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ORIGINAL ARTICLE

A mini-survey of moulds and mycotoxins in locally grown and imported wheat grains in Nigeria

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Abstract A preliminary survey involving limited sample size was conducted to determine the spectrum of moulds and mycotoxins in wheat grains from flour mills and local markets in Nigeria. Fourteen wheat samples were analyzed for moulds using standard mycological methods and for toxic fungal metabolites using a liquid chromatography-tandem mass spectrometric method. *Fusarium* (range of incidence 12.5–61.7%) dominated in the wheat grains though species of *Aspergillus* (range of incidence 2.24–3.86%) were also recovered from the samples. The identified fungal species were *Aspergillus flavus* (7.7%), *Aspergillus niger* clade (2.6%), *Fusarium avenaceum* (10.9%), *Fusarium culmorum* (22.4%) and *Fusarium graminearum* (56.4%). A total of 54 microbial metabolites were detected in the samples at concentration ranging between 0.01 μg/kg for macrosporin and 2560 μg/kg for deoxynivalenol. Among the four mycotoxins addressed by regulations in the European Union (EU) found in the samples, deoxynivalenol (incidence 100%) dominated in the samples and its levels exceeded the maximum acceptable EU limit (750 μg/kg) in 36% of the samples. This report underscores the need for more robust surveys with larger sample sizes and across several agro-ecologies in the country.

Keywords Deoxynivalenol · *Fusarium* · Mycotoxins · Nigeria · *Triticum aestivum*

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Introduction

In Nigeria, wheat constitutes several staple foodstuffs (e.g. bread, noodles and spaghetti). Since these foods are produced industrially, the economic contribution from wheat processing is appreciable. However, Nigeria imports over 90% of wheat for industrial processing from the United States of America (WORC 2002) although the country is the largest wheat producer in West Africa (CIMMYT 2001). CIMMYT (2001) further reported that Nigeria's wheat yield ranged between 2.5 and 3 tons/ha from cultivations in six states: Jigawa, Kano, Borno, Yobe, Gombe and Sokoto. The low yield is mainly due to low quality seed input, pests and diseases prevalent during the growing period which causes injuries that affect plant health and quality; this is critical to minimizing the gap between attainable yield and actual yield.

Wheat can be contaminated by variety of toxigenic fungi during plant growth, harvesting and later in storage (Tancinova et al. 2001). Fungal activities are capable of decreasing the yield at harvest, causing grain discolouration, decreasing the germinability and nutritional value and mycotoxin accumulation in storage; these can

lead to deterioration in baking and milling quality (Magan et al. 2003). Mycotoxins commonly occurring in wheat are the *Fusarium* mycotoxins (e.g. fumonisins; trichothecenes including deoxynivalenol (DON), nivalenol (NIV) and T-2 and HT-2 toxins; and zearalenone (ZEN)) (Gonzalez et al. 2008; Alexa et al. 2013). The contamination levels vary largely between regions, climatic conditions, years, varieties and sowing time.

Regardless of the wide use of wheat in many Nigerian homes, there is paucity of information on the spectrum and co-occurrence of mycotoxins in wheat grown or imported in Nigeria. A previous study by Ezekiel et al. (2008) isolated *Fusarium* species from 50 wheat samples without testing the samples for naturally occurring mycotoxins while Makun et al. (2010) screened 50 wheat samples for only aflatoxin B_1. In accordance with the growing concern for increasing wheat production in the country due to the high susceptibility of other main staples (e.g. groundnut and maize) to aflatoxins and fumonisins, this study was designed. This study assessed the incidence of moulds and spectrum of mycotoxin contamination in wheat grains intended for human consumption. This is a mini-survey with limited number of samples intended mainly to obtain preliminary information on the subject of this paper due to absence of such data.

Materials and methods

Sampling

Fourteen bulk samples (1 kg each) of wheat grains were randomly collected from flour mill industries and markets/farmers' stores in Nigeria. The samples from flour mills (n = 4) were imported while other 10 samples were locally produced. The locally produced samples were either purchased from local markets (n = 6; two samples each from markets in Lagos, Ogun and Oyo) or collected from farmers' (who are wheat vendors) stores (n = 4; two samples each from farmers in Jigawa and Kano states). All the wheat samples were collected as cleaned and ready to be processed (industrial or home) samples. The bulk samples consisted of two subsamples (0.5 kg each) collected from five random points in trader's trays or baskets or from storage bins in industries, and were mixed together. Each sample was comminuted, and 90–100 g representative subsample, obtained after several rounds of quartering, was used for analysis. The representative samples were equally divided into two batches: A for mycological analysis and B for multi-mycotoxin analysis. Representative samples were stored at 4 °C until they were analyzed.

Mycological analyses of wheat grains

Isolation of moulds

One hundred arbitrarily selected grains from each sample were surface-sterilized by dipping them in 1% NaOCl for 30 s. The surface-sterilized grains were then rinsed thrice in sterile distilled water to remove residual chemical which may interfere with mould growth during fungal isolation and were blotted dry on a sterile paper towel. Ten grains per samples were plated out on full-strength potato dextrose agar (PDA) and peptone-pentachloronitrobenzene agar (PPA), a semi-selective medium for *Fusarium* (Nash and Synder 1962). The inoculated PDA plates were incubated unilluminated for 3 days at 31 °C while PPA plates were incubated for 4 days under fluorescent lights on a 12-h day/night schedule at 22–24 °C.

Identification of moulds

Colonies of *Aspergillus* that developed on the grains on PDA plates were carefully purified on fresh full-strength PDA plates, while *Fusarium* colonies on PPA plates were transferred to freshly prepared PPA plates. PDA plates were incubated for 5–7 days at 31 °C, and all isolates were morphologically identified by assessing macroscopic and microscopic characters in line with appropriate keys (Samson et al. 1995; Klich 2002). *Fusarium* isolates were single-spored and incubated on water agar (20 g agar powder/L of distilled water) overnight at 22–24 °C. Germinating spores were maintained on modified Czapek Dox complete medium and then transferred to carnation leaf agar (Fisher et al. 1982) for morphological identification (Nelson et al. 1983). At least 10 single-spore isolates of *Fusarium* species from each sample were identified.

Multi-mycotoxin assessment of wheat grains

Five grams of representative, homogenized samples was weighed into a 50-ml polypropylene tube (Sarstedt, Nümbrecht, Germany) and extracted with acetonitrile/water/acetic acid 79:20:1, *v*/*v*/*v* in a ratio of 4 ml solvent/g sample for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany). Extracts were diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, *v*/*v*/*v*) and injected as described in detail by Sulyok et al. (2007). For spiking experiments, 0.25 g of sample was applied for extraction as described above. Screening of mycotoxins and other microbial metabolites was performed on a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C_{18}-column, 150 × 4.6 mm i.d., 5 μm

particle size, equipped with a C_{18} 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US). The chromatographic method as well as chromatographic and mass spectrometric parameters for 295 of the investigated analytes is as described by Malachova et al. (2014). The accuracy of the method is verified on a routine basis by participation in proficiency testing schemes organized by BIPEA (Bureau Interprofessionnel des Etudes Analytiques, Gennevilliers, France). These include aflatoxins B_1, B_2, G_1, G_2 and M_1; ochratoxin A; fumonisins B_1 and B_2; zearalenone; deoxynivalenol; nivalenol; 3- and 15-acetyldeoxynivalenol; and T-2 and HT-2 toxin. To date, 92% of the 590 results submitted for a broad variety of matrices (including few for which no validation data has been available) are in the satisfactory range. In the case of wheat, 44 out of the 45 submitted results were in the satisfactory range.

Results and discussion

Incidence of moulds in wheat grains

The moulds isolated and identified from the wheat grains are reported in Table 1. A total of 156 moulds were recovered from the samples, of which *Fusarium* (89.7%) dominated. *Aspergillus flavus*, *Aspergillus niger* clade, *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum* were the identified species occurring in the wheat samples. All isolated moulds occurred in all samples except for *F. avenaceum* and *F. culmorum* which were recovered from 64 and 86% of the samples. The dominance of *Fusarium* species in the grains agrees with the report of Ezekiel et al. (2008) of large occurrence of *Fusarium* species on wheat consumed in Nigeria. Furthermore, the diversity of moulds found in our present study agrees with published literature on the occurrence of these fungal species in wheat (Riba et al. 2008; Covarelli et al. 2015).

Overview of metabolite occurrences in wheat grains

A total of 54 microbial metabolites were detected in the samples at concentrations ranging between 0.01 μg/kg for macrosporin and 2560 μg/kg for deoxynivalenol (Tables 2 and S1). Apparent recoveries were above 70% in more than 95% of the 54 analytes.

Metabolites produced by *Claviceps* species and *Fusarium* species were the most prevalent in the samples constituting about 35% each of all metabolites detected (Table S1). Metabolites detected from other fungi include *Alternaria* toxins, *Aspergillus* metabolites (aflatoxins, aflatoxin precursors and metabolites from other species) and *Penicillium* metabolites. Chloramphenicol, a bacterial metabolite, was also detected in the grains. This is similar to the report of Ezekiel et al. (2012) who found this metabolite in fonio millet from Nigeria. Although we did not isolate some of these fungal genera from the wheat grains, probably due to the isolation method (direct plating) or choice of media or a selective subculturing method employed, the detection of their metabolites suggests that these moulds infected and colonized the crop at one time between cultivation and storage.

Table 1 Occurrence of moulds in 14 wheat grain samples from Nigeria

Moulds	Contaminated (%)	Occurrence (%)	Morphological description of fungal species
A. flavus	100.0	7.6	Greenish yellow granular-like obverse colonies with white edge and cream reverse on PDA[a]; biseriate (some isolates were uniseriate) with globose to ellipsoidal conidia
A. niger clade	100.0	2.5	Black granular-like obverse colonies with white colony edge colour on PDA[a]; creamy reverse colour (some colonies showed greenish yellow pigmentation); biseriate with globose condia
F. avenaceum	64.3	11.4	No chlamydospores on CLA[b]; long and slender, thin-walled and straight to slightly curved macroconidia with five septa; long, bent and tapered apical cell; notched basal cell (some isolates had foot-shaped cells); pale orange sporodochia formed on CLA[b] and surface of agar; highly variable microconidia (shape and septation) were sparsely produced by some isolates; presence of both mono- and polyphialides
F. culmorum	85.7	22.8	Abundant chlamydospores formed in chains (some singly) on CLA[b] by 4 weeks; short and stout macroconidia with 3–4 septa formed on monophialides on branched condiophores in abundant orange sporodochia; rounded apical cell and poorly developed foot cell; microconidia not present
F. graminearum	100	55.7	Relatively slender, sickle-shaped and thick-walled macroconidia formed on CLA[b]; macroconidia had 5–6 septa; tapered apical cell and foot-shaped basal cell; microconidia absent; red pigment formed on PDA

[a] Potato dextrose agar

[b] Carnation leaf agar

Table 2 Occurrence and concentrations of four EU-regulated mycotoxins in 14 wheat grain samples in Nigeria

Metabolites	LOD[a]	F (%)[b]	Concentrations (μg/kg) in imported samples (n = 4)			MAL[c] (% samples > MAL)	F (%)[a]	Concentrations (μg/kg) in domestic samples (n = 10)			Percent samples > MAL
			Min	Max	Mean			Min	Max	Mean	
Aflatoxin B_1	0.4	0 (0.0)	–	–	–	2 (0.0)	1 (7.1)	0.3	0.3	–	0.0
Deoxynivalenol	0.8	4 (100.0)	182.5	2557.0	858.7	750 (25.0)	10 (100.0)	118.7	1059.6	517.8	40.0
Nivalenol	0.4	1 (25.0)	31.5	31.5	–	–	4 (40.0)	0.3	12.0	7.5	–
Zearalenone	0.4	1 (25.0)	50.1	50.1	–	200 (0.0)	5 (50.0)	1.4	25.1	14.5	0.0

[a] Limit of detection [S/N = 3:1] expressed as microgram per kilogram sample

[b] Frequency of occurrence (%) of mycotoxins in the wheat grains

[c] Maximum acceptable limit (μg/kg) for mycotoxins stipulated by the European Union (FAO 2004). MAL applies to imported and locally grown wheat samples

Mycotoxins addressed by regulations and their masked/conjugated forms in wheat grains

Four mycotoxins addressed by regulations (aflatoxin B_1, DON, NIV and ZEN; Table 2) and two of their derivatives (DON-3-glucoside and ZEN-14-sulfate; Table S1) were found in the samples at varying occurrences and quantities. All the samples (100%) were contaminated with the trichothecene DON, at a concentration range of 119–2560 μg/kg (mean = 615 μg/kg) making it the most prevalent mycotoxin in the samples. About 36% of these DON concentrations exceeded the European Union recommended maximum acceptable limit of 750 μg/kg (FAO 2004). The conjugated form (DON-glucoside) was found in all samples except one at concentrations up to 200 μg/kg. The imported grain samples (max 2557 μg/kg, mean 858.7 μg/kg) contained higher levels of DON than those obtained from farmers' stores/markets (max 1059.6 μg/kg, mean 517.8 μg/kg). The high DON concentrations in the locally produced grains are unusual due to the tropical climate prevalent in the country; contributing factors need to be further studied. In view of processing effects which tend to lower final levels in finished/consumed products (Lancova et al. 2008), the levels found in the grains may not pose a threat to consumers. However, if the wheat grains are consumed directly, which is very much unlikely, the very high concentrations found in the grains could even lead to exceeding the TDI (as shown in Warth et al. 2013).

Furthermore, the high prevalence of DON found in this study is in agreement with the report of Rodrigues and Naehrer (2012) which established DON as the major mycotoxin in wheat. DON can be produced by any of the two *Fusarium* species (*F. graminearum* and *F. culmorum*), and both species were implicated in the contamination of these samples. To our knowledge, DON is reported for the first time in wheat in Nigeria. The co-occurrence of DON-3-glucoside with the parent toxin, DON, in almost all samples further substantiate the fact that all the DON-contaminated wheat plants attempted to detoxify the parent toxin leading to the glycosylation of DON (Rasmussen et al. 2012; Shin et al. 2012).

NIV and ZEN were both found in less than one half of the analyzed samples at concentrations (max 32 and 50 μg/kg, respectively) below any set limit for these toxins while ZEN-14-sulfate occurred in 57% of the samples. This was caused by the lower detection limit of the method demonstrated by lower concentrations (range 0.02–4 μg/kg). Aflatoxin B_1 occurred in just one sample and at a low concentration (0.3 μg/kg). These mycotoxins (NIV, ZEN and AFB_1) have previously been reported to contaminate wheat although at fairly higher quantities (Magan et al. 2010; Makun et al. 2010). The absence of fumonisins in samples analyzed in the present study agrees with previous reports that fumonisin may not be a problem in wheat as is in corn (Birck et al. 2006; Skrbic et al. 2012), although those reports found fumonisins in very low incidences in wheat.

Other metabolites not regulated in cereals

Alternaria toxins

The five *Alternaria* toxins alternariol, alternariolmethylether, altertoxin-1, macrosporin and tentoxin were detected in 79, 100, 36, 79 and 100% of the analyzed wheat samples, respectively (Table S1). Our report is in agreement with Müller and Korn (2013) who observed co-occurrence of *Alternaria* toxins in wheat samples from Northeast Germany with *Fusarium* toxins. These *Alternaria* toxins have also been found in several cereal crops in Burkina Faso (Warth et al. 2012), Cameroon (Abia et al. 2013) and Nigeria (Ezekiel et al. 2012; Abdus-Salaam et al. 2015), albeit at low concentrations corresponding to findings from the present study. Although we found lower concentrations of *Alternaria* toxins in this

study, the recent association of these toxins with impaired function of human topoisomerase II and inhibition of bacterial gyrase (Jarolim et al. 2016) calls for stricter measures aimed at controlling fungal infection of the grains.

Ergot alkaloids

Ergot alkaloids (EA), produced by *Claviceps* species, contaminated more than 80% of the wheat grains, and as much as 18 EA were detected (Table S1). All the locally produced wheat samples contained this group of toxins and at higher concentrations than one imported sample which was contaminated with the ergot alkaloids. Among the quantified EA in this study, seven (ergovaline, ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocorninine) have been recommended for monitoring by European Food Safety Authority (EFSA 2005). Some countries had set guideline limits for total EA in feed for poultry (100 μg/kg), swine (6000 μg/kg), chicks (9000 μg/kg) in Canada and 450 μg/kg for feeds in Uruguay (WHO 2003). The total EA in one sample exceeded some of these limits, thus suggesting the need for regulations to be in place for EA in Nigeria.

Other Fusarium metabolites

Other important *Fusarium* metabolites due to their prevalence (>70%) in the samples and known toxicological relevance include culmorin and aurofusarin (Table S1). Culmorin co-occurred with DON in all culmorin positive samples. This supports the reports of Ghebremeskel and Langseth (2001) who traced high concentration of the culmorin in wheat sample to the high level of DON during co-occurrence. Aurofusarin, a metabolite of *F. graminearum*, contaminated 93% of the wheat samples at concentrations up to 646 μg/kg. Very recently, it was demonstrated that aurofusarin exhibits pronounced cytotoxicity in Caco-2 cells. Combinations of several other mycotoxins with aurofusarin showed additive effects (Vejdovszky et al. 2016).

Conclusion

This mini-survey with limited sample size suggests that wheat grains available in Nigeria may be contaminated by diverse fungi and consequently, an array of mycotoxins, with the trichothecene DON dominating. Considering that wheat grains undergo processing which include seed coat removal and milling, two processes that are capable of reducing mycotoxin levels, the concentrations of single mycotoxin found in this study may not necessarily pose a threat. However, a potential source of concern to human and animal health may be potential combinatory (additive/synergistic) effects due to co-occurrence of mycotoxins/metabolites whereof many have not been characterized properly so far. Efforts should therefore be put in place to monitor mycotoxin levels in wheat grains and their finished products sold in markets across the country.

Complaince with ethical standard

Conflict of interest None.

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