

**MANAGEMENT OF MYCOTOXIGENIC FUNGI AND ASSOCIATED MYCOTOXINS
IN MAIZE BY USE OF HERMETIC STORAGE**

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DECLARATION

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

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To my parents Albert Maina and Pauline Wachera and siblings Jane Nyambura, Mary Wangare, John Njogu, Teresia Mwihaki, Margret Warweno, Susan Kabura, Magdalene Wairimu, Ishmael Irungu, Daniel Mwangi and Veronica Waithera who have been very supportive of me. God bless you all.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	-	Acquired Immune Deficiency Syndrome
ANOVA	-	Analysis of Variance
CAN	-	Calcium Ammonium Nitrate
CDC	-	Centre for Disease Control and Prevention
CFU	-	Colony Forming Units
CTAB	-	Cetyltrimethyl Ammonium Bromide
DAP	-	Di-ammonium Phosphate
DNA	-	Deoxyribonucleic Acid
DON	-	Deoxynivalenol
EC	-	European Commission
ELISA	-	Enzyme Linked Immunosorbent Assay
FAO	-	Food and Agriculture Organization of the United Nations
FB1	-	Fumonisin B1
FDA	-	Food and Drug Administration
GAP	-	Good Agricultural Practices
GDP	-	Gross Domestic Product
GC	-	Gas Chromatography
HACCP	-	Hazard Analysis and Critical Control Points
HIV	-	Human-Immune Deficiency Virus
HPLC	-	High Performance Liquid Chromatography
IARC	-	International Agency for Research on Cancer
ICIPE	-	International Centre of Insect Physiology and Ecology

ITS	-	Internal Transcribed Sequence
KEBS	-	Kenya Bureau of Standards
KNBS	-	Kenya National Bureau of Statistics
LSD	-	Least Significant Difference
MC	-	Moisture Content
MCIDP	-	Makueni County Integrated Development Plan
MOA	-	Ministry of Agriculture
MS	-	Mass Spectrometry
OARDC	-	Ohio Agricultural Research and Development Centre
OTA	-	Ochratoxin A
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
PICS	-	Purdue Improve Crop Storage
PPB	-	Parts per Billion
PPM	-	Parts per Million
RNA	-	Ribonucleic Acid
RPM	-	Revolutions per Minute
SNA	-	Synthetic Nutrient Agar
TLC	-	Thin Layer Chromatography
USA	-	United States of America
USFDA	-	The US Food and Drug Administration
UV	-	Ultra Violet Radiation
VCGS	-	Vegetative Compatibility Groups

WHO	-	World Health Organization
µg/kg	-	Microgram per kilogram
SSA	-	Sub-Saharan Africa
ZEA	-	Zearalenone

ABSTRACT

Maize is a staple food crop in Kenya with about 90% of the rural households depending on it. However, the grain is vulnerable to degradation by mycotoxin producing fungi in the field and during storage. Mycotoxins produced by these fungi cause significant economic losses and deleterious health effects to humans and animals. Strategies such as storage of maize in hermetic bags have been developed to reduce post-harvest grain losses due to pest attack, fungal growth and mycotoxin contamination. The objective of this study was to determine the effect of hermetic storage on the population of fungal species and levels of aflatoxin and fumonisin in maize. A survey was carried out in October, 2015 to obtain information on maize production practices in Kilala and Mukuyuni Locations of Kaiti District, Makueni County, Kenya. Soil samples were collected at planting and analysed for the population and incidence of mycotoxin producing fungi. Maize grains were sampled at harvest and three months after storage in polypropylene and hermetic bags. Mycotoxin producing fungi in soil and ground maize were isolated and identified based on cultural and morphological characteristics and DNA analysis using ITS1F and ITS4R primers. Aflatoxin and fumonisin in maize grains sampled at harvest and after three months of storage were determined using VICAM method. Over 50% of the farmers in Kaiti District were smallholder who practiced mixed cropping, used simple land preparation methods, applied soil amendments and did not practice crop rotation. Fifty seven percent of farmers stored their maize in modern stores while 43.4% stored maize inside family living houses. Maize was mostly stored in polypropylene bags and the most common storage problem reported by farmers was infestation by insect pests mainly weevils. Eighty seven percent of the farmers had prior knowledge of mycotoxins and considered it a major challenge in maize production. *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. were commonly isolated from

soil and maize grain samples. *Penicillium* spp. was the most prevalent fungal species in soil and maize grains with a mean population of 9.0×10^3 CFU/g soil and 7.0×10^3 CFU/g maize. The population of mycotoxin producing fungi in maize sampled three months after storage was 39.7% higher than at harvest. The type of storage bag had a significant influence ($p \leq 0.05$) on the overall population and diversity of fungal species isolated from maize grains. The population of mycotoxin producing fungi was 78% higher in maize stored in polypropylene bags compared to hermetic bags. The fungal DNA obtained from maize sampled at harvest was positive for 300-bp and 500-bp amplification product while maize sampled three months after storage in the two types of bags generated amplicons of 500-bp amplification product. Total aflatoxin in maize sampled at harvest and after three months storage ranged from <5 to 42.7 ppb with 55% lower aflatoxin content in PICS bags than in PP bags. Fumonisin levels in maize grains sampled at harvest and after three months storage in the two bag types ranged from < 2 to 6.0 ppm. Storage of maize grains in hermetic bags for three months reduced fumonisin levels by 57% compared to polypropylene bags. The hermetic bags effectively reduced fungal population and levels of aflatoxin and fumonisin in maize grains. Therefore, storage of maize in hermetic bags offers an effective and chemical free-method that will enable farmers preserve high quality grains.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Maize (*Zea mays* L.) is an important cereal crop in the world serving different purposes of economic significance (Chilaka *et al.*, 2012). In Sub-Saharan Africa (SSA), maize acts as a source of food and revenue to over 300 million households (Tefera *et al.*, 2011b) and constitutes an important source of carbohydrates, proteins, vitamins and minerals (Makone *et al.*, 2014). With a per capita consumption of between 30 and 34 million bags annually, maize accounts for about 40% of daily calories intake in Kenya (Muiru *et al.*, 2015).

Maize infection by mycotoxigenic fungi is a major challenge worldwide (Lewis *et al.*, 2005) and contamination may occur in the field prior to harvest, at harvest, or postharvest during storage (McMullin *et al.*, 2015). The most important mycotoxin producing fungi of maize are *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. (Nooh *et al.*, 2013). They produce mycotoxins such as aflatoxins, fumonisin, ochratoxins, deoxynivalenol, trichothecenes and zearalenone which cause significant economic losses in crops (Darwish *et al.*, 2014).

Aflatoxins, a group of toxic secondary metabolites are primarily produced by *Aspergillus flavus* and *A. parasiticus* (Okoth *et al.*, 2012). They are the most prevalent mycotoxins that commonly affect maize in Kenya (Hoffmann *et al.*, 2013). Factors such as high temperatures and humidity, high moisture, drought stress (Cotty and Jaime-Garcia, 2007) and delayed harvest predispose maize to *Aspergillus* spp. attack and aflatoxin contamination (Ongoma, 2013). Aflatoxins have been reported to cause human aflatoxicosis, stunted growth in children, immune-system suppression and even death when ingested in food (Shephard, 2008; Wu, 2010).

Fumonisin are also important mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum* (Stack and Carlson, 2003). Infection of maize with *Fusarium* spp. and its contamination by fumonisins is influenced by temperature, moisture, humidity, insect pest infestation and pre- and post-harvest handling (Fandohan *et al.*, 2004). Fumonisin contaminated feed and food have been associated with equine leukoencephalomalacia, porcine pulmonary edema and oesophageal cancer in humans (Stack and Carlson, 2003).

Conventional methods employed in management of mycotoxins such as modifications in cultural practices, use of chemical fungicides and development of resistant cultivars (Palumbo *et al.*, 2008) have only achieved minimal reduction in mycotoxin levels. The traditional storage methods such as use of granaries, gunny bags, polypropylene bags, plastic containers, open cribs (Gitonga *et al.*, 2015; Wambugu, 2009) have been adopted by farmers in Kenya. The extensive use of these conventional storage practices by smallholder farmers results in considerable post-harvest grain losses as a result of fungal growth and mycotoxin contamination (Bankole *et al.*, 2006) which warrant investigations into finding appropriate storage technologies. The triple-hermetic storage bags have become a replacement for conventional storage methods particularly in hot, humid climates because of their chemical-free technology (Villers *et al.*, 2010). The bags are sealed at both ends, creating a high level of gas-tightness which results in an unfavourable modified atmosphere for fungi and insect survival (Edoh Ognakossan *et al.*, 2013). Due to very low levels of O₂ and high levels of CO₂, fungi and insects within the bags become inactive, stop feeding and eventually die of asphyxiation (Moreno-Martinez *et al.*, 2000; Murdock *et al.*, 2012). As a result, mycotoxin production is halted. Adaption of this technology therefore seems to have greater potential in minimizing postharvest losses resulting from fungal growth and subsequent mycotoxin contamination.

1.2 Problem statement

Maize (*Zea mays* L.) is an essential cereal crop in Kenya and acts as a source of food, feed and income. However, contamination of maize with mycotoxins is a serious problem in Kenya. Due to prevailing high temperatures and moisture, high humidity, insect pest damage and poor postharvest grain storage and handling practices (Ongoma, 2013), maize produced in Eastern Kenya is prone to mycotoxin contamination (Okoth *et al.*, 2012). The co-occurrence of aflatoxins and fumonisin can present unacceptable level of toxins in the same grain samples (Ono *et al.*, 2001). Numerous cases of aflatoxicosis outbreak have periodically been reported in Kenya in 1978, 1981, 1982 and 2001 (Muthomi *et al.*, 2012). The worst outbreak occurred in 2004 where 317 patient cases were recorded with 125 deaths mainly in lower Eastern region of Kenya (CDC, 2004). The 2004 outbreak was attributed to inappropriate harvest time, early rains and poor post-harvest storage of maize under moist conditions (Muthomi *et al.*, 2009; Lewis *et al.*, 2005). Despite existence of aflatoxin regulations in Kenya, contamination of food by aflatoxins is still a major challenge in Kenya.

The occurrence of fumonisins in maize from Eastern Kenya has not been widely documented. However, wide spread contamination of maize by fumonisin in Eastern Kenya was reported by Bii *et al.* (2012) and Murithi (2014). The observed high levels of fumonisin in Eastern Kenya strongly suggest exposure to undesirable levels of toxins and therefore extensive research should be done to determine the health effects of fumonisin contamination in Eastern Kenya. According to global statistics on cancer, Kenya is ranked 8th in the world with the highest age-standardized death rate due to oesophageal cancer (Chai and Jamal, 2012). Other studies in Kenya also reported that an increase in esophageal cancer was associated with consumption of food contaminated with fumonisin (Wakhisi *et al.*, 2005; Parker *et al.*, 2010). Despite the frequent

occurrence of fumonisins in maize, no regulatory limits have been set by Kenya Bureau of Standards.

Lack of suitable structures for grain storage often predisposes grains to fungal contamination. Moreover, inadequate and improper drying of maize before storage increases the danger of mycotoxin contamination (Hell *et al.*, 2008). Nevertheless, a modern method that entails the use of triple-layer hermetic bags has been developed and is gaining favour among smallholder farmers given their advantages over the conventional storage methods. The triple-layer hermetic bags provides effective long term preservation of high quality grains compared to traditional storage methods (Hell *et al.*, 2010). The bags employs two layers of air-tight and water-tight high density polyethylene liners and an outer polypropylene bag, within which grains are hermetically stored (Yakubu *et al.*, 2010). Under these storage conditions, the fungal activity is halted thus limiting mycotoxin production.

1.3 Justification

Maize is a staple food and central to household food security for most Kenyans. However, food quality and safety resulting from fungal attack and mycotoxin contamination present a serious threat to food security, improving nutrition and agricultural production. Despite many years of extensive research, contamination of grains with mycotoxin producing fungi and associated mycotoxins has continued to be a serious problem in Kenya. The worst cases of aflatoxicosis outbreaks have occurred in Kenya, where deaths have been reported as a result of aflatoxin exposure (Shephard, 2008). Moreover, during the 2004 to 2006 outbreak, more than 2.3 million bags of maize were condemned due to aflatoxin contamination in Kenya (Atser, 2010).

Progress in reducing post-harvest maize losses from mycotoxins contamination requires the identification and elimination of the constraints to the application of specific technology. Therefore, on-farm safe storage of maize is essential, as it will positively result in improved food and income security for the smallholder farmers (Maria, 2011). The use of triple-hermetic bags that store grains for extended periods is promising to be effective tool for postharvest management of grain loss, and subsequently improving food and income security for poor resource farmers in developing countries (Tefera *et al.*, 2011a). The effect of modified atmospheres could significantly control fungal contamination in stored grains. High levels of CO₂ of >75% within the hermetic bags are required to inhibit growth of mycotoxigenic fungi in moderately dried grains. Triple-bagging technology is sustainable, user and environmental friendly that makes use of chemical pesticides unnecessary (Anankware *et al.*, 2012). This study therefore aimed at determining the diversity of mycotoxigenic fungi in soil and maize and assessing the effect of hermetic storage bags on fungal population, and aflatoxin and fumonisin levels in maize.

1.4 Objectives

The general objective of this study was to determine the effect of modified atmosphere in hermetic bags in management of fungi and mycotoxin contamination in maize in Kaiti District, Makueni County.

The specific objectives of this study were:

- i. To determine the effect of maize production practices on the population of mycotoxin producing fungi in soils.

- ii. To determine the effect of hermetic storage bags on the population of mycotoxin producing fungi in maize.
- iii. To determine the effect of storage of maize in hermetic bags on levels of aflatoxin and fumonisin.

1.5 Hypotheses

- i. The current maize production practices do not encourage the build up of fungal inocula in the soil leading to low population of mycotoxin producing fungi.
- ii. Hermetic storage bags do not affect the population of fungal species leading to low population of mycotoxin producing fungi in maize grains.
- iii. Hermetic storage bags do not influence the levels aflatoxin and fumonisin leading to low mycotoxin contamination in maize.

CHAPTER TWO: LITERATURE REVIEW

2.1 Maize production and importance in Kenya

Maize is a major food crop in Kenya with about 90% of the rural households in the country depending on it (Ouma and De-Groote, 2011). Maize is grown in the highland regions of the Rift valley; in the medium potential regions in Central and Western Kenya; in the marginal areas of Eastern and South Nyanza; in the arid areas of North Eastern and the Coastal lowlands of Kenya (FAO, 2014). With the average annual production and consumption of maize estimated at 2.7 and 3.4 million tons, respectively, there have been fluctuations in maize production over the years which threaten food and income security sources in Kenya (ROK, 2015).

Maize production, which had risen to over 150 kg per capita in the mid-1970s, has dropped gradually ever since to an all-time low of 70 kg per capita (De Groote *et al.*, 2005). This is substantially less than the estimated consumption needs of 103 kg per capita, which necessitates regular imports of large quantities of maize (De Groote *et al.*, 2005). In 2008, 26 million bags of maize were produced in Kenya which was less than a national requirement of 34 million bags (Otunge *et al.*, 2010). In 2013, Kenya produced a total of 38.9 million bags of maize, which was a deficit of 2% compared to 39.7 million bags in 2012 (KNBS, 2014). Due to increasing population which is anticipated to be 43.1 million by the year 2020 and the growing demand for the grain (Otunge *et al.*, 2010), maize deficit will be around 1.2 million metric tonnes in 2020 given that the current maize production rates are low (Kang'ethe, 2011). Maize also makes a large contribution to the economies of developed and developing countries (Nguyen, 2010). It represents 3% of Kenya's Gross Domestic Product (GDP), 12% of the agricultural Gross Domestic Product (GDP) and 21 % of the total value of primary agricultural produce (De Groote

et al., 2005). Maize is also used as animal feed and in industrial production of starch and oil (FAO, 2014).

2.2 Constraints of maize production in Eastern Kenya

Maize production is threatened by a series of production constraints that hamper not only the livelihoods of the smallholder farmers but also meeting of the government objectives for agricultural sector transformation (Ndigwa *et al.*, 2013). These limitations include abiotic and biotic stresses such as temperatures, drought stress, pests and diseases (Mugo and Hoisington, 2002). Environmental factors such as drought, high temperatures, water stresses (Prasad *et al.*, 2008) and season-to-season variability in rainfall distribution are considered the most significant abiotic factors that hamper maize production in Eastern Kenya (Omoyo *et al.*, 2015).

The effect of mycotoxin in maize production in Eastern Kenya is of great concern. The mycotoxins produced by mycotoxigenic fungi affect human and animal health and have been reported to contaminate over 50% of maize grains in cereal crops (Fandohan *et al.*, 2004). Huge economic losses as a result of aflatoxin contamination of maize have also been reported. In 2009, 31,000 and 1,213 bags of maize were condemned in Mbeere, Embu County and Bura Irrigation Scheme in Tana River County, respectively (Nyaga, 2010). Occurrence of *Fusarium* spp. in Kenya also threatens the productivity of major crops like maize (Maina *et al.*, 2009). *Fusarium verticillioides* that causes ear rot in maize is a major contributor to maize yield decline (Maina *et al.*, 2009) in Kenya. In addition to lowering maize quality, *Fusarium* species on maize produce mycotoxins such as trichothecenes, zearalenones and fumonisins which cause severe devastating effects to human and animal health (Logrieco *et al.*, 2002).

2.3 Major mycotoxin producing fungi of maize

Contamination of cereal grains by fungi starts prior to harvest and progressively increases during harvest, drying, storage, transportation and processing (CAST, 2003). Fungal growth and mycotoxin contamination is influenced by high temperature and humidity, high moisture content and mechanical damage (Piotrowska, 2013). Also improper harvest practices, unhygienic conditions during transportation, storage and processing favour fungal growth and mycotoxin production (Wagacha and Muthomi, 2008).

Fungi contaminating grains have been classified as either field or storage fungi (Piotrowska, 2013; Bankole and Adebajo, 2003). The field fungi such as toxigenic *Fusarium* spp. commonly grow and produce mycotoxins at a high moisture content of more than 20% (Logrieco *et al.*, 2003). *Fusarium* spp. normally attack maize causing ear and stalk rots (Whitlow and Hagler, 2005). On the other hand, storage fungi require a moisture content of at least 13% (Atanda *et al.*, 2011) and a relative humidity of 65% and above in cereal grain for growth. The fungal species in the genera *Aspergillus* and *Penicillium* are classified as storage fungi (Bankole and Adebajo, 2003).

2.4 Biology of *Aspergillus* species

Aspergillus species are common soil inhabitants (Bennett, 2010) that grow on a wide array of crops including maize (*Zea mays* L), peanut (*Arachis hypogaea* L), cotton (*Gossypium hirsutum*) and tree nuts (Bennett and Klich, 2003). *Aspergillus*, a genus of *Hyphomycetes* (Pitt and Hocking, 2009) is composed of about 180 anamorphic species with teleomorphs described in nine different genera. Their teleomorphs can be found in the *Ascomycete* structures that produce sexual spores (ascospores), endogenously in a well differentiated ascocarp (Pitt *et al.*, 2000). The *Aspergillus*

genus is also divided into seven subgenera, which in turn are further subdivided into sub-sections (Klich, 2002).

Aspergillus subgenus *Circumdati* section *Flavi* is sub-divided in two groups of species; aflatoxigenic *A. flavus*, *A. parasiticus*, *A. nomius* and *A. bombycis* (Ehrlich *et al.*, 2007). The other group includes non-aflatoxigenic species such as *A. oryzae*, *A. sojae*, and *A. tamarii*, which have been employed in food fermentation processes (Kumeda and Asao, 2001). Other *Aspergillus* species include *A. niger*, *A. carbonarius* and *A. ochraceus* that colonize cereals and cereal-based products in hot and humid regions of the world (Accensi *et al.*, 2004). Soil acts as the main reservoir for *Aspergillus* spp. propagules which grow as saprophytes in soil (Accinelli *et al.*, 2008). Under unfavourable environmental conditions like drought and nutrient stress, the mycelium form compact resistant structures called sclerotia which remain dormant for long periods in soil or crop debris (Wicklow *et al.*, 1993). Under favourable environmental conditions, the sclerotia germinate to produce conidia that are further disseminated in the soil and air (Bennett *et al.*, 1986).

2.5 Biology of *Fusarium* species

The genus *Fusarium* (anamorph) belong to Ascomycota phylum, fungi *Sordariomycetes* class, *Hypocreales* order and also known by its telomorphs *Gibberella* and *Nectria* (Leslie and Summerell, 2006). *Fusarium* species are widespread pathogens of maize in temperate and semi-tropical areas of the world (Logrieco *et al.*, 2002). They are ubiquitous and abundant in soil and play a vital role in saprophytic decomposition, especially cellulolytic materials and nutrient cycling (Maina *et al.*, 2009). They produce dormant structures in the form of chlamydospores in soils, crop residues and organic matter (Pitt and Hocking, 2009). Classification of *Fusarium*

species was traditionally based on morphological characters (Leslie and Summerell, 2006). However, high variability in characteristics of *Fusarium* isolates like pathogenicity and colony morphology has resulted in different classification of species into intra-specific groups (Balali and Iranpoor, 2006). The *Gibberella fujikuroi* species complex was divided into at least eleven different mating populations (Geiser *et al.*, 2005) denoted by letters A through K with populations MP-A (*G. moniliformis*, anamorph *F. verticillioides*), MP-D (*G. intermedia*, anamorph *F. proliferatum*), MP-E (*G. subglutinans*, anamorph *F. subglutinans*) (Moretti, 2009) being most common in maize. These species produce mycotoxins resulting in food contamination (Leslie and Summerell, 2006).

2.6 Mycotoxin contamination of maize

Mycotoxin contamination of maize is a major challenge worldwide (Munkvold, 2003) and the threat of toxin contamination of food in Sub-Saharan Africa is high due to favourable environmental conditions prevailing in these regions (Hell *et al.*, 2010). According to the Food and Agricultural Organization (FAO), about 25% of the world's yearly crop production is contaminated with mycotoxins (Pfliegler *et al.*, 2015). Mycotoxin production may occur prior to harvest, at harvest, during postharvest storage and processing (Whitlow and Hagler, 2005). Fungal species in *Aspergillus*, *Fusarium* and *Penicillium* genera are known to produce mycotoxins (Wagacha and Muthomi, 2008). Mycotoxins cause serious health effects including liver damage, immunosuppression, nephrotoxicoses, neurotoxicoses, hepatotoxicoses, reduced egg and milk production in poultry and dairy animals (IARC, 2002). However, the negative effect of mycotoxins in human and animals depend on the type of mycotoxin, quantity and length of the exposure to the toxins (Chilaka *et al.*, 2012).

2.7 Major mycotoxin contaminants in maize

2.7.1 Aflatoxins

Aflatoxins are the most significant mycotoxins with respect to their occurrence, effect on human health and trade (Gnonlonfin *et al.*, 2013). They are most prevalent in tropical regions where high temperature, moisture and humidity are favourable for toxin production (Bhatt *et al.*, 2010). Aflatoxins are primarily produced by *Aspergillus flavus* and *A. parasiticus* which are ubiquitous and cosmopolitan fungi that contaminate crops including maize (*Zea mays* L), peanut (*Arachis hypogaea* L) and cotton (*Gossypium hirsutum*) (Okoth *et al.*, 2012). Other *Aspergillus* spp. such as *A. nomius*, *A. bombycis* and *A. parvisclerotigenus* are also involved in aflatoxin production (Reiter *et al.*, 2009). Aflatoxins are most abundant in maize and maize products, because maize could be infected even in the field under specific environmental conditions (Krnjaja *et al.*, 2013).

Aspergillus section *Flavi* has the most number of potential aflatoxin producers (Pildain *et al.*, 2008). *Aspergillus flavus*, the major aflatoxin producing fungi, is subdivided into the S and L strains (Cotty, 1994). The S-strain produces copious amounts of B-aflatoxins (Cardwell and Cotty, 2002), while the L-strain produces less B-aflatoxins (Barros *et al.*, 2006; Probst *et al.*, 2011). *Aspergillus parasiticus* forms all major aflatoxin types including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Reiter *et al.*, 2009). The four main aflatoxin types: AFB1, AFB2, AFG1 and AFG2 (Liao *et al.*, 2011) are grouped based on their fluorescence under blue and green UV light (Womack *et al.*, 2013). Aflatoxin B1, the most common mycotoxin has been classified by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen (IARC, 2002). Additionally, aflatoxin M1 (AFM1) a derivative of AFB1 contaminates milk and milk products (Wild and Gong, 2010).

The health implication of aflatoxins is a global concern as it exhibit acute and chronic toxicity effects in human and animals (Womack *et al.*, 2013). Ingestion of large doses of aflatoxin in a short period of time cause acute aflatoxicosis which is evident as hepatotoxicity or fulminant liver failure, impaired digestion, hemorrhage and eventually death (Lewis *et al.*, 2005). Over 40% case-fatalities have been recorded in Eastern Kenya due to consumption of aflatoxin contaminated maize (Daniel *et al.*, 2011). For instance, in 2004, 317 case-patients and 125 deaths were reported in Central and Eastern Kenya (CDC, 2004). Most reported aflatoxicosis outbreaks occurred among subsistence farmers in Eastern Kenya who consumed home grown maize (Daniel *et al.*, 2011).

Chronic aflatoxin exposure involves the consumption of smaller dosages of toxins overtime (Owaga *et al.*, 2011). Chronic aflatoxin exposure has been associated with hepatocellular carcinoma, especially in regions where hepatitis B virus infection is endemic (Lewis *et al.*, 2005), malnutrition, immunosuppression, impaired growth, and diseases such as malaria, human immunodeficiency virus (HIV) and acquired immuno-deficiency syndrome (AIDS) (Strosnider *et al.*, 2006).

2.7.2 Fumonisin

Fumonisin are amongst the most common mycotoxins worldwide that contaminate maize. Fumonisin are *Fusarium* toxins mainly produced by *F. verticillioides* and *F. proliferatum* (Logrieco *et al.*, 2003). *Fusarium verticillioides* primarily infect maize causing ear and stalk rot, whereas *F. proliferatum* attack a wide variety of commodities such as maize (*Zea mays*), asparagus (*Asparagus officinalis*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare* L) (Marin *et al.*, 2010). Factors such as humidity, temperature, maize resistance to infection and

insect pest infestation influence *Fusarium* growth and subsequent fumonisin contamination (Santiago *et al.*, 2015).

Fumonisin B1, B2 and B3 occur in naturally contaminated maize grains (Milićević *et al.*, 2010) with fumonisin B1 being the most abundant, the most toxic and carcinogenic of all the fumonisins (IARC, 2002). Fumonisin-contaminated feeds have been implicated in leucoencephalomalacia in horse, hepatic syndrome in swine, nephrotoxicity and liver cancer in rats (Bankole *et al.*, 2006). Moreover, fumonisin B1 contaminated maize has been linked to oesophageal cancer in humans (Logrieco *et al.*, 2003).

2.7.3 Deoxynivalenol and its acetylated derivatives

Deoxynivalenol (DON) and its acetylated derivatives such as 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and 3, 15-diacetyldeoxynivalenol are the most important trichothecenes with a worldwide distribution (Larsen *et al.*, 2004). Deoxynivalenol is mainly produced by *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*, which commonly contaminate wheat (*Triticum aestivum*), barley (*Hordeum vulgare* L) and maize (*Zea mays* L) (Wu *et al.*, 2011). Deoxynivalenol, are the most prevalent (Bueno *et al.*, 2015) while the acetylated derivatives are detected at low levels where there are high levels of DON. Prolonged exposure to DON in humans' results in anorexia, decreased weight gain, altered immune function and decreased nutritional efficiency (Pestka, 2007). In animals, DON is associated with reduced feed intake, vomiting, digestive disorders, decreased levels of serum protein and oxidative stress (Zhang *et al.*, 2015).

2.7.4 Zearalenone

Zearalenone (ZEA) which contaminates wheat (*Triticum aestivum*), barley (*Hordeum vulgare* L), maize (*Zea mays* L) oats (*Avena sativa*), rice (*Oryza sativa*) and sorghum (*Sorghum Bicolor*) is mainly produced by *F. graminearum* and *F. culmorum*. It has an estrogenous action (Milićević *et al.*, 2010) and significantly affects the productivity of cattle, rather than their general health (Mansfield, 2005). Zearalenone has been associated with infertility and abortions in swine and dairy cattle (OARDC, 2012). Consumption of higher than 12.5ppm of zearalenone reduces conception rates in dairy heifers (OARDC, 2012).

2.7.5 Ochratoxin A

Ochratoxin A is mostly produced by *Aspergillus ochraceus*, *A. carbonarius*, *A. sclerotiorum* and *P. verrucosum* (Wagacha and Muthomi, 2008). *Penicillium verrucosum* is more common in temperate regions of the world at temperatures below 30 °C, while *A. ochraceus* is more prevalent in tropical regions of the world (Magan and Aldred, 2007). The toxin mainly contaminates cereals, coffee, wine, beer and grape juice (Milićević *et al.*, 2010). Ochratoxin A is genotoxic, immunosuppressive, teratogenic and mutagenic, and a group 2B human carcinogenic (IARC, 1993).

2.8 Role of soil and plant debris in accumulation of mycotoxigenic fungi

Filamentous fungi are ubiquitous and abundant in the environment and are responsible for producing mycotoxins in agricultural crops (Venâncio and Paterson, 2007). The spores of these fungi are mainly found in the soil and crop residues and readily contaminate crops in the field (Whitlow and Hagler, 2005). For instance, soil serves as a main reservoir of *Aspergillus* spp. which infects a wide array of agricultural produce. The primary inoculum in the form of spores

of *A. flavus* is propagules in the soil, mycelia that overwinters in crop residues, or sclerotia in the soil (Diener *et al.*, 1987). *Fusarium* species are also important plant pathogenic fungi, and act as opportunistic pathogens of agricultural crops like maize, or as saprophytes on crop residues and cellulosic plant materials (Logrieco *et al.*, 2003). However, intense tillage practices that incorporate debris from previous harvest provide conditions that are less plausible for fungal growth on the crops in the field (Wagacha and Muthomi, 2008).

2.9 Strategies for management of mycotoxins in maize grains

Various pre-harvest and post-harvest mycotoxin management strategies have been developed to counteract both the adverse health effects and economic losses resulting from mycotoxins in foods (Wu, 2014). Pre-harvest interventions include good agricultural practices (GAP) such as early planting, deep tillage and crop rotation (Munkvold, 2003). Other strategies like planting resistant varieties (Munkvold, 2003) and bio-control management practices that make use of competitive exclusion mechanism (Adejumo and Adejoro, 2014) have also been developed. Post-harvest strategies like proper drying and storage of the crops (Darwish *et al.*, 2014), sorting of contaminated products (Palumbo *et al.*, 2008) and use of Hazard Analysis and Critical Control Points (HACCP) (Lopez-Garcia *et al.*, 1999) have been employed to manage mycotoxins in crops. Decontamination processes such as physical, chemical and biological methods have been employed to inactivate, destroy or remove the toxin animal feeds (Hell and Mutegi, 2011).

2.10 Post-harvest maize storage practices in Kenya

Storage of cereals plays an important role in evening out fluctuations in production from one season or year to the other (Kimenju and De Groote, 2010). On-farm storage of maize is vital since it directly contributes to food and income security, poverty alleviation (Tefera *et al.*,

2011a) and also mitigates the impact of dismal and non-consistent harvests (Ndegwa *et al.*, 2013). The major storage facilities used by smallholder maize farmers in Kenya include granaries/cribs, bags, baskets, earthen pots or metal silos (Nduku *et al.*, 2013). Maize is often stored inside the house in sacks, often laid directly on the floor (Aflacontrol, 2012) and this practice is perceived to be secure as grain losses through theft are minimized (Nduku *et al.*, 2013).

However, majority of smallholder farmers in Sub-Saharan Africa still use traditional/old storage methods (Gitonga *et al.*, 2015) which do not safeguard maize from the most common storage pathogens (Tefera *et al.*, 2011a; Maria, 2011) like mycotoxigenic fungi. The use of traditional structures for storage of maize also leads to considerable grain losses. It is estimated that 30 to 40% of the total grain produced in Kenya is lost due to ineffectiveness in postharvest handling and this impacts negatively on farmer's income, market supply, cereal prices and food security (Nduku *et al.*, 2013). Therefore, farmers should sufficiently dry maize before storage and use storage structures which are moisture proof so as to reduce the postharvest losses.

2.10.1 Hermetic storage technology for post-harvest preservation of maize

The essence of good storage structure, condition and hygiene is not only to reduce fungal load on grains but to minimize insect and moisture migration into the bin which could stimulate and ultimately quicken the growth and sporulation of the storage fungi (Enyiukwu *et al.*, 2014). The need for safe storage of maize at farm level has addressed the research on the development of improved storage technology to control fungal attack and mycotoxins contamination (Scarpari *et al.*, 2014). Therefore, a new storage method that involves storage of dry grains in hermetic plastic bags has been developed (Castellari *et al.*, 2010). The storage of maize in hermetic

environment is one of the most important alternatives to conventional storage of cereal grains (Freitas *et al.*, 2011). Sealing the storage system works by controlling grain moisture and reducing the number of insect pests in stored grain (Rickman and Aquino, 2007). Therefore, hermetic storage has become an alternative for conventional/traditional storage methods, chiefly in tropical regions of the world (Villers, 2014).

The need for an effective and efficient storage practice, which is also environmentally and farmer friendly brought to the fore the Purdue Improved Cowpea Storage (PICS) technology which involves storage of grain in a triple-layer bag consisting of an exterior woven bag with two inner airtight hermetic bags (Abudulai *et al.*, 2014). The two inner high density polyethylene (HDPE) bags present substantial barriers for the movement of O₂ across the bag's wall (Bhardwaj, 2015). As the microorganisms and the commodity within the hermetic bags respire they generate a modified atmosphere by utilising oxygen (O₂) and releasing carbon dioxide (CO₂) (Villers, 2014). This drop in O₂ concentration causes the insects and fungi to cease feeding and become inactive which in turn arrests population growth and grain damage (Bhardwaj, 2015). However, the moisture content of maize remains essentially constant under hermetic conditions (Villers *et al.*, 2010). Therefore, accumulation of mycotoxins produced by moulds is inhibited by both the lack of oxygen and lack of high moisture content (Villers *et al.*, 2010). The method is thus effective with 100% grain recovery from storage and also safe as no chemicals are involved (Abudulai *et al.*, 2014).

2.11 Nucleic acid based techniques for detection of mycotoxin producing fungi in grains

The nucleic acid amplification techniques have been developed to detect mycotoxin producing fungi in food and feed (Varga *et al.*, 2004). Polymerase chain reaction (PCR) based technique

has been employed for detection and identification of mycotoxigenic fungi in the genera *Fusarium*, *Aspergillus*, and *Penicillium* (Niessen, 2007). The PCR technique allows for rapid and selective detection of microorganisms in different substrates (Varga, 2003). More improved techniques like quantitative real-time PCR that are robust and highly sensitive have also been developed (Suanthie *et al.*, 2009). However, due to false positive results generated as a result of detection of both living and dead cells with relatively intact DNA, a propagation step is included prior to PCR analysis to overcome these limitations (Varga *et al.*, 2004).

2.12 Methods of detection and quantification of mycotoxins

Various analytical (Pussemier *et al.*, 2006) and immunological techniques (Bueno *et al.*, 2015) have been developed for simultaneous detection and quantification of mycotoxins in food and feed. Analytical techniques include; Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography/Mass Spectrometry (LC/MS) while the immunological techniques include Enzyme-Linked Immunosorbent Assay (ELISA) and Vicam technique (Pascale and Visconti, 2008).

2.12.1 Chromatographic techniques

The main chromatography techniques widely used in routine analysis of mycotoxins are High-performance liquid chromatography (HPLC) and Thin layer chromatography (TLC) (Shephard *et al.*, 2012). Thin layer chromatography is preferred due to simplicity of operation, cost effectiveness and many samples can be analyzed on one plate at a shorter time (Sherma, 2000). Quantification of aflatoxins using TLC is based on immununoaffinity columns which avoid compounds that may interfere with the assay thereby allowing quantification of aflatoxins in levels <1 ng/g (Stroka *et al.*, 2000). High-performance liquid chromatography (HPLC) can be

used both in normal and reverse-phase phase but the reverse-phase is preferred because of accuracy and better separation of aflatoxins (Sahib, 2009). Fluorescence detection is achieved with post-column photochemical derivatisation and time-based programming of the requisite wavelengths (Shephard *et al.*, 2012). More improved techniques such as liquid chromatography coupled with mass spectrometry or tandem mass spectrometry (LC-MS/MS) that have high sensitivity and selectivity have also been developed for multi-toxin analysis (Bueno *et al.*, 2015).

2.12.2 Immunological techniques

The immunological techniques such as Enzyme linked immunosorbent assay (ELISA) and Vicam have been developed for quantification of mycotoxins. Enzyme linked immunosorbent assay (ELISA) is a high throughput and sensitive assay based on the ability of a specific antibody to distinguish a specific mycotoxin (Zheng *et al.*, 2006). The ELISA assays rely on the ability of the toxin from the sample to competitively compete with a toxin–enzyme conjugate for a limited number of antibody-binding sites (Bueno *et al.*, 2015). One disadvantage with ELISA testing is that compounds with similar chemical groups can also interact with antibodies, because the target compounds are mycotoxins, not antigens (Waltman, 2008).

Vicam immunoaffinity chromatography is a quantitative screening method which works on the principle of specific antibody working against a particular mycotoxin. The method involves addition of the sample to the column containing the immunoaffinity complex which consists of a solid phase to which anti-mycotoxin antibodies are covalently coupled (Patel, 2004). The columns are then washed to remove unbound impurities and a developer solution added which reacts with the eluate (Herrman *et al.*, 2014) causing fluorescence that can be measured by HPLC, TLC or fluorimetre.

2.13 Regulation of mycotoxins in food

Various countries in the world have set specific limits for different mycotoxins in food and feed. The maximum tolerable levels have been set by various organisations including European Commission, the US Food and Drug Administration (USFDA) and the Kenya Bureau of Standards (KEBS) on different mycotoxins in food and feed (Table 1). Currently, the US Food and Drug Administration (USFDA) has set a tolerance of 10 µg/kg for aflatoxin B1, the most common form of aflatoxin found in maize grain (Williams *et al.*, 2011), while the European Commission has set maximum permitted levels for aflatoxins (AFB1, 2 µg/kg; total aflatoxins, 4 µg/kg); fumonisins (2000 µg/kg, FB1+ FB2) (Lattanzio *et al.*, 2007). The maximum allowable limit for aflatoxin B1 and total aflatoxins in foods and feeds by the Kenya Bureau of Standards is 5 ppb and 10 ppb respectively (Daniel *et al.*, 2011).

Table 1: Maximum limits of various mycotoxins ($\mu\text{g}/\text{kg}$) in cereals based on European Commission, Food and Drug Administration and Kenya Bureau of Standards

Mycotoxin	Foodstuff	Maximum level ($\mu\text{g}/\text{kg}$)		
		EC	FDA	KEBS
Aflatoxins	All cereals and cereal products	4	20	10
	Maize to be subjected to sorting or other physical treatment before human consumption	10		
Ochratoxin A	Unprocessed cereals	5		
	All cereal products intended for direct human consumption	3		
Deoxynivalenol	Unprocessed maize with the exception of unprocessed maize intended to be processed by wet milling	1750		
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	750	1000	
Zearalenone	Unprocessed cereals other than maize	100		
	Unprocessed maize	350		
	Cereals intended for direct human consumption	75		
	Maize intended for direct human consumption, maize- based snacks and maize-based breakfast cereals	100		
Fumonisin	Unprocessed maize	2000	4000	
	Maize intended for direct human consumption, maize- based foods for direct human consumption	1000	4000	
	Processed maize-based foods and baby foods for infants and young children	200		

EC - European commission; FDA – Food and Drug Administration; KEBS – Kenya Bureau of Standards

Source: EC (2006); FAO/WHO (2002); KEBS (2007).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Effect of maize production practices on fungal population

3.1.1 Description of the study area and selection of maize farmers

This study was conducted in Mukuyuni and Kilala Locations of Kaiti District, Makueni County of Lower Eastern Kenya (Figure 1). Kaiti District lies between latitude 1° 45' 00" S and longitude 37° 42' 00" E. It has a population of about 120,116 and covers approximately 423 Km². The area is semi-arid to arid with temperature range between 18°C to 24°C in the cold seasons and 24°C to 33°C in the hot days (MCIDP, 2013). The rainfall pattern is bi-modal with the long (but unreliable) rains in March to May and the more reliable short rains in October to December. The area receives an annual rainfall of between 800-1200 mm and has an elevation of 600m to 1900m above the sea level (MCIDP, 2013). Residents of Kaiti District rely on subsistence farming as their major source of livelihood. The selection of the study area was based on previous reports of re-current aflatoxicosis outbreaks (Lewis *et al.*, 2005).

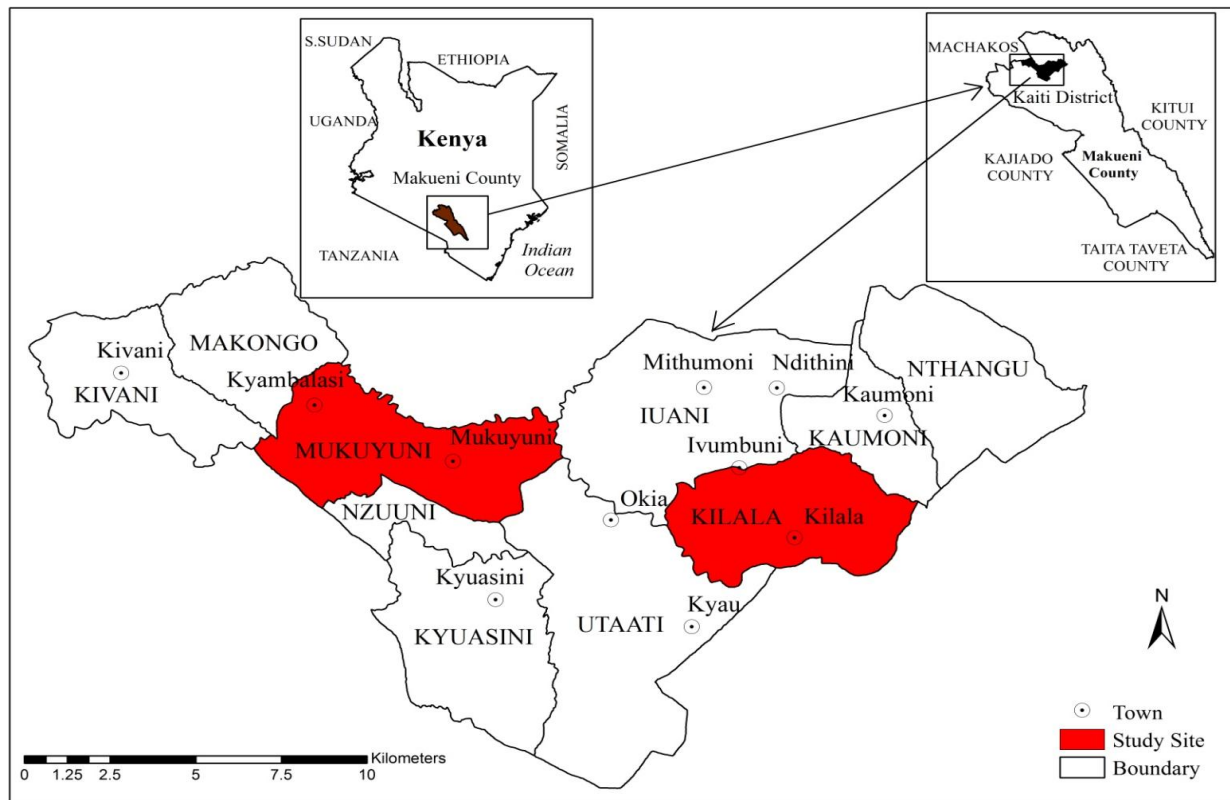


Figure 1: A map of Kenya showing the regions where the study was carried out.

3.1.2 Field survey and sampling design

Field survey and sampling was conducted between October 2015 and January 2016. A field survey involving 30 maize farms selected randomly was carried out in October 2015; 15 maize farms in Mukuyuni and 15 in Kilala Locations of Kaiti District, Makueni County. The selection of farmers was done through the assistance of field extension officers in the Ministry of Agriculture (MoA). A semi-structured questionnaire was used to obtain information on maize production practices such as farm size, maize varieties grown, sources of planting seeds, field preparation practices, cropping systems, storage practices, farmers' knowledge on aflatoxin, type of storage structures, pest problems in storage and duration of grain storage before consumption, selling or planting (Appendix A). Soil sampling was done at planting while maize

grains were sampled after harvest and three months after storage in polypropylene and hermetic bags. A total of 120 samples were collected; 30 soil samples were collected at planting while 30 maize grain samples were obtained after harvest from the farmers storage structures. A further 30 maize grain samples of 6kg each were obtained from the same farmers after harvest and divided into two equal portions for storage. Maize samples were stored in two types of bags: polypropylene bags and hermetic bags for three months. The maize storage bags were kept in storage structures where farmers commonly store their produce. Thirty maize grain samples were obtained from polypropylene bags and a similar sample size was obtained from hermetic bags after three months storage.

3.2 Determination of population of mycotoxin producing fungi in soil and maize and mycotoxin levels in maize

3.2.1 Sampling of soil and maize grains

Soil samples were collected at planting from 30 maize fields. In each farm, a minimum of five sampling points were identified randomly. Approximately 100 g of soil was collected from the top five centimetre layer of each sampling point using a table spoon. The same procedure was repeated in five different randomly selected points in the same farm and at least five meters apart. The sampling spoon was surface sterilized using 70% ethanol to avoid cross contamination when sampling fresh farms. In a paper bag, the samples were thoroughly mixed to make a composite sample. Approximately a 500 g composite soil sample was put in a zip lock bag, labelled and transported to the laboratory within 72 hrs. Each sample was thoroughly mixed then sieved with No. 20 standard testing sieve. The soil was air dried on laboratory benches for 5 days at room temperature and stored in Kraft bags at 25 °C until mycological analysis.

Maize grain samples were collected after harvest from 30 farmers either from the stores or family living house selected randomly. The sampled maize grains were harvested from the same fields where soil was obtained. For each household, shelled grains were randomly taken from different parts of the storage bag. The incremental samples were thoroughly mixed to form a composite sample. A representative sample of approximately 1kg was drawn, packed in a Kraft bag and transported to the laboratory within 72 hrs where they were maintained at 4°C until mycological analysis.

3.2.2 Isolation and enumeration of mycotoxin producing fungi from soil

Isolation of mycotoxin producing fungi from soil was carried out under aseptic conditions using serial dilution and spread plate technique on Potato Dextrose Agar amended with antibiotics (Muthomi, 2001). The PDA was prepared by suspending 39.0 g in one litre distilled water and then autoclaved at 121°C at 15 Pascal for 20 minutes. The PDA was allowed to cool to about 50°C and the following antibiotics added: 50 mg penicillin, 50 mg tetracycline and 50 mg streptomycin. One gram of each soil sample was suspended in 9 ml of sterile distilled water to form a stock solution, vortexed for 30 seconds and serially diluted with sterile distilled water to 10^{-2} of the original concentration. A hundred microlitre of each suspension was plated onto PDA amended with antibiotics and the plates incubated for 5 to 7 days at 25°C. The isolation procedure was carried out in three replicates for each sample. Fungal colonies on each plate were counted and colony counts expressed in colony forming units per gram of soil (CFU/g) as follows:

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

The incidence of each fungal species was calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{Number of isolates of a fungal species}}{\text{Total number of fungal species}} \times 100$$

3.2.3 Isolation and enumeration of mycotoxin producing fungi from maize grains

About 500 g of maize grain samples obtained at harvest was thoroughly mixed and ground in the laboratory using a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two equal sub-samples for microbial and mycotoxin analysis. Isolation of mycotoxin producing fungi was carried out using the serial dilution and spread plate technique on PDA amended with 50 mg penicillin, 50 mg tetracycline and 50 mgs antibiotics (Muthomi, 2001). One gram of each ground maize sample was suspended in 9 ml of sterile distilled water to form a stock solution, vortexed for 30 seconds and serially diluted with sterile distilled water to 10^{-2} of the original concentration. A hundred microlitre of each suspension was spread onto potato dextrose agar amended with antibiotics. The plated cultures were incubated for 5 to 7 days at 25°C. The isolation procedure was carried out in three replicates for each sample. The population and incidence of each fungal species were expressed as described in Section 3.2.2.

3.2.4 Cultural identification of mycotoxin producing fungi

3.2.4.1 Identification of *Aspergillus* species

For identification of *Aspergillus* spp., isolates were isolated on PDA amended with antibiotics and sub-cultured on to 5/2 agar (5% V8 juice and 2% agar, pH 5.2) (Atehnkeng *et al.*, 2008). The cultures on 5/2 agar were incubated at 31°C for 5 days. Isolates that produced small numerous dark sclerotia on 5/2 were identified as *A. flavus* S-strain, while those with yellow to bright green

colony without sclerotia were identified as *A. flavus* L-strain. Isolates that had dark green colonies on 5/2 and produced rough conidia were considered *A. parasiticus* (Atehnkeng *et al.*, 2008). Colonies that were black on the top side, while the bottom side remained pale were identified as *A. niger*. *Aspergillus* spp. were distinguished based on colony colour, shape, elevation, pigmentation, texture and pattern of growth (Klich, 2002).

3.2.4.2 Identification of *Fusarium* species

All *Fusarium* isolates isolated on PDA amended with antibiotics were sub-cultured on PDA and Synthetic Nutrient Agar. The PDA was prepared by weighing 39.0 g of the media into 1 litre of distilled water. The mixture was autoclaved for 15 minutes at 121°C and 15 Pascal. Synthetic Nutrient Agar (SNA) was prepared by weighing 1.0 g KH₂PO₄, 1.0 g KNO₃, 0.5 g MgSO₄.7H₂O, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose and 20 g Agar into 1 litre sterile distilled water (Nirenberg, 1981). The PDA cultures were incubated at 25°C for 7-14 days while the SNA were kept in dark for 14-21 days to induce sporulation (Nirenberg, 1981). Potato Dextrose Agar was used for gross morphological appearance and colony pigmentation while cultures grown on SNA were used for microscopic identification based on conidia shape and size, conidiophore and chlamydospore (Leslie and Summerell, 2006). After incubation, cultures were identified based on growth rate, colony reverse colour, surface texture, colour and shape of aerial mycelia and the development of pigments in agar medium (Nelson *et al.*, 1983; Leslie and Summerell, 2006).

3.2.4.3 Identification of *Penicillium* species

Isolates of *Penicillium* spp. were sub-cultured on PDA and identified to genus level based on colony colour, texture, elevation and production of exudates as described by Pitt and Hocking (2009).

3.2.5 Morphological identification of mycotoxin producing fungi

Microscopic examination of *Aspergillus* spp. and *Fusarium* spp. was done using modified Riddell slides (Riddell, 1950; Murithi, 2014). Slide cultures of *Aspergillus* spp. and *Fusarium* spp. were made by placing 5/2 and SNA agar blocks, respectively on a microscopic slide raised with a V – shaped glass rod in a sterile Petri plate covered with a wet sterile paper towel at the bottom. *Aspergillus* spores and *Fusarium* mycelia were transferred from their isolates to the four edges of the agar block using a sterile inoculating needle. A clean cover slip was placed on the surface of each agar block and the plate partially sealed with parafilm™. Cultures of *Aspergillus* spp. were incubated at 31°C for 5 days while *Fusarium* spp. were placed in the dark for 14-21 days. Slides for light microscopy were prepared by removing the agar block and then adding a drop of either sterile distilled water or lactophenol cotton blue on the slide and cover slip added to cover the growth on the slide. The prepared slides were used for identification and taking images of morphological characteristics of the commonly isolated *Aspergillus* spp. and *Fusarium* spp. All prepared slides were examined under a Light microscope (1000x) and the corresponding images were taken with a camera (LEICA ICC 50, Leica Microsystems, Wetzler, Germany) fitted to a microscope. Microscopic characteristics used in identification of *Aspergillus* spp. were conidial heads, seriation, conidia size, shape and roughness as described by Klich (2002); Pitt and Hocking (2009). The observed features of microconidia, macroconidia and chlamydo spores were used to identify the *Fusarium* spp. as described by Nelson *et al.* (1983); Leslie and Summerell (2006).

3.2.6 Polymerase chain reaction (PCR) based characterization of mycotoxin producing fungi

3.2.6.1 Extraction of fungal genomic DNA

Fungal genomic DNA was extracted from ground maize grains using the Bead Beating method as described by Chang *et al.* (2016) with modification. About 500 g of each maize grain sample was ground in a coffee grinder (Moulinex AR1100, United Kingdom) for 2 minutes to a fine powder and then 0.35 g of each ground maize sample was weighed into a 2 mL microcentrifuge tube. One millilitre of 1x CTAB extraction buffer and 10 of 2 mm glass beads were added to each ground sample and homogenized in a bead beater at an agitation velocity of 5.0 m/s for 3 minutes. Samples were incubated for 5 minutes at 65°C in a water bath, centrifuged at 13000 rpm for 5 minutes and then 500 µL of the supernatant was transferred to a new 2 mL microcentrifuge tube. Chloroform: isoamyl alcohol (24:1; 500 µL) was added to the supernatant and after centrifugation at 13000 rpm for 5 minutes; the aqueous layer was transferred to new 2 mL microcentrifuge tube. About 50 µL aliquot of 3M sodium acetate (NaAc) and 500 µL of isopropanol were added to the supernatant. The solution was mixed gently for 5 minutes at room temperature ($25 \pm 2^{\circ}\text{C}$) and then centrifuged at 13000 rpm for 10 minutes. The supernatant was decanted and the resultant pellet air dried for 5 minutes then resuspended in 500 µL ddH₂O. Phenol: chloroform: isoamyl alcohol (25:24:1; 500 µL) was added to the mixture, vortexed for 3 minutes and then centrifuged at 13000rpm for 5 minutes. A 400 µL aliquot of the supernatant was transferred to a new microcentrifuge tube. A 50 µL of 3M NaAc and 1 ml of 100% ethanol was added to the supernatant and stored at -20°C for about 30 minutes. The mixture was then centrifuged at 13000 rpm for 10 minutes. The resulting pellet was washed with 70% ethanol, air

dried at room temperature and the DNA dissolved in 50 μL ddH₂O. The DNA was then stored at -20°C.

3.2.6.2 The PCR primers and amplification of ITS region

Polymerase Chain Reaction (PCR) analysis was performed by isolating from the DNA the internal transcribed spacer (ITS) regions ITS1 (5'-TCCGTAGGTGAACCTGCGG- 3') and ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') (Ferrer *et al.*, 2001; Beck, 2002), that are highly variable and often used to distinguish taxonomic groups (Suanthie *et al.*, 2009). The primers were obtained from Integrated DNA Technologies and Operon Biotechnology (Huntsville, Alabama, USA) (González-Salgado *et al.*, 2009). The PCR amplification was conducted in 50 μL volume containing 2 μL of template DNA extracted from fungal infected maize grains, 10 μL of 5 \times PCR buffer, 0.3 μL of flexi *Taq* DNA polymerase (recombinant) (Invitrogen Life Technologies, Burlington, Ontario, Canada), 1 μL of 0.5 μM each of the ITS1 and ITS4 primers, 4 μL of 25 mM MgCl₂ and 1 μL of 10 mM each of dNTPs (Invitrogen Life Technologies) and 30 μL of ddH₂O. The PCR amplification protocol was an initial preheat for 5min at 95°C, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1min and 72°C for 10min. PCR products, including the negative control, were analyzed through agarose gel electrophoresis (0.8%) containing ethidium bromide with 1 \times TBE running buffer to reveal the presence/absence of the DNA bands. Images were acquired with a Bio Doc-IT Imaging System (UVP Inc. CA) (Vujanovic *et al.*, 2012). The variability in number and size of fungal DNA bands that resulted from PCR reactions primed by ITS-1 and ITS-4 was used in identification. The PCR amplification of fungal DNA showed clear banding pattern of ITS fragments which indicated the presence of different mycotoxin producing fungi.

3.2.7 Determination of aflatoxin levels in maize grains

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

3.2.8 Determination of fumonisin levels in maize grains

The levels of fumonisin in maize were determined using the VICAM method described by VICAM, (2012) and Atukwase *et al.* (2009) with modification. Five grams from each finely ground maize grain sample was placed in an extraction tube and 10 ml of methanol/water (70:30) added. The mixture was vortexed for 5 min and filtered through a 24 cm fluted filter paper (VICAM, Watertown, USA). A hundred microlitre of Fumo-V Diluent was transferred to the strip test vial and 100 µL of the sample extract added and vortexed for two minutes. A hundred microlitre of the mixture was transferred to the Fumo-V strip tests at a flow rate of one drop per second vertically into the circular opening. The strip tests were allowed to develop for five minutes on a flat surface. Fumo-V strip tests were inserted into the Vertue reader (VICAM,

Watertown, USA) for quantification of fumonisin in parts per million, ppm (Atukwase *et al.*, 2009). The limits of detection were between 2 ppm and 6 ppm.

3.3 Determination of the effect of hermetic storage bags on population of mycotoxin producing fungi and mycotoxin levels

3.3.1 Experimental design

The aim of this study was to determine the effect of hermetic storage bags on population of mycotoxin producing fungi and mycotoxin levels. The storage experiment was carried out in October after harvesting of maize. Approximately 1kg of maize grains was sampled from 30 farmers' storage structures in order to determine the initial fungal population, aflatoxin and fumonisin levels. A similar number of samples were obtained from the same farmers and stored in hermetic bags and woven polyethylene bags. Approximately 6 kg of maize grains was sampled from each household for the storage experiment. The maize samples were thoroughly mixed and divided into two equal portions of approximately 3 kg which was stored in each hermetic bag. Each of the three layers of hermetic bag was tied separately to ensure the bag was air tight. The other 3 kg of maize grains was stored in polypropylene bags and tightly tied. Maize storage bags were stored for a period of three months in storage structures where farmers commonly store their grains.

3.3.2 Determination of population of mycotoxin producing fungi and aflatoxin and fumonisin levels in maize grains

Approximately 1kg of maize grains was sampled from each farmer's store three months after storage to compare the population of mycotoxin producing fungi in maize stored in polypropylene bags and the improved hermetic bags. Households in Kenya commonly use

polypropylene bags to store maize. A total of 60 maize grain samples were collected from 30 farmers' stores; 30 maize samples were obtained from hermetic bags and the other 30 samples from polypropylene bags. Sampling was done from hermetic and polypropylene bags and entailed thoroughly mixing the 3 kg sample and drawing a 1 kg sub-sample. The collected maize grain samples were placed in Kraft bags, transported to the Laboratory within 72 hrs and stored at 4°C until processing. Isolation and identification of mycotoxin producing fungi from maize was carried out following the method described in Sections 3.2.3 and 3.2.4. Aflatoxin and fumonisin levels were determined using the procedure described in Sections 3.2.7 and 3.2.8, respectively.

3.4 Data analysis

The population and incidence of mycotoxin producing fungi in soil and maize was analyzed using the Analysis of Variance (ANOVA) PROC ANOVA procedure of GENSTAT version 15. Frequency data that was not normally distributed was transformed to arcsine before analysis whereas the population data that was not normally distributed was transformed using $\log_{10}x+1$. Least significant difference (LSD) was used to assess the significance of differences between treatment means at 95% confidence level.

CHAPTER FOUR: RESULTS

4.1 Maize production practices in Kaiti District

Eighty seven percent of the farmers in Kaiti District were small scale, 10% were medium scale while only 3.3% were large scale farmers (Figure 2A). Eighty percent of maize farmers owned between one and five acres of land while 13.3% owned between 6 to 10 acres. Only 6.7% of the farmers owned more than 16 acres of land. About 93.3% of area under maize production was between one and five acres. The duration that the farmers had practiced maize production varied from one to forty years (Figure 2B). Forty seven percent of the farmers had been growing maize between 11 and 20 years. Sixty seven percent of the farmers used oxen plough for land preparation compared to 33% who used both oxen plough and hand hoe (Figure 2C). The maize stovers from all the 30 farms were fed to livestock. Farmers reported the use of soil amendments during maize production. Eighty percent of farmers used organic manure while 20 % applied Di-ammonium Phosphate (DAP) at planting. Seventy percent of farmers used calcium ammonium nitrate (CAN) for top dressing.

All the farmers in Mukuyuni Location practiced intercropping while 93.3% of farmers in Kilala Location practiced intercropping (Figure 3A). Maize was intercropped with several crops such as common beans, cowpeas, pigeon peas and green grams. Maize intercropped with beans was common in both Locations while intercropping of maize with pigeon peas or cowpea was common in Kilala. The most common maize varieties grown by farmers were: Pioneer, Duma 43, local (Kinyanya) and DH02 (Figure 3B). Pioneer was the most popular variety which was grown by 36.7% of farmers while DH02 was only grown by 3.3% of farmers. Sixty seven percent of the farmers planted certified maize seeds while 33% planted their own saved seeds

from previous seasons (Figure 3C). Ninety seven percent of farmers determined maize harvesting stage through visual observation (Figure 3D). The crop was considered ready for harvesting when leaves started drying, changing colour from green to yellow, dropping of the cobs and by pricking. Only 3.3% of the farmers used number of days that the crop was in the field to determine the appropriate maize harvesting stage. All farmers manually harvested their maize during dry weather. Fifty three percent of farmers harvested between one and ten 90kg bags per acre while 47% harvested between fifteen and thirty 90kg bags per acre. Seventy percent of the farmers produced maize for both subsistence and commercial purposes while the rest (30%) produced the crop only for subsistence purposes. Besides maize, pumpkins, kales, tomatoes, watermelon, groundnuts, onions, soybeans, cabbage, spinach, bananas, sweet potatoes, oranges and mangoes were grown in the same field (Figure 4).

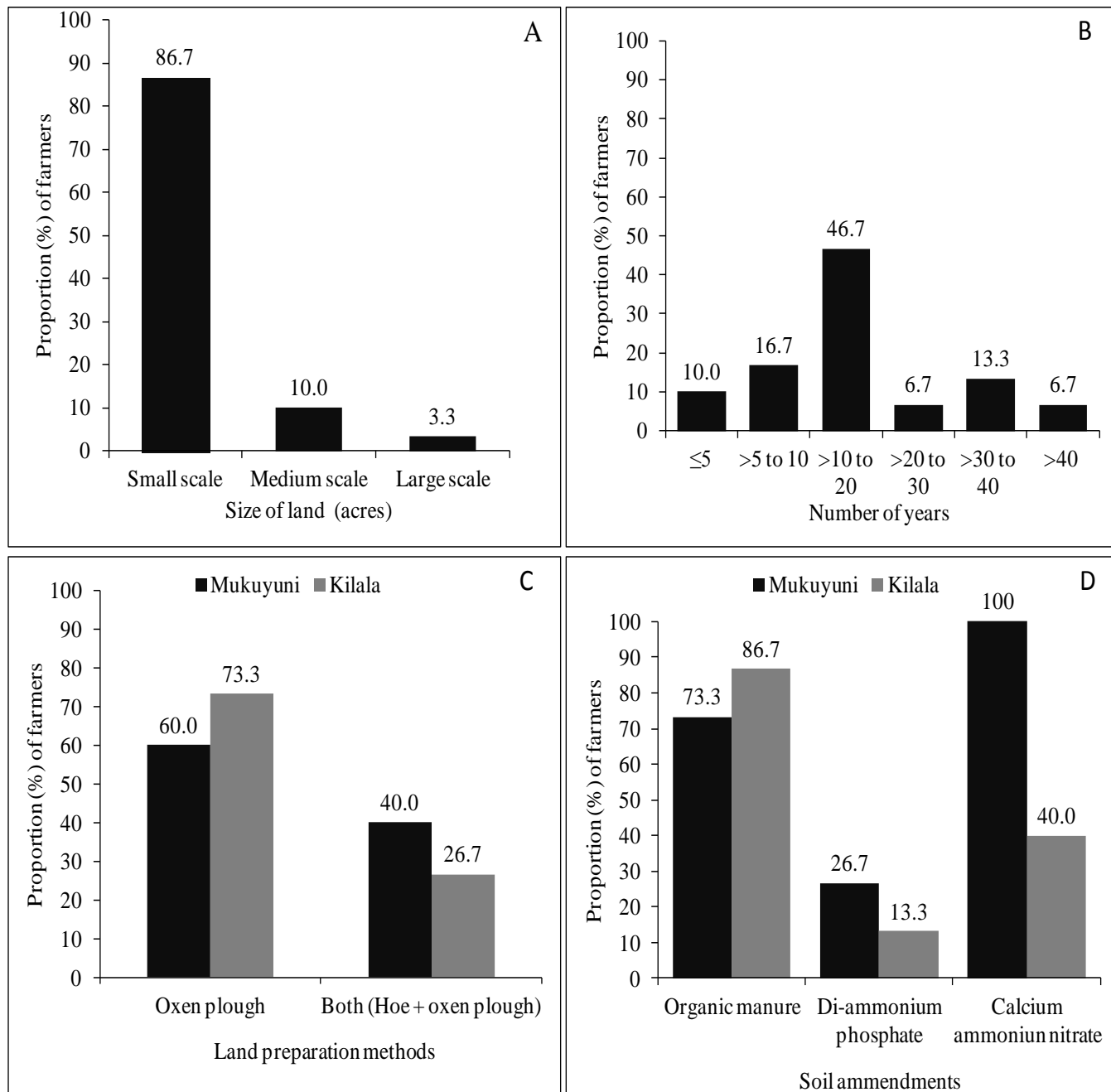


Figure 2: Categories of farm sizes (A), duration of maize production (B), land preparation methods (C) and use of soil amendments (D) by farmers in Mukuyuni and Kilala Locations, Kaiti District.

Small scale: <9 acres; medium scale: 9 – 20 acres; large-scale: >20 acres.

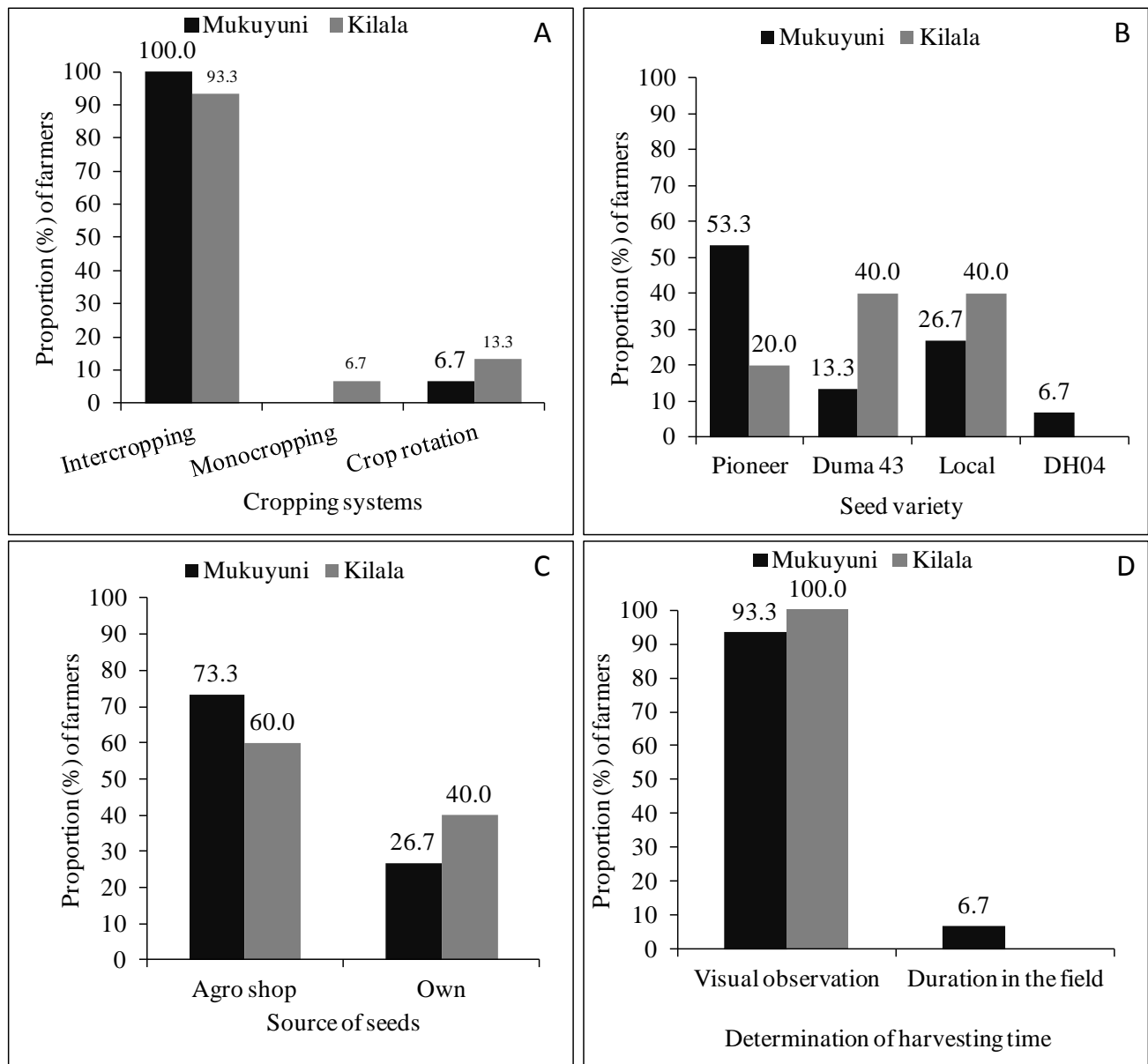


Figure 3: Cropping systems (A), maize varieties planted by farmers (B), source of planting seeds (C), and methods used by farmers to determine when maize is ready for harvesting (D) in Mukuyuni and Kilala Locations of Kaiti District.

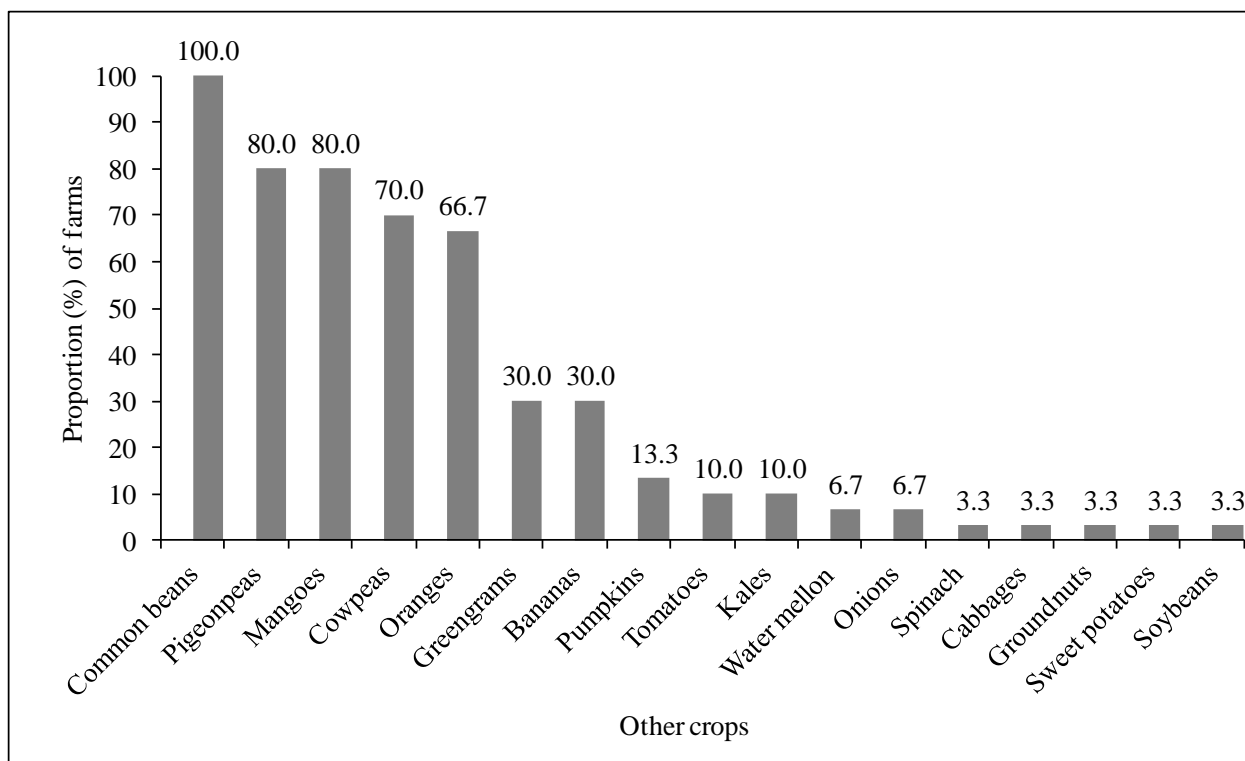


Figure 4: Crops commonly grown by maize farmers either in mixed cropping system or rotation programs in Kaiti District.

After harvest, farmers removed husks from maize cobs before drying. All farmers dried their maize on cobs in the sun immediately after harvest for a duration ranging from one to four weeks. Thirty seven percent of the farmers dried their maize for two weeks while 23.3% dried for four weeks (Figure 5A). The farmers shelled the maize by hand before storage. The storage methods included use of 50 and 90 kg capacity polypropylene bags, sisal bags, and storage of maize in modern granaries and inside the family living house. On average, 56.7% of farmers stored their maize in modern stores made of timber while 43.3% of farmers stored their produce inside the family living house (Figure 5B). Eighty three percent of farmers stored their maize in polypropylene bags (Figure 5C) while only 16.7% used sisal bags.

The common storage challenges encountered by maize farmers were insect damage, mould damage, rodent damage and lack of storage bags (Figure 5D). In this study, farmers treated their maize using commercial insecticides while a few used traditional storage protectants such as ash to control insect pests mainly weevils (*Sitophilus zeamays*). Some farmers used traps to control rodents while all farmers cleaned their storage structures before storage of a new crop. An assessment of the farmers' knowledge on mycotoxin contamination showed high level of awareness and the need for control measures. Eighty seven percent of farmers in Mukuyuni Location considered mycotoxins a major problem compared to 80% of farmers in Kilala Location (Figure 6).

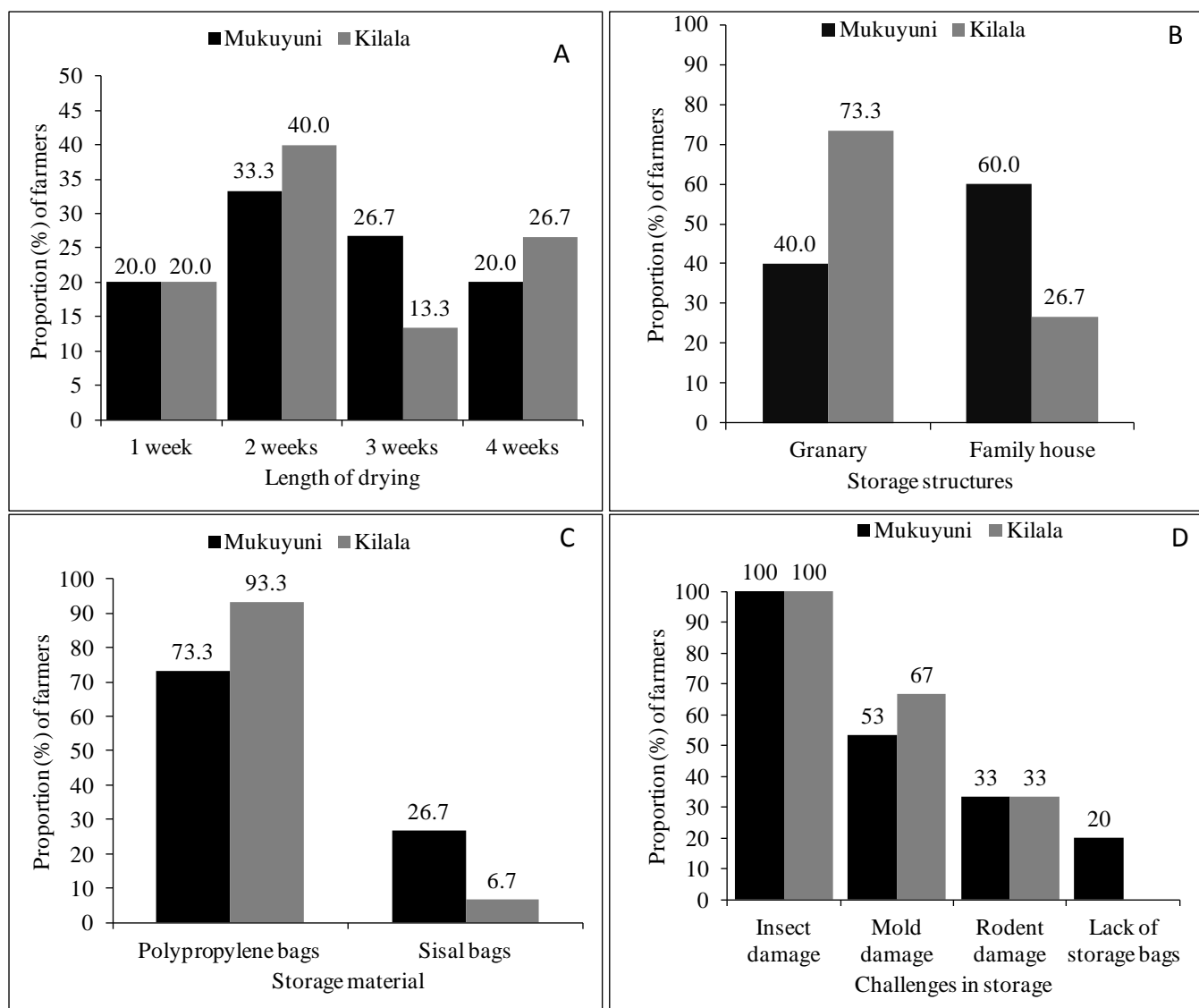


Figure 5: Length of drying period (A), type of storage structures (B), storage methods (C) and challenges encountered by farmers during post-harvest storage of maize (D) in Kaiti District.

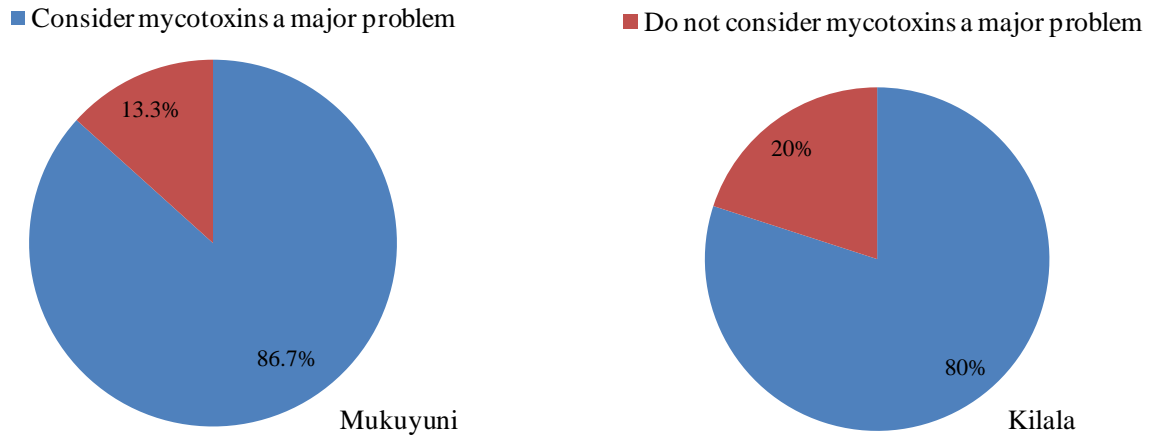


Figure 6: Perceptions of farmers in Mukuyuni and Kilala Locations on effects of mycotoxins in maize production.

4.2 Diversity of mycotoxin producing fungi in soil and maize samples

The major genera of mycotoxin producing fungi isolated from soil and maize grain samples in Kaiti District were: *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. The most common members of *Aspergillus* section *Flavi* isolated from soil and maize grain samples were; *A. flavus* (S and L-strains) and *A. parasiticus* (Figure 7; Figure 8). *Aspergillus niger* was also commonly isolated from soil and maize grain samples. Colonies of *A. flavus* L-strain were yellow to bright green with no sclerotia while *A. flavus* S-strain produced numerous small and dark sclerotia. *Aspergillus parasiticus* produced dark green colonies with rough conidia which were more compact than spores of *A. flavus* L-strain. Colonies of *A. niger* were initially white but soon turned black on the top side, while the bottom side remained pale yellow.

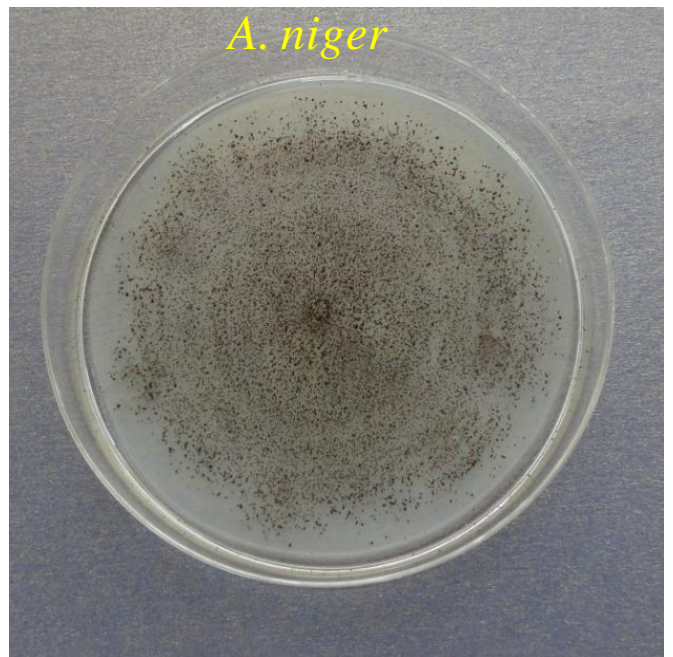
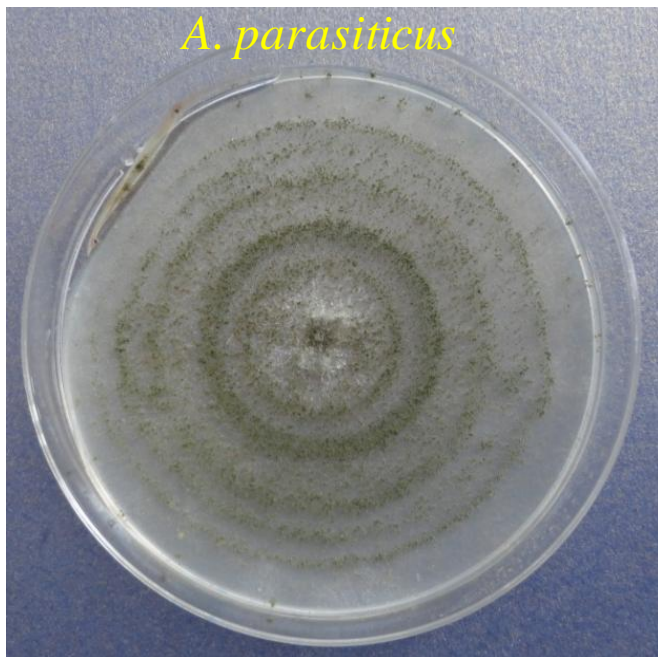
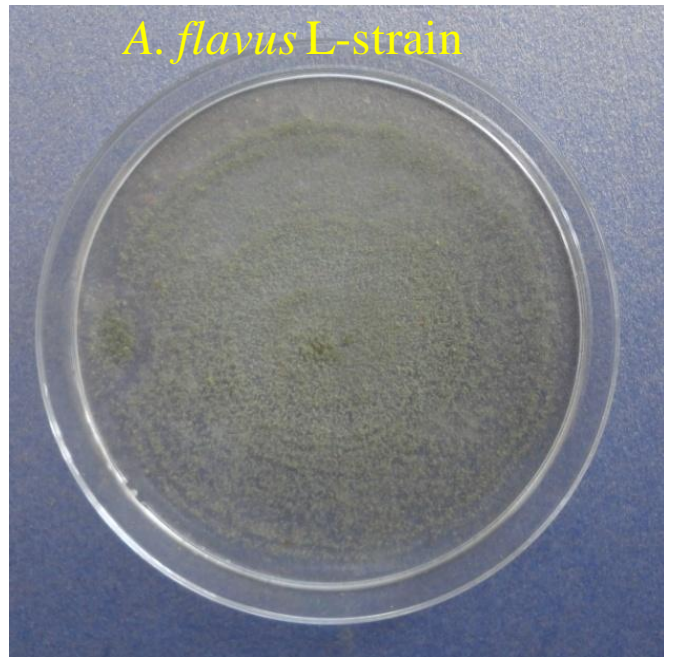
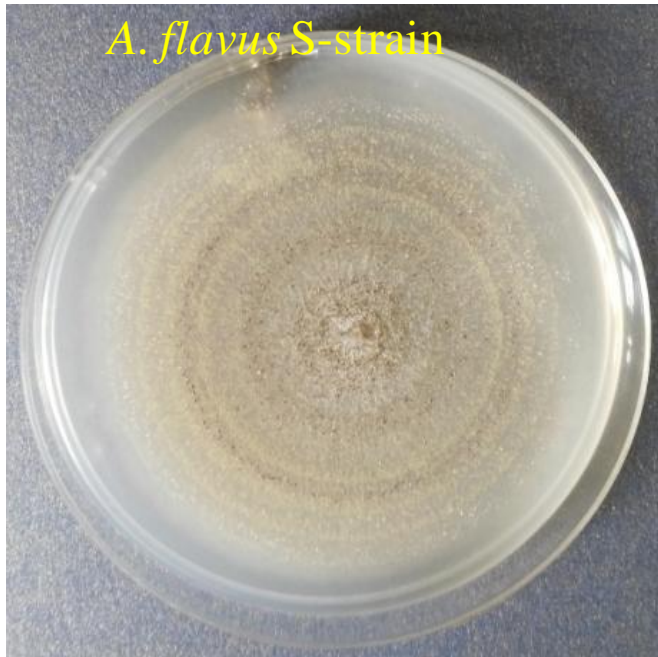


Figure 7: Cultures of *Aspergillus* spp. on 5/2 agar isolated from soil and maize grains from maize fields in Kaiti District

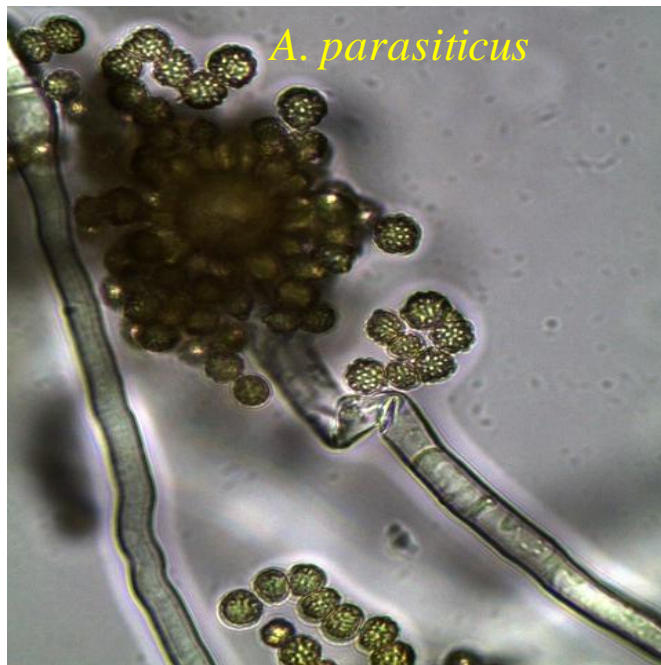


Figure 8: Spores and conidial heads of *Aspergillus* spp. isolated from soil and maize grains from maize fields in Kaiti District. X 1000 magnification

Figure 9 shows the cultural characteristics of *Fusarium* spp. isolated from maize and soil samples. *Fusarium proliferatum* produced white aerial mycelium that grew rapidly and was tinged with purple colour. *Fusarium verticillioides* produced mycelia with white pigmentation. Sporodochia of *F. verticillioides* was dark in colour. *Fusarium oxysporum* produced floccose mycelia that were abundant and white to pale violet and the under surface was pale purple. *Fusarium subglutinans* produced aerial mycelia that grew rapidly and was white in colour while sporodochia was cream in colour. For *F. solani*, growth was rapid and produced abundant aerial mycelia that were cream to purple in colour.

Fusarium proliferatum - the predominant species isolated in maize samples from Kaiti - produced club shaped microconidia that had curved apical end, relatively slender, relatively straight and thin walled (Figure 10). Microconidia of *F. proliferatum* were club shaped, non-septate and with a flattened base. *Fusarium oxysporum* produced non-septate kidney shaped microconidia and slightly curved 3-septate macroconidia. *Fusarium verticillioides* produced club shaped, non-septate microconidia that were in long chains and aggregates (Figure 10). *Fusarium subglutinans* produced oval non-septate microconidia on false heads on the aerial mycelium. *Fusarium solani* produced straight and stout macroconidia and oval shaped non-septate microconidia and thick walled chlamydospores.

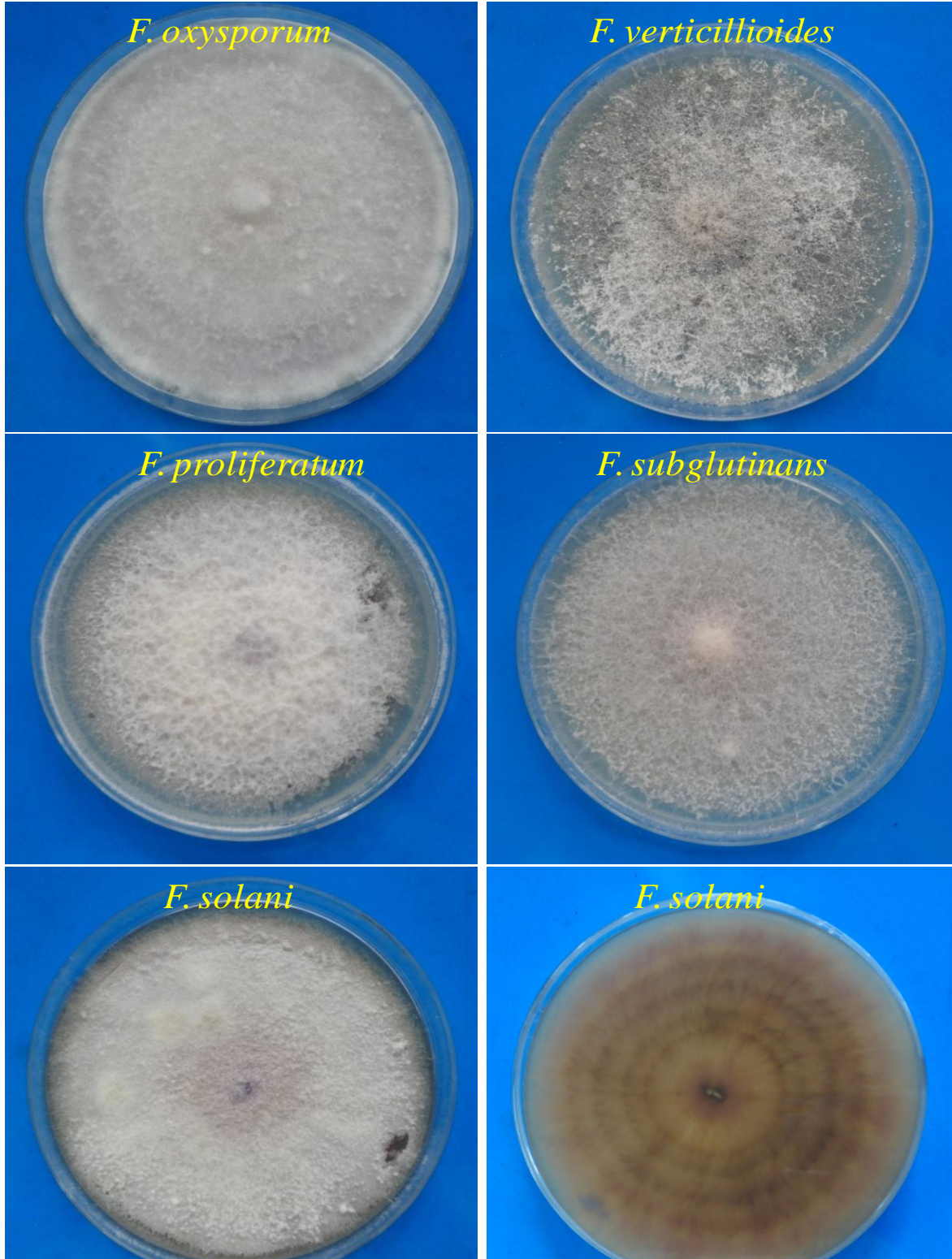


Figure 9: Cultures of major *Fusarium* spp. on potato dextrose agar isolated from soil and maize grains from maize fields in Kaiti District.

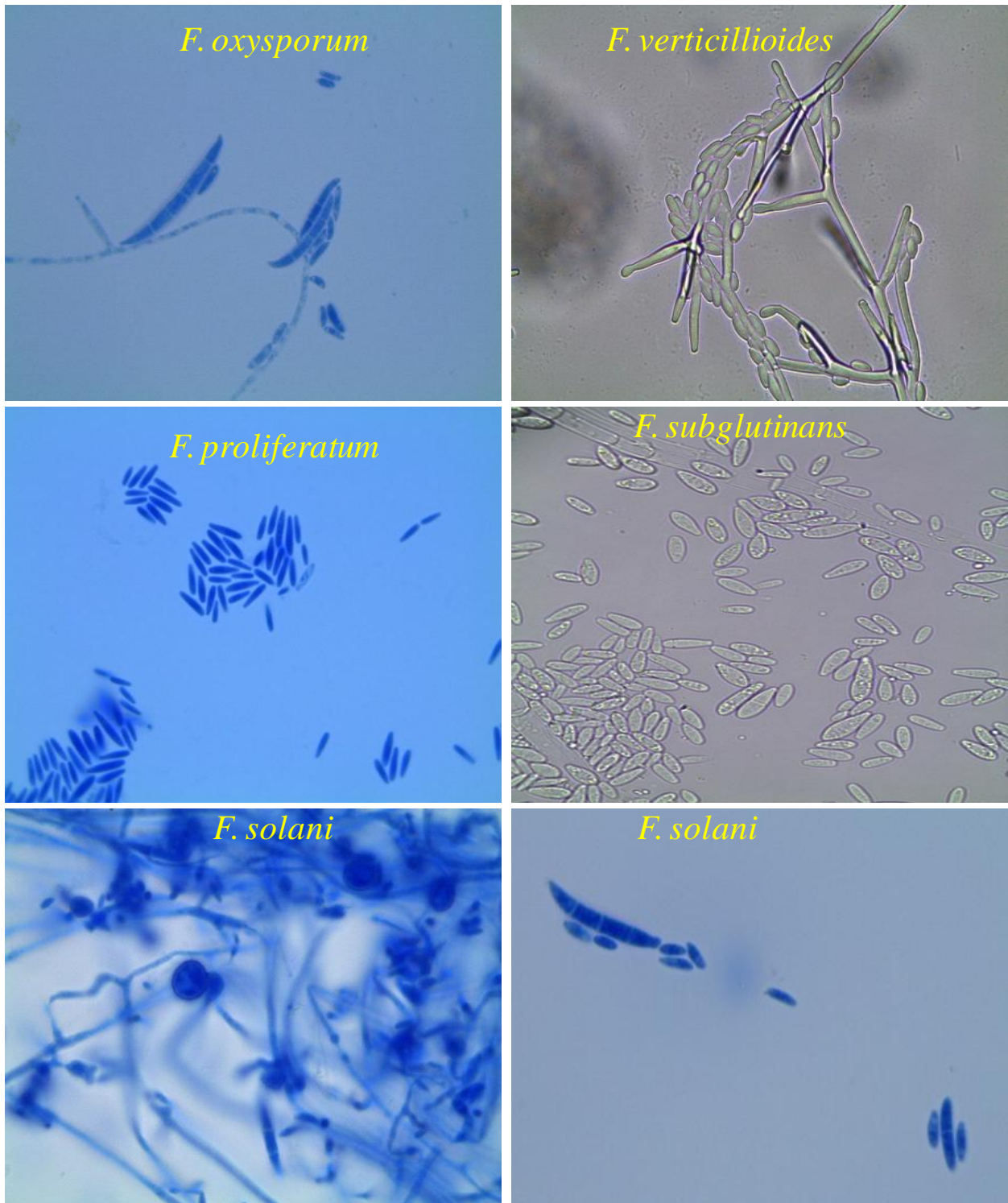


Figure 10: Morphological characteristics of micro- and macro-conidia of major *Fusarium* spp. isolated from soil and maize grains from maize fields in Kaiti District. X 1000 magnification

4.3 Population and incidence of mycotoxin producing fungi in soil

The major mycotoxin producing fungi isolated from soil samples in decreasing incidence were *Penicillium* spp., *Fusarium* spp., and *Aspergillus* spp. (Table 2). *Fusarium* spp. was the most prevalent in Mukuyuni Location while *Penicillium* spp. and *Aspergillus* spp. were more prevalent in Kilala. There was significant difference ($P \leq 0.05$) in the population of other fungal species between Kilala and Mukuyuni. However, the population of *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. in soil was not variable ($p \geq 0.05$) between Kilala and Mukuyuni Locations.

Table 2: Population and incidence of mycotoxin producing fungi in soil sampled from maize fields in Kaiti District

Fungal spp.	Mukuyuni Location		Kilala Location	
	Population (CFU/g)	Incidence (%)	Population (CFU/g)	Incidence (%)
<i>Aspergillus</i> spp.	3422.2a	20.5a	4755.6a	26.6a
<i>Fusarium</i> spp.	4533.3a	28.0a	4044.4a	27.5a
<i>Penicillium</i> spp.	3733.3a	24.7a	5288.9a	30.8a
Other fungi	3488.9a	27.1a	2377.8b	15.1b
Mean	3794.4	25.0	4116.7	25.0
LSD ($p \leq 0.05$)	1537.8	7.6	1330.8	7.1
CV (%)	9.4	1.9	4.4	0.7

Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$). LSD - Least significant difference; CV - Coefficient of variation

Aspergillus species isolated from soil were: *A. flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger* (Table 3). Population of *Aspergillus* spp. in soil varied significantly ($p \leq 0.05$) between Kilala and Mukuyuni Locations (Table 3). *Aspergillus niger* and *A. flavus* L-strain were the most prevalent with a mean population of 2.3×10^3 and 8.4×10^2 CFU/g soil, respectively. Incidence of *A. flavus* L-strain was higher in soil samples from Mukuyuni while the incidence of *A. parasiticus* was significantly higher ($p \geq 0.05$) in soil samples from Kilala Location. There was no significant variation ($p \geq 0.05$) in the incidence of *A. flavus* S-strain in soil sampled from Kilala and Mukuyuni Locations.

Table 3: Population and incidence of *Aspergillus* spp. in soil sampled from maize fields in Kaiti

District	Aspergillus spp.			
	Mukuyuni Location		Kilala Location	
	Population (CFU/g)	Incidence (%)	Population (CFU/g)	Incidence (%)
<i>A. flavus</i> S-strain	533.3bc	10.1b	444.4b	6.6b
<i>A. flavus</i> L-strain	1000.0ab	25.6a	688.9b	16.0b
<i>A. parasiticus</i>	488.9c	7.1a	422.2b	6.2b
<i>A. niger</i>	1400.0a	35.0b	3200.0a	60.1a
Mean	855.6	19.4	1188.9	22.2
LSD ($p \leq 0.05$)	509.3	11.6	679.1	10.4
CV (%)	18.5	47.7	5.5	8.7

Means followed by the same letter(s) within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$). LSD - Least significant difference; CV - Coefficient of variation

The major *Fusarium* spp. isolated from soil were: *F. proliferatum*, *F. oxysporum*, *F. subglutinans* and *F. solani* with *F. proliferatum* being the most commonly isolated from both Mukuyuni and Kilala Locations (Table 4). Although the population of *Fusarium* spp. was not variable ($p \geq 0.05$) in soil samples from the two Locations; there was significant variation ($p \leq 0.05$) in the incidence of *Fusarium* spp. between Kilala and Mukuyuni (Table 4). *Fusarium proliferatum* was the most prevalent in Kilala while *F. oxysporum* was the most prevalent in Mukuyuni Location.

Table 4: Population and incidence of *Fusarium* spp. in soil sampled from maize fields in Kaiti

District	District				
	<i>Fusarium</i> spp.	Mukuyuni Location		Kilala Location	
		Population (CFU/g)	Incidence (%)	Population (CFU/g)	Incidence (%)
<i>F. proliferatum</i>	1533.3a	17.8b	1200.0a	31.2a	
<i>F. oxysporum</i>	1466.7a	35.8a	1044.4a	31.1a	
<i>F. solani</i>	755.6a	13.3b	1133.3a	22.1ab	
<i>F. subglutinans</i>	777.8a	19.8b	688.9a	15.7b	
Mean	1133.3	21.7	1016.7	25.3	
LSD ($p \leq 0.05$)	800.5	12.7	556.4	13.3	
CV (%)	15.7	13.6	21.1	8.5	

Means followed by the same letter(s) within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$). LSD - Least significant difference; CV- Coefficient of variation

4.4 Efficacy of hermetic storage on fungal population in maize grains

4.4.1 Population of mycotoxin producing fungi in maize grains

Nine mycotoxin producing fungi were commonly isolated from maize grains sampled at harvest and three months after storage either in hermetic or polypropylene bags (Table 5). The population of major mycotoxin producing fungi in decreasing order was: *Penicillium* spp. (7.0×10^3 CFU/g), *Fusarium* spp. (6.9×10^3 CFU/g) and *Aspergillus* spp. (2.2×10^3 CFU/g) (Figure 11A). There was significant difference ($p \leq 0.05$) in the population of mycotoxin producing fungi in maize sampled at harvest and three months after storage. The population of mycotoxin producing fungi was 39.7% higher in maize sampled after storage as compared to samples collected at harvest. The type of storage bag had a significant influence ($p \leq 0.05$) on the overall population and diversity of fungal species isolated from maize grains. The population of mycotoxin producing fungi was 78% higher in maize stored in polypropylene bags compared to hermetic bags. However, the population of *Penicillium* spp. was consistently high in the two types of storage bags. On the other hand, maize stored in polypropylene bags had higher diversity of fungal species as compared to hermetic bags.

Aspergillus spp. isolated from maize in decreasing order were: *A. flavus* L-strain (8.7×10^2 CFU/g), *A. flavus* S-strain (6.4×10^2 CFU/g), *A. parasiticus* (3.4×10^2 CFU/g) and *A. niger* (3.3×10^2 CFU/g) (Figure 11B). The population of the aforementioned *Aspergillus* spp. was significantly lower ($p \leq 0.05$) in maize sampled at harvest as compared to three months after storage. The type of storage bag significantly ($p \leq 0.05$) influenced the population of members of *Aspergillus* section *Flavi* - *A. flavus* (S and L strains) and *A. parasiticus*. The population of the three members of *Aspergillus* section *Flavi* was significantly higher ($p \leq 0.05$) in maize stored in

polypropylene bags as compared to hermetic bags. However, the population of *A. flavus* L-strain was consistently higher than that of *A. flavus* S-strain and *A. parasiticus* in the two types of storage bag at the third month of storage (Figure 11B). Overall, the population of *A. flavus* L-strain was 35.0% and 60.4% higher than that of *A. flavus* S-strain and *A. parasiticus*, respectively.

The *Fusarium* spp. isolated from maize kernels were: *F. proliferatum*, *F. verticillioides*, *F. oxysporum* and *F. subglutinans* (Figure 11C). *Fusarium proliferatum* was the most commonly isolated with a mean population of 2.2×10^3 CFU/g of maize, while *F. subglutinans* was the least isolated with a mean of 1.0×10^3 CFU/g of maize. The population of *Fusarium* spp. significantly varied ($p \leq 0.05$) in maize sampled at harvest and three months after storage. The type of storage bag significantly ($p \leq 0.05$) influenced population of the *Fusarium* spp. The population of the four *Fusarium* spp. was significantly higher ($p \leq 0.05$) in maize stored in polypropylene bags compared to hermetic bags.

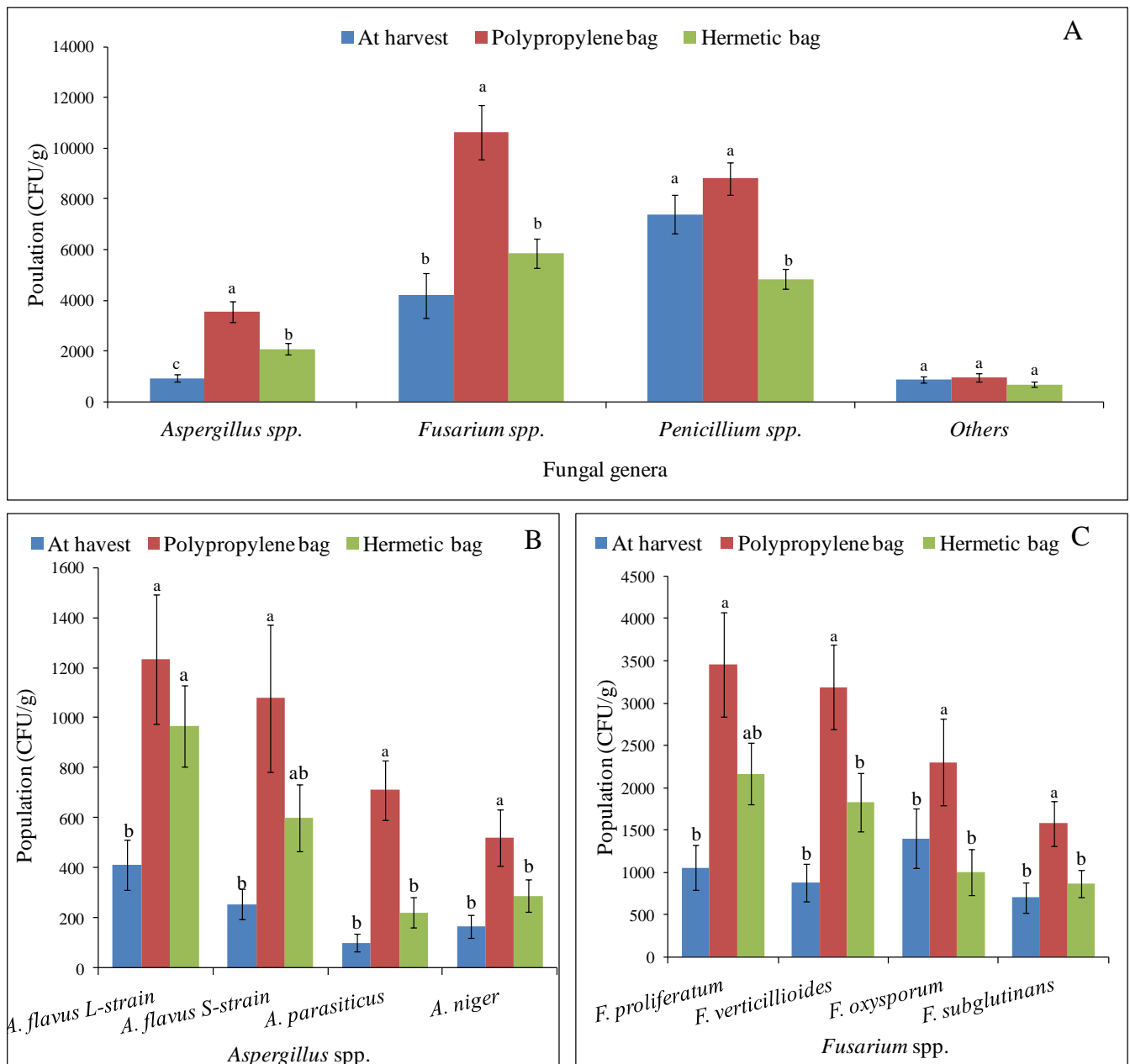


Figure 11: Population (CFU/g of maize) of fungal genera (A), *Aspergillus* spp. (B) and *Fusarium* spp. (B) isolated from maize sampled at harvest and three months after storage in polypropylene and hermetic bags.

Bar graphs accompanied by similar letters are not significantly different ($p \leq 0.05$) for each genus or species/morphotype; Error bars represent standard error of means.

Table 5: The population (CFU/g) of mycotoxin producing fungi in maize grains sampled at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District

Location	Bag type	AFL	AFS	AP	AN	FP	FV	FO	FS	PEN	Others
Mukuyuni	At harvest ^a	511.1b	333.3a	44.4b	88.9b	533.3b	400.0b	444.4b	311.1b	8288.9a	977.8a
	Hermetic bag	1133.3ab	822.2a	288.9b	222.2b	1933.3ab	1711.1ab	955.6ab	866.7ab	5555.5b	622.2a
	Polypropylene bag	1422.2a	1200.0a	800.0a	466.7a	3977.8a	2488.9a	1955.6a	1044.4a	9800.0a	1200.0a
	Mean	1022.2	785.2	377.7	251.9	2148.1	1533.3	1118.5	740.7	7881.5	933.3
	LSD (P ≤ 0.05)	871.1	996.2	300.7	319.9	2095.2	1426.8	1100.9	598.0	2640.4	610.1
	CV%	29.9	19.9	46.7	10.2	15.7	16.7	25.3	17.3	6.2	24.2
Kilala	At harvest ^a	311.1b	177.8b	155.6b	244.4b	1577.8a	1355.6b	2355.6a	1088.9b	6511.1a	755.6a
	Hermetic bag	800.0ab	377.8b	155.6b	355.6ab	2400.0a	1933.3b	1044.4a	866.7b	4133.3b	755.6a
	Polypropylene bag	1044.4a	955.6a	622.2a	600.0a	2333.3a	3888.9a	2644.4a	2111.1a	7822.2a	733.3a
	Mean	718.5	503.7	311.1	400.0	2303.7	2392.6	2014.8	1355.6	6155.6	748.1
	LSD (P ≤ 0.05)	568.8	386.6	326.6	312.3	1354.3	1539.9	1902.7	973.3	2039.4	450.2
	CV%	18.9	24.3	28.6	19.2	14.4	5.4	5.1	14.3	19.6	19.8

Means followed by the same letter(s) within columns in each Location are not significantly different (Fisher's protected LSD at $p \leq 0.05$). LSD - Least significant difference; CV - Coefficient of variation; ^a - Maize grains sampled at harvest.

AFL - *A. flavus* L-strain, AFS - *A. flavus* S-strain, AP - *A. parasiticus*, AN - *A. niger*, FP - *F. proliferatum*, FV - *F. verticillioides*, FO - *F. oxysporum*, FS - *F. subglutinans*, PEN - *Penicillium* spp.

4.4.2 Incidence of mycotoxin producing fungi in maize grains

The incidence of mycotoxin producing fungi varied significantly ($p \leq 0.05$) in maize grains sampled at harvest and three months after storage (Table 6). *Penicillium* spp. was the most predominant fungal genus at harvest (56.8%), while *Fusarium* spp. was the most prevalent in stored maize grains (41.3%) (Figure 12A). The incidence of *Aspergillus* spp. increased through the storage period from 7.5% at harvest to 19.3% after three months of storage.

The most common *Aspergillus* spp. isolated from maize grain samples were: *A. flavus* L-strain (Mean incidence = 23.7%), followed by *A. flavus* S-strain (15.6%), *A. niger* (13.3%) and *A. parasiticus* (12.2%) (Figure 12B). *Aspergillus flavus* L-strain was the most common species in both harvested and stored maize grains. There was no significant difference ($p \geq 0.05$) in the incidence of *A. flavus* S-strain and *A. niger* between the maize grains sampled at harvest and three months after storage. However, the incidence of *A. parasiticus* significantly ($p \leq 0.05$) increased from 5.3% at harvest to 15.7% after three months of storage. The type of storage bag had a significant influence ($p \leq 0.05$) on the incidence of *A. flavus* L-strain and *A. parasiticus* but did not significantly influence ($p \geq 0.05$) the incidence of *A. flavus* S-strain and *A. niger*. The incidence of *A. flavus* L-strain and *A. parasiticus* was significantly higher ($p \leq 0.05$) in maize stored in polypropylene bags than in hermetic bags.

The incidence of *Fusarium* spp. isolated in maize in decreasing order was: *Fusarium proliferatum* (25.4%), *F. verticillioides* (19.7%), *F. oxysporum* (14.8%) and *F. subglutinans* (14%) (Figure 12C). The incidence of *F. proliferatum*, *F. verticillioides* and *F. subglutinans* significantly varied ($p \leq 0.05$) in maize sampled at harvest and three months after storage. However, the incidence of *F. oxysporum* in maize was not variable between harvest and three

months after storage. The type of storage bag had no significant influence ($p \geq 0.05$) on the incidence of *F. proliferatum*, *F. verticillioides* and *F. oxysporum* but had a significant effect on the incidence of *F. subglutinans*. Overall, the incidence of *F. proliferatum* was 3.8% higher in hermetic bag than in polypropylene bag type.

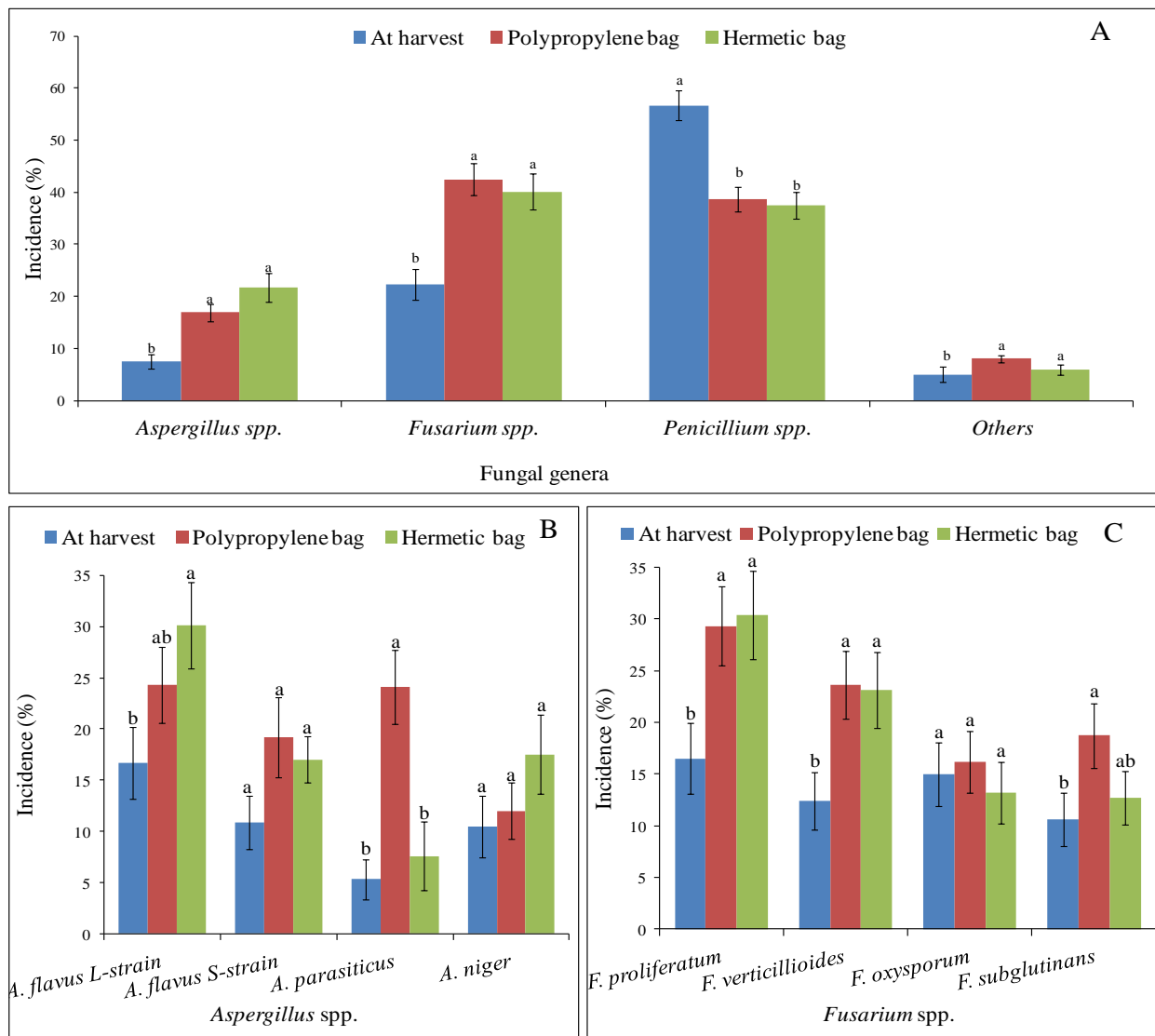


Figure 12: Incidence (%) of fungal genera (A), *Aspergillus* spp. (B) and *Fusarium* spp. (C) isolated from maize grains sampled at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District.

Bar graphs accompanied by similar letters are not significantly different ($p \leq 0.05$) for each genus or species/morphotype.; Error bars represent standard error of means.

Table 6: Incidence (%) of mycotoxin producing fungi in maize grains sampled at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District

Location	Bag type	AFL	AFS	AP	AN	FP	FV	FO	FS	PEN	Others
Mukuyuni	At harvest ^a	20.0a	15.1a	2.2b	7.1a	19.7a	7.6b	6.9a	5.7b	63.7a	4.5a
	Hermetic bag	34.2a	20.5a	10.3b	10.5a	20.8a	24.1a	17.8a	10.7ab	42.3b	6.0a
	Polypropylene bag	27.2a	13.5a	32.6a	7.8a	35.1a	14.4ab	17.7a	17.3a	41.7b	8.2a
	Mean	27.1	16.4	15.1	8.5	25.1	15.5	14.1	11.2	49.2	6.2
	LSD (P ≤ 0.05)	16.6	13.8	12.0	10.7	16.6	12.7	12.4	9.9	14.6	4.6
	CV (%)	3.3	28.8	10.0	12.8	8.6	9.0	2.8	22.2	12.6	18.7
Kilala	At harvest ^a	13.3a	6.7b	8.3a	13.9a	13.2b	17.2b	23.0a	15.5a	49.8a	4.1a
	Hermetic bag	26.1a	13.5ab	4.9b	24.2a	40.1a	22.2b	8.6b	14.8a	32.6b	5.2a
	Polypropylene bag	21.4a	24.6a	15.6a	16.2a	23.6b	32.7a	14.8ab	20.1a	35.8b	7.5a
	Mean	20.3	15.1	9.6	18.2	25.6	24.0	15.5	16.8	39.4	5.6
	LSD (P ≤ 0.05)	13.7	11.5	10.2	14.5	13.3	13.2	11.4	12.2	12.2	4.3
	CV (%)	15.9	23.6	17.7	32.0	21.1	5.5	18.8	4.7	19.5	42.5

Means followed by the same letter(s) within columns in each Location are not significantly different (Fisher's protected LSD at $p \leq 0.05$). LSD - Least significant difference; CV - Coefficient of variation, ^a - Maize grains sampled at harvest.

AFL - *A. flavus* L-strain, AFS - *A. flavus* S-strain, AP - *A. parasiticus*, AN - *A. niger*, FP - *F. proliferatum*, FV - *F. verticillioides*, FO - *F. oxysporum*, FS - *F. subglutinans*, PEN - *Penicillium* spp.

4.4.3 Nucleic acid based characterization of mycotoxin producing fungi

Approximately 500-bp DNA fragment of ITS region from all fungal species was PCR amplified using the ITS-1 and ITS-4 primers (Figure 13). The fungal DNA obtained from maize sampled at harvest generated amplicons with fragments of 500-bp amplification product (Figure 13A). Additionally, fungal DNA obtained from maize sampled at harvest generated 300-bp amplification product (Figure 13A). However, the amplification products were not observed in the negative control lacking template DNA (Figure 13A and Figure 13B). Fungal DNA obtained from maize stored in polypropylene bags was positive for 500-bp amplification product (Figure 13B). Similarly, fungal DNA obtained from all the maize samples that had been stored in hermetic bags generated 500-bp amplification product (Figure 13C).

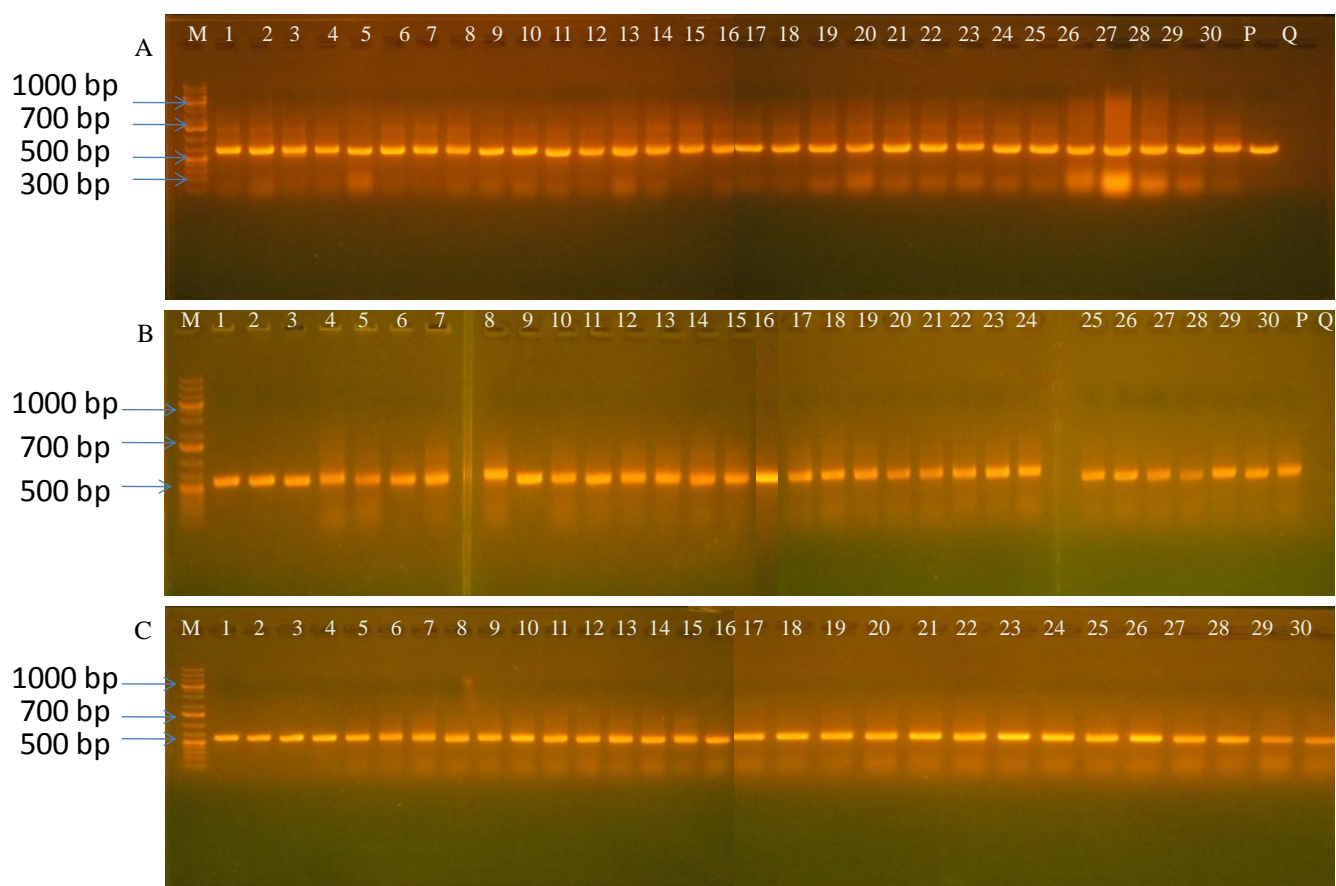


Figure 13: The PCR amplification profile of fungal DNA obtained from maize grains using specific primer sets: ITS-1F/ITS-4R.

Lanes A1 to 30 - Specific amplicons for fungal DNA from maize sampled at harvest; Lanes B1 to 30 - Specific amplicons for fungal DNA from maize stored for three months in polypropylene bags; Lanes C1 to 30 - Specific amplicons for fungal DNA from maize stored for three months in hermetic bags; Lanes M - 1000-bp DNA length ladder; lanes P - positive control; lanes Q - negative control, lacking template DNA

4.5 Efficacy of hermetic storage on aflatoxin and fumonisin levels in maize

Maize grains sampled at harvest and after three months storage in hermetic and polypropylene bags were contaminated with varying levels of aflatoxin (Table 7). The percentage of maize grains sampled at harvest that met different thresholds for total aflatoxin set by various regulatory bodies was as follows: ≤ 4 ppb set by the European Commission (36.7%), ≤ 10 ppb set by the Kenya Bureau of Standards (96.7%) and ≤ 20 ppb set by the US Food and Drug Administration (96.7%). There were variations in the levels of total aflatoxin in maize sampled three months after storage in hermetic and polypropylene bags. The aflatoxin levels in maize stored in hermetic and polypropylene bags ranged from < 5 ppb to 5.76 ppb and < 5 ppb to 42.7 ppb, respectively (Table 7). Maize grains stored in polypropylene bags were more contaminated with total aflatoxin (Mean = 4.7 ppb) than grains stored in hermetic bags (Mean = 2.1 ppb). Maize grains stored in polypropylene bags for three months were 33.4% more contaminated with total aflatoxin than samples stored in hermetic bags. Overall, 50.0% and 90.0% of the maize grains stored in polypropylene and hermetic bags, respectively met the EC standards for total aflatoxin (≤ 4 ppb). Likewise, 96.7% and 100% of the maize grains stored in polypropylene and hermetic bags respectively met the threshold set by KEBS (≤ 10 ppb) and FDA (≤ 20 ppb) (Table 7).

Table 7: Mean proportion (%) of aflatoxin contamination level categories for maize sampled at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District

Location	Bag type	≤ 4	> 4 -10	> 10 - 20	>20	Range (ppb)	Aflatoxin level (ppb) ^b
Mukuyuni	At harvest ^a	66.7	33.3	0.0	0.0	0-4.8	2.2
	PICS bag	100.0	0.0	0.0	0.0	0-3.7	1.5
	PP bag	60.0	40.0	0.0	0.0	0-4.9	2.9
Kilala	At harvest ^a	6.7	86.7	0.0	6.7	0-28.8	7.6
	PICS bag	80.0	20.0	0.0	0.0	0-5.8	7.7
	PP bag	40.0	53.3	0.0	6.7	0-42.7	6.6
	Mean	58.9	38.9	0.0	2.2	0-42.7	4.8

^a – Maize grains sampled at harvest; ^b – Mean aflatoxin concentration; ≤ 4 - EU limit for total aflatoxin, ≤ 10 KEBS limit for total aflatoxin; ≤ 20 FDA limit for total aflatoxin

There was variation in the levels of fumonisin in maize grains sampled at harvest and three months after storage in polypropylene and hermetic bags (Table 8). About 96.7% of the maize grains sampled at harvest met the regulatory threshold set by the European Commission (≤ 2 ppm) for total fumonisin while all the samples met the standard set by the set by the US Food and Drug Administration (≤ 4 ppm). There were variations in the levels of total fumonisin in maize sampled three months after storage in hermetic and polypropylene bags. The fumonisin levels in maize stored in hermetic and polypropylene bags ranged from < 2 ppm to 2.8 ppm and < 2 ppm to 6.0 ppm, respectively (Table 8). Maize grains stored in polypropylene bags were more contaminated with total fumonisin (Mean = 2.1 ppm) compared to grains stored in hermetic bags (Mean= 0.9 ppm) which was 40% more contaminated than samples stored in hermetic bags. Overall, 73.3% and 93.3% of the maize grains stored in polypropylene and hermetic bags respectively met the EC threshold for total

fumonisin (≤ 2 ppm). Likewise, 86.7% and 100% of the maize grains stored in polypropylene and hermetic bags, respectively met the FDA threshold for fumonisin (≤ 4 ppm) (Table 8).

Table 8: Mean proportion (%) of fumonisin contamination level categories for maize sampled at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District

Location	Bag type	≤ 2	$> 2 - 4$	> 4	Range (ppm)	Fumonisin level (ppm) ^b
Mukuyuni	At harvest ^a	93.3	6.7	0.0	0 - 2.3	0.5
	Polypropylene bag	80.0	13.3	6.7	0 - 4.5	1.2
	Hermetic bag	100.0	0.0	0.0	0 - 1.8	0.3
Kilala	At harvest ^a	100.0	0.0	0.0	0 - 1.7	0.5
	Polypropylene bag	66.7	13.3	20.0	0 - 6.0	2.1
	Hermetic bag	86.7	13.3	0.0	0 - 2.8	0.9
	Mean	87.8	7.8	4.5	0 - 6.0	0.9

^a – Maize grains sampled at harvest; ^b – Mean fumonisin concentration; ≤ 2 - EU limit for total fumonisin; ≤ 4 FDA limit for total fumonisin

CHAPTER FIVE: DISCUSSION

5.1 Maize production practices in Kaiti district

Data from the field survey showed that maize farmers in Kaiti District were mostly small scale farmers with over a decade of experience in maize cultivation. Most of the farmers obtained their seeds from the Agro-shop and Pioneer was the most popular maize variety amongst farmers in the region. However, some of the small scale farmers used their own saved seeds from previous seasons. Wambugu *et al.* (2009) observed that 78% farmers save their own maize seeds for the next planting season with a small proportion obtaining their seeds from neighbours, markets and the formal seed sector. Over 50% of the farmers in Kaiti District used simple methods of land preparation such as hoe and oxen ploughs in maize production. This observation concurs with findings in Nepal (Tiwari *et al.*, 2004) and Zimbabwe (Makuvaro *et al.*, 2014) who observed that majority of maize farmers used the ox-drawn plough in land preparation. Tillage practices have a direct impact on fungal growth and accumulation of their inocula. Reduced tillage does not incorporate crop residues into the soil which results in accumulation of fungal inoculum (Dill-Macky and Jones, 2000). Reduced tillage practices could be the reason for the high prevalence of mycotoxin producing fungi in the soil in Kaiti district. Therefore, intense tillage practices that incorporate crop residues (a primary source of inoculum for fungi) should be adopted by farmers.

Maize was mainly intercropped with common beans, pigeon peas and cowpeas in Kaiti District. Atukwase *et al.* (2009) observed that majority of maize farmers in Uganda intercropped maize with beans. Other studies by Cardwell and Cotty (2002) and Makuvaro *et al.* (2014) reported that maize was intercropped with various crops such as sorghum, groundnuts, cowpeas, cotton, melons, pumpkins, sugar beans and pearl millet. Smallholder farmers intercrop maize with common beans to increase soil nitrogen therefore improving

soil fertility (Dwivedi *et al.*, 2015). However, intercropping has been documented as a significant factor that influences fungal growth and mycotoxin contamination. Tédihou *et al.* (2012) reported that intercropping maize with cowpea resulted in reduced population of *A. flavus* and aflatoxin levels. Other reports have also shown that intercropping of maize and common beans resulted in higher inocula of *Fusarium* spp. and subsequent contamination with fumonisins (Atukwase *et al.*, 2009). Intercropping is intended to improve soil fertility since plant nutrient stress predisposes the crop to fungal attack. Therefore, further studies should be done to determine the type of crops to use as intercrops and the ways through which they reduce fungal growth and mycotoxin contamination (Mutinga *et al.*, 2014).

In the current study, over 50% of farmers did not practice crop rotation, with maize being planted continuously in the same fields in Kaiti district. Continuous production of maize in the same fields is the greatest agronomic risk for fungal attack and mycotoxin contamination. Mutegi *et al.* (2012) reported that the incidence of *A. flavus* and *A. parasiticus* was considerably higher in peanut sampled from farmers who did not practice crop rotation compared to those that practiced crop rotation in Western Kenya. In field studies in the USA, counts of *A. flavus* were higher in soils collected from fields where continuous maize production was practiced compared to soils where either cotton or wheat had been grown (Abbas *et al.*, 2004). Studies by Bernhoft *et al.* (2012) reported that lack of crop rotation resulted in a significant increase in the population of *Fusarium langsethiae*, *F. graminearum*, deoxynivalenol and HT-2 toxin. Most fungi survive in soil and on crop residue as saprophytes and therefore rotation of maize with non-host crops breaks the cycle of mycotoxigenic fungi linked with the previous crop (Atukwase *et al.*, 2009; Meissle *et al.*, 2009).

In this study, farmers used soil amendments such as organic manure and chemical fertilizer in maize production. However, farmers in Kaiti District who applied chemical fertilizers, used lower than the recommended application rates. Inadequate fertiliser application by farmers in Kaiti might have therefore contributed to build up of fungal inocula in the soil which resulted to high fungal population. Adequate fertilizer application has been suggested as a cultural practice that alleviates plant nutrient stress and reduces mycotoxin contamination in maize (Munkvold, 2003). Studies by Waliyar *et al.* (2008) reported that application of farm yard manure effectively reduced *A. flavus* contamination in peanuts. Hell *et al.* (2010) reported that application of low levels of nitrogen fertilizer promoted *A. flavus* infection in fertility stressed maize plants while Okoth and Siameto (2010) reported that the population of *Fusarium* spp. was evidently controlled in soils treated with nitrogen fertilizer and organic manure in Taita Taveta County, Kenya. Other studies by Blandino *et al.* (2008) in Italy reported that application of slow-release of nitrogen fertilizer led to considerable increase in population of *Fusarium* spp. in maize. In this regard, a balanced N fertilizer application minimizes plant stress during seed development thereby preventing fungal attack and subsequent mycotoxin contamination.

Most farmers in Africa do not harvest maize based on physiological maturity (7 to 8 weeks after maize flowering) period, but employ traditional practices to determine readiness of maize for harvesting by pricking of kernels and drooping of cobs (Akowuah *et al.*, 2015). The aforementioned practices were employed by maize farmers in Kaiti district to determine the harvesting time. These practices are not reliable and accurate; thus, harvested grains may still contain high moisture content, therefore predisposing maize to fungal attack and aflatoxin contamination (Hell *et al.*, 2008). The most suitable time for maize harvesting is at physiological maturity (Kaaya *et al.*, 2006; Hell and Mutegi, 2011). Kaaya *et al.* (2006) reported that levels of aflatoxin in maize increased by four folds at the third week after the

recommended harvesting time and by seven fold when harvesting was delayed for four weeks. Delayed harvesting has also been positively correlated with *Fusarium* growth and fumonisin contamination (Atukwase *et al.*, 2009). Thus, harvesting crops at physiological maturity and adequate drying of the produce play a vital role in reducing fungal contamination of maize grains (Bankole and Adebajo, 2003; Atanda *et al.*, 2013; Adegoke and Letuma, 2013). Despite the fact that majority of smallholder farmers are well-informed of the importance of harvesting at appropriate time, inadequate storage space, unpredictable weather, theft of the produce and rodent damage force farmers to harvest at unsuitable time (Bankole and Adebajo, 2003).

Most of the farmers used polypropylene bags to store maize, while a few used sisal bags. Maize was either stored inside the living house or the granary. A study by Gitonga *et al.* (2015) reported that 60% of smallholder farmers stored maize inside the house while 17% used modern granaries. Other studies in Zambia (Kankolongo *et al.*, 2009) and Tanzania (Shabani *et al.*, 2015) reported that smallholder farmers stored their maize in polypropylene bags inside the family living house. Fandohan *et al.* (2006) reported that in most Sub-Saharan African countries, maize is usually stored in cob form either under the roofs of farmers' houses, or on the floor of their houses and in wooden granaries. In this study, storage of maize in polypropylene bags and family house might have contributed to high population of fungal species. It has been reported that different storage structures vary in their ability to protect grains from fungal contamination (Fandohan *et al.*, 2006). Hell *et al.* (2000) reported that some storage structures provided conditions that were more favourable for fungal growth and aflatoxin contamination than others in West Africa found. Kaaya *et al.* (2006) also reported that storage of maize in improved granaries in Uganda was associated with reduced aflatoxin contamination. However, use of improved granaries by smallholder farmers to store maize is uncommon due to theft of the produce (Aflacontrol, 2010).

The most common storage challenges reported by maize farmers were insect pest infestation and rodent damage. Studies carried out by Hell *et al.* (2008) in Benin and Shabani *et al.* (2015) in Tanzania, reported that maize farmers complained about insect pests infestation and rodents damage at various stages of storage. Hell *et al.* (2000) observed that farmers complained about insect pest infestation during two to five months of storage. Storage insect pests such as *Sitophilus zeamais* (Coleoptera: curculinidae), play a key role in the contamination of foods with mycotoxin producing fungi (Lamboni and Hell, 2009). Insect pest infestation is influenced by improper harvest and storage conditions which also predispose maize to mycotoxin contamination (Atanda *et al.*, 2013).

Application of chemical pesticides to control storage insect pests was extensively practiced by maize farmers involved in this study. This finding was consistent with previous reports that farmers apply synthetic chemical insecticides to maize grains before storage to control storage insect pests (Hell *et al.*, 2000; Kaaya *et al.*, 2006; Shabani *et al.*, 2015). Application of chemical pesticides is a significant factor in *A. flavus* and *A. parasiticus* management (Garcia and Heredia, 2006). A previous study by Plasencia (2004) reported that control of insect pest populations with chemical insecticides in stored maize grains significantly reduced *A. flavus* and *A. parasiticus* contamination. Likewise, De la Campa *et al.* (2005) observed that application of chemical insecticides to protect cereals against insect pest infestation was effective in reducing fumonisin contamination. However, insecticides are considered too expensive for subsistence farmers and therefore, farmers in Kaiti District did not use the recommended application rates. In addition, application of insecticides is labour intensive since farmers have to apply them after every three months.

5.1.1 Effects of maize production practices on population of mycotoxin producing fungi in soil

The fungi isolated from soil samples were: *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. implying that soil in Kaiti District was contaminated with mycotoxin producing fungi. The fungal propagules in the soil and crop residues which act as the major source of primary inoculum could have contributed to high rate of fungal proliferation. The high population of these fungal species implies that maize grown in these fields is under risk of contamination with associated mycotoxins. A study by Muthomi *et al.* (2009) identified *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. in soil sampled from Eastern Kenya. Other studies also identified *Aspergillus*, *Fusarium* *Penicillium* and *Trichoderma* species in soil from Argentina (Nesci *et al.*, 2006) and Mysore district, India (Sharma and Raju, 2013). In the current study, *Penicillium* spp. was the most commonly isolated fungal pathogen in soil similar to findings by Muthomi *et al.* (2009) in Eastern Kenya. *Penicillium* spp. are mycotoxin producers, thus their high incidence in the maize fields pose a risk of contaminating the grains with mycotoxins such as ochratoxins, citrinin and patulin (Pitt and Hocking, 2009).

Aspergillus spp. isolated from soil were: *Aspergillus flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger*. In a similar study, Karanja (2013) isolated different members of *Aspergillus* section *Flavi* from soil sampled from Eastern Kenya. *Aspergillus* spp. in soil especially members of *Aspergillus* section *Flavi* act as a primary inocula for contamination of maize with aflatoxin when conditions are favourable. Horn (2003) reported that soil act as a reservoir for *A. flavus* and *A. parasiticus* that produce different aflatoxin types in agricultural produce. Previous studies have reported that *Aspergillus* spp. form sclerotia that allows the fungus to survive as saprophytes for longer periods in the soil and maize residues (Accinelli *et al.*, 2008; Wagacha and Muthomi, 2008). Moreover, the sclerotia in soil produce

conidiophores and conidia in subsequent season to infect the crop via silk (Scheidegger and Payne, 2003). Thus, removal of infected crop residues (which serve as *Aspergillus* inoculum sources) from the previous harvest may reduce contamination of subsequent crops (Strosnider *et al.*, 2006).

The major *Fusarium* spp. isolated from soil were: *F. proliferatum*, *F. oxysporum*, *F. subglutinans* and *F. solani*. Similar spectrum of *Fusarium* spp. was identified by Maina *et al.* (2009) in soil sampled from Taita Taveta County, Kenya. Similarly, a study by Latiffah *et al.* (2010) identified *F. solani*, *F. oxysporum*, *F. semitectum* and *F. proliferatum* from soil in Malaysia. In the current study, *F. proliferatum* and *F. oxysporum* were the predominant species in soil. *Fusarium* spp. are economically important plant-pathogenic fungi of maize with some species producing mycotoxins which are dangerous to human and animal health and reduce both crop yield and quality (Maina *et al.*, 2009). These mycotoxins and the corresponding *Fusarium* spp. producing them include: fumonisins (*F. verticillioides* and *F. proliferatum*), fusaproliferin (*F. subglutinans* and *F. proliferatum*), moniliformin and beauvericin (*F. oxysporum* and *F. subglutinans*) (Logrieco *et al.*, 2002).

5.2 Efficacy of hermetic storage bags on fungal population in maize grains

In this study, potentially mycotoxigenic fungi in the genera *Aspergillus*, *Fusarium* and *Penicillium* were isolated from maize grains sampled at harvest. A study by Wagara *et al.* (2008) reported *Fusarium*, *Aspergillus* and *Penicillium* genera from maize grains sampled from Western Kenya. The high population of mycotoxin producing fungi in maize sampled at harvest could be attributed to high temperatures with drier conditions in Makeni County which predisposes maize to the moulds at pre-harvest stage in the field (Okoth *et al.*, 2012). Contamination of maize with mycotoxins depends on the availability of susceptible hybrids

and environmental conditions that encourage proliferation of mycotoxigenic fungi (Blandino *et al.*, 2009).

Aspergillus spp., *Fusarium* spp., *Penicillium* spp. and other fungal species were also isolated from maize grains stored in polypropylene and hermetic bags for three months. The population of mycotoxin producing fungi increased during storage in polypropylene bags by 83.5% compared to only 2% increase in hermetic bags. The high contamination of maize by the aforementioned mycotoxin producing fungi implies high exposure of the grains to mycotoxins associated with the pathogens and consequently to farmers and consumers of the maize and maize products. A study by Viebrantz *et al.* (2016) identified *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. while evaluating mortality of insects and quality of maize grains in hermetic and non-hermetic storage for 50 days. Castellari *et al.* (2010) identified *A. flavus*, *A. parasiticus* and *F. verticillioides* from maize stored in silo-bags with moisture content ranging from 14% to > 20%. Other studies have reported isolation of *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. from stored maize grains (Kankolongo *et al.*, 2009; Lamboni and Hell. 2009; Krnjaja *et al.*, 2013).

In this study, the amplified fungal DNA obtained from maize grains yielded positive products of 300- bp and 500-bp with the universal ITS1 and ITS4 fungal primers. The amplification of ITS region implied the presence of mycotoxin producing fungi in maize grains. A report by Romanelli *et al.* (2010) indicated that the ITS region can be amplified from a broad spectrum of fungi with ITS-1 and ITS-4 primers and can generally be recovered in a single PCR, since the amplicon is usually ~300 to 700 bp in length. A study by Suanthie *et al.* (2009) which also used PCR primers ITSPF and ITSPR, reported that pathogenic fungi of genera *Aspergillus*, *Fusarium*, *Penicillium* and other fungal species gave an amplification product of about 300-bp. Other studies by Gautam and Bhadauria (2011) observed variation in the

number and sizes of these ITS regions (ITS1 and ITS4) with different band patterns. Since morphological and colony appearance alone are insufficient to accurately identify isolated species, DNA isolation and PCR amplification of ITS region was used to support morphological identification of these species (Majid *et al.*, 2015). However, the results of this work did not identify specific mycotoxin producing fungi. Therefore sequencing should be done to further confirm the identity of the isolates that were morphologically identified.

In this study *Penicillium* spp. was the most commonly isolated fungal pathogen from maize followed by *Fusarium* spp. and *Aspergillus* spp. The high incidence of *Penicillium* spp. could be attributed to its high occurrence in the soils in the study area. Predominance of *Penicillium* spp. in maize grains was however, contrary to the findings by Bii *et al.* (2012) who reported that the most frequently isolated fungal genera from maize were: *Aspergillus* (35.8%), followed by *Fusarium* (15.5%) then *Penicillium* (9.2%) and *Rhizopus* (5.3 %) while the incidence of other fungal species was 34.4%. Muthomi *et al.* (2009) also identified *Fusarium* spp. as the most commonly isolated fungal species in maize from Eastern Kenya. This high diversity of mycotoxigenic fungi in maize grains poses a health risk of exposing consumers of maize and maize products to mycotoxins (Wagacha *et al.*, 2013).

The population of mycotoxin producing fungi in maize grains increased by 78% and 0.5% in polypropylene and hermetic bags, respectively after three months storage. The increase in fungal population during storage in polypropylene bags could be attributed to the availability of conducive environment favourable for fungal growth. Previous studies have reported that the population and incidence of mycotoxin producing fungi in maize grains increased with increase in storage period (Sobowale *et al.*, 2013). Tanaka *et al.* (2001) reported the incidence of *Aspergillus* spp. and *Penicillium* spp. increased as the storage period increased. Overall, contamination of maize by mycotoxin producing fungi in Kaiti District was 39.7% higher in

stored maize grains than in the samples collected at harvest. These findings are in agreement with the report by Tsedaley and Adugna (2016) that the populations of *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. were higher in stored maize samples in Ethiopia compared to maize samples collected at harvest. The composition of fungal species established in the field and effects of rain prior to harvest or during storage significantly influence the development of mycotoxigenic fungi during storage (Hirooka *et al.*, 2007).

Aspergillus flavus S-strain, *A. flavus* L-strain, *A. parasiticus* and *A. niger* were isolated from maize grains sampled at harvest. This could be attributed to high population of *Aspergillus* spp. resident in the soil in the study area. A study by Murithi (2014) isolated *A. flavus* L-strain, *A. flavus* S-strain and *A. parasiticus* in maize from Eastern Kenya. Other studies on maize microflora from Western Kenya by Nyukuri (2007) reported that *A. parasiticus*, *A. flavus*, *A. niger* as well as other *Aspergillus* spp. were isolated from harvested maize grain samples. Similar spectrum of *Aspergillus* spp. reported at harvest was also observed in maize grains sampled three months after storage in polypropylene and hermetic bags. This could be explained by the occurrence of correspondingly high population of *Aspergillus* spp. resident in maize sampled at harvest which influences the population in storage. In a similar study, Okoth *et al.* (2012) reported that *A. flavus* and *A. parasiticus* were recovered from stored maize kernels from Nandi and Makueni regions of Kenya. In the current study, *A. flavus* was the most prevalent *Aspergillus* spp. in maize grains sampled at harvest and in storage while *A. niger* was the least predominant species. A study by Muthomi *et al.* (2012) reported that *A. flavus* was the most predominant in maize harvested from Eastern and Nandi regions of Kenya. Gachara (2015) reported that *A. flavus* (82%) was the most predominant followed by *A. niger* at 49% in stored maize from Eastern and Rift valley regions of Kenya.

In this study, the population of *Aspergillus* spp. in maize increased from harvest to sampling after three months of storage. This agrees with reports by Wagacha *et al.* (2013) that the population of *A. flavus* and *A. parasiticus* in peanuts progressively increased during storage and was significantly higher at the third month of storage. Domenico *et al.* (2016) reported that there was an increase in the population of *Aspergillus* spp. in maize after three months of storage with a progressive increase until nine months. A previous study by Hell *et al.* (2003) also observed higher frequencies of *A. flavus* in stored maize in Benin compared to maize obtained at harvest.

The most common *Fusarium* spp. isolated from maize sampled at harvest in this study in decreasing incidence were: *F. proliferatum*, *F. verticillioides*, *F. oxysporum* and *F. subglutinans*. *Fusarium* spp. are mainly considered field fungi thus the high occurrence at harvest was probably because maize was contaminated before harvest. Similarly, *F. proliferatum*, *F. verticillioides*, *F. oxysporum* and *F. subglutinans* were isolated from stored maize grains. The high population of *Fusarium* spp. in maize sampled at harvest might have influenced the population in storage. Similar to findings of this study, Murithi (2014) and Bii *et al.* (2012) isolated *F. verticillioides*, *F. proliferatum* and *F. oxysporum* from maize grains sampled at harvest and in storage in Eastern Kenya. In the study by Bii *et al.* (2012), *F. verticillioides* (40%) was the most prevalent followed by *F. proliferatum* (15%). In the current study, *F. proliferatum* was the most predominant *Fusarium* spp. in maize grains. *Fusarium proliferatum* and *F. verticillioides* mainly produce fumonisins in maize grains (Millicivec *et al.*, 2010) and therefore the predominance of these *Fusarium* species in Kaiti District might therefore have influenced fumonisin contamination of maize.

The population of *Fusarium* spp. increased from harvest to three months after storage in polypropylene and hermetic bags. Similar to findings of this study, Viebrantz *et al.* (2016)

reported an increase in the incidence of *Fusarium* spp. after 30 days of storage. Other studies have reported that incidence of *Fusarium* spp. increased with increase in storage period from two weeks to two months (Atukwase *et al.*, 2012; Sobowale *et al.*, 2013). In contrast, Santin *et al.* (2009) reported that the incidence of *Fusarium* spp. in maize kernels stored in steel mesh silos, decreased during storage because of low oxygen levels.

The population of mycotoxin producing fungi in maize grains stored in polypropylene bags was 78% higher compared to maize stored in triple-layer hermetic bags. Factors conducive for fungal growth such as high moisture content, high relative humidity and aeration of the grains in polypropylene bags might have contributed to high fungal population. On the other hand, low oxygen concentration, high carbon dioxide levels and low relative humidity could have contributed to low fungal population in maize stored in hermetic bags (Moreno-Martinez *et al.*, 2000). Gregori *et al.* (2013) observed that maize stored in silo bags had lower number of fungal counts and therefore suitable for safe cereal storage as compared to conventional bags. However, most smallholder farmers still rely on polypropylene bags for storage of maize grain while these bag types are neither airtight nor insect resistant and therefore readily absorb moisture and heat from the ambient environment compared to hermetic bags (Hell *et al.*, 2000; Shabani *et al.*, 2015). The maize grains are therefore still susceptible to fungal attack and mycotoxin contamination (Nyukuri, 2007).

The population of *Aspergillus* spp. was 71% higher in maize stored in polypropylene bags than in hermetic bags at the third month of storage. The storage conditions in polypropylene bags favoured proliferation of *Aspergillus* spp. Previous studies have reported that the fungal counts in peanuts stored in aerated bags were higher than in hermetically sealed bags after 90 days of storage (Villers, 2014). Domenico *et al.* (2016) reported that the population of *Aspergillus* spp. in maize stored in conventional bags increased steadily and peaked at the third month while in hermetic bags the population increased gradually and peaked at the sixth

month of storage. The present study also showed that the population of *Fusarium* spp. was 74.6% higher in polypropylene bags compared to hermetic bags. In a related study, Domenico *et al.* (2016) observed that *Fusarium* counts in samples stored in conventional bags increased up to the third month, and then gradually reduced in number while in the hermetic bags, counts fluctuated, and decreased after the twelve months of storage. Viebrantz *et al.* (2016) reported that in non-hermetic system, a higher incidence of *Fusarium* spp. ranging from 0 to 16% was observed, while in the hermetic system, it varied from 0 to 4.1% during the storage period.

Maize grains stored in hermetic bags were generally less contaminated with mycotoxin producing fungi than from the polypropylene bags due to diminished oxygen and high concentration of CO₂ within the bags (IFPRI, 2010). Viebrantz *et al.* (2016) reported that although initial growth was observed, for hermetic and non-hermetic systems, the growth as well as the final incidence was lower in the hermetic system, indicating that low oxygen rates reduced the growth of microorganisms. Studies by Bartosik *et al.* (2008) reported that elevated levels of CO₂ (about 30%) in presence of 21% O₂ concentrations within hermetic bags resulted in a decrease in fungal population. Edo Ognakosan *et al.* (2013) stated that insect, fungi and grain respiration is crucial in reducing the O₂ levels within the hermetic bags to below 2% therefore inhibiting their growth. Thus hermetic bag protects maize grains better than conventional storage bags (Baoua *et al.*, 2014) and therefore can be used for effective grain storage.

5.3 Efficacy of hermetic storage bags on aflatoxin and fumonisin levels in maize grains

The current study investigated the levels of total aflatoxin and fumonisin in maize grains sampled from Kaiti District at harvest and three months after storage. Sixty three percent of maize grains collected at harvest had total aflatoxin levels above the acceptable limits set by

the European Commission (≤ 4 ppb) while only 3.3% exceeded the limits set by Kenya Bureau of Standards (≤ 10 ppb) and the US Food and Drug Administration (≤ 20 ppb). In a similar study in Kenya, Mwihia *et al.* (2008) reported that 35.5% of maize sampled from Makueni County at harvest had aflatoxin levels above FDA maximum limit of 20 ppb. The high levels of total aflatoxin contamination in maize sampled at harvest could be attributed to *A. flavus* attack of maize prior to or during harvest (Bankole and Mabekoje, 2003).

Maize stored in polypropylene bags was 33.4% more contaminated with aflatoxin compared to samples stored in hermetic bags. The high aflatoxin levels in polypropylene bags could be attributed to retention of high moisture and heat (Nyukuri, 2007; Wagacha *et al.*, 2013) which favour fungal growth and aflatoxin contamination. A study by Domenico *et al.* (2016) reported the mean levels of total aflatoxin of 85 and 85.4 $\mu\text{g}/\text{kg}$ in maize stored in hermetic and conventional bags, respectively. Overall, 90% and 100% of maize samples stored in hermetic bags in this study met the Kenyan regulatory threshold of ≤ 10 ppb and FDA standard of ≤ 20 ppb for total aflatoxin. Hermetic bags effectively reduced aflatoxin levels by 55.3% after three months of storage which could be attributed to low O_2 content $< 3\%$ and elevated CO_2 levels in hermetic bags which hinder the growth of fungal and production of aflatoxin (Moreno-Martinez *et al.*, 2000). Hockings (2003) reported that carbon dioxide enrichment hinders aflatoxin formation in the substrate. Studies by Bartosik *et al.* (2008) reported that the ability of *A. flavus* to produce aflatoxin in groundnuts was significantly reduced with the raise in CO_2 and decline in O_2 concentrations. This implies that the storage of maize in hermetic bags provided conditions that were unfavourable for fungal growth and aflatoxin contamination.

The levels of fumonisin in all the maize samples obtained at harvest were less than 4 ppm. In a similar study, Bii *et al.* (2012) reported that the mean fumonisin content in maize samples

from Makueni and Kitui Districts was 1.2 µg/g and 0.9 µg/g, respectively. There was substantial increase in the levels of fumonisins in maize after three months of storage. Maize grain samples stored in polypropylene bags were 40% more contaminated than samples stored in hermetic bags. About 93% and 100% of the maize stored for three months in hermetic bags in this study met the European Commission and the US Food and Drug Administration threshold for total fumonisins of ≤ 2 ppm and ≤ 4 ppm, respectively. From this study, hermetic bags effectively reduced fumonisin levels by 57.1 % as compared to polypropylene bags. This was due to creation of a modified atmosphere within the hermetic bags as a result of oxidative metabolism by fungi, insect pest and the stored grain leading to diminished O₂ and high CO₂ levels (Quezada *et al.*, 2006). Samapundo *et al.* (2007) reported that the ability *F. verticillioides* and *F. proliferatum* to produce fumonisin in maize stored in sealed bags was inhibited by high CO₂ concentration of 30%.

Maize grains stored in hermetic bags were generally less contaminated with aflatoxin and fumonisin than in polypropylene bags. This could be ascribed to changes in internal gas composition (decrease in oxygen and increase in CO₂) surrounding the grain in hermetic bags that suppress the capacity of fungi to reproduce and/or develop (Villers *et al.*, 2010) and produce mycotoxins. The triple layer hermetic bags also maintain the initial grain moisture content and protect it from changes associated with seasonal variation in humidity (Baoua *et al.*, 2014). Hermetic bags also protect the maize grains from insect pests that may introduced mould spores from the outside (Baoua *et al.*, 2014) leading to high toxin levels.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Maize production practices by farmers in Kaiti District increased the risk of fungal attack and mycotoxin contamination of maize. Most maize farmers did not practice crop rotation, used simple land preparation methods that did not bury previous crop residues and used minimal amount of soil amendments. These agronomic practices contribute to accumulation of fungal propagules in the soil which act as a primary source of inoculum of fungi that infect the crops when conditions are favourable and in turn result in mycotoxin contamination. Lack of timely harvesting and improper storage practices also predisposed the maize to fungal attack and mycotoxin contamination.

Aspergillus spp. *Fusarium* spp. and *Penicillium* spp. were the major mycotoxin producing fungi isolated from soil and maize grains. The predominance of fungal species in the soil indicated that the soil was contaminated with mycotoxin producing fungi which acts as a reservoir of fungal propagules. The population of mycotoxin producing fungi increased by 83.5% and 2% in polypropylene and hermetic bags, respectively during the three months storage period. Therefore, storage of maize in hermetic bags effectively reduced the population of mycotoxin producing fungi. Hermetic bags preserved the grains from changes related to variation in moisture content and humidity which predispose the grain to fungal attack.

Although maize grains were contaminated with varying levels of aflatoxin, 96.7% of maize grains sampled at harvest had total aflatoxin levels within the limits set by Kenya Bureau of Standards (KEBS) and the Food and Drug Administration (FDA). Storage of maize for three months in hermetic bags reduced aflatoxin levels by 55% as compared to storage of the grains in polypropylene bags. The results showed that 90% of the maize samples stored in

hermetic bags had aflatoxin levels that were within the acceptable limits set by the EC, while 100% met the threshold set by KEBS and FDA. On the other hand, 96.7% of maize sampled at harvest met the limit set by EC for fumonisin while 100% met the FDA threshold. Storage of maize grains in hermetic bags for three months reduced fumonisin levels by 57% compared to polypropylene bags. About 93.3% of the maize samples stored in hermetic bags had fumonisin levels that were within the limits set by EC while all the samples met limits set by FDA. Storage of maize in hermetic bags effectively reduced aflatoxin and fumonisin contamination of maize grains. It is therefore evident that hermetic storage provides good protection against fungal growth and mycotoxin contamination of maize grains. Therefore, adoption of hermetic storage technology by smallholder farmers will provide an effective option for managing fungal and mycotoxin contamination of maize grains as well as maintaining grains of high quality.

6.2 Recommendations

- i. Maize farmers should be trained on good agronomic practices particularly on intense tillage, crop rotation, application of soil amendments, proper disposal of plant residues and timely harvesting.
- ii. Additionally, farmers should be trained on proper drying of maize before storage. Drying on bare ground should be avoided since contact with the soil leads to fungal contamination. Maize should also be dried to $\leq 13\%$ moisture content before storage.
- iii. Storage of maize in appropriate storage structures that are well ventilated should be encouraged. Use of modern granary reduces the chances of maize contamination with mycotoxin producing fungi.
- iv. There is need for raising awareness among maize farmers on effects of mycotoxins on human and livestock health, and on trade. Moreover, there is need for continuous

monitoring and surveillance programmes on levels of mycotoxins to ensure compliance with the standards set by the Kenya Bureau of Standards.

- v. Public demonstrations on use of hermetic bags should be conducted to raise awareness among maize farmers on proper use of the hermetic bags for storage of high quality maize grains.
- vi. The government should also provide subsidies to farmers to ensure that the hermetic bags are cost-effective. Moreover, private business enterprises should create a functional supply chain by increasing the retail network to improve availability of the bags to farmers.
- vii. Further research should be conducted to evaluate the effect of hermetic bags on fungal population and toxin levels in maize grains stored for longer periods beyond the three months investigated in the current study. Investigations on the effect of hermetic bags on storage insect pests should be done. Studies are also recommended to establish the role of weather conditions on quality of grains stored in hermetic bags.

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APPENDICES

Appendix A: Questionnaire on maize production practices in Kaiti District

Section I: Basic information

Survey code _____ Date of Interview _____

Enumerator _____ Sub-County _____

Division _____ Village _____

Section II: Respondent's personal detail

Name of the farmer _____ Age _____

Gender: Male Female Highest level of education _____

Marital status _____ Household size _____

Section III: Information on farming practices

1. How many Acres of land do you own? _____
2. How many Acres of land are under maize production? _____
3. How many years have you practiced maize production? _____
4. Why do you practice maize production? a) Subsistence b) Commercial
c) Others (specify) _____
5. Which varieties of maize do you grow? _____
6. Where do you get the maize seeds from? a) Own b) Neighbour c) Local
Market d) Agro-shop e) other (specify) _____
7. Which land preparation method do you employ? a) Hoe b) Oxen plough
c) Tractor
8. Do you use any soil amendments in maize production? a) Yes b) No
9. If yes, which type and at what growth stage? a) _____
b) _____ c) _____

10. What other crops do you grow in the farm? a) _____ b) _____ c) _____ d) _____
 e) _____ f) _____ g) _____
11. Is the current maize crop intercropped with any other crop(s)? a) Yes b) No
12. If yes, which crop(s)? a) _____ b) _____ c) _____
 d) _____ e) _____

Section IV: Harvesting of maize

13. How do you know your maize is ready for harvesting? _____
14. On average, how much maize (in 90kg bags) do you harvest per Acre? _____
15. When do you harvest your maize? a) During rainy weather b) During wet weather
 c) During dry weather d) Others (specify) _____
16. Which harvesting method do you employ? a) Hand b) Machine

Section V: Drying of maize

17. When is the maize dried? A) Immediately after harvest b) Sometime after harvest
 (how many days?) _____
18. Where is maize dried? a) On the field b) c) Maize is brought back to the home
19. Is the maize dried on cob prior to shelling? a) Yes b) No
20. How do you dry your maize after harvesting? a) Drying in the sun b) Hanging
 outside c) Drying in the house d) stacking in the field d) Others (specify) _____
21. How many days does it take to completely dry your maize? _____
22. How do you shell your maize? a) Manually b) Use of machine
23. How do you determine your maize is dry enough (moisture content) for storage? a)
 Kernel hardness (biting kernels) b) Sound of kernel when shaking in hand
 c) Moisture meter d) Visual observation e) Others (specify) _____

24. What challenges do you face when drying maize? a) Rain during drying b) Lack of drying space c) Destruction by livestock while drying d) Theft of grain e) Contamination from dust f) Others (specify) _____

Section VI: Maize storage practices and challenges

25. In what form do you store your maize? a) Maize cobs b) Maize grains

27. Do you apply/sprinkle any chemical on your maize before you store it? a) Yes b) No

28. Which storage material do you use to store your maize? a) Sisal sacks b) Polypropylene bags b) Plastic container d) Paper bags e) Bucket f) Clay pot e) others (specify) _____

29. What storage structure do you use to store your maize? a) Traditional granary b) Modern store c) Hanging in the house d) In the house on canvas d) In the house on the floor e) others (specify) _____

30. On average, how long do you store your maize before consumption? _____

31. On average, how long do you store your maize before selling? _____

32. Which challenges do you encounter during maize storage? a) Lack of enough storage space b) Lack of storage bags c) Insect damage d) Mold damage e) Rodent damage f) others (specify) _____

33. Have you observed spoilage of your maize during storage? a) Yes b) No

34. Have you observed insect infestation during storage? a) Yes b) No

35. What do you do when your grain is infested by insects/rodents in storage? a) Throw away b) Feed to livestock c) Sell d) Consumption by Household

36. What do you do when your grain goes moldy in storage? a) Throw away b) Feed to livestock c) Sell d) Consumption by Household e) Others (specify) _____

37. Have you received any training on how to properly dry and store grain? a) Yes
b) No

Section VII: mycotoxin contamination

38. Have you heard of mycotoxin contamination of maize? a) Yes b) No
39. Do you consider this to be a major problem? a) Yes b) No
40. What is the local name of aflatoxin? _____
41. When is the problem of aflatoxin contamination seen? _____
42. How do you identify the problem? _____

Section VIII: Management practices

43. What methods do you use to reduce mycotoxin contamination of your maize in the field and storage? a) Chemical b) Crop rotation c) Resistant varieties
d) Bio-control e) Intercropping f) Cultural e) Others (Specify) _____
44. Where do you get information on farming practices? a) Self b) Neighbours
c) Agricultural extension workers d) Media e) NGO f) Reading
g) Middlemen Others (Specify) _____
45. Any further comments?

Thank you for your responses