

Evaluation of five major mycotoxins co-contaminating two cereal grains from Nigeria

Egbuta Mary Augustina^{1,2}, Mwanza Mulunda^{1,2}, Dutton Micheal Francis²

1. Faculty of Agriculture, Science and Technology, North-West University, Mafikeng campus, South Africa.

2. Food, Environmental and Health Research Group, Faculty of Health Sciences, University of Johannesburg, Doornfontein campus, South Africa.

Corresponding author:

Email: donmartinni@yahoo.com

ABSTRACT

Aims-The aim of this study was to determine the co-occurrence of five major mycotoxins in two Nigerian cereal grains destined for human consumption.

Study design-Nigeria experiences high humidity and high temperatures for up to three-quarters of the year; and these are some of the conditions that promote mycotoxin production by filamentous fungi in food commodities. In this regard, rice and maize samples were collected from stores and markets in South Eastern and South Western Nigeria and evaluated for occurrence of five major mycotoxins- aflatoxins (AFs), ochratoxin A (OTA), fumonisin B₁ (FB₁), Fumonisin B₂ (FB₂), deoxynivalenol (DON) and zearalenone (ZEA).

Place and duration of study- The study was part of a Masters dissertation developed in the Food, Environment and Health Research Group, Faculty of Health Sciences, University of Johannesburg.

Methodology-Imploring three different extraction procedures, mycotoxin occurrence and levels were determined via thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Results-Results showed high incidences of all five mycotoxins in both samples with OTA highest in rice samples (92.7%), while AFs and FB₁ had highest incidence in maize samples (94.9%). Data from HPLC analysis of rice and maize samples showed different concentration range of mycotoxins AFs (0.01-6.50µg/kg and 0.07-109.78µg/kg); OTA (0.7-180.9µg/kg, 0.6-79.0µg/kg; FB₁ (0.9-59.6µg/kg, 10.0-3644.0µg/kg); ZEA (0.7-570.6µg/kg, 1.8-652.3µg/kg) and DON (0.1-0.7µg/kg, 0.1-0.7µg/kg) respectively.

Conclusion-Mycotoxins concentration determined in the food samples were above Nigerian and European Union (EU) set limits. The co-occurrence of these mycotoxins in food samples analysed in this study gives further awareness to the health risks consumers of these food commodities are exposed to. The study also contributes to research being done in Nigeria in other to understand the degree of mycotoxins contamination of food consumed by the populace.

Keywords: Mycotoxins, Maize, Rice, Nigeria

1 INTRODUCTION

Cereal grains, maize and rice are some of the staple foods consumed in most parts of sub-Saharan Africa and the world over [1]. Rich in different nutrients such as carbohydrates, proteins and vitamins, these cereal grains are also susceptible to fungal infection both on the growing fields and in the stores [2, 3]. Subsequent production of mycotoxins by fungi contaminating cereals is a fact that cannot be disputed because mycotoxins are secondary metabolites synthesized by filamentous fungi during their stationary phase of growth [4, 5]. These mycotoxins which are secondary metabolites have very negative health effects on humans and animals [6] inducing carcinogenic, teratogenic, mutagenic and immuno suppressive effects [7].

In Nigeria, especially in the rural areas most of the food commodities consumed are staple food with less consumption of processed food. Maize and rice are some of the food crops widely consumed across the country. Rice is cultivated in the country as well as imported in other to meet the high demand for rice in the country. It was reported that up to 32.9kg of rice was consumed per person on a yearly basis between 2007 and 2010 in the country [8], with an assumption that this value must have increased between 2010 and now. Maize on the other hand, is one food crop that is consumed in different forms in the country. It can be cooked, roasted or processed into a porridge meal (“ogi”) that is at most times used as a weaning food for infants. Most of these food crops are cultivated by subsistence farmers who do not implore high tech farming techniques. Also, because food crops are cultivated in seasons they are usually stored in proper and improper storage houses for availability throughout the year.

Mycotoxin production by filamentous fungi mainly *Aspergillus*, *Fusarium* and *Penicillium* species is usually triggered by specific conditions which range from environmental, climatic and human [9]. Extremely high and low temperatures, humidity, improper handling and improper storage have been contributory to mycotoxin production by filamentous fungi in most food crops either on the field, in storage or during transportation [9]. There are two major seasons in Nigeria: the rainy and dry (harmattan) seasons, with high temperatures almost all through the year. These climatic conditions alongside other conditions are some of the challenges faced by farmers in this part of the world which has prompted the need for research into mycotoxin contamination of a variety of food crops cultivated in the country [10].

As a result of the susceptibility of maize and rice crops to mycotoxin producing fungi [11, 12], there are possible health risks consumers of this food crops would be exposed to. Mycotoxins have been mentioned in association with some negative health conditions in both humans and animals such as immune suppression, carcinogenesis, tumourgenesis, mutagenesis and reproductive related diseases [7]. Aflatoxin B₁ has been reported to act in synergy with the hepatitis B virus [13, 14], thereby contributing to hepatocarcinogenesis in humans. Some of these major mycotoxins (ochratoxin A and aflatoxin B₁) have been classified by the International Agency for Research on Cancer as human carcinogens [15-17].

In the past years, due to the prevalence of *Aspergillus* species contaminating most food commodities in Nigeria, much emphasis had been on aflatoxins, fumonisins and ochratoxin A studies in comparison to other mycotoxins but currently, mycotoxin research has gone beyond aflatoxins and ochratoxin A to other less common mycotoxins produced by filamentous fungi [18, 19]. Imploring different methods of extraction and two types of validation technique to determine mycotoxin contamination, this study was done in contribution to other studies being carried out in Nigeria in a bid to understand the extent of exposure of consumers to some mycotoxins that are known to contaminate cereal grains with the main aim of screening maize and rice samples for different mycotoxins co-contamination.

2 MATERIALS AND METHODS

2.1 Sampling

Maize samples (39) were collected from markets and stores in south-eastern Nigeria whereas rice samples (41) were collected from south-eastern and south-western Nigeria. They were transported sealed in sterile sample collection bags and transported to the Food, Environment and Health Research Laboratory in the University of Johannesburg, South Africa following strict custom duty procedures. Samples were milled in the laboratory upon arrival using a sterilized laboratory mill (IKA M20, Merck, Darmstadt, Germany) and stored at -20°C prior to mycotoxin analysis.

2.2 Mycotoxin extraction

2.2.1 Multi-mycotoxin extraction

Dichloromethane (DCM), acetonitrile, 4% potassium chloride (KCl), saturated sodium hydrogen bicarbonate (NaHCO_3), sodium sulphate (Na_2SO_4), acetone, 250ml separating funnels, 250ml conical flasks, round bottom flasks, 1ml screw cap amber vials, glass funnels, Whatmann no. 1 filter paper, dialysis tubing, boiling test tubes, rotary vacuum evaporator (Buchi Laboratory-techniques Ltd., Flawil, Switzerland).

2.2.2 Fumonisin extraction

Strong anion exchange (SAX) cartridge (ANATECH, Gauteng, South Africa), methanol (CH_3OH), distilled water, acetonitrile, acetic acid (CH_3COOH), sodium hydroxide (NaOH), Whatmann no. 1 filter paper, 250ml conical flasks, pH meter (MicroSet MS 324), sterile pipette tips, 1 ml amber vials.

2.2.3 Immuno-affinity extraction

Immuno-affinity columns (VICAM), fluted filter papers, microfiber filter papers, 250ml plastic disposable beakers, disposable plastic pipette, HPLC grade methanol, acetonitrile, PBS, tween-20 solution, sodium bicarbonate, disposable plastic pipette, HPLC grade methanol, acetonitrile, PBS, tween-20 solution, sodium bicarbonate

2.2.4 TLC (thin layer chromatography)

mobile phase- Dichloromethane/Ethyl-acetate/Propan-2-ol (90:5:5 v/v/v) (DEP), Toluene/Ethyl-acetate/Formic acid (6:3:1 v/v/v) (TEF), Butanol/Water/Acetic acid (12:5:3 v/v/v) (BWA), Dichloromethane/Acetone (90/10 v/v) (DA); aluminium backed thin layer chromatography plates (20 x 20cm) pre-coated with silica gel G (Merck, Germany), reversed-phase (C18) tlc plates (Merck, Germany), capillary pipettes, chromatography tanks (Camag Ltd), variable hot air drier, UV light in viewing cabinet, Spray gun, fume hood, paper towelling, oven at 120°C ; *derivatizing and spraying reagents*- chromotropic acid (CTA), diazotised benzidine and anisaldehyde reagent.

2.2.5 HPLC (high performance liquid chromatography)

HPLC grade acetonitrile, HPLC grade methanol, sodium di-hydrogen phosphate, potassium bromide (KBr), nitric acid (HNO_3), o-phthalaldehyde (OPA) reagent prepared by dissolving 40 mg of OPA in 1 ml CH_3OH and diluted with 5 ml 0.1M sodium tetraborate ($\text{Na}_2\text{B}_2\text{O}_4$) and 50 μl mercapthoethanol. HPLC Spectra Physics SCM400 SYSTEM (Waters, Milford,

MA, USA), Shimadzu Corporation (Kyoto, Japan) LC-20AB liquid chromatograph equipped with CBM-20A communication bus module, LC-20AB degasser, CTO-20A column oven, Nova-Pak 4mm C18 reversed phase analytical column (250×4.6 mm, 5µm), SIL-20A auto sampler, RF-10AxL fluorescence detector, RID-10A refractive index detector and SPD-M20A photodiode array detector linked to LC solutions version 1.22 Software Release. Kobra cell (DR Weber Consulting, Germany).

2.2.6 Mycotoxins standards

Aflatoxins B₁, B₂, G₁, G₂, ochratoxin A(OTA), fumonisin B₁, B₂, zearalenone (ZEA) and deoxynivalenol (DON) were procured from the Medical Research Council (MRC), South Africa.

2.3 Multi-mycotoxin extraction procedure

Following the method implored by Patterson and Roberts [20] and Mwanza [21] aflatoxins, ochratoxin A, zearalenone and deoxynivalenol were extracted from 25g of food samples using isooctane and DCM through an anhydrous Na₂SO₄ bed. Extracts clean-up was achieved through a dialysis clean up step where extracts were left to shake overnight in a dialysis tube inserted in a test tube containing 30% acetone and further extracted with DCM. Neutral fractions and acid fractions were extracted, dried using nitrogen gas stream at 60°C and stored at 4°C for further analysis.

2.4 Fumonisin extraction procedure

The method of clean-up and extraction of using solid phase extraction (SPE) isolate strong ion exchange (SAX) columns (International Sorbent Technology, UK) was implemented for all the food commodities following the method of Shephard and Sewram [22] with some modifications. Ten (10) grams of each sample was extracted using 100ml of Methanol/water (70/30, v/v) and pH of filtered extract adjusted between 5.9 and 6.5. This step was followed by diluting extracts with 4 ml methanol/water (70/30) and loaded unto SAX columns which had been pre-conditioned with methanol and methanol/water (70/30). Fumonisins were eventually eluted with methanol/acetic acid (99/1, v/v) after columns had been washed with methanol/water (70/30) and methanol. Extracts were dried under a stream of nitrogen gas at 60°C and stored at 4°C for further analysis.

2.5 Immuno-affinity clean up and extraction procedure

Following the methods Implemented by Mwanza [23] with slight modifications, clean-up and extraction of each mycotoxin (OTA, AFs, FBs) using immuno-affinity columns was done following the procedure of the manufacturers (VICAM Ltd. Watertown, USA). In this procedure, extracts from food samples were applied to the column and mycotoxins acted as antigens binding to the antibodies present in the column. The mycotoxins were then eluted using an eluting solution which was passed through the column. Final mycotoxin extracts from the columns were dried and stored at 4°C for further analysis.

2.6 Thin layer chromatography of mycotoxin extracts

Using silica gel coated aluminium backed 20cm by 20cm TLC plates and specific mobile phases mycotoxin extracts were analysed to determine the presence of toxins investigated [23], [24]. Dried neutral and acid fraction extracts were dissolved in 200µl of acetonitrile, thoroughly mixed and 20 µl of extracts spotted on silicon backed tlc plates. Extracts were run alongside known concentration of standards in different mobile phases and mycotoxins that could florescence were viewed under UV light. Other mycotoxins like ZEA and DON which did not florescence were sprayed with derivatizing solutions (dianisidine and chromotropic acid respectively) and viewed.

Fumonisin extracts were dissolved in 200 µl of methanol, vortexed and 20 µl spotted on tlc plates and run in a tlc tank containing a mobile phase (butanol/water/acetic acid 12/5/3, v/v/v). Thin layer chromatography plates were dried, derivatized using anisaldehyde solution, put in an oven for a few minutes and viewed.

2.7 High Performance Liquid chromatography analysis of food extracts

Acid and neutral fractions of mycotoxin extracts from the food samples were dissolved in 500µl of hplc grade acetonitrile prior to hplc analysis, whereas fumonisin extracts were suspended in 500µl hplc grade methanol. Following the methods of Shepherd, et al. [25], Reiter, et al. [26] and Abdulkadar, et al. [27], concentrations of AFs, OTA, ZEA and FBs were determined at different emission and excitation wavelengths alongside standards of known concentrations.

2.8 Recoveries

Samples with known mycotoxin concentrations were spiked with the following different mycotoxin standards of known concentration- (AFs-100µg/ml; OTA-50µg/ml; FBs-100µg/ml; ZEA-50µg/ml and DON-50µg/ml). In otherto confirm the effectiveness of the methods

implemented for extraction of mycotoxins from samples, mycotoxins were extracted and final mycotoxin concentration determined using the hplc equipment.

2.9 Statistical analysis

Using the IBM SPSS statistical software version 16, descriptive statistics and a one-way analysis of variance (ANOVA) was performed to derive mean values, which were compared by least significant difference using all pairwise multiple comparison procedures. Analysis was done in three replications and mean values among treatment groups were deemed to be different if the level of probability was ≤ 0.05 .

3 RESULTS AND DISCUSSION

Data obtained from the different analysis showed mycotoxin occurrence in both food samples analysed at varying concentrations. Results showed some of the food samples being contaminated by all five mycotoxins investigated. Thin layer chromatography analysis was able to determine to some extent the occurrence of some of the mycotoxins (AFs, OTA and FBs) as it is illustrated in table 1. High performance liquid chromatography analysis was able to detect mycotoxin contamination at very low concentrations in the food samples analysed. Incidences of all five mycotoxins were determined in both food samples up to 94.9%. Data obtained from the analysis (table 2 and 3) showed the occurrence of aflatoxins and ocratoxin A in both rice and maize samples in comparison to the other three mycotoxins. The concentration of the different mycotoxins detected in the food samples via HPLC were based on the chromatograms of AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁, FB₂, ZEA and DON standards used as well as calibration curves plotted from the areas of peaks of standards and concentration of standards.

Table 1. Percentage incidence of major mycotoxins in rice and maize samples determined by thin layer chromatography

Mycotoxins	Food samples	
	Rice (n=41)	Maize n= (39)
AFs	14.6	23.1
OTA	4.9	2.6
FBs	n.d	15.4
ZEA	n.d	n.d
DON	n.d	n.d

n- no. of samples, n.d- none detected

Table 2: Analyses of mycotoxins in rice samples as determined by high performance liquid chromatography

Mycotoxins	% incidence	Range ($\mu\text{g/kg}$)	Mean	Std. Dev.
AFs	87.8	0.01-6.50	0.76	3.10
OTA	92.7	0.7-180.9	18.2	112.3
FB ₁	90.2	0.9-59.6	18.8	112.6
FB ₂	n.d	n.d	n.d	n.d
ZEA	80.5	0.7-570.6	203.9	440.0
DON	70.7	0.1-0.7	0.3	1.6

Mean*- mean of positive samples. % incidence- no. of positive samples in percentage. Std. Dev.- standard deviation.

Table 3: Analyses of mycotoxins in maize samples as determined by high performance liquid chromatography

Mycotoxins	% incidence	Range ($\mu\text{g/kg}$)	Mean	Std. Dev.
AFs	94.9	0.07-109.78	10.24	24.92
OTA	92.3	0.6-79.0	14.8	16.7
FB ₁	94.9	10.0-3644.0	645.2	919.1
FB ₂	66.6	15.2-1123.9	137.8	252.4
ZEA	64.1	1.83-652.3	139.0	155.8
DON	69.2	0.1-0.7	0.3	0.2

Mean*- mean of positive samples. % incidence- no. of positive samples in percentage. Std. Dev.- Standard deviation

Statistical analysis of results from HPLC analysis showed ranges of concentration of the different mycotoxins screened for. The highest range of mycotoxin contamination in rice samples was ZEA ranging from 0.7- 570.6µg/kg as indicated in table 2. Maize samples had highest concentration range of fumonisin B₁ (10- 3644µg/kg) with a mean value of 645.2. table 3 shows the different concentration range of all mycotoxins screened for in the maize samples along with the mean and standard deviation values derived. Different concentration ranges were recorded for mycotoxins in rice and maize samples from the two collection areas as illustrated in table 4 and 5. Table 6 and 7 shows concentration range of mycotoxins determined by all three method implored and correlation co-efficient (r) analysis of results derived from multi-mycotoxin extraction and SAX columns against results got from VICAM immuno- affinity columns.

Table 4: Distribution of mycotoxin contamination in rice samples from both collection areas

Mycotoxins	Stores		Markets	
	Range (µg/kg)	Mean	Range (µg/kg)	Mean
AFs	0.01-0.18	0.06	0.04-6.50	1.26
OTA	0.7-180.9	24.1	0.8-92.2	9.2
FB ₁	0.9-44.9	18.3	4.4-59.6	15.7
FB ₂	0	0	0	0
DON	0.1-0.6	0.4	0.1-0.7	0.3
ZEA	0.7-317.6	97.7	0.7-570.6	271.8

Table 5: Distribution of mycotoxin contamination in maize samples from both collection areas

Mycotoxins	Stores		Markets	
	Range ($\mu\text{g/kg}$)	Mean	Range ($\mu\text{g/kg}$)	Mean
AFs	0.18-109.78	11.62	0.07-30.85	8.94
OTA	0.6-79.0	15.0	2.5-46.5	13.1
FB ₁	10.0-2401.5	630.3	22.2-3643.6	610.7
FB ₂	15.2-686.6	245.2	27.8-1123.9	145.0
DON	0.1-0.7	0.4	0.1-0.7	0.3
ZEA	88.1-652.3	251.4	1.83-132.6	36.7

Table 6: Mycotoxin HPLC concentration range ($\mu\text{g/kg}$) in food extracts by multi-mycotoxin, SAX column and VICAM immuno affinity column extraction procedure

Mycotoxins	Rice			Maize		
	Multi-Mycotoxin	SAX column	Immuno-affinity	Multi-Mycotoxin	SAX column	Immuno-affinity
AFs	0.01-6.50		4.76-15.70	0.20-109.78		0.91-123.62
OTA	0.7-180.9		10.4-220.40	0.6-40.3		7.8-60.1
FB ₁		0.90-59.60	3.7-62.0		10.0-3643.6	16.3-3722.7
FB ₂		n.d	n.d		15.2-1123.9	32.1-1343.8

n.d- none detected

Table 7: Correlation co-efficients (r) for mycotoxins detected from multimycotoxin and SAX column in comparison with VICAM extraction.

Food samples	Correlation co-efficient (r)			
	AFs	OTA	FB ₁	FB ₂
Maize	0.970	0.969	0.990	-
Rice	0.932	0.992	1.0	0.982
Cocoa	0.996	1.0	-	-
Cocoa beverage	0.992	0.984	-	-

3.1 Mycotoxin contamination of food samples

Food contamination by mycotoxins is a major problem in the tropics and sub-tropics due to climatic conditions and other conditions that favour the growth of fungi and subsequent production of mycotoxins by these fungi [28]. Nigeria is not left out of this situation because of its adverse weather conditions, farming practices, storage conditions and mode of transportation of food crops. Out of over 20 commonly occurring mycotoxins in food and agricultural products [29], the aflatoxins, ochratoxins, fumonisins, zearalenone and the trichothecenes have been reported to be major mycotoxins occurring in food.

Rice grain is a highly suitable substrate for the production of the five major occurring mycotoxins mentioned as these mycotoxins have been reported to occur in rice grains at different stages of production worldwide. Mycotoxin contamination of rice often occurs in the field prior to harvest, while post-harvest contamination can occur if drying was delayed and during storage, if moisture content is allowed to exceed critical values for mould growth [28]. Results obtained from the mycotoxin screening of rice samples also agree with this views about mycotoxin contamination of rice crops. Although lower incidences were obtained when samples were analysed using TLC which could be attributed to the detection limit of thin layer chromatographic techniques [30-32], very high incidences were determined through HPLC analysis. The occurrence of these mycotoxins AFs, OTA, FBs, DON and ZEA evaluated in this food sample could be attributed to contamination of the samples by mycotoxigenic fungi *Aspergillus*, *Fusarium* and *Penicillium*[33-35].

Maize is also another food commodity that act as a good substrate for growth of toxigenic fungi producing mycotoxins. One major mycotoxin associated with maize is fumonisin which is produced mainly by *F.verticillioides* and *F. proliferatum*[36-38]. Other mycotoxins reported to be associated with contamination of maize include OTA, ZEA, AFs and the trichothecenes. Thin layer chromatography data from maize samples showed incidences for AFs, OTA and FBs at 23.1%, 2.6% and 15.4% whereas ZEA and DON were not detected. High performance liquid chromatography analysis data also indicated high incidences of all five mycotoxins with varying concentration ranges. Out of all five mycotoxins present in the maize samples, fumonisins B₁ and B₂ was detected to be of highest concentration. This could very well be attributed to the susceptibility of maize crops to the fungi specie *F.verticillioides* and *F. proliferatum* as was earlier mentioned.

There have been reports of filamentous fungi prevalence and mycotoxin occurrence in many food commodities originating from Nigeria in recent years [33, 34, 39-41], and many food commodities (unprocessed or processed) are being screened continually for these fungal metabolites. The findings from this study are in accordance with most studies from other parts of the world where mycotoxin analysis has been done on cereal crops and grains. In this study, OTA was found to occur in most samples at lower concentration although some samples had high concentrations of OTA which was above the set regulatory limit of 5µg/kg for the EU and Nigeria. Ochratoxin A mostly produced by *A. ochraceus* and *P. viridicatum* has been mentioned as a predominant mycotoxin alongside the aflatoxins contaminating many Nigerian food commodities from farm to fork [33, 40]. This can be due to the high temperature and humidity of the country which favours the production of these mycotoxins by fungi [9]. One other observation from the study was that samples that were highly contaminated with OTA had lower concentration of AFs and vice versa. This could be explained by the inhibitory effect of ochratoxin A to the production of aflatoxins by *A. parasiticus* reported by Dimitrokallis, et al. [42], although there is no evidence of this inhibitory effect on the production of aflatoxins by *A. flavus*. Another reason could be the divergent uptake behaviour of mycotoxins which indicates the transport of OTA being different from that of AFs in food commodities as a result of chemico-physical effects [43].

Fumonisin contamination of both rice and maize samples with concentration above the EU regulatory set limit of 1000µg/kg was recorded in this study. There was also the prevalence

of DON and ZEA in both samples. As it is, most mycotoxin studies on Nigerian food commodities have concentrated on the fumonisins, ochratoxin A and aflatoxins with very little attention given to other mycotoxins even though it is well known that these mycotoxins have very adverse health effects both in humans and animals. The high levels of DON and ZEA in the food samples analysed could be because cereals are good substrates for contamination by DON and ZEA producing fungi [3, 44, 45]. Also, ZEA is a heat and pH stable mycotoxin and so can withstand very harsh weather conditions [46, 47].

There was a distinctive difference in mycotoxin contamination of food samples from stores and markets. All rice samples collected from the markets were contaminated with high concentrations of mycotoxins in comparison to rice samples collected from d stores. A reason for this variation in mycotoxin contamination could be attributed to the means of transportation and handling of the samples at the market places. Most of these food samples are displayed in big containers or in bags at the market places which are left open for buyers to see and purchase. With the presence of filamentous fungi in the air and soil, some of the food samples in the market places would have been more contaminated by the mycotoxin producing fungi and subsequent mycotoxin production. Evaluation of mycotoxins co-occurring in the selected food samples showed a prevalence of all five mycotoxins at varying concentration levels which is something to taken seriously.

4 CONCLUSION

In this study, we were able to evaluate the incidence of five major mycotoxins in the food samples. These mycotoxins were found to co-occur at different concentrations above the set limits which are a cause for concern in terms of mycotoxin control strategies in Nigeria. As was mentioned earlier much emphasis has been given to aflatoxins, ochratoxin A and fumonisins in Nigerian foods with little attention given to other less common mycotoxins which also have negative health effects. The high incidence of both DON and ZEA in the food commodities analysed is a cause for concern and reason for more research into other mycotoxins contamination of Nigerian food commodities as well control strategies in order to combat mycotoxin contamination.

5 ACKNOWLEDGEMENT

We wish to thank the following institutions for their contributions to the success of this study: University of Johannesburg and North-West University, Mafikeng campus.

6 CONFLICT OF INTEREST

The authors declare no conflict of interest.

7 REFERENCES

1. Chauvin ND, Mulangu F, Porto G. Food production and consumption trends in sub-Saharan Africa: Prospects for the transformation of the agricultural sector. United Nations Development Programme; 2012:4-29.
2. Piotrowska M, Ślizewska K, Biernasiak J. *Mycotoxins in Cereal and Soybean-Based Food and Feed*; 2013.
3. Pereira VL, Fernandes JO, Cunha SC. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. *Trends Food Sci. Technol.* 2014;36(2):96-136.
4. Ndlovu CS. Fungi and mycotoxins in South African forage crops and silage [Dissertation]. University of Johannesburg; 2008.
5. Devi P, D'Souza L, Kamat T, Rodrigues S, Naik CG. Batch culture fermentation of *Penicillium chrysogenum* and a report on the isolation, purification, identification and antibiotic activity of citrinin. *Indian J. Mar. Sci.* 2009;38(1):38-44.
6. Edite Bezerra da Rocha M, Freire FdCO, Erlan Feitosa Maia F, Izabel Florindo Guedes M, Rondina D. Mycotoxins and their effects on human and animal health. *Food Control.* 2014;36(1):159-65.
7. Richard JL. Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol.* 2007;119(1–2):3-10.
8. Cadoni P, Angelucci F. Analysis of incentives and disincentives for rice in Nigeria. Technical notes series. Rome: MAFAP, FAO; 2013:8-9.
9. Wagacha JM, Muthomi JW. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *Int. J. Food Microbiol.* 2008;124(1):1-12.
10. Atanda O, Makun HA, Ogara IM, Edema M, Idahor KO, Eshiett ME, et al. Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds. In: Makun HA, ed. *Mycotoxin and Food Safety in Developing Countries*. Croatia: Intech; 2013:3-38.
11. Amadi JE, Adeniyi DO. Mycotoxin production by fungi isolated from stored grains. *Afr. J. Biotechnol.* 2009;8(7):1219-21.
12. Egbuta MA, Mwanza M, Njobeh PB, Phoku JZ, Chilaka CA, Dutton MF. Isolation of filamentous fungi species contaminating some Nigerian food commodities. *J. Food Res.*; Vol. 4, No. 1; 2015. 2015;4(1):38-50.
13. Wild CP, Montesano R. A model of interaction: Aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. *Cancer Lett.* 2009;286(1):22-28.
14. Kew MC. Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. *Liver Int.* 2003;23(6):405-09.
15. IARC. Ochratoxin A. Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. Lyon, France: International Agency for Research on Cancer; 1993b:489-521.
16. IARC. Traditional herbal medicines, some mycotoxins, naphthalene and styrene. Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer; 2002:82-171.
17. IARC. Aflatoxins. Monographs on chemical agents and related occupations: International Agency for Research on Cancer; 2012:225-48.
18. Fapohunda SO, Anjorin ST, Akueche EC, Harcourt B. Multi mycotoxin profile of gamma-radiated sesame seeds from Abuja markets, Nigeria using LC-MS/MS. *Nature and Science.* 2012;10(10):127-34.

19. Rubert J, Fapohunda SO, Soler C, Ezekiel CN, Mañes J, Kayode F. A survey of mycotoxins in random street-vended snacks from Lagos, Nigeria, using QuEChERS-HPLC-MS/MS. *Food Control*. 2013;32(2):673-77.
20. Patterson DS, Roberts BA. Mycotoxins in animal feedstuffs: sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin. *J. Assoc. Official Analytical Chems.* 1979;62:1265-67.
21. Mwanza M. An investigation in South African 461 domesticated animals, their products and related health issues with reference to mycotoxins and fungi [Masters]. University of Johannesburg; 2007.
22. Shephard GS, Sewram V. Determination of the mycotoxin fumonisin B1 in maize by reversed-phase thin-layer chromatography: a collaborative study. *Food Addit. Contam.* 2004;21:498-505.
23. Mwanza M. A survey of fungi and mycotoxins with respect to South African domestic animals in Limpopo Province [Dissertation]. University of Johannesburg; 2007.
24. Njobeh PB, Dutton MF, Koch SH, Chuturgoon AA, Stoev SD, Mosonik JS. Simultaneous occurrence of mycotoxins in human food commodities from Cameroon. *Mycotox. Res.* 2010;26:47-57.
25. Shepherd GS, Thiel PG, Sydenham EW, Vleggaar R, Alberts JF. Determination of the mycotoxin fumonisin B1 and identification of its partially hydrolysed metabolites in the faeces of nonhuman primates. *Food Chem. Toxicol.* 1994;32:23-29.
26. Reiter E, Zentek J, Razzazi E. Review on sample preparation strategies and methods used for the analysis of aflatoxins in food and feed. *Mol. Nutr. Food Res.* 2009;53(4):508-24.
27. Abdulkadar AHW, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. Mycotoxins in food products available in Qatar. *Food Control*. 2004;15(7):543-48.
28. Kumar V, Basu MS, Rajendran TP. Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Protect.* 2008 27:891-905.
29. Steyn PS. Mycotoxins, general view, chemistry and structure. *Toxicol. Lett.* 1995;82/83:843-51.
30. Xu B-j, Jia X-q, Gu L-j, Sung C-k. Review on the qualitative and quantitative analysis of the mycotoxin citrinin. *Food Control*. 2006;17(4):271-85.
31. Ono EYS, Silva Md, Ribeiro RMR, Ono MA, Hayashi L, Garcia GT, et al. Comparison of thin-layer chromatography, spectrofluorimetry and bright greenish-yellow fluorescence test for aflatoxin detection in corn. *Braz. Arch. Biol. Techn.* 2010;53:687-92.
32. Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: A review. *Anal. Chim. Acta.* 2009;632(2):168-80.
33. Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, et al. Fungal and aflatoxin contamination of some human food commodities in Nigeria. *Afr. J. Food Sci.* 2010;4(4):127-35.
34. Adejumo TO, Hettwer U, Karlovsky P. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. *Int. J. Food Microbiol.* 2007;116(3):350-57.
35. Mounjouenpou P, Gueule D, Fontana-Tachon A, Guyot B, Tondje PR, Guiraud J-P. Filamentous fungi producing ochratoxin a during cocoa processing in Cameroon. *Int. J. Food Microbiol.* 2008;121(2):234-41.
36. Dutton MF. Fumonisin, mycotoxins of increasing importance: Their nature and their effects. *Pharmacol. Ther.* 1996;70(2):137-61.
37. Kpodo K, Thrane U, Hald B. *Fusaria* and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. *Int. J. Food Microbiol.* 2000;61:147-57.

38. Sweeney MJ, Dobson AD. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Int. J. Food Microbiol.* 1998; 43:141-58.
39. Adejumo TO, Hettwer U, Karlovsky P. Survey of maize from south-western Nigeria for zearalenone, α - and β -zearalenols, fumonisin B1 and enniatins produced by *Fusarium* species. *Food Addit. Contam.* 2007;24:993-1000.
40. Egbuta MA, Chilaka CA, Phoku JZ, Mwanza M, Dutton MF. Co-contamination of Nigerian cocoa and cocoa-based powder beverages destined for human consumption by mycotoxins. *Ethno Medicine.* 2013; 7(3):187-94.
41. Bankole SA, Schollenberger M, Drochner W. Survey of ergosterol, zearalenone and trichothecene contamination in maize from Nigeria. *J. Food Compos. Anal.* 2010;23(8):837-42.
42. Dimitrokallis V, Meimaroglou DM, Markaki P. Study of the ochratoxin A effect on *Aspergillus parasiticus* growth and aflatoxin B1 production. *Food Chem. Toxicol.* 2008;46:2435- 39.
43. Raters M, Matissek R. New examinations of mycotoxin carryover to cocoa beans. *Mycotox. Res.* 2007;23:39-42.
44. Van Der Fels-Klerx HJ, Klemsdal S, Hietaniemi V, Lindblad M, Ioannou-Kakouri E, Van Asselt ED. Mycotoxin contamination of cereal grain commodities in relation to climate in North West Europe. *Food Addit. Contam.: Part A.* 2012;29(10):1581-92.
45. Bernhoft A, Eriksen GS, Sundheim L, Berntssen M, Brantsæter AL, Brodal G, et al. Risk assessment of mycotoxins in cereal grain in Norway. *Norwegian Scientific Committee for Food Safety (VKM)*; 2013:5-25.
46. Yazar S, Omurtag G. Fumonisin, Trichothecenes and Zearalenone in Cereals. *Int. J. Mol. Sci.* 2008;9(11):2062-90.
47. Ryu D, Hanna MA, Eskridge KM, Bullerman LB. Heat Stability of Zearalenone in an Aqueous Buffered Model System. *J. Agric. Food Chem.* 2003;51(6):1746-48.