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Assessment of aflatoxin-producing fungi strains and contamination levels of aflatoxin B1 in groundnut, maize, beans and rice

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ABSTRACT

Aflatoxins are polyketide-derived, toxic and carcinogenic secondary metabolites produced by some species of *Aspergillus* and other fungi on food crops and feed. Aflatoxin B1 is classified as the most toxic of the aflatoxins, responsible for not only great economic loss but is also the most potent naturally occurring chemical liver carcinogen known. Random samples of groundnut, maize, beans and rice were collected from the Kumasi Central Market and analyzed for their aflatoxin levels using High Performance Liquid Chromatography (HPLC). Furthermore, molecular assessment of aflatoxin-producing fungi in the grain samples involving five pairs of universal and eight specific aflatoxin primers were carried out. The contamination levels found ranged from trace amount to 31.11 ppb, with groundnut registering the highest aflatoxin content. A further microbial culture examination revealed that most of the crop samples, especially groundnut and maize were susceptible to various species of aflatoxigenic, *A. flavus, A. paraciticus, A. tamarii, P. expansum, Mucor hiemalis, A. niger, P. citrinum, Moniliellaspp*. and other toxigenic fungi. Colony forming units per gram (CFU/g) from the microbial cultures ranged from 4.3×10^6 to 2.1×10^3. However, a poor correlation existed between the aflatoxin contamination level and the CFU/g per sample. A consistent correlation could not be made between the molecular analysis and microbial results. Just as in *A. versicolor*, four universal primers, 0817F/1196R, U1/U2, FF2/FR1 and ITS1/IST2, and one specific aflatoxin producing fungi primer, Nor1/Nor2, was able to show positive bands on *A. versicolor*. These suggest that particular fungi have the gene to produce aflatoxin; however, it could not produce detectable aflatoxin by the HPLC. The results showed the aflatoxin levels were within acceptable limit for consumption and exportation (0-20 ppb) by Codex.

Keywords: Aflatoxin, beans, groundnuts, maize, rice, PCR, HPLC, microbiological culturing.
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INTRODUCTION

Aflatoxins are naturally occurring mycotoxin that is largely produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi. This is a highly toxic secondary metabolite that contaminates a number of crops, causing a great economic loss (Cary et al., 2000; CAST, 2002). Several other moulds and fungi species also produce aflatoxin and these include *A. nomius, A. pseudotamarii* and *A. bombycis* (Peterson et al., 2001). The biosynthetic pathway of aflatoxin in *A. flavus* and *A. parasiticus* are similar and well characterized (Cary et al., 2000; Yu et al., 2004). Aflatoxins are produced in certain foods and feeds, and undoubtedly the best known and most intensively researched mycotoxins in the world. It has been associated with various diseases, such as aflatoxicosis in livestock, domestic animals and humans throughout the world (Bhatnagar et al., 2003). Certain environmental factors influence the occurrence of aflatoxin; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of crop commodities to

Table 1. Sample collection and size.

Food crops	Number of batches	Number of samples per batch	Sample size (kg)	Number of replicates for analysis
Groundnut	3	1	5	3
Maize	3	1	5	3
Beans	3	1	5	3
Rice	3	1	5	3

fungal invasion during pre-harvest, storage or processing periods (Bhatnagar et al., 2003).

Food security on the African continent has been worsening and deteriorating in recent decades. The proportion of the malnourished population has remained predominant in most Sub-Saharan African countries (FAO, 1996). To eradicate or mitigate this situation, an increase in productivity of agricultural food crops, especially groundnut, maize, rice millet and beans, are not only necessary as these crops form part of the staple foods and the main cash crops of most of these countries, but must go hand-in-hand with better farming practices and post-harvest handling.

These agricultural products are very prone to aflatoxin contamination, particularly during growth periods, harvesting, threshing and drying (Siriacha et al., 1989). Contamination can also occur when grains are poorly stored, which can cause pest infestation and poor conditions that lead to an accelerated growth rate of *Aspergillus* and other fungi species (WHO, 2006). Aflatoxin-producing species of *Aspergillus* are common and widespread in nature. Grains are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high-humid environment, or damage from stressful conditions (Udoh et al., 2000).

Mycotoxins affect the nutritional and economic value of staple foods and cash crops, especially in developing countries like Africa. Of the many mycotoxins, aflatoxin is of major concern, especially in countries where agricultural practices are not strictly controlled, and human and animal exposure to mycotoxins is very high (Wagacha *et al.,* 2008). Attention is only paid to meet export criteria while the effects of aflatoxin on the health of the local consumers are not prioritized. The contamination of foods with aflatoxin has in recent times created a great alarm on food security in Africa (Leslie, 2005). It has caused massive economic losses on export and import markets, and diseases such as impaired immune system, cancer and stunted growth in infants (Williams et al., 2004).

In Ghana, groundnut, maize and other cereals and legumes are sold in the open market with less or no regulation of quality. Most of the contaminated foods find their way into households and restaurants and patronized by unsuspecting consumers. The assessment of the levels of aflatoxin in food crops and identification of fungi responsible for their contamination will inform policymakers to improve upon proper handling of grains to reduce the toxin in foods. The main objective of this study was to evaluate the diversity of Aflatoxin-producing fungi and levels of Aflatoxin B1 in selected cereals and legumes from Kumasi Central Market in Ghana using HPLC, PCR and microbial culture techniques.

MATERIALS AND METHODS

Sample collection and preparation

Raw samples of groundnut, maize, beans, and rice were collected from different sales points in Kumasi Central Market-Ghana (Table 1). Batches were collected from the same sales points in an interval of two weeks, which is the maximum time limit for a consignment to be completely sold. Sampling was done according to the sampling protocol for Official Control of Mycotoxins in Food (EU, 2003) to give a representative sample which was then put in sealed bags and transported to the Biotechnology Laboratory, Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Ghana.The entire primary samples were ground to powder by milling and homogenization. Thereafter, sub-samples were made into different portions for the different tests HPLC, microbiological culture and molecular analysis. Samples were stored at -20ºC until analyzed.

Aflatoxin HPLC procedure

A total of 25 g of each ground sample of groundnut, maize, beans and rice was weighed into independent and labeled blender jars, and 5 g of Sodium Chlorine (NaCl) was added to each. Then 125 ml of 70% methanol was added to each set-up and blended at high speed of 15,000 rpm for 2 min. The content was subsequently filtered with a Whatman No1 filter paper and the filtrate collected into a clean beaker. The purpose of this step was to dissolve the analyte in the solvent. A total of 15ml of each filtrate or extract was pipetted into another beaker and diluted with 30 ml of distilled water, followed by thorough mixing by a vortex. Each diluted extract was filtered through a glass microfiber filter and 15 ml loaded

into the glass syringe (AOAC, 2005). Each loaded glass syringe (equivalent to 1 g of sample) was passed through the AflaTest column. The AflaTest column was washed with 10 ml of distilled water twice. The aflatoxin was then eluted with 1.0 ml methanol (HPLC grade) and was collected into a glass cuvette. The aflatoxin analysis was conducted with a fluorescence detector wavelength of 360nm excitation and 440 nm emission. A column specification of 30 cm long x 4.6 mm wide, 5 um Supelco C-18. The mobile phase pumped at a flow rate of 1ml/min and consisted of water: methanol of 60:40, with the addition of 120 mg of potassium bromide and 350 µl of nitric acid. The injection volume of both the analyte and the standard was 100µl. The column oven temperature was 40°C, and the lower detection limit was 0.5 ppb while the limit of quantification was 1.0 ppb, Ahsan et al., (2010).

Microbial culture procedure

A 10 ml of peptone water was pipetted into a sterile test tube labeled 1, and 9 ml into other test tubes, labeled 2 and tube 3. Then 1g of each sample was weighed into test tube 1 and shaken vigorously to dissolve the sample. Thereafter, 1ml of the mixture was serially diluted in test tube 2 and test tube 3. Finally, 1ml from each dilution factor was cultured by spread plate technique on a correspondingly labeled agar plate that contained Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), DRBC agar, 100 mg/l of chloramphenicol and 50mg/l chlortetracycline hydrochloride. The antibiotics suppressed the growth of bacteria incubated at room temperature for 5-7 days. Fungal growth was observed within the 5-7 days in plate colonies. After the incubation period, colonies were sub-cultured to the appropriate media and further incubated to attain pure cultures of each fungal species. After obtaining colonies of pure isolates, they were identified by observing them phenotypically and verified microscopically by examining colony color, size, appearance and cell morphology (Hogg, 2005). The colonies were further counted using a colony counter. Fungal loads were calculated in colony forming units per gram of sample (CFU/g) as:

$$\text{CFU/g} = \frac{\text{number of colonies x reciprocal of the dilution factor}}{\text{Plating volume (1 ml)}}$$

Molecular characterization of fungal species

Isolates of the various fungus strains from samples were sub-cultured to pure isolates and incubated for 6 days at room temperature. Fungal hyphae were collected, centrifuged and washed with water once to rinse out the spores, followed by drying under a laminar hood. About 100 mg of mycelia from each strain were freeze-dried with liquid nitrogen and ground with a mortar and pestle. A 900 µl of 2% CTAB pre-warmed extraction buffer was added, mixed and incubated at 65°C for one hour. Again, 900 µl phenol: chloroform of 1:1 was added. The mixture was homogenized by vortexing for 30 seconds and phases were separated by centrifugation at 10,000 rpm for 10 mins. A 500µl of the supernatant was pipetted in a fresh 2 ml tube and 2 µl RNase (5 µg/ml) was added and incubated at 37°C for 30 mins. An exact 750 µl of chloroform was then added with quick vortexing (5 seconds) and then re-centrifuged at 10,000 rpm for 10 mins. Furthermore, 250 µl of 7.5 M NH_4OAc and 1ml of ethanol (99%) was added to the 500 µl of the supernatant in the same 2ml tube and kept on ice for over an hour. The tube was finally centrifuged at 12,000 rpm for 15 min. followed by decanting of all the supernatant; another quick centrifuge at same speed for 5 seconds, followed by pipetting out of the remaining liquid was done. Tubes were then dried in the laminar flow hood for 45 min.. The dried pellets were dissolved in 45 µl of Tris-HCl EDTA buffer with pH 8.0. Concentration and the purity of DNA were checked by nanodrop and gel electrophoresis according to the method of Stephen and Lewis, (2001). Primers were hydrated with molecular biology grade water to their respective volumes as directed by the manufacturer (Biolegio), to give a stock concentration of 100 µM, and centrifuged for about 5 seconds. To prepare the working concentration(10 µM),10 µl was pipetted from both forward and reverse primer stock into a new microfuge tube, followed by dilution with 180µl molecular biology grade water and was vortexed. A master mix, consisting of primer sets, dNTPs, 10X buffer, H_2O and Taq polymerase, was prepared. A 9.0 µl of the master mix was dispensed into a 200 µl PCR microfuge tube, and 1.0 µl of DNA added and spun for 30 seconds. All the tubes were positioned in the thermal cycler and all cycling conditions were optimized. About 3-4 h later, the PCR was completed, and 5 µl was loaded and ran 2% agarose gel and visualized with UV. Detailed PCR conditions and reagent volumes, primer sequences and gel electrophoretic procedures are available upon request.

Statistical analysis

All results generated from the experiments were analyzed using GraphPad Prism (V.5) for the effects of various factors on the results at 5% probability and 95% confidence interval.

RESULTS

Levels of aflatoxin in food samples

Although the focus was on AFB1, all four types of aflatoxins (AFB1, AFB2, AFG1, and AFG2) were

Table 2. Aflatoxin B1 contamination levels as detected by HPLC.

AFB1 contamination level in crops P,C,N,R				
Crops	**P**	**C**	**N**	**R**
1	2.20	8.79	-	-
2	-	3.15	0.0006	-
3	31.11	-	-	0.0001
Lsd	6.04	1.388	0.0001199	0.0001998

P:groundnut, C: maize, N: beans, R: rice, P1, C2, N1: sample batch number, -:negative, AF:aflatoxin, Lsd: less significant difference.

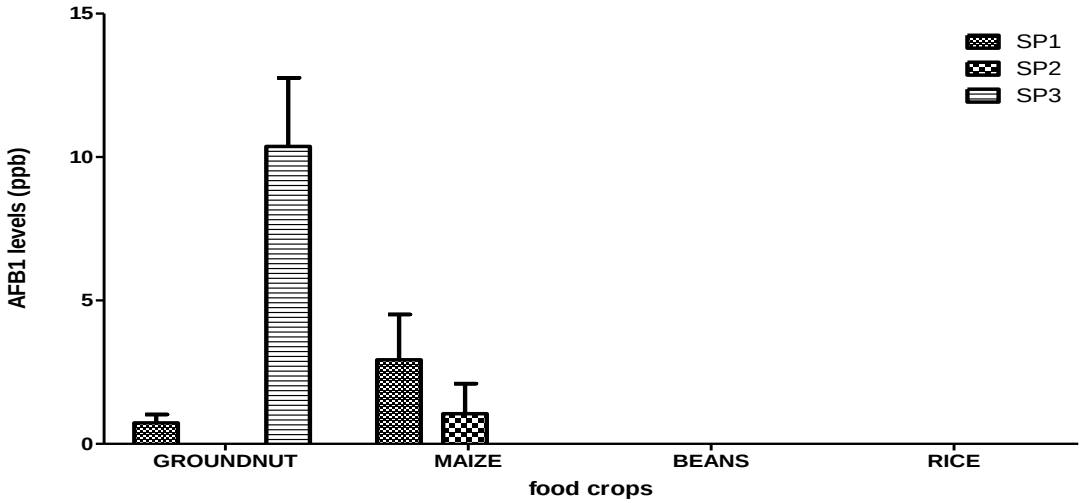

Figure 1. Aflatoxin levels in food samples at various sales points (SP).

observed (Table 2). Out of the twelve samples, only two groundnut samples (P1 and P3,) and two maize samples (C1 and C2), recorded significant amounts of AFB1, ranging from 2.20 to 31.11 ppb. A beans sample (N2) and a rice sample (R3), showed aflatoxin contamination but was too low to pose a health hazard. Moreover, there was no significant difference (p>0.05) between sales point (SP) SP1 and SP2 in terms of groundnut and maize (Figure 1). There was, however, the highly significant difference (p<0.001) between SP1 and SP3 with respect to groundnut. Aflatoxin levels for grain samples from SP1 showed no significant difference (p>0.05) between maize and beans, as well as maize and rice. Furthermore, no significant difference (p>0.05) was found between beans and rice in all SPs. The analyses, however, revealed highly significant difference (p<0.001) between groundnut and all the rest of the crops (maize, beans and rice) from SP3.

Diversity of fungi observed on each sample

The commonest fungi genera found in the food samples were *Aspergillus* and *Penicillium* (Table 3). *A. flavus* and *A. paraciticus* were the predominant *Aspergillus* species. In general, 11, 10,8 and 5 different moulds species were found to be present in groundnut (P), maize (C), bean (N) and rice samples, respectively. The samples had different levels of microbial contamination (Table 4). The Groundnut (P) samples recorded the highest level of microbial contaminants, ranging from 3.8x105 cfu/g to 4.3×106 cfu/g compared to Rice (R) which had the lowest microbial loads ranging from 2.1×10^3 cfu/g to 4.3×10^3 cfu/g. Maize (C) had microbial loads ranging from $4.6x10^4$cfu/g to $3.4x10^6$cfu/g whilst the microbial loads recorded for Beans (N) ranged from $2.9x10^4$cfu/g to $5.5x10^4$cfu/g. The standard microbial count range within 25-250 CFU/g (Zipkes *et al.,* 1981). Some mould species were predominant on particular samples. For instance, *Aspergillus* and *Penicillium* species, such as *A. flavus, A. paracitius, A. terreus, P. citrinum P. expansum* as well as *Emericellanidulans*, dominated on all groundnut and maize samples.

Molecular analysis of aflatoxin-producing fungi

Molecular characterization of observed fungal species

Table 3. Mouldsidentified on various samples.

Crops	Samples	Fungi identified
Groundnut (P)	P1	*A. flavus, A. niger, P. citrinum, Moniliellaspp.*
	P2	*A. flavus, A. terreus, Emericellanidulans, P. expansum*
	P3	*A. flavus, A. paracitius, P. italicum, Absidiacorymbifera, Aureobasidium spp.*
Maize (C)	C1	*A. paracitius, A. tamarii, P. expansum, Mucor hiemalis*
	C2	*A. paracitius, Emericellanidulans, Rhizoctoinia solani*
	C3	*A. flavus, A. terreus, P. citrinum, A. versicolor*
Beans (N)	N1	*A. niger, P. expansum, Fusarium spp.*
	N2	*P. citrinum, Eurotium amstelodami*
	N3	*Mucorhiemalis, Cladosporium spp., Botrytisspp.*
Rice (R)	R1	*P. italicum, Cladosporium spp.*
	R2	*Moniliella spp.,Aureobasidium spp.*
	R3	*Eurotium amstelodami*

P: Groundnut, C:Maize, N: Beans, R: Rice, (P1, C2, N3 ..) sample with batch number *spp*: species.

Table 4. Microbial Count (CFU/g ± SD) of the samples.

Crops	Samples	Contamination level (CFU/ g)	Standard deviation (SD)
Groundnut	P1	3.8×10^{5}	3.74
	P2	5.3×10^{5}	0.16
	P3	4.3×10^{6}	0.49
Maize	C1	3.4×10^{6}	0.29
	C2	3.4×10^{5}	0.44
	C3	4.6×10^{4}	3.09
Beans	N1	5.5×10^{4}	0.24
	N2	3.2×10^{4}	2.44
	N3	2.9×10^{4}	0.24
Rice	R1	4.3×10^{3}	4.92
	R2	2.7×10^{3}	0.17
	R3	2.1×10^{3}	0.04

P:Groundnut, C:Maize, N:Beans, R: Rice, (P1, C2, N3...): sample with batch number, CFU/g: Colony forming unit per gram, Dilution factor 10^{-3}

was carried out by PCR amplification of various DNA markers using both universal and specific primers, with subsequent resolution of PCR products on agarose gel (Figure 2, Table 5). The identities of many fungal species were confirmed through these molecular techniques.

DISCUSSION

Employing HPLC, microbial culture and PCR techniques, it has shown that some grains from Kumasi Central market have aflatoxin concentrations that can impact negatively on human health. The most significant contaminations were observed on groundnut and maize while beans and rice recorded very low aflatoxin levels. The low aflatoxin levels, as well as the insignificant difference in aflatoxin levels between SP1 and SP2 as far as groundnut and maize were concerned, may have resulted from the almost same hygienic practices at both SPs; the saleswomen were continuously sorting out damaged grains at the times of research visits. The grains at these SPs were also exposed to the heat of the sun while on sale, a process known to inhibit aflatoxin production (Wu et al., 2010). It has been shown that proper heat drying can effectively limit the spread of harmful fungi that produce different mycotoxins, especially aflatoxin (Magan et al.,2007). Different storage conditions could also account for why SP3 differed greatly in its aflatoxin level for groundnut as compared to the rest of the two Sales Points. This great difference exhibited by SP3 is further clarified in groundnut sample P3, where groundnut samples registered the highest

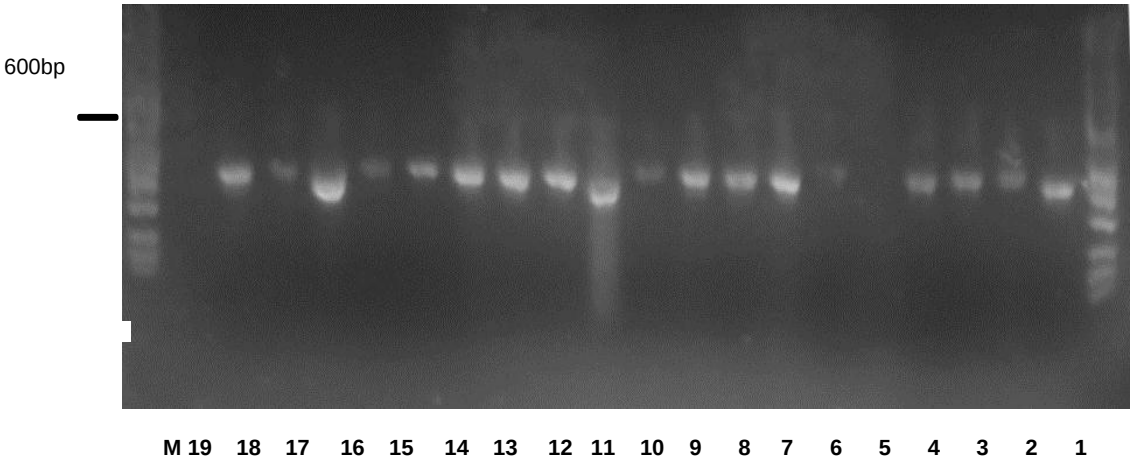

Figure 2. Amplification of DNA markers by universal primer set (FF2 & FR1) on fungal isolates. Scoring of bands that represent PCR products showed that lane 2,5,6,10,16 and 18 were negative with respect to 600bp locus whereas lane 1,3,4,7,8,9,11,12,13,14,15, 17 and 19 were positive. M:DNA ladder.

Table 5. Loci scores for various primer pairs on different samples.

Primer no.		Fungal isolates 1-19 arranged accordingly as represented in figure 2																	
	Primers(F&R)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
1	F: 0817F R: 1196R	+	+	+	+	-	+	+	-	+	+	+	+	-	+	-	+	-	+	+
2	F: P1 R: P2	+	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	+
3	F: U1 R: U2	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+
4	F: FF2 R: FR1	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	-	+
5	F: ITS5 R: IST2	-	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+
6	F: nor1 R: nor2	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	F: Ver1 R:Ver2	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	+
8	F: Omt1 R: Omt2	-	+	-	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-
9	F: VER-496 R: VER-1391	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
10	F: APA-450 R: APA-1482	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	F: OMT-208 R: OMT-1232	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
12	F: AflR620 R: AflR1249	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
13	F: Ord1508 R: Ord2226	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
Suspected/similar fungi		8	3	2	n	n	n	11	1	n	n	7	n	n	n	n	16	n	18	n

NB: -: absent, +: present, F: forward primer, R: reverse primer, n: not identical with any fungi.

aflatoxin level probably due to prolonged storage of the samples under unhygienic conditions. Bad agronomical practices in the field, such as continuous cropping and breaking of the shell during harvesting, could also account for the contamination (Hell et al., 2000).

A. flavus is the most common species associated with

aflatoxin contamination of crops (Cotty, 1997). The present study observed that *A. flavus and A. paraciticus* were present and dominant in all groundnuts and maize samples but absent from the rice and beans samples. These findings are in agreement with observations elsewhere which stated that the two fungi (*A. flavus* and *A. paracitius*) are commonly found in maize, cotton, tress, and groundnuts; they are seldom found on rice (Samuel et al., 2013; Chen et al., 2013). There was no sign of the presence of *A. flavus and A. paraciticus* on all bean samples; only the black-spored *A. Niger* appeared in one of the bean samples. *A. tamarii* was part of the isolates identified alongside *A. paraciticus* in a maize sample (C1). *A. tamarii* constituted 26% of the total fungi in this particular sample. The total CFU/g of fungi in this sample was $3.4x10^6$, which was higher than the acceptable limits of $<10^5$ CFU/g (Abadias et al., 2008). The total level of aflatoxin contamination in the maize sample was 8.72 ppb. Despite *A. Parasiticus* being a known aflatoxin producer, it showed negative to all the specific primers except Ver1/Ver2. Some of the molecular markers U1/U2 and ITS5/ ITS2 has shown some positivity in *A. tamarii* which shows that the fungi is capable of producing aflatoxin. This is in concordance withan earlier observation that *A. tamarii* had been found for the first time to produce aflatoxin (Dorner, 1983).

A. versicolor is a common indoor fungus in damp houses, carpet, dust etc. It is capable of producing Sterigmatocystin, which is a precursor of aflatoxin biosynthesis (Yu et al., 2004). *A. versicolor* is among the major producers of hepatotoxic and carcinogenic mycotoxins sterigmatocystin (Barnes et al., 1994). However, the acute and chronic toxicities effects of sterigmatocystin from *A. versicolor* are significantly lower (Scudamore et al., 1996).*A. versicolor* represented about 16% of the total fungi isolated by the present study, scoring $4.6x10^4$ CFU/g with *A. flavus, A. terreus, and Penicillium citrinum*in the maize (C3) sample. Even though the CFU/g was high enough for *A. versicolor*with the presence of *A. flavus,* no aflatoxin contamination was recorded by the HPLC. Four universal primers (0817F/1196R, U1/U2, FF2/FR1 and ITS/IST2) and one specific aflatoxin producing fungi primer (nor1/nor2) were able to show positive bands for *A. versicolor*. This suggests*. versicolor* has the gene to produce aflatoxin; however, it could not produce detectable levels of aflatoxin. This presupposes that the presence of aflatoxin-producing fungi in grains does not necessarily mean there will be aflatoxin contamination. This observation is corroborated by a study which established poor correlations between infection percentages and aflatoxin contamination (Cole et al., 1983). It has been shown that infection of groundnuts by *A. flavus*, *A. parasiticus,* and other mycotoxins-causing fungi was not a prerequisite for aflatoxin contamination. It is, thus, possible to detect significant aflatoxins levels in biochemically clean seeds while an infested looking seed may not contain any amount of aflatoxin. It is also difficult to use percentage pathogenic infections as a guide to aflatoxin build-up since even disinfected seeds may still carry the fungal propagules and continue to sporulate in the due cause (Dorner et al., 2003).

Plant seeds contain some proteins which may act as inhibitors of fungal infection and growth during storage and germination (Guo et al., 1999). When these proteins are concentrated in the seed, it may prevent fungal invasion and reproduction, by exhibiting bioactivity against the growth of *A. flavus*, and other mycotoxin-causing fungi. In the present study, some of the samples may have recorded no aflatoxin contamination probably due to the interactive effects of the seed inhibiting proteins (phytoalexins) and competition among the fungi.

Penicillium species are very commonly found in the soil, on decaying vegetation and compost, on wood, dried foodstuffs, spices, dry cereals, fresh fruit, vegetables, etc. They are also one of the best lipase and alpha-amylase producers among fungi in arctic tundra (Bancerz et al., 2005). *Penicillium* fungi are versatile, opportunistic and many species are one of the most common causes of fungal spoilage in fruits and vegetables, and are also post-harvest pathogens. Some *Penicillium* species produce mycotoxins, for example, *P. expansum* produces Patulin (ICMSF, 1998). *P. expansum* was positive to three of the universal primers and two of the specific aflatoxin-producing fungal primers (Ver1/Ver2 and omt1/omt2), suggesting that *P. expansum* was capable of producing aflatoxin.

Fusarium species are widely distributed in soil, buried or subterranean, and aerial plant parts, plant debris, and other organic substrates (Nakar et al., 2001) and are present in water worldwide as part of water structure biofilms (Elvers et al., 1998). The widespread distribution of *Fusarium* species may be attributed to their ability to grow on a wide range of substrates and their efficient mechanisms for dispersal (Burgess et al., 1981). *Fusarium* species are important plant pathogens causing various diseases such as crown rot, head blight, and scab on cereal grains. They are capable of producing mycotoxins such as *Fumonisin* or *Tricchothecenes*which are responsible for diarrhoeal diseases (Nakar et al., 2001); they may occasionally cause infection in animals **(**Ferrer et al., 2005**).** In humans, *Fusarium* species cause a broad spectrum of infections almost exclusively in severely immune-compromised patients.*Fusarium* was found only in beans samples; it is also possible that the species observed were non-aflatoxin producers, as it was negative to all the specific primers. The same was true for *Botrytis* and*Eurotiumamstelodami*. Some species of *Moniliella* can cause disease in humans (Guarro et al., 1999) and in cats (McKenzie et al., 1984); three of the specific primers suggested this fungus may also produce aflatoxins.

The presence or lack of mRNA of an aflatoxin gene could permit direct differentiation between fungal species (Mayer et al., 2003). After testing 19 fungi isolates with five universal primers and eight specific aflatoxin-producing primers by the present study, *Rhizoctoiniasolani,Botrytisspp*,*Eurotiumamstelodami, A. terreus, A. versicolor and A. tamarii* showed negative results for all the specific primers.This suggests that all these six fungi are non-aflatoxigenic mould. The rest of the fungi displayed different bands with various primers. Some known aflatoxin-producing fungi, such as *A. flavus,* showed no band with the primer nor1/nor2*.* This may have resulted from a mutation in this strain of the fungi, which may include substitution of some bases (Geisen, 1996). Various physiological conditions and nutrient source can also affect aflatoxin biosynthetic pathway. It is therefore practically possible to isolate aflatoxigenic fungi by PCR, yet the fungi would not be able to produce aflatoxin.

Conclusion

The aflatoxin level found in almost all the 12 samples were within the range of the acceptable limit for consumption and exportation (0-20 ppb) by Codex Alimantarius commission. A consistent correlation could not be established between the aflatoxin contamination levels and the CFU/g recorded by the microbiological cultures per sample. Therefore, the presence of an aflatoxin-producing fungus on a food commodity, as detected by PCR, does not necessarily mean the fungus is contaminating the food commodity with an aflatoxin.

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