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Fungal isolates and metabolites in locally processed rice from five agro-ecological zones of Nigeria

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ABSTRACT

This study reports the distribution of fungal isolates and fungal metabolites that naturally contaminate locally processed rice from five agro-ecological zones of Nigeria. The fungal species were isolated by the dilution plate technique and identified by appropriate diagnostics, while metabolites were determined by a liquid chromatographic tandem mass spectrometric method. *Aspergillus* and *Penicillium* species were the predominant isolates found in the rice samples while *Fusarium* spp. were not isolated. The mean fungal count differed significantly ($p < 0.05$) across the zones and ranged from 9.98×10^2 cfu g^{-1} in the Southern Guinea Savannah to 96.97×10^2 cfu g^{-1} in the Derived Savannah. For 16 fungal metabolites, selected from 63 positively identified fungal metabolites based on their concentration and spread across the zones, an occurrence map was constructed. The Northern Guinea Savannah recorded the highest contamination of fungal metabolites while the Sudan Savannah zone recorded the least.

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Locally processed rice; agro-ecological zones; fungal metabolites; metabolite occurrence map

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world population (FAO 2004) and provides 20% of the world's dietary energy supply, followed by wheat (19%) and maize (5%). Nigeria is currently the largest rice producer in West Africa, with an annual production of 4.7 million tonnes of paddy rice and the second largest in Africa, after Egypt (FAO 2008). It is also the largest importer of rice in the World (Olorunmowaju et al. 2014). The principal factors driving increased rice production in Nigeria are population growth and urbanisation (WARDA 1996). Rice is commonly eaten as boiled rice and in the northern part of the country; it is also taken as a paste ("Tuwo"), fermented bread ("Masa") and as unleavened bread named "Waina" (Olorunmowaju et al. 2014). The Hausas also use it in the preparation of a local snack called "Nakiya".

The Food and Agricultural Organization of the United Nations has estimated that 25% of the world food crops are contaminated with mycotoxins (Negedu et al. 2011). Mycotoxins are secondary metabolites produced by toxigenic fungi, mainly belonging to the genus *Aspergillus, Penicillium* and *Fusarium*, which cause diseases in humans and animals with a wide range of severity (Bennett & Klich 2003). They are responsible for many different toxic effects including the induction of cancer, kidney and nerve defects. The mycotoxin problem is particularly relevant to human health in tropical areas such as sub-Saharan Africa where crops including rice, due to the climatic conditions of the cultivated areas, are susceptible to contamination by several major mycotoxins, comprising aflatoxins (AFs) and fumonisins. In Nigeria, where the weather is generally hot and humid with an average annual temperature of 31.7°C and a relative humidity of 51.6% rice production faces these problems as well (Abdus-Salaam et al. 2015). Only a few studies have been conducted on fungi and mycotoxin contamination of locally processed rice in Nigeria (Makun et al. 2007, 2011; Ayejuyo et al. 2008; Somorin et al. 2010; Olorunmowaju et al. 2014) despite the fact that it is a highly consumed cereal in the country. In addition, Abdus-Salaam et al. (2015) reported the mean and maximum concentration of microbial metabolites of rice in different agro-ecological zones (AEZs) of Nigeria but did not monitor the distribution pattern. Most developed countries of the world have occurrence

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maps that provide information to government, researchers and policy makers about the prevalence of toxins in different regions of their country thus allowing formulation of appropriate intervention strategies (Adetunji et al. 2014a). Therefore, this paper reports the distribution of fungal isolates and metabolites contaminating locally processed rice from five AEZs of Nigeria to fill this information gap.

Materials and methods

Sample collection and preparation

Samples of locally milled rice were aseptically collected from five out of the seven AEZs of Nigeria between November 2011 and February 2012. These AEZs were Sudan Savannah (SS), Northern Guinea Savannah (NGS), Southern Guinea Savannah (SGS), Derived Savannah (DS) and Humid Forest (HF). Samples were not collected from the Sahel and Mid-Altitude zones of the country due to the security challenges of these zones. Geographical location, temperature and rainfall patterns of these zones have been documented by previous workers (Udoh et al. 2000; Atehnkeng et al. 2008; Adetunji et al. 2014a). Briefly, the SS zone lies between latitudes 12°2′ and 13°8′N and longitudes 3°9′and 13°9′E, with a unimodal average annual rainfall of between 650 and 1000 mm and maximum temperatures varying from 30 to 40°C and the NGS zone lies within latitudes 9°10′ and 11°59′N and longitudes 3°19′ and 13°37′E and has a unimodal average rainfall distribution of between 900 and 1000 mm annually, with maximum temperatures varying from 28 to 40°C (Udoh et al. 2000). The SGS zone lies within latitudes 8°4′ and 11°3′N and longitudes 2°41′ and 13°33′E, with a bimodal average rainfall of between 1000 and 1300 mm per year and with maximum temperatures varying from 26 to 38°C. The DS zone lies within latitudes 6°8′ and 9°30′N and longitudes 2°40′ and 12°15′E and has a bimodal average rainfall distribution of between 1300 and 1500 mm annually, with maximum temperatures varying from 25 to 35°C (Athenkeng et al. 2008). The HF zone lies within latitudes 6°4′ and 7°5′N and longitudes 3°5′ and 8°8′E and is characterised by an annual rainfall of 1300–1500 mm in the west and over 2000 mm in the east, with a humidity of over 80% and maximum temperatures ranging from 26 to 28°C Adetunji et al. 2014a). Sample collection and preparation were as reported by Abdus-Salaam et al. (2015). Different processing centres, where rice is traditionally soaked, parboiled, dried and milled (dehulled), were selected in states of the federation within the five AEZs and milled rice samples collected from available commercial processors in these centres. Samples were further pooled together to form a composite sample per centre. The number of samples collected per state was dependent on the number of available processors, thus a total of 38 composite samples comprising of SS = 7, NGS = 4, SGS = 4, DS = 11 and HF = 12 from 143 processors were analysed. The samples were kept in labelled sterile polyethylene bags and transported to the laboratory for analysis. The samples were hand-mixed with the aid of a sterile glove, coarse-ground and allowed to pass through a No. 14 mesh screen taking appropriate aseptic precautions. Sub-samples of 500 g were taken from each lot, ground with a milling machine (Greiffenberger Antriebstenchnic, Marktredwitz, Germany) and further sieved with a 1-mm mesh. Sub-samples of 50 g were taken and placed in zip-lock envelopes for fungal and metabolite analyses. Samples which were not immediately processed were stored at −20°C prior to analyses.

Moisture content

Moisture content was determined out according to A.O. A.C (2000) method. Briefly, samples (5 g) were oven-dried in a pre-weighed dish at 105°C for 4 h and cooled in a desiccator continuously until a constant weight was obtained. The moisture content was calculated from reduction in weight and expressed as a percentage of the original weight.

Isolation of fungal isolates

Fungal species were isolated from 50 g sub-samples of powdered rice by the dilution plate technique (Samson et al. 1995). Ten grammes of each sub-sample was diluted in 90 ml of 0.1% sterile peptone water and the mixture was vortexed for 2 min by vigorous hand inversion. Aliquots (0.1 ml) were inoculated by surface plating on half strength Potato Dextrose Agar (PDA) plates supplemented with 0.01% chloramphenicol. Isolations were made in two sets from each sub-sample and each set contained triplicate inoculated PDA plates. The first set was incubated at 30°C for 3 days for enumeration of *Aspergillus* species, while the second set was incubated at 25°C for 7 days for enumeration of *Fusarium* and *Penicillium* species. Yeasts of both sets were enumerated from PDA plates.

Identification of fungal isolates

Colonies of the isolates were identified as reported by Adetunji et al. (2014c). Prior to morphological identification of the isolates, colonies that bore resemblance to *Aspergillus* and *Penicillium* species were transferred to

full strength PDA. The *Aspergillus* and *Penicillium* cultures on PDA were incubated unilluminated at 30 and 25°C, respectively, for 7 days. All isolates were identified on the basis of morphological characteristics (macro: colony colour, morphology and size; micro: conidia morphology and size) and comparison with appropriate keys in literature (Klich 2002; Ehrlich et al. 2007; Pitt & Hocking 2009). The identified *Aspergillus* isolates were further maintained on PDA slants by the single colony transfer technique at 4°C.

Sample preparation for fungal metabolite analysis

Procedures were carried out as reported by Abdus-Salaam et al. (2015): 5 grammes of each sample were weighed into a 50-ml polypropylene tube (Sarstedt, Nümbrecht, Germany) and 20 ml of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) were added. Samples were extracted for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany), diluted with the same volume of extraction solvent and injected into the liquid chromatography–mass spectrometry (LC–MS/MS) (Sulyok et al. 2007). Apparent recoveries of the analytes were cross-checked by spiking a non contaminated sample with mycotoxins by means of a multi-analyte standard on 1 concentration level. The spiked sample was stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and the sample. The accuracy of the applied method is further verified by participation in proficiency tests organised by the Bureau Interprofessionnel des Etudes Analytiques (BIPEA, Gennevilliers, France). *Z*-scores for all submitted results were within the satisfactory range ($-2 < z < +2$).

LC–MS/MS method

LC–MS/MS screening of target microbial metabolites was performed with a QTrap 5500 LC–MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with Turbo Ion Spray electrospray ionisation and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25°C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA). LC–MS/MS method parameters are given by Vishwanath et al. (2009).

Occurrence map for metabolites

A total of 16 fungal metabolites, selected from the database of 63 positively identified fungal metabolites (Abdus-Salaam et al. 2015) based on concentration and spread across the AEZs, was used to construct occurrence maps. The global positioning system coordinates obtained from the AEZs were tabulated against the metabolite concentration in a Microsoft Excel spread sheet. The resultant table was converted into Text Tab Delimited to enable importation into the geographic information system (GIS) environment. The file was added as an event theme to create a database layer in the base map (administrative boundaries of Nigeria). Charts for metabolite concentration were created using ArcView GIS 3.2 into various thematic maps of metabolite concentration across the states and AEZs.

Statistical analysis

The obtained data were subjected to statistical analysis of variance using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Means were separated using Duncan's Multiple Range Test. Simple descriptive analysis was used to evaluate occurrence, concentration and distribution of metabolites across the AEZs. The concentration of individual AFs and fumonisin types were also compiled as total AF ($\sum AFB_1$, AFB_2, AFG_1 and AFG_2) and total fumonisin ($\sum FB_1$, FB_2, FB_3), respectively.

Results

Moisture content and fungal colony counts

Table 1 shows that mean moisture content of the locally processed rice was not significantly different across the AEZs, with a minimal value of 12.50% recorded in the DS zone and a maximum value of 13.50% in the SGS zone. Table 1 also shows that mean fungal colony counts of the rice samples were significantly different ($p < 0.05$) across the zones. The lowest value was found in the SGS zone (mean = 9.98×10^2 cfu g^{-1}), the highest in the DS zone (mean = 96.97×10^2 cfu g^{-1}).

Distribution of fungal isolates

Table 2 shows the distribution of the fungal isolates that colonised the investigated rice samples. There were significant differences ($p < 0.05$) in the distribution of the fungal isolates across the zones. The major groups of fungi found in the milled rice were *Aspergillus, Penicillium* and yeast, while *Fusarium* spp. were surprisingly not detected. Of the suspected mycotoxigenic fungi in the milled rice, *Penicillium* species had the highest occurrence in the zones with the exception of the SGS zone where *Aspergillus flavi* had

Table 1. Moisture content and fungal colony counts of locally processed rice from five Nigerian AEZs.

AEZ	Mean moisture content (%)	Moisture content range (%)	Mean fungal colony count ($\times 10^2$ cfu g^{-1})	Range of fungal colony count ($\times 10^3$ cfu g^{-1})	*Aspergillus* spp. ($\times 10^2$ cfu g^{-1})	*Aspergillus section Flavi* ($\times 10^2$ cfu g^{-1})	*Aspergillus section Nigri* ($\times 10^2$ cfu g^{-1})	*Penicillium* spp. ($\times 10^2$ cfu g^{-1})	Yeast ($\times 10^2$ cfu g^{-1})	Other fungal isolates ($\times 10^3$ cfu g^{-1})
SS	12.75^a	9.4–13.8	17.02^b	1.20–6.69	5.20^c	1.20^b	ND	10.20^b	1.60^b	6.69^c
NGS	13.00^a	12.8–13.2	21.67^c	3.20–4.69	8.20^d	6.00^d	3.20^c	29.20^e	46.90^d	3.65^b
SGS	13.50^a	12.8–13.2	9.98^a	2.00–2.03	3.60^b	20.30^e	2.00^b	3.30^a	27.00^c	0.37^a
DS	12.50^a	11.0–13.4	96.97^e	0.30–37.90	17.80^e	3.90^c	0.30^a	25.40^d	154.70^e	37.90^e
HF	13.02^a	11.2–17.6	44.24^d	0.9–19.71	3.20^a	0.90^a	ND	18.50^c	1.50^a	19.71^d

AEZ: Agro-ecological zone; SS: Sudan Savanna; NGS: Northern Guinea Savanna; SGS: Southern Guinea Savanna; DS: Derived Savanna; HF: Humid Forest; ND: not detected.
Mean values with different superscripts along the columns are significantly different ($p < 0.05$).

Table 2. Distribution of fungal isolates colonising locally processed rice from five Nigerian AEZs.

AEZ	SS	NGS	SGS	DS	HF
Aspergillus spp.	6.11^a	6.31^a	6.01^a	3.05^b	1.45^c
Aspergillus section *Flavi*	1.41^c	4.62^b	33.89^a	0.67^d	0.4^d
Aspergillus section *Nigri*	ND	2.46^b	3.34^a	0.05^c	ND
Penicillium spp	11.99^b	22.46^a	5.51^d	4.38^e	8.36^c
Yeast	1.88^d	36.08^b	45.06^a	26.80^c	0.68^e
Other fungal isolate	78.61^b	28.08^d	6.18^e	65.02^c	89.10^a

AEZ: Agro-ecological zone; SS: Sudan Savanna; NGS: Northern Guinea Savanna; SGS: Southern Guinea Savanna; DS: Derived Savanna; HF: Humid Forest; ND: not detected.
Mean values with different superscripts along the rows are significantly different ($p < 0.05$).

the highest occurrence (33.9%). Furthermore, *Aspergillus nigri* was not isolated in rice samples from the SS and HF zones. The rice samples were also contaminated with fungal species belonging to the genera *Alternaria, Rhizopus, Cladosporium* and *Eurotium* (data not shown).

LC/MS/MS method quality assurance

The performance characteristics for the analytical method used in this study as established from the spiked blank samples are shown in Table 3 and the precision of the method is in the range of 5–10%.

Distribution of fungal metabolites across Nigerian AEZs

Figure 1 shows the geospatial distribution of major metabolites contaminating rice in Nigeria. The NGS zone had a high concentration of mycotoxins such as total AF (7.30 µg kg^{-1}), zearalenone (ZEN) (464 µg kg^{-1}), nivalenol (NIV) (36.3 µg kg^{-1}) and sterigmatocystin (42.0 µg kg^{-1}). In addition, the SS zone had the highest concentration of fumonisins (23.4 µg kg^{-1}) while the SGS zone had the least contamination of ZEN (4.11 µg kg^{-1}), NIV (2.72 µg kg^{-1}) and sterigmatocystin (0.27 µg kg^{-1}). Deoxynivalenol (DON) was detected only in the NGS (5.76 µg kg^{-1}) and DS (2.40 µg kg^{-1}) zones while Ochratoxin A was detected in the NGF (µg kg^{-1}) and HF (1.46 µg kg^{-1}) zones. Locally processed rice samples from the NGS zone also recorded the highest level of contamination by beauvericin (BEA) (34.7 µg kg^{-1}), kojic acid (2224.00 µg kg^{-1}) and equisetin (834.00 µg kg^{-1}). Similarly, moniliformin (MON) (51.70 µg kg^{-1}) and 3-nitropropionic acid (565.00 µg kg^{-1}) contamination was highest in the DS zone while the least concentration of kojic acid (21.8 µg kg^{-1}), 3-nitopropionic acid (3.28 µg kg^{-1}), MON (9.78 µg kg^{-1}) and equisetin (2.66 µg kg^{-1}) was recorded in the SS zone.

Table 3. LC/MS/MS method performance characteristics of fungal analytes in locally processed rice from five Nigerian AEZs.

	Analyte	Limit of detection (μg kg^{-1})	Apparent recovery (%)
Major mycotoxins	Aflatoxin B_1	0.15	94.8 ± 4.1
	Aflatoxin B_2	0.2	91.4 ± 4.1
	Aflatoxin G_1	0.2	93.70 ± 3.8
	Fumonisin B_1	3.0	100.0
	Fumonisin B_2	1.5	100.0
	Fumonisin B_3	2.0	100.0
	Nivalenol	0.5	85.5 ± 12
	Zearalenone	0.3	106.4 ± 9.3
	Sterigmatocystin	0.01	103.8 ± 2.4
Aspergillus metabolites	Kojic acid	10.0	84.4 ± 1.6
	3-Nitropropionic acid	10.0	84.4 ± 1.6
Fusarium metabolites	Beauvericin	0.002	109.1 ± 12.4
	Moniliformin	0.5	82.8 ± 6.4
	Equisetin	0.05	124.5 ± 23.5

Discussion

The mean moisture content of milled rice was within the allowable limit of 11–13% for stored grains (Mohale et al. 2013) and below 15% legal limit for rice as recommended by Codex Alimentarius (1995). This may have been responsible for the low fungal load observed in this study and may have resulted from processing (soaking, parboiling, drying and dehulling/milling) steps as usually employed during rice production. Most critical are the dehulling and drying steps which are usually performed under high temperature and which may generate heat thus leading to low fungal counts (Taligoola et al. 2011). In addition, rice processing involves floatation during which damaged and infected grains are usually removed. Therefore, the occurrence of fungal propagules in the analysed rice samples may most likely be the result of initial infection of the crop on the field. The presence of *Aspergillus* and *Penicillium* species (potential mycotoxin producers) in the rice samples corroborates the report of Aydin et al. (2010) who also found these as the predominant genera of fungi in Turkish rice. Previous studies by Reddy et al. (2004) and Makun et al. (2007) have implicated various species of *Fusarium, Aspergillus* and *Penicillium* as the contaminants of rice. The higher incidence of *Penicillium* with respect to *Aspergillus* in the SS and NGS zones also corroborate the reports of Park et al. (2005) and Aydin et al. (2010) that *Penicillium* species were the predominant fungi contaminating rice in northern regions of Korea and Turkey, respectively. The SS and NGS zones are known to have an annual unimodal rainfall distribution and maximum temperature ranges between 28 and 40°C (Atehnkeng et al. 2008), thus favouring the growth of *Penicillium* spp.

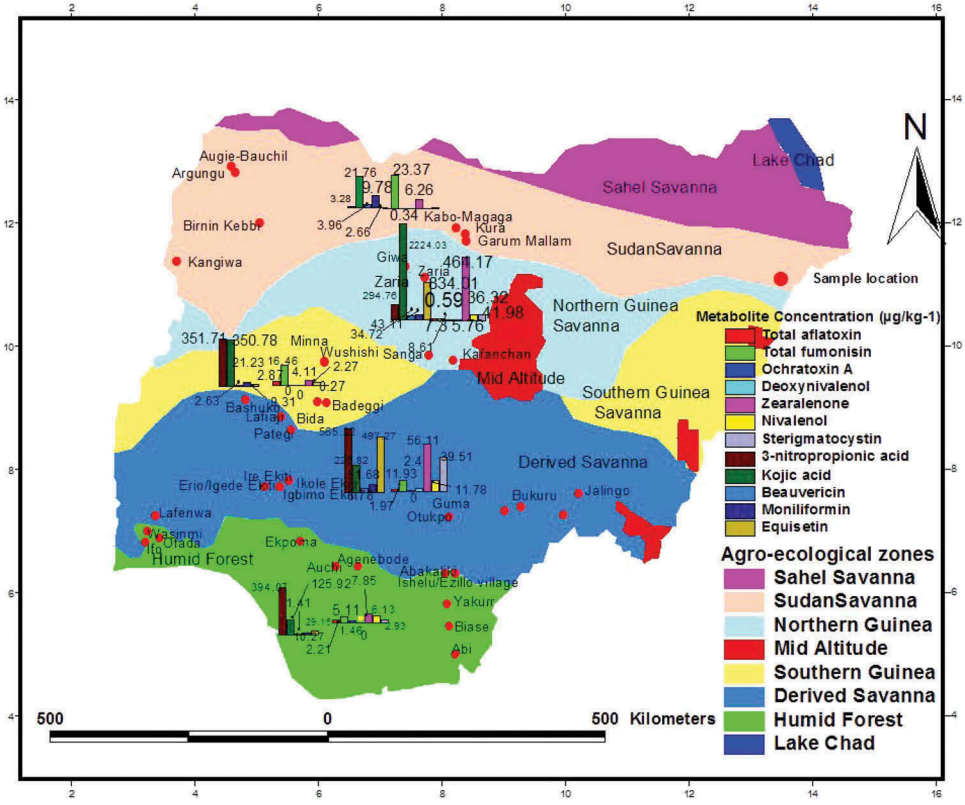

Figure 1. Distribution of major metabolites contaminating rice in Nigeria.

The high incidence of *Aspergillus* and in particular *A. flavi* in the SGS zone is in agreement with the work of Atehnkeng et al. (2008) and Adetunji et al. (2014b). Last cited authors further postulated that high incidence may be due to favourable weather conditions (long period of rainfall and high humidity) in the zone. Makun et al. (2007) also reported severe fungal infection in the wetter zones than in the drier zones of Niger state (SGS zone). The relatively low moisture content of the rice grains may be responsible for the non-detection of *Fusarium* spp. *Fusarium*, a field fungus (Park et al. 2005; Hedayati et al. 2010) has been found to grow better at higher moisture content of 20–25% (Abdel-Hafiz et al. 1992). Taligoola et al. (2011) also reported that species of *Aspergillus and Penicillium* occurred more frequently than *Fusarium* in imported rice grains stored in Uganda. Furthermore *Fusarium* growth was also found to be inhibited at a low moisture content of 14.48% in rice grains (Taligoola et al. 2011). In contrast, Abdel Hafez et al. (1992) and Mazen et al. (1993) found that increase in moisture content and storage period caused an increase in *Fusarium* infection incidence of paddy grains in Egypt.

The relatively low moisture content of rice samples from the SS zone may be responsible for the low concentration of AFs in rice grains from this zone, in contrast to the higher amount found in the NGS zone. The incessant climate change has brought an increase in global temperature which is predicted to reach 4.8°C by 2100 (Kovalsky 2014). This affects temperature and water availability, which are two main factors underlying mycotoxins production by fungi. High temperatures and dry conditions are known to favour infection by *Aspergillus* fungi and subsequent development of AF is frequently accompanied by heat and drought stress (Adetunji et al. 2014b). Among the AFs, AFB_1 is the most potent for mammals and presents hepatotoxic, teratogenic and mutagenic properties, causing damage such as toxic hepatitis, haemorrhage, oedema, immunosuppression and hepatic carcinoma (Speijers & Speijers 2004). The relatively high AF concentration in the NGS zone, exceeding the maximum limit (4 µg kg^{-1}) as set by the European Commission (EC 2006) for total AFs in food, may be attributable to the agricultural practices in this zone. It has a unimodal rainfall pattern and high temperature, hence, rice production is sustained by irrigation. Additionally, this zone includes Kaduna state which is the main traditional rice growing state in Nigeria, also gaining the highest yield (Olorunmowaju et al. 2014). Furthermore, the mean concentration of total AFs in the NGS zone (7.30 µg kg^{-1}) was higher than 5.15 µg kg^{-1} as reported by Madbouly et al. (2012) for marketed rice from the major five zones of the province of Cairo, Egypt and 1.6 µg kg^{-1} reported by Rahmani et al. (2011) for 256 rice samples collected from retail markets in 30 provinces of Iran between October 2007 and July 2008.

The occurrence of *Fusarium* mycotoxins (Fumonisins B_1, B_2 and B_3, DON, ZEN, NIV, BEA, MON and Equisetin) at varying concentrations in the rice samples despite the fact that *Fusarium* species were not isolated corroborates the report of Madbouly et al. (2012), who reported a value of 1014 µg kg^{-1} for marketed rice in Cairo, Egypt despite the absence of *Fusarium moniliformes* (a major fumonisin producer) in most of the rice samples. This observation may be explained by the fact that the *Fusarium* isolates might have infected the rice grains while on the field, produced the mycotoxins therein and died off before harvest and or during subsequent rice processing operations. Since field fungi colonise rice grains only when the water activity, temperature and relative humidity are high (Magro et al. 2010), the irrigation practise of the NGS zone may also be responsible for the relatively high concentration of *Fusarium* toxins in the zone. In addition, toxic fungal metabolites can translocate through xylem vessels from the stem base (Winter et al. 2013), while distribution and level of contamination of toxigenic fungi can be affected by several factors such as processing, water content and availability of nutrients like carbon and nitrogen and the presence of competitors. Many *Fusarium* and *Paecilomyces* species (Jestoi 2008) have been implicated in the diseases of rice on the field (Desjardins et al. 2000) including the production of BEA, which has been found to induce genotoxic and cytotoxic effect in human lymphocytes and animal species, respectively. It also induces chromosomal aberrations, sister-chromatid exchange and micronuclei formation (Çelik et al. 2010). Abdus-Salaam et al. (2015) reported a 100% incidence of BEA in locally processed rice from five AEZs of Nigeria with mean and maximum values of 7.32 µg $kg^{-1}$and 131.00 µg kg^{-1}, respectively. In cereals, the most common family of *Fusarium* toxins produced in the field are trichothecenes, particularly DON. The low incidence of DON in this study corroborates with a previous report of Ok et al. (2009), in which rice-based products had the lowest contamination (3.40 µg kg^{-1}), out of four cereal-based products investigated in Korea. In another study, Ok et al. (2014) did not find DON to be prevalent in freshly harvested rice. Furthermore, Makun et al. (2011) did not detect DON in 5 marketed rice samples but found it in 1 out of 10 field rice samples from Niger state, Nigeria. DON affects animal and human health causing vomiting, acute temporary nausea, diarrhoea, abdominal pain, headache, dizziness and fever (Sobrova et al. 2010). A commonly found trichothecene in cereals, NIV which occurred in all

the AEZs sampled in this study has been shown to have a stronger toxic effect on rat intestinal epithelial cells compared to DON (Bianco et al. 2012). Although mean values of DON and NIV in this study were low, Bony et al. (2007) reported a genotoxic potential associated to low NIV levels. The enhancement of intestinal inflammation by low doses of DON has also been reported (Vandenbroucke et al. 2011). Except under extreme conditions, the concentration of fumonisin does not increase during grain storage. Formation of fumonisin in the field usually correlates with the occurrence of *Fusarium verticillioides* and *Fusarium proliferatum* (Bolger et al. 2001), which predominate during late maturity. Mean ZEN level in the NGS zone was above the regulatory limits of 75 and 20 μg kg^{-1}, respectively, as proposed by the EC (2007) for cereals intended for direct human consumption, cereal flour, bran and germ as end products marketed for direct human consumption and processed cereal-based foods for infant and young children. ZEN, an estrogenic compound, may induce lower fertility, foetal wastage and lower hormonal levels in animals and humans (Sirot et al. 2013). It is classified by the International Agency for Research on Cancer in group 3 (IARC 2002). Nagaraj et al. (1996) reported that another *Fusarium* metabolite, MON is a potent inhibitor of the pyruvate dehydrogenase complex that induces acidosis, muscular weakness and cardiotoxicity. Sterimagocystin (STC) is an intermediary metabolite in the AF biosynthetic pathway, being the penultimate AFB_1 precursor. It is produced by more than 50 fungal species, including *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus versicolor* and *Aspergillus nidulans* (Keller et al. 1994). Mean STC concentrations in the NGS and DS zones were unexpectedly high, as most STC was expected to be removed by the milling process (Takahashi et al. 1984).

Conclusion

Aspergillus and *Penicillium* spp. were the prevalent fungal isolates contaminating milled rice in Nigeria while *Fusarium* species were not detected, despite the occurrence of *Fusarium* metabolites in the zones. The NGS zone recorded the highest occurrence of fungal metabolites while the SS zone recorded the least. Constant monitoring of the levels of metabolite contamination and implementation of good agricultural and processing practices are essential when considering the high consumption rate of rice in Nigeria.

Disclosure statement

No potential conflict of interest was reported by the authors.

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