Journal of Stored Products Research 69 (2016) 119–128

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Effect of triple-layer hermetic bagging on mould infection and aflatoxin contamination of maize during multi-month on-farm storage in Kenya

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
22 July 2016
Accepted 26 July 2016

Keywords:
Maize
On-farm storage
Hermetic bags
Mould
Aflatoxin

ABSTRACT

Field trials were conducted in small-scale farmers' grain stores in an aflatoxin endemic region to assess the effect of storing maize in triple layer hermetic (PICS™) bags on aflatoxin contamination. Shelled maize grain was purchased from farmers, and filled into PICS bags, woven polypropylene (PP) and jute bags and kept in the farmers' own stores for 35 weeks. Grain moisture content, total mould count and mould incidence levels were examined at onset and after every 7 weeks during the 35 weeks of storage. Aflatoxin contamination was examined at onset, and after 14, 28 and 35 weeks. Ambient temperature and r.h. in the trial site and in all the bags, as well as oxygen and carbon dioxide levels in the PICS bags were also monitored. Initial moisture content (m.c.) of maize varied from farmer to farmer and ranged between 12.4 and 15.0%. The m.c. of maize stored in PICS bags remained significantly higher ($P < 0.05$) than in PP and jute bags in the last 14 weeks of storage. Total mould count and aflatoxin contamination of maize stored at an initial m.c. < 13% and 13% ≤ m.c. ≤ 14% increased significantly in PP and jute bags but not in PICS bags. After 35 weeks, total aflatoxin of maize stored in the PICS bags at an initial m.c. < 13% and 13% ≤ m.c. ≤ 14% did not change where as it increased 5–8 folds in the PP and jute bags. Total mould count and aflatoxin contamination of maize stored at an initial m.c. > 14% increased profusely in the three types of bags. Our findings demonstrate that storing maize in PICS bags can prevent accumulation of aflatoxin in rural farmers' stores if grain moisture is <14%.

1. Introduction

Maize (*Zea mays* L.) is a main food and income crop for many households in Sub-Saharan Africa. As a food resource, it accounts for 40% of total dietary intake in Eastern and Southern Africa (Doss et al., 2003; Kimanya et al., 2008). The bulk of production is carried out by small-scale farmers who cultivate less than 5 ha of the crop annually due to resource constraints. However, biotic and abiotic factors, especially after harvesting, contribute to losses in quantity, quality (safety and nutritional value), and economic value of the grain available for consumption or trade (World Bank, 2010). A main biotic cause for postharvest losses in maize is mould infection. Maize becomes infected at any stage of production including cultivation, harvesting, drying, storage, transportation, and marketing. A variety of moulds such as *Fusarium*, *Aspergillus*, and *Penicillium* spp are often involved (Quezada et al., 2006; Blandino et al., 2009; Chulze, 2010). The infection not only reduces quality of the maize through discoloration and reduction of nutritional value (Ehrlich, 2007), but also culminates in deposition of toxic metabolites when the colonizing fungi are mycotoxigenic, and the conditions favour production of the toxins (Bennet and Klich, 2003; Wagacha and Muthomi, 2008).

Stored maize may be infected by three main aflatoxigenic species of the genus *Aspergillus*, namely, *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius* (Peraica et al., 1999; Guo, 2000). Aflatoxin contamination of maize is almost exclusively by *A. flavus*, which produces aflatoxin B1 and B2 (Mutungi et al., 2008). Typically, *A. flavus* grows optimally at 25 °C with a minimum water activity (a_W) of 0.75 (Parry, 1990; Oladiran and Iwu, 1993), but the

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http://dx.doi.org/10.1016/j.jspr.2016.07.005
0022-474X/

optimal conditions for subsequent production of aflatoxin include moisture content above 14%, temperature of 28–30 °C, and a_W of 0.83–0.97 (Oladiran and Iwu, 1993). The oxygen - carbon dioxide ratio, physical integrity of the grain, initial level of *A. flavus* infection, presence of competing moulds, pest activity, and genetic properties of the grain have also been reported to determine the degree of contamination and subsequent aflatoxin contamination (Diener et al., 1987).

Contamination of maize and other food commodities with aflatoxins is of great public health concern because of the ability of aflatoxins to cause human and animal diseases (CDC, 2004; Gong et al., 2004). Aflatoxin has been implicated in acute and chronic aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of the immune system, aggravation of kwashiorkor, and impaired childhood growth (Hall and Wild, 1994). In Kenya, outbreaks of acute human aflatoxicosis occur frequently especially with respect to maize, the dietary staple to over 85% of the population, and are well documented (Ngindu et al., 1982; CDC, 2004; Azziz-Baumgarter, 2005; Lewis et al., 2005). In particular, aflatoxin contamination is more prevalent in the tropical and subtropical regions due to the warm humid conditions (Choudhary and Sinha, 1993; Cotty et al., 1994). Aflatoxigenic fungi may infect the maize crop before harvest and remain associated with the kernel through harvesting and storage (Cotty, 1990). Thus, contamination is likely to continue in the postharvest stage if the produce is not handled or stored properly to minimize the growth of these fungi (Wilson and Abramson, 1992).

Chemical-free hermetic storage technologies that have less destructive impact to environment and human health may offer safe and cost-effective protection of stored grains against mould infection and aflatoxin contamination (Williams et al., 2014). One such technology is the Purdue Improved Crop Storage (PICS®) triple-layer hermetic storage bag which applies a two-layer envelope made of 80 μm thick high density polyethylene (HDPE) liners inserted in an outer woven polypropylene sack. The HDPE liners have low permeability to air, and are thus able to secure a modified low oxygen and high carbon dioxide atmosphere generated by respiration of the grain, insects and other life-forms enclosed when the bag is sealed. This action stops damage of the stored produce by insect pests (Murdock et al., 2012). A concern regarding hermetically stored maize, relates to proliferation of moulds leading to aflatoxin contamination because of the possibility of moisture build-up in the impermeable enclosures during multi-month storage. Some findings reported that under hermetic storage, fungistatic effect is induced when oxygen concentration drops to 1% or below (Richard-Molard, 1988). Other findings, however, reported that mycotoxigenic fungi can develop in maize samples (m.c. 13–25.1%) stored in hermetic plastic bags with the potential risk of contamination with aflatoxins and fumonisins (Castellari et al., 2010). The aim of this study was to investigate the effect of PICS bag storage on stored maize quality, based on mould proliferation and aflatoxin contamination. Mould infection and total aflatoxin levels of maize packed in PICS, PP and jute bags were compared during long-term storage under farm conditions in an area that is endemic to aflatoxin contamination.

2. Materials and methods

2.1. Trial site, timing and experimental conditions

Storage trials were conducted with individual small-scale farmers in 9 villages of Kibwezi (1036 M, 02° 22.888′S, 37° 57.088′E), Machinery (1004 M, 02° 54.078′S, 37° 28.337′E), and Makindu divisions (1019 M, 02° 18.464′S, 37° 49.772′E) in Makueni County, Eastern Kenya. The trial site was selected because it is a hotspot for aflatoxin outbreaks in Kenya. The region receives a bimodal rainfall pattern in March–May (long rains; harvesting, July–August) and October–December (short rains; harvesting, February–March). The annual rainfall ranges between 200 and 700 mm while day time temperatures range between 20 and 30 °C. The trials were conducted over a period of 35-weeks beginning May 2014 to February 2015, and covered the typical maize storage cycle which spans 8–9 months starting shortly after the short rains harvest season. A total of 33 farmers (3–4 farmers in each village) who had a harvest of about five 90 kg bags of maize, and who also expected to store part of it, were recruited to participate in the trials. A rapid appraisal using semi-structured questionnaire was conducted to capture data on storage practices of the farmers.

2.2. Materials

One bag of 100 kg of shelled maize grain which had not been treated with insecticide or mixed with indigenous grain admixtures (wood ashes, animal dung, and botanical protectants) was purchased from each participating farmer. Each farmer also provided storage structure in the homestead. Jute and PP bags of 50 kg capacity were purchased from a grain dealer in Nyamakima market in Nairobi, Kenya. The PICS™ bags (50 kg) were supplied by Lela Agro Industries Limited (Kano, Nigeria).

2.3. Bagging, storage and sampling

Each 100 kg bag of maize was sieved through a 2 mm aperture sieve to remove any insects, dirt and other debris, and subdivided into three equal portions by weight. The three portions were randomly filled into PICS™, PP or jute bags. An EL-USB-2 data logger (Lascar electronics Inc., Pennsylvania, USA), programmed to record data every 1 h, was placed in each of the storage bag to record the temperature, r.h. and dew point conditions during the storage period. The bags were then sealed by firmly twisting the open end, and fastening with sisal twine, and placed on wooden planks in the farmer's store. To record the temperature, r.h., and dew point conditions of the local environment, another EL-USB-2 data logger was placed at an open strategic place in the compound of at least one farmer in each village.

Sampling was done during trial set-up (baseline data) and subsequently at seven-week intervals. Before opening the PICS bags, oxygen and carbon dioxide levels were measured using a portable Mocon Pac Check Model 325 oxygen/carbon dioxide analyzer (MOCON Inc., Minneapolis, USA) fitted with a 20-gauge hypodermic needle for sampling inside the bag. To take gas composition measurements, the inner HDPE liner was punctured with the analyzer needle at the top, middle and bottom. Needle holes were then immediately sealed with plastic adhesive tape after taking the readings. Subsequent measurements were performed from the same spot by lifting and replacing the tape. To obtain samples for examination of quality parameters, the bags were opened and a composite sample of 500 g of maize from each storage bag was drawn from five random points by pushing a two-inch diameter hollow tube sampler from the top of the bag. The 500 g sample from each storage bag was thoroughly mixed and about 125 g sub-sample was randomly separated by coning and quartering method to be used in determination of total mould counts and mould incidence levels. The remaining portion of the sample (about 375 g) was used to determine moisture content after which it was milled into a fine powder using a laboratory-scale Knife Mill Cup KM 400 MRC Lab (MRC International, Westminster, UK). A portion of milled sample (100 g) was drawn and stored at −15 °C awaiting aflatoxin analysis.

2.4. Determination of grain moisture content

A Dickey-John mini GAC® plus moisture tester (DICKEY-john Corporation, Illinois, USA) calibrated on the basis of U.S. Federal Grain Inspection Service (FGIS) moisture content meters calibration was used. Maize grain sample was filled into the tester cup, levelled off and the moisture content read directly and recorded.

2.5. Determination of total mould count and mould incidence levels

Total mould count was determined using dilution plating (Pitt and Hocking, 1997) on Sabourand Dextrose Agar, SDA: (enzymatic digest of casein 5 g, enzymatic digest of animal tissue 5 g, dextrose 40 g, agar 15 g in 1000 mL distilled water; pH 5.6 ± 0.2 at 25 °C) modified with 20 mg chloramphenicol (SDA-C). Maize kernels (10 g) were added in 90 mL of sterilized peptone water in 200 mL conical flask and mixed thoroughly by shaking. Then, 1 mL was drawn and added into 9 mL sterile peptone water and serially diluted to a dilution of 10^{-4}. Duplicate of 0.1 mL aliquots of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were spread-plated on SDA-C Agar and incubated at 25 °C for 3 d. The number of colonies in plates bearing 10–100 were enumerated and reported as number of colony forming units per gram (cfu/g).

Determination of mould incidence levels was done using direct plating technique for internal infestation (Pitt and Hocking, 1997) on Czapek-dox Agar: (Sucrose 30 g, Sodium nitrate 2 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g, agar 15 g in 1000 mL distilled water; pH 7.3 ± 0.2 at 25 °C). One hundred maize kernels were randomly taken from each sample. The kernels were surface-sterilized for 2 min in NaOCl (2%) and rinsed twice with sterile distilled water. The kernels were then plated on Czapek-dox Agar plates (7–10 kernels per plate). The plates were incubated at 25 °C for 5 d, and the number of kernels showing growth of fungal species in each Petri dish counted. Fungal colonies were then isolated and subcultured on Czapek-dox Agar for 5 d and identified based on cultural and morphological characteristics as described by Watanabe (1994). The percentage of grains infected by each fungi species was calculated to determine their incidence on maize kernels.

2.6. Determination of aflatoxin contamination

Ridascreen® ELISA (Enzyme-Linked Immunosorbent Assay) kit for total Aflatoxin (R-Biopharm AG, Darmstadt, Germany) was used for quantification. The preparation procedure was as follows: milled maize samples (2 g) were weighed into a 50 mL screw cup centrifuge tube and mixed with 10 mL of methanol/distilled water (70/30 v/v). The mixture was agitated gently on a vortex mixer at room temperature for 10 min, centrifuged at 3000 × *g* and the supernatant recovered. The supernatant was diluted appropriately while ensuring that the final extract contained 10% v/v methanol. Aliquots (50 μL) of the dilute extract and equal volumes of the calibrated aflatoxin standards (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, and 4.05 ppb) were added in separate duplicate wells of anti-aflatoxin antibody coated microtitre plate. In to each well, 50 μL of enzyme conjugate was added followed by another 50 μL of antibody solution and mixed gently by tapping the plate manually. The plate was covered with aluminum foil and incubated for 30 min at room temperature (20–25 °C) in a dark cabinet after which the liquid in the plate wells was poured off and the wells filled with 250 μL washing buffer (10 mM phosphate buffer, pH 7.4 containing 0.05% Tween 20). The washing procedure was repeated twice and the wells semi-dried by tapping the plate gently on adsorbent paper. A hundred (100) μL of substrate/chromogen solution was added to each well, and after mixing gently the plate was incubated for 15 min at room temperature in a dark cabinet following which 100 μL of stop solution (1 mol/L sulfuric acid) was added. Absorbance of liquid in each well was measured at 450 nm using a UT-6100 auto microplate reader (MRC International, UK) within 20 min of adding the stop solution. Aflatoxin concentrations of samples were determined from a calibration curve prepared from the known standards.

2.7. Statistical analysis

To stabilize variances mould count and aflatoxin data (x) were log transformed ($Y = \log_e(x+1)$) whereas percentage data (P) (moisture content and mould incidence levels) were arcsine transformed ($(Y = \sin^{-1}\sqrt{P})$), where Y is the result of transformation. The transformed data were then subjected to analysis of variance (ANOVA) using Stata SE version 12 (StataCorp LP, Texas, USA). Further due to inherent limitations of ANOVA in describing difference in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was also applied to test effects of treatment and storage duration, and the interaction effects. When the coefficient of the interaction term was significant ($P < 0.05$), it was concluded that there was a significant difference between treatments over the storage period. One-way ANOVA was performed where treatment outcomes at a specific point in storage time needed to be compared. Means were separated using Bonferroni adjustment at 95% confidence level.

3. Results

3.1. Maize storage practices of farmers

Average maize production of farmers recruited in the present trial varied widely but majority of farmers (46.7%) harvested between 11 and 20 bags of 90 kg. At least 30% of the farmers harvested more than 20 bags while 23.3% had a small harvest of between 1 and 10 bags. Prior to shelling and storage, almost three quarters of the farmers (70%) dried their maize for approximately one week while 30% of the farmers dried for approximately 1–2 weeks in the sun. However, more than three quarter of farmers (80%) dried the maize within their homesteads while a small proportion (20%) dried at the farm and then brought the dried maize to the homestead. Slightly more than half of the farmers (53.3%) harvested and stored traditional maize varieties (*kinyanya*) whereas 36.7% of the farmers had pure improved varieties. A small proportion (10%) cultivated both traditional and improved varieties. Most of the farmers (90%) stored their maize as shelled grain while 10% stored as both dehusked cobs and shelled grain. The quantity of grain reserved for household consumption varied from farmer to farmer. More than half of the farmers (63.3%) stored between 6 and 10 bags, 20% stored between 1 and 5 bags while 16.7% stored more than 10 bags but this primarily was found to be dependent on household size. About three quarters of farmers (73.3%) stored maize for a period exceeding 7 months. A small proportion (26.7%) of farmers stored their maize for a period of less than 6 months.

Majority of farmers (66.7%) who stored shelled maize packed the grain in woven PP bags, which were then placed in granaries (*ikumbi*), but about a third of the farmers (33.3%) preferred to store maize in special rooms in the living house. The granaries were mainly raised structures constructed using wooden slats or sisal stems with either grass thatch (traditional granaries, 42.1%) or iron sheet roofing (improved granaries, 57.9%). The special rooms used for maize storage by the farmers were mainly brick wall rooms with concrete floor (100%) but farmers habitually installed raised

wooden platforms on which the bags were laid. Among the farmers who participated in the storage trial, 60% were aware of aflatoxin poisoning in the area. However, only a small proportion (10%) of these farmers attributed mould infection to storage losses. This small proportion of farmers who reported loss due to mould infections noticed mouldy grains during harvesting, drying and storage, and used it to feed chicken and livestock or disposed it together with other household wastes. Farmers who participated in this trial were also aware of good storage sanitation practices. All the farmers removed the old stock and cleaned their stores before introducing new harvest.

3.2. Moisture content of stored maize

The initial moisture content (m.c.) of maize varied from farmer to farmer. Three m.c. levels were identified as follows: m.c. < 13%, 13% ≤ m.c. ≤ 14%, and m.c. > 14%. Data was clustered into these initial m.c. levels for purpose of analysis and interpretation. Fig. 1 shows the progression of grain m.c. in PICS, PP, and jute bags over the 35 weeks of storage. For m.c. < 13%, (n = 7) maize, the average m.c. was 12.7 ± 0.1% (range: 12.4–12.9%) at start of experiment. Maize stored in PICS bags retained this m.c. throughout the storage time ($F = 0.95$; $df = 5, 36$; $P = 0.463$). Contrastingly, m.c. of maize stored in PP and jute bags started to decline from the 14th week, and reached levels that were significantly lower than in PICS bags from the 21st weeks of storage onwards ($F = 16.91$; $df = 2, 18$; $P < 0.001$). Throughout the entire storage period, m.c. of maize stored in PP and jute bags were not different (Fig. 1) and the lowest m.c. levels reached for the two types of bag were 11.1 ± 0.2% and 10.9 ± 0.2%, respectively.

For maize stored at initial 13% ≤ m.c. ≤ 14% (n = 13), the average m.c. at the start of experiment was 13.3 ± 0.1% (range: 13.0–13.8%). Maize stored in PICS bags generally retained its m.c. throughout the storage period ($F = 0.58$; $df = 5, 72$; $P = 0.712$) at about 13.3 ± 0.1%. On the contrast, m.c. of maize stored in PP and jute bags started to decline from the 7th week, and reached levels that were significantly lower than in PICS bags from the 14th weeks of storage onwards ($F = 9.16$; $df = 2, 36$; $P < 0.001$). Likewise, the m.c. of maize packed in PP and jute bags did not differ significantly throughout the entire storage period and the lowest m.c. levels attained were 11.7 ± 0.2% and 11.5 ± 0.2%, respectively.

The maize with an initial m.c. > 14% (n = 7), had an average m.c. of 14.4 ± 0.1% (range: 14.2–15.0%) at start of the experiment. As with other m.c. levels, maize stored in PICS bags retained its m.c. throughout the storage period ($F = 0.86$; $df = 5, 36$; $P = 0.517$) whereas m.c. of maize stored in PP and jute bags continued to decline reaching levels that were significantly lower than in PICS bags (13.1 ± 0.3% and 13.3 ± 0.2% respectively) in the 35th week of storage ($F = 3.72$; $df = 2, 18$; $P < 0.045$). There was also no significant difference in the m.c. of maize stored in PP and jute bags throughout the entire storage period. ANCOVA tests revealed that interaction effect between type of bag and storage period was significant for the three m.c. levels (m.c. < 13%: $F = 7.57$; $df = 10, 108$; $P < 0.001$, 13% ≤ m.c. ≤ 14%: $F = 9.61$, $df = 10, 216$, $P < 0.001$; m.c. > 14%: $F = 2.37$; $df = 10, 108$; $P < 0.014$).

3.3. Gas composition in PICS bags

Fig. 2 shows the mean oxygen and carbon dioxide concentrations in the PICS bags containing maize at three levels of m.c. From the atmospheric oxygen and carbon dioxide concentrations, that is, 21% and 0.03% respectively, the oxygen levels in PIC bags containing maize stored at an initial m.c. < 13%, dropped to 4.7 ± 0.7% in the first 7 weeks of storage whereas carbon dioxide increased to 11.2 ± 1.5%. During the rest of storage period, oxygen concentration increased gradually to 10.6 ± 0.5% while carbon dioxide averaged 8.7 ± 0.8% at 35 weeks of storage. Similar trends were observed in PICS bags containing 13% ≤ m.c. ≤ 14% maize, where oxygen levels dropped to 5.2 ± 0.2% in the first 7 weeks of storage while carbon dioxide increased to 11.0 ± 0.6%. During the rest of storage period, oxygen concentration increased gradually and averaged 10.6 ± 0.4% while carbon dioxide stabilized at 11.1 ± 0.7% in 35 weeks of storage. For the maize with an initial m.c. > 14%, oxygen levels dropped to 4.1 ± 0.6% while carbon dioxide increased to 10.2 ± 0.3% in the first 7 weeks of storage. During subsequent weeks of storage oxygen concentration increased gradually to 9.8 ± 0.4% while carbon dioxide stabilized at 12.9 ± 0.8% in 35 weeks of storage. ANCOVA results showed significant differences in oxygen ($F = 2.59$; $df = 10, 144$; $P < 0.007$) and carbon dioxide ($F = 2.22$; $df = 10, 144$; $P < 0.019$) progression patterns at the three levels of moisture.

3.4. Temperature, relative humidity and dew point condition in storage bags

Fig. 3 shows mean temperature, r.h. and dew point conditions prevailing in the trial site and in the storage bags over the 35 weeks

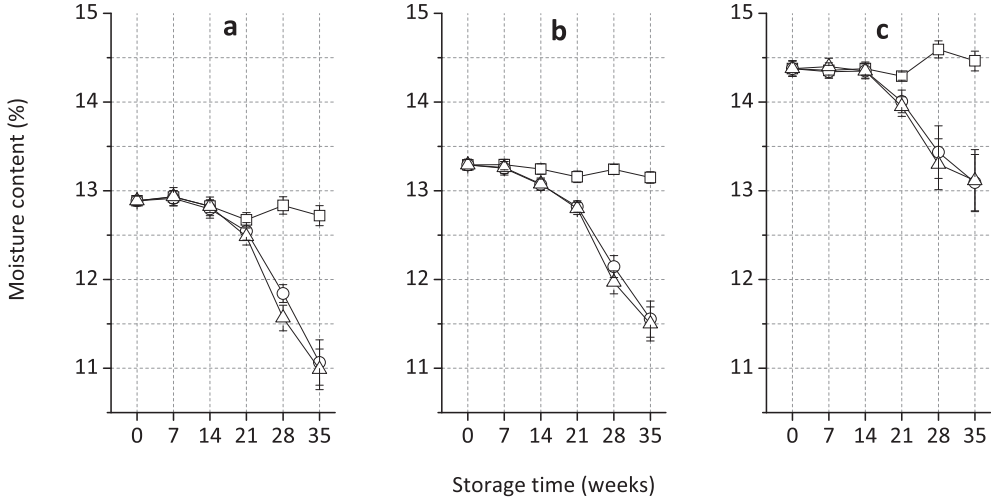

Fig. 1. Moisture content of maize stored in PICS (□),PP (○) and jute bags (Δ) at initial moisture contents of (a) m.c. < 13%, (b) 13% ≤ m.c. ≤ 14%, and (c) m.c. > 14% for 35 weeks. Storage trials were conducted in May 2014 to February 2015.

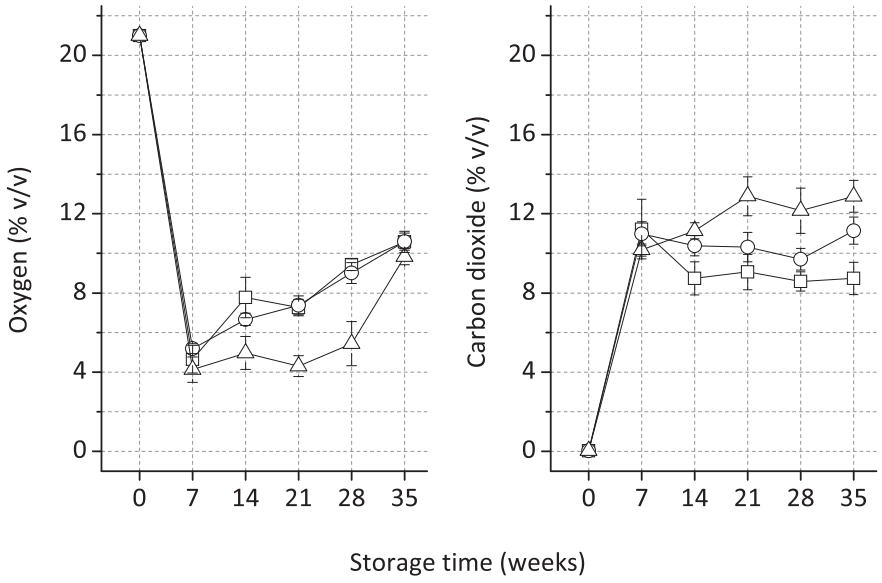

Fig. 2. Oxygen and carbon dioxide levels in PICS bags containing maize grain stored with initial moisture contents of m.c. < 13% (□); 13% ≤ m.c. ≤ 14%, (○), and m.c. > 14% (Δ) for 35 weeks. Storage trials were conducted in May 2014 to February 2015.

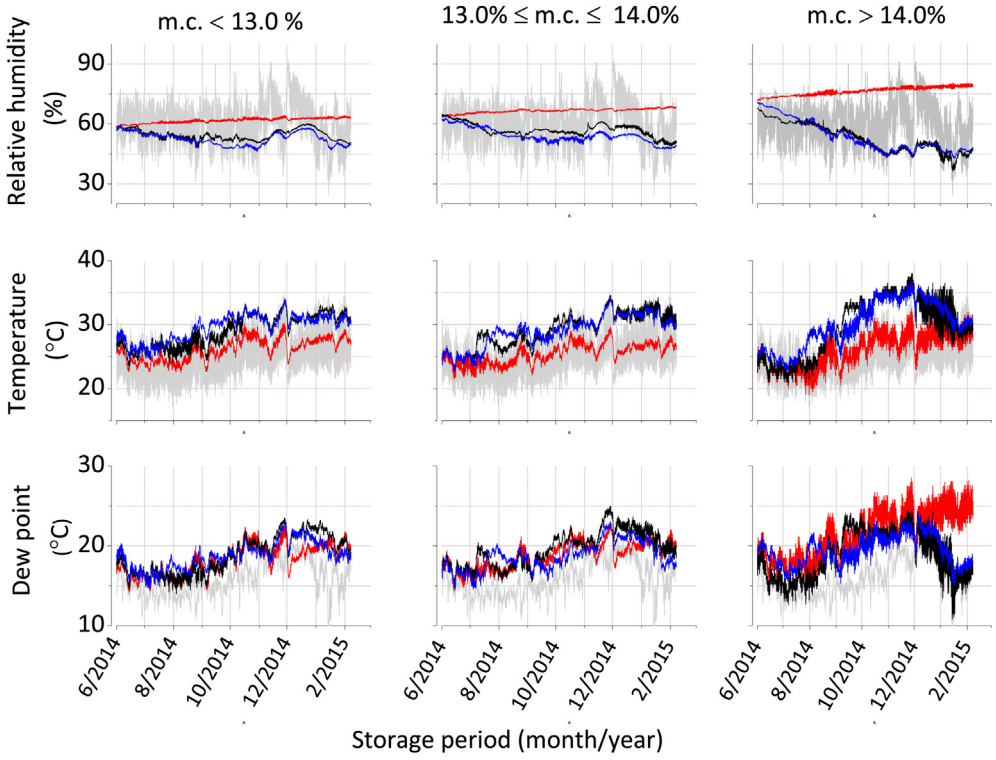

Fig. 3. Relative humidity, temperature and dew point conditions in the trial site (grey), and relative humidity, temperature and dew point conditions prevailing in PICS (red), PP (black) and Jute bags (blue) filled with maize having moisture contents of m.c. < 13%; 13% ≤ m.c. ≤ 14% and m.c. > 14%, and stored for a period of 35 weeks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of storage. The mean atmospheric temperature, r.h. and dew point were 23.9 ± 3.2 °C, 59.9 ± 11.1% and 15.9 ± 2.2 °C, respectively. These patterns were characterized by wide ranges between 17.2 and 35.2 °C (temperature), 24.4–91.5% (r.h.) and 7.9–20.7 °C (dew point). In the storage bags, temperature varied with varying atmospheric temperature. For maize stored at initial m.c. < 13%, average temperature in the PICS bags was 26.1 ± 0.4 °C. On the other hand, temperature prevailing in PP and jute bags averaged 29.9 ± 0.2 °C and 29.6 ± 0.5 °C, respectively. These temperature conditions were similar to those prevailing in bags containing maize stored at initial 13% ≤ m.c. ≤ 14%, which averaged 25.4 ± 0.5 °C in PICS bags, 29.1 ± 0.4 °C in PP bags and 29.0 ± 0.3 °C in jute bags. Regarding maize stored at initial m.c. > 14%, temperature in the PICS bags averaged 26.1 ± 0.3 °C whereas the mean temperatures were 29.7 ± 0.4 °C and 30.1 ± 0.5 °C in PP and jute bags, respectively. Generally, temperature conditions in PICS bags

remained lower than in PP or jute bags.

Relative humidity in the storage bags also varied considerably. Fairly steady r.h. levels were maintained in the PICS bags. Relative humidity of maize packed in the PICS bags at initial m.c. < 13% increased from 58.7 to 63.8% (mean 61.7 ± 1.8%) whereas the r.h. in the PICS bags containing maize at initial 13% ≤ m.c. ≤ 14% and m.c. > 14% increased from 62.9 to 68.3% (mean 66.0 ± 1.9%) and 71.5–80.5%, (mean 76.4 ± 1.9%), respectively. In each of the three storage moisture categories, r.h. was higher in PICS bags compared to PP or jute bags, in which the r.h. decreased steadily, consistent with declining moisture contents of the maize.

With regard to dew point, the temperature at which moisture condensation would occur, the maize stored at initial m.c. < 13% had dew point temperatures below 20 °C. These averaged 18.1 ± 0.3 °C (range: 14.5–22.5 °C) in PICS bags, 18.8 ± 0.3 °C (range: 14.5–22.6 °C) in PP bags, and 18.7 ± 0.4 °C (range: 15.1–22.7 °C) in jute bags. Similarly, the mean dew point temperatures in bags containing maize stored at 13% ≤ m.c. ≤ 14% were 18.7 ± 0.3 °C (range: 14.6–23.0 °C) in PICS bags, 19.0 ± 0.3 °C (range: 15.3–24.9 °C) in PP bags, 18.7 ± 0.2 °C (range: 14.9–24.0 °C) in jute bags. A significant difference among the bags occurred in the maize stored at initial m.c. > 14% where due point temperature exceeded and remained about 25 °C in the PICS bags starting from the 20th–21st weeks of storage onwards, suggesting greater likelihood of moisture to condense at ambient conditions.

3.5. Effect of storage bag on mould infection

Table 1 shows total mould counts on maize stored in PICS, PP, and jute bags in the three storage moisture levels. At onset, mould infection was three times higher in maize with m.c. > 14% than the maize at m.c. < 13%. Throughout the 35 weeks of storage, mould infection levels did not change significantly in the maize stored in PICS bags at initial m.c. < 13% ($F = 0.06$; $df = 5, 36$; $P = 0.997$) and 13% ≤ m.c. ≤ 14% ($F = 0.13$; $df = 5, 72$; $P = 0.985$). In contrast, mould count in PP and jute bags increased up to six-fold reaching levels that were significantly higher than in PICS bags (m.c. < 13%: $F = 4.51$; $df = 2, 18$; $P = 0.025$; 13% ≤ m.c. ≤ 14%: $F = 10.32$; $df = 2, 36$; $P = 0.003$) at the end of storage. For maize having m.c. > 14%, the total mould counts were not significantly different in the various storage bags ($F = 1.97$; $df = 2, 18$; $P = 0.169$).

Moulds of the genera *Aspergillus, Fusarium*, and *Pencillium* were isolated at higher frequencies. Fig. 4 shows the internal mould incidence levels in different storage bags on maize containing initial moisture of 13% ≤ m.c. ≤ 14%. Interaction effect between type of bag and storage duration was significant for *Aspergillus* spp. ($F = 2.31$; $df = 10, 162$; $P = 0.014$) and *Pencillium* spp. ($F = 3.30$; $df = 10, 162$; $P < 0.001$). In the PICS bags, incidences of *Aspergillus* spp. (9–16%) and *Penicillium* spp. (3–6%) did not change significantly with storage time ($F = 0.60$; $df = 5, 54$; $P = 0.699$; $F = 0.48$; $df = 5, 54$; $P = 0.790$). In the PP and jute bags, however, incidence levels increased up to five-fold (*Aspergillus* spp.) and seven-fold (*Penicillium* spp.), and reached significantly higher incidence levels than in PICS bags at the end of storage period ($F = 11.12$; $df = 2, 27$; $P = 0.003$; $F = 21.37$; $df = 2, 27$; $P < 0.001$). For *Fusarium* spp. there was no significant interaction effect between type of bag and storage duration ($F = 1.36$; $df = 10, 162$; $P = 0.202$). Further analysis of the main effects showed that both storage duration ($P = 0.004$) and the type of storage bag ($P = 0.007$) were significant.

Table 1
Total mould counts ($\times 10^3$ cfu/g) of maize grain stored in PP, jute and PICS bags for 35 weeks.

Treatment	Storage duration (weeks)					
	0	7	14	21	28	35
m.c. < 13%						
PICS	19.4a	19.0a	19.2a	20.4a	22.1a	21.6a
PP	19.4a	45.1a	59.8b	74.1b	91.4b	126.3b
Jute	19.4a	62.2a	70.7b	93.2b	99.2b	115.6b
13% ≤ m.c. ≤ 14%						
PICS	31.6a	34.3a	30.6a	29.1a	31.8a	25.8a
PP	31.6a	46.2a	66.7a	77.2b	111.3b	119.6b
Jute	31.6a	57.1a	68.7a	99.5b	121.2b	154.0b
m.c > 14%						
PICS	59.8a	65.9a	47.6a	67.9a	105.7a	160.3a
PP	59.8a	88.7a	111.4b	162.1b	178.5b	201.4a
Jute	59.8a	89.8a	122.9b	132.7b	198.5b	215.7a

Data are means values (m.c. < 13% n = 7, 13% ≤ m.c. ≤ 14% n = 13, m.c. > 14% n = 7). Entries in the same column followed by same letters are not significantly different ($P > 0.05$). Means were separated using Bonferroni adjustment. Storage was conducted between May 2014 and February 2015.

3.6. Effect of storage bag on aflatoxin contamination

The results of aflatoxin contamination are presented in Table 2. For maize stored at initial m.c. < 13%, interaction effect between storage duration and storage bag was not significant ($F = 0.54$; $df = 6, 72$; $P = 0.799$). However, analysis of the main effects showed that storage duration ($F = 3.13$; $df = 3, 80$; $P = 0.040$) and the type of storage bag ($F = 11.07$; $df = 2, 81$; $P = 0.001$) were significant. For the maize stored at 13% ≤ m.c. ≤ 14%, a significant interaction effect between type of bag and storage duration was observed ($F = 2.47$; $df = 6, 144$; $P = 0.026$), and as with maize stored at m.c. < 13%, no significant change in aflatoxin contamination was noticed in the PICS bags throughout the 35 weeks of storage ($F = 0.24$; $df = 3, 48$; $P = 0.865$). In contrast, aflatoxin contamination increased in PP and jute bags and reached levels that were significantly higher than in PICS bags from the 14th week onwards. In addition, aflatoxin contamination levels in PP and jute bags did not differ significantly (Table 2). In maize stored at initial m.c. > 14%, interaction effect between the type of bag and storage duration was not significant ($F = 0.14$; $df = 6, 72$; $P = 0.991$). Analysis of the main effects showed that storage duration was significant ($P < 0.001$) but the type of storage bag was not ($P = 0.525$). Thus, aflatoxin contamination increased significantly with storage time (PICS: $F = 4.60$; $df = 3, 25$; $P = 0.011$; PP: $F = 4.91$; $df = 3, 24$; $P = 0.008$; jute: $F = 3.52$; $df = 3, 24$; $P = 0.030$) but did not significantly differ with type of storage bag ($F = 0.48$; $df = 2, 81$; $P = 0.621$).

Overall, there was a significant correlation between aflatoxin contaminations and total mould count ($r = 0.677$; $P = 0.001$), incidence of *Aspergillus* spp. ($r = 0.640$; $P = 0.001$), and incidence of *Penicillium* spp. ($r = 0.298$; $P = 0.002$). Also a significant correlation was found between total mould count and incidences of *Aspergillus* spp. ($r = 0.802$; $P = 0.001$) and *Penicillium* spp. ($r = 0.339$; $P = 0.001$).

4. Discussion

In many rural villages, small-scale farmers store varying quantities of grain for subsistence and other reasons. Farmer practices, storage duration and storage structures have been linked with aflatoxin contamination of maize (Hell, 1997). In the present study, virtually all farmers relied on ordinary drying in the sun which was done by exposing the dehusked cobs on bare ground, sometimes on the farm. Sun drying, is slow in case of inadequate sunshine or intermittent exposure, and could expose the maize to saprophytic fungal inoculums surviving in the soil or on decaying crop residues if done on unprotected ground. Typical storage periods were found to be long and some farmers stored the maize in living houses. Longer storage periods are linked to high mould and aflatoxin incidence (Hell et al., 2000; Kaaya and Kyamuhangire, 2006) whereas poor ventilation in living houses is associated with humid and warm

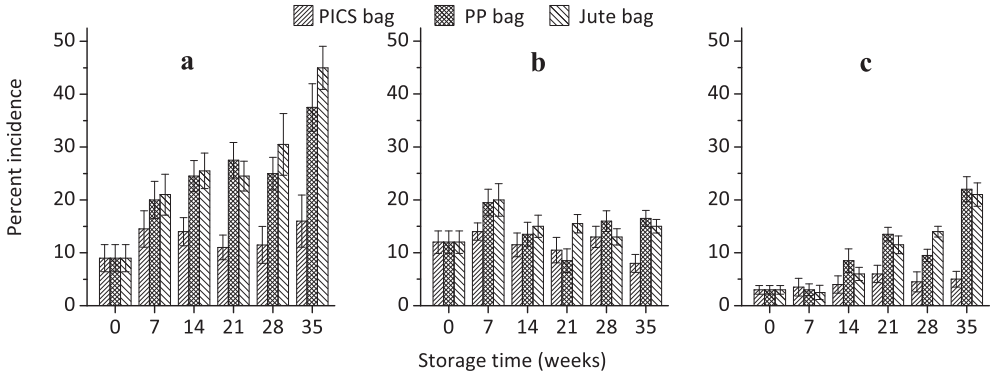

Fig. 4. Percentage incidence levels of moulds that were frequently isolated in maize grain (13% ≤ m.c. ≤ 14%) stored in PP, jute and PICS bags for 35 weeks: *Aspergillus* spp. (a); *Fusarium* spp. (b); *Penicillium* spp. (c).

Table 2
Total aflatoxin concentration (μg/kg) of maize grain stored in PP, jute and PICS bags for 35 weeks.

Treatment	Storage duration (weeks)			
	0	14	28	35
m.c. < 13%				
PICS	62.6 ± 13.2a	50.7 ± 14.5a	51.7 ± 12.7a	53.3 ± 15.7a
PP	62.6 ± 13.2a	158.5 ± 57.9b	182.8 ± 65.1b	306.8 ± 116.3b
Jute	62.6 ± 13.2a	221.5 ± 73.8b	253.5 ± 71.8b	393.8 ± 132.4b
13% ≤ m.c. ≤ 14%				
PICS	64.7 ± 17.5a	66.9 ± 18.4a	48.9 ± 15.2a	59.1 ± 14.3a
PP	64.7 ± 17.5a	143.5 ± 37.3b	201.8 ± 51.8b	414.9 ± 134.4b
Jute	64.7 ± 17.5a	167.2 ± 39.5b	211.9 ± 49.8b	492.7 ± 141.9b
m.c. > 14%				
PICS	107.2 ± 39.3a	89.6 ± 29.4a	254.6 ± 94.7a	630.9 ± 158.6a
PP	107.2 ± 39.3a	159.7 ± 34.6a	354.5 ± 117.4a	864.4 ± 208.6a
Jute	107.2 ± 39.3a	177.5 ± 49.6a	407.9 ± 127.8a	823.5 ± 198.5a

Data are means ± standard errors (m.c. < 13% n = 7, 13% ≤ m.c. ≤ 14% n = 13, m.c. > 14% n = 7). Entries in the same column followed by same letters are not significantly different ($P > 0.05$). Means were separated using Bonferroni adjustment. Storage was conducted between May 2014 and February 2015.

conditions that favour mould growth (Hell et al., 2000). Other malpractices such as failure to separate chaff or visibly mouldy grains, and poor store sanitation practices could encourage mould infection. Whereas farmers who participated in this trial reported being aware of good storage hygiene and cleaned their stores before introducing newly harvested maize, we did not verify the hygiene status of storage structures as this was beyond our scope.

Grain moisture is an important factor that needs to be controlled when storing maize in hermetic containers. From farmer to farmer, the initial moisture content of maize varied (12.4–15.0%), and was above 14% for slightly more than a quarter of the farmers recruited in this study. The reason for this variation is that many farmers lack tools for objective verification of grain moisture before or during storage. Instead farmers rely on subjective judgements such as the rattling sound of grains or hardness of the germ. For short-term storage, moisture of 14% is considered safe, but moisture content of 13.5% or below is recommend for long-term storage of maize (KEBS, 2014). On the one hand, many farmers are accustomed to storing maize in woven PP and jute bags. These bags allow the grain to continue drying during storage although excessive drying could translate to economic loss due to loss of sellable weight (Compton et al., 1998). We observed that the moisture content of maize stored in PP and jute bags decreased with time due the low moisture barrier properties of the bags considering that the trial proceeded during the dry weather season. Similarly, Baoua et al. (2014) in storage trials involving traders, marketing cooperatives, private seed companies, and private food processors reported on average 24% m.c. loss of maize stored in woven bags as compared to maize stored in PICS bags for 6.5 months. During a two months laboratory trial, Williams et al. (2014) observed moisture loss on maize stored in woven bags as compared to maize stored in PICS bags and attributed this to dry environment of the room in which they were stored. An important observation, however, is that the final moisture content reached was dependent on the initial moisture content of the grain, implying that safe storage moisture level may not be reached so rapidly where moisture of the grain at storage is too high. A protracted drying period can increase the likelihood of deterioration since both field and storage fungi can proliferate during this period and contaminate the maize with mycotoxins. In other instances, where r.h. of ambient air is high, dried maize may gain moisture when stored in PP and jute bags. Other factors that can cause moisture gain are high insect activity and heavy fungal growth especially on insect damaged grains due to breakdown of organic matter to yield carbon dioxide, heat, and water as reported by Compton et al. (1998) and Njoroge et al. (2014). Maize stored in PICS bags neither gained nor lost moisture content except later in storage when the grain moisture was higher than 14%. In the latter, the moisture gain could have been due to fungal growth during later stages of storage.

With regard to oxygen and carbon dioxide levels in the PICS bags, extremely low oxygen levels were not attained unlike in some other studies (Murdock et al., 2012; Baoua et al., 2013; Williams et al., 2014). It has been argued that low oxygen and high carbon dioxide levels in hermetic storage systems could control mould proliferation (Richard-Molard, 1988; Williams et al., 2014). The drop in oxygen and rise in carbon dioxide observed when maize was stored in PICS bags was as the result of aerobic metabolism of life forms enclosed together with the maize (Murdock et al., 2012) and could be influenced by elements of the storage system such as insect populations, moisture content of grain, fungal inoculums, quality of the grain, and gas-tightness of the hermetic package (Moreno-Martinez et al., 2000). Thus oxygen depletion and carbon dioxide build-up may be slow in grains that are well dried, and free from insects and moulds. However, oxygen and carbon dioxide levels of about 4–5% and 10–11% respectively were evident during the first 7 weeks of storage in the three levels of m.c., reaching concentrations of 5.4–9.4% and 8.5–12.2%, respectively, in 28 weeks (9.8–10.6% and 8.7–12.8%, respectively, in 35 weeks). These results compare closely with those of Baoua et al. (2014) who reported oxygen and carbon dioxide concentrations of 6.1–12.4% and 3.1–7.7%, respectively, in PICS bags packed with naturally infested maize stored at 10.3–13.5% moisture content for 6.5 months in storage trials involving traders, marketing cooperatives, private seed companies, and private food processors. Williams et al. (2014) reported lack of significant oxygen depletion in maize stored at

12% m.c. but a depletion of up to 0–1% in maize conditioned at m.c. of 15, 18, and 21% during one month storage in PICS bags, indicating the role of grain moisture. Similarly, Murdock et al. (2012), observed a rapid drop in oxygen levels to about 1–2% with a concomitant rise in carbon dioxide to 9% within 24 h of closing PICS bags filled with highly infested cowpeas. Seemingly, however, as observed in our results, the modified gas conditions in the bags could be lost overtime. A similar observation was reported by Baoua et al. (2012a) where oxygen levels dropped to range 2–3% within 12 d before gradually rising to 12–15%, while carbon dioxide rose to 5% before gradually decreasing again. It is reasoned that during protracted storage, oxidative metabolism is severely attenuated, and as oxygen consumption drops, the concentration of oxygen around individual grains tends to increase as air proceeds to leak slowly through the partially impermeable HDPE liners following concentration gradient (Baoua et al., 2012b). Thus, carbon dioxide and oxygen concentrations in the hermetic bags were dependent on the balance between respiration, the entrance of external oxygen to the system, and the loss of carbon dioxide to the ambient air.

It was expected that packing maize in PICS bags would alter the course of mould proliferation by creating a modified storage microenvironment. High mould counts were determined in all maize samples at the onset of the storage trials. This observation might be related to an interaction between the ubiquitous nature of fungi associated with maize and agro-climatic conditions of the trial site. The fungi usually form sclerotia that allow saprophytic survival for extended periods in the soil, maize residue and maize cobs (Wagacha and Muthomi, 2008), while high temperatures and drier conditions in semi-arid areas predispose maize to mould infections at pre-harvest stage in the field and post-harvest stage during storage (Okoth et al., 2012). Moreover, maize grains that are internally infected with fungi, when left to germinate, could give rise to plants that are internally infected with the same fungi (Mycock et al., 1992). The present trials demonstrated that maize stored in PICS bags with m.c. < 14% can be successfully kept without further mould infection during typical storage periods in rural households. Mould infection on maize stored in PP and jute bags, nevertheless, increased with increasing storage duration irrespective of the initial storage moisture. Magan and Lacey (1988) observed that mycoflora development in stored cereals is influenced by environmental factors, especially temperature, a_W and gas atmosphere. In present study maize stored in PICS bags with m.c. $\leq$ 14% did not show an increase in mould infection although it is unlikely that the oxygen/carbon dioxide environment achieved in the PICS bags could inhibit mould development. According to Richard-Molard (1988) fungistatic effect is induced when oxygen concentration drops to 1% or below. Early works by Magan and Lacey (1984) reported that decreasing oxygen to <0.14% is required before mould growth can be substantially reduced and increasing carbon dioxide to >50% is required for inhibition of mycelial growth. Other studies also reported the effect of modified atmospheres in controlling fungal growth and mycotoxin production in stored products (Dixon and Kell, 1989; Ellis et al., 1993). Studies on modified atmospheres with different carbon dioxide levels balanced with oxygen and nitrogen showed that *A. flavus* grew on wheat and rye with up to 75% carbon dioxide (Suhr and Nielsen, 2005). On maize, Giorni et al. (2008) indicted that treatment with 25% carbon dioxide reduced *A. flavus* development, but at least 50% carbon dioxide was necessary to reduce aflatoxin synthesis.

Fungi of the genera *Aspergillus, Fusarium* and *Pencillium* were frequently isolated. Of the three genera, *Aspergillus* had the highest frequency of isolation in the three bag types. According to Abbas (2005), fungi of the genera *Aspergillus* and *Pencillium* are often classified as storage fungi that can survive and grow on a variety of substrates and under a wide range of environmental conditions. The two mould spp. increased during storage in PP and jute bags but not in PICS bags. Infection by the genus *Fusarium* decreased during storage. Fandohan et al. (2005) noted that genus *Fusarium* generally may decrease with duration of storage as moisture content and a_W of the grain declines. Previously, Bii et al. (2012) found that moulds belonging to the genus *Aspergillus* were most frequently isolated (35.8%) in Eastern Kenya. Other genera including *Fusarium, penicillium*, and *Rhizopus* were isolated at frequencies of 15.5%, 9.2%, and 5.3%, respectively. In a similar study, Muthomi et al. (2012) reported high incidence levels of *Aspergillus* species isolated from soil samples, whole maize grain, and maize products in the Eastern region of Kenya. The pervasive nature of *Aspergillus* spp. and their high ability to colonize diverse substrates (Muthomi et al., 2009) may be reason for high occurrence in the maize samples.

In order to minimize mould proliferation, m.c. of maize to be packed in PICS bags should not exceed 14%. For long term storage, m.c. of 13–13.5% is recommended (KEBS, 2014) to avoid mould growth. However, a better indicator of the likelihood for moulds to colonize stored products is a_W which, in addition to m.c., is related to temperature (Mahmoud et al., 1992). Water activity (a_W) is a measure of the fraction of water content which is free and therefore available for fungal growth (Reichmuth, 2008), and is equivalent to equilibrium relative humidity expressed as a fraction. The growth limit for most fungi during storage of durable products is a_W of 0.65–0.70 (Reichmuth, 2008). For maize at 26 °C, the average temperature recorded in the PICS bags, a_W of 0.7 corresponds to moisture content of 14% (ASAE, 1995), although slight variations may occur depending on variety. Relative humidity in PICS bags packed with maize at m.c. of 14% or less, did not exceed 70% (Fig. 3). The r.h. measured in the bags may be regarded as the equilibrium r.h. or a_W of the enclosed maize. Accordingly, mould counts on maize in these bags did not increase as a_W did not exceed 0.7. However, for the PICS bags packed with maize at an initial m.c > 14% the r.h. exceeded 70% (71.5–80.5%; Fig. 3) representing $a_W > 0.7$. This explains the steady increase in mould infection. Lacey and Magan (1991) reported that commodities stored at r.h. > 75% and m.c. > 15% are susceptible to fungal attack within normal storage time. Moreover, studies have shown that the less xerotolerant fungi such as *A. ochraceous* and *A. versicolor* also begin to grow at moisture of 14% thus increasing mould infection (Wilson and Abramson, 1992).

A main observation made during this trial was the high insect population and damage of maize stored in the PP and jute bags by insects. The results are published elsewhere (Ng'ang'a et al., 2016). Insects' role in mould infection of stored maize was reviewed extensively; they are able to physically disseminate conidia in stored grain lots during movement and feeding, and also deposit them via defecation (Barry, 1987; Diener et al., 1987). Furthermore, damage inflicted by feeding insects, and the heat and moisture generated could enhance mould growth (Wright, 1992). These reasons related to profuse insect activity probably explain the increase in total mould count on maize stored in PP and jute bags even when m.c. was within the limit for safe storage, that is, below 14%. Moreno-Martinez et al. (2000) also reported low *A spergillus chevalieli* invasion on maize stored in hermetic containers as compared to maize stored in non-hermetic ones, and attributed the difference to high insect activity in the non-hermetic containers.

Similar to mould infection, initial aflatoxin contamination of maize quantified in this study was high, suggesting field or pre-storage contamination. In maize agro-ecological zones characterized by dry hot seasons such as in the present study area, spore populations of *A. flavus* increase on crop debris leading to high levels of mould propagules in the air (Wilson and Payne, 1994). Thus, heavy *A. flavus* innoculums may have been introduced to the

crop during growth and maturation or during pre-storage handling. Drought stress and delayed harvesting also increase the risk of field contamination with aflatoxins (Wagacha and Muthomi, 2008). However, our findings demonstrate that PICS bags can prevent further aflatoxin accumulation in maize during postharvest storage provided the maize is dried to below 14% m.c. Das et al. (2012) noted that *A. flavus* is a mesophilic fungus which grows optimally at a temperature of 30 °C and r.h. above 80%. Lacey and Magan (1991) stated that the minimum a_W for germination and growth of *A. flavus* is 0.78 which corresponds to m.c. of 16% at 27 °C or 15.5% at 32 °C. Other researchers, (Fernandez-Pinto et al., 1991) observed that minimal aflatoxin production by *A. flavus* occurred at a_W of 0.85 when temperature is about 20 °C, while maximum toxin production required a_W of 0.95 and temperature of 35 °C. Likewise, Faraj et al. (1991) reported maximal colony growth of *A. flavus* and aflatoxin production at 30 °C and 0.98 a_W, suggesting that a combination of fairly warm and humid conditions is necessary. Eventually, in the PICS bags, aflatoxin accumulation was observed when moisture of stored maize exceeded 14% in which r.h. and temperature in the bags averaged 76% (71.5–80.5%; Fig. 3) and 26 °C, respectively. According to Sumner and Lee (2012) development of the aflatoxin-producing moulds usually stops when moisture of the maize is below 12–13.5% and a_W is below 0.70. While aflatoxin accumulation was not observed in maize stored in PICS bags at m.c. of 14% or below, for the maize stored in PP and jute bags containing similar m.c. the aflatoxin accumulation was observed probably because of the influence of insect infestation in these bags (Ng'ang'a et al., 2016). Wilson and Abramson (1992) indicated that pest activity may increase the extent of aflatoxin contamination as insects break the physical integrity of grains, and could create localised spots of high moisture and temperature in the grain lot. Other earlier studies also associated insect damaged maize with increased risk of aflatoxin contamination (Diener et al., 1987; Mcmillian et al., 1987; Sinha and Sinha, 1991). Our results concur with the findings of Baoua et al. (2014) in West Africa, who reported lower levels of aflatoxin in 10–13.5% m.c. maize stored in PICS bags as compared to PP bags.

5. Conclusion

Storage losses due to insect pest infestations and mould infection are a serious problem that threatens the food security, nutrition, and livelihood of rural farmers who rely on traditional storage systems. As a storage solution, hermetic technologies are being promoted; they do not require use of chemicals, although fears abound regarding their moisture barrier properties that could affect quality and safety of the stored grain. This study has demonstrated that triple layer hermetic (PICS) bags are capable of maintaining the quality of maize with respect to mould and aflatoxin contamination so long as the storage moisture does not exceed 14%. Pre-storage precautions should thus emphasize proper drying and training or provision of grain moisture verification tools, as the subjective methods used for by some farmers may not be accurate.

Acknowledgements

C. Mutungi is thankful to Grand Challenges Canada for a grant (GCC-0423-01) to conduct this study. The authors thank the International Centre of Insect Physiology and Ecology (*icipe*) for institutional support.

References

Abbas, K.H., 2005. Aflatoxins and Food Safety. CRC press, Taylor and Francis Group, New York, p. 67.
ASAE, 1995. ASAE data D245.4-moisture relationships of grains. In: Standards, Engineering Practices, and Data, 42nd Ed. (Am. Soc. Agric. Eng. St. Joseph MI).
Azziz-Baumgartner, E., 2005. Case-control study of an acute aflatoxicosis outbreak - Kenya, 2004. Environ. Health Perspect. http://dx.doi.org/10.1289/ehp.8384.
World Bank, 2010. Missing Food: the Case of Postharvest Grain Losses in Sub-Saharan Africa, p. 116. Washington, DC.
Baoua, I.B., Margam, V., Amadou, L., Murdock, L.L., 2012a. Performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. J. Stored Prod. Res. 51, 81–85.
Baoua, I.B., Amadou, L., Margam, V., Murdock, L.L., 2012b. Comparative evaluation of six storage methods for postharvest preservation of cowpea grain. J. Stored Prod. Res. 49, 171–175.
Baoua, I.B., Amadou, L., Murdock, L.L., 2013. Triple bagging for cowpea storage in rural Niger: questions farmers ask. J. Stored Prod. Res. 52, 86–92.
Baoua, I.B., Amadou, L., Ousmane, B., Baributsa, D., Murdock, L.L., 2014. PICS bags for post-harvest storage of maize grain in West Africa. J. Stored Prod. Res. 58, 20–28.
Barry, D., 1987. Insects of maize and their association with aflatoxin contamination. In: Zuber, M.S., Lillehoj, E.B., Renfro, B.L. (Eds.), Aflatoxin in Maize, pp. 201–211.
Bennet, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 3, 497–516.
Bii, F., Wanyoike, W., Nyende, A.B., Gituru, R.W., Bii, C., 2012. Fumonisin contamination of maize (*Zea mays*) in aflatoxin "hot" zones in Eastern province of Kenya. Afr. J. Health Sci. 20, 28–36.
Blandino, M., Reyneri, A., Vanara, F., 2009. Effect of sowing time on toxigenic fungal infection and mycotoxin contamination of maize kernels. J. Phytopathol. 157, 7–14.
Castellari, C., Marcos Valle, F., Mutti, J., Cardoso, L., Bartosik, R., 2010. Toxigenic fungi in corn (maize) stored in hermetic plastic bags. In: 10th International Working Conference on Stored Product Protection. Julius-Kuhn Archiv, 425.
CDC (Centers for Disease Control and Prevention), 2004. Outbreak of aflatoxin poisoning -eastern and central provinces, Kenya, January – July, 2004. MMWR Morb. Mortal. Wkly. Rep. 53, 790–792.
Choudhary, A.K., Sinha, K.K., 1993. Competition between atoxigenic *Aspergillus flavus* strain and other fungi on stored maize kernels. J. Stored Prod. Res. 29, 75–80.
Chulze, S.N., 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Add. Contam. 27 (5), 651–657.
Compton, J.A.F., Floyd, S., Magrath, P.A., Addo, S., Gbedevi, S.R., Agbo, B., Bokor, G., Amekupe, S., Motey, Z., Penni, H., Kumi, S., 1998. Involving grain traders in determining the effect of post-harvest insect damage on the price of maize in African markets. Crop Prot. 17, 483–489.
Cotty, P.J., 1990. Effect of atoxigenic strains of *Aspergillus flavus* on aflatoxin contamination of developing cotton seed. Plant Dis. 74, 233–235.
Cotty, P.J., Bayman, P., Egel, D.S., Elias, K.S., 1994. Agriculture, aflatoxins and *Aspergillus*. In: Powel, K.A., Renwick, A., Peverdy, J.F. (Eds.), The Genus *Aspergillus*: from Taxonomy and Genetics to Industrial Application. Plenum Press New York, pp. 1–27.
Das, A., Angayarkanni, J., Bhattacharya, S., Palaniswamy, M., 2012. Evaluation of process parameters influencing aflatoxin B1 synthesis from *Aspergillus flavus* MTCC 2798 using rice straw under submerged fermentation. Int. J. Pharm. Biol. Sci. 2, 94–105.
Diener, U. l., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, S.L., Klich, M.L., 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev. Phytopathol. 25, 249–270.
Dixon, N.M., Kell, D.B., 1989. The inhibition by CO_2 of growth and metabolism of micro organisms. J. Appl. Bacteriol. 67, 109–136.
Doss, C.R., Mwangi, W., Verkuijl, H., de Groote, H., 2003. Adoption of Maize and Wheat Technologies in Eastern Africa: a Synthesis of the Findings of 22 Case Studies. CIMMYT, Mexico, D.F. CIMMYT Economics Working Paper 03–06.
Ehrlich, K.C., 2007. Aflatoxin-producing *Aspergillus* species from Thailand. Int. J. Food Microbiol. 144, 153–159.
Ellis, W.O., Smith, J.P., Simpson, B.K., Khanizadeh, S., Oldham, J.H., 1993. Control of growth and aflatoxin production of *Aspergillus flavus* under modified atmosphere packaging (MAP) conditions. Food Microbiol. 10, 9–21.
Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F., Wingfield, M.J., 2005. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, west Africa. Int. J. Food Microbiol. 99, 173–183.
Faraj, M.K., Smith, J.E., Harran, G., 1991. Interaction of water activity and temperature on aflatoxin production by *Aspergillus flavus* and *A. parasiticus* in irradiated maize seeds. Food Add. Contam. 8 (6), 731–736.
Fernandez-Pinto, V.E., Vaamonde, G., Montani, M.L., 1991. Influence of water activity, temperature and incubation time on the accumulation of aflatoxin B1 in soybeans. Food Microbiol. 8, 195–201.
Giorni, P., Battilani, P., Pietro, A., Magan, N., 2008. Effect of a_W and CO_2 level on *Aspergillus flavus* growth and aflatoxin production in high moisture maize postharvest. Int. J. Food Microbiol. 122, 109–113.
Gong, Y., Hounsa, A., Egal, S., Sutcliffe, A.E., Hall, A.J., Cardwell, K.F., Wild, C.P., 2004. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, west Africa. Environ. Health Perspect. 112, 1334–1338.
Guo, B.N., 2000. Control of pre-harvest aflatoxin contamination in corn: fungus plant- insect interactions and control strategies. Recent Res. Dev. Agric. Food Chem. 4, 165–176.
Hall, J.A., Wild, P.C., 1994. Epidemiology of aflatoxins related disease. In: Eaton, L.D., Groopman, J.D. (Eds.), The Toxicology of Aflatoxins. Academic Press, Inc,

London, pp. 233–258.
Hell, K., 1997. Factors Contributing to the Distribution and Incidence of Aflatoxin Producing Fungi in Stored Maize in Benin. University of Hannover, Germany, p. 143. PhD Thesis.
Hell, K., Cardwell, K.F., Setamou, M., Poehling, H., 2000. The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa. J. Stored Prod. Res. 36, 365–382.
Kaaya, A.N., Kyamuhangire, W., 2006. The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. Int. J. Food Microbiol. 110, 217–223.
Kenya Bureau of Standards (KEBS), 2013. In: List of Standards Approved by 102nd Standards Approval Committee Meeting on 10th April 2014 KS EAS 2, second ed. Kenya standard maize grains specification.
Kimanya, M.E., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., van Camp, J., Kolsteren, P., 2008. Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages in Tanzania. Food Add. Contam. 25, 1353–1364.
Lacey, J., Magan, N., 1991. In: Chelkowski, J. (Ed.), Fungi in Cereal Grains: Their Occurrence and Water and Temperature Relationships in Cereal Grain; Mycotoxins, Fungi and Quality in Drying and Storage. Elsevier, Amsterdam, pp. 77–118.
Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., luber, G., Nyamongo, S.J., Baker, L., Dahiye, A.M., Misore, A., Kevin, D.R., the Kenya aflatoxin investigating group, 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ. Health Perspect. 113, 1763–1767.
Magan, N., Lacey, J., 1984. Effects of gas composition and water activity on growth of field and storage fungi and their interactions. Trans. Br. Mycol. Soc. 82, 305–334.
Magan, N., Lacey, J., 1988. Ecological determinants of mould growth in stored grain. Intl. J. Food Microbiol. 7, 245–256.
Mahmoud, M.I., El-Bazza, Z.E., Mohamed, Z.G., 1992. Aflatoxin production at different relative humidities on gamma-irradiated herbs used as Egyptian drinks. Egypt J. Pharm. Sci. 33, 21–30.
Mcmillian, W.W., Widstrom, N.W., Wilson, D.M., 1987. Impact of husk type and species of infesting insects on aflatoxin contamination in pre-harvest corn at Tifton, Georgia. J. Entomol. Sci. 22, 307–310.
Moreno-Martinez, E., Jimenez, A.S., Vazquez, M.E., 2000. Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically. J. Stored Prod. Res. 36, 25–36.
Murdock, L.L., Margam, V.M., Baoua, I., Balfe, S., Shade, R.E., 2012. Death by desiccation: effects of hermetic storage on cowpea bruchids. J. Stored Prod. Res. 49, 166–170.
Muthomi, J.W., Njenga, L.N., Gathumbi, J.K., Chemining'wa, G.N., 2009. The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in eastern Kenya. Plant Pathol. J. 8, 113–119.
Muthomi, J.W., Mureithi, B.K., Chemining, G.N., Gathumbi, J.K., Mutit, E.W., 2012. *Aspergillus* species and aflatoxin B1 in soil, maize grain and flour samples from semi-arid and humid regions of Kenya. Int. J. Agri. Sci. 2, 22–34.
Mutungi, C., Lamuka, P., Arimi, S., Gathumbi, J., Onyango, C., 2008. The fate of aflatoxins during processing of maize to muthokoi - a traditional Kenyan food. Food Control 19, 714–721.
Mycock, D.J., Rijkenberg, F.H.J., Berjak, P., 1992. Systemic transmission of *A. flavus var. columnaris* from one maize seed generation to the next. Seed Sci. Technol. 20, 1–13.
Ngindu, A., Kenya, P.R., Ocheng, D.M.I., 1982. Outbreak of a cute hepatitis by aflatoxin poisoning in Kenya. Lancet 319, 1346–1348.
Ng'ang'a, J., Mutungi, C., Imathiu, S., Affognon, H., 2016. Low permeability triple-layer plastic bags prevent losses caused by insects on maize in rural on-farm stores. Food Sec. http://dx.doi.org/10.1007/s12571-016-0567-9.
Njoroge, A.W., Affognon, H.D., Mutungi, C.M., Manono, J., Lamuka, P.O., Murdock, L.L., 2014. Triple bag hermetic storage delivers a lethal punch to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in stored maize. J. Stored Prod. Res. 58, 12–19.
Okoth, S., Beatrice, N., Vincent, A., Erastus, K., Hannu, K., Vesa, J., 2012. Toxigenic potential of *Aspergillus* species occurring on maize kernels from two agro-ecological zones in Kenya. Toxins 4, 991–1007.
Oladiran, A.O., Iwu, L.N., 1993. Studies on fungi associated with tomoto fruit rots and effect of environmental factors on storage. Mycologia 121, 157–163.
Parry, D., 1990. Plant Pathology in Agriculture. Cambridge University Press, Cambridge, p. 385.
Peraica, M., Radic, B., Lucic, A., Pavlovic, M., 1999. Toxic effects of mycotoxins in humans. Bull. World Health Organ. 77, 754–766.
Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage, second ed. Blackie Academic and Professional, London, UK.
Quezada, M.Y., Moreno, M.E., Vázquez, J., Mendoza, M., Méndex-Albores, A., Moreno-Martínez, E., 2006. Hermetic storage system preventing the proliferation of *Prostephanus truncatus* Horn and storage fungi in maize with different moisture contents. Postharvest Biol. Technol. 39, 321–326.
Reichmuth, C., 2008. Microscopic fungi and related mycotoxins. In: Reichmuth, C., Hansen, L.S., Wildey, K., Hamel, D., Pelz, H.-J., Camon, T., Kroos, G., Perez, G.H., Betsche, T.P., Ducom, J., Davis, R. (Eds.), Health Risks and Safety Hazards Related to Pest Organisms in Stored Products: Guidelines for Risk Assessment, Prevention and Management. Council of Europe Public Health Committee. Council of Europe Publishing, Strasbourg Cedex, France, pp. 79–109.
Richard-Molard, D., 1988. General characteristics of the microfrola of grain and seeds and the principal resulting spoilage. In: Mutton, J.C. (Ed.), Preservation and Storage of Grain Seeds and Their By-products. Lavoisier, New York, pp. 226–242.
Sinha, K.K., Sinha, A.K., 1991. Effect of *Sitophilus oryzae* infestation on *Aspergillus flavus* infection and aflatoxin contamination in stored wheat. J. Stored Prod. Res. 27, 65–68.
Suhr, K.I., Nielsen,., P.V., 2005. Inhibition of fungal growth on wheat and rye bread by modified atmospheres packaging and active packaging using volatile mustard essentials oil. J. Food Sci. 70, 37–44.
Sumner, P.E., Lee, D., 2012. Reducing Aflatoxin in Corn during Harvest and Storage. The University of Georgia, Georgia College of Agriculture and Environmental Sciences, Atlanta, GA.
Wagacha, J.M., Muthomi, J.W., 2008. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol. 124, 1–12.
Watanabe, T., 1994. Pictorial atlas of soil and seed fungi. In: Morphologies of Cultured Fungi and Key to Species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C, p. 410.
Williams, S.B., Baributsa, D., Woloshuk, C., 2014. Assessing Purdue Improved Crop Storage (PICS) bags to mitigate fungal growth and aflatoxin contamination. J. Stored Prod. Res. 59, 190–196.
Wilson, D.M., Abramson, D., 1992. Mycotoxins. In: Sauer, D.B. (Ed.), Storage of Cereal Grains and Their Products. American Association of Cereal Chemist, Inc., St. Paul, Minnesota, pp. 341–391.
Wilson, D.M., Payne, G.A., 1994. Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops. In: Eaton, L.D., Groopman, J.D. (Eds.), The Toxicology of Aflatoxins. Academic Press, INC. San Diego. New York. Boston. London. Sydney. Tokyo. Toronto, pp. 309–325.
Wright, V.F., 1992. Assessment of insect infestation in stored maize and their relationship to *Aspergillus flavus* contamination. In: Semple, R.L., Frio, A.S. (Eds.), Mycotoxin Prevention and Control in Food Grains. UNDP/FAO REGNET and ASEAN Grain Postharvest Programme, Bangkok, Thailand, pp. 110–116.