



Multi-mycotoxins and Fungal Contaminants of Wheat from Anambra State, Nigeria

R.N. Amasiani¹, C.A Oyeka²

^{1 & 2} Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

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Corresponding author: C.A Oyeka

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Abstract

This study was carried out to determine the fungal and mycotoxins contamination of 36 Wheat (*Triticum aestivium*) samples purchased randomly from the seller of the agricultural produce in local markets of Anambra State, Nigeria. Results from the studies showed that two hundred and three fungal isolates consisting of 18 species of moulds and 5 species of yeasts contaminated the wheat samples at varying degrees. For moulds, *Aspergillus species* contaminated the samples mostly with (28) isolates followed by *Penicillium species* (19) isolates while *Verticillium species* and *Cladosporium species* had equal least contaminations with (3) isolates each. Among the yeast species, *Candida rugosa* had the highest number of contamination with (37) isolates followed by *Cryptococcus laurentii* (31) isolates while *Candida stellatoidea* (9) isolates had the least contamination. Twenty-four fungal metabolites were also recovered. The concentration of trichothecene mycotoxin Deoxynivalenol (2067µg/kg), a protein synthesis and cell proliferation inhibitor in animals exceeded the maximum acceptable limits for human consumption. It can be deduced therefore that wheat circulating in Anambra State, Nigeria are variously contaminated with different xerophilic moulds and mycotoxins which can exert adverse health problems to consumers.

Keyword: *Wheat samples, fungal contaminants, multi-mycotoxins and market zones*

Introduction

Wheat (*Triticum spp*)(order poales), is a cereal crop belonging to the family poaceae and is ranked as the third main staple food and industrial cash crop after maize and rice in Nigeria (Tanno and Willcox, 2006, Kolawole, *et al.*, 2013). Wheat is widely cultivated in North Africa and in Sudan and Ethiopia and it is grown on around 10 million ha in Africa and it is also an imported commodity in all of Africa. African countries are the world's biggest wheat importer with more than 45 m t in 2013 at around 15

billion US\$ Wheat imports account for 60% of African's wheat consumption and 80% of Sub-Saharan (SSA) countries (Macauley, 2015).

Wheat consumption is steadily increased in all African countries from the past 20 years as a result of growing population, changing food preferences and socioeconomic change associated with urbanization. In rapidly urbanizing in sub-Saharan Africa, wheat consumption is expected to grow to 38% by 2023 with imports already at 23 m tons of wheat

in 2013 at a cost of \$7.5 billion. With the growing importance of wheat in food security in Africa, the African Union Heads of State in January 2013, endorsed to add wheat to the list of strategic crops for Africa (Macauley, 2015). Wheat grains are milled and are used to produce thick porridges and “swallows”, which are known by various names in different parts of African continents (Ukwuru *et al.*, 2018). Wheat is not widely grown in Nigeria and it serves as additional food or feed supplement that provides vegetable protein, minerals and vitamins to humans and livestock (Kolawole *et al.*, 2013). It is a major component of many other foods including breads, porridge, cracker and biscuits (Tanno and Willcox, 2006, Kolawole, *et al.*, 2013).

However, the practices associated with production, processing and post processing handling of wheat, may exacerbate fungal contamination. Thus, the fungi contaminate the wheat samples on field, indeed accompany them to their storage points and may express the capacity to produce associated mycotoxins thus leading to a progressive deterioration in food quality, leading to alarming economic loss reflected as reduced productivity, discolouration, production of off-odours, direct commodity loss, livestock losses due to death and lower growth rates and human illness (Ogiehor and Ikenebomeh, 2006, Kolawole, *et al.*, 2013). Mycotoxins are natural products (poison) produced by filamentous fungi which evoke toxic responses when introduced in low concentrations to higher vertebrates by natural routes (Oyeka, 2003). Mycotoxins are produced by fungi for a variety of reasons (1) to attack or gain access to hosts by helping to dissolve cell membranes. (2) As a protective measures against encroaching organisms (Zain, 2011). The occurrence of mycotoxins within the fungus and commodities depend on the particular crops, the environmental conditions under which a particular crop is grown, harvested, stored and or from the raw materials used in processing them which can be controlled. Other extrinsic factors like climate or intrinsic factors such as fungal

strain, specifically strain variation, the particular enzymes of the fungus and instability of toxigenic properties which are more difficult to control (Zain, 2011). The fungi that produce mycotoxins do not have to be present to cause harm because they may be killed by heat (Oyeka, 2003) but the mycotoxins they produced on foods are stable under most food processing conditions and therefore, persist to the final products. It is therefore, difficult to eliminate them once the food stuffs are contaminated and usually there is no treatment for mycotoxin poisoning (Zain, 2011)

All mycotoxins are of fungal origin but not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolites are both important (Zain, 2011). Local food markets in developing countries like Nigeria are usually characterized by the absence of regulatory agencies and limited information on food quality; hence the constant selling of these fungal contaminated grains to consumers. Poverty, coupled with ignorance about mycotoxin contamination is also very important predisposing factors to consumers of contaminated food (Oyeka *et al.*, 2019). Lack or absence of routine laboratory checks and diagnosis of mycotoxicosis has been complicated by a disregard on awareness of mycotoxin effects, acceptance of chronic low productivity and disease they cause. Also, lack of feed samples, sampling difficulties, analytical complications, species specific responses, nonspecific effects of mycotoxins on various body systems, universal symptoms that may be similar to other disorders and a cascading of animal responses (as seen with immune suppression) resulting in disorders not thought to be associated with mycotoxins hinder diagnosis of mycotoxins (CAST, 2003, whitlow and Hagler, 2005).

The survey for mycotoxigenic fungal contaminants of wheat consume in Anambra State, Nigeria would no doubt give an insight into the possible mycotoxins which can be produced in nature and their toxicological roles in human and animal health.

Materials and Methods:

Study Location:

Study Area

This study was carried out in local markets of Anambra State. The local markets were divided into six zones viz: Zone 1- Aguata/Orumba North and South, Zone 2- Awka North /South, Zone 3- Nnewi North/South/Ihiala Ekwusigo, Zone 4- Ogbaru/Onitsha North/South, Zone 5- Anaocha/. Idemili North/South, Zone 6- Njikoka/Anyamelum/Oyi/Anambra East.

Sample collection

A total of thirty six (36) wheat samples (*Triticum aestivium*) sample lots were purchased at random from six local market zones of Anambra State, Nigeria. The wheat samples were securely packaged in polythene bags, labeled properly and transported to the laboratory, stored under 4°C for later use for research analysis.

Processing of samples

The fungi were isolated using modified standard methods of Dubey and Maheshwari (2005). Duplicate samples of surface sterilized, blended, and serially diluted wheat samples were cultured in potato dextrose agar fortified with 0.5mg/ml chloramphenicol. These were incubated for 5-7 days and regularly examined daily for fungal growth. The remaining portions of the wheat powder were stored for mycotoxins quantification. Twenty (20) grams of twelve selected stored samples of ground wheat sample lots were chosen at random, packaged in zip lock paper envelopes, properly labeled and sent to Center for Analytical Chemistry, Department of Agro Biotechnology, University of Natural Resources and Life Sciences, Tulln, Austria for quantification of mycotoxins.

Identification of isolates

Identification of the isolates was based on extensive study of macroscopic and microscopic morphologies which was compared with standard texts of (Buhmer 1978; Frey *et al* 1979; De Hoog and Guarro 1995; Summerbell 1996;

David *et al*,2007). Moulds isolates were identified with Slide culture technique of (Errol *et al* 2012) while yeasts isolates were identified using Sugar fermentation tests of (Chessbrough 2004), urease test of (Mcginnis 1980), germ tube tests of (Chessbrough 2004), Corn meal agar test of (Acumedia 2011) and Chromogenic agar tests of (Massoud 2010).

Chemicals and reagents

Chemicals and reagents were obtained from Romer Labs Inc. (Tulln, Austria) and Sigma-Aldrich (Vienna, Austria). Standards of fungal metabolites were obtained from Iris Biotech GmbH (Marktred-Witz, Germany), Axxora Europe (Lausanne, Switzerland), and LGC (Promochem GmbH, Wesel, Germany).

Quantification of mycotoxin from wheat samples

Duplicate portions of the 12 randomly selected ground representative wheat samples were used for quantification of mycotoxins using Liquid chromatography tandem Mass Spectrometry (LC-MS/MS) method of (Malachova *et al* 2014). Five (5) grams of each representative samples were weighed into a 50-ml polypropylene tube (Sarstedt, Nümbrecht, Germany) and 20ml of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) added. Samples were extracted for 90 minutes on a GFL 3017 rotary shaker (GFL, Burgwedel,) and later diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) and subsequently centrifuged for 2 minutes at 3000 rpm (radius 15 cm) on a GS-6 centrifuge (Beckman Coulter Inc., Fullerton, CA). After appropriate mixing, 50µl of the diluted extracts were injected into the LC-MS/MS system without further pre-treatment.

Spiking of model matrices

For spiking the model matrices, 0.25g of a representative of the samples that were not contaminated with mycotoxins were weighed into 50ml of polypropylene tube. Multi – analyte standard in one concentration level was added in each of the tubes. The samples were placed in

darkness to avoid analyte degradation and stored overnight at room temperature to allow evaporation of the solvent and to establish equilibrium between analytes and samples. The concentration range of the spiked samples were chosen to cover the respective limits of detection of each toxins, estimation linear calibration range, legislation limits of regulated toxins as well as the levels of commonly found toxins in naturally contaminated samples. External calibration was prepared by dilution of appropriate amounts of the final working solution with acetonitrile/water/acetic acid (49.5/49.5/1, v/v/v) at level corresponding to those of the spiked samples. The corresponding peak areas of the spiked samples were used for the estimation of apparent recoveries by comparison to a multi-analytes standard on one concentration level prepared and diluted in neat solvent. All concentrations of the naturally contaminated samples were corrected by a factor equivalent to the reciprocal of apparent recovery (i.e $1/R_A$, where R is the apparent recovery of each analyte) Malachova *et al* 2014.

$R_A = \text{Average area of spiked samples} \times 100$

Average area (neat solvent standard)

LC-MS/MS parameters

Determination and quantification of target mycotoxins was performed with a QTrap 5500MS/MS system (Applied Biosystems, Foster City, CA) equipped with a TurboV electrospray ionization (ESI) source and a 1290 series HPLC system (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed at 25°C on a Gemini®C18-column, 150 × 4.6 mm i.d., 5µm particle size, equipped with a C₁₈ security guard cartridge, 4 × 3 mm i.d. (all from Phenomenex, Torrance, CA, US). ESI-MS/MS was performed

in the time scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per samples by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte was set to its expected retention time ±27 and ±48 in the positive and negative modes respectively. Confirmation of positive analyte identification of two MRMs per analyte (with the exception of Nitropropionic acid, which exhibited only one fragment ion) allowed confirming the identity of the positive results (Commission decision, 2002, Malachova *et al* 2014, Adetunji *et al*, 2014).

STATISTICAL ANALYSIS

The data collected were analysed with SPSS (Statistical Package for Social Sciences) version 21 (SPSS Inc., Chicago, IL, USA).

Results

A total of two hundred and three (203) fungal isolates were recovered from the study and *Aspergillus species* (28) had the highest number of moulds isolates followed by *Penicillium species* (19) isolates while the least *Verticillium sp.* and *Cladosporium carrionii* had 3 isolates each. In addition to moulds recovered, the study showed that yeasts were also very important fungal contaminants of the samples screened. Among the yeast species, *Candida rugosa* had the highest number of contamination with (37) isolates followed by *Cryptococcus laurentii* (31) isolates while *Candida stellatoidea* had the least number of contamination with (9) isolates. Wheat from zone (1) had the highest fungal contamination with (76) isolates followed by those from zone (5) with (50) isolates while zone (2) had the least fungal contamination with (12) isolates. Statistical result showed that the frequency of moulds recovery was significant $P < 0.05$, whereas the recovery of yeasts was not significant $P > 0.05$ (Table 1)

Table 1: Fungi isolated from wheat samples in six market zones of Anambra State

Isolated organisms	Market zones						frequency
	1	2	3	4	5	6	
<i>Aspergillus niger</i>	0	0	1	4	0	0	5(2.46%)
<i>Aspergillus flavus</i>	5	0	0	0	0	0	5(2.46%)
<i>Aspergillus fumigatus</i>	0	1	0	2	0	0	3(1.50%)
<i>Aspergillus versicolor</i>	0	5	0	0	0	0	5(2.46%)
<i>Aspergillus glaucus</i>	0	0	0	0	5	0	5(2.46%)
<i>Aspergillus terreus</i>	0	0	4	0	0	1	5(2.46%)
				Sub-total			28(13.79%)
<i>Penicillium chersanum</i>	6	0	2	0	0	0	8(3.94%)
<i>Penicillium verrucosum</i>	5	0	0	0	3	0	8(3.94%)
<i>Penicillium marneffeii</i>	0	0	3	0	0	0	3(1.50%)
				Sub-total			19(9.36%)
<i>Fusarium oxysporium</i>	0	0	0	3	3	2	8(3.94%)
<i>Fusarium solani</i>	1	0	1	2	0	0	4(1.97%)
<i>Fusarium aquaeductuum</i>	0	0	0	0	5	0	5(2.46%)
				Sub-total			17(8.37%)
<i>Rhizopus oryzae</i>	0	0	0	2	0	3	5(2.46%)
<i>Verticillium sp.</i>	0	0	0	0	3	0	3(1.50%)
<i>Alternaria infectoria</i>	0	0	0	0	7	0	7(3.45%)
<i>Malbranchea sp.</i>	0	0	0	0	8	0	8(3.94%)
<i>Cladosporium carrionii</i>	0	3	0	0	0	0	3(1.50%)
<i>Trichoderma sp.</i>	4	0	0	0	0	0	4(1.97%)
<i>Cryptococcus laurentii</i>	5	3	2	0	16	5	31(15.27%)
<i>Candida stellatoides</i>	0	0	9	0	0	0	9(4.43%)
<i>Candida rugosa</i>	37	0	0	0	0	0	37(18.23%)
<i>Saccharomyces cerevisiae</i>	13	0	0	0	0	0	13(6.40%)
<i>Candida tropicalis</i>	0	0	0	0	0	19	19(9.36%)
Total	76	12	22	13	50	30	203(100%)

P=0.021(Fungal isolation from grain samples were significant), p=0.013(Moulds isolation was significant), P=0.558(Yeast isolation not significant). 1 - AON-Aguata/Orumba North & South Market zone, 2- ANS-Awka North & South market zone, 3 - NNSIE-Nnewi North & South/Ihiala/Ekwusigo market zone, 4 - OONS-Ogbaru/Onitsha North & South market zone, 5 - AINS- Anaocha/Idemili North & South market zone, 6 - NAOA Njikoka/Ayamelum/Oyi/Anambra East market zone.

Twenty-four fungal metabolites were recovered from the studied wheat samples with 14 *Fusaria* metabolites being the highly recovered followed by 5 *Alternaria* metabolites and 1 *Penicillium* metabolite as the least fungal metabolite recovered. Analytical results of wheat samples showed high percentage recoveries (77.6–202.4%) of fungal metabolites. The minimal quantification concentrations of fungal metabolites in the analyzed wheat samples ranged between 0.01 µg/kg (enniatin B3) and 409.8 µg/kg (Deoxynivalenol) (Table 2).

Table 2: Table2: LC-MS/MS Method performance characteristics of Analytes in Wheat samples

Metabolite category	Analyte	Retention time (minute)	Spiked sample LOD µg/kg	Net standard LOD µg/kg	Apparent Recovery (%)
<i>Fusarium</i> toxin	15-hydroxyculmorin	10.2	165.6	158.77	104.3
<i>Aspergillus</i> toxin	3-Nitropropionic acid	3	12.08	13.51	89.4
<i>Alternaria</i> toxin	Alternariol	11	1.5048	1.56	96.5
<i>Alternaria</i> toxin	Alternariolmethylether	12.8	0.7147	0.71	100.7
<i>Aspergillus</i> toxin	Asperglaucide	11.6	14.896	14.9	100
<i>Fusarium</i> toxin	Beauvericin	14.4	0.8296	0.86	96.5
Unspecific metabolite	Brevianamid F	7.4	2.6384	2.64	100
<i>Fusarium</i> toxin	Culmorin	12.2	23.8	22.45	106
<i>Fusarium</i> toxin	Deoxynivalenol	5.8	409.8	382.95	107
<i>Fusarium</i> toxin	Deoxynivalenol-3-Glucoside	5.6	19.704	19.66	100.2
<i>Penicillium</i> toxin	Emodin	14.3	0.8136	1.06	81.4
<i>Fusarium</i> toxin	Enniatin A	14.9	2.8168	2.78	101.3
<i>Fusarium</i> toxin	Enniatin A1	14.6	19	18.38	103.3
<i>Fusarium</i> toxin	Enniatin B	14	22.432	22.8	98.4
<i>Fusarium</i> toxin	Enniatin B1	14.3	46.536	48.63	95.7
<i>Fusarium</i> toxin	Enniatin B2	13.7	1.2128	1.33	91
<i>Fusarium</i> toxin	Enniatin B3	13.2	0.01	0.01	100
<i>Fusarium</i> toxin	Equisetin	15.1	0.8504	0.42	202.4
<i>Alternaria</i> toxin	Infectopyron	9.4	329.04	329.04	100
<i>Fusarium</i> toxin	Nivalenol	4.8	6.1744	7.96	77.6
<i>Alternaria</i> toxin	Tentoxin	10.3	16.8	13.94	120.5
<i>Alternaria</i> toxin	Tenuazonic acid	9.6	101.12	53.47	189.1
Unspecific metabolite	Tryptophol	7.8	298.8	304.9	98
<i>Fusarium</i> toxin	Zearalenone	11.9	5.6728	5.47	103.7

LOD - limit of detection (S/N=3:1) expressed in µg/kg, RA (%) -calculated from spiked experiment of a single wheat sample compared with the neat standard.

Quantification of metabolites

There was recoveries of regulated mycotoxins and non –regulated fungal metabolites from the analysed wheat samples; the regulated mycotoxins recovered include Deoxynivalenol and Zearalenone while the non-regulated fungal metabolites include 3-nitropropionic acid, alternariol, alternariol-methylether, asperglaucide, beavericin, deoxynivalenol-glycoside, nivalenol, 15-hydroxyculmorin, , brevianamid F, culmorin, emodin, enniatin A,

enniatin A1, enniatin B, enniatin B1, enniatin B2, enniatin B3, equisetin, infectopyron, tentoxin, tenuazonic acid, and tryptophol. The regulated mycotoxins recovered from the wheat samples had 100% contaminations while the non – regulated fungal metabolites recovered had moderate (50-75%) to low (33 – 42%) contamination ranges with the exception of nivalenol that had 100% contamination of the wheat samples analyzed (Table 3

Table 3: Regulated and non-regulated microbial metabolites recovered in wheat samples.

Metabolites	n ^c	p ^c (%)	Concentration (µ/kg)					Proportion of contaminated wheat samples across the six market zones						
			Min	Max	Median	Mean	SD	1 (n=2)	2 (n=2)	3 (n=2)	4 (n=2)	5 (n=2)	6 (n=2)	
Regulated mycotoxins														
Deoxynivalenol ^s	12	100 12	409.8 100	2067 409	1030.7 76	1100.2 2067	640 1030.69	2 1100.2	2 639.8	2 2	2 2	2 2	2 2	1
Zearalenone ^{SS}	12	100	5.67	7.82	6.75	6.56	0.79	2	2	2	2	2	2	2
Non regulated														
3-Nitropropionic acid ^s	7	58.3	12.08	14.29	12.62	12.91	0.89	1	1	1	1	2	1	
Alternariol ^{SS}	6	50	1.5	18.33	13.7	12.44	5.71	1	0	2	1	1	1	
Alternariol- methylether ^{SS}	5	41.7	0.71	0.92	0.71	0.78	0.1	1	1	1	0	2	0	
Asperglaucide ^{SS}	7	58.3	14.9	22.53	18.78	18.19	3.42	1	2	2	0	1	1	
Beavericin ^{SS}	8	66.7	0.83	0.96	0.87	0.88	0.05	2	2	2	1	1	0	
Deoxynivalenol glycoside ^{SS}	8	66.7	19.7	33.9	23.6	25.65	6.58	2	2	2	1	1	0	
Nivalenol ^{SS}	12	100	6.17	10.9	8.07	8.22	1.84	2	2	2	2	2	2	
15-hydroxyculmorin ^s	8	67	165.6	268	182.06	203.5	43.53	2	1	2	1	1	1	
Brevianamid F ^s	12	100	2.64	2.7	2.67	2.67	0.3	2	2	2	1	2	1	
Culmorin ^s	7	58	23.8	25.4	23.9	24.08	1.14	1	2	2	1	1	0	
Emodin ^s	9	75	0.8	1.8	0.81	1.04	0.42	1	2	2	1	2	1	
Enniatin A ^{SS}	8	67	2.82	4.76	2.82	3.2	0.7	2	2	2	1	1	0	
Enniatin A1 ^s	7	58	19	33.6	25.72	26.16	5.42	2	2	1	1	0	1	
Enniatin B ^s	5	42	22.43	50.8	31.97	36.04	12.94	1	1	1	0	2	0	
Enniatin B1 ^s	4	33	46.54	80.2	46.55	54.02	14.81	1	1	1	1	0	0	
Enniatin B2 ^s	6	50	1.21	1.66	1.2	1.23	0.6	2	1	1	0	2	0	
Enniatin B3 ^s	5	42	0.01	0.04	0.01	0.02	0.01	1	1	1	0	1	1	
Equisetin ^{SS}	5	42	0.85	2.4	0.87	1.36	0.72	1	1	1	1	1	0	
Infectopyron ^{SS}	6	50	329	364.6	343.51	343.95	14.36	2	1	2	0	0	1	
Tentoxin ^{SS}	8	67	16.8	29.6	24.2	23.3	5.6	2	1	2	1	2	0	
Tenuazonic acid ^s	8	67	101.1	264.7	201.1	190.9	64.9	2	2	1	1	1	1	
Tryptophol ^s	5	42	298.8	316.5	305.9	306.7	7.7	1	1	1	0	1	1	

n = number of samples examined

n^c number of samples contaminated

^c -percentage of contaminated samples

<LOD - less level of detection

Market zones: 1 (Aguata/Orumba North and south), 2 (Awka north and south), 3 (Nnewi north/south Ihiala/Ekwusigo),

4 (Ogbaru/ Onitsha north/south), 5 (Anaocha/Idemili north/south) 6 (Njikoka/Ayamelum/Oyi/Anambra East

The superscript ^s indicates mycotoxins with significant differences (P≤0.05) among wheat samples screened

The superscript ^{SS} indicates mycotoxins with significant differences (P≤0.05) among wheat samples and market zones

Discussions

The genus *Aspergillus* dominated other mould species recovered from wheat samples in this study; this was followed by *Penicillium*, *Fusarium*, *Malbranchea*, *Alternaria*, *Rhizopus*, *Trichoderma*, *Cladosporium* and *Verticillium*. Many factors in field conditions and during storage act together in the contamination of crops and production of mycotoxins by these fungi, (Enyiukwu *et al.*, 2014). Important

mycotoxins producing fungi belonging to the genus *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* are the major contributor of food contamination. Elaaraj *et al.* (2015) reported *Aspergillus* as the most predominantly isolated fungal organisms in wheat samples followed by *Penicillium*, *Fusarium*, *Alternaria* and *Cladosporium*. Some of the mould species obtained in this study from wheat samples belong to genera they isolated. The fungi

isolated from the six market zones of Anambra State showed that wheat bought from market zone 1 had the highest fungal isolates (76), followed by those from zone 5 (50) isolates while the least was from market zones 2 (12 isolates) (Table 1). Yeasts also implicated as contaminants of the samples metabolize some food components and produce metabolites which cause the physical, chemical, and sensible properties of a food to change and thus food spoilage (Fleet and Praphailong 2001) though they do not produce mycotoxins. *Saccharomyces cerevisiae* is common food and environmental saprophyte and was isolated in this study, *Candida tropicalis* is found as part of the normal human mucocutaneous flora, its environmental isolations may be that the samples were contaminated with faeces, and contaminated soil (David *et al.*, 2007). *Candida tropicalis* and *Saccharomyces cerevisiae* were recovered by Desiye and Abegas, 2013 from teff batter (Ethiopian pancake). Environmental isolations of *Candida Stellatoides* and *Candida.rugosa* may be that the sources of the samples are contaminated by human or animal excreta, polluted water, soil, air and plants. *Candida rugosa* has been associated with catheter related fungemia, (David *et al.*, 2007). Wheat samples sold in Anambra State local markets were unique; rare mycotoxins were recovered from the samples. The conventional mycotoxins (Aflatoxins, Ochratoxins and Fummosins) were not recovered in the study. This may be because wheat are not produced in Anambra State but are bought from the northern part of the country and they are usually stored for short period in bags in the market places by the traders thus limiting the conditions that favour the mycotoxigenic fungi that produce Aflatoxins, Fummosin and Ochratoxins (Oyeka *et al.*, 2019).

Anambra State is in the rain forest zone with high rain fall pattern about (1300 - 2000mm), suitable temperature and high relative humidity which favours the growth of mycotoxigenic fungi (Udo *et al.*, 2000, Oyeka, *et al.* 2019). *Fusarium* fungal metabolites dominated other

fungal metabolites recovered in this study. *Fusarium* being field fungi may have contaminated the samples in the field and accompanied the samples to the storage and market places. The short storage of the samples by the traders gave room to *Fusarium* fungal metabolites to dominate the storage fungal metabolites of *Penicillium sp* and *Aspergillus sp*) Oyeka *et al.*, 2019).

Zearalenone, deoxynivalenol and nivalenol had 100% contamination of all the wheat samples. The concentration of regulated mycotoxin deoxynivalenol (409.8 - 2067 μ g/kg) in the studied samples exceeded maximum acceptable limits (MAL) for human consumption (European Commission (EC, 2006). Deoxynivalenol is a trichothecene; immunosuppressive and protein synthesis inhibitor in animals and humans (CAST, 2003). Deoxynivalenol glycoside; a masked form of deoxynivalenol contaminated the samples with concentration range of (19.7- 33.9 μ g/kg). Regular consumption of wheat containing low levels of deoxynivalenol and its masked form toxin may lead to acute toxicity to human and animals. Nivalenol, another type B immunosuppressive, protein inhibitor trichothecene and a non-regulated fungal metabolite contaminated the samples with concentration range of (6.2 -10.9 μ g/kg) and mean level of (8.22 μ g/kg) respectively. The metabolite nivalenol, is as toxicity as deoxynivalenol, therefore, any synergistic reaction of nivalenol and deoxynivalenol recovered in this study in animals will be a public health risk significance Oyeka *et al.*, 2019..

The oestrogenic mycotoxin zearalenone implicated in alterations in humans and animals reproductive tract, decrease in fertility or infertility, increase in number of foetal resorptions and implantation failure, reduced litter size, permanent damage to reproductive tracts, human cervical cancer, and outbreaks of precocious pubertal changes in children among other problems (CAST, 2003, Oyeka, 2003) contaminated the samples with the

concentrations (5.7–7.8 µg/kg) which are within the acceptable limits of human consumption (European Commission (EC, 2006).

Enniatins (A, A1, B, B1, B2, and B3) are similar to Beauvericin in function and toxicity. Enniatins (A, A1, B, B1, B2, and B3 and Beauvericin are mixtures of isomeric and homologous cyclodepsipeptides (Taylor, 1970). Enniatins were thought to be similar but are chemically distinct antibiotics. They are mixtures of isomeric and homologous cyclodepsipeptides to which the general name “enniatin” is applied. Enniatins are powerfully bioactive, apparently by interaction with membrane components, these properties they shared to some extent by the related cyclo-octadepsipeptide- valinomycin but there are differences, notably with regard to ion-selectivity (Audhya and Russel (1974). Beauvericin contaminates cereals and is genotoxic and can induce apoptosis in several human cells including lymphocytes (Adetunji *et al.*, 2014), Oyeka *et al.*, 2019. Considering the percentage contamination and different concentrations of these seven fungal metabolites in the studied wheat samples, they may be toxic to human and animal especially the Beauvericin (66.7%), Enniatin A (67%) and Enniatin BI which recorded higher concentration range of 46.54 -80.2µg/kg.

Postulation has been made on Equisetin on its possible role in human leukomogen and also a strong antibiotic to certain Gram positive bacterial. It is phytotoxic to plants and inhibits growth of various dicotyledonous and monocotyledonous plants. Roots, shoots and coleoptiles growth of these plants is usually inhibited by the microbial metabolite, at concentration of 10µg/kg for 72 hrs, thus permanently damage the plants seedlings though the concentrations (2.4µg/kg) recorded in this study were below 10µg/kg (Wheeler *et al.*, 1999). Much have not been documented about the effects of the microbial metabolites in human but they may work in synergy with other known *Fusarium* mycotoxins to cause harm to human and animals when continuous consumption of contaminated wheat with the *Fusarium*

metabolites and other known *Fusarium* toxins take place Oyeka *et al.*, 2019.

Penicillium metabolites Emodin has antibiotic property and had 75% contamination of the samples. Emodin has been reported to be toxic to cells (Ezekiel *et al.*, 2012).

Alternaria metabolites, Infectopyron, Tentoxin and Tenuazonic acid had moderate percentage occurrence in the samples. Concentration of Tenuazonic acid (101.1-265µg/kg, median = 201.1µg/kg) in the wheat samples studied was higher than what was reported in rye flour (57µg/kg) in Germany by Asam *et al.*, 2012. The co-presence of Alternariol, Alternariol-methylether, Tentoxin with Tenuazonic acid further confirms the infestation of the samples by *Alternaria* fungal species. From toxicological stand point, Tenuazonic acid is known to be toxic to chick embryo and some animal species inducing haemorrhages in vital organs principally through the inhibition of protein synthesis by suppression of the release of newly formed proteins from the ribosomes into the supernatant fluid, (Ezekiel *et al.*, 2012). Acute toxicosis by Tenuazonic acid may pose a threat to consumers though chronic exposure of the toxin has not yet been investigated; toxic situations may be more complicated due to synergism by many metabolite combinations (Ezekiel *et al.*, 2012).

Brevianamid F and Tryptophol fungal metabolites of many species of fungi also contaminated the samples with 100% and 42% contamination respectively. Much has not been known about the toxicity of these two metabolites in human and animals. Ezekiel *et al.*, 2012 reported 100% contamination of all the fonio and sesame samples by these unspecific fungal metabolites. Misihairedgwi *et al.* (2019) also found brevianamid (100%) and tryptophol (74%) in vegetables in Namibia. Oyeka *et al.*, 2019 recovered these unspecific fungal metabolites (Brevianamid F (100%) and Tryptophol (70%) in maize in Anambra State, Nigeria.

Conclusion

The co-presence of mycotoxigenic fungi, the studied mycotoxins and other fungal metabolites in the samples as demonstrated in this study implies that the samples could contain fungal toxins that can cause human and animal mycotoxicosis. The concentration of the immunosuppressive deoxynivalenol (409.8 - 2067 µg/kg) above the acceptable limit of human consumption in this study is a source of worry. Therefore, the consumption of the contaminated samples could have adverse consequences on national livestock production, human and economy.

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Disclosure statement

The authors alone are responsible for the content and writing of this manuscript. Therefore, there is no potential conflict of interest by the authors.

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