Sitophillus zeamais* and *Prostephanus truncatus* were obtained from the Entomology Laboratory of Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences (CBAS), University of Ghana (UG), Legon. Insects were cultured to obtain insect population of known age at culture conditions of 28±2 ^{0}C, 65 % Relative humidity and 12:12 LD photo regime (Bonu-Ire, 2001). About 500 unsexed adult *S. zeamais* and 500 unsexed adults *P. truncatus* were introduced into separate glass jar containing 1 kg of sterilized maize. The grains were sterilised in a refrigerator for 24 hours and in an oven at 40 ^{0}C for six hours (Bonu-Ire, 2001). After 15 days, all the insects were removed and the maize grains kept in the same condition for 2 months. After this period, the adult insects that emerged from the culture were used for the experiments.

3.2.2 Experimental procedure

Impurities and broken kernels were removed by screening the maize. Prior to filling the triple-layer hermetic bags, they were tested for hermetic seal by filling each of the two inner high-density polyethylene (HDPE) envelopes with air to ensure freedom from tears and leakages. Each of the 5 kg triple-layer hermetic bags (TLHB) and the polypropylene bags (PPB) was filled with 2.5 kg of maize and grouped into three sets.

In the first sets, 50 unsexed adults of *S. zeamais* from the laboratory culture were introduced into the maize with a camel brush, to simulate pre-storage infested maize. Each bag was gently pressed to evacuate all air present and tied quickly with cotton rope after the expulsion of air. This was labelled TLHB1 and PPBI respectively.

In the other sets of 5 kg TLHB and PPB bags, 50 unsexed adults of *Prostephanus truncatus* was introduced into each of the maize samples, following the same procedure as stated above. This was also labelled TLHB2 and PPB2 respectively.

The third set of bags contained a mixture of the *S. zeamais* and *P. truncatus* in the same proportion i.e. 50 adult insects from each. These set of bags were also tied and stored.

The last sets of TLHB and PPB bags were filled with 2.5 kg maize sterilized at 60 ºC for 3 hours to kill all insects present. The bags were gently pressed to remove all air present and tied immediately with cotton ropes after the evacuation of air. These sets were labelled TLHB4 and PPB4.

Each treatment was replicated three (3) times and stored in an improved crib at the University of Ghana campus farm, Accra for six months.

In all, there were 144 experimental units. Destructive and replacement sampling was done at one month interval (i.e. 24 bags per month) for six months.

Germination percentage was assessed prior to storage, 3 and 6 months after storage. Moisture content was recorded monthly for six months. Percentage insect damage and Aflatoxin levels were assessed before, 1, 3 and 6 months after storage.

Plate 3. 1: 2.5kg triple-layer hermetic bag used for the study

Plate 3. 2: Maize stored in triple-layer and polypropylene bags in crib on the farm

3.3 Determination of aflatoxin level of insect-free maize and insect infested maize in the triple layer hermetic bags.

3.3.1 Determination of percentage damage

A random sample of 50 grams of maize was taken from each storage bag using cone and quarter method prior to storage, 1, 3 and 6 months after storage. Bored grains were separated from whole grains and their number counted.

The percentage damage was calculated using the method described by Adams and Schulten (1978) and Duna (2003).

$$\% \text{ Damaged grains} = \frac{\text{number of bored grain}}{\text{Total number of grains samples}} \times 100$$

3.3.2 Sampling for initial levels of aflatoxin in maize

Maize transported to the experimental site was sun dried to moisture content of between 12%-13% and mixed thoroughly in a container to obtain a uniform sample. Four composite samples, each weighing 2 kg was taken from the mixed sample, by sub-sampling from the different parts of the container. The initial average aflatoxin content of maize was obtained from the composite samples at the Ghana Standard Authority.

3.4 Determination of temperature and relative humidity

A thermo hydrometer data logger (EL-USB-2, LASCAR electronics) was used to measure the temperature and relative humidity in the hermetic bags, polypropylene bag and surrounding environment for the duration of the study. The thermo hydrometer logger was programmed to continuously measure the temperature and relative humidity

every thirty minutes. Data logger was inserted into the triple-layer hermetic bag and the conventional bag before they were tied to measure the internal temperature and relative humidity. Similarly, the external environmental conditions of the storage structure were also measured by placing the data logger on the platform of the storage structure.

3.5 Determination of oxygen (O_2) and carbon dioxide (CO_2)

Prior to the opening of the triple-layer hermetic bags, oxygen and carbon dioxide levels were measured with the aid of GrainPro oxygen analyser (SCY-2A, MAOAN) butterfly needle and epoxy glue. The instrument, calibrated in nitrogen gas to 21% was used to measure the oxygen level in the hermetic bags. To take measurements the inner HDPE lining was punctured with the analyser needle at the top, middle and bottom. All punctures on the bag walls were sealed with epoxy glue. Subsequent measurement was performed from the same spot by simply opening and closing the lid of the butterfly needle. This was done immediately after the set up and repeated daily.

3.6 Isolation and identification of fungus

Isolation and identification of fungal pathogens from the samples was carried out in the Pathology Laboratory, Crop Science Department in the School of Agriculture, CBAS, Legon on potato dextrose agar (PDA) before, during (3 months) and after storage (6 months). Potato dextrose agar was prepared by dissolving 3.9 grammes of PDA powder in 100 ml of distilled water in a 250 ml conical flask. The conical flask together with the content was carefully swirled, covered with aluminum foil and autoclaved at 1.05 kg/cm^2 pressure and 121 ^{o}C for 15 minutes. The prepared PDA was poured in 9 cm sterilized petri dishes and allowed to solidify. Surface sterilization of well mixed, random sampled

maize was done in 1% sodium hypochlorite for 30 seconds, blotted dry with filter paper and the sterilized grains placed at a rate of 10 grains per petri dish on cooled potato dextrose agar. The morphological identification of fungus associated with maize grains was done by scraping mycelia plugs advancing from margins of the grains with flamed scalpel. The plugs were mounted on slides for microscopic examination using distilled water. The prepared slides were examined under a compound microscope. Identification of the isolates was done based on colour, morphology of mycelial, conidia and sporulating structures as described by Alasduro (1970), Agrios (2005) and, Barnett and Hunter (2006).

3.7 Assessment of levels of temperature effective to control aflatoxin in the triple-layer hermetic bags.

A factorial treatment combination of three (3) temperature levels (16^{o}C, 30^{o}C and 38^{o}C) and two (2) bagging technologies (triple-layer hermetic bag and polypropylene interwoven bag) were used for the experiment in a completely randomized design.

Sample of maize from obatanpa variety was screened to remove foreign materials. The grains were sterilized at a temperature of 60 ºC for 3 hours and allowed to cool. A five kilogram hermetic and polypropylene bags were filled with 2.5 kg grains and grouped into three batches representing the experimental treatments (temperature at 16 ºC, room temperature (30 ºC), and 38 ºC).

The first batch of maize samples was stored in an incubator in the Entomology Laboratory, Crop Science Department, School of Agriculture, and CBAS at temperature

of 16 º C. This batch was labelled HT1 and PT1 The second batch of maize samples was stored at a room with an average temperature of 30 ºC at the Africa Regional Postgraduate Programme in Insects Science (ARPPIS)-West Africa Center, University of Ghana, Legon. This batch was labelled HT2 and PT2

The last batch of maize samples was also stored at warm room at temperature of 38 ºC at ARPPIS University of Ghana, Legon. The last batch was labelled HT3 and PT3.

Each treatment was replicated three (3) times and stored for three months. In all, there were 18 experimental units. Sampling of maize for analysis of moisture content and aflatoxin levels was done before storage and 3 months after storage.

3.8 Analysis of maize for aflatoxins levels

3.8.1 Sampling

In the first month of storage, insects in maize stored in the different storage bags were sieved-out to obtain clean maize samples. The clean maize grains were thoroughly mixed to obtain a uniform sample. A 1 kg subsample was drawn from each of the 2.5 kg of maize in the storage bags for aflatoxins analysis. The above procedure was repeated for subsequent months for aflatoxins analysis.

3.8.2 Determination of aflatoxins content in stored maize

The aflatoxin levels were determined at the Mycology Laboratory of the Ghana Standard Authority, Accra, according to ISO 16050: 2003 test methods. The 1 kg sub-sample maize was ground with laboratory mill and blender and mixed thoroughly before sampling into sample containers. Two grams (2 g) of sodium chloride and 100 ml of

extraction solvent were added to 20 g of milled maize. The solution was shaken and homoginised for 3 minutes. The extract was filtered using a paper filter and 20 ml of the filtrate was diluted with 60 ml of PBS, mixed well and filtered again using glass microfiber filter. Purification was carried out by passing the extract through immunoaffinity columns (IAC) containing antibodies specific for aflatoxin B_1, B_2, G_1 and G_2. The aflatoxins were eluted with 1.0 ml of methanol and quantified by reverse-phase High Performance Liquid Chromatography (HPLC) with spectrofluorometric detector. Aflatoxins were derivatised in a post-column reaction chamber (Kobra cell) by adding potassium bromide of 0.119 g to 1L of the mobile phase followed by fluorescence detection. Aflatoxins are identified by comparing the retention time of the peak detected in the chromatogram of the test solution with the retention time of the peaks of the standard for aflatoxins.

3.9 Determination of viability and germination rate

Seed viability test was conducted in the laboratory in petri dish and on the field by sowing in the soil. The test was conducted on samples of maize seeds from the various treatments. The test was done before, 3 and 6 months after storage. Fifty (50) seeds was randomly selected from various bags and sown in the field. Another batch of 50 randomly selected seeds was cultured in petri dishes lined with filter paper which was moistened with distilled water. Both laboratory and field experiments were watered periodically and monitored for seven days for seed emergence. On the seventh day the germinated seeds from each test were counted. The viability or germination potential was calculated using the formula:

$$\text{Germination potential (Gp) \%} = \frac{Ng}{Nt} \times 100$$

Ng= number of germinated seeds; Nt= total number of seeds in the sample or initial number of seeds in the sample

The results of the test were compared to determine whether length of storage and treatment conditions inside the hermetic storage bags affected the germination capacity of maize seeds during storage.

3.10 Analysis of Data

Descriptive statistical analysis of survey data was carried out using Statistical Package for Social Scientist (SPSS) software (version 16). All other data were subjected to Analysis of Variance procedure in GENSTAT statistical package (version 12) and Microsoft Excel. Fischer's protected LSD was used to separate the means when there were significance differences.

CHAPTER FOUR

4.0 RESULTS

4.1 Social acceptability, benefits and challenges of using the triple layer hermetic bag by farmers in Techiman Municipality in the Brong Ahafo Region of Ghana.

The outcome of the questionnaire administered to farmers in Forikrom, Fiaso, Tanoso and Techiman all in the Techiman Municipality of the Brong Ahafo Region of Ghana, on the level of social acceptability, opportunities/benefit and challenges encountered in the utilization of the triple layer hermetic bag is presented below.

Out of a total of one hundred and seven (107) farmers who benefited from the triple-layer hermetic technology, fifty eight (58) farmers representing 54.2% were women, with men constituting 45.8%. The predominant household size within the communities ranged from 4-7. Majority (53.3%) of farmers interviewed belonged to farmer based organization as shown in Table 4.1

Table 4.1: Demographic data of respondents

Variable	Description	Response (%)
Gender	Male	45.8
	Female	54.2
Household size	0-3	32.7
	4-7	49.5
	8-11	16.9
	>11	0.9
Association/Organization	Yes	53.3
	No	46.7

There was great disparity in the level of education of respondents. Greater proportion (40%) of respondent had no formal education, with sizeable numbers of (29%) and (22%) having primary and junior high school education respectively as shown in Figure 4.1.

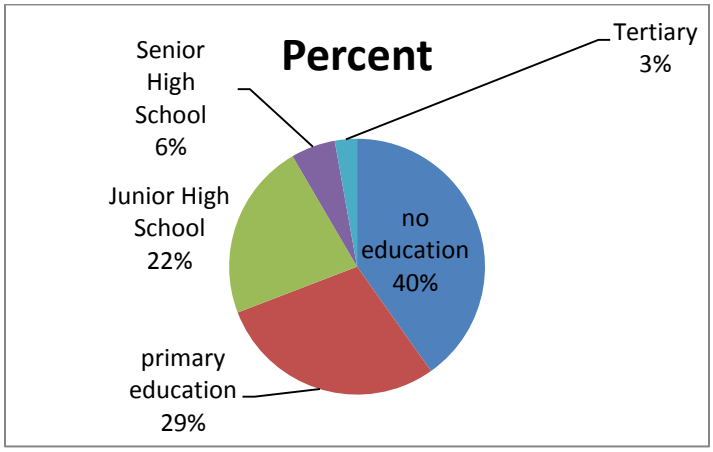

Figure 4. 1: Level of education of farmers

Also, the survey proved that most of the beneficiaries of the intervention were first introduced to the technology through agricultural-based NGOs (Concern Universal and ABOFAP) working within the municipality (Figure 4.2).

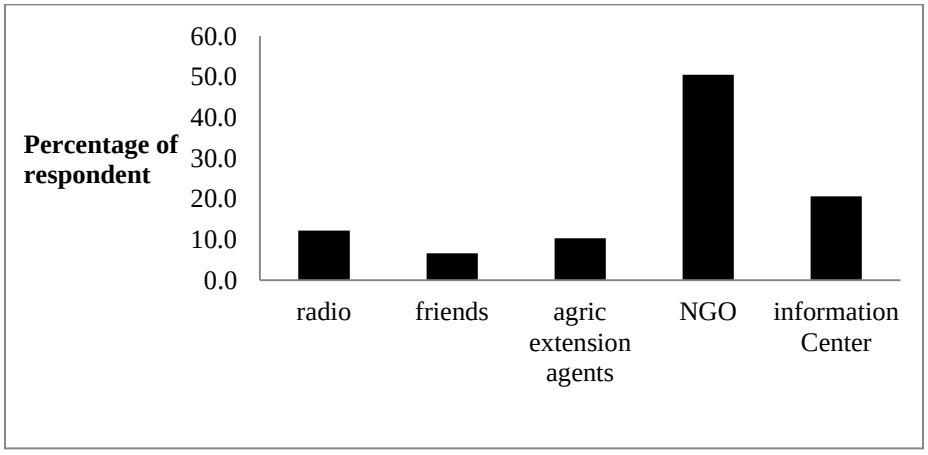

Figure 4. 2: Farmers first source of information about the triple-layer hermetic bag technology

4.1.1 Level of social acceptability of the triple-layer hermetic bags

A large proportion (75.7%) of the beneficiaries accepted the technology without much hesitation (Table 4.2). When probed on their reasons behind such a decision, farmers expressed varied reasons (Figure 4.3).

Table 4.2: Percentage respondent of farmer's initial reception to the triple-layer hermetic bag technology

Variable	Description	Percentage of response %
Level of acceptance of the hermetic bag	Yes	75.7
	No	24.3

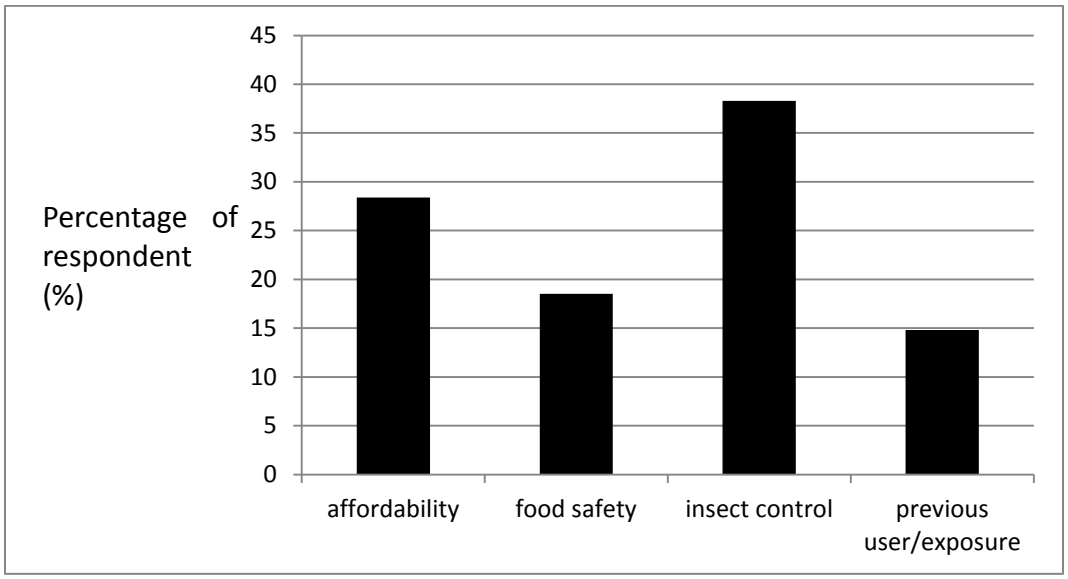

Figure 4. 3: Farmers reasons for accepting the triple layer hermetic technology bag after introduction

Farmers who later adopted the intervention gave different reasons for their initial hesitation. The issue of cost was the predominant reason, followed by their doubt of the effectiveness of the technology and its durability (Figure 4.4).

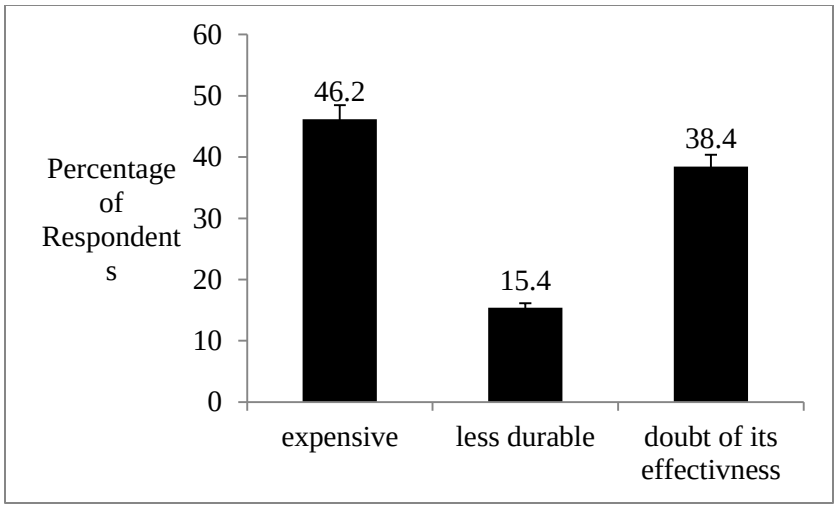

Figure 4. 4: Reasons for farmer's reluctance in accepting the triple-layer hermetic bag technology after introduction

4.1.2 Benefits gained by farmers from the triple-layer hermetic bag technology

Higher percentage of farmers (38.2%) adopted the technology with the aim of protecting their farm produce from insect damage. When asked whether their expectation were met, all the farmers responded that the intervention protected their farm produce from insects attack. When further enquired about the length of time they did store their farm produce following the adoption of the hermetic bag technology, more than 40% of beneficiaries said they did store their produce for at least six (6) months with 35.5% and 18.7% storing their produce between 7-12 months and 1-2 years respectively. All farmers interviewed responded that the technologies have helped improve food security in their household. Aside the improvement in farmer's household food security, greater proportion of 34.8% said storing their produce in the bags enabled them to take advantage of good prices in the course of storage (Figure 4.5). The survey also revealed that beside maize, farmers

used the hermetic bags to preserve other agricultural commodities and a non-agricultural item (cloths) as shown in Table 4.3.

Table 4.3: Length of storage and other alternate use of the triple layer bags by farmers

Variables	Description	Percent %
Length of storage	≤ 6 months	45.8
	7-12 year	35.5
	1-2 years	18.7
Other commodities stored with the triple-layer hermetic bag by farmers	Cowpea	48.4
	Groundnut	9.3
	Cassava powder	19.4
	rice	14.4
	Clothes	8.5

Plate 4. 1 Clothes kept in hermetic bag in Forikrom, Techiman Municipality

Source: From survey, 2015

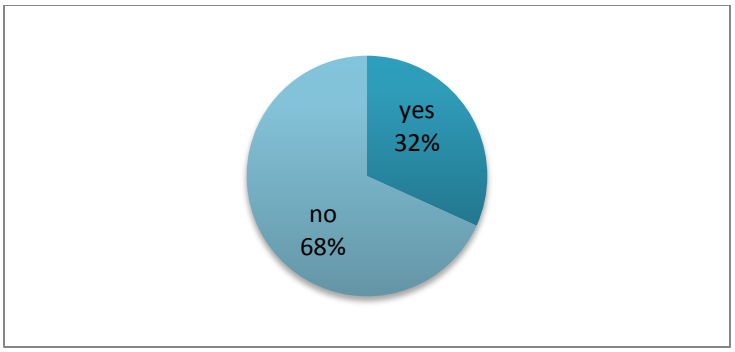

Figure 4. 5: Percentage of respondent farmers who took advantage of good prices for their produce after using the hermetic bag

4.1.3 Concerns of farmers on the triple-layer hermetic technology bags

When asked about their concerns regarding the intervention, 67.9% of respondents said they have no concerns after using the triple layer hermetic bags (Table 4.4). Nonetheless, the few concerns expressed by farmers ranged from leakage of the HDP bags and the continuous rise in the price of the bag as showed in Figure 4.6.

Another concern worth-mentioning is the situation where the cost of replacing a torn HDP bag is the same as a complete PICS bag. Lastly, when farmers were ask about where they could get the hermetic bags to buy in case they lose what they have, 3.7% farmers expressed lack of information on where to get a new bag in case the need arose, whereas 96.3% of farmers know where to get replacement for their torn bags.

Table 4.4: Proportion of farmers with concerns and information of sale point of the triple-layer hermetic bag

Variables	Description	Percentage (%)
Concerns	Yes	67.9
	No	32.1
Information on triple-layer technology sale point	Yes	96.3
	No	3.7

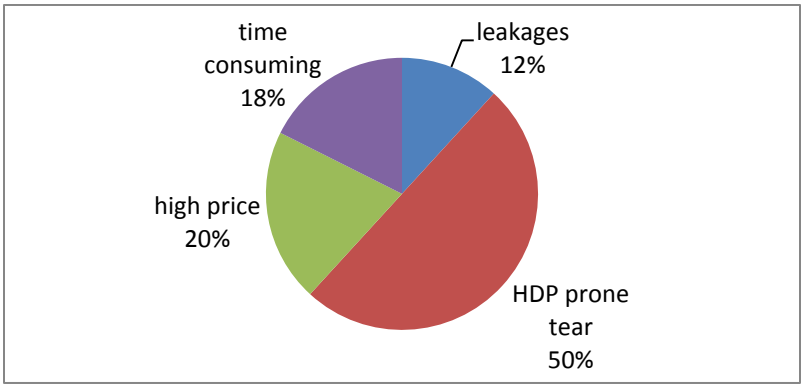

Figure 4. 6: Concerns of farmers on triple-layer hermetic bag technology

4.2 Changes in temperature, relative humidity and dew point within the storage structure, hermetic bag and polypropylene bag.

Temperature, relative humidity and dew point fluctuated both within the storage structure and the different storage bag technology during the storage period. A maximum and minimum temperature of 36.5 ^{o}C and 22 ^{o}C were recorded within the crib whilst a maximum and manimum relative humidity and dew point of 98.5 % to 50% and 30.5 ^{o}C and 19.7 ^{o}C were respectively recorded as shown in Figure 4.7. Similarly, internal

temperature, relative humidity and dew point within the triple-layer hermetic bag and the polypropylene bag fluctuated as the season changes (Figure 4.8).

A minimum and maximum temperature of 23.0 ^{o}C and 33.5 ^{o}C were recorded in the hermetic bag with a corresponding minimum and maximum relative humidity of 68.5% and 79.5% were respectively recorded. At the same period of storage, the highest and lowest dewpoint recored were 28.7 ^{o}C and 17.8 ^{o}C respectively. Also, the three abiotic factors varied significantly within the polypropylene interwoven bag with a minimum and maximum temperature of 30.5 ^{o}C and 36.5 ^{o}C repectively. The minimum and maximum relative humidity recorded in the conventional bag during the period of storage were 68.5% and 79.5% respectively whilst the highest dew point recorded in the same period was 32.5 ^{o}C as shown in Figure 4.9.

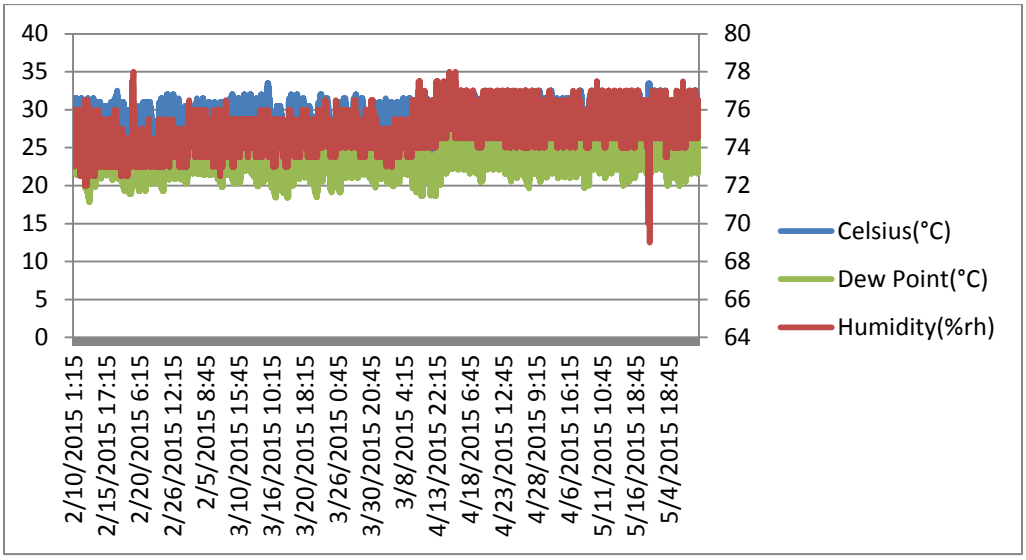

Figure 4. 7: Daily Temperature, dew point and relative humidity in the maize storage structure

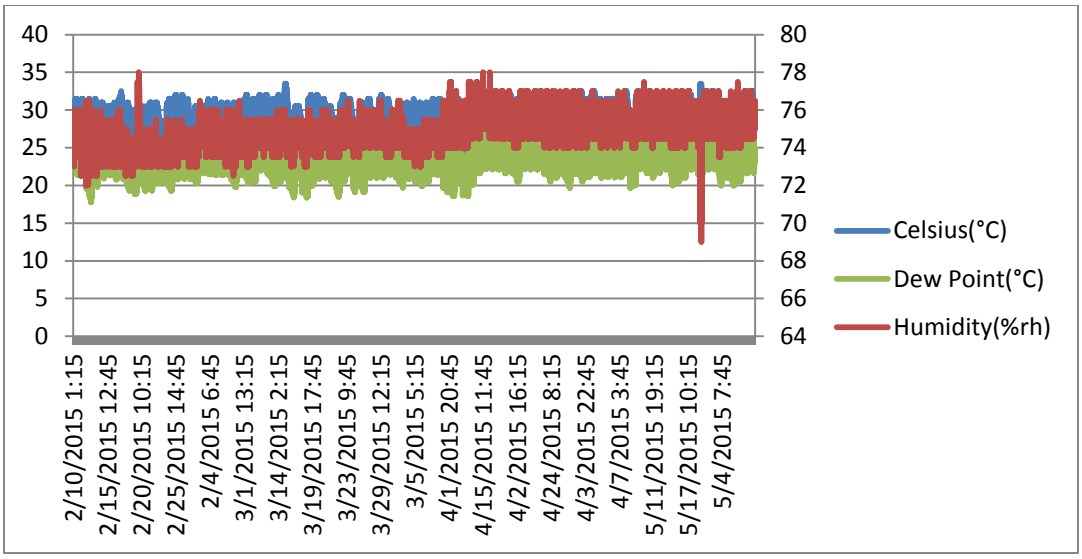

Figure 4. 8: Internal daily temperature, dew point and relative humidity in the triple-layer hermetic bag

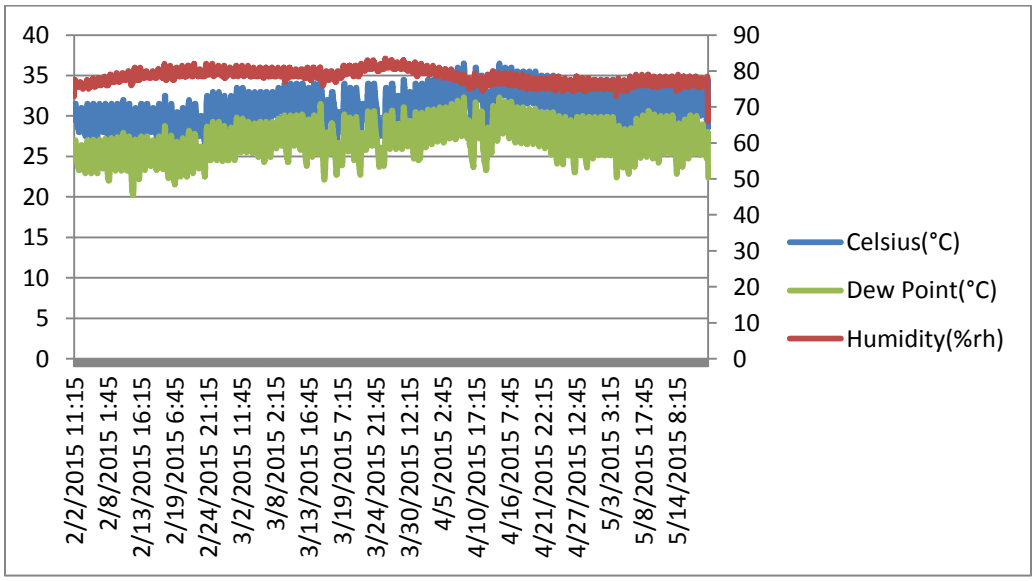

Figure 4. 9: Internal daily Temperature, dew point and relative humidity in the polypropylene bag

4.3 Changes in oxygen and carbon dioxide concentration in the triple-layer hermetic bag

Figure 4.10 and 4.11 show the changes in the atmospheric gas content in insect-free and insect-infested maize in the triple-layer hermetic bag. During the first couple of days (1 to 4) after sealing the hermetic bags the initial oxygen of 21% in the insect-infested maize dropped greatly to 13.5%. The corresponding carbon dioxide concentration (7.2%) was recorded after four days of storage. Similar trend was observed in the insect-free maize with oxygen dropping from 21% to13.5% after 5 days of storage. However the levels of oxygen depletion and carbon dioxide elevation remained relatively constant after 20 days of storage in the hermetic bags, regardless of the presence or absence of insect on the maize. The lowest concentration of oxygen and highest carbon dioxide of 4.6% and 16.7% was recorded respectively after 23 days for maize stored in the triple-layer hermetic bags.

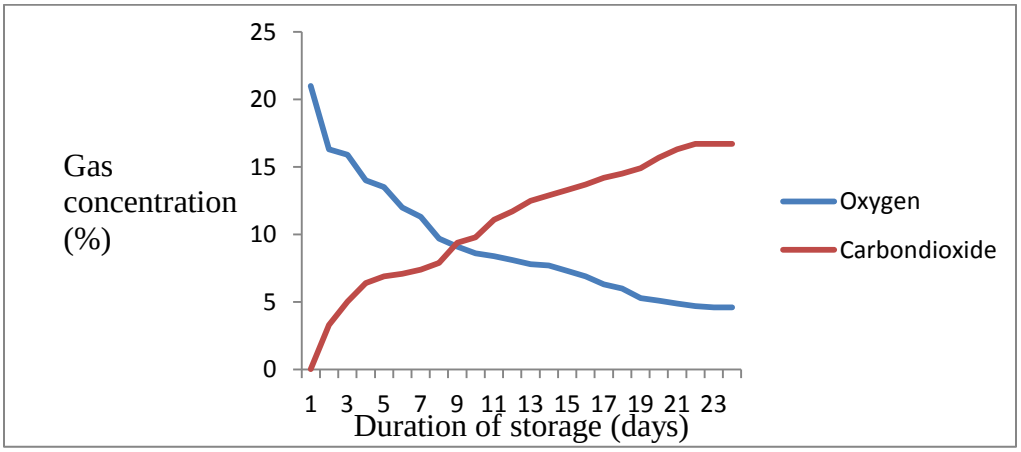

Figure 4. 10: Changes in atmospheric gas concentration in insect-free maize in triple-layer hermetic bag

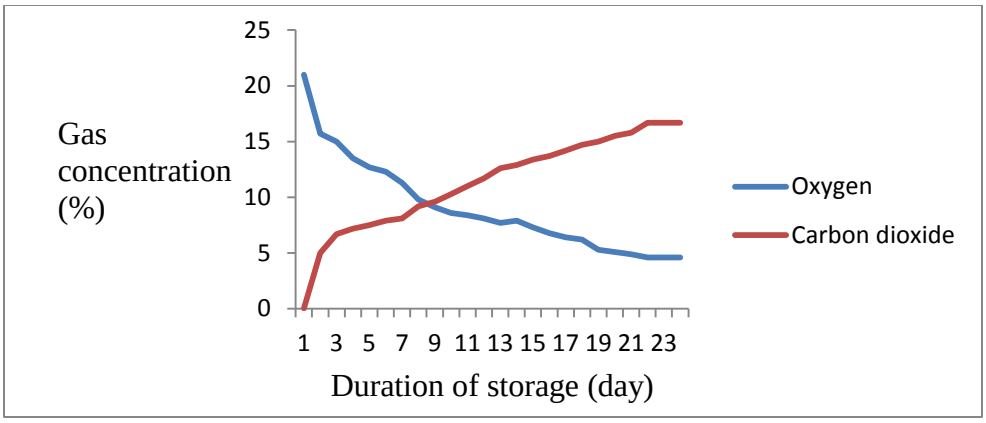

Figure 4. 11: Changes in atmospheric gas concentration in insect-infested maize in triple-layer hermetic bag

4.3 Changes in moisture content of maize grain in different storage bag technologies.

During the period of storage, changes in the moisture content of grains in relation to the storage time, interactions of the storage bag technologies (triple layer hermetic bag and polypropylene interwoven bag) and insects, showed significant differences ($p<0.05$) as shown in Figure 4.12. The initial mean moisture content (12.2%) of maize generally remained relatively stable in the hermetic bag after one (1) month, but dropped slightly below the initial mean moisture level after two (2) months of storage to 11.1% in insect-free maize in hermetic bag. On the contrary, the moisture content in the polypropylene interwoven bags increased from the initial 12.2% to 14.4% after one month and reduced to near the initial moisture level after 2 months of storage. Likewise, the moisture content of maize in the hermetic bags, the moisture level of insect-free maize in the conventional storage bag reduced after one month to 11.9%, after which it remained relatively stable until the third month of storage. Overall, there was a surge in grain moisture from the third month (March) of storage with analysis of variance showing significant difference

($p<0.05$) in moisture contents of maize stored in either storage bag technologies. The highest moisture content of 16.7% was recorded in maize infested with *S. zeamais* in polypropylene bag at the sixth month with 14.3% recorded in insect-free maize in the triple-layer hermetic bag.

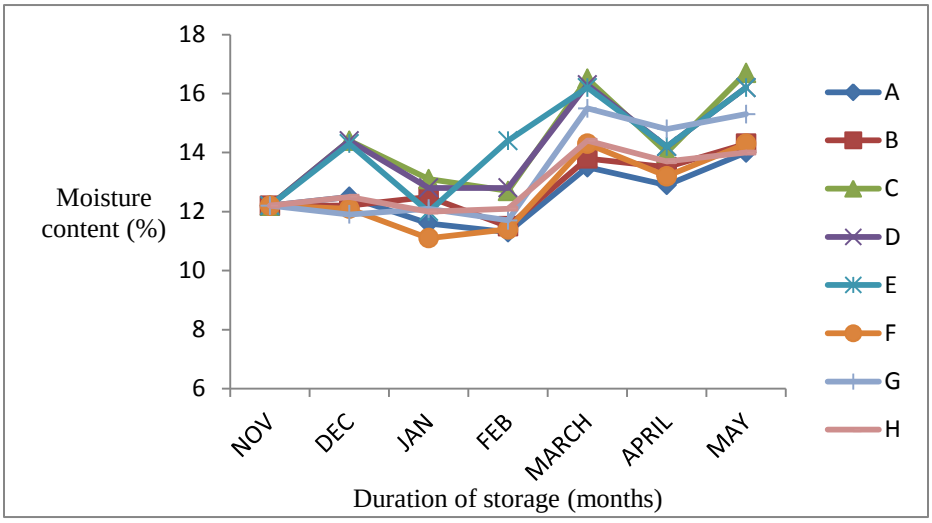

Figure 4. 12: Changes in moisture content of maize stored in hermetic and polypropylene bags

A= *S. zeamais* in hermetic bag, B= *P. truncatus* in hermetic bag, C= *S. zeamais* in polypropylene bag, D= *P. truncatus* in polypropylene bag, E= *S. zeamais* and *P. truncatus* in hermetic bag, F= insect-free maize in hermetic bag, G= insect-free maize in polypropylene bag, H= *S. zeamais* and *P. truncatus* in polypropylene bag

4.3 Levels of insect damage and aflatoxin contamination

4.3.1 Percentage damage of stored maize due to insect infestation.

Percentage damage by insects and the resultant levels of aflatoxins produced are presented in Table 4.5-4.8. Analysis of variance results showed significant differences ($p<0.05$) in the percentage damage between insect infested grains in polypropylene bag

and hermetic bag. Whilst percentage damage in both insect-infested and insect-free maize in hermetic bags remains fairly constant over time, the level of damage of insect-infested maize in polypropylene bags continued to increase over time at an exponential rate. The mean level of damage of 1.3% at the inception of storage increased significantly to 97.9% in the *P. truncatus* infested maize grains in the polypropylene bag after the six (6) months of storage. There was however no significant difference ($p<0.05$) in the levels of damage in insect-infested maize in polypropylene bags over the 6 months storage period.

4.3.2 Changes in aflatoxin concentration in stored maize in triple-layer hermetic bag and polypropylene interwoven bag.

Analysis of variance results showed significant differences ($p<0.05$) in the levels of aflatoxins in relation to treatment received and the storage period. The aflatoxin level of 38.2 µg/kg detected in the maize sample prior to storage increased over the course of storage to 321µg/kg.

The level of aflatoxin G_2 (AFG_2) was 0.0 µg/kg for the first one month in both insect-infested maize and insect-free maize stored in the different storage bag technologies. Apart from *P. truncatus* infested maize in polypropylene bag whose level of AFG_2 changed (2.4 µg/kg) during the third month of storage, all other treatments showed no AFG_2. Also, regardless of the kind of treatment, the last month of storage produced AFG_2 that was significantly different ($p<0.05$).

Aflatoxin G_1 level at the beginning of storage was 0.0 µg/kg which remained constant until month 6 when the highest level of 22.20 µg/kg was recorded in maize grain

devastated by the cumulative activities of *S. zeamais* and *P. truncatus* in polypropylene bag.

There was significant ($p<0.05$) variation in the level of aflatoxin B_2 from 4.2 µg/kg prior to storage, to 24.1 µg/kg in *S. zeamais* infested grains in polypropylene bags at the last month of storage. There was no significant difference ($p<0.05$) in the level of AFB_2 in the insect infested grains and the insect-free maize stored in hermetic bag at the third month of storage. On the contrary, significant differences ($p<0.05$) were observed in insect-damaged grains and insect-free maize in the different storage bags at the same month. Analysis of variance showed significant difference ($p<0.05$) in the accumulation of AFB_2 after six months of storage, with *P. truncatus*-infested grain in hermetic bag recording the lowest AFB_2 of 7.9 µg/kg.

The level of aflatoxin B_1 was the highest (34.0 µg/kg) prior to maize storage. The level of AFB_1 continued to increase over the course of storage to 293.1 µg/kg as the percentage damaged by insect increased. There was an exponential increment of AFB_1 in insect-damaged grains in polypropylene interwoven bags, with *S. zeamais* infested-grains recording the highest level of AFB_I. The lowest level of AFB_1 (64.5 µg/kg) was recorded in *S. zeamais* infested grains in hermetic bag during the sixth months. Analysis of variance showed no significant difference ($p<0.05$) in the level of aflatoxin B_1 in *P. truncatus*-infested maize, combined activities of the two insects and insect-free maize by month six in hermetic bags. Also there was no significant difference ($p<0.05$) in the total level of aflatoxin accumulated both in insect-infested and insect-free maize in the hermetic bag at the end of storage. On the contrary, there were significant differences

($p<0.05$) in the total level of aflatoxin accumulated in polypropylene bags (with or without insects) with *S. zeamais*-infested maize recording the highest level of aflatoxin of 321.8µg/kg.

Table 4.5: Effect of insect damage on aflatoxin levels in maize at time of storage

Storage treatment	Initial level of damage (%)	**Aflatoxins (µg/kg)**				
		G2	**G1**	**B2**	**B1**	**TOTAL**
Weevil (HB)	1.3	0.0	0.0	4.2	34.0	38.2
LGB(HB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil+LGB (HB)	1.3	0.0	0.0	4.2	34.0	38.2
IFM (HB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil (PB)	1.3	0.0	0.0	4.2	34.0	38.2
LGB (PB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil+LGB (PB)	1.3	0.0	0.0	4.2	34.0	38.2
IFM (PB)	1.3	0.0	0.0	4.2	34.0	38.2

HB-hermetic bag; LGB-larger grain borer; IFM- insect-free maize; PB-polypropylene bag

Table 4.6: Effect of insect damage on aflatoxin levels in maize after one month of storage

Storage treatment	Damage level (%) month 1	**Aflatoxins (µg/kg)**				
		G2	**G1**	**B2**	**B1**	**TOTAL**
Weevil(HB)	5.9b	0.00a	0.00a	5.4bc	62.0b	67.4b
LGB (HB)	4.0b	0.00a	0.00a	5.9b	57.3bc	63.2b
Weevil+LGB (HB)	2.8b	0.00a	0.00a	5.6b	62.6b	68.2b
IFM (HB)	0.0	0.00a	0.00a	3.9d	48.2d	52.1d
Weevil (PB)	59.7b	0.00a	0.00a	5.0c	52.6cd	57.6c
LGB (PB)	66.6b	0.00a	0.00a	10.4a	93.8a	104.2a
Weevil+LGB (PB)	63.3b	0.00a	0.00a	4.8cd	36.6e	41.4e
IFM (PB)	0.00	0.00a	0.00a	5.0c	39.0e	44.0e

[a-e] Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
HB-hermetic bag; LGB-larger grain borer; IFM- insect-free maize

Table 4.7: Effect of insect damage on aflatoxin levels in maize after three months of storage

Storage treatment	Damage level (%) month 3	Aflatoxins (µg/kg)				
		G2	**G1**	**B2**	**B1**	**TOTAL**
Weevil (HB)	4.3b	0.0b	0.0a	7.3c	60.1d	67.4d
LGB (HB)	5.7b	0.0b	0.0a	5.1c	56.6d	61.7d
Weevil+LGB (HB)	4.7b	0.0b	0.7a	6.7c	63.6d	71.9d
IFM (HB)	0.0	0.0b	0.0a	6.7c	54.0d	60.7d
Weevil (PB)	92.2a	0.0b	0.0a	17.9a	268.4a	286.3a
LGB (PB)	94.2a	2.4a	0.0a	11.7b	133.5b	147.6b
Weevil+LGB (PB)	94.2a	0.0b	0.0a	10.0b	112.1c	122.1c
IFM (PB)	0.0	0.0b	0.0b	10.2b	50.6d	60.8d

[a-e] Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
HB-hermetic bag; LGB-larger grain borer; IFM- insect-free maize; PB-polypropylene bag

Table 4.8: Effect of insect damage on aflatoxin levels in maize after six months of storage

Storage treatment	Damage level month 6 (%)	Aflatoxins (µg/kg)				
		G2	**G1**	**B2**	**B1**	**TOTAL**
Weevil(HB)	2.3b	0.0d	1.2c	8.0e	64.5f	73.7e
LGB (HB)	4.8b	0.0d	0.0d	7.9e	75.7ef	83.6e
Weevil+LGB (HB)	2.4b	1.4c	0.0d	8.5de	81.1e	89.6e
IFM (HB)	0.0	0.0d	0.0d	10.4cd	75.6ef	86.0e
Weevil (PB)	96.8a	4.6a	0.0d	24.1a	293.1a	321.8a
LGB (PB)	98.0a	3.4b	5.1b	18.9b	200.3b	227.7c
Weevil+LGB (PB)	97.77a	1.9c	22.2a	20.0b	264.7b	308.8b
IFM (PB)	0.00	2.8b	0.0d	11.5c	124.1d	138.4d

a-f Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
HB-hermetic bag; LGB-larger grain borer; IFM- insect-free maize; PB-polypropylene bag

Plate 4. 2: State of *S. zeamais*-infested maize after three (3) months of storage in triple-layer hermetic bag

Plate 4. 3: State of *S. zeamais*-infested maize after three (3) months of storage in polypropylene interwoven bag

4.4 Fungi identified in maize samples before and after storage

The experiment showed that maize grains used for the study were previously infected with the aflatoxigenic fungi; *Aspergillus flavus*. Apart from *Rhizopus stolonifier* which

was also recorded during the study, *Aspergillus flavus* and *Aspergillus niger* were the predominant fungi in all aflatoxin contaminated maize after storage. Pictures of all the three fungi identified under the microscope are presented in Plate 4.4-4.6.

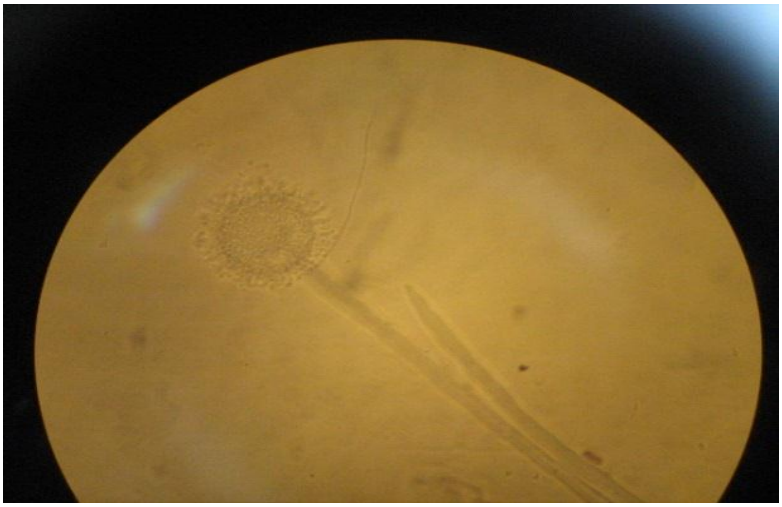

Plate 4. 4: *Aspergillus flavus*

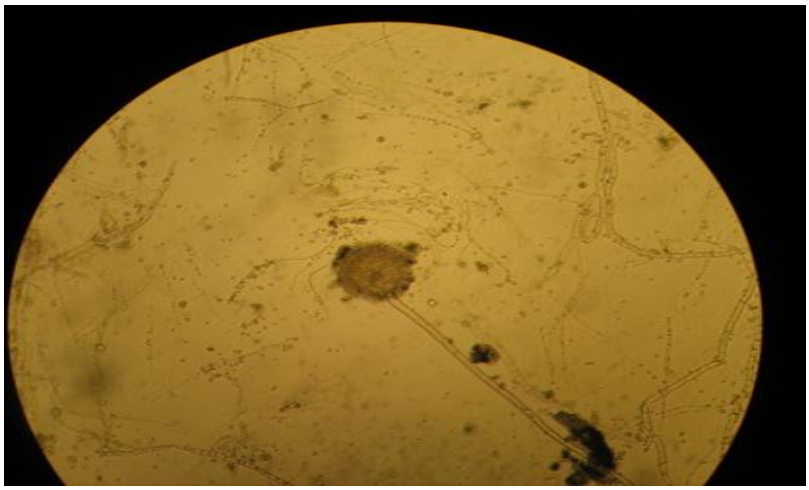

Plate 4. 5: *Aspergillus niger*

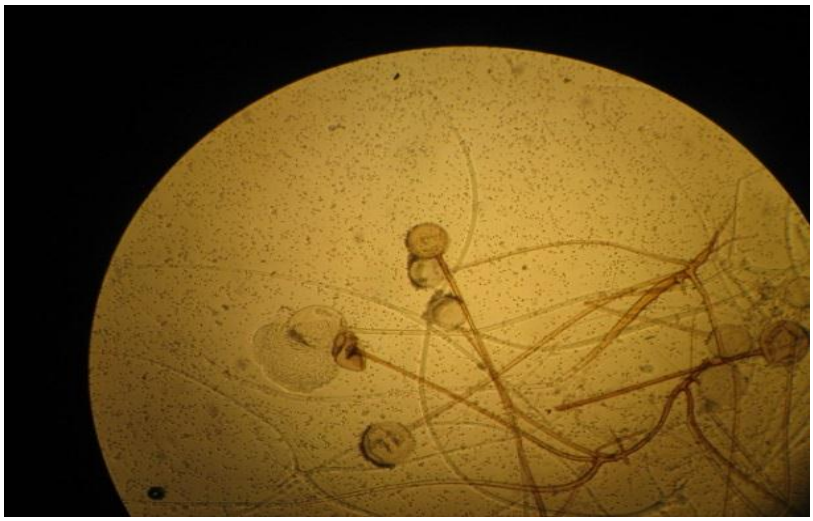

Plate 4. 6: *Rhizopus stolonifier*

4.5 Effect of moisture content and relative humidity on the degree of accumulation of aflatoxin at different levels of temperature

The result showed significant differences ($p<0.05$) in moisture content and the accumulation of aflatoxins under the different temperature conditions in relation to the storage bag technologies (Table 4.9-4.12) and (Figure 4.13). The initial mean moisture content of 12.3% increased to 14.1% after three months of storage of grain in the hermetic bag under low temperature condition (16 ^{o}C). On the contrary, the initial moisture content reduced significantly to 9.9% after three months of storing maize grains in the polypropylene bag at 38^{o}C. The relative humidity of all the temperature levels remained relatively stable during the experiment. The lowest (45.7%) and highest (78.9%) mean relative humidity was recorded at temperatures 38^{o}C and 16 ^{o}C respectively. On the other hand a moderate level of relative humidity (66%) was recorded at room temperature.

There was no contamination of AFG_1 over the three (3) months of maize storage in the triple-layer hermetic and polypropylene bag. Prior to storage the maize sample was found to contain no AFG_2. But by month three (3) a mean Aflatoxin G_2 of 0.6 µg/kg was detected in maize samples stored in polypropylene bag at 30 ^oC. Among all the various treatments, maize sample stored in hermetic bag at 16 ^oC accumulated the highest level of aflatoxin B_2 during the three months of storage. There were significant differences ($p<0.05$) in the contamination level of AFB_1 irrespective of the kind of treatment received by the maize.

Generally, as moisture content of grains increases the level of aflatoxins accumulated in the grains also increased. This trend was profoundly evident when AFB_1 in the hermetic bag increased considerably from the initial 34.0 µg/kg to 71.6 µg/kg within three (3) months of storage under 16^o C following a drastic increased of grain moisture content from 12.3% to 14.4%. Conversely, as moisture content dropped from the initial level of 12.3% to 9.9% in the polypropylene bag at 38^{o}C the lowest level of aflatoxin B_1 (34.6 µg/kg) was recorded.

Table 4.9: Effect of temperature level and changes in moisture content on aflatoxin G_1 content before and after storage

Bag type	Temperature (^oC)	Initial-MC (%)	Initial-AFG_1 (µg/kg)	Month-3 MC (%)	Month-3 AFG_1(µg/kg)
TLH Bag	16	12.3a	0.0a	14.4a	0.0a
	30	12.3a	0.0a	12.2a	0.0a
	38	12.3a	0.0a	11.8b	0.0a
Polypropylene	16	12.3a	0.0a	13.9b	0.0a
Bag	30	12.3a	0.0a	11.5b	0.0a
	38	12.3a	0.0a	9.9c	0.0a

[a-c] Values in the same column not sharing a common superscript are significantly different at LSD ($p<0.05$)
TLH= triple-layer hermetic

Table 4.10: Effect of temperature level and changes in moisture content on aflatoxin G_2 content before and after storage

Bag type	Temperature (^oC)	Initial-MC (%)	Initial-AFG_2 (µg/kg)	Month-3 MC (%)	Month-3 AFG_2 (µg/kg)
TLH Bag	16	12.3a	0.0a	14.4a	0.0b
	30	12.3a	0.0a	12.2a	0.0b
	38	12.3a	0.0a	11.8b	0.0b
Polypropylene	16	12.3a	0.0a	13.9b	0.0b
Bag	30	12.3a	0.0a	11.5b	0.6a
	38	12.3a	0.0a	9.9c	0.0b

[a-c] Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
.TLH= triple-layer hermetic

Table 4.11: Effect of temperature level and changes in moisture content on aflatoxin B_2 content before and after storage

Bag type	Temperature (^oC)	Initial-MC (%)	Initial-AFB_2 (µg/kg)	Month-3 MC (%)	Month-3 AFB_2(µg/kg)
TLH Bag	16	12.3a	4.2a	14.4a	14.7a
	30	12.3a	4.2a	12.2a	5.8c
	38	12.3a	4.2a	11.8b	4.7c
Polypropylene	16	12.3a	4.2a	13.9b	9.3b
Bag	30	12.3a	4.2a	11.5b	8.2b
	38	12.3a	4.2a	9.9c	4.2c

[a-c] Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
TLH= triple-layer hermetic

Table 4.12: Effect of temperature level and changes in moisture content on aflatoxin B_1 content before and after storage

Bag type	Temperature (°C)	Initial MC (%)	Initial-AFB_1 (µg/kg)	Month-3 MC (%)	Month-3 AFB_1(µg/kg)
TLH Bag	16	12.3a	34.0a	14.4a	71.6a
	30	12.3a	34.0a	12.2a	44.3d
	38	12.3a	34.0a	11.8b	39.2e
Polypropylene	16	12.3a	34.0a	13.9b	67.8b
Bag	30	12.3a	34.0a	11.5b	47.7c
	38	12.3a	34.0a	9.9c	34.6f

[a-f] Values in the same column not sharing a common subscript are significantly different at LSD ($p<0.05$)
TLH= triple-layer hermetic

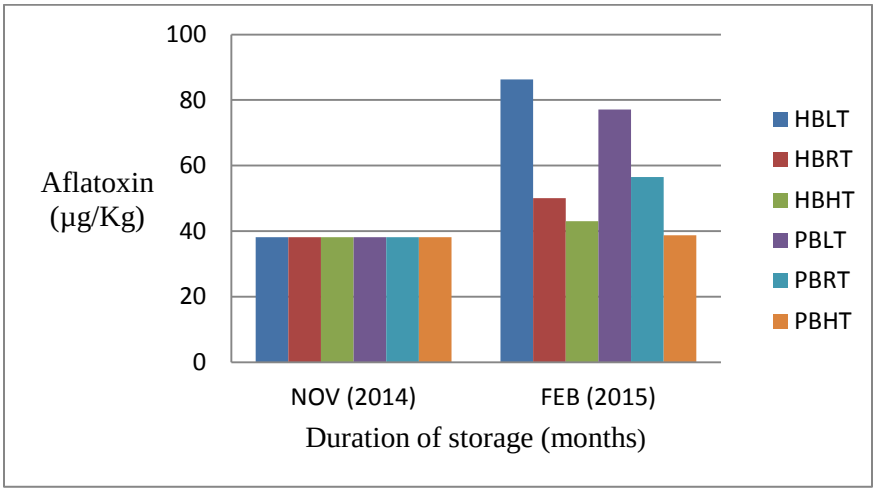

Figure 4. 13: Effect of temperature level on aflatoxin (G1+G2+B2+B1) before and after storage of maize

HBLT-hermetic bag under low temperature, HBRT-hermetic bag under room temperature, HBHT-hermetic bag under high temperature, PBLT- polypropylene bag under low temperature, PBRT- polypropylene bag under room temperature, PBHT-polypropylene bag under high temperature

4.6 Changes in germination capacity of maize stored in the triple-layer hermetic bag and polypropylene interwoven bags after storage at farm level

The average germination capacity of seed maize used in the study prior to storage was 71%. Analysis of the germination potential of seed maize showed significant differences ($p<0.05$) with regard to the type of treatment and the location of germination test (laboratory or field) as shown in Figure 4.14 and Figure 4.15. The initial germination percentage (71.7%) of insect-infested maize and insect-free maize in hermetic bag conducted in petri dish dropped marginally to 67% and 70% respectively after three months of storage. In contrast, there was significant difference ($p<0.05$) in the laboratory germination test on insect-infested maize and insect-free maize in the polypropylene bags after the same (3) months of storage. Similarly, there was significant difference ($p<0.05$) in the germination percentage of maize stored in either storage bag technology regardless of the present or absent of insect. While the insect-infested and insect-free seed maize in the hermetic bag continues to record germination after the six months storage period, seed maize from insect-infested maize stored in propylene bags failed to germinate after the storage period.

There were significant differences ($p<0.05$) in the germination capacity of seed maize tested on the field from hermetic and polypropylene interwoven bag with or without insect infestation. While the percentage germination in the insect-free maize in the polypropylene interwoven bags dropped from the initial mean germination percentage of 71% to 0% in six months, the triple layer hermetic bag maintained relatively the germination capacity during the storage period by dropping from 71% to 60% after the

six months storage. Like the laboratory test, there was no germination of insect-damaged grain in the polypropylene bags during the field germination test.

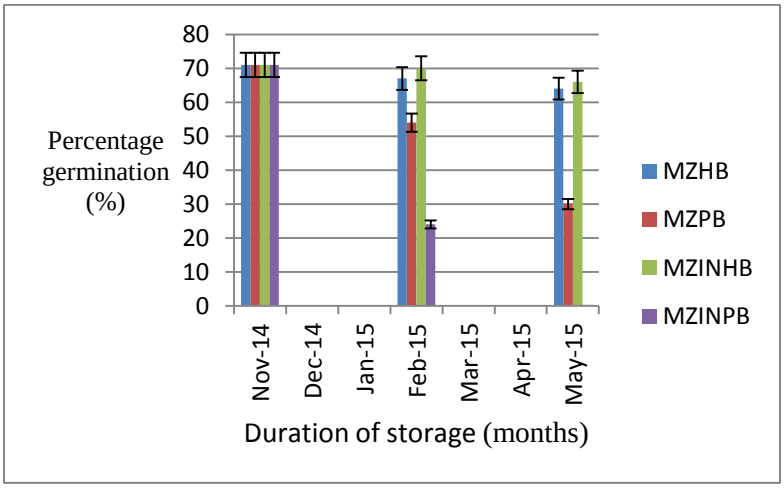

Figure 4. 14: Change in germination capacity of seed maize during laboratory germination test

MZHB= maize in hermetic bag, MZPB= maize in polypropylene bag, MZINHB= maize infested with insect in hermetic bag, MZINPB= maize infested with insects in polypropylene bag

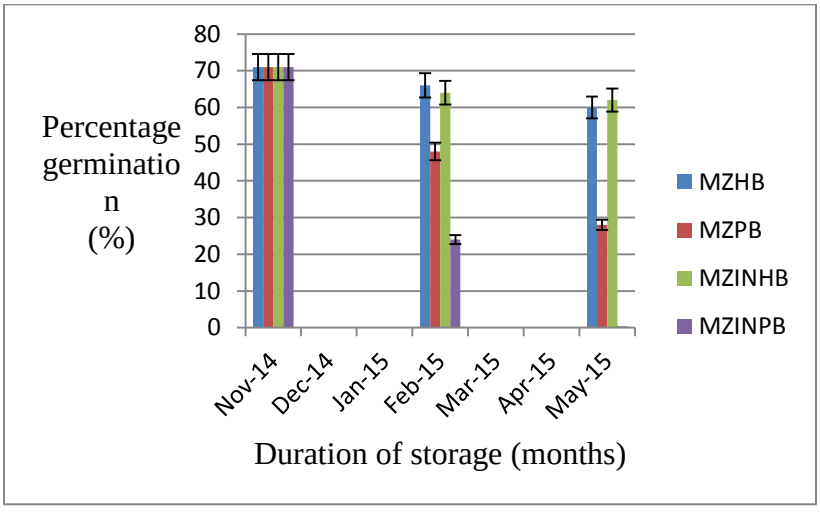

Figure 4. 15: Change in germination capacity of seed maize during field germination test

MZHB= maize in hermetic bag, MZPB= maize in polypropylene bag, MZINHB= maize infested with insect in hermetic bag, MZINPB= maize infested with insects in polypropylene bag

CHAPTER FIVE

5.0 DISCUSSION

5.1 The level of social acceptability, opportunities/benefits and challenges of using triple-layer hermetic bags by farmers in the Techiman Municipality of the Brong Ahafo Region.

The purpose of this study was to assess farmer's acceptance of the triple layer bag technology, the benefits they have reaped from the technology and their challenges. The results showed that majority of respondents who adopted the hermetic bag technology from the study were females. This may be attributed to the fact the most of the post-harvest activities in most sub-Saharan African countries are carried out by women. In most developing countries, women are reported to usually make the final decision about postharvest production especially storage, processing and marketing (Obeng- Ofori and Boateng, 2008). Beside, previous report on adoption of the triple layer bag in West and Central Africa showed that male respondents were less likely to adopt the technology (Moussa *et al.*, 2014). The survey also revealed that most of the farmers who adopted the technology belong to local farmer based organization. Previous studies on social network and technology adoption by farmers revealed that, farmers who belong to association are more likely to adopt technology because they are more exposed to information (Bandeira and Rasul, 2003).

Although education had been highlighted in numerous studies to have greater influence on the adoption of most technologies (Oyadele and Yahaya, 2009), the findings of this survey shows that farmers level of education had minimum bearing on the level of

acceptability and adoption of the triple-layer hermetic bag. Chetini and Mushenge (2014) showed that the level of awareness created prior to the introduction of a new technology has greater influence on farmer's decision to accept or reject a technology regardless of their level of education. Creating awareness prior to the introduction of a new technology to farmers is deemed important for farmer's acceptance or rejection of the technology. Farmers' source of information about the hermetic bag technology varied widely from the study. NGOs were reported as the predominant source of information, followed by community information centers, radio, Agricultural extension agents and friends. It came out from the research that the NGOs (Concern Universal and ABOFAP) involved in disseminating the triple layer technology were community based. The demonstrations, radio programmes and their reputation in the community might have influenced farmer's decision in accepting the intervention. The personal characteristic of the agent of change (NGO, extension worker etc.) such as credibility, good relationship with farmers, intelligence, and ability to communicate with farmers, persuasiveness and development orientation as reported by Thi *et al.* (2002) are the key ingredients that influence farmers to accept a new technology by any agent of change. Agents of change such as Catholic Relief Service and other medium such as radio were reported to be used in disseminating the triple layer bags and plastic jug in other countries in the West Africa sub-region (Moussa *et al.*, 2014).

In general, diffusion of innovation would take place only within groups of people who are homogenous in terms of problems, aspiration and needs (Valera and Plopino, 1987). The study proved that almost all the farmers that welcomed the technology were maize

farmers with a common problem of insect infestation, insecticides residue on produce (issues of food safety) with a common aspiration of ensuring household food security and taking advantage of premium prices at some point of their farm business. In addition to farmers believe that the technology would address their primary concern of insect pests, the affordability of the intervention might have influenced majority decision in adopting the triple-layer hermetic bag without much hesitation. As pointed out by Scandizzo and Savastano, (2010) and Soleri *et al.* (2008), small scale and risk averse farmers would be ready to accept a new technology provided there was an element of affordability, durability, financial and health benefit associated with it. From the investigation, sizeable number of farmers was found to have previously used or exposed to the triple-layer hermetic bags prior to their introduction by the NGOs. These were migrant farmers from Northern Ghana, where the intervention might have earlier been introduced.

Farmers interviewed reported of reaping benefits from the technology. They buttressed their response by pointing out to the increment in the length of storage of their farm produce and premium prices they obtained from their produce in the course of storage which hitherto was not possible due to insect damage. Cowpea farmers from seven West African countries including Ghana also reported that storing their produce in the triple-layer hermetic bag improved their household food availability and higher prices for their produce after storage (Moussa *et al.,* 2014).

Some maize farmers in Techiman Municipality are also taking advantage to exploit the underlying principle of the triple-layer hermetic bag by storing other commodities such as cassava powder, groundnut, cowpea and clothes. The triple-layer hermetic bag system

has proven effective in storing a variety of crops including cowpeas, maize, peanuts, sorghum, wheat, and common beans against insect pests (Mutungi *et al.,* 2014; Njoroge *et al.,* 2014; Anankware *et al.,* 2013; Baoua *et al.,* 2014; Vales *et al*., 2014; Murdock *et al*., 2012).

The most reported concern of farmers was the easily tearing of the HDP bags followed by cost, time consuming nature of technology and leakages. Out of thirty four (34) farmers that registered their concerns, seven (7) of them representing 20% stated cost as their concern on the usage of the technology. Mousa *et al.* (2014) reported that only 1% out of 232 farmers in the Volta Region of Ghana, cited cost as their reason for not adopting and using the triple-layer hermetic bag technology.

It was also observed from the survey that there were inconsistency in the texture and diameter of the HDP bags used in the Techiman municipality. This might have accounted for tearing and leakages observed by some farmers. Besides, some farmers stored the hermetic-bagged produce in their traditional structures such as cribs and barns which normally have sharp surfaces thus accounting for the frequent tearing of the bags. A significant minority of farmers after introduction accepted the technology mainly due to its affordability. Many of these same farmers are presently reporting the high cost of replacing their torn bags or buying additional bags as a source of concern. The worse scenario they reported was where the cost of replacing a torn HDP bag was the same as a new PICS bag. Boys and Lowenberg-deboer (2007) report of the failure of the sealed metal drum technology in storing grains in Senegal due to the high cost of replacing it with a newer drum. To achieve the full benefit of the technology three plastic bags are

required; this unfortunately has been a source concern by some farmers because of the relatively longer period it took for them to fill the three plastic bags. Their comparison of the triple-layer hermetic bag to other bagging technologies such as the polypropylene and jute sack might have resulted in this concern.

5.2 Changes in moisture content of maize grains in storage

The experiment revealed fluctuation in moisture content of grains stored at farm level in the crib during the period of storage. The increase in moisture content in the triple-layer hermetic bags could be attributed to the extreme temperature fluctuation experienced during the first-three month after storage (November-February). The frequent temperature fluctuations that characterized this period (harmattan) led to the formation of condensed water at the sides of the hermetic bag that trickled down the bottom of the bags. This concurs with Obeng-Ofori and Boateng (2008) who reported that grains closer to the walls and bottom of the hermetic bags absorbed the water to increase its moisture content.

There was sharp rise in the moisture content of maize stored in the polypropylene interwoven bag from the initial mean moisture content of 12.2% to 14.4% after two months of storage. It then increased to a staggering level of 16.7% after six (6) months of storage. The poor barrier protection of the polypropylene interwoven bags against water, oxygen and sun created optimum conditions to elevate insect metabolic activities. The increased metabolic activities of insects in polypropylene interwoven bags were accompanied with heat and moisture that was absorbed by the grains leading to the rise in moisture content, hot spot and subsequent caking of the grains (Obeng-Ofori and

Boateng, 2008). The better barrier provided by the triple-layer hermetic bag against oxygen, moisture and insects might have accounted for the relatively lower moisture of maize in the hermetic bags compare with polypropylene interwoven bags. Similar incidence was reported by William *et al.* (2014) when maize stored in the triple-layer hermetic bag maintained its moisture content close to the initial moisture content of the maize.

Maize is hygroscopic in nature and hence the general rise in its moisture content after February was due to the absorption of moisture from the storage environment due to the high relative humidity experienced following the onset of rain from March to May. Moisture content as reported by Obeng-Ofori and Boateng (2008) is affected by the prevailing relative humidity in the storage environment.

5.3 Changes in Temperature, dew point and relative humidity of the storage environment

The three abiotic factors (temperature, dew point and relative humidity) fluctuated significantly within the storage structure and inside the two storage bag technologies throughout the period of storage. The relatively higher variability in temperature, dew point and relative humidity within the crib were due to poor protection offered by crib against these climatic factors. This was consistent with the report by Olakojo and Akinlosotu (2004) that despite the more ventilation capacity of the crib, it does not provide full protection to stored product against harsh environmental conditions. The significant difference in the abiotic factors in the triple-layer hermetic bag and the polypropylene interwoven bag might also be due to the multiple covering of the hermetic

bag which minimizes the amount of heat radiation from the sun on the hermetic bag (Jonfia-Essien *et al*., 2010).

5.4 Effect of insect damage and aflatoxin contamination of maize stored in the triple-layer hermetic bag and polypropylene interwoven bag

The study showed that as the level of insect damage increased the levels of aflatoxin concentration also increased. Insect devastation was profoundly evident in maize stored in the polypropylene interwoven bags during storage. The unrestricted amount of oxygen in the conventional bag provided an ideal condition for the metabolic activities of insects leading to the exponential increment of aflatoxin during the period of storage. The high metabolic activities of insects increased temperature, relative humidity and moisture content of grains, creating fertile grounds for *Aspergillus species* proliferation and aflatoxin contamination (Sauser and Burrough, 1980; Mill, 1983; Sinha and Sinha, 1991; Beti *et al.,* 1995).

Also, Beti *et al.* (1995) reported that maize kernel infested with *Aspergillus flavus* contaminated with *Sitophilus zeamais* had higher levels of aflatoxin than maize without insects. Even though the level of insect damage of grains in the triple-layer hermetic bags remained relatively stable during the storage period, the level of aflatoxin accumulation after storage was relatively higher than the average initial amount. This may be due the surge in the grains moisture content as result of temperature fluctuation during storage. The condensed water at the walls of the bag at daytime settled at the bottom of the bags at night when the temperature fell, and may have favoured the growth of aflatoxigenic fungus present in the grain before storage. Barney *et al*., (1995) and Rees (2004) reported

that fungal growth in stored grain in the tropical countries is mainly associated with increases in grain moisture contents, and fluctuation in temperatures resulting in unsafe storage of high-moisture grain and moisture migration and condensation. Similar conclusion was also drawn by Ojeda *et al.* (2009) that *Aspergillus flavus* grew on oxo-biodegradable polyethylene film as used in the triple-layer bagging technology, while moisture content increased to produce aflatoxins.

The general rise in the level of aflatoxins in both bag technologies may have been significantly influenced by the change in season (harmattan and rain) and length of storage. Aflatoxins levels in insect-free maize in the polypropylene remained relatively stable for the first-three month due to the marginal reduction of the moisture content of grains as a result of the dry, windy and hazy weather experienced during the harmattan. Following the onset of the raining season in March, the previously dried grain in hermetic and polypropylene bags absorbed moisture from the humid air to create suitable conditions for aflatoxins contamination. This was confirmed by Choudary and Sinha (1993) in India when they observed that aflatoxin accumulation was highest in maize stored for 52 weeks during the monsoon, a season with high relative humidity. The same authors reported of a decline of 33% in the level of aflatoxin B_1 in the winter when relative humidity was low. Seasonal and diurnal temperature difference between stored grains and surrounding environment can result in moisture translocation or migration among quantities of bulk grains or in condensation of moisture on the grain (FAO, 1979).

The present study showed that the crib storage structure may be effective to limit aflatoxin production in the dry season due to its high ventilation qualities but may not be

appropriate in the raining season. The crib failed to protect the different storage bag technology from rainstorm from March-May leading to significant accumulation of aflatoxins. After reporting that aflatoxin accumulation increase with storage time, Hell *et al.* (2000b) concluded that, the level of aflatoxins contamination after harvest or during storage is dependent on the type of storage structure. Several other researchers also observed that aflatoxins concentration was related to storage structure (Ansah, 2012; Ahmad, 1993 and Prasad *et al*., 1987), storage time (Lillehoj and Zuber, 1988) and storage insect pests (Sinha and Sinha, 1992).

The level of oxygen and carbon dioxide achieved in the triple-layer bags after 24 days of storage was <5% and 16.7% respectively. This was in contrast with the findings of Jonfia-Essien *et al.* (2010) and Murdock *et al.* (2003) who recorded a level of 0.0% oxygen concentration. This may be due to the difference in thickness of the bag used. The high density polypropylene bag (150 µm thick) used for the research was quite thicker, tougher and somewhat inflexible that might have resulted in ingress of air through the entrance of the bag. The level of gases achieved in the hermetic bag in this study, according to previous studies by Anankware *et al*. (2013) was enough to kill all insects present. But unlike insects, many storage fungi are capable of growing in low pressure of oxygen (O_2) and reduction of oxygen is often not sufficient to prevent moulding (Hocking, 1989). Decreasing oxygen to <0.14% and elevating carbon dioxide to >50% is required for complete inhibition of growth and subsequent aflatoxin contamination by aflatoxigenic fungi (Magan and Lacey, 1984).

5.5 Effect of temperature levels on aflatoxin contamination of maize stored in the triple layer hermetic bag and polypropylene interwoven bag.

Moisture content and temperature are the two key environmental factors that influence growth of moulds and fungi (Alborch *et al.,* 2011). The current research revealed that different levels of temperature had significant impact on the percentage moisture in the grain and the relative humidity of the storage environment in relation to the different bag technologies. Maize grains stored in the triple-layer hermetic and the polypropylene interwoven bags had an average initial moisture content of 12.3%. The average initial moisture content changed steadily after three months with respective to the levels of temperature and type of storage bag. The grains stored at warm temperature of 38 ^{o}C recorded the lowest moisture content 9.9% after three months. The good permeability of warm air into the polypropylene interwoven bags might have resulted in moisture level below the average initial moisture content. This was consistent with other studies that concluded that the minimum restriction offered by the polypropylene bag to air leads to the reduction in grain moisture content (Marin *et al.,* 2012; Othman and Al-Deluing, 2012). The well-dried grains in the polypropylene bags did not support mould growth and aflatoxin production since free water is indespensable for aflatoxigenic fungi to contaminate grains with aflatoxin (Hell *et al.,* 2008). The lowest aflatoxin level recorded in the polypropylene interwoven bag at 38^{o}C might also be due the low relative humidity (45.7%) recorded in the storage environment. Relative humidity of below 65% decreases the population density of aflatoxigenic fungi (Atanda, *et al.,* 2011). The interactions between moisture and relative humidity have been reported to have significant influence

on the level of accumulation of aflatoxin in storage. Moisture content below 10% and relative humidity below 70% are inimical to destructive biotic agents such as fungi, insects, mites, etc. (Obeng-Ofori and Boateng, 2008).

There was a general rise in moisture content of maize grain stored in the hermetic and polypropylene interwoven at 16 ^{o}C. The rise in temperature within the bags was as result of metabolic activities of grains and lower temperature condition in the storage environment (incubator) that might have resulted in the generation of condensed water on the walls of the bag. Some of the condensed water wet the grains close to the wall and bottom of the bag to increase their moisture content for subsequent aflatoxin contamination by aflatoxigenic fungi present in the grain before storage (Obeng-Ofori and Boateng, 2008). The average high relative humidity (78.9%) in the storage environment coupled with high moisture content of grains within the different bag technologies created suitable condition for the proliferation and contamination of maize by aflatoxigenic fungi present in stored maize (Cotty and Jaime-Garcia, 2007).

The change in aflatoxins levels from 38.2 µg/kg to 50.1 µg/kg in the hermetic bag and 38.2 µg/kg-56.2 µg/kg in the polypropylene bag at room temperature may be due to the stable environmental conditions within the store room. This was consistent with the report by Ansah, (2012) that when environmental conditions are stable within storage structures, the initial levels of aflatoxin concentration would be contained in the triple-layer hermetic bag and polypropylene interwoven bag.

The differences in aflatoxin accumulation in the two (2) storage bag technologies at room temperature may be due to amount of oxygen and carbon dioxide present in each of them. This validates the study by Sadini *et al*. (2014) that the free atmospheric air couple with the presence of aflatoxigenic fungi in cloth bags accounted for the higher level of aflatoxin in cloth bag than triple-layer bag in peanut stored at room temperature. The percentage change (31.2%) of aflatoxin accumulated after three months of storage in the hermetic bag at room temperature was within the acceptable limit of 15 µg/kg set by the Codex Alimentarius Commission and the Ghana Stanadard Authority (Dohlman, 2003).

5.6 Effect of hermetic condition on the germination capacity of seed maize

The findings of this experiment showed that seed maize germination potential could be substantially preserved in the triple-layer hermetic than polypropylene interwoven bags regardless of the present or absent insect in the bags. The elevation of carbon dioxide 0.03% to 16.7% and the drastic fall of oxygen from 21% to 4.6% may have resulted in the cessation of insects destructive activities in the hermetic bag. Ideally, grain should be kept with low oxygen content and high concentration of carbon dioxide to control insects and fungi that are major cause of increase in temperature of the grains, decreasing its germination capacity (Govender *et al.*, 2008). Nonetheless, the slight reduction in the germination capacity of seeds observed in the triple-layer hermetic bag could be attributed to the increased in grain moisture content, thus resulting in the growth of some fungi on the germ of the grain thus reducing its germination capacity (Copeland and McDonald, 1995; Pradhan and Badola, 2012; Ansah, 2012). The moderate permeability of the polypropylene interwoven bag to oxygen and moisture might have accounted for

the rise in insect destructive activities and subsequent damage of the seed maize. The high infestation of seed maize by insects in the conventional bags resulting in the damage of the germ of the seeds led to a loss of seed viability which concurs with other findings (Quazeda *et al.,* 2006; Anankware and Bonu-Ire, 2013; Ansah, 2012; William *et al*., 2014). The difference in germination capacity of seed maize in the laboratory and field may be attributed to variations in the environmental condition during the germination test. Similar conclusion was drawn by Copeland and McDonald (2001) that environmental conditions influence the germination potential of seeds.

The poor protection offered by the storage structure (crib) might also have affected the germination potential of the seed maize most especially in the polypropylene interwoven bag. Most traditional storage structure according to Olakojo and Akinlosotu (2004) provide better aeration but often exposed the stored product to unfavourable environmental conditions such as rain and sun. The overall decrease of the mean percentage germination from 71% to 60% in the field and from 71% to 64% in the laboratory after six months of storage in the hermetic bag was an acceptable decrease in germination compared to insect infested maize in the conventional storage bag, as the acceptance percentage germination is 70% according to the Plant Protection Act (1976).

CHAPTER SIX

6.0- CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Farmers in Techiman municipality who adopted the triple-layer hermetic bag technology have improved food security in their household and obtained higher prices for their produce during the lean season. Leakages, wear and tear of the high density polypropylene bag and the same price paid by farmers to replace a torn HDP and a complete triple-layer bag were the major challenges faced by farmers following their adoption of the technology.

The findings of the study established the association between insect and fungus on stored maize. Insect infested stored grains are more susceptible to *Aspergillus flavus* infection and hence aflatoxin contamination than insect-free maize. Insects also act as vectors by transporting large amount of spores of aflatoxigenic fungi to contaminate the grains with aflatoxins.

The oxo-biodegradable liner of triple-layer hermetic bag increased the moisture content of grains stored in areas of extreme temperature fluctuations and thus contributing to fungal growth and subsequent accumulation of aflatoxin.

Regardless of the type of storage bag technology (triple- layer hermetic bag or polypropylene bag) once the moisture content of stored maize (<11%) is maintained during storage period aflatoxin concentration could be controlled.

Storage structures of high ventilation capacity and better protection for stored produce from harsh environmental condition could limit aflatoxin contamination in well-dried maize in the tropics. The crib storage structure facilitates the production of aflatoxin during the rainy season but ensures that aflatoxin levels were maintained at relatively lower level during the dry season.

6.2 Recommendations

The research on the effectiveness of the triple-layer hermetic bag on stored maize at the farm level in the tropics has revealed that:

I. Maize grains to be stored in the triple-layer hermetic bag should be dried to a safe moisture content of ≤11% and stored in cool dry environment to prevent the growth of aflatoxigenic fungi.

II. Farmers should be encouraged to store aflatoxin-free maize at safe moisture level.

III. Crib storage structure should be used to store maize in the dry season due to its high ventilation capacity and not the rain season due to its poor protection against rainstorm and high relative humidity.

IV. Further studies should be conducted on the effectiveness of constant warm temperature conditions on aflatoxin and major quality parameters of maize stored in the triple-layer hermetic bag.

V. Further research should focus on the specific tolerance level of maize grain to low oxygen and high carbon dioxide atmospheres as well as the potential for anaerobic fungi to cause damage in the biodegradable triple-layer bag for its effective use.

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APPENDICES

APPENDIX 1

A survey on social acceptability, opportunities/benefits and concerns of the PICS/triple-layer hermetic bags in the Techiman Municipality, Brong Ahafo, Region, Ghana.

A. Demographic data of respondents

1. Age of respondent
   - 18-28
   - 29-39
   - 40-50
   - 51-61
   - > 61
2. Gender of respondent
   - Male
   - Female
3. Marital status respondents
   - Married
   - Single
   - Divorced
4. Household size of respondent
   - 0-3
   - 4-7
   - 8-11
   - > 11
5. What is your level of education?
   - No education
   - Primary education
   - Junior High School
   - Senior High School
   - Tertiary
6. Do you belong to any farmer based organization?
   - Yes
   - No

B. Level of social acceptability of the triple-layer hermetic bags by respondents

7. Have you heard about the triple-layer hermetic bag?
   - Yes
   - No

8. If yes, where did you hear about the triple-layer hermetic bag?

- On radio
- Friends
- Television
- Agric Extension officer
- NGO
- Information center

9. Were you hesitant in accepting and using the hermetic bags?
   - Yes
   - No
10. If yes, what were your reasons? List.....

11. If no, what were your reasons? List.....

12. Do you know where the triple-layer hermetic bags are sold?

- Yes
- No

13. Do you know how to fill the bag with your commodity?

- Yes
- No

14. If yes, who taught you how to fill the bag?

- Vendor
- AEA
- Other farmers
- NGO
- self

C. Respondents benefits from the triple-layer hermetic bag

15. How many months did store your maize in the hermetic bag?

- ≤ 6 months
- 7-12 months
- 1yr.-2yrs
- >2

16. Was the hermetic bag able to protect you maize?

- Yes
- No

17. Apart from maize, have you protected any other commodity with the hermetic bag?

- Yes
- No

18. If yes, which of the following commodities?

- Cowpea
- Groundnut
- Rice
- Cassava powder
- clothes

19. On a scale of 1-5, choose your perception about the initial price of the bag?

- Very cheap
- Cheap
- Normal
- Expensive
- Very expensive

19. Have your household food security improve following your usage of the hermetic bag?

- Yes
- No

20. Have you taken advantage of higher prices for your commodities in your usage of the triple-layer hermetic bag?

- Yes
- No

D. Respondents concerns relating to the triple-layer hermetic bag

21. Do you have any concerns regarding the triple-layer hermetic bag?

- Yes
- No

22. If yes, what are your concerns? List.....

APPENDIX 2: ANOVA for aflatoxin in insect-infested and insect-free maize in triple-layer and polypropylene.

Aflatoxin G2 initial level

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.	0.		
Residual	16	0.	0.		
Total	23	0.			

Aflatoxin G2 month 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	0.	0.		
Residual	16	0.	0.		
Total	23	0.			

Aflatoxin G2 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	14.49656	2.07094	73.40	<.001
Residual	16	0.45140	0.02821		
Total	23	14.94796			

Aflatoxin G2 month 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	65.1807	9.3115	50.25	<.001
Residual	16	2.9648	0.1853		
Total	23	68.1455			

Aflatoxin G1 initial

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.	0.		
Residual	16	0.	0.		
Total	23	0.			

Aflatoxin G1 month 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.	0.		
Residual	16	0.	0.		
Total	23	0.			

Aflatoxin G1 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	1.1667	0.1667	1.00	0.466
Residual	16	2.6667	0.1667		
Total	23	3.8333			

Aflatoxin G1 month 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1243.3515	177.6216	445.48	<.001
Residual	16	6.3795	0.3987		
Total	23	1249.7310			

Aflatoxin B2 initial level

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.000	0.000	0.00	1.000
Residual	16	30.914	1.932		
Total	23	30.914			

Aflatoxin B2 Month 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	81.7242	11.6749	41.22	<.001
Residual	16	4.5314	0.2832		
Total	23	86.2556			

Aflatoxin B12 Month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	368.385	52.626	19.62	<.001
Residual	16	42.924	2.683		
Total	23	411.309			

Aflatoxin B 2 month 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	881.584	125.941	97.97	<.001
Residual	16	20.567	1.285		
Total	23	902.152			

Aflatoxin B1 initial level

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.00	0.00	0.00	1.000
Residual	16	265.04	16.56		
Total	23	265.04			

Aflatoxin B1 month 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	6738.41	962.63	73.27	<.001
Residual	16	210.22	13.14		
Total	23	6948.64			

Aflatoxin B1 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	116950.06	16707.15	257.35	<.001
Residual	16	1038.70	64.92		
Total	23	117988.76			

Aflatoxin B1 month 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	179656.14	25665.16	670.58	<.001
Residual	16	612.37	38.27		
Total	23	180268.51			

Total initial level of aflatoxin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.00	0.00	0.00	1.000
Residual	16	476.99	29.81		
Total	23	476.99			

Total aflatoxin month 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	8145.92	1163.70	93.22	<.001
Residual	16	199.73	12.48		
Total	23	8345.65			

Total aflatoxin month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	121743.55	17391.94	224.44	<.001
Residual	16	1239.82	77.49		
Total	23	122983.37			

Total aflatoxin month 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	230022.92	32860.42	996.82	<.001
Residual	16	527.45	32.97		
Total	23	230550.36			

APPENDIX 3: ANOVA for aflatoxin for aflatoxin contents at different temperatures in triple-layer and polypropylene bag

Aflatoxin G2 initial

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.	0.		
Residual	12	0.	0.		
Total	17	0.			

Aflatoxin G2 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	1.1762	0.2352	0.91	0.511
Residual	11	2.8566	0.2597		
Total	16	4.0328			

Aflatoxin G1 initial

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.	0.		
Residual	12	0.	0.		
Total	17	0.			

Aflatoxin G1 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.	0.		
Residual	12	0.	0.		
Total	17	0.			

Aflatoxin B2 intial

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.0000	0.0000	0.00	1.000
Residual	12	1.8012	0.1501		
Total	17	1.8012			

Aflatoxin B2 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.0000	0.0000	0.00	1.000
Residual	12	1.8012	0.1501		
Total	17	1.8012			

Aflatoxin B1 initial

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.0	0.0	0.00	1.000
Residual	12	1934.6	161.2		
Total	17	1934.6			

Aflatoxin B 1 month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	3507.693	701.539	181.87	<.001
Residual	12	46.289	3.857		
Total	17	53.982			

Total initial aflatoxin level

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.00	0.00	0.00	1.000
Residual	16	476.99	29.81		
Total	23	476.99			

Total aflatoxin month 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	5457.017	1091.403	146.44	<.001
Residual	12	89.436	7.453		
Total	17	5546.453			

Relative humidity, temperature in storage environment

Storage bag	Temperature levels (^{o}C)	Relative humidity (%)
hermetic	16	78.9
	30	66
	38	45.7
Polypropylene	16	78.9
	30	66
	38	45.7