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Evaluation of aflatoxin contamination of stored maize in the Brong-Ahafo region of Ghana

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Abstract

This study assessed the aflatoxin contamination and the presence of fungi in three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) stored using different storage methods namely storage in hermetic bags, woven polypropylene sacks and local crib in the Nkoranza-South district of the Brong-Ahafo region of Ghana. A factorial design arrangement was laid out in a randomized complete block design (RCBD). The isolation and identification of fungal pathogens associated with maize samples before and after storage were carried out on potato dextrose agar (PDA). Total flatoxin levels in the three maize varieties was determined by the use of enzyme-linked immunosorbent assay (ELISA) at 450 nm wavelength. Six fungi species were identified in the maize namely: *Aspergillus flavus, Penicillium* sp, *Fusarium* sp., *Lasiodiplodia theobromae*, *Colletotrichum gleosporioides* and *Rhizopus*. Before storage, *Abontem* variety recorded significantly higher ($p < 0.05$) total aflatoxin levels (113.56 ppb) compared to *Obatanpa* (2.91 ppb) and Aburohemaa (2.96 ppb). Maize samples stored in the polypropylene sack established significantly higher ($p < 0.05$) total aflatoxin levels of 82.9 ppb compared to hermetic bags (48.9 ppb) and local crib (48.9 ppb) after storage for six months. Aflatoxin levels under the interactive effect of variety and storage method was significant ($p < 0.05$). Overall storage of maize in hermetic bags significantly reduced aflatoxin levels hence the need to encourage maize farmers and traders to adopt hermetic bag storage technology.

Key words: aflatoxin, fungi, maize varieties, *Obatanpa*, *Abontem*, *Aburohemaa*, hermetic bag, polypropylene sack, local crib.

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops grown globally, and it is the third after wheat and rice in total food grain production (Anupama *et al*., 2005). It has a very high adaptability and productivity hence it is produced in most countries of the world (Dlamini *et al*., 2012). Maize is a staple food for an estimated 50% of the population of sub-Saharan Africa (FAOSTAT, 2006). The crop is grown in all the six agro-ecological zones of Ghana and has a cultivated area of 1,023459 ha and an average yield of 1.72 tonne per hectare, making it the major cereal crop (MoFA-SRID, 2015). New varieties with improved quality have been developed in Ghana to increase output. Some improved maize varieties available in Ghana include *Abeleehe, Aburotia, Dobidi, Dorke, Kawanzie, Kwadaso local, Obatanpa, Okomasa, Mamab*a, *Abontem*, and *Aburohema* (Manga, 2010; Tweneboah-Koduah, 2013).

The quality of grain is usually assessed by its germination capacity, weight, microbial contamination, insect infestation and nutritional content. Grain quality is affected by temperature, moisture content, relative humidity, storage period, and several other biological factors (Jayas and White,

2003; Chattha *et al*., 2014). Fungal infestation is the major microbial contamination in stored grains which leads to the production of mycotoxins that subsequently reduces the quality of the grains. Mycotoxins are metabolites of certain fungi. They are toxic to humans and other animal groups even at very low concentrations and are frequently responsible for health-related problems in many countries (Morris *et al.,* 2001). The FAO estimates that, 25% of agricultural crops worldwide is contaminated by mycotoxins especially aflatoxins (Shekhar *et al.,* 2011). Aflatoxins are harmful toxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Omari and Amoah, 2015). It was first isolated in the early 1960s and found to be the most potent naturally formed carcinogenic compounds (Shekhar *et al.,* 2011). The types of aflatoxins produced by these species are B1, B2, G1 and G2. Generally, *A. parasiticus* strains produce B1, B2, G1 and G2 while *A. flavus* produces only B1 and B2 (CAST, 2003; Abbas *et al.,* 2006). The intake of higher doses of aflatoxin can cause acute aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of immune system and impaired childhood growth (Fung and Clark, 2004).

Maize is a staple food of most Ghanaian communities and therefore its quality and quantity must not be compromised. Maize has the potential of becoming a non-traditional export commodity of Ghana considering its high productivity but unacceptable aflatoxin levels could be a huge setback. Aflatoxin contamination has been found to occur both at pre- and post-harvest stages of the production chain. Many studies have, however, shown that the bulk of the aflatoxin contamination in Ghana occur at the postharvest stages mainly due to improper food handling and storage practices (Omari and Amoah, 2015). In most developing countries such as Ghana, health issues related to aflatoxin contamination of food stuffs are more problematic since no proper food safety regulations have been established and/or rigorously enforced. The majority of maize produced in Ghana is either used for home consumption or sold in the local markets. Thus, the human health impact will be greatest if there is no monitoring and control mechanisms for aflatoxin contamination of food. For both food safety and economic reasons, there is the need to develop effective ways to mitigate the high and unacceptable levels of aflatoxins in food as it is becoming a serious public health and economic concern throughout the world.

Investigations on aflatoxin contamination of maize have been carried out in different parts of Ghana (Kpodo, 1996; Akrobortu, 2008; Amankwa, 2009; Tweneboah-Koduah, 2013). Most of the investigations focused on the type of storage methods that could be used for managing aflatoxin contamination of maize on cobs. However, most farmers and traders remove the grain from the cob before storage. Maize in this state is generally stored in hermetic bags, polypropylene sacks, jute sacks and mud silos. Tweneboah-Koduah (2013) reported lower concentrations of aflatoxins in *Golden crystal* i.e. yellow maize (19.9 pbb) compared to *Abasa* (24.5 pbb) and *Obatanpa* (27.9 pbb) maize varieties grown and stored in the Central Region of Ghana. To the best of our knowledge, no work has been done on the levels of aflatoxins in maize grown and stored by farmers and traders in the Nkoranza South District of the Brong-Ahafo Region of Ghana.

The Nkoranza-South District is one of the major producers of maize in Ghana. It is the highest maize-producing district in the Brong-Ahafo Region. The main maize varieties cultivated in this district include *Obatanpa*, *Abontem* and *Aburohemaa*. Communities in the district are also predominantly rural with high illiteracy rate, hence, most farmers and traders employ traditional storage methods for their maize. Some of the storage methods coupled with conducive climatic conditions of high rainfall and high temperatures in the region promote fungal growth and the production and accumulation of aflatoxins in maize. Since aflatoxin contamination cannot be assessed visually and also its effect is not immediate, farmers and traders are usually less concerned about it. Furthermore, maize from the district is distributed throughout Ghana hence, high mycotoxin levels in the grains will impact negatively on the human and animal health. Thus, it is very necessary to find ways to reduce aflatoxin levels in this commodity to acceptable levels. The objectives of the study were therefore to identify the presence of aflatoxin-producing fungi species in three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) stored using three storage methods (hermetic bag,

polypropylene sack and local crib), and assess the aflatoxin levels in the three maize varieties stored at ambient conditions under the three storage methods.

2. Materials and methods

Study site

The field work was conducted at four communities (Nkoranza, Bibiani, Akumsa Dumase and Brahoho) in the Nkoranza South District of the Brong-Ahafo Region of Ghana between September 2015 and April 2016. (Figure 1). Nkoranza South District is one of the twenty-two administrative districts in the Brong-Ahafo Region of Ghana. It is located in the middle portion of Brong-Ahafo Region. It lies within longitudes 1° 10'W and 1° 55'W and latitudes 7° 20'N and 7° 55'N covering a total area of about 920 km^2. The district has about 105 settlements, which are mostly rural. It shares boundaries with Nkoranza North District to the North, Techiman Municipality to the West, both in the Brong-Ahafo Region and Offinso North District to the South and Ejura-Sekyeredumase to the South-East in the Ashanti Region (MoFA, 2011). The district lies within the wet semi-equatorial region, having a mean annual rainfall level ranging between 800-1,200 mm. It has its major rainy season from March to June, experiencing her minor rains in September to November. The month of August experiences a short dry season, with the prolonged one in the months of December to March. The district has an average annual temperature of about 26 °C (MoFA, 2011). The dominant occupation of people in the district is agriculture, the proportion of which is about 82% of the district's labour force. It is one of the major producers of maize in the country.

Figure 1: Maps of Brong-Ahafo region (top image) of Ghana and Nkoranza South district (bottom image). Insert in top figure is the map of Ghana.

Experimental setup in the four communities

The experiment was conducted in four communites (Nkoranza, Bibiani, Akumsa Dumase and Brahoho) in the Nkoranza South district of the Brong-Ahafo region from September 2015- April 2016. Three varieties of maize namely *Aburohemaa* (AB), *Abontem* (AN) and *Obatanpa* (OB) commonly grown in the district and three storage methods namely Hermetic bags (HT), Local crib (LC) and Woven polypropylene sack or Poly sack (PS) that are commonly employed by farmers and traders in the district were used for the study. Each maize variety (25 kg) was collected after harvest and stored for six months in each of the storage systems in the four communities. Maize in hermetic bags and woven polypropylene sacks were stored in rooms. Relative humidity and temperature in the storage systems were measured at 30 min intervals using EL-USB LCD 2 thermo-hydrometer data loggers. To determine the impact of storage method on fungal growth and aflatoxin contamination, 1 kg of the maize samples were taken out o the 25 kg sample and analysed for aflatoxin levels and fungal growth prior to storage. The analysis was repeated after six months of storage. A factorial treatment arrangement was used for the study with storage methods (Hermetic, Local crib, Poly sack) and maize varieties (*Aburohemaa*, *Abontem*, *Obatanpa*) being the main factors. The treatment combinations were; (AB×HT, AB×LC, AB×PS, AN×HT, AN×LC, AN×PS, OB×HT, OB×LC, OB×PS). The factorial treatment combination was laid out in a randomized complete block design (RCBD) with the four communities serving as replications.

Isolation and identification of fungi associated with maize samples

Isolation of fungal pathogens associated with maize samples before and after six months storage was carried out on potato dextrose agar (PDA). The PDA was prepared by dissolving 3.9 g of the maize powder in 100 mL of distilled water in a 250 mL conical flask. The conical flask was shaken well together with its content to form a uniform solution. It was covered with aluminium foil and autoclaved at 1.05 kg/cm^2 pressure and 121 °C for 15 min. The PDA was poured into 9 cm petri dishes and allowed to cool. Surface sterilization was done to each sample in 1% w/v sodium hypochlorite for 30 sec and blotted dry with lint-free paper. Five sterilised grains were plated in each Petri dish and incubated at room temperature for five days to induce growth of fungi. Morphological identification of fungus associated with maize grains was done by scraping mycelia plugs advancing from the margins of the grains with a scalpel that is flamed. The mycelia plugs were mounted on slides for microscopic examination using distilled water. Compound microscope at low and high powers was used to examine the prepared slides. Identification of the isolates was based on colour,

morphology of mycelia, conidia and sporulating structures (Agrios, 2005; Barnett and Hunter, 2006). Micrographs were taken using a digital camera.

Determination of levels of total aflatoxins in maize

Enzyme-linked immunosorbent assay (ELISA) was carried out to determine the levels of total aflatoxin in the three maize varieties before and after storage. Celer AFLA ELISA Test Kits (Tecna S.r.l., Trieste, Italy) was used for the ELISA. The kit reagents include premixing microtitre plate (non-coated wells), microtiter plate (coated with anti-aflatoxin antibody), total aflatoxin standards (0, 2, 8, 30 and 80 ppb), enzyme conjugate, washing-buffer 10X, developing solution and stop solution. Sodium chloride and methanol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Aflatoxin analysis

To extract aflatoxins from maize samples, the procedure as reported in the Techna Total Aflatoxin Kit (code MA210/MA211) was followed. Briefly, 50 g of finely ground maize sample and 10 g of NaCl were mixed in 250 mL of 70% v/v methanol. The mixture was vortexed for 10 min and filtered using Whatman Filter Paper No. 1 and the extract (filtrate) was used for the analysis. The assay kit stored in the refrigerator was allowed to thaw to room temperature before analysis. To each premixing well was added 100 μL of enzyme conjugate followed by 50 μL of each standard/sample. The components in the premixing well were thoroughly mixed and 100 μL was transferred into a corresponding anti-aflatoxins antibody coated microwell. The mixture was incubated for 10 minutes at ambient temperature after which the wells were emptied followed by washing with 1X working buffer. A 100 μL of developing solution was added to each well, thoroughly mixed and incubated for 5 minutes followed by addition of 50 μL of stop solution. The absorbance was measured at 450 nm on a Readwell Strip ELISA Analyser (Robonik) plate reader. From a calibration curve of the standard solutions, the concentration of total aflatoxins in maize was determined.

3. Results

Effect of storage method on fungi infestation of maize grains

The results obtained after culturing, isolation and identification of fungi associated with the maize grains before and after storage indicated that the maize samples were infested with different species of storage fungi. Fungi isolated on grains before storage were *Aspergillus flavus, Colletotrichum gleosporioides, Fusarium* sp., *Lasiodiplodia theobromae*, *Penicillium* sp., and *Rhizopus* sp. Six different storage fungi species were again isolated from the maize samples after six months of storage namely, *A. flavus, A. niger, Fusarium* sp., *L. theobromae, Penicillium* sp., and *Rhizopus* sp. No *Colletotrichum gleosporioides* was found in the maize after storage for six months irrespective of the storage method. On the other hand, *Aspergillus niger*, which was not present in the grains before storage was identified in all maize varieties after six months of storage.

Total aflatoxin levels in maize varieties before and after storage

Aflatoxin levels in *Obatanpa*, *Abontem* and *Aburohemaa* maize varieties before storage varied significantly ($p < 0.05$). Aflatoxin levels in *Obatanpa* (2.91 ppb) and *Aburohemaa* (2.96 ppb) were similar compared to *Abontem* (113.56 ppb), which had a significantly high level of aflatoxin ($p < 0.05$) among the three varieties. **S**imilarly, there were significant differences in aflatoxin levels ($p < 0.05$) in all the three maize varieties after six months of storage in three different storage methods. *Obatanpa* and *Aburohemaa* varieties established significantly lower ($p< 0.05$) aflatoxin contamination levels of (5.0 ppb) and (6.6 ppb), respectively compared to *Abontem* (169.3 ppb) as shown in Table 1. Aflatoxin levels among the storage methods also varied significantly. Grains stored in the hermetic bag and local crib had aflatoxin levels of 48.9 ppb each which were significantly lower ($p < 0.05$) than the levels in grains stored in the polypropylene sack (82.9 ppb).

Table 1. Mean aflatoxin levels (ppb) in three maize varieties stored for six months under three storage methods under ambient conditions

Variety (V)	Storage method (SM)			
	Hermetic	Polypropylene sack	Local crib	**Mean**
Obatanpa	4.0	05. Apr	05. Apr	**5.0**
Abontem	138.0	236.1	133.8	**169.3**
Aburohemaa	5.0	7.3	7.6	**6.6**
Mean	**49.0**	**82.9**	**48.9**	
LSD (0.05)	**V**	**SM**	**V*SM**	
	24.88	**24.88**	**43.10**	

Aflatoxin levels under the interactive effect of variety and storage methods was significant ($p < 0.05$). The trend shows that the three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) had low aflatoxin levels when stored in the hermetic bags. The contamination levels increased in poly sack storage but reduced in all the three varieties when stored in the local crib. After storing the three maize varieties in hermetic bags, *Obatanpa* and *Aburohemaa* established contamination levels of 4.1 ppb and 4.7 ppb, respectively which was significantly lower ($p < 0.05$) than contamination levels in *Abontem* variety (137.9 ppb). Aflatoxin levels for the three maize varieties followed the same trend when stored in polypropylene sack and local crib. Contamination levels in *Obatanpa* (5.4 ppb) and *Aburohemaa* (7.3 ppb) when stored in polypropylene sack were similar and significantly ($p < 0.05$) lower than contamination levels in *Abontem* variety (236.1 ppb). Maize varieties stored in the local crib had contamination levels of 5.4 ppb for *Obatanpa* and 7.6 ppb for *Aburohemaa* which were significantly ($p < 0.05$) lower than *Abontem* (133.8 ppb).

4. Discussion

Six different fungi species were isolated on the maize samples before and after storage for six months. The fungal growth could be due to late harvesting of maize by farmers which predisposed the maize grains. It has been shown that maize grains are infested with microorganism right from the field and late harvesting is a contributory factor to field infestation (Widstrom, 1992).

There was varied aflatoxin levels among *Obatanpa*, *Abontem*, and *Aburohemaa* maize varieties before storage. The differences in contamination levels could be due to varied infection levels of the aflatoxin-producing fungus, *A. flavus* in maize grains on the field even before harvest. *Aspergillus flavus* infection can occur at pre-harvest, especially when the crop is in the field (Kuchareck and Raid, 2000; Hurburgh *et al*, 2005). Agriculture in Ghana is rain-fed. Coupled with high temperatures and unavailability of regular rains, the crop is left under stress. Aflatoxin presence at pre-harvest is a common phenomenon when high temperature and drought stress are present during the growth cycle (Cotty and Jaime-Garcia, 2007). This may explain the presence of aflatoxins on maize grains before storage. *Abontem* variety is an extra early maturing maize variety compared to early maturing and intermediate maturing varieties of *Aburohemaa* and *Obatanpa*, respectively. Most farmers do not cultivate one variety and also delay harvest because they want uniform drying of their cobs. Thus, *Abontem* variety will stay on the field for extra days after maturity than *Aburohemaa* and *Obatanpa* before they are harvested together. This might have predisposed that particular variety to moisture and lodging thereby promoting the infection and growth of *Aspergillus*. Different maize varieties have different susceptibilities to microbial attacks (Lokesha *et al*., 1987). Yellow maize is more susceptible to microbial and fungal attack than white maize which could be due to the high nutritive content of the yellow maize variety (Nwogu *et al.,* 1979). This could also explain the high aflatoxin levels in *Abontem* variety before storage.

The high levels of aflatotoxins in *Abontem* variety after six months of storage could be due to the high levels of the mycotoxin in the maize grains prior to storage. Aflatoxin once produced is very stable and storage conditions can only prevent further accumulation but do not reduce aflatoxin concentrations (Bani, 2014).

Maize stored in the woven polypropylene bags had the highest aflatoxin levels after six months of storage, confirming the findings of Udoh *et al.* (2000). Maize stored by traders in Uganda (majority in woven polypropylene bags) for six to seven months had mean aflatoxin levels of 107 ppb which suggests the grains were not suitable for the export and local markets (Kayaa and Warren, 2005). The possible reason for this observation may be the high temperature and high moisture content recorded in the polypropylene sack. Major factors reported for aflatoxin production in maize seeds include moisture content (Manoch *et al*., 1988), relative humidity and temperature in storage (Moreno and Kang, 1999), storage period (Chattha *et al*, 2014) and storage types (Roy and Chourasia, 2001). Grains should therefore be stored at 20 °C, 40-50% relative humidity and 11.5% grain moisture content in order to maintain grain quality (Abba and Lovato, 1999). The most important function of any storage structure is to provide hermetic conditions to the stored product and also give high protection from pests and fungi (Chattha *et al*, 2015). That could have contributed to the low aflatoxin levels in the grains stored in the hermetic bags. The interactive effect of storage method and maize varieties on aflatoxin contamination was significant with the hermetic bag storage giving comparatively lower aflatoxin levels in *Obatanpa* and *Aburohemaa* maize varieties. *Abontem* maize variety, however, had the lowest aflatoxin levels when stored in the local crib compared to the other two storage methods. This could be attributed to the efficiency of the local crib in terms of air flow and circulation which give grains lower moisture contents and subsequently reducing the development of aflatoxins. Low temperatures recorded in the local crib during storage period could also be contributory factor to the low aflatoxin levels observed in *Abontem* variety.

5. Conclusions

Quantitative studies carried out on three maize varieties from the Nkoranza-South District of the Brong-Ahafo Region of Ghana has shown that there are high levels of aflatoxins in the maize. Storage under hermetic conditions drastically reduced the levels of aflatoxins in maize. Improper storage practices such as storage in polypropylene sacks and storage in cribs promote the growth of aflatoxin-producing fungi. There is the need to intensify education for all stakeholders involved in the maize supply chain on aflatoxin contamination in maize and its negative effects on the health of humans and animals and national economy. Most farmers in Ghana are peasant and live below the poverty line. Embracing proper handling and storage practices will reduce post-harvest losses due to fungal infection and improve their income levels. Contaminated grains can be sold to ethanol producers since aflatoxins are eliminated during ethanol production. Farmers should also be educated on timely harvesting of maize and proper drying before storage. Hermetic bags are recommended for the storage of maize since they result in lower aflatoxin levels.

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Effect of Cold Plasma on Storage Toxigenic Fungi - *Aspergillus flavus*

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