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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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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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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.