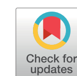
Food Control 104 (2019) 152–156

Contents lists available at ScienceDirect

Food Control

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

Prevalence of fungi and aflatoxin contamination in stored groundnut in Ghana



Kwabena Asare Bediako[a,b,*], Daniel Dzidzienyo[a], Kwadwo Ofori[a], Samuel Kwame Offei[a], James Yaw Asibuo[b], Richard Adu Amoah[c], Joshua Obeng[d]

[a] West Africa Centre for Crop Improvement, University of Ghana, Legon, Accra, Ghana
[b] CSIR-Crops Research Institute, P. O. Box 3785, Kumasi, Ghana
[c] CSIR-Plant Genetic Resources Research Institute, P. O. Box 7, Bunso, Ghana
[d] CSIR-Oil Palm Research Institute, P. O. Box 74, Kade, Ghana

ARTICLE INFO

Keywords:
Aspergillus
Groundnut aflatoxin
Contamination
Fungal species

ABSTRACT

Fungal species associated with stored groundnuts together with incidence of aflatoxin contamination in four major growing regions of Ghana were investigated in this study. *Aspergillus niger* (39.9%) and *A. flavus* (26.3%) were the predominant species recovered respectively from 73.3% to 83.3% of 60 groundnut samples used for microbial study. *A. flavus* was found in equal proportion in the four regions under investigation. Other fungal species identified in the study were *Colletotrichum* (13.3%), *Rhizopus* (14.8%), *Penicillium* (5.4%), *Curvularia* (0.2%) and *A. ochraceus* (0.1%). A total of 120 samples were assayed for total aflatoxin using HPLC with fluorescence detection. High contamination of groundnut samples (LOD-928.7, mean = 25.0 ng/g) was detected across the four regions with 20.8% exceeding the threshold limit of 20 ng/g total aflatoxin set by the Ghana Standards Authority. A significant positive correlation (0.59, $p < 0.01$) was observed between *A. flavus* and total aflatoxin in groundnut samples, an indication that *A. flavus* was the major causal agent of aflatoxin contamination of groundnut samples in this study. Further, other potential mycotoxigenic fungi were found in groundnut samples suggesting the need to investigate groundnuts in Ghana for contamination by other mycotoxins.

1. Introduction

Aflatoxins are secondary metabolites produced mainly by the ubiquitous fungi, *Aspergillus flavus* and *A. parasiticus* (Mutegi, Ngugi, Hendriks, & Jones, 2012). Aflatoxin has received global attention due to its potential threat to human and animal health (Waliyar et al., 2016). Consumption of low doses of aflatoxin over extended periods may cause liver cancer, immune systems suppression, poor nutrient absorption, retarded growth in children and malnutrition. Acute aflatoxicosis, liver failure and death can result from exposure to higher concentrations of aflatoxin (Mahuku et al., 2019). The deleterious effects of aflatoxin at low or high levels have prompted many countries to set maximum limits of total aflatoxin in groundnuts and groundnut products intended for direct human consumption. For example, maximum acceptable limits of 4 ng/g for European Union (EU), 20 ng/g for United States (US) and Ghana, and 10 ng/g for Kenya have been documented (Asare Bediako et al., 2019). Thus, these limits inflict strong potential economic impact on nations intending to trade in countries with stringent requirements such as the European countries.

Groundnut is an essential cash crop and a significant component of the diet of many households in developing countries, including Ghana. In Ghana, groundnut is grown in all agro-ecological zones of the country with 70% of production concentrated in the Northern, Upper East and Upper West regions (Oteng-Frimpong, Sriswathi, Ntare, & Dakora, 2015). In 2015, farmers in Ghana produced 417,000 metric tons of groundnuts from 336,000 ha (MoFA-SRID, 2016, pp. 12–13). Groundnut is consumed widely in raw, roasted or boiled state or processed forms such as oil, cookies, flakes and candies. About 80% of Ghanaians consume groundnut and/or groundnut products at least once a week and 32% at least thrice a week. By virtue of its high protein content, groundnut is promoted by nutritionists and agriculturists in Ghana as a suitable supplement to animal protein (Jolly et al., 2008).

Despite the importance of groundnut in Ghana, the crop is prone to infection by *A. flavus* or *A. parasiticus* and subsequent aflatoxin contamination during production and storage. Subsistence farmers in Ghana usually subject harvested groundnuts to heap drying in the field

* Corresponding author. West Africa Centre for Crop Improvement, University of Ghana, Legon, Accra, Ghana.
E-mail address: asbed85@yahoo.com (K. Asare Bediako).

https://doi.org/10.1016/j.foodcont.2019.04.034
Received 15 February 2019; Received in revised form 17 April 2019; Accepted 26 April 2019
Available online 27 April 2019
0956-7135/ © 2019 Elsevier Ltd. All rights reserved.

prior to storage. This process could generate warm and moist conditions resulting in further contamination in the presence of pre-harvest *Aspergillus* infected groundnuts. Further, freshly harvested groundnuts are stored in recycled storage bags which serve as agents of cross contamination (Masters, Ghosh, Daniels, & Sarpong, 2013, pp. 51–52). Aflatoxin accumulation proceeds in store under poor storage practices (Florkowski & Kolavalli, 2013) along with high humidity and temperature which are common in the tropics (Hell & Mutegi, 2011). The potential for aflatoxin buildup under storage conditions prior to marketing demands urgent assessment for aflatoxin contamination of stored groundnuts for timely interventions. Immediate interventions will limit aflatoxin in groundnuts to levels safe for human consumption and acceptable for trade in lucrative markets of the EU and US. The objectives of the study were: 1) to identify fungi and determine the incidence of aflatoxin in stored groundnut samples originating from four major growing regions in Ghana and 2) to determine the association between the fungal species and total aflatoxin detected in groundnut samples.

2. Materials and methods

2.1. Sample collection and processing

Purposive sampling was adopted in the selection of regions and locations based on groundnut production potential. In-shell groundnuts were collected from farmers' stores in 20 locations across four regions (5 locations per region) from March to April 2017 after being stored for approximately 4–6 months. During sample collection, data were collected on the latitude, longitude and altitude of the sampled sites. Samples were either stored in polyethylene or jute bags or granaries across the regions examined in the study. Sampling was done from two different stores in three sites at each location, corresponding to a total of 120 samples and 60 sites. Samples were shelled, stored in plastic bags and kept in refrigerator at 4 °C prior to microbial and aflatoxin analyses. All precautions to avoid cross contamination during shelling, including use of hand gloves and hand sterilization between samples using 70% ethanol were strictly adhered to.

2.2. Fungal isolation and identification

Samples from the two stores in each site were bulked together to constitute a sample. A total of 60 samples (15 per region) were used for fungal isolation. Fifty kernels per sample were randomly selected, surface sterilized in 10% sodium hypochlorite for 1 min and rinsed three times in sterile distilled water. Surface sterilized kernels were plated (5 per Petri plate) on full strength potato dextrose agar amended with chloramphenicol (500 mg/l of medium) and incubated at 25 ± 2 °C for 5–7 days. Colonies of fungi growing on each Petri plate were identified to the genus level and those belonging to *Aspergillus* were identified to the species level (Barnett & Hunter, 1972; Klich & Pitt, 2002, pp. 5–67; Mathur & Kongsdal, 2003; Singh, Frisvad, Thrane, & Mathur, 1991).

2.3. Aflatoxin analysis

A total of 120 groundnut samples (30 per region) were used for aflatoxin analysis. Aflatoxin content of samples was determined using high performance liquid chromatography (HPLC).

Standards of AFB_1, B_2, G_1 and G_2 were obtained from Romer Labs®, Austria. Sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$), acetonitrile and methanol (HPLC grade) were purchased from Merck, USA.

The extraction of aflatoxins in groundnut samples was carried as described by Sirhan, Tan, Al-Shunnaq, Abdulra'uf, and Wong (2014) with some modifications. Briefly, samples were milled and homogenized using a Preethi Mixer Grinder. Two grams of sample was weighed into a 15 ml centrifuge tube, 5 ml of distilled water was added and the tube was shaken for 1 min. The solution was allowed to stand for 5 min. A volume of 5 ml 1% (v/v) acetic acid in acetronitrile solution was added. The resultant mixture was thoroughly mixed using Genie Vortex machine for 3 min. A mass of 1.32 g of anhydrous $MgSO_4$ and 0.2 g of NaCl were added to the mixture and then shaken for 1 min. 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 50 μl of the filtered extract was injected into the HPLC.

HPLC analysis was done according to AOAC Official Method 2005.08 (AOAC, 2006) substituting Photochemical Reactor for Enhanced Detection (PHRED) with Kobra Cell for post-column derivatisation. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360 nm, Em: 440 nm) with YMC C18 Column (150 × 4.60 mm, 5 μm) was used for aflatoxin analysis. 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 L of mobile phase were added 119 mg of potassium bromide and 350 μl of 4 M nitric acid (required for post column electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng/g) were prepared from Romer Labs® aflatoxin standard of 5.02 ng/μl in acetronitrile. Aflatoxins in samples were detected by using the retentions of the standard solution run and quantification done using the calibration curves of respective toxins. Limits of detection (LOD) for AFB_1 and total aflatoxin were established at 0.2 and 0.5 ng/g, respectively. Limits of quantification (LOQ) were 0.5 ng/g for AFB_1 and 1 ng/g for total aflatoxin. All samples were analyzed for aflatoxins in triplicates.

2.4. Method performance

Recovery studies were carried out to check for precision and trueness by spiking blank samples with aflatoxins standard. For aflatoxin (AF) B_1, five replicated groundnut samples were spiked at 5, 10 and 40 ng/g with average recoveries with their standard deviations of 95 ± 1.10%, 96 ± 1.00% and 98 ± 1.19% respectively. A six-point calibration curve for AFB_1 showed good linearity with coefficient of determination (R^2) of 0.996. For total aflatoxin, five replicated groundnut samples were spiked at 13, 26 and 104 ng/g with average recoveries with their standard deviations of 89 ± 1.61%, 93 ± 1.33% and 99 ± 1.87% respectively. Blank samples that were run periodically contained no detectable amount of aflatoxin. Further validation was done using an in-house reference material (naturally contaminated groundnut). The value obtained was 24 ± 1.92 ng/g from ten replicates within the recommended range of the certified value of 26.00 ± 3.12 ng/g. Coefficient of variation was less than 15% for replicates.

2.5. Statistical analyses

For microbial data, colonies of fungi growing on each Petri plate were counted, pooled and isolation frequency for each fungus computed as percent of total fungi obtained. Percent samples infected was determined as number of samples infected expressed as percent of total samples assayed. Pearson's correlation coefficients and the respective *p* values were generated using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to establish the association between fungi frequencies and total aflatoxin in groundnut samples. Values for fungal frequencies were square root transformed (√value) while aflatoxin data were log transformed (value + 1) for analysis.

For aflatoxin data, samples were categorized into four based on their aflatoxin levels: samples with levels: < 0.5 ng/g (limit of detection) = Not detected, ≤ 4 ng/g = European Union Standard, ≤ 20 ng/g = Ghana Standards Authority Standard, and > 20 ng/g = Not fit for human consumption in Ghana.

3. Results

3.1. Fungal species associated with groundnuts

A total of 1237 isolates belonging to seven fungal species were identified. *Aspergillus* species accounted for 66.3% of the total fungi recovered while *Colletotrichum*, *Rhizopus*, *Penicillium* and *Curvularia* species constituted the remaining 33.7%. *Aspergillus niger* (39.9%) and *A. flavus* (26.3%) were predominant among the fungal isolates, followed by *Rhizopus* spp. (14.8%), *Colletotrichum* spp. (13.3%), *Penicillium* spp. (5.4%) and the less prevalent fungi, *Curvularia* spp. (0.2%) and *Aspergillus ochraceus* (0.1%). Out of 60 groundnut samples, 50 samples representing 83.3% were infected with *A. flavus* while *A. niger* was found in 44 (73.3%) samples. *Colletotrichum* spp., *Curvularia* spp. and *Aspergillus ochraceus* contaminated 43.3% (26), 3.3% (2) and 1.7% (1) of the samples respectively. Each of *Rhizopus* and *Penicillium* species occurred in 23.3% (14) of the entire samples.

3.2. Distribution of fungi in groundnut samples

Curvularia spp. and *A. ochraceus* showed up only in samples from Ashanti region. *A. flavus* was discovered in nearly equal proportion in Ashanti (21.23%), Northern (24.92%), Upper East (29.23%) and Upper West (24.62%) regions. *A. niger* and *Colletotrichum* spp. were present in all regions with the highest abundance in Ashanti (52.43%) and Northern (51.83%) regions respectively. *Rhizopus* and *Penicillium* species were present in all regions but predominantly in samples from Upper West in proportions of 43.17% and 41.79% respectively.

3.3. Aflatoxin levels in different locations within a region

The occurrence of aflatoxins in groundnuts from four major growing regions comprising of 20 sampling locations is presented in Table 1. Wide variation in aflatoxin B_1 and total aflatoxin ranging from LOD to 916.8 ng/g and LOD to 928.7 ng/g, respectively was observed among sampling locations across the regions under study. Within the same region, both high and mean aflatoxin concentrations were detected in samples. For example, aflatoxins were not detected in samples from Kaleo and Piisie in Upper West region while Naaha within the same region recorded high aflatoxin contamination (mean = 246.9 ng/g) in samples. Additionally, while Gukpegu registered no aflatoxin (< LOD) in Northern region, Mosifong belonging to the same region recorded 86.7 ng/g mean total aflatoxin.

3.4. Prevalence of aflatoxin contamination in groundnuts

The aflatoxin contents measured in the groundnut samples were categorized as shown in Table 2. All samples from Ashanti region had detectable levels of aflatoxin in contrast to samples from Northern, Upper East and Upper West regions which recorded 53.3%, 53.3% and 80% non-detectable aflatoxin levels, respectively. In general, more than 50% of samples from Northern, Upper East and Upper West regions gave aflatoxin levels within 4 ng/g whereas only 13.3% of samples from Ashanti region attained the aflatoxin limit of 4 ng/g. Categorization of total aflatoxin according to limit imposed by Ghana Standards Authority (≤20) revealed 66.7%, 83.3%, 76.7% and 90% of samples collected respectively from Ashanti, Northern, Upper East and Upper West regions. The samples beyond 20 ng/g where highest in Ashanti (33.3%), followed by Upper East (23.3%), Northern (16.7%) and Upper West (10%). Across the study regions, aflatoxins were not detected in 46.7% of the entire samples. However, 56.7% had aflatoxins within 4 ng/g while 79.2% met the aflatoxin limit of 20 ng/g. Furthermore, 20.8% of the samples were contaminated with aflatoxin at concentrations higher than 20 ng/g.

Table 1
Incidence and range of aflatoxins (ng/g) in groundnuts sampled from four major growing regions in Ghana.

Region	Aflatoxin B_1			Total aflatoxin	
	N	Mean	Range	Mean	Range
Ashanti	30	5.1	LOD to 92.4	19	1.5–141.8
Northern	30	18.1	LOD to 264.9	21.4	LOD to 313.1
Upper East	30	0.2	LOD to 2.2	9	LOD to 62.5
Upper West	30	49.3	LOD to 916.8	50.5	LOD to 928.7
Overall	120	18.2	LOD to 916.8	25	LOD to 928.7

LOD: Limit of detection = 0.2 ng/g for AFB_1 and 0.5 ng/g for total aflatoxin.

Table 2
Aflatoxin distribution in groundnuts by category in four major growing regions of Ghana.

Total aflatoxin category (ppb)	Percent samples (number of samples)				
	Ashanti	Northern	Upper East	Upper West	Total
ND	0 (0)	53.3 (16)	53.3 (16)	80 (24)	46.7 (56)
≤4	13.3 (4)	70 (21)	56.7 (17)	86.7 (26)	56.7 (68)
≤20	66.7 (20)	83.3 (25)	76.7 (23)	90 (27)	79.2 (95)
> 20	33.3 (10)	16.7 (5)	23.3 (7)	10 (3)	20.8 (25)

ND = Not detected; limit of detection = 0.5 ng/g ≤4 = European Union Standard; ≤20 = Ghana Standards Authority Standard; > 20 = Not fit for human consumption in Ghana.

3.5. Correlation between fungal species and aflatoxin contamination

There was significant positive correlation ($p < 0.01$) between *A. flavus* incidence and total aflatoxin recorded in samples from 20 sampling locations in four regions of Ghana (Table 3). On the other hand, no significant correlation ($p > 0.05$) was found between *A. niger*, *Colletotricum* spp., *Rhizopus* spp., *Penicillium* spp. incidences and total aflatoxin in samples.

4. Discussion and conclusion

About seven fungal species were identified in groundnut samples collected from 20 locations spanning four regions in Ghana. The detection of *Aspergillus, Penicillium*, and *Rhizopus* species is consistent with findings of similar studies on groundnut kernels from Brazil (Atayde, Reis, Godoy, Zorzete, & Reis, 2012; Nakai et al., 2008), China (Xing et al., 2016) and India (Bhattacharya & Raha, 2002). The frequency of *Aspergillus* spp was higher in groundnut kernels because of the great adaptation of these fungi to this substrate, particularly during storage (Nakai et al., 2008). Of the seven species identified, *A. niger* and *A. flavus* were the most prevalent. Prevalence of *A. flavus* and its presence in 83.3% of groundnut samples implies that groundnuts in Ghana are prone to aflatoxin contamination. *A. flavus* and *A. parasiticus* are most commonly implicated in aflatoxin contamination of agricultural crops (Torres, Barros, Palacios, Chulze, & Battilani, 2014). Nonetheless, in the present study, *A. parasiticus* was not recovered. This observation is not surprising due to rare occurrence (0–1%) of *A. parasiticus* in stored groundnuts in a wider coverage study conducted in three agro-ecological zones in Ghana (Agbetiameh et al., 2018). The authors' report buttressed the opinion of Awuah and Kpodo (1996) that *A. parasiticus* is a minor problem on stored groundnut in Ghana due to its infrequent occurrence. Also in Brazil, *A. parasiticus* was not recovered from stored groundnut kernels and hulls (Nakai et al., 2008). Although *A. niger* is not a known aflatoxin producer, it possesses the ability to produce other toxins such as ocratoxin A and malformins (Wagacha, Mutegi, Karanja, Kimani, & Christie, 2013). The high incidence of *A. niger* and the

Table 3
Pearson's correlation coefficients of association between fungal incidence and total aflatoxin.

	% *A. flavus*	% *A. niger*	% *Colletotricum* spp.	% *Rhizopus* spp.	% *Penicillium* spp.
Sample aflatoxin (ng/g)	0.59**	0.32ns	−0.09ns	0.05ns	−0.35ns

Correlation significance, ** significant at p = 0.01, ns = not significant at p = 0.05; n = 20.
Percent data were square root transformed prior to analysis.
Total aflatoxin was log transformed (value + 1) prior to analysis.

recognition of its presence in groundnut samples (73.3%) demands further investigations into other mycotoxins existing in Ghanaian groundnuts and action for their control. Furthermore, the low abundant *Penicillium* spp. (5.4%) surfacing in 23.3% of groundnut samples merits attention due to an array of mycotoxins such as ochratoxins, patulin, citrinin, penicillic acid, cyclopiazonic acid and rubratoxin associated with this genus (Wagacha et al., 2013). Youssef, El-Maghraby, and Ibrahim (2008) documented the co-occurrence of aflatoxins and other mycotoxins such as sterigmatocystin, ochratoxin, diacetoxyscirpenol and zearalenone in groundnut, a situation suspected to arise from simultaneous colonization by other non-aflatoxigenic fungi. Another important observation in this study is the detection of *Colletotrichum* species, an economically important seed-borne pathogen reported as causal agent of anthracnose in groundnut. Anthracnose disease is associated with reduced seedling emergence and seed germination (Rajeendran, Nulit, Yien, Ibrahim, & Kalhori, 2017). Identification of *Colletotrichum* species in all four study regions together with the proportion of groundnut samples (43.3%) contaminated by this fungus suggest potential for yield reduction in the major groundnut growing regions in Ghana, particularly Northern region.

All seven fungal species except *Curvularia* spp. and *A. ochraceus* were found in varying proportions in the four regions under study. *Curvularia* spp. and *A. ochraceus* occurred exclusively in Ashanti region and at low frequency in the present study. This presupposes that these fungi may not be of importance in stored groundnut contamination in Ghana. On the other hand, the widespread distribution of *A. flavus*, *A. niger*, *Rhizopus* spp., *Colletotrichum* spp. and *Penicillium* spp. suggests their importance on stored groundnuts in Ghana. This finding points out suitable focal areas for targeted research or possible fungal and mycotoxin interventions. For example, even distribution of *A. flavus* across the sampling locations implies that research or management interventions targeting *A. flavus* and its associated problems should focus on all four regions under investigation. However, Ashanti and Upper East regions should be of relevance to investigators targeting *A. niger*. It also gives an indication of severity and incidence of health and/or economic hazards posed by various fungi in the regions under consideration.

Co-occurrence of high and low mean aflatoxin levels among samples within regions were frequently encountered in all four regions. Ordinarily, sampling locations within these regions fall in the same agro-ecological zone and therefore are exposed to similar climatic conditions. This observation coupled with the revelation of even *A. flavus* distribution across sampling locations suggest possible varietal differences among groundnut samples which can be harnessed for groundnut improvement in Ghana. Varietal differences in *A. flavus* infection and aflatoxin production have been documented (Okello, Monyo, Deom, Ininda, & Oloka, 2013; Upadhyaya, Nigam, Mehan, Reddy, & Yellaiah, 2001; Waliyar et al., 2016). In addition, differences in management practices among farmers in the locations being investigated could have accounted for the wide variation in aflatoxin levels observed. Management practices such as timely planting, avoidance of drought stress, adequate weed control, crop rotation, use of improved varieties, and post-harvest sorting and drying are possible remedies for aflatoxin reduction (Bhatnagar-Mathur, Sunkara, Bhatnagar-Panwar, Waliyar, & Sharma, 2015; Guchi, 2015; Mutegi et al., 2012; Park, 2002). A detailed study in the sampling locations would reveal the different management approaches adopted by farmers in their bid to reduce aflatoxin contamination in groundnut. This will to a large extent contribute to aflatoxin reduction in locations that recorded high aflatoxin levels.

Aflatoxin levels in samples collected across the four regions of Ghana were categorized based on European Union (EU) import restriction limit (≤4 ng/g) and allowable safe limit established by the Ghana Standards Authority and the U.S. Food and Drug Administration (≤20 ng/g). Aflatoxins were detected in all samples from Ashanti region with few samples (13.3%) qualifying for export to the European countries whereas over 50% of the samples from Northern, Upper East and Upper West regions met the most stringent EU requirement for export. Albeit a significant proportion of samples from each region is safe for human consumption in Ghana (≤20 ng/g), there still exist some component exceedingly higher than 20 ng/g. The high levels of aflatoxin present in the samples could be attributed to improper post-harvest drying followed by aflatoxin buildup in store. Most of the groundnut samples collected for this study were harvested around October and November 2015 and stored for 4–6 months prior to sampling. Following harvesting, groundnuts are usually heaped by farmers for field drying/curing preceding home drying (Guchi, 2015). This farmers' traditional practice usually exposes groundnuts to warm and moist conditions leading to rapid fungal invasion and subsequent aflatoxin accumulation in store. Aflatoxin contamination could possibly have been exacerbated in some locations in Northern, Upper East and Upper West regions by a characteristic late season drought along with high temperature during the cropping season. Torres et al. (2014) were of the opinion that plant stress resulting from high soil temperatures and drought influences pre-harvest infection by aflatoxigenic *Aspergilli*. The highly contaminated samples (>20 ng/g) occurring in the study regions, although a small proportion, are capable of contaminating clean samples during handling, storage and processing (Florkowski & Kolavalli, 2013). Eventually, groundnuts with unsafe levels of aflatoxin penetrate local and major markets of these four regions which serve as main outlets for groundnuts in Ghana. It is not surprising that Awuah and Kpodo (1996) found high levels of aflatoxins (5.7–22,168 ng/g) in market groundnut samples in Ghana. In general, 79.2% of groundnut samples collected across all four regions in Ghana are considered safe for human consumption in Ghana and fit for export to the United States if we consider 20 ng/g as the safe limit. Nonetheless, per groundnut aflatoxin regulation in Ghana, the present study indicates that aflatoxin contamination is occurring at unacceptable levels in 20.8% of the entire samples. Agbetiameh et al. (2018) established that aflatoxin levels in about 11.5% of groundnut samples in Ghana exceeded 20 ng/g. Following a recent report by the investigators, we report a much higher proportion of groundnut samples (20.8%) with aflatoxin concentrations higher than 20 ng/g. This calls for an urgent need to roll out aflatoxin management interventions to keep groundnut samples in Ghana under acceptable aflatoxin threshold.

In the present study, only *A. flavus* showed significant positive correlation with total aflatoxin recorded groundnut samples. Mutegi et al. (2012) discovered a significant positive correlation between incidence of *A. flavus* and aflatoxin contamination in groundnut samples, a finding which is in line with our observation. This posits that *A. flavus*

was the only aflatoxin producer among the fungi identified. Our finding is in agreement with those of Klich (2007) and Torres et al. (2014) who described *A. flavus* as a major producer of aflatoxin in seeds of agricultural crops including groundnut. Additionally, we suspect that most of the isolates of *A. flavus* identified in the study were aflatoxigenic and were implicated in aflatoxin contamination of the groundnut samples. This inference is supported by Nakai et al. (2008) who found 93.8% of *A. flavus* strains isolated from stored groundnuts in Brazil to be toxigenic.

The study has demonstrated that *A. flavus* is the primary causal agent of aflatoxin contamination of the groundnut samples. However, the occurrence of other toxigenic fungi signals the need to screen groundnuts for other mycotoxins co-occurring with aflatoxins. Wide variation in groundnut aflatoxin contamination among the sampling locations was observed amid uniform *A. flavus* distribution. Further studies should be conducted to investigate the management practices and varieties adopted by farmers in these locations. In addition, the results have shown that groundnuts in Ghana are contaminated with aflatoxin and could potentially reach alarming levels if aflatoxin management strategies are not urgently implemented. Currently, practices such as crop rotation, timely planting and harvesting, post-harvest sorting and windrow drying, among others could be adopted as more effective complemental approaches such as aflatoxin resistance breeding and biocontrol are underway.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors gratefully acknowledge the DAAD for the financial support. We appreciate the assistance of extension agents in the locations sampled for the study. We are also grateful to Messrs. William Ofori Appaw and Festus Acheampong of KNUST for their assistance.

References

Agbetiameh, D., Ortega-Beltran, A., Awuah, R. T., Atehnkeng, J., Cotty, P. J., & Bandyopadhyay, R. (2018). Prevalence of aflatoxin contamination in maize and groundnut in Ghana: Population structure, distribution, and toxigenicity of the causal agents. *Plant Disease, 102*, 764–772.

AOAC (2006). AOAC official method 2005.08 aflatoxins in corn, raw peanuts and peanut butter liquid chromatography with post-column photochemical derivatization. *Journal of AOAC International, 89*(3), 678–692.

Asare Bediako, K., Ofori, K., Offei, S. K., Dzidzienyo, D., Asibuo, J. Y., & Adu Amoah, R. (2019). Aflatoxin contamination of groundnut (*Arachis hypogaea* L.): Predisposing factors and management interventions. *Food Control, 98*, 61–67 November 2018.

Atayde, D. D., Reis, T. A., Godoy, I. J., Zorzete, P., & Reis, G. M. (2012). Mycobiota and aflatoxins in a peanut variety grown in different regions in the state of São Paulo, Brazil. *Crop Protection, 33*, 7–12.

Awuah, R. T., & Kpodo, K. A. (1996). High incidence of *Aspergillus flavus* and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis. *Mycopathologia, 134*, 109–114.

Barnett, H. L., & Hunter, B. B. (1972). *Illustrated genera of imperfect fungi* (3rd ed.). Minneapolis, Minnesota: Burgess Publishing Company.

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating *Aspergillus flavus* and aflatoxin contamination in crops. *Plant Science, 234*, 119–132.

Bhattacharya, K., & Raha, S. (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia, 155*, 135–141.

Florkowski, W. J., & Kolavalli, S. (2013). *Aflatoxin control strategies in the groundnut value chain in Ghana. IFPRI Ghana Strategy Support Program.* Washington, DC, USA: International Food Policy Research Institute Working paper 33.

Guchi, E. (2015). Aflatoxin contamination in groundnut (*Arachis hypogaea* L.) caused by *Aspergillus* species in Ethiopia. *Journal of Applied & Environmental Microbiology, 3*(1), 11–19.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of sub-Saharan Africa. *African Journal of Microbiology Research, 5*(5), 459–466.

Jolly, C. M., Awuah, R. T., Fialor, S. C., Agyemang, K. O., Kagochi, J. M., & Binns, A. D. (2008). Groundnut consumption frequency in Ghana. *International Journal of Consumer Studies, 32*(6), 675–686.

Klich, M. A. (2007). *Aspergillus flavus*: The major producer of aflatoxin. *Molecular Plant Pathology, 8*(6), 713–722.

Klich, M. A., & Pitt, J. I. (2002). *A laboratory guide to common Aspergillus species and their teleomorphs.* North Ryde, Australia: Commonwealth Scientific and Industrial Research Organization.

Mahuku, G., Sila, H., Mutegi, C., Kanampiu, F., Narrod, C., & Makumbi, D. (2019). Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. *Food Control, 96*(August 2018), 219–226.

Masters, W. A., Ghosh, S., Daniels, J. A., & Sarpong, D. B. (2013). *Comprehensive assessment of the peanut value chain for nutrition improvement in Ghana* Final report (September).

Mathur, S. B., & Kongsdal, O. (2003). *Common laboratory seed health testing methods for detecting fungi* (1st ed.). Bassersdoff, Switzerland: International Seed Testing Association.

MoFA-SRID (2016). *Agriculture in Ghana: Facts and figures (2015). Ghana: Ministry of food and agriculture (MoFA), statistics.* Research and Information Directorate (SRID).

Mutegi, C. K., Ngugi, H. K., Hendriks, S. L., & Jones, R. B. (2012). Factors associated with the incidence of *Aspergillus* section Flavi and aflatoxin contamination of peanuts in the Busia and Homa bay districts of western Kenya. *Plant Pathology, 61*(6), 1143–1153.

Nakai, V. K., Rocha, L. O., Gonçalez, E., Fonseca, H., Ortega, E. M. M., & Corrêa, B. (2008). Distribution of fungi and aflatoxins in a stored peanut variety. *Food Chemistry, 106*, 285–290.

Okello, K. D., Monyo, E., Deom, C. M., Ininda, J., & Oloka, H. K. (2013). *Groundnuts production guide for Uganda, recommended practices for farmers.* Entebbe, Uganda: National Agricultural Research Organization.

Oteng-Frimpong, R., Sriswathi, M., Ntare, B. R., & Dakora, F. D. (2015). Assessing the genetic diversity of 48 groundnut (*Arachis hypogaea* L.) genotypes in the Guinea savanna agro-ecology of Ghana, using microsatellite-based markers. *African Journal of Biotechnology, 14*(32), 2484–2493.

Park, D. L. (2002). Effect of processing on aflatoxin. *Advances in Experimental Medicine & Biology, 504*, 173–179.

Rajeendran, A., Nulit, R., Yien, C., Ibrahim, M., & Kalhori, N. (2017). Isolation and molecular identification of Colletotrichum gloeosporioides from infected peanut seeds. *International Journal of Physical and Social Sciences, 19*(2), 1–8.

Singh, K., Frisvad, J. C., Thrane, U., & Mathur, S. B. (1991). *An illustrated manual on identification of seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins* (1st ed.). Hellerup, Denmark: Danish Government Institute for Seed Pathology for Developing Countries.

Sirhan, A. Y., Tan, G. H., Al-Shunnaq, A., Abdulra'uf, L., & Wong, R. C. S. (2014). QuEChERS-HPLC method for aflatoxin detection of domestic and imported food in Jordan. *Journal of Liquid Chromatography & Related Technologies, 37*, 321–342.

Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International, 62*, 11–19.

Upadhyaya, H. D., Nigam, S. N., Mehan, V. K., Reddy, A. G. S., & Yellaiah, N. (2001). Registration of *Aspergillus flavus* seed infection resistant peanut germplasm ICGV 91278, ICGV 91283, and ICGV 91284. *Crop Science, 41*, 559–600.

Wagacha, J. M., Mutegi, C., Karanja, L., Kimani, J., & Christie, M. E. (2013). Fungal species isolated from peanuts in major Kenyan markets: Emphasis on *Aspergillus* section Flavi. *Crop Protection, 52*, 1–9.

Waliyar, F., Kumar, K. V. K., Diallo, M., Traore, A., Mangala, U. N., Upadhyaya, H. D., et al. (2016). Resistance to pre-harvest aflatoxin contamination in ICRISAT's groundnut mini core collection. *European Journal of Plant Pathology, 145*, 901.

Xing, F., Ding, N., Liu, X., Selvaraj, J. N., Wang, L., Zhou, L., et al. (2016). Variation in fungal microbiome (mycobiome) and aflatoxins during simulated storage of in-shell peanuts and peanut kernels. *Scientific Reports, 6*(April), 25930.

Youssef, M. S., El-Maghraby, O. M. O., & Ibrahim, Y. M. (2008). Mycobiota and mycotoxins of Egyptian peanut (*Arachis hypogaea* L.) seeds. *International Journal of Botany, 4*(4), 349–360.