Food Control 104 (2019) 91–98

Contents lists available at ScienceDirect

Food Control

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

Distribution of *Aspergillus flavus* and aflatoxin accumulation in stored maize grains across three agro-ecologies in Ghana

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ARTICLE INFO

Keywords:
Maize
Aspergillus
Aflatoxin
Mould
Contamination

ABSTRACT

Aflatoxins are carcinogenic secondary metabolites produced mainly by two species of *Aspergillus* fungi, (*A. flavus* and *A. parasiticus*). The toxin contaminates maize grains during the developmental stage as well as in storage. Grains with contamination levels above 20 ng/g are usually destroyed in the USA while the European Union and Japan allow 2–4 ng/g and nil respectively. Ghana lacks regulatory infrastructure for monitoring fungal contamination and detection of aflatoxin in grains prior to market. Therefore, this study assessed the extent of *A. flavus* contamination together with other mould on maize and total aflatoxin accumulation among stored maize grains across 34 towns and villages under three agro-ecologies in Ghana during the minor crop season of 2015–2016. *A. flavus,* was identified as the most predominant contaminant and recorded average percentage contamination of 56.7%, 30.6%, 53.5% and 45.6% on grains sampled across four communities (Fumesua, Wenchi, Ejura and Akomadan), respectively. Total aflatoxins recorded in the samples per community were in the range of below limit of detection (LOD) to 692 ng/g, 23 ng/g, 945 ng/g and 112 ng/g for Fumesua, Wenchi, Ejura and Akomadan, respectively. These dangerous upper limits demand urgent attention in the area of aflatoxin resistance breeding in maize to help address the aflatoxin menace in Ghana.

1. Introduction

Aflatoxins are the most carcinogenic natural compound found in nature. This fungal secondary metabolite is lethal to humans and livestock in amounts measured in parts per billion (Varga et al., 2015). Two major species of *Aspergillus* (*flavus* and *parasiticus*) are implicated for the biosynthesis of the toxin, and these food contaminants are closely monitored in oily-seeded crops and dairy products (Gourama & Bullerman, 1995; Payne, 1992). Studies have shown that aflatoxin B1 is normally predominant in laboratory cultures and in food products (Oliveira & Germano, 1997). Aflatoxins M1 and M2 are hydroxylated forms of B1 and B2 respectively (Dors et al., 2011), and when B1 in contaminated feed or foodstuffs is ingested by domestic animals, such as dairy cows, the toxin undergoes liver biotransformation and is converted into aflatoxin M1, a hydroxylated form of B1, and is secreted in the milk. Aside from aflatoxins, A. *flavus* and some fungi produce cyclopiazonic acid, a toxin with the ability to induce various pathological lesions in test animals (Burdock & Flamm, 2000).

A. flavus is also confirmed as the main species that attacks maize and subsequently contaminates it with aflatoxin. Due to the toxic nature and health threat aflatoxins pose to human and livestock, the developed countries such as the USA and the EU have set aflatoxin permissible limits in interstate commerce of food. The USA allows a limit of < 20 ng/g while that of the EU ranges between 2 and 4 ng/g and 0 ng/g for Japan (USDA-FAS report 2010).

On the contrary, most African countries and other developing nations do not have proper regulatory bodies to check mould contamination and levels of aflatoxin accumulation in farms and stored food products and therefore continue to trade and consume high levels of aflatoxin contaminated products.

In Africa, the earliest reported case of aflatoxicosis was in 1977 in Kenya, where several dogs and poultry died in the Coast and Rift Valley Provinces, Nairobi after feeding on poorly stored grains, while in 1978, samples of human and dog food (flour) were found to be contaminated with aflatoxin (MOA, 2008). The worst case of aflatoxicosis was reported when consumption of aflatoxin contaminated

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https://doi.org/10.1016/j.foodcont.2019.04.035
Received 9 February 2019; Received in revised form 17 April 2019; Accepted 26 April 2019
Available online 27 April 2019
0956-7135/

maize affected 317 people with 125 deaths in 2004 (KEPHIS, 2006). In Ghana, early surveys conducted by Kpodo (1996, p. 33) indicated that maize samples from silos and warehouses in Ejura contained aflatoxin levels in the range of 20–355 ng/g; while fermented maize dough collected from major processing sites contained aflatoxin levels of 0.7–313 ng/g. James et al. (2007) also found high average aflatoxin levels in maize samples collected from North Kwahu (153 ng/g), Ejura Sekyerdumasi (121 ng/g) and Nkoranza (134 ng/g), considerably beyond the approved limits recommended by the USA and the European Union.

Recently Perrone et al. (2014) reported high aflatoxins on maize grains collected from open markets of Ghana and Nigeria and also isolated both *A. flavus and A. parasiticus* from the samples.

The previous reports did not include some major areas where maize is actively cultivated in Ghana. Furthermore, they did not consider the extent of *A. flavus* contamination together with other mould on maize grains and the extent of aflatoxin accumulation in the stored maize across the major maize producing agro-ecologies to elucidate an appropriate control measure.

It is thus still vitally important to determine the presence, relative abundance of *A. flavus sp*, and level of aflatoxin accumulation in maize grains across towns and villages in Ghana where maize is actively cultivated. The specific objectives were to (1) determine the relative abundance and distribution of *Aspergillus flavus* and other fungal contaminants in maize samples across 34 towns and villages within three agro-ecologies in Ghana; and (2) determine prevalence as well as levels of aflatoxin found in stored maize ready for the market.

2. Materials and methods

2.1. Field survey locations

Field surveys were carried out in three main agro-ecological zones in Ghana where maize is widely cultivated. The agro-ecological zones fall within four districts [Fumesua (6°41North, 1°28West.), Wenchi (07°34′38″North, 01° 55′ 45″ West), Akomadan (7° 24′ 0″ North, 1° 57′ 0″ West), and Ejura (7° 23′ 0″ North, 1° 22′ 0″ West)]. The average yearly min and maximum temperature of the agro-ecological zones were as follows; Fumesua 21 °C min and 31 °C max, Wenchi 20 °C min and 35 °C max, Akomadan 21 °C min and 33 °C max while Ejura was 24 °C min and 33 °C max.

In each of the four districts, varied number of storage barns and silos (corresponding to the towns and villages visited) were sampled totalling 34 silos and barns. The sampling sites were radially separated by at least 10–20 km from each other within a district.

2.2. Maize sample

Maize grain samples which had not exceeded two months in storage were collected from storage barns and silos at harvest time in Ghana during the 2015/2016 growing season. Five cobs each were collected randomly from at least three silos per town or village and bulked to represent the town or village. Samples were then placed in sterile plastic bags and stored at 4 °C until processed.

Each sample was divided equally into two sub-samples. One subsample was evaluated mycologically while the other was ground in a Model MLI-204 Bühler mill (Milan, Italy) for mycotoxin analysis. Subsamples were stored in plastic bags at 4–6 °C until analyzed to prevent further fungal growth and mycotoxin biosynthesis prior to testing (Atehnkeng et al., 2008). Mycotoxin analyses were based on toxins present in the collected material and not on the potential of the identified fungi to produce mycotoxins.

2.3. Isolation and identification of moulds

One hundred maize kernels (randomly selected) from a sample lot from each of the 34 locations were assayed in two replicates for mould using direct plating technique for internal infestation (Pitt & Hocking, 1997). Kernels were surface sterilized for 1 min in 2.5% NaOCl, washed in three changes of sterile distilled water, and plated sparsely (10 kernels per Petri plate) directly onto the surface of 1/4 strength Potato Dextrose Agar (PDA) containing 9.75 g/l Potato Dextrose Broth (Difco) and 20 g/l agar, amended with 2 ml/l lactic acid to suppress bacterial contamination. The plated kernels were incubated at 31 °C for 3 days. The number of grains on which fungal growth exhibiting morphologies consistent with *Aspergillus, Fusarium, Penicillium,* and *Rhizopus, species* were counted and recorded in percentage as described by Mathur and Kongsdal (2003).

2.4. Aflatoxin analysis

Aflatoxin was extracted using methods described by Sirhan, Tan, and Wong (2013) with modifications. Samples were milled using a Preethi Mixer Grinder into homogenized flour. A weight of 2 g of slurry was weighed into a 15 ml centrifuge tube and topped-up with a 4 ml of 60:40 (v/v) methanol:acetronitrile solution. The resultant mixture was vortexed using Genie Vortex machine for 3mins. 1.32 g of anhydrous $MgSO_4$ and 0.2g of NaCl were added to the mixture and then vortexed for 1min. The tube was centrifuged for 5 min at 4000 rpm and the upper organic layer filtered through a 0.45 µm nylon syringe prior to injection. A volume of 100 µl of the filtered extract was injected into the HPLC. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360 nm, Em: 440 nm) with Phenomenex HyperClone BDS C18 Column (150 × 4.60 mm, 5um). The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1 ml/min with column temperature maintained at 40 °C. To 1 liter of mobile phase were added 119 mg of potassium bromide and 350 µl of 4 M nitric acid (required for postcolumn electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6 ng/µL in methanol. Concentration of B_1 and G_1 were 0.5, 1, 2, 8, 16 ng/g per 100 µl injection.

Concentration of B_2 and G_2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100ul injection. Limit of Detection and Limit of quantification of total aflatoxin were established at 0.5 ng/g and 1 ng/g respectively. Aflatoxin concentration was estimated as, ng/g = A x (T/I) x (1/W).where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (µl), I = volume eluate injected into LC (µl), W = mass (g) of commodity represented by final extract.

2.4.1. Validation of HPLC method

Recovery studies were conducted to check for precision and accuracy. Blank samples were spiked at 5 (five) replicated maize samples at 13 ng/g, 26 ng/g and 104ug/g with recoveries 91 ± 1.75%, 98 ± 1.33% and 102 ± 1.87% respectively. Blanks that were run periodically contained no detectable amount of target analyte. Trueness was further validated using a certified reference material (TR-A1000) from Triology laboratory, USA. The value obtained was 20.17 ± 1.14 µg/kg from ten replicates was within the recommended range of the certified value of 21.0 ± 2.9ug/kg. Coefficient of variation was less than 15% for replicates.

2.5. Data analysis

Analysis of variance (ANOVA) was performed on percentage contaminations of grains using Genstat 12th edition copyright 2009, VSN International Ltd. Data on fungal contamination were $\log_{10}$

Table 1
Relative frequency of *A. flavus* and other fungi found in stored maize samples within Fumesua and surrounding communities.

location	*A. flavus*		*A. niger*		*A .ochraceus*		*Rhizopus sp.*		*Fusarium sp.*		*Pennicilium sp.*	
	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans
Jiachie	15.5	(3.2)	0.0	(0.7)	0.0	(0.7)	51.0	(5.8)	42.5	(5.0)	46.5	(5.2)
Krapa	14.7	(3.3)	10.5	(2.9)	0.0	(0.7)	0.0	(0.7)	50.0	(7.0)	12.0	(2.9)
Abenase	44.7	(6.7)	14.5	(3.1)	0.0	(0.7)	3.0	(1.6)	2.5	(1.5)	34.5	(4.5)
Kwaso	51.0	(7.2)	41.5	(6.4)	0.0	(0.7)	17.5	(3.3)	26.0	(5.1)	2.0	(1.4)
Kwamo	58.1	(7.6)	17.7	(3.7)	10.0	(2.6)	0.0	(0.7)	27.0	(5.2)	0.0	(0.7)
Jiachie No 2	70.0	(8.4)	18.5	(4.4)	0.0	(0.7)	0.0	(0.7)	2.5	(1.5)	35.0	(7.0)
Kwadaso A.S	71.0	(8.5)	0.0	(0.7)	0.0	(0.7)	11.7	(2.8)	20.0	(4.5)	18.0	(3.4)
Boankra	85.5	(9.2)	3.0	(1.6)	0.0	(0.7)	0.0	(0.7)	13.5	(3.6)	19.0	(3.5)
Kwadaso R.A	99.5	(10.0)	69.5	(8.2)	0.0	(0.7)	1.0	(1.1)	0.5	(1.0)	1.0	(1.1)
%CV		**22.2**		**9.2**		**19.7**		**18.7**		**23.0**		**19.6**
Lsd (0.05)		**3.9**		**4.7**		**ns**		**3.3**		**4.7**		**2.3**
Range		**3.2–10.0**		**0.7–8.2**		**0.7–2.6**		**0.7–5.8**		**1.0–7.0**		**0.7–7.0**

Digits in parenthesis represent transformed data.
Trans – Transformed (square root).
Un-trans –Untransformed.
CV – Co-efficient of variation.
Lsd – Least significant difference.
ns – Not Significant.

transformed to reduce the heterogeneity of variance of contamination levels.

3. Results

3.1. Distribution of Aspergillus sp. and other fungi in 34 maize samples collected across farming communities within Akumadan, Ejura Fumesua and Wenchi

Aspergillus sp and other fungi that were found to contaminate maize samples within the study area indicated that *A. flavus* was the most predominant and abundant organism found in the maize samples examined across 34 towns within the four main districts (communities) under review.

Organisms identified alongside *A. flavus* species included *A. niger, Rhizopus sp, Fusarium moniliforme, Penicillium sp., Verticillium sp., Curvularia lunata, Tricoderma sp., Bipolaris sp. Trichothecium sp., Botryodiplodia sp., and Nigrospora sp*. Among the fungal groups identified, five (*A. flavus*, *A. niger, Rhizopus sp. Penicillium sp. and Fusarium sp.*) were commonly found on the samples in most of the locations surveyed. Aside the predominance of *A. flavus* in almost all locations, the presence and relative distribution of other fungi varied, depending on the location where the samples were collected (Table 1).

For instance, the means of percentage abundance of fungus identified within Fumesua and its environs varied from one town to the other within the community (Table 1) Similar trend was observed for the towns and villages within Wenchi, Ejura and Akomadan (Tables 2–4) respectively.

In general, samples collected from the Ejura state farms (main station) were heavily contaminated with several fungi relative to other samples within the area. Incidence of *Rhizopus sp.* was noted in only three samples (Ejura state farms, Ashakoko 2 and Roadside) within the Ejura area (Table 3).

3.2. Relationship between fungal contaminants on maize samples

Fig. 1, *A. flavus* (area labelled B) was seen to compete with *Rhizopus sp.* (area labelled A) on the same grain. In Fig. 2, *A. niger* was seen to have engulfed the grain (as seen in the area labelled C) while pockets of *A. flavus* and *Rhizopus* growth seems to have been limited (and confined to the area labelled E). The area labelled D is the pericarp of the grain. In Fig. 3, the region F represents the radicle of the grain colonized mainly by *A. flavus* (the majority of region G) whilst the region H displays limited growth of *Fusarium sp.*

3.3. Types and levels of aflatoxin contamination in stored maize across 34 towns within the major maize growing communities

High performance liquid chromatography (HPLC) analysis showed the presence and varied concentrations of aflatoxin B1, B2, G1 and G2 in the samples. Aflatoxin B1 was the most predominant type found in most of the locations where samples were collected, followed by B2, G1 and G2 respectively (Table 5). Aflatoxin concentration in maize samples collected from farmers' storage silos across 34 towns during the 2015–2016 minor crop season showed varied concentrations of toxins among samples collected within and between different communities. The concentration of aflatoxin B1 (most toxic) was comparatively high and variable within eight towns in the Akomadan area (Table 6A). On the contrary, low levels of aflatoxin G2 was detected in only two towns, Mbredani (1 ng/g) and Tanokwaem (1 ng/g). Aflatoxin G1 was detected across four localities within the same study area with varied concentrations between towns and villages.

In Ejura, samples analysed revealed that aflatoxin G2 was absent in all the locations sampled whilst G1, B2 and B1 were detected in some villages and town within the area. Extremely high levels of B1 (821.4 ng/g) and B2 (107.4 ng/g) were recorded in samples obtained from the main Ejura State farms storage silos which stocks huge volumes of maize in Ghana. Outside of this site, the rest of the locations recorded relatively lower aflatoxin concentration (Table 6A).

Further analysis of the samples collected for aflatoxin within Wenchi and Fumesua communities showed relatively similar concentration and distribution pattern as observed in previous towns. In Wenchi, aflatoxin G2 detection was uncommon compared to G1 which was found in Subinso and Amponsahkrom.

Aflatoxin B2 and B1 were found in 2 and 4 samples respectively across Wenchi (Table 6B). Total aflatoxin in the samples from this area ranged from < LOD to 23 ng/g with Duaponko samples recording the highest level. Fumesua samples on the other hand did not record aflatoxin G2, meanwhile GI, B2 and B1 were relatively common in the area. Aflatoxin G1 ranged from < LOD to 5 ng/g whilst B2 ranged from < LOD to 61 ng/g. Aflatoxin B1 ranged from < LOD to 631 ng/g with

Table 2
Relative frequency of *A. flavus* and other fungal organisms found in stored maize sample within Wenchi and surrounding communities.

Location	*A. flavus*		*A. niger*		*A .ochraceus*		*Rhizopus sp.*		*Fusarium sp.*		*Pennicilium sp.*	
	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans
Amponsah K	3.0	(1.6)	3.5	(1.7)	0.0	(0.7)	46.0	(5.2)	15.0	(3.8)	20.5	(3.6)
Main Station	12.5	(3.4)	0.5	(1.0)	0.0	(0.7)	25.0	(3.9)	14.0	(3.0)	43.5	(5.0)
Aframso	14.0	(3.7)	1.5	(1.3)	0.0	(0.7)	15.0	(3.1)	21.5	(4.1)	14.0	(3.0)
Duaponko2	32.7	(5.7)	9.5	(2.6)	0.0	(0.7)	4.5	(1.9)	16.5	(4.1)	14.0	(3.0)
Subinso No. 1	38.5	(6.1)	21.1	(4.5)	0.0	(0.7)	2.0	(1.4)	3.0	(1.6)	1.5	(1.3)
Duaponko	41.3	(6.4)	16.0	(4.0)	0.0	(0.7)	10.0	(2.6)	26.5	(5.2)	6.5	(2.2)
Animal H	72.3	(8.5)	12.0	(2.8)	0.0	(0.7)	0.5	(1.0)	20.0	(3.5)	18.0	(3.4)
% CV		**13.6**		**23.3**		0.0		**22.0**		**16.4**		**12.7**
Lsd(0.05)		**3.5**		**ns**		**ns**		**3.8**		**3.1**		**2.3**
Range		**1.6–8.5**		**1.0–4.5**		**0.0**		**1.0–5.2**		**1.6–5.2**		**1.3–5.0**

Digits in parenthesis represent transformed data.
Trans – Transformed (square root).
Un-trans –Untransformed.
% CV – Co-efficient of variation.
Lsd – Least significant difference.
ns – Not Significant.

Boankra recording the highest aflatoxin concentration in the area (Table 6B).

4. Discussions

In the quest to improving food security in the sub region, Ghanaian maize farmers continue to cultivate different maize varieties, which can boost economic development. However, the challenge of aflatoxin contamination counteracts these improvements, as most varieties grown in Ghana are susceptible to aflatoxin contamination and accumulation emanating from *Aspergillus flavus* infection that usually begins from infested soils where conditions are favourable (Abbas, Zablotowicz, Bruns, & Abel, 2006; Cotty, 2006; Okun et al., 2015). Surveys and analysis of stored maize samples indicated the heavy presence of several mould fungi, especially the L-type *A. flavus* and others such as *Rhizopus*, *Fusarium sp* and *Penicillium sp.*

The presence of these moulds on the grain confirmed earlier observation made by Atehnkeng et al. (2008) and Perrone et al. (2014) who identified similar fungi during fungal isolation from cereals and grains purchased from the open market in Ghana and Nigeria. This observation clearly shows that most of the grains stored in structures and ready for the market are likely to be unwholesome due to probable contamination with several fungi which secrete various mycotoxins that causes illness and in some cases death to consumers (Azziz-Baumgartner et al., 2005). Although this study did not focus much on the determination of the presence and levels of other mycotoxins produced alongside aflatoxin, the available reports in previous studies (Shephard, Kimanya, Kpodo, Gnonlonfin, & Gelderblom, 2013; Shirima et al., 2013) clearly prove that *Fusarium sp.* produces fumonisins, a powerful cancer promoter which could increase the risk of cancer in humans, especially, if ingested alongside aflatoxin contaminated maize. *Penicillium sp.* is also known to produce high levels of harmful ochratoxins, together with other substances that suppress or even eliminate the growth of bacteria (Haas et al., 2013).

Humidity (especially just before harvest and post-harvest) promotes growth of fungus (Payne, 1998), while drought suppressed the ability of the maize plant to fight fungal growth. The average maximum and minimum temperature conditions of Ejura was close to the preferred ideal condition which probably enhanced *A. flavus* contamination rate and distribution in the area. On the contrary, high *A. flavus*

Table 3
Relative frequency of *A. flavus* and other fungal organisms found in stored maize sample within Ejura and surrounding communities.

Location	*A. flavus*		*A. niger*		*A .ochraceus*		*Rhizopus sp*		*Fusarium sp*		*Pennicilium sp*	
	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans
Ashakoko	63.0	(7.9)	4.0	(2.0)	0.0	(0.7)	0.0	(0.7)	28.2	(5.3)	28.0	(5.3)
Babasotown	16.5	(4.1)	0.5	(0.7)	0.0	(0.7)	0.0	(0.7)	48.5	(7.0)	0.0	(0.7)
Expt Station	51.3	(7.2)	38.5	(6.2)	0.0	(0.7)	0.0	(0.7)	13.0	(3.6)	25.5	(5.0)
E. Main Farms	87.0	(9.3)	0.0	(0.7)	0.0	(0.7)	36.5	(6.0)	22.5	(4.7)	41.5	(6.4)
Roadside	29.3	(5.4)	17.0	(4.1)	0.0	(0.7)	4.5	(2.1)	16.2	(4.0)	39.5	(6.3)
Ashakoko2	74.0	(8.6)	28.0	(5.3)	0.0	(0.7)	10.0	(3.2)	28.4	(5.3)	28.5	(5.3)
% CV		**5.7**		**15.1**		**0.0**		**16.7**	**23.6**			**18.6**
Lsd(0.05)		**0.5**		**4.3**		**ns**		**4.6**	**8.5**			**8.2**
Range		**4.1–9.3**		**0.7–6.2**		**0.0**		**0.7–6.0**	**3.6–7.0**			**0.7–6.4**

Digits in parenthesis represent transformed data.
Trans – Transformed (square root).
Un-trans –Untransformed.
CV – Co-efficient of variation.
Lsd – Least significant difference.
ns – Not Significant.

Table 4
Relative frequency *of A. flavus* and other fungal organisms found in stored maize sample within Akomadan and surrounding Communities.

Location	*A. flavus*		*A. niger*		*A .ochraceus*		*Rhizopus sp*		*Fusarium sp*		*Pennicilium sp*	
	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans	Un-trans	Trans
Abrofia	21.0	(4.5)	30.5	(4.5)	0.0	(0.7)	0.5	(0.9)	49.5	(5.3)	2.00	(1.4)
Apatem	20.5	(4.6)	20.0	(4.2)	0.0	(0.7)	3.0	(1.6)	19.5	(4.5)	8.00	(2.4)
Apatem2	49.0	(6.9)	1.0	(1.1)	0.0	(0.7)	0.0	(0.7)	57.5	(7.3)	7.50	(2.3)
Main Station	52.0	(7.1)	14.5	(3.8)	0.0	(0.7)	0.0	(0.7)	26.0	(5.1)	11.50	(2.8)
Mankrampso	39.9	(6.4)	8.5	(2.5)	0.0	(0.7)	1.0	(1.1)	34.5	(5.9)	1.50	(1.3)
Mbredani	35.0	(6.7)	36.0	(5.1)	0.0	(0.7)	0.0	(0.7)	88.0	(9.3)	11.50	(2.2)
Mfanti	55.5	(7.3)	33.5	(5.8)	0.0	(0.7)	2.0	(1.4)	17.0	(4.2)	5.50	(2.1)
Mpeapem	25.6	(4.8)	16.9	(4.0)	0.0	(0.7)	0.0	(0.7)	41.0	(6.2)	9.50	(2.6)
Nkubesa	54.0	(7.3)	28.0	(4.7)	0.0	(0.7)	10.5	(2.7)	40.5	(6.2)	35.50	(4.6)
Serwia	50.0	(6.8)	10.5	(2.9)	0.0	(0.7)	0.5	(0.9)	6.5	(2.2)	8.50	(2.5)
Srentiatia	96.5	(9.9)	11.4	(2.8)	0.0	(0.7)	0.5	(0.9)	15.5	(4.0)	1.50	(1.3)
Tanokwaem	48.5	(7.0)	50.0	(7.1)	0.0	(0.7)	9.0	(2.5)	19.0	(4.4)	1.50	(1.3)
CV%		**3.3**		**33.2**		**0.0**		**16.5**		**20.2**		**21.3**
Lsd(0.05)		**3.9**		**4.5**		**ns**		**ns**		**4.7**		**3.3**
Range		**4.5–9.9**		**1.1–7.1**		**0.0**		**0.7–2.7**		**2.2–9.3**		**1.3–4.6**

Digits in parenthesis represent transformed data.
Trans – Transformed (Square root).
Un-trans –Untransformed.
CV – Co-efficient of variation.
Lsd – Least significant difference.
ns – Not Significant.

contamination was also noticed among Fumesua samples although the average minimum temperature was lower than 35 °C. This observation may infer that *A. flavus* is capable of adapting to relatively wide range of average temperature conditions. It is therefore possible that the rain forest, which is relatively humid supported the rate of contamination and growth of *A. flavus* as well as other fungi such as *Fusarium sp* and *Penicillium sp*.

The analysis of total aflatoxin accumulation in the maize samples revealed concentrations of moderate to dangerously high toxin levels, far beyond acceptable limits recommended by the EU and USA. This has serious implications on the trade and consumption of maize grains to these areas. The upper limits in some communities exceeded levels of (490.6 ng/g, 120.5 ng/g) previously reported by James et al. (2007), indicating that not much progress has been made over the years to keep the levels in check.

The high variation observed for aflatoxin B1 among samples within and between districts/communities infers that environmental conditions in some locations such as Wenchi were probably not very conducive compared to others where extremely high concentration was noticed. It was interesting to note that the high B1 concentrations were found among samples collected from towns within the rain forest and forest transition zones. This observation implies that aflatoxin biosynthesis is wide spread and does not entirely depend on drier environment as reported in some studies (Hamidou et al., 2014; Sibakwe et al., 2017).

Aflatoxin G1 was the second most important toxin detected across the four main communities (Ejura, Akomadan, Fumesua and Wenchi) although mycological isolation and identification of the fungus did not reveal the presence of other *Aspergillus sp* that could be responsible for the production of the G type. This may be due to the direct plating technique which may have preferentially transferred L-type *A. flavus* for identification and that other *Aspergillus sp.* responsible for aflatoxin G

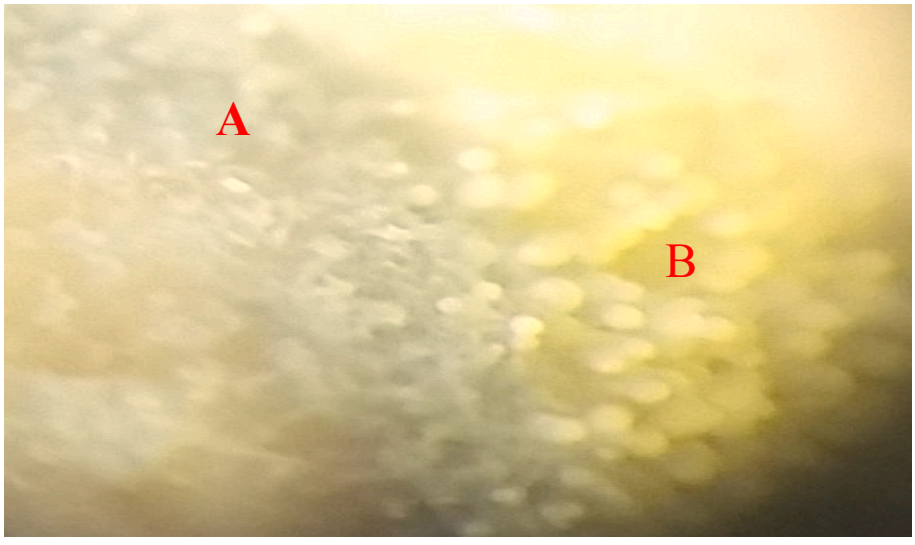

Fig. 1. Maize grain contaminated with *Aspergillus flavus* and *Penicillium sp.*

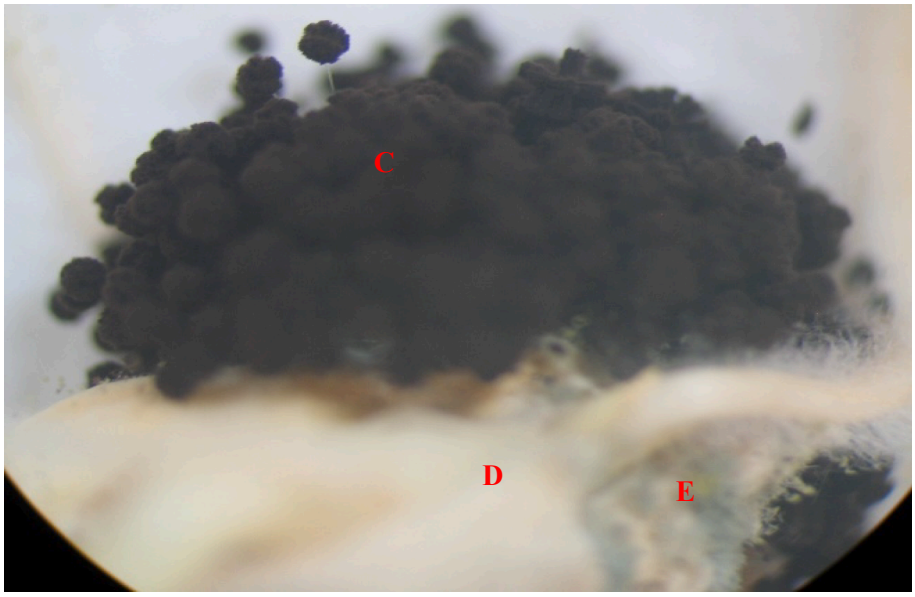

Fig. 2. Maize grain contaminated with *A.niger, A, flavus* and *Rhizopus sp*. Area labelled C represent *A. niger* growth whilst area labelled E is a mixture of *A. flavus* and *Rhizopus sp.* Area D is the pericarp.

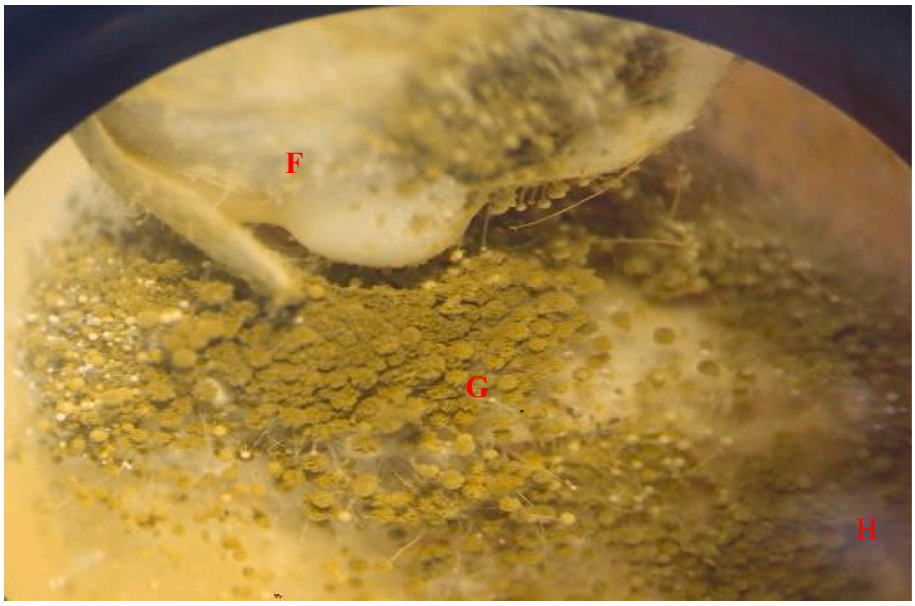

Fig. 3. Maize grain contaminated with *A. flavus* and *Fusarium sp.* Region F represents the radicle of the grain colonized mainly by *A. flavus* (as well as the majority of region G) whilst the region H displays limited growth of *Fusarium sp.*

might have existed transiently but could not be detected by the plating method (Cotty & Cardwell, 1999). Aflatoxin B2 was particularly high only in Fumesua, and aflatoxin G2 was the least aflatoxin detected among the four types studied.

The high incidence of aflatoxin contamination did not corroborate with the fungal accumulation levels. High aflatoxin contamination was expected for samples from areas where high *A. flavus* growth was recorded (Shapira et al., 1996; Lund and Frisvad, 2003) but this was not always the case in this study.

The variation observed in terms of the relative abundance, distribution and aflatoxin bio-synthesis may in part be due to the variation in the climatic and storage conditions which may have aided or suppressed aflatoxin synthesis.

5. Conclusions

The study identified *A. flavus, A. niger, Rhizopus sp, Penicillium sp* and *Fusarium sp* as the major fungal contaminants on stored maize grains in the major growing areas of Akumadan, Wenchi, Ejura and Fumesua. *Apergillus flavus* was the most common contaminant among the fungi identified. Grains produced from the rainforest zones and the forest transition recorded a relatively high *A. flavus* contamination compared to grains collected from the transitional savanna zone. High *A. flavus* contamination per sample did not corroborate with high aflatoxin levels.

Aflatoxin B1, highly toxigenic compound was the most common aflatoxin type detected across the four main communities studied. Aflatoxin contamination in stored maize grains was wide spread across

Table 5
Percentage mean infection and aflatoxin contamination in stored maize samples collected from 34 farmers across four districts within 3 main agro-ecologies in Ghana.

District	Percentage contamination of grains with Aflatoxin per district/community				Temp °C (Average Min &
	G2	G1	B2	B1	Max)
Wenchi (SVT)					
Contamination/Location	1/7 (14.3%)	2/7 (28.6%)	3/7 (42.9%)	4/7 (57.4%)	
Mean	< LOD	1.5	2.0	4.1	
Range	< LOD - 0.4	< LOD -7.1	< LOD -13.3	< LOD - 23.0	20.0–35.0
Akomadan (FRT)					
Contamination/Location	2/12(16.7%)	5/12 (41.7%)	5/12 (41.7%)	8/12 (66.7%)	
Mean	< LOD	1.2	1.6	10.0	
Range	< LOD -1	< LOD -6	< LOD -11	< LOD - 101	21.0–33.0
Ejura (FRT)					
Contamination/Location	–	4/6 (66.7%)	5/6 (83.3%)	3/6 (50.0%)	
Mean	–	4.3	19.0	138.0	
Range	–	< LOD - 16.5	< LOD - 107.4	< LOD - 821.4	24.0–33.0
Fumesua (RFR)					
Contamination/Location	–	3/9 (33.3%)	4/9 (44.4%)	6/9 (66.7%)	21.0–31.0
Mean	–	< LOD	8.0	81.4	
Range	–	< LOD - 5	< LOD - 61	< LOD - 630.6	

AEZ refers agro-ecological zone; SVT = Savana Transition, FRT = Forest Transition and RFR = Rain Forest. LOD = Limit of detection. Dash (−) = not detected or below limit of detection (LOD).

Table 6A
Concentration of aflatoxin (ug/g) in 18 maize samples across Akomadan and Ejura.

Towns/Villages	Aflatoxin Concentration				
	G2	G1	B2	B1	Total Aflatoxin
AKOMADAN (FRT)					
Nkubesa	–	–	4	6	10
Mankrampso	–	–	–	–	–
Abrotia	–	2	1	1	4
Apatem	–	–	–	1	1
Srentiatia	–	–	–	1	1
Serwia	–	–	–	1	1
Mfanti	–	6	–	3	9
Apatem	–	–	–	1	1
Main Station	–	–	–	–	–
Tanokwaem	1	4	–	–	5
Mpaepaem	–	–	11	101	112
Mbredani	1	2	–	–	3
EJURA (FRT)					
Ashakoko	–	–	–	1	1
Main Farms	–	17	107	821	945
Expt. Station	–	2	1	–	3
Babaso Town	–	4	1	–	5
Ashakoko 2	–	3	1	–	4
Roadside	–	–	4	5	9

AEZ refers agro-ecological zone; FRT = Forest Transition. Dash (−) = not detected or below limit of detection (LOD).

Table 6B
Concentration of aflatoxin (ug/g) in 16 maize samples across Wenchi and Fumesua.

	Aflatoxin Concentration				Total Aflatoxin
Towns/Villages	G2	G1	B2	B1	
WENCHI (SVT)					
Aframso	–	–	1	1	2
Main Station	–	–	–	–	–
Duaponko	–	–	–	–	–
Subinso No. 1	–	7	–	3	10
Duaponko				23	23
Animal Hubandry	–	-	13	2	15
Amponsahhkrom	1	3	-	-	4
FUMESUA (RFR)					
Jiachie	–	–	-	-	–
Boankra	–	–	61	631	692
Kwadaso-Agric Station	–	–	-	15	15
Kwaso	–	–	-	2	2
Kwadaso Roundabout	–	-	4	57	61
Abenase	–	–	-	-	–
Kwamo	–	1	–	4	5
Krapa	–	5	4	–	9
Jiachie 2	–	–	3	24	27

AEZ refers agro-ecological zone; SVT = Savana Transition and RFR = Rain Forest. Dash (−) = not detected or below limit of detection (LOD).

the ecological zones where some communities displayed dangerously high levels. Furthermore, the heavy presence of other fungi suggested the possibility of a complex toxic contamination of grains in Ghana which needs immediate attention.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding

USAID Funded the PhD studies and research through the West African Centre for Crop Improvement (WACCI) as a scholarship package.

Acknowledgements

The authors would like to thank USAID for the sponsorship and the West Africa Centre for Crop Improvement for the PhD training in Plant Breeding. We would also like to thank all the staff of Plant health Division of CSIR –Crops Research Institute Kumasi, Ghana for their

support. The help of Messers Akyeampong and Obeng Josuah, William Appaw is deeply appreciated. Special thanks to Dr. Stephen Koranteng for reviewing the article.

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