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# Assessment of Farmers Maize Production Practices and Effect of Triple-Layer Hermetic Storage on the Population of *Fusarium* Spp. and Fumonisin Contamination

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**Abstract** Fumonisin contamination of maize by *Fusarium* spp. is a major risk in food security, human and animal health. A study was carried out in Kaiti District, Makueni County in Kenya, to assess the effectiveness of triple-layer hermetic (PICS™) bags in the management of *Fusarium* spp. and fumonisin contamination of stored maize grains. Maize production practices including scale of production, methods of land preparation, variety grown and storage methods were obtained with a questionnaire. *Fusarium* spp. in soil and maize were isolated by dilution-plating method and fumonisin content in maize was measured. Majority (86.7%) of the farmers were smallholders who mostly stored maize in polypropylene (PP) bags. *Fusarium proliferatum* was predominant in soil (1.4 x $10^3$ CFU/g of soil) and stored grain (2.7 x $10^3$ CFU/g of maize) while *F. oxysporum* was predominant in freshly harvested grain (1.4 x$10^3$ CFU/g of maize). The population of *Fusarium* spp. was 74.6% higher in PP than in PICS bags after three months of storage. Total fumonisin in maize grains sampled at harvest and after three-months storage ranged from < 2 to 6.0 ppm and was 57.1% lower in PICS bags than in PP bags. The population of *Fusarium* spp. in maize was positively correlated with fumonisin levels. The findings of this study demonstrate that PICS bags can effectively manage the population of *Fusarium* spp. and accumulation of fumonisin in stored maize.

***Keywords:*** *Fumonisin, Fusarium spp., hermetic storage, maize, polypropylene bags*

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## 1. Introduction

Maize (*Zea mays* L.) is the key staple food crop in Kenya [1] contributing 65% of staple food calories and 36% of the total caloric intake [2]. A major constraint in maize production in Kenya is contamination by *Fusarium* spp., which lowers grain yield and indirectly affects quality through production of mycotoxins [3].

*Fusarium* spp. are economically the most important plant-pathogenic fungi of maize [3]. They are ubiquitous and abundant in soil of temperate, semi-tropical as well as tropical regions of the world [4]. The most important *Fusarium* species that infect maize include *F. verticillioides*, *F. proliferatum*, *F. graminearum*, *F. oxysporum* and *F. sporotrichioides* [5]. It is estimated that more than 50% of maize grain is lost as a result of *Fusarium* diseases and subsequent mycotoxin contamination [6]. *Fusarium verticillioides* and *F. proliferatum*, which cause maize ear rot disease [3,5] are the primary producers of copious amounts of the mycotoxin fumonisin [7]. Although *Fusarium* spp. are mostly considered field fungi [8], it has been reported that *F. verticillioides* and *F. proliferatum* are common contaminants of stored maize grains [9,10].

Among the most common mycotoxins produced by *Fusarium* spp., fumonisins are the most prevalent toxins that contaminate maize and maize based products [6]. Fumonisins are a group of toxic secondary metabolites produced by *Fusarium* spp. in the Liseola section [7]. Fumonisins B1 (FB1), B2 (FB2) and B3 (FB3) occur in naturally contaminated maize grains [7], with fumonisin B1 being the most abundant and most toxic accounting for about 70-80% of total fumonisin in infected maize [11]. Fumonisin-contaminated feeds have been implicated in leucoencephalomalacia in horses, porcine pulmonary edema and hepatic syndrome in swine and liver cancer in rats [7]. In humans, consumption of food contaminated with fumonisin B1 has been associated with high rates of esophageal cancer [12] and increased risk for neural tube defects (NTDs) [13].

There are several reports that document fumonisin contamination of maize in Kenya. Widespread contamination of maize with fumonisin in Eastern Kenya has been reported [14,15]. A survey in Western Kenya also reported

high prevalence of fumonisin in maize grains [16], while another reported fumonisin B1 (FB1) levels that ranged from 39 to >5000 µg/kg of maize [17]. These high levels of fumonisin strongly suggest a risk of unacceptable exposure, which is detrimental to human health. Previous studies in Kenya also indicate an increase in esophageal cancer that correlates with consumption of fumonisin contaminated foods [18]. Despite the widespread occurrence of fumonisins in maize in Kenya, no regulatory standards have been set by the Kenya Bureau of Standards (KEBS).

Maize production in developing countries is commonly done by smallholder farmers who lack access to efficient farming systems and modern storage technologies and facilities. Of major concern and critical to food and income security are post-harvest losses due to fungal attack and mycotoxin contamination [19]. These losses are aggravated by inadequate and inefficient storage facilities and technologies among smallholder farmers [1]. In Sub-Saharan Africa, most farmers often store their maize under varying and often poor storage conditions before consumption or sale [19]. Such storage facilities include woven polypropylene bags, jerrycans, sisal bags and baskets [1,20]. These storage conditions are associated with frequent rewetting of grains, high temperature and humidity and insect infestation predisposing maize to fungal infection and subsequent mycotoxin contamination [19].

The need for effective post-harvest management of maize led to the development of Purdue Improved Crop Storage (PICS$^{TM}$) triple-layer hermetic bag that offers a safe and cost-effective protection of stored grains against insect infestation [21]. The airtight condition created by the two inner hermetic bags suppresses the growth and development of living organisms within the enclosed storage space [21,22]. Adoption of the PICS technology by smallholder farmers has been promoted as an option that will improve food and income security, and alleviate poverty for the smallholder farmers [22]. Questions have arisen about the effects of hermetic bags on *Fusarium* growth and fumonisin contamination in stored maize. The objective of this study was therefore to assess the effect of hermetic bags on the population of *Fusarium* spp. and levels of fumonisin in stored maize. The population of *Fusarium* spp. and total fumonisin levels of maize stored in PICS and PP bags were compared after three months of on-farm storage.

# 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°C to 24°C in the cold seasons and 24°C to 33°C in the hot days (Table 1). 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 [23]. 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 [24].

## 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 size of land, land preparation methods, variety of maize planted and type of storage methods. Soil samples were collected before planting 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 and stored at room temperature until mycological analysis.

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

| Month | Minimum temperature (°C) | Maximum temperature (°C) | 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.

Maize grain samples were collected after harvest from 30 farmers; 15 of whom were randomly selected from each of the two administrative Locations. The sampled maize was 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 same bag. 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 the storage experiment. 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°C until mycological analysis.

### 2.3. Isolation and Enumeration of *Fusarium* spp. from Soil and Maize Grains

Isolation and enumeration of *Fusarium* spp. from soil and ground maize grain was carried out aseptically by serial dilution and spread plate technique on potato dextrose agar (PDA) medium amended with 50 mg penicillin/L, 50 mg tetracycline/L and 50 mg streptomycin/L antibiotics [25]. One kilogram of maize grain sample was mixed thoroughly and ground with a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two equal sub-samples for microbial and fumonisin analysis. *Fusarium* spp. were isolated and enumerated from soil and ground-maize samples by suspending 1g of sample in 9 mL of sterile distilled water, which was thoroughly shaken and serially diluted up to $10^{-2}$. A 100 µL aliquot of each suspension was plated onto PDA medium amended with antibiotics and incubated for 5 days at 25°C. Fungal colonies were identified and classified and colony counts of *Fusarium* species recorded. The isolation and enumeration of *Fusarium* spp. was carried out in triplicates for each soil and grain sample.

### 2.4. Identification of *Fusarium* Species

All *Fusarium* isolates were sub-cultured on PDA and Synthetic Nutrient Agar (SNA). SNA was prepared by weighing 1.0 g $KH_2PO_4$, 1.0 g $KNO_3$, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose and 20.0g Agar into one litre of distilled water [26]. The cultures on PDA were incubated at 25°C for 7-10 days and SNA cultures were kept in the dark for 14-21 days to induce sporulation [25]. Potato Dextrose Agar medium was used for gross morphological appearance and colony pigmentation based on growth rate, colony reverse color, surface texture, colour and shape of aerial mycelium, and the development of pigments in agar medium [4,27]. The cultures grown on SNA were used for microscopic identification based on microconidia, macroconidia, phialides, conidiophore and chlamydospore as previously described [4,27].

### 2.5. Determination of Fumonisin Levels in Maize Grains

Fumonisin levels were determined using the VICAM method [28,29] 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 and100 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 1 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 Vertu lateral flow reader (VICAM, Watertown, USA) for quantification of fumonisin in parts per million (ppm) [29].

### 2.6. Data Analysis

Data on the population and incidence of *Fusarium* sp. in soil and maize grains were analyzed with the Analysis of Variance (ANOVA) PROC ANOVA procedure of GENSTAT version 15. Frequency data that were 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 Farmer Practices in Kaiti District

A majority (86.7%) of maize farmers were smallholder who owned between one and 10 acres of land while 10% were medium scale farmers with between 11 and 20 acres of land. Only 3.3% were large scale with over 20 acres of land (Figure 1A). Most (66.7%) of the farmers used oxen plough for land preparation while 33.3% used both oxen plough and hand hoe (Figure 1B). A majority (66.7%) of farmers planted improved maize varieties obtained from agro-stockists while 33.3% planted traditional varieties (locally known as *Kinyanya*) saved from previous seasons (Figure 1C). Most (83.3%) farmers packed their shelled grains in polypropylene bags while a few (16.7%) stored the grains in sisal bags (Figure 1D).

### 3.2. Population and Incidence of *Fusarium* spp. in Soil

*Fusarium* spp. isolated from soil were: *F. proliferatum*, *F. oxysporum*, *F. subglutinans* and *F. solani* (Table 2). *Fusarium proliferatum* (Mean = 1.4 x $10^3$ CFU/g soil) was the most predominant followed by *F. oxysporum* (Mean = 1.3 x $10^3$ CFU/g soil), *F. solani* (Mean = 9.4 x $10^2$ CFU/g soil) and *F. subglutinans* (Mean = 7.3 x $10^2$ CFU/g soil) (Table 2). Population of *Fusarium* spp. in soil was not significantly different ($p \geq 0.05$) between Kilala and

Mukuyuni Locations. The incidence of *Fusarium* spp. isolated from soil was significantly different ($p \leq 0.05$) and in decreasing order: *F. oxysporum*, *F. proliferatum*, *F. subglutinans* and *F. solani*. The incidence of *F. proliferatum* and *F. solani* were significantly ($p \leq 0.05$) higher in soil samples from Kilala Location while the incidence of *F. oxysporum* was consistently higher in soil sampled from the two Locations.

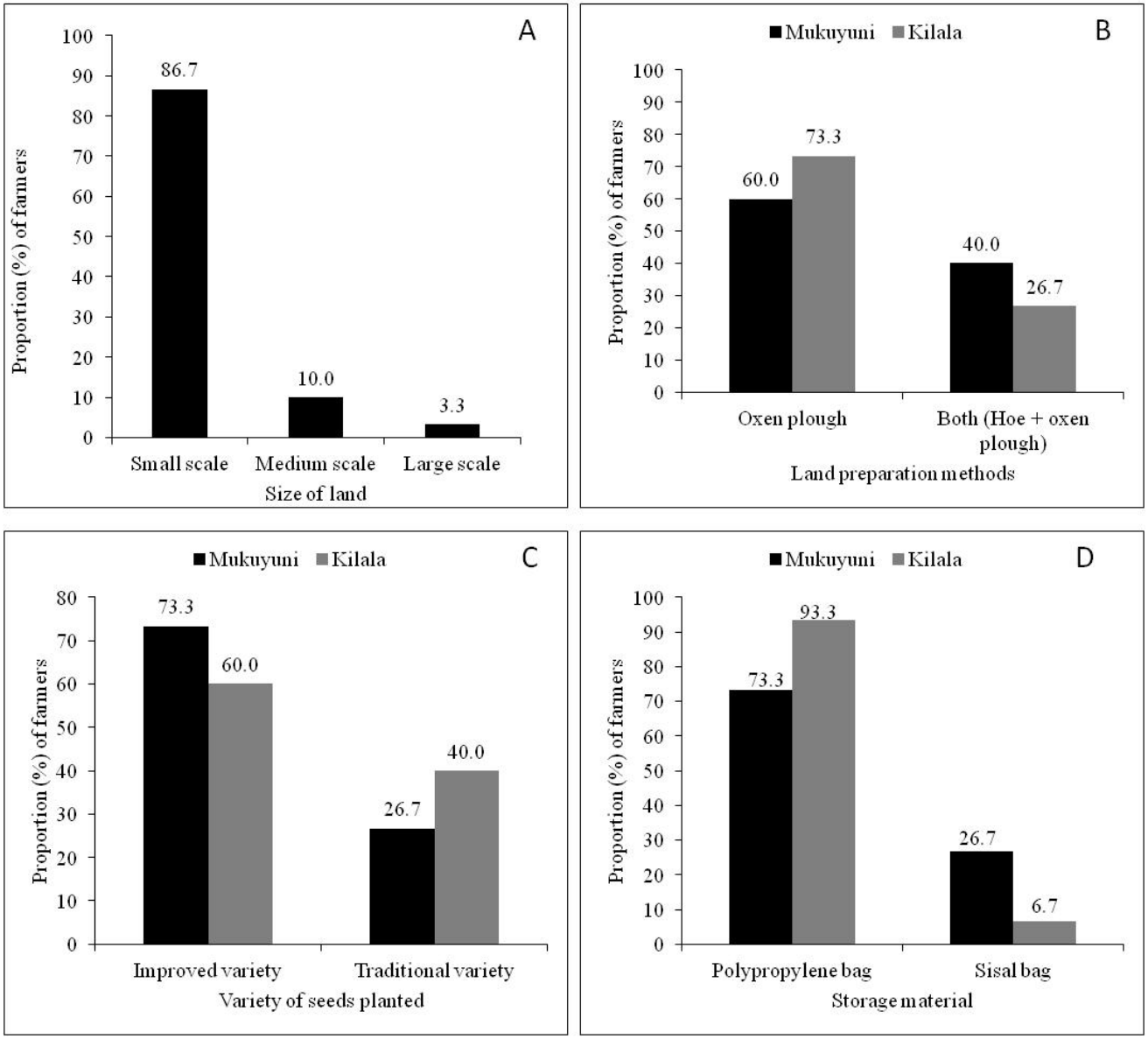


**Figure 1.** Categories of farm sizes (A), land preparation methods (B) variety of maize seeds planted (C) and maize grain storage methods (D) used by farmers in Kaiti District

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

| *Fusarium* spp. | Mukuyuni Location | | Kilala Location | |
|---|---|---|---|---|
| | Population (CFU/g) | Incidence (%) | Population (CFU/g) | Incidence (%) |
| *F. proliferatum* | $1.5\times 10^3 \pm 471.0^a$ | $17.8 \pm 4.0^b$ | $1.2 \times 10^3 \pm 175.8^a$ | $32.2 \pm 5.3^a$ |
| *F. oxysporum* | $1.5 \times 10^3 \pm 194.9^a$ | $35.8 \pm 5.6^a$ | $1.0 \times 10^3 \pm 152.3^a$ | $31.1 \pm 5.1^a$ |
| *F. solani* | $7.6 \times 10^2 \pm 206.4^a$ | $13.3 \pm 3.5^b$ | $1.1 \times 10^3 \pm 280.0^a$ | $22.1 \pm 4.6^{ab}$ |
| *F. subglutinans* | $7.8 \times 10^2 \pm 161.9^a$ | $19.8 \pm 4.9^b$ | $6.9 \times 10^2 \pm 161.5^a$ | $15.7 \pm 3.9^b$ |
| 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.

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

| Location | Bag type | *F. proliferatum* | *F. verticillioides* | *F. oxysporum* | *F. subglutinans* |
|---|---|---|---|---|---|
| Mukuyuni | At harvest [a] | $5.3 \times 10^2 \pm 163.9^b$ | $4.0 \times 10^2 \pm 169.4^b$ | $4.4 \times 10^2 \pm 251.4^b$ | $3.1 \times 10^2 \pm 148.5^b$ |
| | PICS bag | $1.9 \times 10^3 \pm 576.8^{ab}$ | $1.7 \times 10^3 \pm 521.9^{ab}$ | $9.6 \times 10^2 \pm 277.8^{ab}$ | $8.7 \times 10^2 \pm 228.3^{ab}$ |
| | PP bag | $4.0 \times 10^3 \pm 1141.0^a$ | $2.5 \times 10^3 \pm 685.8^a$ | $2.0 \times 10^3 \pm 565.8^a$ | $1.0 \times 10^3 \pm 248.1^a$ |
| | Mean | 2148.1 | 1533.3 | 1118.5 | 740.7 |
| | LSD ($P \leq 0.05$) | 2095.2 | 1426.8 | 1100.9 | 598.0 |
| | CV% | 15.7 | 16.7 | 25.3 | 17.3 |
| Kilala | At harvest [a] | $1.6 \times 10^3 \pm 507.0^a$ | $1.4 \times 10^3 \pm 413.4^b$ | $2.4 \times 10^3 \pm 621.3^a$ | $1.1 \times 10^3 \pm 322.3^b$ |
| | PICS bag | $2.4 \times 10^3 \pm 455.3^a$ | $1.9 \times 10^3 \pm 459.9^b$ | $1.0 \times 10^3 \pm 482.0^a$ | $8.7 \times 10^2 \pm 221.6^b$ |
| | PP bag | $2.3 \times 10^3 \pm 382.4^a$ | $3.9 \times 10^3 \pm 716.5^a$ | $2.6 \times 10^3 \pm 865.2^a$ | $2.1 \times 10^3 \pm 454.2^a$ |
| | Mean | 2303.7 | 2392.6 | 2014.8 | 1355.6 |
| | LSD ($P \leq 0.05$) | 1354.3 | 1539.9 | 1902.7 | 973.3 |
| | CV% | 14.4 | 5.4 | 5.1 | 14.3 |

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. PICS- Purdue Improved Crop Storage bags, PP- woven polypropylene bags.

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

| Location | Bag type | *F. proliferatum* | *F. verticillioides* | *F. oxysporum* | *F. subglutinans* |
|---|---|---|---|---|---|
| Mukuyuni | At harvest [a] | $19.7 \pm 5.8^a$ | $7.6 \pm 4.6^b$ | $6.9 \pm 3.4^a$ | $5.7 \pm 2.7^b$ |
| | PICS bag | $20.8 \pm 5.6^a$ | $24.1 \pm 5.8^a$ | $17.8 \pm 4.7^a$ | $10.7 \pm 2.9^{ab}$ |
| | PP bag | $35.1 \pm 6.3^a$ | $14.4 \pm 4.2^{ab}$ | $17.7 \pm 4.9^a$ | $17.3 \pm 4.6^a$ |
| | Mean | 25.1 | 15.5 | 14.1 | 11.2 |
| | LSD ($P \leq 0.05$) | 16.6 | 12.7 | 12.4 | 9.9 |
| | CV (%) | 8.6 | 9.0 | 2.8 | 22.2 |
| Kilala | At harvest [a] | $13.2 \pm 3.5^b$ | $17.2 \pm 4.7^b$ | $23.0 \pm 5.0^a$ | $15.5 \pm 4.5^a$ |
| | PICS bag | $40.1 \pm 6.2^a$ | $22.2 \pm 4.7^{ab}$ | $8.6 \pm 3.6^b$ | $14.8 \pm 4.3^a$ |
| | PP bag | $23.6 \pm 4.2^b$ | $32.7 \pm 4.7^a$ | $14.8 \pm 3.5^{ab}$ | $20.1 \pm 4.2^a$ |
| | Mean | 25.6 | 24.0 | 15.5 | 16.8 |
| | LSD ($P \leq 0.05$) | 13.3 | 13.2 | 11.4 | 12.2 |
| | CV (%) | 21.1 | 5.5 | 18.8 | 4.7 |

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

## 3.3. Efficacy of PICS Bags on the Population of *Fusarium* spp. in Maize

Four *Fusarium* spp. were commonly isolated from maize grains sampled at harvest and after three months of storage in PICS and PP bags (Table 3). *Fusarium* spp. isolated from maize grains in order of decreasing population were: *F. proliferatum* (Mean = 2.1 $x10^3$ CFU/g), *F. verticillioides* (Mean = 2.0 $x10^3$ CFU/g), *F. oxysporum* (Mean=1.6 $x10^3$ CFU/g) and *F. subglutinans* (Mean = 1.0 $x10^3$ CFU/g) (Table 3). The population of *Fusarium* spp. was significantly ($p \leq 0.05$) higher in maize sampled after storage compared to samples obtained at harvest. The type of storage bag significantly influenced ($p \leq 0.05$ the population of *Fusarium* spp., which was 74.6% higher in maize stored in PP bags than in PICS bags.

## 3.4. Efficacy of PICS Bags on the Incidence of *Fusarium* spp. in Maize

The incidence of *Fusarium* spp. isolated in maize sampled at harvest and three months after storage in decreasing order was: *Fusarium proliferatum* (Mean incidence = 25.4%), *F. verticillioides* (Mean incidence = 19.7%), *F. oxysporum* (Mean incidence = 14.8%) and *F. subglutinans* (Mean incidence = 14%) (Table 4). *Fusarium proliferatum* (Mean incidence = 16.5%) was the most prevalent in harvested and stored maize grains (Mean incidence = 30%). The incidence of *Fusarium* spp. increased by 45.3 % and 60.6% in PICS and PP bags, respectively after three months of storage. There was significant variation ($p \leq 0.05$) in the incidence of *F. proliferatum*, *F. verticillioides* and *F. subglutinans* in maize sampled at harvest and after

three months storage. However, the incidence of *F. oxysporum* was not variable between harvested and stored maize grains. The type of storage bag had a significant influence ($p \leq 0.05$) on the incidence of *Fusarium* spp. which was 28.4 % higher in PP bag than in PICS bag type. Overall, the incidence of *F. proliferatum* was 3.8% higher in PICS bag than in PP bag type.

### 3.5. Efficacy of Hermetic Storage on Fumonisin Levels in Maize

Fumonisin levels in maize grains sampled at harvest and after three months storage in PICS and PP bags ranged from < 2 to 6.0 ppm (Table 5). The percentage of maize grains sampled at harvest that met different thresholds for total fumonisin set by various regulatory bodies was as follows: ≤ 2 ppm set by the European Commission (EC) (96.7%) and ≤ 4 ppm set by the US Food and Drug Administration (FDA) (100%). Maize grains stored in PP bags were more contaminated with total fumonisin (Mean = 2.1 ppm) compared to grains stored in PICS bags (Mean = 0.9 ppm). Storage of maize in PICS bags reduced fumonisin contamination by 57.1% compared to PP bags. Seventy three percent and 93.3% of the maize grains stored in PP and PICS bags, respectively met the EC threshold for total fumonisin (≤ 2 ppm). On the other hand, 86.7% and 100% of the maize grains stored in PP and PICS bags, respectively met the FDA threshold for total fumonisin (≤ 4 ppm) (Table 5).

### 3.6. Correlation between the Population of *Fusarium* spp. and Fumonisin Levels in Maize

There was a positive significant ($p \leq 0.05$) correlation between the population of *F. proliferatum* and fumonisin levels, and a highly significant ($p \leq 0.001$) positive correlation between the population of *F. verticillioides* and fumonisin levels in maize sampled at harvest (Table 6). There was a highly significant positive correlation ($p \leq 0.001$) between the population of *F. verticillioides* and fumonisin levels in maize obtained three months after storage (Table 6). A significant positive correlation ($p \leq 0.05$) between the population of *F. subglutinans* and fumonisin levels in maize sampled after storage was also observed (Table 6).

**Table 5. Fumonisin contamination in maize at harvest and three months after storage in polypropylene and hermetic bags in Kaiti District**

| Region | Bag type | Percent of samples in each range | | | Range (ppm) | Fumonisin level (ppm)[b] |
|---|---|---|---|---|---|---|
| | | < 2 | 2- 4 | > 4 | | |
| Mukuyuni | At harvest [a] | 93.3 | 6.7 | 0.0 | 0 - 2.3 | 0.5 |
| | PP bag | 80.0 | 13.3 | 6.7 | 0 - 4.5 | 1.2 |
| | PICS 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 |
| | PP bag | 66.7 | 13.3 | 20.0 | 0 - 6.0 | 2.1 |
| | PICS bag | 86.7 | 13.3 | 0.0 | 0 - 2.8 | 0.9 |
| | Mean | 87.7 | 7.8 | 4.5 | 0 - 6.0 | 0.9 |

[a] – Maize grains sampled at harvest; [b] – Mean fumonisin concentration; ≤ 2- European commission limits for total fumonisins; ≤ 4 – Food and Drug Administration limits for total fumonisin; PICS- Purdue improved crop storage bags, PP- woven polypropylene bags.

**Table 6. Correlation between the population of *Fusarium* spp. and fumonisin levels in maize grains sampled at harvest and after three months storage**

| | | *F. proliferatum* | *F. verticillioides* | *F. oxysporum* | *F. subglutinans* | Fumonisin |
|---|---|---|---|---|---|---|
| At harvest | *F. proliferatum* | - | | | | |
| | *F. verticillioides* | 0.7354** | - | | | |
| | *F. oxysporum* | 0.7569** | 0.5771** | - | | |
| | *F. subglutinans* | 0.3665* | $0.1555^{ns}$ | 0.5411** | - | |
| | Fumonisin | 0.3616* | 0.4665** | $0.3047^{ns}$ | $-0.0151^{ns}$ | - |
| Three months storage | *F. proliferatum* | - | | | | |
| | *F. verticillioides* | $0.0035^{ns}$ | - | | | |
| | *F. oxysporum* | $-0.1115^{ns}$ | $0.0207^{ns}$ | - | | |
| | *F. subglutinans* | $0.0269^{ns}$ | $0.3101^{ns}$ | 0.4449* | - | |
| | Fumonisin | $0.3598^{ns}$ | 0.4755** | 0.5313** | 0.3793* | - |

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

## 4. Discussion

Data from the field survey showed that maize farmers in Kaiti District were mostly smallholder who owned between one and 10 acres of land. Studies in Eastern Kenya [30] and in Ghana [31] reported that majority (> 50%) of farmers were smallholder with an average farm size of 2 ha. Over 50% of the farmers in Kaiti District used oxen ploughs in land preparation while a few used hand hoes. This observation concurs with findings in Zimbabwe [32] that majority of maize farmers used conventional tillage methods where ox-drawn ploughs

were used in land preparation. Tillage practices have a direct impact on fungal survival and accumulation of their inocula. Reduced tillage leaves some crop residues on the soil surface which results in accumulation of fungal inoculum [33]. Reduced tillage practices such as the use of hand hoe could be the reason for the high occurrence of *Fusarium* spp. in the soil in Kaiti district. Therefore, intense tillage practices that incorporate crop residues (a primary source of inoculum for fungi) in the soil should be adopted by farmers.

Most of the farmers obtained their maize seeds from the Agro-shop. However, some of the small scale farmers used their own saved seeds from previous seasons. Studies carried out in Ghana reported that most maize farmers were smallholder who saved seeds as planting material for the following season [31]. Other studies in Western Kenya reported that most (78%) farmers saved their own seed for planting the next season while a small proportion obtained their seeds from markets, neighbours and the formal seed sector [1]. In this study, most farmers used polypropylene bags to store maize, while a few used sisal bags. This concurs with previous studies that reported that smallholder farmers pack their dry maize grains in woven polypropylene bags after harvest [20]. Studies have reported that sack storage method was inadequate for protecting maize against fungal infection because of buildup of high humidity and moisture content [34]. In this study therefore, packing maize in woven polypropylene bags might have influenced infection of stored maize grains with *Fusarium* spp. This could be attributed to retention of heat and moisture associated with polypropylene bags which influence *Fusarium* growth and fumonisin contamination.

The major *Fusarium* spp. isolated from soil were: *F. proliferatum, F. oxysporum, F. subglutinans* and *F. solani*. A similar diversity of *Fusarium* spp. was reported [3] in soils sampled from Taita Taveta County, Kenya. The high population of *Fusarium* spp. such as *F. proliferatum* in soils from Kaiti district implies that they are important pathogens that infect maize and consequently play a major role in contamination of maize with mycotoxins mainly fumonisins. In the current study, *F. oxysporum* and *F. proliferatum* were the most prevalent *Fusarium* spp. in soil. A previous study in Eastern Kenya reported that *F. oxysporum* was the most prevalent (60.9%) in soil, followed by *F. solani* at 16.3% [35]. The high prevalence of *Fusarium* spp. could be explained by the fact that *Fusarium* spp. can survive as thick-walled chlamydospores in soil [4], which act as a source of primary inocula when moisture and temperature are favorable for fungal infection of the crop.

*Fusarium proliferatum, F. verticillioides, F. oxysporum* and *F. subglutinans* were isolated from maize grains sampled at harvest. Similar observations were made in maize sampled from Eastern Kenya [15]. *Fusarium* spp. are mainly considered field fungi and thus their high occurrence in maize obtained at harvest could be attributed to pre-harvest infections, improper drying as well as unhygienic conditions during handling. Likewise, *F. proliferatum, F. verticillioides, F. oxysporum* and *F. subglutinans* were isolated from maize grains sampled three months after storage in PICS and PP bags. The high population of *Fusarium* spp. in maize sampled at harvest might have influenced the population in storage. A similar diversity of *Fusarium* spp. was observed in stored maize grains sampled from Nandi County, Kenya [36]. In the current study, *F. proliferatum* and *F. verticillioides* were the most predominant *Fusarium* spp. in harvested and stored maize grains. A study in Western Kenya reported that *F. verticillioides, F. graminearum* and *F. subglutinans* were the most prevalent *Fusarium* spp. in maize grains sampled at harvest [17]. In a study in Eastern Kenya [14], *F. verticillioides* (39.9%) was the most prevalent followed by *F. proliferatum* (15%) in stored maize grains. Other studies on maize mycoflora in South Africa also reported that *F. verticillioides* was the most frequently isolated species occurring in stored maize grains [37]. *Fusarium proliferatum* and *F. verticillioides* are the major producers of fumonisins in maize grains and therefore their predominance in Kaiti district might have influenced fumonisin contamination of maize. This concurs with previous reports that associated the aforementioned *Fusarium* spp. isolated from maize grains to fumonisin contamination [7,9,10].

In this study, the population of *Fusarium* spp in maize increased from harvest to sampling after three months of storage in PP and PICS bags, however the population was significantly higher in maize stored in PP bags compared to PICS bags. This concurs with previous studies that reported an increase in the population of *Fusarium* spp. in both hermetic and non-hermetic systems, although the increase was less in the hermetic system [38]. A previous study [39] reported that *Fusarium* counts in conventional storage bags increased up to the third month of storage, followed by a progressive reduction in counts while for the hermetic bags, the counts oscillated followed by a decrease in the number after twelve months of storage. The high population of *Fusarium* spp. in PP bags implies that despite *Fusarium* being a field fungus it continues to grow in storage and could produce mycotoxins when conditions are conducive.

The incidence of *Fusarium* spp. increased from harvest to sampling after three months of storage in PICS and PP bags, however the incidence was significantly lower in PICS bags compared to PP bags. Previous studies have reported that the incidence of *Fusarium* spp. increased with increase in storage period from two weeks to two months [40]. Similarly, in non-hermetic system, a higher incidence of Fusarium spp. was observed, ranging from 0 to 16%, while in the hermetic system, it varied from 0 to 4.1% during 30 days of storage [38]. Other findings have reported a higher frequency of *Fusarium* spp. (40%) in maize grains stored in hermetic bags [9]. In contrast, the incidence of *Fusarium* spp. in maize kernels stored in steel mesh silos, decreased after harvest and during storage, with loss of inoculum viability at 112 days after harvest because of low oxygen rates [41]. In this study therefore, the low population and incidence within the hermetic bags could be attributed to an increase in $CO_2$ concentration and a drop in $O_2$ which creates bio-generated atmospheres unfavorable for fungal growth and survival [21].

There were varying levels of fumonisin contamination in maize grains sampled at harvest and three months after storage in PICS and PP bags. About 96.7% of the maize grains sampled at harvest met the regulatory threshold set by the European Commission ($\leq$ 2 ppm) for total fumonisin while all the samples met the standard set by

the US Food and Drug Administration (≤ 4 ppm). In a similar study, the mean fumonisin content in maize samples from Makueni and Kitui Districts was 1.2 µg/g and 0.9 µg/g, respectively [14]. The contamination of maize grains sampled at harvest with fumonisin could be attributed to prevailing environmental conditions and initial *Fusarium* infection of maize prior to harvest. Other factors such as delayed harvesting and improper harvest practices might have increased the risk of maize contamination with fumonisin [29].

There was significant increase in the levels of fumonisin in maize sampled after three months of storage in PICS and PP bags in the current study. This concurs with findings in Argentina indicating an increase in fumonisin levels in stored maize [42]. In contrast a decrease in fumonisin levels in maize grains stored for 6 months was reported [10]. Maize stored in woven PP bags was 40% more contaminated with fumonisins compared to samples stored in PP bags. The high levels of fumonisin in PP bags could be attributed to pre-storage contamination by *Fusarium* spp. [8]. Moreover, poor storage conditions associated with high temperature and high moisture content of grains contributed to high levels of fumonisin contamination [42]. 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, PICS bags effectively reduced fumonisin levels by 57.1 % as compared to PP bags. This could be attributed to decrease in oxygen levels in hermetic bags, as the grain, fungi and other microorganisms respire in the confined environment [21]. Studies have shown that fumonisin production by *F. verticillioides* and *F. proliferatum* on maize is inhibited by 30% $CO_2$ over a range of moisture (aw) levels, in sealed storage systems [43].

Results of this study demonstrate that triple-layer hermetic (PICS) bags were effective in reducing *Fusarium* growth and fumonisin contamination compared to the commonly used woven PP bags. As fungi and grains respire within PICS bags, the levels of oxygen reduce drastically while carbon dioxide ($CO_2$) levels rise thus hindering development of the pathogens [22]. An increase in $CO_2$ concentration from 3% to 30% and a drop in $O_2$ in hermetic bags create conditions that are inhospitable for fungal growth and mycotoxin contamination [21]. Studies have demonstrated that low oxygen concentration and elevated levels of carbon dioxide within the hermetic bags reduced the growth of *Fusarium* spp. as well as fumonisin contamination [38]. Likewise it was previously reported [44] that elevated level of $CO_2$ was effective in reducing the population of *Fusarium* spp. in stored maize grains. Thus, a drop in $O_2$ concentration and rise in $CO_2$ levels within the enclosed system brings about the oxidative stress which reduces the rate of fungal growth and subsequent mycotoxin contamination [21]. In this study PICS bags protected maize grains better than PP bags and therefore provide an alternative management option for reducing the population of *Fusarium* spp. and fumonisin contamination in stored maize grains.

There was a positive significant correlation between the population of *F. proliferatum* and fumonisin levels, and *F. verticillioides* and fumonisin levels in maize sampled at harvest. A significant correlation between fumonisin levels and *Fusarium* spp. was observed in maize grains sampled at harvest [10]. Other studies in Poland reported that the levels of fumonisin B1 in maize grain were positively correlated with the occurrence of *F. verticillioides* [47]. There was a significant positive correlation between the population of *F. verticillioides* and fumonisin and *F. subglutinans* and fumonisin levels in maize sampled after storage. A similar positive significant correlation between *F. verticillioides* and fumonisins was reported [37]. The positive correlation could be as a result of favourable environmental conditions, initial contamination of maize by *Fusarium* spp. and poor storage conditions which promote *Fusarium* growth and fumonisin contamination.

## 5. Conclusion

*Fusarium* growth and fumonisin contamination of maize results in huge quantity and quality loses when storage conditions are sup-optimal. Storage of maize in PP bags did not suppress *Fusarium* growth and fumonisins contamination since storage conditions within PP bags were favourable for fungal growth. Storing maize in PICS bags reduced the population of *Fusarium* spp. and levels of fumonisin. This implies that the hermetic storage conditions were effective in reducing the population of *Fusarium* spp. and levels of fumonisin in maize grains. Therefore, triple-layer hermetic bags are a good option for long term storage of maize and protection against fungal attack and mycotoxin contamination. Smallholder farmers in Kenya should therefore be encouraged to store their maize in PICS bags as adoption of this technology will offer an effective and long term preservation of high quality grains.

## Competing Interests

The Authors have no competing interest.

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