Mycotoxicology Society of Nigeria

***Mycotoxicology*, 2014, 1: 46-56**

ORIGINAL RESEARCH

Simultaneous Occurrence of Aflatoxin and Ochratoxin A In Rice From Kaduna State, Nigeria

Olorunmowaju, Y. B, Makun, H. A*, Mohammed, L. H., Adeyemi, H. R H, Ifeji, E, Muhammad H. K and Mailafiya, S. C

Department of Biochemistry, Federal University of Technology, Minna, Nigeria.
***Corresponding author:** hussaini.makun@futminna.edu.ng*

ABSTRACT

Eighty six samples of field, stored and marketed rice (*Oryza sativa*) collected from the traditional rice growing areas of Kaduna state, were analyzed for aflatoxins (AFs) and ochratoxin A (OTA) by high-performance liquid chromatography (HPLC). Aflatoxins were detected in 74.4% of the samples, AFB_1 was found at concentrations between 4-292µg/kg and AFB_2 between 0.4-27.2µg/kg. OTA was found at concentrations between 0.2 µg/kg and 35.6µg/kg but at higher prevalence than aflatoxins. Co-contamination with AF and OTA was common; thirty seven (37) of the rice samples contained both aflatoxins and ochratoxin A. The AFB_1 and OTA levels in 100% and 58% of the rice samples were regarded as unsafe based on Nigerian and European Union maximum permissible levels of 2µg/kg and 5µg/kg respectively. The presence of these toxins at unacceptable concentrations and their multi-occurrences in the rice samples which might exert either additive or synergistic toxic effects in human beings raise concern with respect to public health.

Keywords: Mycotoxins, Aflatoxin, Ochratoxin A, Rice, Nigeria.

1.0 Introduction

Mycotoxins are toxic secondary metabolites produced by fungi and they contaminate different agricultural commodities before or under post-harvest conditions. They are mainly produced by fungi in the *Aspergillus, Penicillium* and *Fusarium* genera (CAST, 2003). When mycotoxins are ingested, inhaled or absorbed through the skin, they cause lowered performance, sickness or death on humans and animals (CAST, 2003). Exposure to mycotoxins can produce both acute and chronic toxicities ranging from deleterious effects upon the central nervous to death, cardiovascular and pulmonary systems, and upon the alimentary tract (CAST, 2003). Other mycotoxins occurring in food have longer term chronic or cumulative effects on health, including the induction of cancers and immune deficiency (CAST, 2003). Mycotoxins may also be carcinogenic, mutagenic, teratogenic and immunosuppressive. The ability of some mycotoxins to compromise the immune response and, consequently, to reduce resistance to infectious disease is now widely considered to be the most important effect of mycotoxins, particularly in developing countries (Coker, 1997). Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade. It has been estimated by Miller (1993), for example, that annual losses in the USA and Canada, arising from the impact of mycotoxins on the feed and livestock industries, are of the order of $5 billion. In developing countries, where the food staples (e.g. rice, maize and groundnuts) are susceptible to contamination, it is likely that significant additional losses will occur among the human population because of morbidity and premature death associated with the consumption of mycotoxins (Miller, 2003).

Rice is one of the most important staple foods in the world. The world has increased its rice production by 27%, or 155 million tonnes, at the current estimate of 482 million tonnes (723 million tonnes of paddy), world rice production would be 3.4 percent larger than in 2010, reflecting a combination of good weather and attractive prices, which encouraged producers to expand the area under rice by an estimated 2.4 percent to 165 million hectares (FAO, 2011). The increase in world production is anticipated to be concentrated in Asia, where the five top rice producing countries: Bangladesh, China, India, Indonesia and Viet Nam, are all heading towards record output (FAO, 2011). The FAO forecast for production in Africa has changed little since September 2011, remaining in the order of 17.0 million tonnes, which is 2.6 percent more than in 2010. Many western countries are implementing expansionary rice production policies.

In particular, output is set to rise vigorously in Benin, Ghana, Mali, Nigeria and Sierra Leone, amid attractive market prices. Nigeria is the largest rice producing country in the West African region and also the largest importer of rice in the world. Rice production rose gradually over the years with area expansion to surpass major rice producing countries like Cote d Ivoire and Sierra Leone (WARDA, 1996). The principal factors driving increased rice production in Nigeria is population growth and urbanization. (WARDA, 1996). The annual demand for rice in the country is estimated at 5 million tonnes. In Nigeria, The North Central zone is the largest producer of rice accounting for about 47% of the total rice output in 2000. This was followed by Northwest (29%) Northeast (14%) southeast (9%) and the least (the southwest (4%) (Nweke *et al,.*1999). Kaduna state is the largest rice producing state in the country accounting for about 22% of the country's rice output, followed by Niger State (16%), Benue State (10%) and Taraba State (7%). (Nweke *et al,.*1999).

Kaduna State is the main traditional rice growing area in Nigeria with the highest yield (Erenston and Lacon, 2003). Rice is commonly eaten as boiled rice and in the northern parts of the country it is taken as paste "tuwo", fermented breads (‘masa') and as unleavened bread (‘Waina'). The Hausa also use it in preparation of a local snack called "nakiya". Due to the fact that rice is a highly consumed cereal and little has been done on the fungi and mycotoxin contaminating it in Kaduna state, this study was undertaken to determine the level and extent of contamination by ochratoxin A, Aflatoxin B_1 and Aflatoxin B_2 under natural conditions in Kaduna state, a leading rice producer. Many scientists in Nigeria (Okoye, 1992) and from other parts of the world (Taligoola *et al*., 2004) have studied and reported the fungi and mycotoxins contaminating rice but there seem to be little or no reports on the fungal and mycotoxin profile of rice in Kaduna State.

Table 1: Kaduna State Local Government Areas (LGAs) according to microclimatic zones

	Zone	Annual Rainfall Range (mm)	LGAs
1	(wettest)	> 1600	Kachia, Sanga, Kaura, Jama’a, Zango/Jaba
2	(Wet)	1200 – 1600	Kagarko, Birni Gwari, Kaduna North, Kaduna south, Chikun, Saabon- Gari
3	(Dry)	1000 – 1200	Zaria, Ikara, Kudan, Makarfi, Soba, Igabi, Kubaun, Lerea, Giwa
4	(Driest)	<1000	Kauru

2.0 MATERIALS AND METHODS

All chemicals used were of Analar grade and manufactured by May and Baker LTD (Dagenham England unless otherwise stated.). Silica gel 60-120 mesh, petroleum spirit (60-80^{oc}), n -Hexane, Orthophosphoric acid, methanol, sodium sulphate anhydrous, sulphuric acid, sodium hydrogen carbonate, methylene chloride. Mycotoxin standards of aflatoxins (B_1

and B_2) and OTA standards were obtained from Sigma, St. Louis, Mo., USA. HPLC was fitted with ZORBAX Eclipse XDB-C18, 4.6mm X 150mm, 3.5µm column.

2.1 Sampling

Dry sample of rice were randomly collected during the rainy season (April to October) from the twenty two local government areas of Kaduna state (Table1). Stored, marketed and field samples were collected. The field samples were collected shortly before the harvest period, the stored samples were collected from traditional storage facility called rumbu (a locally built mud barns) and the marketed samples were collected from the rice sellers in the various markets. About 1.0 kilograms of each sample were collected, labeled, packaged in a plastic bottle which were properly sealed and taken to the laboratory. In the laboratory, the samples were ground into fine powder with the aid of an electric blender, the powder were stored in the cupboard for mycotoxin analysis.

2.2 Analysis of mycotoxins.

The samples were screened and analyzed for aflatoxin B_1, B_2 and ochratoxin A using a multi-mycotoxin assay method (Ehrlich and Lee, 1984) without modification. In the method, methylene chloride and phosphoric acid were used for the simultaneous extraction of AFB_1, B_2 and OTA. A separate portion of the initial methylene chloride/phosphoric acid extract was subjected to a specific clean-up procedure for each mycotoxin.

2.2.1 Extraction of Mycotoxins

About 50g portion of pulverized rice samples was weighed into 500ml Erlenmeyer flask and 25ml 1M-phosphoric acid and 250ml of methylene chloride were added. The flask was shaken for 30 minutes using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 200ml of the filtrate was collected and from this, 50ml aliquot each was placed in separate 100ml Erlenmeyer flasks with glass stoppers, for AF and OTA assay.

The fractions for aflatoxin analysis were subjected to a specific column chromatographic clean up method. To this end, a column was set up with glass wool, 150ml dichloromethane (DCM) poured into the column and emptied half way. Anhydrous sodium sulphate (Na_2SO_4) was added, the sides of the column were washed with DCM. Silica gel was added to green line of column and 80ml DCM added again, and this was allowed to settle half way. Three scoops of sodium sulphate (Na_2SO_4) was added and drained off to top of column. About 50ml of the filtrate was added and drained off to top of column. The filtrate was defatted with 130ml hexane and 130ml ether sequentially and each fraction drained to dump. Aflatoxins were extracted into 130ml ether: methanol: water (96:3:1) that was collected off column in a new beaker. The extract was evaporated to near dryness, put into vials and stored at 0^oC until used for analysis.

A different clean up method to that of aflatoxin was used for ochratoxin A. Using separatory funnel the toxin was extracted into aqueous sodium bicarbonate solution (4gm $NaHCO_3$/100ml distilled water) which was acidified to pH 2 with H_2SO_4 to obtain an acid fraction. OTA was further extracted from the acid fraction into dichloromethane (by rinsing with 25ml of DCM thrice). The pooled DCM fractions was drained through Na_2SO_4, evaporated and stored in amber glass vials at 0^oC until used for analysis.

2.2.2 High Pressure Liquid Chromatographic Technique

Aflatoxins were analyzed on on Cecil 1100 series HPLC with UV detection as described by Cora *et al*. (2005) at wavelength of 365nm. The altraspher ODS column, 4.6mm x 25cm was used at ambient temperature of 25^oC. Acetonitrile : water and acetic acid in ratio 10:50:40 v/v/v respectively was used as mobile

phase at flow rate of 0.8ml/min. The injection volume was 20 µl.

The analyses were carried out with aflatoxins standards (Sigma Chemical Company, St. Louis, MO, USA) of known concentrations with $AFB_1$and AFB_2 eluting at distinct retention time of 1.673 and 1.524 respectively. Calibration curves with correlation factors of 0.91 and 0.99 were obtained for $AFB_1$and AFB_2, respectively using series of dilutions containing 0.005µg/ml, 0.01µg/ml, 0.015µg/ml, 0.02µg/ml and 0.025µg/ml of each of the standard. The detection limit of the machine with regards to the toxins was 0.21µg/kg. About 10 µg/ml of AFB_1 and AFB_2 were spiked in 3 samples of rice in order to determine the recovery rates. The mean ± standard deviation obtained for the two toxins were 98.5±3.2% and 89.3±2.5% respectively.

OTA was quantified on same HPLC machine with UV detection as described by Engstrom, Richard and Cysewski (1977) at wavelength of 254nm. The operating temperature was ambient temperature of 25^{o}C. Acetonitrile : water and acetic acid in ratio 50:48:2 respectively was used as mobile phase at flow rate 1ml/min. The injection volume was 60 µl. Calibration curve with a correlation factor of 0.925 was determined using series of dilutions containing 0.023 µg/ml, 0.018 µg/ml, 0.014 µg/ml, 0.009 µg/ml and 0.004 µg/ml. The retention time for OTA was 1.11 minutes while the detection limit of the machine with regards to the toxin was 0.1 µg/kg. 10µg/ml of OTA was spiked in 3 samples of each food commodity and recovery rates determined. The Mean ± standard deviation recovery rates obtained for OTA was 99.1±6.1 The observed recoveries indicate that the sensitivity and reliability of the methods employed were sufficient for evaluation of aflatoxins and OTA in rice. The concentrations reported were adjusted based on recovery rates obtained.

3.0 Results

3.1 Mycotoxin Contamination of Rice

Aflatoxin B_1 and B_2 were detected in the samples from all the twenty two local government areas. Eighty six rice samples were analyzed for aflatoxin B_1 and 64 were contaminated with the toxin at concentrations between 4-292µg/kg with a mean value of 66.028µg1kg, Kauru had the highest occurrence of aflatoxin B_1 (157.34µg/kg) and lowest incidence was observed in Giwa. (14.66µg/kg). Similarly, of the 86 rice samples analyzed for Aflatoxin B_2, 41 were contaminated with the toxin at concentrations of between 0.4-27.2µg/kg with a mean value of 5.168µg/kg. The occurrence was highest in Makarfi (27.2µg/kg) with lowest incidences in Giwa and Birnin Gwari..

The results of mycotoxin determination according to the four microclimatic zones of Kaduna State as well as the sample sources are presented on Tables 3 and 4. Aflatoxin B_1 was a common contaminant of rice from all the four microclimatic zones of the state. The mycotoxin contents were significantly higher in samples from the driest part of the State (zone 4) than those from the other zones. There were no significant ($p<0.05$) differences between aflatoxin B_1concentrations in samples from zone I, II and III. Similarly, higher incidence of the toxin was recorded in zone IV (100%) than zones I (75%), II (66.66%) and III (78.13%). The incidence of the mycotoxin was lowest in the field samples (45.45%) when compared with the marketed (70.83%) and stored (69.44%) samples. However, there were no significant differences between the aflatoxin B_1 concentrations of the marketed, stored and field samples.

The rice samples collected were also analyzed for aflatoxin B_2, 41 of the 86 samples analyzed contained the toxin (0.4-27.2 µg/kg). There were no significant differences ($p<0.05$) in both the concentrations and incidence of aflatoxin B_2 present in the 41 positive sample analyzed from the marketed stored and field and from the four zones. The incidence and concentrations of the mycotoxins determined are presented on Table 2. Ochratoxin A was detected in samples from twenty two local government areas of Kaduna state.

Table 2: Summary of the Incidence of Mycotoxins Contamination of Rice in Kaduna State According to the 22 Local Govt. Areas

		OCTRATOXIN A				AFLATOXIN B_1				AFLATOXIN B_2			
S/N	**LGA**	**NSA**	**NPS**	**RANGE**	**MEAN± S.D**	**NSA**	**NPS**	RANGE	MEAN± S.D	NSA	NPS	RANGE	MEAN± S.D
1.	Birni Gwari	3	3	2.4-19.2	11.47±2.45	3	3	8-108	49.34±15.06	3	1	0.8	0.8±0.003
2.	Chikun	6	5	0.4-11.2	4.00± 0.95	6	3	40-80	64.0± 6.11	6	2	1.6-4.8	3.2±0.800
3.	Igabi	7	6	1.6- 4.8	5.80 ±0.59	7	6	20-140	66.66±10.49	7	3	1.6-7.2	374±0.880
4.	Ikara	2	2	1.2- 2.0	2.60 ± 0.70	2	2	20-204	112±46.00	2	2	0.4-2.4	1.4±0.500
5.	Jamaa	6	4	1.6 – 24.4	11.00 ±2.83	6	5	32-116	68.0±7.01	6	3	2.4-22.4	13.6±2.9
6.	Kaduna South	5	4	4.0 – 20.0	11.50 ±1.89	5	4	4-140	80.0±14.17	5	3	4.8-12.0	7.74±1.09
7.	Kaduna North	2	1	0. 2	0.20 ± 0.00	2	1	68	68.0±0.00	2	0	-	-
8.	Kachia	6	6	0.4 – 15.2	7.86 ±1.14	6	4	4-76	49±7.80	6	2	2	0.8-9.6	5.2±2.20
9.	Kagarko	6	5	0.2.- 22.4	6.04 ± 2.09	6	5	4-120	68.0±10.83	6	4	0.8-9.6	3.48±1.02
10.	Kaura	6	4	4.4-16.8	9.20±1.43	6	5	8-76	48.0±6.16	6	4	1.6-4.8	2.8±0.383
11.	Kauru	3	3	10.4-18.0	14.54±1.11	3	3	56-292	157.34±35.07	3	2	4.8-12.8	8.8±2.00
12.	Zangol Jaba	4	4	6.4-35.6	17.10±3.20	4	2	76-160	118±21.00	4	1	3.6	3.6±0.00
13.	Kubaun	2	2	6.4-10.4	8.40 ±1.00	2	2	40-72	56.0±8.00	2	2	0.8-4.4	2.6±0.90
14.	Kudan	2	2	1.6-4.0	2.80±0.60	2	1	48	48.0±0.00	2	1	1.6	1.6±0.00
15.	Lerea	4	4	0.4-14.0	6.10±1.44	4	2	40-76	58.0±9.00	4	2	0.8-2.4	1.6±0.40
16.	Sanga	2	2	0.2-4.8	2.50±1.15	2	2	60-108	84.0±12.00	2	1	14.4	14.4±0.00
17.	Sabon Gari.	5	4	0.4-18.8	7.80±2.22	5	2	60-108	84.0±12.00	5	1	2.4	2.4±0.00
18.	Zaria	7	6	0.2-16.24	11.20±1.16	7	6	4-144	68.0±11.88	7	3	3.6-15.2	7.76±1.86
19.	Makarfi	3	2	7.2-18.8	13.00±2.90	3	3	24-56	44.0±5.03	3	1	27.2	27.2±0.00
20.	Soba	2	2	7.2-11.2	9.20±1.00	2	0	-	-	2	0	-	-
21.	Giwa	3	1	3.2	3.20±0.00	3	3	8-56	14.66±8.62	3	3	0.4-1.6	0.8±0.01
	Total	**86**	**72**	**0.2-35.6**	**8.83±0.43**	**86**	**64**	**4-292**	**66.03±3.08**	**86**	**41**	**0.4-27.2**	**5.17±0.43**

Key: NSA- Number of samples analyzed, NPS- Number of positive samples

Of the 86 rice samples analyzed for OTA, 72 were contaminated with the toxin at concentrations of between 0.2-35.6µg/kg with a mean value of 8.832µg/kg. The occurrence of ochratoxin A. in rice was highest in Zangon Jaba (17.1µg/kg) with lowest incidences observed in Kaduna North (0.2µg/kg). The ochratoxin A concentrations in samples from the four microclimatic zones were not significantly ($p<0.05$) different, however, higher incidence of the toxin was recorded in zone IV (100%) than zones I (83.33), II (81.48) and III (84.38). There were no significant differences between the concentrations of ochratoxin A from the marketed, stored and field samples but the incidence of the mycotoxin was lowest in stored samples (66.67%) when compared with the marketed (81.25%) and field (81.8) samples.

Out of the 86 rice samples analyzed, aflatoxin B_1, and B_2 occurred together in forty five (45) samples, thirty seven (37) samples contained both the aflatoxins and ochratoxin A and 43 samples were contaminated with ochratoxin A alone.

4.0 Discussion

Rice (*oryza sativa*), is highly cultivated and consumed worldwide and this makes it one of the most important principal sources of mycotoxins to human beings and animals in the world. According to data tracked by the Food and Agricultural organization (FAO, 2011), world rice production is expected to increase to 456.2 million tonnes while consumption is expected to rise to 455.2 million tonnes. Aflatoxins and ochratoxin A are among the five most significant and abundant mycotoxins contaminating foods and feed stuffs in the world (Bhat and Vasanthi, 2003), and the results obtained in this study indicate that they are also major contaminants (Ochratoxin A, Aflatoxin B_1 and Aflatoxin B_2 in decreasing order of prevalence) of rice in Kaduna State, Nigeria.

The insignificant differences in incidence and concentrations of toxins observed due to geographical locations and types of samples i.e. field store and market samples might be as results of the seeds' microclimatic and physiological conditions. Aflatoxin B_1,and B_2 incidences and concentrations were higher in stored and marketed samples than field samples because crops are mostly infected with fungi from field due to environmental factors, farming system or insect infestation; these field fungi persist and proliferate with consequence increase in mycotoxin formation during storage when favourable conditions persist (Miller, 1995). This might have led to high incidence of aflatoxins recorded in this work in stored rice samples (store and market) than in field samples.

Although ochratoxin A incidence and contents exhibited a decreasing order from field to market and then store, the decreasing trend could be attributed to increased effectiveness of the traditional storage facilities "rumbu" (in Hausa) against ochratoxin producing fungi (Udoh *et al*., 2000), that are built on raised platforms that prevent rodent and insect attack, moisture from getting to grains and also provide anaerobic conditions within it. Such conditions can reduce fungal growth and consequently mycotoxin production (Javis, 1971). It could also be as a consequence of the pre-storage sun drying of newly harvested grains on dry surfaces rocks (Awuah and Ellis, 2002) by farmers as observed by the researchers during sampling. These processes significantly reduce the fungal and mycotoxin contamination (Hell *et al*., 2000) and might therefore, account for the consistently lower incidence and mycotoxin contents in stored samples compared to those from the field and market. Of the four microclimatic zones, a general higher incidence of toxins was observed in the driest zone (Zone IV). It could be that the stress on the crop due to excessive heat, agricultural management practices such as: irrigation, crop rotation, methods of harvesting in the zone have created unique ecological niches that promote the toxigenic potential of strains of the species of fungi isolated (Bilgrami *et al*. 1981).

Table 3: Summary of the Incidence of Mycotoxins Contamination of Rice in Kaduna State According to Microclimatic Zones

Microclimatic Zone		aflatoxin B_1	Aflatoxin B_2	Ocharatoxin A
Zone I	Mean±S.D	65.56± 4.38 [a]	7.32±1.10 [a]	10.08± 0.99 [a]
		4- 160	0.8-22.4	0.2-35.6
	Range	18/24	11/24	20/27
	nc/ ns			
Zone II	Mean±S.D	68.66±4.38 [ab]	4.26±0.57 [ab]	7.28±0.80 [ab]
		4-140	0.8-12.0	0.2-22.4
	Range	18/27	11/27	22/27
	nc/ns			
Zone III	Mean±S.D	60.96±5.01 [abc]	4.52±0.8[abc]	7.46±0.49 [abc]
		4-204	0.4-27.2	0.4-18.8
	Range	25/32	17/32	27/32
	nc/ns			
Zone IV	Mean±S.D	157.34±35.07 [d]	8.80±1.16 [abcd]	14.54±1.11 [abcd]
		56-292	4.8-12.8	10.4-18
	Range	3/3	3/3	3/3
	nc/ns			

abcd: Means with different letters (superscripts) along column were significantly different from each other ($P<0.05$)

Aflatoxin data found here when compared with those of other studies are in conformity with the findings of Tanaka *et al*. (2007) that mycotoxin contamination is less commonly reported for rice than other crops. Such postulation is based on data reported here and others (Opadokun and Ikeorah, 1979; Ibeh *et al.,* 1991; Obidoa and Gungani, 1992, Ikeorah and Okoye, 2005; Atehnkeng *et al*., 2008), which reveal much lower levels in Nigerian rice (maximum of 174 µg/kg) than other crops especially maize, groundnuts (range: 2.2 to 2000 µg/kg). The higher seed coat integrity of rice seed acts as a barrier against fungal invasion (Stossel, 1986), thus limiting fungal growth and consequent mycotoxin production in rice relative to that of maize, groundnut and others that have less formidable coat and hence are excellent substrates for mycotoxin production.

Aflatoxin B_1 is the most toxic amongst the two aflatoxins studied ($B_1$and B_2), it is an important contaminant of food and feed crops before, during and after harvest (Shananah *et al*., 2003) and it is well establish that it is both carcinogenic and cytotoxic. The findings in this study showed that the rice samples had aflatoxins B_1: 100%, and B_2 : 22.09% and with levels exceeding acceptable limits (2 and 4µg/kg respectively) set by the Nigeria and European Union. The unwholesome quantities of aflatoxins found in the rice samples (Aflatoxin B_1 = 4 - 292µg/kg and B_2 = 0.4 - 27.2µg/kg) which though are all lower than levels (1600 – 12,000 µg/kg) that caused deaths in the two fatal outbreaks of AF poisoning in Kenya (Afla-guard, 2005), could when ingested chronically, synergistically interact with other cancer promoters especially hepatitis B virus to elicit high primary liver cancer incidence observed in Nigeria (Fakunle *et al.* 1977), which has previously been identified as the most common malignant tumour seen in medical wards (Olubuyide *et al*., 1986). It is reported to be the commonest cause of death from cancer in the middle aged (Junid, 1979) and elderly populations (Olubuyide and Solanke, 1990) in the country. Apart from causing liver cancer, continuous intake of AF at low doses could increase still-births and neonatal mortality (Maxwell *et al.*, 1998), immunosuppression with increased susceptibly to infectious diseases such as pneumonia (Oyelami *et al*.1997) and HIV/AIDS (Lane, 2005). Intake of AF has also been associated with stunted growth (Gong *et*

al., 2002, 2003, 2004) and aggravation of protein malnutrition in children (Adhikari *et al*, 1994).

Ochratoxin A contamination of cocoa and cocoa products in Nigeria has been well documented (Bankole and Adebanjo, 2003) but very few reports of its incidence in other crops from the country are available. High level of up to 150 µg/kg of the toxin was detected in maize (Sibanda *et al*., 1997) and mouldy rice (Makun *et al*., 2007 and Makun *et al.*, 2011)) from Northern Nigeria. Ayejuyo et al. (2008) found very low levels of OTA (maximum: 2.18 µg/kg) in samples of imported rice marketed in Lagos metropolis but the range observed in this study was (0.2 - 35.6 µg/kg).

The OTA contents in imported rice were all below the international regulatory limit of 5 µg/kg than those found in the present study (58.02% unsafe), which could be because there must have been compliance to the international regulatory limits at the point of processing, packaging and import. This evaluation of mycotoxins in Nigerian rice gives the quality of the cereal with regards to its acceptability for human and animal consumption. Ochratoxin A is nephrotoxic, teratogenic, carcinogenic and immuno-suppressive in many animal species (Stoev, 1998 and International Agency for Research on Cancer (IARC),1993). The international Agency for Research on Cancer has classified OTA as possibly carcinogenic in humans (group 2B carcinogen) (IARC, 1993). The demonstrated presence of AFs and OTA at concentrations above the limits acceptable to world mycotoxin regulatory agencies and the co-occurrences of toxins with possible toxic synergistic effects make these studied rice samples of low quality for human and animal consumption and in fact raises preliminarily national public health concerns.

With such simultaneous occurrences of unrelated mycotoxins (Rizzo *et al*. 2004) in similar samples, this will certainly increase the severity of health-related problems generated from consumption of such contaminated food products as consumption of multiple mycotoxin in foods may exert both synergistic and additive effects (Placinta *et al*. 1999; Casado *et al*. 2001; Creppy *et al*. 2004; Speijer and Speijer 2004; Luongo *et al*. 2008) in both animal and man.

Sedmikova *et al.*, (2001) reported the possibility of OTA increasing the mutagenic ability of aflatoxin B_1 in cases of simultaneous occurrence of the two mycotoxins in the same crop.

Table: 4. Summary of the Incidence of Mycotoxin Contamination of Rice in Kaduna State According Sample Sources

Sample source		**Aflatoxin B_1**	**Aflatoxin B_2**	**Ocharatoxin A**
Market	Mean±S.D	75.18± 4.39 [a]	5.34±0.72 [a]	8.72± 0.65 [a]
		4-292	0.4-27.2	0.2-35.6
	Range	34/48	20/48	39/48
	nc/ ns			
Store	Mean±S.D	69.92±5.20 [ab]	6.08±0.86 [ab]	7.14±0.56 [ab]
		4-204	0.4-22.4	0.2-15.2
	Range	25/36	16/36	24/36
	nc/ns			
Field	Mean±S.D	42.40±12.48 [abc]	3.54±0.46 [abc]	10.54±1.08 [abc]
		4-140	1.6-6.4	0.4-20.0
	Range	5/11	5/11	9/11
	nc/ns			

abc: Means with different letters (superscripts) along columns were significantly different from each other (P<0.05)

5.0 Conclusion

The demonstrated presence of aflatoxins and ochratoxin A in this highly consumed foodstuff at unsafe levels renders them the problematic mycotoxins in Nigerian rice. Therefore, rice can be regarded as a major source of mycotoxin exposure in Nigeria. In view of the forgoing, it is recommended that studies to elucidate the possible aetiologic roles of AFs and OTA in the increased incidences of liver cancer and nephropathy should be conducted in Nigeria. Regulating these toxins in foods in Nigeria is therefore also an imperative.

REFERENCES

Amadi, J.E. and Adeniyi ,D.O. (2009).Mycotoxin production by fungi isolated from stored grains. *Africa. Journal of Biotechnol*. *8*, 1219-1221.

Atehnkeng, J., Ojiambo, P.S.,Donner, M., Ikotun, K., Sikora, R.A.,Cotty, P.J. and Bandyopadhyay, R. (2008). Distribution and toxicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones of Nigeria. *International Journal of Food Microbiology 122*, 74-84.

Awuah RT, Ellis WO (2002). Effects of some groundnut packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi. *Mycopathologia* 154: 29-26.

Bankole S.A, Adebanjo A (2003). Aflatoxin contamination of dried yam chips marketed in Nigeria. *Tropical. Science*. **43** (3/4).

Bankole, S.A. and Adebanjo, A. (2003).Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. African Journal of Biotechnology. *2*, 254-263.

Bhat, R V, Beedu, S R, Ramakrishna, Y and Munshi, K L (1989) Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir Valley, India. *Lancet* **1**: 35-37.

CAST, (2003). Mycotoxins - risks in plant, animal and human systems, Task Force Report, No. 139. Council for Agricultural Science and Technology, Ames, Iowa. 1-191.

Coker, R D (1997). Mycotoxins and their control: constraints and opportunities. NRI Bulletin 73. Chatham, UK: Natural Resources Institute.

Darling SJ (1963).Research on aflatoxin in groundnuts in Nigeria. Paper No 13. Institute of Agric. Research, Ahmadu Bello University, Samaru, Zaria.

Diener,U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S. and Klich, M.A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annual. Review of Phytopathology. *25*, 249-270.

Engstrom, GW. Richard, J.L and Cysewski, S.J (1977). High Pressure liquid chromatographic method for detection and resolution of rubratoxin, aflatoxin and other mycotoxins *Journal of Agricultural Food Chemistry*, **25,** 833-836

Erenstein,O. and Lancon, F. (2003). The Nigerian rice economy in a competitive world. Constraints, opportunities and strategic choices. Report of the final technical workshop held in IITA on August 20-21, 2003.

FAO (Food Agriculture Organization).(2011) Year End Global Rice Production Report, Plus Rice Production this Decade, by K.M. on November 16th, 2011.

FAO (Food Agriculture Organization). (2008). Paddy rice production (000t), by country and geographical regions: 1961-2007. FAO Statistics Division.

Food Nutrition and Agriculture (FAO), (1991). Food for the Future. FAO 1.

Hell K, Cardwell KF, Setamou M, Poehling HM (2000) The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Production Research*. **36**: 365-382.

Horn, B.W. and Dorner, J.W. (1999). Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of Aspergillus flavus along a transect within the United States. *Applied. Environmental Microbiology*. *65*, 1444-1449.

Jaime-Garcia, R. and Cotty, P.J. (2004). *Aspergillus flavus* in soils and corncobs in south Texas: Implications for management of aflatoxins in corn-cotton rotations. Plant Distribution. *88*, 1366-1371.

Javis B (1971). Factors affecting the production of mycotoxins. *Journal of Applied. Bacteriology.* 34(1):199- 213.

Jones, R.K., Duncan, H.E. and Hamilton, P.B. (1981). Planting date, harvest date and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology 71*, 810-816.

Klich, M.A. (2002). Introduction; economic and medical importance of *Aspergillus*. In *Identification of Common Aspergillus Species* pp. 1-16.

Makun, H. A., Gbodi, T. A., Akanya, H. O., Sakalo, A. E., and Ogbadu, H. G. (2007). Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger state, Nigeria. *African Journal of Biotechnology.* **6**(2): 99-108.

Miller, J. D. (1993). The toxicological significance of mixture of fungal Toxins, In proceedings of the Pan African Newsletter of occupational Health and Safety, Wild, C. P. (Ed). *Institute of Occupational Health, Helsinki,* 32-38

Nesci, A. and Etcheverry, M. (2002). *Aspergillus* section Flavi populations from field maize in Argentina. *Letter of Applied Microbiology*. *34*, 343-348.

Ngala, G.N. (1983). *Sarocladium attenuatum* as one of the causes of rice grain spotting in Nigeria. *Plant Pathology*. *32*, 289-293.

Nweke F I, B.O Ugwu, A.G.O.Dixon, C.L.A. Asadu and O Ajobo (1999), Cassava production in Nigferia: a function of farmer access to markets and to improved production and processing technologies, COSCA working Paper No. 20, International Institute of Tropical Agriculture Ibadan, Nigeria.

Okoye ZSC (1992). An overview of Mycotoxins likely to contaminate Nigerian staple food stuff. A paper presented at the first National Workshop on Mycotoxins held on 29th November, 1990 at University of Jos. Book of proceeding . 9-27

Olubuyide, I.O. and Solanke, T.F. (1990). The causes of death in an elderly African population. *Journal of Tropical.Medicine and Hygiene*. *93*, 270-274.

Ominski, K.H., Marquardt, R.R., Sinah, R.N., Abramson, D., (1994). Ecological aspects of growth and mycotoxin production by storage fungi. In:Miller, J.D., Trenholm, H.L. (Eds.), Mycotoxins in Grain-compounds other than Aflatoxin. Eagan Press, St. Paul, 287-312.

Oyelami OA, Maxwell SM, Adelusola KA, Aladekoma TA, Olyelese AO (1997) Aflatoxins in the lungs of children with

kwashiorkor and children with miscellaneous diseases in Nigeria. *Journal of Toxicology and Environmental Health* 51, 623-628.

Park, J.W., Choi, S.Y.,Hwang, H.J. and Kim, Y.B. (2005). Fungalmycoflora andmycotoxins in Korean polished rice destined for humans. *International Journal of Food and Microbiology*. *103*, 305-314.

Pitt, J.I. and Hocking, A.D. (1997). Primary keys and miscellaneous fungi. In *Fungi and Food Spoilage*, 2nd Ed. (••ed.) pp. 59-171, Blackie Academic and Professional, London,

U.K.,Weinheim, Germany,NewYork,NY, Tokyo, Japan, Melbourne,Australia,Madras, India.

Reddy, K.R.N., Reddy, C.S., Mangala,U.N. and Muralidharan, K. (2006). Site of infection of *Aspergillus* sp. in seeds of rice cultivars. *Journal of Mycology and Plant Pathology*. *36*, 271-277.

Sedmikova, M., Reisnerora, H., Dufkova, Z., Burta,I.and Jilek, F. (2001). Potential hazard of simultaneous occurrence of aflatoxins B1 and ochratoxin A, *Veterinary Medicine* **46** :169-174.

Sibanda L, Marovatsanga LT, Pestka JJ (1997) Review of mycotoxin work in sub-Saharan Africa. *Food Control* 8:21-29

Stoev,S.D (1998).The role of ochratoxin A as a possible cause of Balkan Endemic Nephropathy and its risk evaluation, *Journal of Veterinary and Human Toxicology* 40: 352-360.

Sudakin, D.L. (2003) Trichothecenes in the environment: Relevance to human health. *Toxicological Letter*. **143**: 97-107.

Taligoola, H. K., Ismail, M. A., and Chebon, S. K. (2004). Mycobiota associated with rice grains marketed in Uganda. *Journal of Biological Science.* **4**(3): 271-278.

Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H., and Kushiro, M. (2007). Mycotoxins in rice. *International Journal of Food Microbiology.* **119**: 59-66.

Udagawa, S. (1976). Distribution of mycotoxin-producing fungi in foods and soil from New Guinea and Southeast Asia. *Proceedings of Japanese Association.Mycotoxicology*. 2: 10-15.

Uraguchi, K. and Yamazaki, M. (1978). Toxicology: Biochemistry and Pathology of Mycotoxins, Halsted Press, Japan pp. 1-278,

USDA. (2008). Import of milled rice by country and geographical region: 1961-2008. http://beta.irri.org/solutions/images/ stories/wrs/wrs_jul08_2009_table10_usda_import.xl.

Warda (1996) Rice trends in sub Saharan Africa: A synthesis of statistics on rice production, trade and consumption (1973 - 1992), West Africa Rice Development Association, Bouake, Cote d' Ivoire

Zinedine, A., Soriano, J.M.,Molto, J.C. and Mañes, J.(2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenicmycotoxin. Food Chemistry and Toxicology. *45*, 1-18.