Incidence of aflatoxins, fumonisins, trichothecenes and ochratoxins in Nigerian foods and possible intervention strategies

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Abstract

Mycotoxins are toxic secondary metabolites produced by some species of fungi. Aflatoxins, fumonisins, trichothecenes and ochratoxins are the common mycotoxins in Nigeria. Aflatoxin is the most frequently reported in literatures, with trichothecenes being the least, they cause yield loss to farmers as well as constituting major health risk to humans. The occurrence of mycotoxins in food is a serious problem that Nigeria is facing presently, as it continues to pose threat to feed and food safety of animals and humans. There is the need to seek for approaches that would lead to reduction in their toxicity. The practice of good sanitary measures right from the farm to storage, creation of awareness campaign to indicate the toxic effects associated with mycotoxin poisonings in humans and livestock, and proper evaluation of food crops for its presence can go a long way in achieving the target reduction in incidence of mycotoxins in Nigeria.

1.0   Introduction

Mycotoxins are secondary metabolites of fungal origin which produce toxic responses when ingested by animals or humans. The word 'mycotoxin' is a combination of a Greek word, 'mykes' meaning 'fungus' and a Latin word 'toxicum' meaning 'by poison' (Rai *et al.,* 2012). Mycotoxicosis is a term used to denote the diseases that result from the ingestion of mycotoxin by animals and humans (Nierman *et al*., 2008). The acuteness of a specific mycotoxicosis may vary in different victims. Various factors determine the severity of a mycotoxin; among them are the level of toxicity, dosage, age, nutritional and immunity status of the victim (Peraica *et al.,* 1999).

The general attraction to the study of mycotoxins started in 1960 when more than 100,000 turkeys died in London, England after consuming contaminated groundnut meal that was imported from Brazil (Blount, 1961). The disease that resulted was called 'Turkey X' disease. *Aspergillus flavus* was later isolated from the groundnut meal and the toxic agents named 'aflatoxin' (*A. flavus* toxin) accordingly (Splensley, 1963; Kensler *et al*., 2011). The word 'mycotoxin' was then coined in 1962 after the Turkey X incident.

Most mycotoxins of concern are produced by three genera of fungi, namely, *Aspergillus*, *Penicillium*, and *Fusarium.* These are the predominant fungal genera associated with food grains during storage (CAST, 2003). These genera are considered significant because of their ubiquity in the environment and their ability to produce various types of mycotoxins like aflatoxins, fumonisins and ochratoxins (Juan *et al*., 2010). However, the presence of these fungi does not necessarily imply the presence of toxins.

The mycotoxigenic fungi can be classified as either field or storage fungi. It should be noted that a fungus may produce several mycotoxins and a mycotoxin may be produced by different fungi (Fernandez-Cruz *et al*., 2010). There are intrinsic and extrinsic factors which influence the production of mycotoxins. The intrinsic factors include pH, water activity (a_w) and redox potential of the substrate while the extrinsic factors are the relative humidity, temperature and oxygen availability of the environment where the commodities are produced, stored or processed (Whitlow and Hagler, 2004; Nagwa *et al*., 2013).

Over 400 types of mycotoxins have been reported (Bhat *et al*., 2010), but only four (aflatoxins, fumonisins, trichothecenes and ochratoxins) will be discussed in this review. While the other mycotoxins are important for studies, aflatoxins, fumonisins, trichothecenes and ochratoxins are of greater interests because of their prevalence in agricultural produce and the harmful effects they exert when they are consumed by animals and humans (Richard, 2007).

 The Food and Agriculture Organization (FAO) estimated that about 25% of the world's agricultural produce is contaminated with mycotoxins which cause huge losses for farmers (Wu, 2007). These losses are measured in reduced crop yields, lower quality, reduced animal performance and reproductive capabilities, and increased disease incidence.

The biochemical properties of mycotoxins are diverse, and their toxic effects are exceedingly variable. Mycotoxins are carcinogenic, tremorogenic, haemorrhagic, genotoxic, teratogenic, nephrotoxic, hepatotoxic and

immunotoxic (Refai, 1988; Hosseini and Bagheri, 2012). Mycotoxins usually enter the body via ingestion of contaminated foods, but inhalation of toxigenic spores and direct dermal contact are also important routes (Omar, 2013).

Nigeria enjoys the humid tropical climate type and experiences consistent high temperatures all year round. The tropical climate is characterized by the hot and wet conditions associated with the movement of the Inter-Tropical Convergence Zone (ITCZ) north and south of the equator. This produces two seasons: the rainy or wet season that lasts from mid-March to November in the southern parts of the country and from May to October in the northern parts of the country; and the dry season that fills up the rest of the year (Oyenuga, 1967).

 Iloeje (2001) classified Nigerian agroecological zones into two broad categories: forest and savannah zones. Forest is the ecological zone in which trees are the dominant species while savannah is a grassland area with no forest cover. The important agroecological zones for mycotoxin studies in Nigeria are humid forest, derived savannah and Guinea savannah zones**.** The humid forest zone is characterized by two growing seasons, starting from April to November with an annual rainfall of 1,500 mm and 2,000 mm, average annual temperature of 24.5 to 27.5°C, and mean relative humidity of 78 to 100% (Afolabi *et al.,* 2013). Cash crops such as oil palm, cocoa and kolanut, and staple crops such as maize, rice, beans and groundnut are found in this region. Derived Savannah is the transition between the rainforest and guinea savannah zones. It has an annual rainfall range of 1, 200mm to 1, 700 mm, average annual relative humidity range of 66 to 78% and mean temperatures of 26 to 27 °C; maize, cassava, yam and rice are the common crops grown in this region. The Guinea Savannah is the most extensive ecological zone in Nigeria, occupying almost half the area of the country. The zone is sometimes subdivided into the Southern and Northern Guinea savannah. It is has a wet season which lasts from May to October, with an annual rainfall of 1, 000mm to 1, 200 mm, relative humidity of 57 to 66%, and average annual temperature of 25.5 to 26.5 °C (Afolabi *et al*., 2013). Crops in areas like this are more prone to contamination with mycotoxin-producing fungi because of the high temperature and humidity (Sherif *et al.,* 2009).

In Nigeria, mycotoxin contamination of cereals (rice), grains (maize) and seeds (cocoa) has raised a lot of concern for food safety (Kumar *et al*., 2008) as these foods, especially rice and maize are not only eaten directly, but are also used in the production of various forms of indegenous foods like *ogi*, *eko*, *tuwo*, *kunu*, *donkwa*, and *masa* among others. Bankole and Adebanjo (2003) noted that the mycotoxin that has received the most attention in scientific literature in food products from West African sub-region is aflatoxin, while there are few researches carried out on other mycotoxins like fumonisin and ochratoxin A. However, there have been some recent studies on the mycotoxins present in food products from Nigeria, especially maize, rice, groundnuts, guinea corn, sorghum, cocoa and cocoa-based beverages (Adejumo *et al*., 2007a, 2007b; Ayejuyo *et al*., 2008; Dongo *et al*., 2008; Amadi and Adeniyi, 2009; Makun *et al*., 2010; Olayiwola *et al*., 2013).

2.0   Mycotoxins
2.1   Aflatoxin
Aflato**x**ins are a group of toxic and carcinogenic secondary metabolites of fungal origin. They are produced by strains of *Aspergillus flavus*, *A. parasiticus* and, in rare cases, *A. nominus* and *A. pseudotamari* (Mazaheri, 2009). Among the 18 different types of aflatoxins identified the four naturally occurring ones are Aflatoxin B_1 (AFB$_1$), Aflatoxin B_2 (AFB$_2$), Aflatoxin G_1 (AFG$_1$) and Aflatoxin G_2 (AFG$_2$) (Filazi and Sireli, 2013). Aflatoxin B_1 and B_2 were named because of the strong blue fluorescence under ultraviolet light, hence the 'B', while Aflatoxin G_1 and G_2 are so named because of their greenish yellow fluorescence (Kensler *et al*., 2011). The subscript designates relative chromatographic mobility during thin-layer chromatography, '1' and '2' indicate major and minor compounds respectively (Zain, 2011).

Apart from the four major aflatoxins, there are two other metabolites, designated as M_1 and M_2. Aflatoxin M_1 and M_2 are metabolites of Aflatoxin B_1 and B_2 respectively and they may be present in milk, eggs or blood of animals that are fed with AFB$_1$ and AFB$_2$-contaminated feed (IARC, 2002; CAST, 2003). Toxigenic *A. flavus* generally only produces AFB$_1$ and AFB$_2$ while
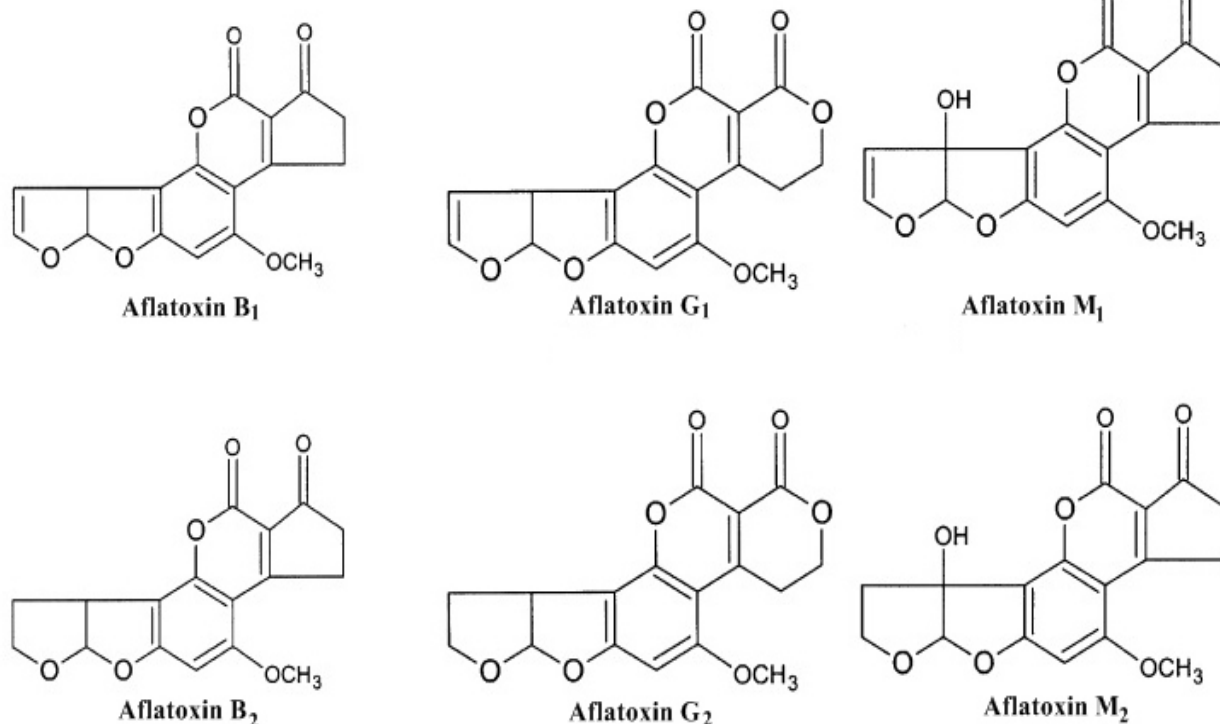
Figure 1. Chemical structures of AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2 (Zain, 2011).

A. parasiticus (the most toxigenic species) produce AFB_1, AFB_2 AFG_1 and AFG_2 (Davis and Diener, 1983; Van Egmond, 1989). The order of toxicity in these aflatoxins is

$AFB_1>AFG_1>AFB_2>AFG_2$; AFB_2 and AFG_2 are typically nontoxic except they are metabolized into AFB_1 and AFG_1 inside the cells (Kensler *et al*., 2011; Filazi and Sireli, 2013). AFB_1 is always genotoxic *in vitro* and *in vivo* (EFSA, 2007).

Aflatoxins are among the most powerful carcinogenic, mutagenic and teratogenic compounds that occur naturally (Jackson and Al-Taher, 2008). In view of this, the International Agency for Research on Cancer (IARC) classified aflatoxin as a Class I human carcinogen (IARC, 1993).
Aflatoxicosis is the disease associated with the ingestion of aflatoxins. Outbreaks of aflatoxicosis have been reported in Kenya, India and Thailand, but none has been reported so far in Nigeria. The outbreak which occurred in Kenya is one of the largest and most acute outbreaks of aflatoxicosis ever documented (CAST, 2003), with 317 cases reported and 125 deaths recorded (CDC, 2004). Investigations revealed that the outbreak was due to the consumption of maize contaminated with extremely toxic AFB_1. The case-control studies carried out by Lewis *et al*. (2005) showed that aflatoxicosis in the affected area was associated with eating home-grown maize stored under humid conditions. During the outbreak, individual daily exposure of AFB_1 was estimated to be 50mg/day (Probst *et al.*, 2007).
Bankole and Adebanjo (2003b) reported that many studies on aflatoxin had been carried out in West Africa. This includes investigation to know the levels of aflatoxin in rice, maize, groundnuts, guinea corn, sorghum and cocoa, as well as impacts of aflatoxin on human beings. McDonald (1964) reported that groundnuts cultivated in the northern parts of Nigeria were contaminated with aflatoxin levels of up to 2000µg/kg. Salifu (1981) studied fungal and mycotoxin contamination of sorghum in Nigeria, and reported that aflatoxin levels ranged from 10 to 80 µg/kg[-1]. In a recent report by Ezekiel *et al*. (2013), about 90% (26/29) of peanut cake samples had aflatoxin concentrations exceeding the maximum limit of 20 mg/kg set by USDA (FAO, 2004) (Table 1).

2.1.1   Health Risk of Aflatoxins to Humans and Animals
The primary disease associated with the ingestion of aflatoxins is hepatocellular carcinoma (liver cancer) (Williams *et al*., 2004), which according to WHO (2008) is the third-leading cause of cancer worldwide, with about 600,000 fresh cases each year. Aflatoxin contamination has been shown to reduce feed intake, increase liver and kidney weights of farm animals, as well as induce immunosuppression and hepatitis in them; all of which contribute to increased mortality in farm animals (Hussein and Brussel, 2001; Zain, 2011).

Smith *et al.* (1992) reported that feeding broilers with a combined high level (3.5mg/kg) feed of aflatoxin blend (AFB$_1$: 79%; AFB$_2$: 4%; AFG$_1$: 16% and AFG$_2$: 1%) caused a reduction in body weight, and an increase in weights of liver and kidney. In cattle, increasing aflatoxin to levels such as 10, 26, 56.4, 81.1 and 108.5µg/kg has been shown to reduce feed intake depending on the dosage (Choudhary *et al.*, 1998). The quality of milk produced by these cattle is also affected by the aflatoxins (Zain, 2011).

Report on aflatoxin-related human health problems in Nigeria is limited. Aflatoxin M$_1$ has been reported in breast milk of lactating mothers in Ogun State, with 85% of the breast milk screened

Table 1. Incidence of Aflatoxin-contamination of Common Nigerian Foods

Commodities	Region	Type of Aflatoxin	Incidence Rate	Range µg/kg	References
Rice	Niger State, Nigeria	AFB1 AFB$_2$ AFG$_1$ AFG$_2$ Total AF	100% 21/21	4.1-30.9 1.3 – 24.2 5.5-76.8 3.6-44.4 28-372	Makun et al., 2011.
Dried Yam Chips	South-western Nigeria	AFB$_1$ AFG$_1$ AFB$_2$	54.2% 5.2% 32.3%	4-186 4-18 2-55	Bankole and Adebanjo, 2003a.
White Yam Flour	South-western Nigeria	AFB$_1$ AFG$_1$	57% 21%	0.02-3.2 0.05-3.5	Somorin et al., 2012
Water Yam Flour	South-western Nigeria	AFB$_1$	32%	0.02-0.6	Somorin et al., 2012
Kulikuli	Oyo State, Nigeria.	AFB$_1$	90% (18/20) 100% (29/29)	20-455 13-2824	Akano and Atanda, 1990; Ezekiel et al., 2012.
Dried Mushrooms	Lagos State, Nigeria.	ND*			Ezekiel et al., 2013.
Tiger nuts	Nigeria		35%	10-120	Bankole and Eseigbe, 1996
Beans Cassava flour Semovita Yam Wheat meal "Gari"	Ogun State, Nigeria	AFB$_1$		nd-0.89 nd-0.07 nd-0.17 nd-0.27 nd-0.06 nd-0.69	Adejumo et al., 2013

Table 1. Incidence of Aflatoxin-contamination of Common Nigerian Foods Contd.

Commodities	Region	Type of Aflatoxin	Incidence Rate	Range µg/kg	References
Maize	Niger State, Nigeria	AFB$_1$ AFB$_2$		0-1874 0-608	Atehnkeng et al., 2008
Palm oil	Ibadan, Nigeria	AFB$_1$	ND*	ND*	Olorunfemi et al., 2014
Wheat **Millet** **Guinea corn** **Breadfruit** **Groundnut**	Niger Delta Region, Nigeria.	AFB$_1$	100% 100% 100% 100% 100%	17.01-20.53 34.00– 40.30 27.22-36.13 40.06-48.59 74.03-82.12	Odoemelam and Osu, 2009
Yam chips	Ibadan, Oyo State.	AFB$_1$	97.5%	11-196	Abiala et al., 2011

Fresh milk / Nono / Kindirmo	Bida, Niger State	AFM_l	100% 100% 100%	0.407-0.952µg/l 0.248-2.510µg/l 0.139-1.238µg/l	Okeke et al., 2012
Powdered milk	Lagos, Lagos State	AFM_1	19%	0.02 - 0.41µg/l	Makun et al., 2010
Beans / Wheat	Minna, Niger State	AFB_1	58% 54%	63.5 - 106.2 102.9 - 198.4	Makun et al., 2010
Breast milk	Ogun State	AFM_1	85%	3.49-35ng/l	Adejumo et al., 2013

nd – Not Determined

*ND – Not Detected

contaminated with AFM_1 (3.49-35 ng/l) and 16% of the breast milk samples exceeding the European Union (EU) limit of 25 ng/l (Adejumo *et al.,* 2013).

Uriah *et al.* (2001) reported that the blood and semen in infertile men attending the University of Benin Teaching Hospital had aflatoxin levels which ranged from 700 to 1392ng/ml and 60 to l48ng/ml respectively and these values were significantly higher than the concentrations of the toxin in fertile men. Twenty-two out of the 60 samples (37%) of serum and semen of infertile men had aflatoxin B_1 ranging from 60ng/ml to 148ng/ml. Although, their studies indicated that aflatoxins might have a negative effect on fertility, more studies are needed to ascertain the universality of this connection.

Aflatoxins were reported in the autopsy kidney specimens of 58% of children with kwashiorkor at Obafemi Awolowo University Teaching Hospital, Ile-Ife (Oyelami *et al.*, 1998). In a similar study by Onyemelukwe *et al.* (2012), aflatoxins were found in both the sera and the blood of children with kwashiorkor. Aflatoxins were present in the sera of 88.9% of the children with a range of 5.3 to 7646µg/l, and in the urine of 84.6% of the children with a range of 15 to 361µg/l. The study data indicate that higher aflatoxins were more frequently detected in sera and urine of patients with kwashiorkor, as compared to the healthy control within a similar age-group (Hendrickse, 1983).

2.2   Fumonisins

Fumonisins are fungal metabolites produced by various Fusarium species (Senyuva *et al*., 2010), primarily by *Fusarium verticillioides,* formerly *F. moniliforme* (Seifert *et al.,* 2003)*,* and *Fusarium proliferatum,* which are global contaminants of maize and maize products (Adejumo *et al*., 2007a; Somorin *et al*., 2012). Recently, it was discovered that fumonisins are also produced by *Aspergillus niger* (Soares *et al*., 2013). The fumonisins were first isolated from cultures of *F. moniliforme* strain MRC at the South African Medical Research Council by Gelderblom *et al.* (1988). The structures of Fumonisins B_1 and B_2 (Figure 2) were first elucidated by Bezuidenhout *et al.* (1988).

Fumonisins, primarily FB_1, FB_2 and FB_3, are afluorescent cancer-promoting metabolites that have a long-chain hydrocarbon unit which is similar to that of sphingosine and sphinganine, which plays a role in their toxicity (Marin *et al.,* 2013). Maize is the major commodity affected by fumonisins, although some occurrences have been reported in sorghum and rice (Hosseini and Bagheri, 2012). Fumonisins are formed in maize prior to harvest or during the early stage of storage. Except under extreme conditions, their concentrations do not increase during storage (Marin *et al*., 2013).
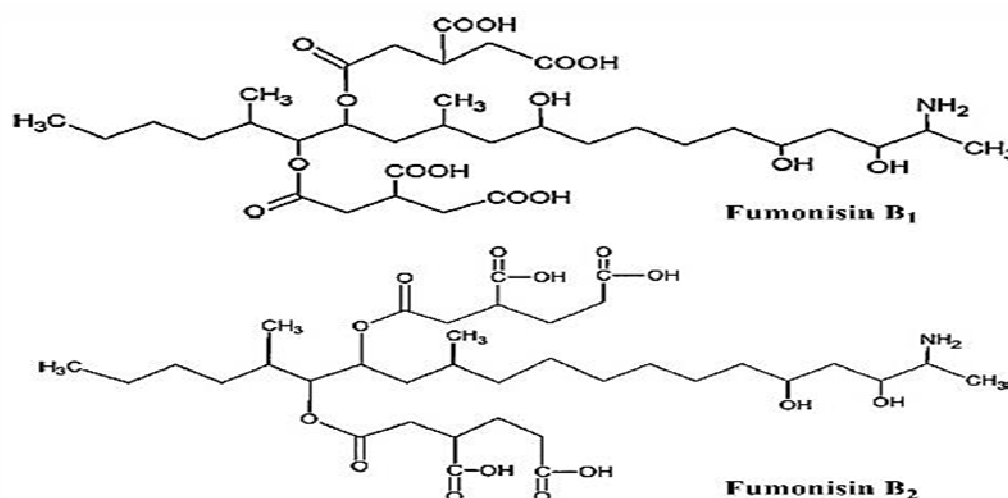
Figure 2. Chemical Structures of Fumonisin B_1 and B_2 (Zain, 2011; Marin *et al*., 2013)

Among fumonisins, FB_1 is the most toxic, and has been proven to stimulate tumour growth in rats, cause leukoencephalomalacia in horses, pulmonary oedema in pigs and brain haemorrhage in rabbits (Gelderblom *et al*., 1988; Marasas, 1995; Hussein and Brasel, 2001). They have also been linked with high incidence of human oesophageal cancer in some regions of South Africa and China (Sydenham *et al*., 1990; Sun *et al*., 2007).

Missmer *et al.* (2006) reported that fumonisin exposure increases the risk of neural tube defects (with respect to dose) up to a threshold level, at which point foetal death may be more likely to occur. In Nigeria, Bankole *et al*. (2003) detected fumonisin B_1 in 51% (55 out of 104 samples) of maize samples analysed with concentration range of 65 to 1830µg/kg, and mean value of 390µg/kg (Table 2). In a similar study on the natural occurrence of fumonisins in pre-harvest maize in south western Nigeria, Bankole and Mabekoje (2004) reported *F. verticillioides* occurring in 89.3% of samples. Fumonisin B_1 was reported to be the predominant toxin (frequency at 76.8%, concentration between 70 and 1780µg/kg, and mean of 495µg/kg), while Fumonisin B_2 was detected in 66% of the samples with a mean of 114 µg/kg.

Avantaggio *et al*. (2002) reported that insect damage was a good predictor of *Fusarium* mycotoxin contamination. The insect damage to maturing maize ears allows the strains of *Fusarium* to enter the ears and kernels. *Fusarium* species are always present in the ears and kernels of maize and fumonisins have even been shown to be present in symptomless kernels of maize in Nigeria (Thomas and Buddenhagen, 1980). Adejumo *et al*. (2007b) reported FB_1 in 73% of maize samples from four South-western states of Nigeria (mean117µg/kg; range 10 to 760µg/kg). In a later study, the authors also investigated 80 maize samples collected from South-western Nigeria for fumonisin B_1 content (Adejumo *et al.,* 2009), and reported FB_1 in 55 (68.7%) of the total samples (range: 10 to 126.7 µg/kg; mean: 98.5 µg/kg). Somorin *et al*. (2012) reported that low levels of fumonisins were detected in yam flours which were being used in making *amala* in south western Nigeria, FB_1 was in 32% of white yam flour samples (range: 0.5 to 90 µg/kg; mean 5 µg/kg), and 5% of water yam samples (range 0.5 to 2µg/kg). Makun *et al*. (2011) reported FB_1 and FB_2 at frequencies of 14.3% and 4.8% respectively in rice samples from Niger State.

Most of these occurrences of fumonisins in Nigerian commodities are still within the acceptable limit of <1000 µg/kg for fumonisins (CAST, 2003), although there is still the need for frequent monitoring of foods for fumonisin contamination.

2.3   Trichothecenes

Trichothecenes are a group of structurally related compounds with a common tetracyclic sesquiterpenoid 12, 13-epoxytrichothec-9-ene ring system. The trichothecenes are chemically the most diverse of all the mycotoxins with over 200 identified (Grove, 2007). Some trichothecenes are among the most toxic non-nitrogenous compounds known to man. They are powerful inhibitors of eukaryotic protein synthesis, insecticidal, phytotoxic, and toxic to animals (Grove, 2007).

Table 2. Incidence of fumonisin-contamination of common Nigerian foods and feeds

| Commodity	Region	Type	Incidence	Range (µg/kg)	Mean (µg/kg)	References
Rice	Niger state	FB1 FB2	14.3% 4.8%	0.4-4.4 132.5–132.5		Makun et al., 2011
White yam flour	South western Nigeria	FB1 FB2	32% 12%	0.5-91 1-32	5 ND	Somorin et al., 2012
Water Yam Flour	South western Nigeria	FB1	5%	0.5-2		Somorin et al., 2012
Maize	South western Nigeria	FB1 FB_1 FB_1	51% 73% 68.7%	65-1830 10-760 10-714	390 117 98.5	Bankole et al., 2003 Adejumo et al., 2007b Adejumo et al., 2009
Maize (Pre-harvest)	South western, Nigeria	FB_1 FB_2	78.6% 66%	70-1780 70-1780	495 114	Bankole and Mabekoje, 2004
Dried Meat (Tinko)	Ibadan, Nigeria	FB_1 FB_2	10% 10%	0-1.91 0-1.04	ND ND	Oladejo and Adebajo, 2011
Animal feed	Nigeria	FB_1 FB_2	89% 89%	20-2860 20-855	1092 338	Rodrigues et al., 2011

ND - Not Determined

The trichothecenes are grouped into four types (A, B, C and D), named according to their characteristic functional groups with types A and B trichothecenes being the most common (Krska *et al.,* 2007). Examples of type A trichothecenes are HT-2 toxin (HT2), T-2 toxin (T2) 4,15-diacetoxyscirpenol, 15-monoacetoxyscirpenol, T-2 triol, T-2 tetraol, neosolaniol, 4,15-diacetylverrucarol, and verrucarol; while examples of type B are deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, nivalenol (NIV) and fusarenon-X (FUS-X) (Krska *et al.*, 2007;

Deoxynivalenol (Type B)   **Diacetoxyscirpenol**
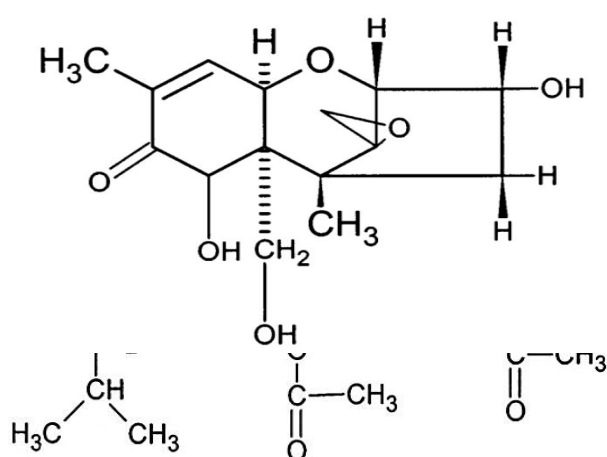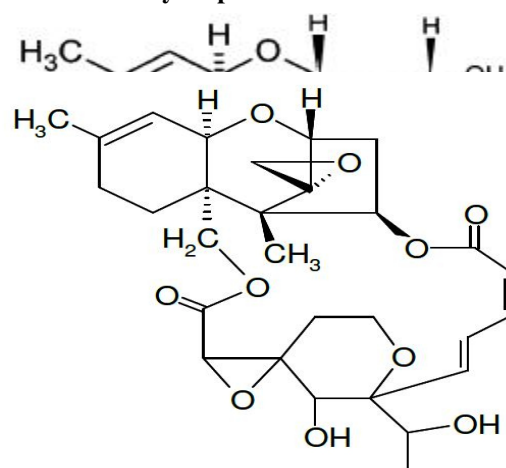
T-2 Toxin (Type A)   **Satratoxin G (Type D)**

Figure 3. Chemical structures of deoxynivalenol, diacetoxyscirpenol, T-2 toxin and satratoxin G (Hussein and Brasel, 2001; Gottschalk et al., 2009)

Gottschalk *et al.*, 2009; Marin *et al.*, 2013). The C trichothecenes include crotocin and baccharin, while type D trichothecenes are verrucarin, satratoxin G and H, and roridin A (Sudakin, 2003). However, type C and D trichothecenes are of lesser importance (Marin *et al.*, 2013).

The simple type A and B trichothecenes are produced by a variety of different *Fusarium* species (Thrane, 2001), although a number of other genera including *Trichoderma*, *Stachybotrys*, *Myrothecium*, and *Trichothecium* are also known to produce them (Sweeny and Dobson, 1998). However, the most important T-2 and HT-2-producing species are *F. sporotrichioides, F. langsethiae, F. acuminatum,* and *F. poae*. The main producers of DON are *F. graminearum, F. culmorum, and F. cerealis* (Liddell, 2003; Marin *et al.*, 2013). Type D trichothecenes are

Table 3. Trichothecenes-producing fungi in food commodities

Trichothecenes	Fungi	Reference
Type A and B trichothecenes	*Fusarium* species, *Trichoderma*, *Stachybotrys*, *Myrothecium* and *Trichothecium.*	Sweeny and Dobson, 1998; Thrane, 2001
T-2 Toxin (Type A)	*F. sporotrichioides, F. langsethiae, F. acuminatum* and *F. poae.*	Liddell, 2003; Marin *et al.*, 2013
HT-2 (Type A)	*F. sporotrichioides, F. langsethiae, F. acuminatum* and *F. poae.*	Liddell, 2003; Marin *et al.*, 2013
DON (Type B)	*F. graminearum, F. culmorum* and *F. cerealis.*	Liddell, 2003; Marin *et al.*, 2013
Roridin A and verrucarin A (Type D)	*Myrothecium* spp.	Ueno, 1983
Satratoxin G and H (Type D)	*Stachybotrys* spp.	Ueno, 1983

metabolites of *Myrothecium* spp. (e.g. roridin A, verrucarin A) and *Stachybotrys* spp. (e.g. satratoxin G and H) (Ueno, 1983).

There is paucity of information on contamination of Nigerian commodities trichothecenes. A few notable studies include the one conducted by Bankole *et al.* (2010) between 2005 and 2006 on Nigerian maize, with a reported 15-monoacetoxyscirpenol (15MAS) in one out of 32 samples at concentration of 4 µg/kg and T-2 teraol in two out of 32 samples at levels of 73 and 280 µg/kg. The authors concluded from the study that trichothecenes toxins do not appear to be major contaminants of Nigerian maize. However, Adejumo *et al.* (2007a) reported the screening of 180 maize samples meant for human consumption for twelve major trichothecenes (deoxynivalenol (DON), 3, mono-acetyldeoxynivalenol 93-AcDON), 15, mono-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), HT-2 toxin (HT-2), neosolaniol (NEO), T-2 toxin (T-2), T-2 tetraol and T-2 triol, diacetoxyscirpenol (DAS), MAS-monoacetoxyscirpenol (MAS) and fusarenone-X), and showed that sixty-six (36.3%) samples were contaminated with trichothecenes -DON, 3-AcDON, and DAS at different ranges. Authors of the report claimed that it was the first comprehensive report of the natural occurrence of trichothecenes in maize for direct human consumption in Nigeria.

A similar study was conducted on rice by Makun *et al.* (2011) who detected DON in 23.8% of rice sampled from Niger State, at a mean concentration of 18.9µg/kg and range of 0 to 112.2µg/kg. The reports on maize by Adejumo *et al.* (2007a) and Bankole *et al.* (2010) were followed up by Afolabi *et al.* (2013), who reported the detection of T-2 toxin in 36% of total maize samples; 29µg/kg, 13µg/kg and 12µg/kg were the maximum, mean and median values for T-2 toxin respectively.

2.4   Ochratoxins

Ochratoxins are toxic fungal metabolites produced by various *Aspergillus* species, *Penicillium verrucosum* and *P. nordicum* (Frisvad *et al.*, 2004). They are named after *A. ochraceus* from which the compound was first isolated (van der Merwe et al., 1965). Several ochratoxins occur in nature, namely Ochratoxin A (OTA), Ochratoxin B (dechlorinated OTA), and Ochratoxin C (ethylated OTA) which are often co-produced (Reddy and Bhoola, 2010). However, OTA is the most toxic and most prevalent member of the ochratoxins (Marin *et al.*, 2013). Ochratoxin A is nephropathic (Kumar *et al.*, 2008), immunosuppressive, teratogenic, genotoxic, hepatotoxic and

Table 4. Incidence of Trichothecene Contamination of Nigerian food commodities

Commodity	Region	Type	Frequency	Mean and Range	Reference
Maize	Nigeria	15 MAS T-2 teraol	1/32 2/32	Mean:4µg/kg 73 µg/kg and 280µg/kg	Bankole et al., 2010
Maize	Southwestern Nigeria	DON 3-AcDON DAS	22% 17% 9%	Mean: 226.2µg/kg Range: 9.6-745.1µg/kg Mean: 17.3µg/kg Range: 0.7-72.4µg/kg Mean: 16.0µg/kg Range: 1.0-51.0µg/kg	Adejumo et al., 2007a
Maize	Nigeria	T-2	36%	Mean:13µg/kg Range:0-29µg/kg	Afolabi et al., 2013
Rice	Niger State.	DON T-2	23.8% 4.76%	Mean: 18.9µg/kg Range:0-112µg/kg ND	Makun et al., 2011
Processed Cocoa samples	South western Nigeria.	DON	59%	Mean:3.3µg/kg Range:0.2-8.5µg/kg	Egbuta et al., 2013
Cocoa-Based Powder Beverage	South western Nigeria.	DON	32%	Mean:2.2µg/kg Range:0.1-7.6µg/kg	Egbuta et al., 2013
Animal feed	Nigeria	DON NIV AcDON	56% 2% 4%	Mean: 181 µg/kg Range: 50- 451 µg/kg Mean: 4 µg/kg Range: 50-186 µg/kg Mean: 9 µg/kg Range: 50-239 µg/kg	Rodrigues et al., 2011

ND – Not Determined

mutagenic (Qi *et al*., 2014). Presently, there is no conclusive evidence of its carcinogenicity in human beings, but there is sufficient evidence in experimental animals. In view of this, the International Agency for Research on Cancer (IARC) has classified OTA in Group 2B as a possible carcinogen to humans (IARC, 1993).

OTA is found as natural contaminants of many foodstuffs including cereals, dried fruits, cocoa and cocoa products, cassava flour, grains, wine, poultry eggs, and milk (Bankole and Adebanjo, 2003b; Belli *et al.,* 2004; Gollucke *et al.,* 2004; Dongo *et al.,* 2008). These toxins are generally associated with grains stored in temperate climates of Europe and North America and it is not considered a major problem under Nigeria's tropical climate, although it has been reported in some food products (Adebajo, 1993; Steyn, 1995; Bankole and Adebajo, 2003b). The origin of OTA in warm temperate and tropical zones is commonly associated with *A. ochraceus* and the black Aspergilli (Accensi *et al.,* 2004). OTA production by *A. ochraceus* is usually at a temperature ranging from $12^{o}C$ to $37^{o}C$ with an optimum of $31^{o}C$ and water activity level of 0.80 (Adebajo *et al*., 1994).

Ochratoxin A is fairly stable to heat; in cereal products, up to 35% of the toxin survives autoclaving for up to 3 hours (IARC, 1976), hence OTA is not easily destroyed by common food preparation methods (Boudra *et al*., 1995). The European Food Safety Authority (EFSA) has, on a request from European Commission (EC) derived a tolerable weekly intake (TWI) of 120ng/kg body weight (EFSA, 2006).

High frequency of OTA (66.7%) was reported in rice from Niger State, with a mean concentration of 141.9µg/kg (Makun *et al.,* 2011). The occurrence of OTA in the rice samples exceeded the maximum acceptable levels (CAST, 2003; EC 2006) in cereals for human consumption. Co-occurrence of OTA with AFB_1 has been reported to increase the mutagenicity of the latter (Sedmikova, *et al*., 2001). Dried mushroom (Ezekiel *et al*., 2013) and yam flour (Somorin *et al*., 2012) from Nigeria were screened for the presence of Ochratoxin A, but they were not detected in any of the samples. This shows that OTA is not a likely contaminant of these food products. It has been observed that whenever OTA is detected in high levels in human breast milk, AFB_1 is absent or present at very low levels and vice versa. This relationship has also been reported in peanut cakes (Banu and Muthumary, 2008). These suggest some kind of competition between the toxins at either the production level in the substrate or their rate of absorption in the human gastrointestinal tract (Zain, 2011).
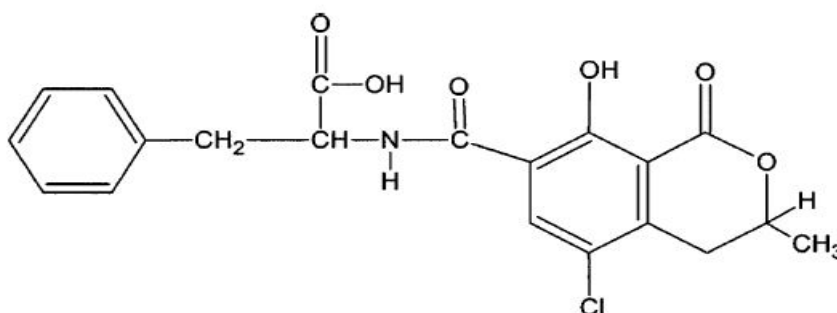
Figure 5. Chemical Structure of Ochratoxin A (Zain, 2011)

3.0 Intervention Strategies

It is important to know that complete elimination of mycotoxin-contaminated products/commodities is not feasible. In view of this, good agricultural practices (GAP) represents a primary line of defense against contamination of cereals with mycotoxins, followed by implementation of good manufacturing practices (GMP) during the handling, storage, processing, and distribution of cereals for human food and animal feed (Codex Alimentarius Commission, 2003; Atanda *et al.*, 2013). In addition, employing food safety practices like Hazard Analysis and Critical Control Point (HACCP) system can be useful in preventing and reducing mycotoxin contamination of agricultural produce (Adegoke and Letuma, 2013). Many intervention strategies to the problem of mycotoxins abound, out of which some of them itemized below can be found suitable to Nigeria's situation.

3.1 Good Agricultural Practice

3.1.1 Crop Rotation

A crop rotation routine should be developed and sustained to ensure that the same crop is not planted in the same field for two consecutive years. Crops that have been identified to be susceptible to toxigenic fungi should not be used in rotation with each other. For example, wheat and maize have been identified to be susceptible to toxigenic *Fusarium* spp, and should not be used in rotation with each other (CAC, 2003; Munkvold, 2003).

Table 5. **Incidence of Ochratoxin in Nigerian foods**

Commodities	Region	Type	Incidence Rate	Range µg/kg	Mean (µg/kg)	References
Tiger nuts	Nigeria	OTA	44.4%	10-80		Adebajo, 1993
Rice	Niger State, Nigeria	OTA	66.7%	0-341.3	141.7	Makun et al., 2011.
Cocoa seeds extract	South western Nigeria	OTA	88.2%	0.7-525.4	88.8	Egbuta et al., 2013.
Cocoa based powder beverage	South western Nigeria	OTA	56%	0.3-884.8	80.8	Egbuta et al., 2013.
Cocoa beans	Cross River, Edo and Ondo States	OTA	90%	1.0-277.5	37.7	Dongo et al., 2008
Cocoa beans	South	OTA	34.4%	0.05-	0.31	Aroyeun et al.,

	western Nigeria			2.70ng/ml		2007.
Peanut cake	Five Nigerian states	OTA	13.8%	-	4.0	Ezekiel et al., 2012.
'Tinko'	Ibadan, Nigeria	OTA	13.3%	0-2.13	ND	Oladejo and Adebayo (2011).

Table 5. Incidence of Ochratoxin in Nigerian foods contd.

Commodities	Region	Type	Incidence Rate	Range µg/kg	Mean (µg/kg)	References
White Kolanut	Ibadan, Oyo State	OTA	96%	13.9-65.3	30.2	Dongo et al., 2007.
Red Kolanut	Ibadan, Oyo State	OTA	100%	0.8–19.1	11.3	Dongo et al., 2007.
Poultry feeds	South western, Nigeria	OTA	98%	2.0-14.2	11.22	Ezekiel et al., 2011.
Maize	Minna, Niger State	OTA	98.2%	0-139.2	-	Makun et al., 2013.
Millet				10.20- 46.57		
Sorghum				0-29.50		
Sesame				1.90-15.66		
Acha				1.38-23.90		
Garri				3.28-22.75		

ND – Not Determined

3.1.2   Sanitation
Basic sanitation measures such as removal and destruction of debris from previous harvest would help in minimizing infection and infestation of produce in the field. Cleaning of the stores before loading new produce has been shown to be correlated with reduced aflatoxin levels (Hell *et al*., 2000).

3.1.3   Insect Management
The level of insect damage influences the extent of mycotoxins contamination (Bankole and Adebanjo, 2003b). Insect damage of maize has been found to be good predictor of *Fusarium* mycotoxins contamination (Avantaggio *et al.*, 2002). Insects carry spores of mycotoxigenic fungi from plant surfaces to the interior of the stalk, kernels or create infection wounds through their feeding habits, and promote fungal growth (Munkvold, 2003; Ariyo *et al*., 2013). Therefore, proper management of insect pests through any appropriate control strategy would reduce mycotoxins contamination.

3.1.4   Proper Irrigation and Fertilization

Proper irrigation and fertilization should be carried out to reduce plants' stress which is sometimes responsible for mycotoxin development. The need to apply fertilizer and other soil conditioners to assure adequate soil pH and plant nutrition is critical (CAC RC, 2003; Munkvold, 2003).

3.1.5   Early harvesting

Early harvesting reduces fungal infection of crops in the field, as well as contamination of the harvested produce. In Nigeria, unpredictable weather, lack of storage space, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate times (Amyot, 1983). Rachaputi *et al.* (2002) reported that early harvesting and threshing of groundnuts resulted in lower aflatoxin levels and higher gross returns of 27% than in delayed harvesting.

3.1.6   Proper drying

Rapid drying of agricultural products to low moisture level is critical as it creates less favourable conditions for fungal growth and proliferation, insect infestation and helps produce keep longer (Lanyasunya *et al.*, 2005). In order to reduce or prevent the production of most mycotoxins, drying should take place soon after harvest, and as rapidly as possible. The critical water content for safe storage corresponds to water activity (a_w) of about 0.7. Maintenance of feeds below 0.7 a_w is an effective technique used throughout the world for controlling fungal spoilage and mycotoxin production in foods (Ariyo *et al.*, 2013). A community-based intervention trial in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in mean aflatoxin levels in intervention villages (Turner *et al.*, 2005). During storage, transportation and marketing, maintenance of low moisture levels should be maintained by avoiding leaking roofs and condensation arising from inadequate ventilation (Bankole and Adebanjo, 2003b).

3.1.7   Physical Separation

Physical treatments implemented through sorting, winnowing, washing, crushing and de-hulling are effective in reducing mycotoxin concentration according to Fandohan *et al.* (2005).

3.2   Biological Control

Biological control of mycotoxins may be a product of many factors which include competition for space and nutrients, production of anti-mycotoxigenic metabolites by co-existing microorganisms, changes in pH of the substratum, or a combination of these factors (Bianchini and Bullerman, 2010). In the presence of cell free supernatant of *Bacillus pumilus*, Munibubazi and Bullerman (1998) reported that 98% inhibition in aflatoxin production due to *A. parasiticus* and 53% inhibition of mould growth.

One of the biocontrol management strategies that had been developed is that which make use of competitive exclusion mechanism. The principle is utilized by the field application of atoxigenic strains that outcompete the toxigenic ones upon introduction, thereby shifting the fungal population from toxigenic to atoxigenic. Dorner and Cole (2002) reported a field application of non-toxigenic strains of *A. flavus* and *A. parasiticus* that reduced post-harvest aflatoxin contamination by 95.9%. This control method has also been successfully used in the USA and Nigeria as claimed by the authors (Atanda *et al*., 2013, Bandyopadhyay and Cotty, 2013).

In Nigeria, the International Institute of Tropical Agriculture (IITA) has pioneered this technique with the production of Aflasafe[TM]. There are single strain and multistrain kinds of this product, with the multistrain Aflasafe[TM] having the advantage of displaying both immediate and long term efficacy in various environments (Probst *et al*., 2011). The multistrain Aflasafe[TM] contains a mixture of four native atoxigenic strains specifically targeted for a particular country or agroecosystem. The authors reported 50-99% reduction in aflatoxin contamination in maize field trials (Bandyopadhyay and Cotty, 2013).

3.3   Chemical Control

The application of fungicides and insecticides controls transmission of spores of mycotoxigenic fungi, fungal growth and minimize insect infestation of crops (Munkvold, 2003). However, poor education background of farmers often leads to misuse of pesticides. Hundreds of people have died in Nigeria as a result of consumption of cowpea treated with inappropriate pesticides (Bankole and Adebanjo, 2003b). In 2008, 120 students of Government Girls' Secondary School, Dome, Gombe State were rushed to the hospital after consuming a meal of beans that was suspected to have been preserved with poisonous chemicals, 10 of the students were reported to be in critical condition (Gwary *et al.*, 2012). Analysis of the cooked and uncooked samples of the beans revealed that they contained high levels of Lindane, a banned pesticide (NAFDAC, 2004). In 2011, it was reported that 112 people were hospitalized in Calabar and 2 children confirmed dead, while 20 people were hospitalized in Oshogbo and 10 people died after eating a bean delicacy (Gbadeyan, 2010). Analyses revealed that the beans became poisonous because more pesticide was added than normal. In view of this growing

concern for food safety and potential health and environmental hazards associated with these chemicals, their use is being discouraged (Zain, 2011). Adejumo (2012) tested five botanicals against the maize mycotoxigenic fungus, *Fusarium verticillioides* and reported that *Piper guineense, Garcinia kola* and *Aframomum melegueta* had the potentials to be successfully used as sustainable alternative biopesticides, as a result of their cheap price, effectiveness, availability and environmental-friendliness.

3.4   Breeding for Resistance

This is one of the most promising intervention strategies to mycotoxin menace in Nigeria. Munkvold (2003) reported that sources of resistance to *A. flavus* and *Fusarium* spp., particularly *F. verticillioides* had been identified and had already been incorporated into public and private breeding programs. Genetic resistance is another approach. The genes for resistance to *Aspergillus* and *Fusarium* have been identified in cereals and peanuts, with prototypes of these crops containing genes encoding fungal growth inhibitors for reducing fungal infection. Scientists at United States Department of Agriculture have identified two maize lines that are resistant to *A. flavus* and *F. verticillioides* (Hamiton, 2000).

3.5   Detoxification and Decontamination

Detoxification involves the systemic degradation of mycotoxins into less toxic products. It was reported by Duvick *et al*. (2006) that the fungus *Expoliata spinnifera* was able to grow on fumonisin B_1 as a sole of carbon source (The hydrolysis of fumonisin B_1 yields free tricarboxylic acid and aminopental, the intermediate aminopentol undergo oxidative deamination). In similar circumstances, *Agrobacterium-Rhizobium* strain E3-39 isolated from soil samples effectively converted deoxynivalenol into 3- keto-deoxynivalenol (which is less toxic) under aerobic conditions (Shima *et al*., 1997). Zhou *et al*. (2008) isolated a bacterium strain Barpee from soil samples enhanced with *F. graminearum* infected maize. The strain showed strong DON transforming activity, transforming DON into two major products: a stereo-isomer of DON and 3-keto-DON, under aerobic conditions.

Another detoxification technique that is currently under investigation is the use of enterosorption, which is based on the introduction of harmless phyllosilicate clay (Hydrated Sodium Calcium Alumino Silicates (HASCAS)), which is widely used as an anticaking agent in animal feed, into animal diets. (Phillips, 1997). HSCAS is capable of tightly and selectively absorbing aflatoxins *in vitro* and *in vivo* (Bankole and Adebanjo, 2003). There are different adsorption agents and their efficacies in preventing mycotoxicosis vary. In enterosorption, there is a risk that non-specific adsorption agents may prevent uptake of micronutrients from the food (Zain, 2011).
Decontamination as an intervention strategy seeks to reduce or eliminate contamination by mycotoxigenic fungi. The essential oils and aqueous extracts of *Aframomum danielli* have been reported to reduce ochratoxin A by between 64% and 95% in spiked cocoa powder. Although, ochratoxin molecule is stable, the authors stated that 40 to 90% of OTA got destroyed during roasting of coffee beans (Aroyeun and Adegoke, 2007).
Irradiation is also a useful tool in inactivating some mycotoxins. Herzallah *et al*. (2008) showed that exposure to sunlight (solar radiation), γ-radiation (^{60}Co), and microwave heating were efficient in decontaminating aflatoxin residues in poultry feeds. The effect of irradiation depends on the fungus, application dose, moisture content, composition of food and storage conditions (Kabak and Var, 2005; Aziz *et al*., 2006). There is a theoretical risk, however, of mycotoxins increasing after irradiation. One theory is that the higher radio-resistance of moulds and yeasts compared with bacteria results in a loss of competitive inhibition of mould and yeast growth. Any mould surviving under treatment with irradiation may be expected to grow more rapidly in the absence of competitors and eventually dominate the mycoflora (Temur and Tiryaki, 2013), although their potential to produce mycotoxins is the major concern.

3.6   Modification of Diet

One of the factors that impair food safety in Africa is the reliance on a single crop, especially cereals such as maize (Adebayo *et al.,* 2010). With high daily intake of the cereal, only moderate mycotoxin contamination levels are required to exceed recommended tolerable intake, thereby predisposing people to mycotoxicosis (Bryden, 2007). Bandyopadhyay *et al.* (2007) reported that Nigerian maize was more heavily colonised by aflatoxigenic strains of *Aspergillus* spp. than sorghum and millet, with corresponding higher aflatoxin levels. Consumption of risky foods such as maize was then discouraged by the authors in favour of less risky ones such as sorghum and millet.

3.7   Education and Awareness Creation

Due to the fact that the risks posed by mycotoxins are largely known to the scientific researchers and not to the general populace (Bankole and Adebanjo, 2003b), there is the need to properly inform people, especially farmers, on the economic importance of mycotoxins and the dangers they pose to human health. Due to limited time and resources, the entire populace cannot be realistically reached; people who are trained and given

orientation about mycotoxins should therefore be advised to train others by sharing their knowledge with them. This effectively creates an advantageous ripple effect. The national bodies and societies should sensitize people on mycotoxins. For instance, the National Agency for Food and Drug Administration and Control (NAFDAC) could partner with a society like Mycotoxicology Society of Nigeria (MYCOTOXSON) on ways to educate the general public on mycotoxins. This can be carried out on media outlets such as television and radio stations, newspapers and on internet blogs and social media. WHO (2006) reported that field projects, strengthening surveillance and awareness raising and educating consumers on matters related to mycotoxins were part of WHO's agenda for Africa.

3.8   Legislation

In controlling mycotoxin contamination of food and feeds, certain legislation needs to be put in place. In most countries, regulations are established to control the contaminants in foodstuffs to protect human health. These regulations may include specific maximum limits for several contaminants for different foods and a reference to the sampling methods and performance criteria of analysis to be used (Kubo, 2012). In the Codex Alimentarius Standard, aflatoxin limits are 0.05µg/l for milk and milk products, 4 to 5µg/kg for beans, 10µg/kg in nuts such as peanuts and almonds, and 20µg/kg for cereals. The acceptable limit for fumonisins is <1000 µg/kg (CAST, 2003). These limits are adopted in Nigeria. The EU set a limit of 0.5µg/kg OTA in raw cereals, with a tolerable weekly intake (TWI) of 0.12µg/kg, while maximum DON limits are set between 200µg/kg for processed cereal-based foods and baby foods for infants and young children, and 1750µg/kg for unprocessed wheat, oats and maize (EC, 2006). However, there is presently no maximum tolerable limit for trichothecenes in Nigeria. The responsible authority for these regulations in Nigeria is the National Agency for Food and Drug Administration and Control (NAFDAC). Official regulations concerning maximum acceptable limits for mycotoxins in Nigerian foods are urgently needed.

In addition to the intervention strategies discussed above, Atanda *et al.* (2013) suggested the following methods for the control of the hazards posed by mycotoxins in Nigeria: This include collection of a database of predominant fungi and mycotoxin in Nigeria, establishment of a mycotoxin occurrence map to know the areas prone to mycotoxin contamination and the establishment of a permanent culture collection media.

4.0   CONCLUSION

A considerable number of studies have been done on the problems of mycotoxins in Nigeria and the ways by which they can be solved or managed. Some techniques for management of mycotoxin contamination may be in usual practice in developed countries, in order to reduce or eliminate contamination of agricultural products meant for export and local use, and to protect Nigerians from the harmful effects that can result from consuming mycotoxin-contaminated food, we can adopt some of the methods described in this review. It is very important to properly educate Nigerian farmers on the implementation of good methods of cultivation and storage of agricultural products that would not promote fungal contamination. This review has shown that there are food products in Nigeria with mycotoxin levels above the maximum acceptable limits discussed earlier. Unfortunately, majority of the populace do not know the inherent dangers of consuming mouldy products (with possible contamination by mycotoxigenic fungi) because of their lack of knowledge on mycotoxin. In view of this, the general public needs to be educated on the economic and health hazards posed by mycotoxins. Control measures such as physical selection, proper washing and cooking practices of food commodities should be emphasized. There is a large pool of information on aflatoxins and ochratoxin A contamination in food commodities, but few on other major mycotoxins (fumonisins, trichothecenes and zearalenone) occurring naturally in food commodities. It is therefore advised that wider range of agricultural produce and food commodities be screened for all major mycotoxins to improve food safety in Nigeria.

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