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Postharvest Practices of Maize Farmers in Kaiti District, Kenya and the Impact of Hermetic Storage on Populations of *Aspergillus* Spp. and Aflatoxin Contamination

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Abstract

Aflatoxin contamination in maize by *Aspergillus* spp. is a major problem causing food, income and health concerns. A study was carried out in Kaiti District in Lower Eastern Kenya to evaluate the effect of three months storage of maize in triple-layer hermetic (PICS™) bags on the population of *Aspergillus* spp. and levels of aflatoxin. Postharvest practices by maize farmers including time of harvesting, drying and storage methods were obtained with a questionnaire. *Aspergillus* spp. in soil and maize were isolated by serial dilution-plating and aflatoxin content was measured using Vicam method. Maize was mostly stored in woven polypropylene (PP) and sisal bags within granaries and living houses. *Aspergillus flavus* L-strain was the most predominant isolate from soil (Mean = 8.4 x10^2 CFU/g), on the harvested grain (4.1 x 10^2 CFU/g) and grain sampled after three months of storage (1.1 x 10^3 CFU/g). The type of storage bag significantly ($P \leq 0.05$) influenced the population of members of *Aspergillus* section *Flavi*, with *A. flavus* (S and L strains) and *A. parasiticus* being 71% higher in PP bags than in PICS bags. 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. After storage, the population of *Aspergillus* section *Flavi* was positively correlated with aflatoxin levels. The results of this study demonstrate that PICS bags are an effective management option for reducing population of toxigenic *Aspergillus* spp. and aflatoxin in stored maize.

Key words: aflatoxin, *Aspergillus* spp., hermetic bag, maize, polypropylene bag

1. Introduction

Maize (*Zea mays* L.) is a staple food crop in Kenya accounting for about 40% of daily calories with per capita consumption of 98 kilograms per annum (Muiru, Charles, Kimenju, Njoroge, & Miano, 2015). With about 90% of the rural households in Kenya depending on maize, this grain dominates all national food security considerations (Ouma & De-Groote, 2011). An important challenge for maize production in Kenya is contamination with aflatoxin, which poses negative health effect to humans and animals and causes huge economic losses (Okoth et al., 2012). Aflatoxins are toxic secondary metabolites produced by several *Aspergillus* spp. Aflatoxins have been associated with stunted growth in children, immune-system suppression, cancer and even death in humans (Strosnider et al., 2006). In animals, aflatoxin contaminated feeds have been associated with aflatoxicosis, impaired growth, immunosuppression, liver and kidney tumors in rodents and reduced quality of milk and milk products because of the presence of aflatoxin M1, a derivative of aflatoxin B1 (Lizárraga-Paulín, Moreno-Martínez, & Miranda-Castro, 2011).

Numerous recurrent cases of aflatoxicosis as a result of consumption of maize contaminated with aflatoxin have been reported in Kenya since 1981 (Wagacha & Muthomi, 2008). The worst outbreak occurred in 2004 where 317 patient cases were recorded with 125 deaths mainly in lower Eastern regions of Kenya (Center for Disease Control and Prevention [CDC], 2004). The 2004 outbreak was attributed to inappropriate harvest time, early rains and poor post-harvest storage of maize under moist conditions (Muthomi, Njenga, Gathumbi, & Chemining'wa, 2009; Lewis et al., 2005). In 2009, 31,000 and 1,213 bags of maize contaminated with aflatoxins

were condemned in Mbeere, Embu County and Bura Irrigation Scheme in Tana River County, respectively (Nyaga, 2010). Due to deleterious health effects associated with aflatoxin consumption, the Kenya Bureau of Standards (KEBS) set the limit for total aflatoxin level in maize at 10 μg/kg (Kenya Bureau of Standards [KEBS], 2007). Despite the existence of such regulations, contamination of food by aflatoxins is still a major challenge in Kenya.

Despite its importance in the country's economy, maize is liable to infections by mycotoxin producing fungi at pre-harvest, harvest and post-harvest stages (Atanda et al., 2011). Colonization of maize by *Aspergillus* spp. is a major challenge in maize production due to aflatoxin contamination (Wagacha & Muthomi, 2008). *Aspergillus* species are ubiquitous in the environment such as soil, air and debris and are widely distributed in tropical and sub-tropical environments (Klich, 2002). They grow as saprophytes in the soil which serves as the main reservoir of their propagules and as a source of primary inocula (Scheidegger & Payne, 2003). Factors such as high temperatures and moisture, drought stress and delayed harvesting predispose maize to infection by *Aspergillus* spp. and consequently aflatoxin contamination (Atanda et al., 2011). Aflatoxins, the most prevalent mycotoxins in Kenya are mainly produced by *A. flavus* and *A. parasiticus* (Okoth et al., 2012) and other less common *Aspergillus* species such as *A. nomius*, *A. bombycis* and *A. parvisclerotigenus* (Reiter, Zentek, & Razzazi, 2009). There are two major morphotypes of *Aspergillus flavus*; S and L strains (Cotty, 1994). *Aspergillus flavus* S-strain produces small numerous dark sclerotia and is considered the most toxigenic since it produces high levels of B type aflatoxins (Cotty & Cardwell, 1999). *Aspergillus flavus* L-strain produce yellow to bright green colonies with fewer sclerotia and less B-aflatoxins (Probst, Bandyopadhyay, Price, & Cotty, 2011). *Aspergillus parasiticus* which is distinguished by dark green colonies and rough conidia (Atehnkeng et al., 2008) produces aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) (Reiter et al., 2009).

In maize production, post-harvest practices, such as proper drying and storage, are key areas along the maize value chain to maintain grain quality, quantity and safety (Akowuah, Mensah, Chan, & Roskilly, 2015). However, poor storage practices often adopted by smallholder farmers play a major role in fungal growth and aflatoxin contamination (Wagacha et al., 2013). Most smallholder farmers use different packaging/storage materials that include polypropylene bags and giant woven baskets for packing and storing their maize grains (Hell, Cardwell, Setamou, & Poehling, 2000; Shabani, Kimanya, Gichuhi, Bonsi, & Bovell-Benjamin, 2015). These packaging/ storage materials provide optimal conditions for fungal growth and aflatoxin contamination (Hell et al., 2000). Moreover, insect pest disseminate spores of *Aspergillus* spp. in storage increasing the chances of aflatoxin contamination (Hell at al., 2008). However, application of chemical insecticides to protect grains against storage pests and other pathogens has yielded minimal results. Moreover, lack of suitable and affordable grain storage technologies often compel majority of the smallholder farmers to sell their produce immediately after harvest (Gitonga, De Groote, & Tefera, 2015).

A modern method that entails the use of Purdue Improved Crop Storage (PICS™) triple-layer hermetic bags for grain storage has been developed and is gaining favor among many farmers given its advantages when compared with the traditional storage methods (Hell, Mutegi, & Fandohan, 2010). This technology relies on creation of bio-generated atmospheres that hinder survival of microorganisms including fungi (Anankware, Fatunbi, Afreh-Nuamah, Obeng-Ofori, & Ansah, 2012). As a result of fungal, insect and grain respiration within the hermetic bags, oxygen levels drop significantly while carbon dioxide levels increase (Baoua, Amadou, Baributsa, & Murdock, 2014). This creates an unfavorable atmosphere for survival of these organisms within the enclosed system. Triple-bagging technology is therefore sustainable, cost effective and effectively maintains high quality maize grains for longer period of time (Anankware et al., 2012). A frequently asked question by maize producers is what impact the hermetic bags have on mycotoxigenic fungi. The objective of this study was therefore to evaluate the effect of PICS bags on the population of *Aspergillus* spp. and levels of aflatoxin in maize grains stored for three months. The population and incidence of *Aspergillus* spp. and total aflatoxin levels of maize stored in PICS and woven polypropylene (PP) bags were compared after three months storage under farmers' storage conditions.

2. Materials and Methods

2.1 Description of the Study Area

The study was conducted in Mukuyuni and Kilala Locations of Kaiti District, Makueni County of Lower Eastern Kenya. Kaiti District lies between latitude 1 °45' 00" S and longitude 37° 42' 00" E. The area is semi-arid to arid with a temperature range between 18 ℃ to 24 ℃ in the cold seasons and 24 ℃ to 33 ℃ in the hot days (Table 1). The rainfall pattern is bi-monsoonal with the long but unreliable rains in March to May and the more reliable short rains in October to December (Makueni County Integrated Development Plan [MCIDP], 2013). 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 and mixed farming as their major source of livelihood. Maize is the primary dietary staple and the main crop produced. The selection of the study area was based on previous reports of re-current aflatoxicosis outbreaks (Lewis et al., 2005).

Table 1. Monthly temperature (℃), precipitation (mm) and relative humidity (%) data of Kaiti District for the year 2015

Month	Minimum temperature (℃)	Maximum temperature (℃)	Precipitation (mm)	Minimum RH (%)	Maximum RH (%)
January	17.6	30.4	0.0	36.7	85.3
February	18.3	31.6	0.2	35.5	87.8
March	18.5	31.2	0.4	35.6	87.8
April	19.5	29.3	9.3	51.0	95.1
May	18.7	27.5	0.7	56.6	94.9
June	17.5	26.5	0.0	55.3	94.4
July	17.5	26.4	0.0	53.0	95.0
August	17.6	25.9	0.1	50.6	93.4
September	17.9	28.4	0.0	42.2	91.1
October	20.0	29.4	0.5	43.5	92.0
November	20.1	28.5	6.2	58.4	96.3
December	19.8	29.1	5.3	54.6	94.8

Source: (awhere, Inc, 2015); RH – Relative humidity

2.2 Field Survey and Sampling

Field survey and sampling were conducted between October 2015 and January 2016. A field survey involving 30 maize farms selected randomly was carried out in October 2015; 15 farms in Mukuyuni and 15 farms in Kilala administrative Locations of Kaiti District, Makueni County. A semi-structured questionnaire was administered to the farmers to collect data on maize production, handling and storage. Questions were designed to determine the time of harvesting, length of maize drying period, storage practices, farmers' knowledge on aflatoxin, type of storage structures, problems associated with storage and duration of grain storage before consumption, selling or planting. Soil samples were collected at planting time from 15 maize fields in each of the study locations. In each farm, a minimum of five sampling points at least 5 m apart were identified randomly. Approximately 100 g of soil was collected from the top 5 cm horizon at each sampling point, thoroughly mixed to make a composite sample from which a 500 g sample was drawn. Soil was put in a zip lock, plastic bag, transported to the laboratory within 72 h of sampling. The soil was air dried on laboratory benches for five days and stored in Kraft bags at room temperature (23 ± 2 ℃) until mycological analysis.

Dry maize grain samples were collected after harvest from 30 farmers; 15 of whom were randomly selected from each of the two Locations. The sampled maize grains were harvested from the same fields where soil had been sampled at the time of planting. From each household, shelled grains were randomly taken from different parts of the storage bag or container. The incremental sample was thoroughly mixed to form a composite sample from which 1 kg was drawn. A further 30 maize grain samples of 6 kg each were obtained from the same farmers after harvest and divided into two equal portions for storage. The samples were separately stored in woven polypropylene bags (PP) and PICS bags for three months in farmers' storage structures. Sampling from PICS bags and PP bags entailed thoroughly mixing the 3 kg grain sample and drawing a 1 kg sub-sample. The collected maize grain samples were placed in Kraft bags and transported to the laboratory within 72 h of sampling and stored at 4 ℃ until mycological analysis.

2.3 Isolation and Enumeration of Aspergillus Spp. from Soil and Maize Grains

Isolation and enumeration of *Aspergillus* spp. from soil and ground maize grains was carried out aseptically using serial dilution and spread plate technique on potato dextrose agar (PDA) medium amended with antibiotics: 50 mg penicillin/L, 50 mg tetracycline/L and 50 mg streptomycin/L (Muthomi, 2001). One kilogram of maize grain sample was mixed thoroughly and ground using a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two equal sub-samples for microbial and aflatoxin analysis. *Aspergillus* spp. were isolated and enumerated from soil and ground maize grain samples by suspending 1g of sample in 9 mL of sterile distilled water, which was thoroughly shaken and serially diluted up to 10^{-2} of the original concentration.

A 100 μL aliquot of each suspension was plated onto PDA medium amended with antibiotics and incubated for 5 days at 25 °C. The isolation and enumeration of *Aspergillus* spp. was carried out in triplicates for each soil and maize grain sample. Fungal colonies from soil and maize were identified and classified and colony counts of *Aspergillus* species expressed in colony forming units per gram of soil (CFU/g) as follows:

$$\text{CFU/g sample} = \frac{\text{Number of colonies of a fungal species}}{\text{Amount plated} \times \text{Dilution factor}} \quad (1)$$

The incidence of each fungal species was calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{Number of isolates of a fungal species}}{\text{Total number of fungal species}} \text{X } 100 \quad (2)$$

2.4 Identification of Aspergillus Species

Single colonies of *Aspergillus* spp. identified in PDA medium amended with antibiotics were sub-cultured onto 5/2 media (5% V8 juice and 2% agar, pH 5.2) (Atehnkeng et al., 2008). The cultures were incubated at 31 °C for 5 days. *Aspergillus* spp. were identified based on colony colour, shape, pigmentation, texture and pattern of growth (Klich, 2002). Isolates that produced numerous small dark sclerotia on 5/2 media 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 formed dark green colonies on 5/2 media and produced rough conidia were considered *A. parasiticus* (Atehnkeng et al., 2008). Colonies that were black on the top surface, while the underside remained pale, were identified as *A. niger*. Microscopic examination of *Aspergillus* spp. was done with modified Riddell slides (Riddell, 1950). All prepared slides were examined using a light microscope (x1000 magnification) (LEICA DM500, Leica Microsystems, Switzerland*)* and images recorded using a camera (LEICA ICC 50, Leica Microsystems, Wetzler, Germany) mounted on the microscope. Microscopic characteristics used in identification of *Aspergillus* spp. were conidial heads, seriation, conidia size, shape and roughness as described by Klich (2002).

2.5 Determination of Aflatoxin Levels in Maize Grains

Detection of aflatoxin levels in maize grains was performed using VICAM (Milford, MA, USA) protocol (Vicam, 2013; Herrman, Lee, Jones, & McCormick, 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 (Vicam, 2013). The limits of detection were between 5ppb and 100ppb.

2.6 Data Analysis

Data on the population and incidence of *Aspergillus* spp. in soil and maize grains were analyzed with the Analysis of Variance (ANOVA) PROC ANOVA procedure of GENSTAT version 15. Frequency data that was not normally distributed were transformed to arcsine before analysis whereas the colony forming units data that was not normally distributed were transformed as $\log_{10}(x+1)$. Least significant difference (LSD) was used to assess the significance of differences between treatment means at 95% confidence level.

3. Results

3.1 Maize Production Practices in Kaiti District

A majority (96.7%) of farmers determined the maize-harvesting stage by visual observation techniques (Figure 1A). The crop was considered ready for harvesting when leaves started drying, changed color from green to yellow, drooping of the cobs and by pricking kernels. Only 6.7% of the farmers in Mukuyuni used number of days that the crop was in the field to determine the appropriate maize harvesting stage. Farmers harvested their maize by hand after which they 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. Most (36.7%) of farmers dried their maize for two weeks while 23.3% dried it for four weeks. The rest (20%) of the farmers dried their maize for one and three weeks, respectively (Figure 1B). A majority (56.7%) of farmers stored their maize in a granary while 43.3% of farmers stored their produce inside the family living house (Figure 1C). The granaries were mainly raised wooden structures with iron sheet roofing (improved granaries). Most (83.3%) of the farmers packed their shelled grain in woven polypropylene bags while only 16.7% used sisal bags to store

their maize (Figure 1D).

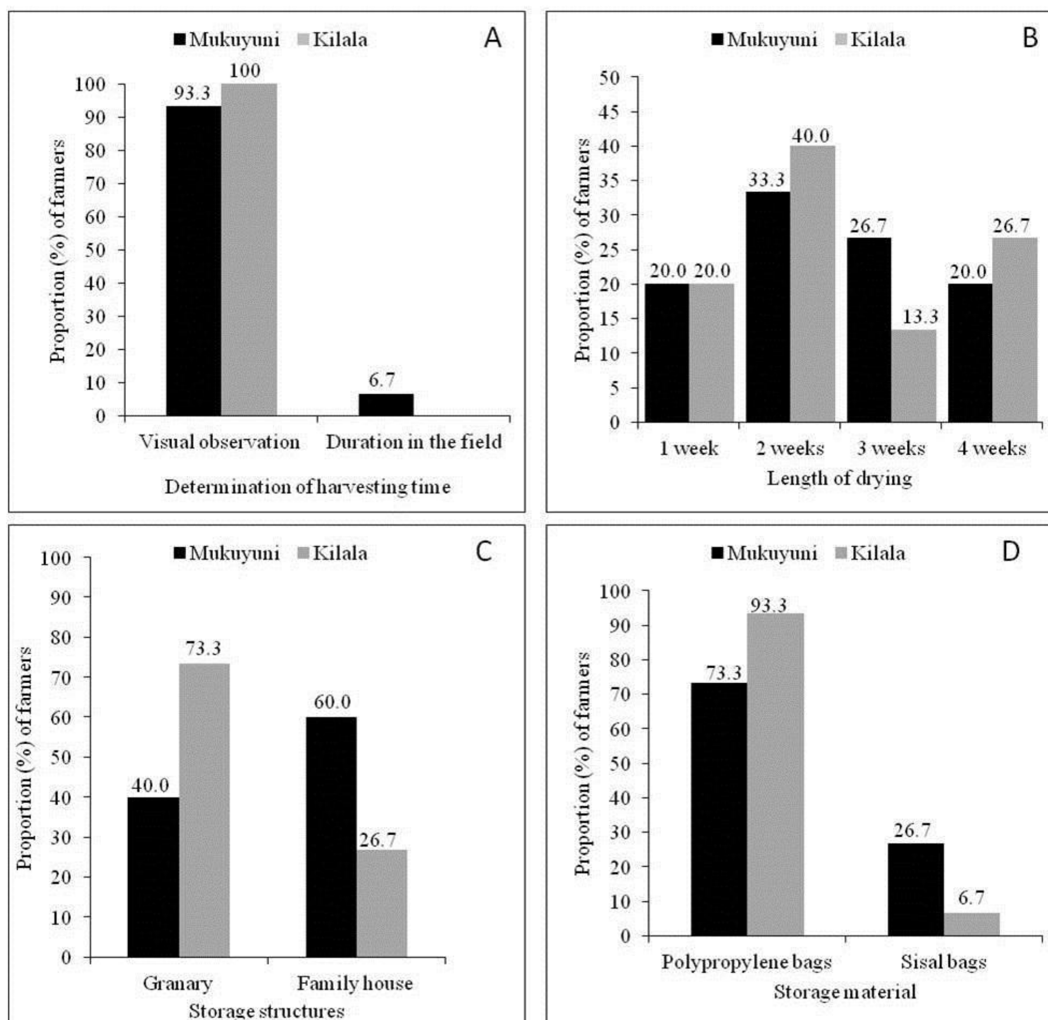

Figure 1. Methods used by farmers to determine when maize is ready for harvesting (A), length of drying period (B), type of storage structures (C) and storage methods (D) in Kaiti District.

The common storage challenges encountered by maize farmers in decreasing importance were insect damage, mold damage, rodent damage and lack of storage bags (Figure 2A). All farmers shelled their maize by hand and treated the grains with chemical insecticides before storage. Most farmers treated their maize to control insects, mainly weevils, with commercial insecticides, however, a few used traditional storage protectants such as ash (Figure 2B). Some farmers used traps to control rodents while all farmers cleaned their storage structures before storage of a new crop. About 87% of farmers in Mukuyuni Location considered mycotoxins a major concern compared to 80% of farmers in Kilala Location (Figure 2C). The majority (70%) of farmers obtained information on good farming practices and aflatoxin contamination from agricultural extension workers while a few relied on their own knowledge (16.7%) (Figure 2D).

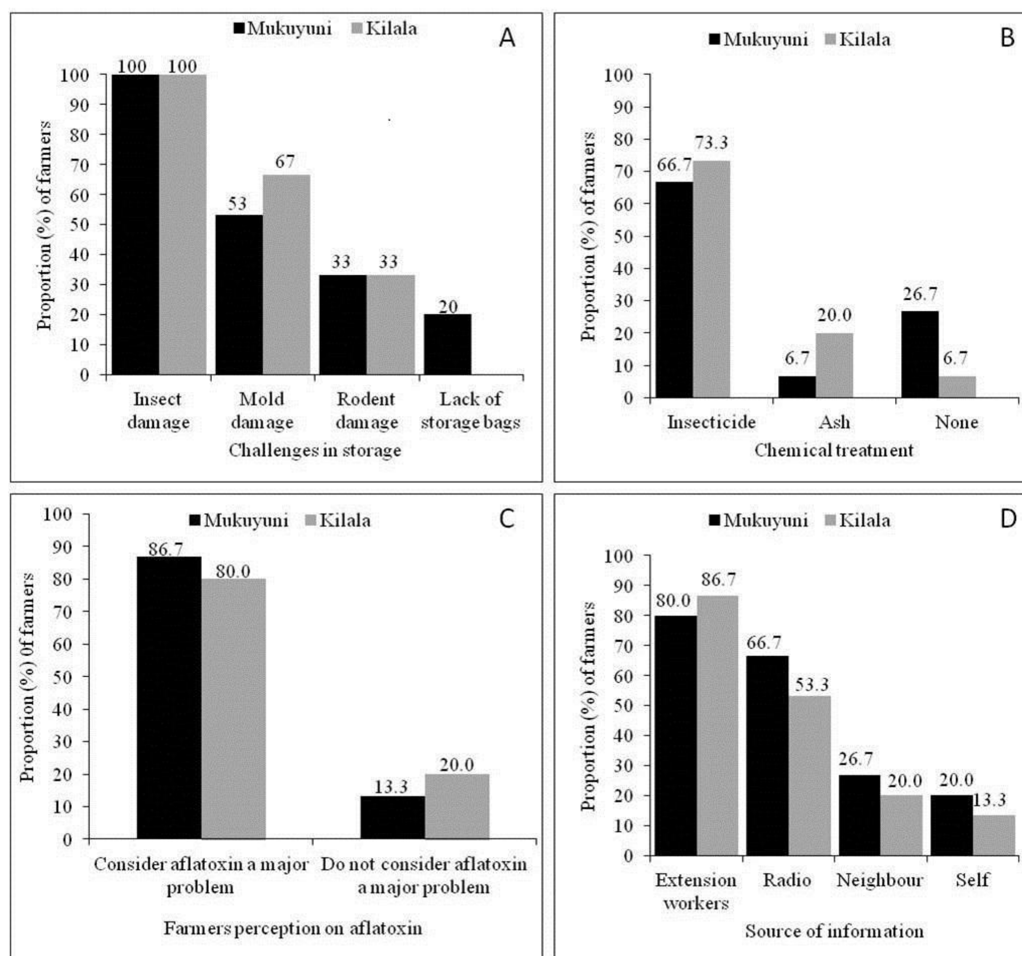

Figure 2. Challenges encountered by farmers during post-harvest storage of maize (A), grain treatment during storage (B), perceptions on aflatoxins in maize (C) and sources of information (D) in the Kaiti District.

3.2 Population and Incidence of Aspergillus Spp. In Soil

Aspergillus species isolated from the soil were: *A. flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger* (Table 2). Among the members of *Aspergillus* section *Flavi*, *A. flavus* L-strain was the most predominant (Range = 0 - 6.0 x 10^3 CFU/g soil), followed by *A. parasiticus* (Range = 0 - 4.0 x 10^3 CFU/g soil) and *A. flavus* S-strain (Range = 0 - 3.0 x10^3 CFU/g soil). Population of *Aspergillus* spp. in soil varied significantly ($p \leq 0.05$) between Kilala and Mukuyuni Locations (Table 2). Incidence of *A. flavus* L-strain was higher in soil samples from Mukuyuni while the incidence of *A. parasiticus* was significantly higher ($p \leq 0.05$) 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 the two Locations.

Table 2. Population and incidence of *Aspergillus* spp. in soil sampled from maize fields in Kaiti District

Aspergillus spp.	Mukuyuni		Kilala	
	Population (CFU/g)	Incidence (%)	Population (CFU/g)	Incidence (%)
A. flavus S-strain	5.3 x 10^2 ±137.1^{bc}	10.1 ±3.1^{b}	4.4 x 10^2 ±121.3^{b}	6.6 ±1.9^{b}
A. flavus L-strain	1.0 x 10^3 ±165.1^{ab}	25.6 ±4.8^{a}	6.9 x 10^2 ±141.5^{b}	16.0 ±3.9^{b}
A. parasiticus	4.9 x 10^2 ±147.8^{c}	7.1 ±2.1^{b}	4.2 x 10^2 ±121.0^{b}	6.2 ±2.0^{b}
A. niger	1.4 x 10^3 ±273.3^{a}	35.0 ±5.7^{a}	3.2 x 10^3 ±430.6^{a}	60.1 ±5.7^{a}
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	7.7	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.

3.3 Efficacy of PICS Storage Bags on the Population of Aspergillus Spp. in Maize

Four *Aspergillus* spp. were commonly isolated from maize grains sampled at harvest and after three months of storage in PICS and PP bags (Table 3). The population of *Aspergillus* spp. in maize sampled at harvest and three months after storage was significantly different ($p \leq 0.05$) and in decreasing order: *A. flavus* L-strain (Mean = 8.7 x 10^2 CFU/g), *A. flavus* S-strain (Mean = 6.4 x10^2 CFU/g), *A. parasiticus* (Mean = 3.4 x10^2 CFU/g) and *A. niger* (Mean = 3.3 x10^2 CFU/g). The population of the aforementioned *Aspergillus* spp. was significantly ($p \leq 0.05$) higher in maize sampled after storage compared to samples collected at harvest. The population of *Aspergillus* spp. in maize increased up to three fold from 24.9% at harvest to 74.7% 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* – which was 71% higher in maize stored in PP bags than in PICS bags. Overall, the population of *A. flavus* L-strain was 35% higher than that of *A. flavus* S-strain.

Table 3. Population (CFU/g) of *Aspergillus* spp. in maize grains sampled at harvest and three months after storage in PP and PICS bags in Kaiti District

Location	Bag type	AFL	AFS	AP	AN
Mukuyuni	At harvest [a]	5.1 x 10^2 ±167.0[b]	3.3 x 10^2 ±100.5[a]	4.4 x 10^1 ±31.0[b]	8.9 x 10^1 ±42.9[b]
	PICS bag	1.1 x 10^3 ±270.0[ab]	8.2 x 10^2 ±242.6[a]	2.9 x 10^2 ±87.8[b]	2.2 x 10^2 ±89.3[b]
	PP bag	1.4 x 10^3 ±435.2[a]	1.2 x 10^3 ±553.8[a]	8.0 x 10^2 ±163.9[a]	4.7 x 10^2 ±170.0[a]
	Mean	1022.2	785.2	377.7	251.9
	LSD ($P \leq 0.05$)	871.1	996.2	300.7	319.9
	CV%	29.9	19.9	46.7	10.2
Kilala	At harvest [a]	3.1 x 10^2 ±109.3[b]	1.8 x 10^2 ±65.82[b]	1.6 x 10^2 ±63.2[b]	2.4 x 10^2 ±78.9[b]
	PICS bag	8.0 x 10^2 ±184.2[ab]	3.8 x 10^2 ±106.8[b]	1.6 x 10^2 ±83.8[b]	3.6 x 10^2 ±96.2[ab]
	PP bag	1.0 x 10^3 ±277.0[a]	9.6 x 10^2 ±203.4[a]	6.2 x 10^2 ±172.0[a]	6.0 x 10^2 ±147.0[a]
	Mean	718.5	503.7	311.1	400.0
	LSD ($P \leq 0.05$)	568.8	386.6	326.6	312.3
	CV%	18.9	24.3	28.6	19.2

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,* PICS - Purdue improved crop storage bags, PP - woven polypropylene bags, CFU - colony forming units.

3.4 Efficacy of PICS Storage Bags on the Incidence of Aspergillus Spp. in Maize

Table 4. Incidence (%) of *Aspergillus* spp. in maize grains sampled at harvest and three months after storage in PP and PICS bags in Kaiti District

Location	Bag type	AFL	AFS	AP	AN
Mukuyuni	At harvest [a]	20.0 ±5.5[a]	15.1 ±4.6[a]	2.2 ±1.6[b]	7.1 ±3.8[a]
	PICS bag	34.2 ±6.4[a]	20.5 ±5.3[a]	10.3 ±3.6[b]	10.5 ±4.4[a]
	PP bag	27.2 ±5.7[a]	13.5 ±4.8[a]	32.6 ±6.2[a]	7.8 ±3.2[a]
	Mean	27.1	16.4	15.1	8.5
	LSD ($P \leq 0.05$)	16.6	13.8	12.0	10.7
	CV (%)	3.3	28.8	10.0	12.8
Kilala	At harvest [a]	13.3 ±4.3[a]	6.7 ±2.4[b]	8.3 ±3.6[ab]	13.9 ±4.6[a]
	PICS bag	26.1 ±5.5[a]	13.5 ±4.2[ab]	4.9 ±2.7[b]	24.2 ±6.3[a]
	PP bag	21.4 ±4.9[a]	24.6 ±5.2[a]	15.6 ±4.4[a]	16.2 ±4.5[a]
	Mean	20.3	15.1	9.6	18.2
	LSD ($P \leq 0.05$)	13.7	11.5	10.2	14.5
	CV (%)	15.9	23.6	17.7	32.0

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,* PICS- Purdue improved crop storage bags, PP- woven polypropylene bags.

The incidence of *Aspergillus* section *Flavi* isolated from maize grain sampled at harvest and after three months of storage in decreasing order was: *A. flavus* L-strain (Mean incidence = 23.7%), *A. flavus* S-strain (15.6%) and *A. parasiticus* (12.2%) (Table 4). *Aspergillus flavus* L-strain was the most prevalent in harvested (Mean incidence = 16.7%) and stored maize grains (Mean incidence = 27.2%). The incidence of the aforementioned *Aspergillus* spp. was significantly ($p \leq 0.05$) different and 84.8% higher in maize obtained after three months of storage compared to samples collected at harvest. The type of storage bag had a significant influence ($p \leq 0.05$) on the incidence of members of *Aspergillus* section *Flavi* - *A. flavus* (S and L strains) and *A. parasiticus*. The incidence of *A. flavus* S-strain was 12% higher in maize stored in PP bags than in PICS bags while there was no significant variation ($p \geq 0.05$) in the incidence of *A. flavus* L-strain stored in the two bag types.

3.5 Efficacy of PICS Bags on Aflatoxin Levels in Maize

The levels of total aflatoxin in maize grains sampled at harvest and after three months of storage in PICS and PP bags ranged from < 5 ppb to 42.7 ppb (Table 5). 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%). Maize grains stored in PP bags were more contaminated with total aflatoxin (Mean = 4.7 ppb) than grains stored in PICS bags (Mean = 2.1 ppb). Storage of maize in PICS bags reduced aflatoxin contamination by 55% as compared to PP bags. Overall, 50% and 90% of the maize grains stored in PP and PICS bags, respectively met the EC standards for total aflatoxin (≤ 4 ppb). Likewise, 96.7% and 100% of the maize grains stored in PP and PICS bags, respectively met the threshold set by KEBS (≤ 10 ppb) and FDA (≤ 20 ppb) (Table 5).

Table 5. Mean proportion (%) of aflatoxin contamination level categories for maize sampled at harvest and three months after storage in PICS and PP 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

3.6 Correlation between the Population of Aspergillus Section Flavi and Aflatoxin Levels in Maize

There was a positive significant correlation ($p \leq 0.05$) between the population of *A. parasiticus* and aflatoxin levels in grains sampled after storage (Table 6). However, there was no significant correlation ($p \geq 0.05$) between the population of *A. flavus* (L and S strains) and aflatoxin levels during the two sampling regimes.

Table 6. Correlation between the population of *Aspergillus* section *Flavi* and aflatoxin levels in maize grains sampled at harvest and after three months of storage

	Aspergillus spp.	*A. flavus* S-strain	*A. flavus* L-strain	*A. parasiticus*	Aflatoxin
At harvest	*A. flavus* S-strain	1			
	A. flavus L-strain	0.71**	1		
	A. parasiticus	0.26[ns]	0.25[ns]	1	
	Aflatoxin	-0.18[ns]	-0.10[ns]	0.01[ns]	1
Three months storage	*A. flavus* S-strain	1			
	A. flavus L-strain	0.07[ns]	1		
	A. parasiticus	-0.17[ns]	0.01[ns]	1	
	Aflatoxin	0.04[ns]	0.11[ns]	0.43*	1

**Correlation coefficient significant at $p \leq 0.01$; *correlation coefficient significant at $p \leq 0.05$; ns - not significant.

4. Discussion

Most smallholder farmers do not harvest maize based on physiological maturity, but employ traditional practices such as observing the dried tassels of cobs and drooping of cobs to determine readiness of maize for harvesting (Akowuah et al., 2015). The aforementioned practices were employed by farmers in Kaiti District to determine the harvesting time of maize. A study by Hell et al. (2000) reported that farmers in Benin left maize in the field for 2 to 3 weeks after physiological maturity before harvest. Akowuah et al. (2015) observed that majority (69%) of farmers in Ghana harvested their maize beyond the physiological maturity period, thus late harvesting was identified as a common practice. These techniques are not accurate and therefore, harvested maize may still have high moisture content, thereby making the grains highly susceptible to fungal growth and aflatoxin contamination (Hell et al., 2008). The most suitable time for maize harvesting is at physiological maturity (Kaaya, Harris, & Eigel, 2006; Hell & Mutegi, 2011). Kaaya et al. (2006) observed that delayed harvesting was positively correlated to increase in aflatoxin levels by about four times by the third week after the recommended harvesting time and more than seven times when maize harvest was delayed for four weeks. Thus, timely harvesting and adequate drying of crops can play a role in reducing fungal contamination of crops (Bankole & Adebanjo, 2003; Atanda et al., 2011). However, lack of storage space, unpredictable weather, labour constraint, theft of the produce, rodent damage and other animals compel farmers to harvest at inappropriate time (Bankole & Adebanjo, 2003).

Most of the farmers used woven polypropylene (PP) bags to store maize, while a few used sisal bags. Maize was stored either inside the family house or the granary. Gachara (2015) observed that most farmers in Eastern and Rift valley regions of Kenya first packed their maize in polypropylene bags after which they stored it in the granaries. Studies in Zambia (Kankolongo, Hell, & Nawa, 2009) and Tanzania (Shabani et al., 2015) reported that smallholder farmers stored their maize grains in polypropylene bags inside the family living house. A study by Gitonga et al. (2015) showed that most (60%) smallholder farmers used space in the family living house and improved granaries (17%) to store maize after harvest. Fandohan, Gnonlonfin, Marasas and Wingfield (2006) reported that in most Sub-Saharan African countries, maize is generally stored in cob form either in wooden granaries, under the roofs of farmers' houses, or on floor in houses. In the current study, storage of maize in polypropylene bags and family house might have contributed to high population of *Aspergillus* species. Storage structures differ in their ability to protect grains from fungal contamination. In a study in West Africa, Hell et al. (2000) reported that some types of farmers' storage structures provided conditions that were more conducive to fungal infection and aflatoxin contamination than others. Kaaya et al. (2006) also reported that storage of maize in improved granaries in Uganda was related to reduced aflatoxin contamination. However, use of improved granaries by smallholder farmers to store maize is uncommon due to risk of theft of the produce.

The most common storage problems reported by maize farmers in Kaiti District were infestation by insects and mold damage. Studies carried out by Hell et al. (2008) in Benin and Shabani et al. (2015) in Tanzania, reported that insects and rodents were common maize storage problems. Storage pests, in particular *Cathartus quadricollis* and *Sitophilus zeamais*, play an important role in the contamination of foods with fungi, especially those that produce toxins (Lamboni & Hell, 2009). Pest infestation is largely due to improper post-harvest and storage conditions and the level of insect damage influences the extent of mycotoxin contamination (Atanda et al., 2011).

Application of chemical insecticides to control storage pests was extensively practiced by maize farmers involved in this study an observation consistent with previous reports (Hell et al., 2000; Kaaya et al., 2006; Shabani et al., 2015). Application of insecticides is a significant factor in *A. flavus* and *A. parasiticus* management (García & Heredia, 2006). A previous study by Plasencia (2004) reported that control of insect populations with insecticides in maize storage environments reduced *A. flavus* and *A. parasiticus* contamination. Insecticides are considered too expensive for subsistence farmers and therefore, farmers in Kaiti District did not use the recommended application rates. Use of wrong insecticides might have also contributed to high occurrence of pests during storage. In addition, application of insecticides is labour intensive since farmers have to apply them after every three months.

Aspergillus flavus L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger* were isolated from soil. In a similar study, Karanja (2013) isolated different members of *Aspergillus* section *Flavi* from soil sampled from Eastern Kenya. Horn (2003) reported that soil serves as a reservoir for *A. flavus* and *A. parasiticus* that produce different aflatoxin types in agricultural commodities. Under adverse environmental conditions, *Aspergillus* spp. form sclerotia that allow the fungus to survive as saprophytes for extended periods in the soil, maize residue and maize cobs (Wagacha & Muthomi, 2008). Likewise, the sclerotia survive in soil and produce conidiophores and conidia during subsequent seasons to infect the crop via silk (Scheidegger & Payne, 2003). The propagules in the

soil and crop debris act as the primary source of contamination with *Aspergillus* spp. infecting maturing maize crops (Atehnkeng et al., 2008). Thus, elimination of *Aspergillus* spp. inoculum sources such as infected debris from the previous harvest may prevent infection of the subsequent crops (Strosnider et al., 2006).

Aspergillus flavus S-strain, *A. flavus* L-strain, *A. parasiticus* and *A. niger* were isolated from the maize grains sampled at harvest. A similar diversity has been reported in maize sampled from Eastern (Murithi, 2014) Kenya at harvest. The high population of *Aspergillus* spp. in maize sampled at harvest could be attributed to the dry weather conditions associated with high temperatures in Makueni County which predispose maize to the fungi at pre-harvest stages in the field (Okoth et al., 2012). Also, improper harvest practices such as delayed harvesting and throwing cobs on the ground during and after harvesting might have contributed to *Aspergillus* spp. contamination of maize. Similar spectrum of the aforementioned *Aspergillus* spp. was also observed in maize grains sampled three months after storage in PP and PICS 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.

Of all the *Aspergillus* section *Flavi* isolated in maize grains sampled at harvest and after storage in the current study, *A. flavus* L-strain was the most prevalent while *A. parasiticus* was the least predominant species. The current findings on the abundance of *A. flavus* L-strain contradict those of Okoth et al. (2012) who reported that *A. flavus* S-strain was the most predominant in maize obtained from Makueni region of Kenya. A previous study in Eastern Kenya by Probst, Njapau and Cotty (2007) observed a higher incidence of *A. flavus* S-strain (71.8%), followed by *A. flavus* L -strain (28.2%) and lastly *A. parasiticus* (2.1%). Predominance of *A. flavu*s L-strain in the current study could be attributed to the fact that the climatic conditions during the study period were not harsh to favour *A. flavu*s S-strain over other members of *Aspergillus* section *Flavi*. The high occurrence of *Aspergillus* section *Flavi* in this study indicates a risk of aflatoxin poisoning when conditions are favorable as observed by Probst et al. (2007) since *Aspergillus* section *Flavi*, have been reported as the main contaminants of maize from Eastern regions, Kenya (Okoth et al., 2012).

In this study, the population of *Aspergillus* spp. in maize increased from harvest to sampling after three months of storage in PP and PICS bags. This agrees with reports by Domenico et al. (2016) who observed 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, Cardwell and Poehling (2003) also reported higher frequencies of *A. flavus* in stored maize in Benin compared to maize obtained at harvest. In this study, the population of *Aspergillus* spp. was 71.1% higher in maize stored in woven PP bags than in PICS bags at the third month of storage. Factors conducive for fungal growth such as high moisture content, high relative humidity and aeration of the grains in woven polypropylene bags might have contributed to high population of *Aspergillus* spp. Previous studies have reported that fungal counts in peanuts stored in aerated bags were higher than in hermetically sealed bags after 90 days of storage (Navarro, Navarro, & Finkelman, 2012). Domenico et al. (2016) reported an increase in the population of *Aspergillus* spp. in conventional bags and hermetic bags with respective peaks at three and six months, followed by stability in counts. Furthermore, Viebrantz, Radunz and Dionello (2016) reported that although initial growth of *Aspergillus* spp. 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.

The population of *Aspergillus* spp. increased up to two-fold in PICS bags compared to up to three-fold increase in PP bags after three months storage. Maize grains stored in PICS bags were less contaminated with *Aspergillus* spp. than from the woven PP bags possibly due to reduced oxygen (hypoxia) and elevated CO_2 levels (hypocarbia) (International Food Policy Institute [IFPRI], 2010) within hermetic storage that hinders the development of the pathogens (Baoua et al., 2014). Bartosik, Cardoso and Rodr ǵuez (2008) observed that increasing CO_2 concentration from 3% to 30% (even with O_2 concentrations of 21%) resulted in a reduction of fungal counts. Similarly, Hocking (2003), found that growth of *Aspergillus* spp. was significantly reduced but not prevented by storage in an atmosphere of 0.1% O_2 and 21% CO_2. Other studies by Giorni, Battilani, Pietro and Magan (2008) reported that treatment with 25% carbon dioxide reduced *A. flavus* development; however concentration of at least 50% carbon dioxide was required to reduce aflatoxin formation in maize. Changes in internal gas composition due to decrease of oxygen and increase of carbon dioxide within the hermetic bags affect the oxidative metabolism thus fungi and other microorganisms do not generate metabolic water for support of growth and cellular integrity (Mutungi, Affognon, Njoroge, Baributsa, & Murdock, 2014). Low O_2 and high CO_2 concentration reduces the rate of growth of fungi, degree of sporulation, respiratory rate and their ability to attack grain tissues (Moreno-Martinez et al., 2000). Thus hermetic bag protects maize grains better than conventional storage bags (Baoua et al., 2014) and can therefore be used for effective grain storage.

There were varying levels of aflatoxin contamination in maize grains sampled 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 the Kenya Bureau of Standards (≤ 10 ppb) and the US Food and Drug Administration (≤ 20 ppb). Mwihia *et al*. (2008) reported that 35.5% of maize sampled from Makueni in Eastern Kenya at harvest had aflatoxin levels above FDA maximum limit of 20 ppb, while Muthomi et al. (2009) reported aflatoxin levels of up to 160 $\mu g\ kg^{-1}$ in maize samples from areas with high prevalence of *A. flavus* in Eastern Kenya. The high levels of aflatoxin contamination in maize sampled at harvest indicated that *A. flavus* infection of maize had already occurred in the field prior to or during harvest. Moreover, drought stress, delayed harvesting, improper harvest practices and spreading of maize on the ground for drying before storage might have increased the risk of maize contamination with *Aspergillus* spp. (Wagacha & Muthomi, 2008).

Maize stored in woven PP bags was 33.4% more contaminated with aflatoxin compared to samples stored in PICS bags. The high aflatoxin levels in PP bags could be attributed to high temperature, relative humidity and moisture conditions (Wagacha et al., 2013) that promote fungal growth and aflatoxin contamination. A study by Domenico et al. (2016) observed that the mean levels of total aflatoxins in kernels stored in conventional bags, hermetic bags and silos were 85.4, 85 and 91.2 µg/kg, respectively. Overall, 90% and 100% of maize samples stored in PICS bags in this study met the Kenyan regulatory threshold of ≤ 10 ppb and FDA standard of ≤ 20 ppb for total aflatoxins. Storage of maize in PICS bags effectively lowered aflatoxin levels by 55.3% after three months of storage which could be attributed to low O_2 content and elevated CO_2 levels in hermetic bags (Moreno-Martinez et al., 2000), which limit fungal growth and toxin production. Hocking (2003) reported that carbon dioxide enrichment hinders aflatoxin formation in the substrate. Other findings demonstrated that the ability of *A. flavus* to produce aflatoxin in groundnuts was substantially reduced with the increase in CO_2 and decrease in O_2 concentrations (Bartosik et al., 2008). Magan & Aldred (2007) observed that aflatoxin production decreased by 25% when CO_2 was elevated to 20% although it had no visible effect on growth and sporulation of fungi.

In this study, there was a significant positive correlation between the population of *A. parasiticus* and aflatoxin levels in maize sampled three months after storage. The positive correlation could be as a result of the presence of sub-optimal storage conditions (high humidity, moisture and ambient temperature) favorable for growth of *A. parasiticus* and aflatoxin contamination. However, Probst et al. (2007) observed no correlation between aflatoxin content and the incidence of *A. parasiticus* in maize. Other studies by Hell et al. (2000) observed a positive correlation between aflatoxin levels and *Aspergillus* spp. in stored maize flour in Benin. In this study therefore, *A. parasiticus* greatly contributed to total aflatoxin in stored maize.

5. Conclusion

The high population of *Aspergillus* spp. in maize stored in polypropylene bags implies a serious health concern to consumers when temperature, relative humidity and storage conditions are favorable for aflatoxin contamination. From the study, the triple-layer hermetic bags effectively suppressed the growth of *Aspergillus* spp. Likewise, hermetic bags provided conditions that were unfavorable for aflatoxin contamination. It is therefore evident that hermetic bags provide good protection against fungal growth and aflatoxin contamination of stored maize grains. Moreover, storage of maize in hermetic bags offers a cost effective and chemical free-method that will enable farmers to preserve high quality grains. Therefore, adoption of hermetic storage technology by smallholder farmers will provide an effective option for managing fungal and aflatoxin contamination of maize grains as well as maintaining grains of high quality.

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Conflict of Interests

The authors have not declared any conflict of interests.

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