IMPROVING PUBLIC HEALTH AND INTERNATIONAL TRADE THROUGH MYCOTOXIN CONTROL

By

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INTRODUCTION

Fungi are ubiquitous plant pathogens and are major spoilage agents of foods and feedstuffs. They grow on many foods and feed. The optimal condition for growth differs for several fungi, nevertheless, it has been reported by Ominiski *et al* (1994) that most fungi that are toxigenic do better at temperature between 24 ^{o}C - 28 ^{o}C and moisture content of the substrate above 17.5%. Their best substrates are those that provide oil and carbohydrate as an energy source, and proteins, minerals etc. The infection of plants by various fungi not only results in reduction in crop yield and quality, with significant economic losses, but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins. The ingestion of such mycotoxin-contaminated grains by animals and human beings has enormous public health significance, because these toxins can cause diseases in man and animals (Bhat and Vasanthi 2003).

There are over three hundred mycotoxins found in foods, but those that have the most impact on agriculture and public health and consequently economy are aflatoxins (AFs), trichothecenes [e.g. deoxynivalenol (DON), T-2 toxin], fumonisins (FBs), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA) (CAST, 2003). The AFs, especially, AFB_1, are potent hepatotoxins and hepatocarcinogens, which were implicated in the death of 215 people in Kenya who consumed highly AF-contaminated maize meals in 2004 (Makun *et al.*, 2012). Trichothecenes are a group of about 150 related compounds that are protein inhibitors with consequent immunosuppressive effects, causing severe damage to the digestive tract and death due to intestinal haemorrhage. The commonest trichothecenes are DON and T-2 toxin (CAST, 2003). Fumonisins, especially fumonisin B_1 (FB_1) cause liver and kidney cancer, and neural tube defects in rodents, leukoencephalomalacia in horses and pulmonary oedema in (Marasas *et al.*. 1988). Of major concern is the association of FB_1 with elevated incidence of human oesphageal cancer in parts of South Africa, North Eastern Iran and China, upper gastrointestinal tract cancer in Northern Italy (Dutton, 1996) and neural tube defects in human babies (Hendricks, 1999). ZEA, an oestrogenic toxin that causes infertility in animals, is associated with outbreaks of precocious pubertal changes in children in Puerto Rico, and has been suggested to have a possible involvement in human cervical cancer (Zinedine *et al*., 2007). OTA causes kidney and liver impairment in animals (especially pigs) and man (Stoev and Stefan, 2013).

Other emerging mycotoxins that are currently having global attention as reviewed by Njobeh *et al.* (2010) include patulin, moniliformin, penicillic acid, cyclopiazonic acid, ergot alkaloids, sterigmatocystin, ergot alkaloids, citrinin, *Alternaria* toxins and rubratoxins. The IARC (1987) classified sterigmatocystin as group 2B, which means it is carcinogenic in other species and is possibly carcinogenic to humans. Ergot alkaloids may cause strange hallucinations, the feeling of itchy and burning skin, gangrene, loss of hands and feet, and even death. Moniliformin causes cardiac permeability in young rats and ducklings, suggesting a mechanism for inducing Keshan disease in humans. Patulin elicits nausea, vomiting and gastrointestinal disturbances in human being and is classified by the International Agency for Research on Cancer in category 3 as a not classifiable toxic compound regarding its carcinogenicity to humans (IARC, 1993). Citrinin is a nephrotoxin while Cyclopiazonic acid (CPA) causes focal necrosis in most vertebrate inner organ in high concentrations and affects the ducts or organs originating from ducts (Huang *et al*., 2014).

Penicillic acid may cause hepatotoxicity, mutagenotoxicity, genotoxicity, in mice while alternaria mycotoxins affect adversely the liver and kidney and may be a factor in the aetiology of oesophageal cancer in Linxian, China (Liu *et al*. 1992). Rubratoxins are hepatotoxic mycotoxin found in cereals they have been responsible for outbreak of toxicosis in the U.S (Farlex, 2012).

Historical Background

Although the involvement of fungi and their toxins in causing disease to man and animals dates to the period when the Dead Sea Scrolls were written (Richard, 2007), it seems the evidence for their historic occurrence and impact were not obvious until the Middle Ages, when ergot alkaloids poisoning outbreaks in Europe were responsible for the death of thousands of people. Ergotism, also known as Saint Anthony's Fire, a disease that had its origin in the ingestion of rye and other grains infested with the mould, *Claviceps purpurea* was the first known mycotoxicosis that killed tens of thousands of people in Europe for over 300 years between A.D 900 and 1300. The last epidemics of ergotism were in 1825. However, serious outbreaks did occur in Russia in 1926-7 and in England in 1928; in France in 1951 and Ethiopia in the 1970's resulting in nearly 50 deaths. The disease caused losing of extremities; fingers and limbs.". Subsequently, between 1940s and 1950s a lethal human disease caused by *Fusarium* toxins and referred to as 'Alimentary Toxic Aleukia' was reported in Russia (Smith and Moss, 1985). Similarly, in 1938 Japan, *Penicillium* species were responsible for the colouring of rice that erratically led to the fatal human cardiac syndrome called 'yellow rice disease' (Uraguchi and Yamazaki, 1978). The livestock industry was also affected since 1822, the New Zealand sheep industry was devastated by facial eczema, a fungal infection caused by *Pithomyces chartarum.* Other deadly animal syndromes arising from fungal infections and termed differently as equine leukoencephalomalacia (1930s to 1970s in USA), stachybotryotoxicosis (1930s in USSR), red mould diseases (1945-1947 in Japan), and red clover disease, vulvovaginitis and mouldy corn toxicosis (1920s to 1950s in USA) plagued the world (Gbodi and Nwude, 1988). Despite these grave episodes, little attention was paid to fungal diseases. However, in 1960, when the Turkey X disease killed thousands of poultry animals in Britain (Blount, 1965); the world became fully aware of the potential hazard of mycotoxins and responded to the disaster by a systematic and multidisciplinary approach which led to the discovery of aflatoxins. Following the discovery of aflatoxins, at least three hundred mycotoxins have been shown to occur in nature. But those that pose the greatest risk to human and animal health are

aflatoxins (AFs), trichothecenes (deoxynivalenol and T-2 toxin), fumonisins, zearalenone, patulin and ochratoxin A (CAST, 2003) and of these AFs are the most notorious in the food trade because of their high prevalence and severity of health impact and are therefore the most studied.

Prevention and Control of Mycotoxins

The health hazards of mycotoxins translate to food insecurity and financial burden on the national health sector and this is complicated by rejection of export food commodities from international trade which further adversely affect national economy. For these reasons, there are recommended preharvest and postharvest mycotoxin intervention strategies. Preharvest measures include planting fungi resistant cultivars on appropriate soil types and tillage method that reduces fungi inoculum (Jouany *et al*., 2007). Crop rotation with non-host crops like beets, vegetable interrupts the production of infectious crop debris and survival chance of *Fungi* inoculum for the next season crop. Sowing should be done on dates such that anthesis coincides with the time of release of spore (Eeckhout *et al.*, 2013). Biocontrol techniques which aim at outcompeting toxigenic strains and inhibiting mycotoxins synthesis are effective (IARC, 2015). Management strategies that improve plant nutrition, health and therefore resistance to diseases including fungal infestation such as appropriate use of fertilizer, irrigation, weed and other pesticide control are recommended. Harvesting should be done at maturity and in low moisture conditions (Jouany, 2007) using appropriate harvesting equipment that will result in minimal damage of grains as damaged grains allow for increased fungal colonization and mycotoxin synthesis is recommended (CAC, 2016).

Postharvest strategies like removing diseased and damaged kernels before and after storage. Storage under constant low temperature 15^{o}C and "safe" moisture level of <14% profoundly reduce fungi species in agricultural produce (Jouany, 2007). Thermal treatment, irradiation, chemical decontamination and bio-decontamination using microorganisms or enzymes and absorption of mycotoxins in gastrointestinal tract by absorbents are effective in removing mycotoxins from contaminated commodities (EFSA, 2009). The adoption of the principles of Hazard Analysis Critical Control Point (HACCP) into mycotoxin control scheme has led to an integrated approach. The integrated mycotoxin management system, which looks through the farm value chain and identify all critical points where control can be implemented, has generated better results. While all these methods of prevention, reduction and detoxification of mycotoxins can

significantly deplete mycotoxins in foods and feeds, it should be borne in mind that absolute elimination of these toxins from the value chain is impossible.

OUR CONTRIBUTIONS TO MYCOTOXIN MITIGATION

Our contribution to the prevention and control of these menaces to mankind is in identifying and measuring the extent of exposure and risks associated to the presence of mycotoxins in Nigerian food system, and invariably deriving intervention strategies against the food borne hazards.

Risk Assessment of Human Exposure to Mycotoxins in Nigeria

Risk assessment is a scientifically based process consisting of hazard identification, hazard characterization, exposure assessment and risk characterization. The process requires that the hazard that is identified and whose nature of toxicity characterized on dose-response basis must have adverse health effect. Exposure assessment will therefore be the amount of the toxin taken via our foods while risk characterization on the other hand is the qualitative or quantitative estimation of the possibility of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification and characterization and exposure assessment.

Hazard Identification

In accordance with risk assessment principles, we isolated and identified fungi from our crops (Table 1 and 2). The toxins secreted by representative isolates of the fungi were injected into mice to demonstrate their ability to be harmful to mice and therefore human beings. The mice were observed for signs of toxicity for 14 days. The toxicity of the extract was arbitrarily classified into four categories: very toxic (If all of the three extract treated mice died), moderately toxic (two of the three of the extract treated mice died), mildly toxic (If the one of the three mice was killed) and Non – toxic (If none of the three extract treated mice died) (Makun *et al*, 2010). Using this method, we identified the toxic fungi contaminating maize, rice or sorghum. Toxicity screening of fungi isolated from maize revealed that of the 30 isolates screened, 19 produced toxic metabolites (Makun *et al.*, 2010). Of these toxic nineteen isolates, 6 were very toxic, 4 were moderately toxic while 9 were mildly toxic. Of the nine isolates that produced mildly toxic metabolites, six were *Aspergillus* species and the other three were *Fusarium* spp. and *Mucor* spp. Two *Aspergillus* spp,

one each of *Rhizopus* and *Fusarium* Species constituted the moderately toxic isolates. Of the six very toxic isolates, three were *Aspergillus* Species, two *Penicillium* spp and one *Fusarium* spp. Toxicity screening results of fungal isolates from millet samples of the wet season showed that 15 of the 22 screened isolates produced toxic metabolites. The *Helminthosporium* spp. were highly toxic (4.5%) while the *A. niger, Penicillium spp* and *R. stolonifer* were moderately toxic (18.2%). The ten fungal isolates that produced mildly toxic metabolites were *Aspergillus flavus, A. fumigatus, A. glaucus, A. parasiticus, Fusarium* spp., *F. equiseti, F. trincintum* and *Syncephalastrum* spp. Seven isolates were found to be non-toxic (31.8%) and they include two isolates of *Mucor* and *Penicillium* spp., and one each of *Phoma* spp., *R. stolonifer* and *Syncephalastrum* spp (Makun *et al*., 2010).

One hundred and forty-eight fungal isolates from both guinea corn (67) and rice (81) were tested for toxicity (Tables 1 and 2) (Makun *et al*., 2009). Of all these, 95 were found to produce toxic metabolites that were lethal to mice and these were *Aspergillus* spp (41), *Fusarium* spp (14), *Penicillium* spp (10), *Trichoderma* spp (8), *Syncephalastrum* spp (4), three each of *Alternaria* spp, *Phoma* spp and *Curvularia lunata.* Others include two each of *Colletotrichum* spp, *Geotrichum candidum* and *Helminthosporium* spp, and one each of *Cladosporium werneckil, Cryptococcus neoformis* and *Mucor* spp. A few of the fungi which have not been known to produce mycotoxins were found to be toxigenic. For example, *Syncephalastrum* spp isolates from both guinea corn and rice were found to be moderately and mildly toxic.

Following the demonstration that these fungi contaminating Nigerian crops produce toxic metabolites, we attempted to identify the mycotoxins they produce. Makun *et al*. (2011) showed that all strains of *A. flavus* (aflatoxins B_1 and B_2), *A. parasiticus* (aflatoxins B_1, B_2, G_1 and G_2), *A. ochraceus* (ochratoxin A), *F. proliferatum* and *F. verticillioides* (fumonisins B_1 and B_2) isolated from rice, were excellent producers of their respective mycotoxins. Patulin was produced by *A. terreus*, whereas deoxynivalenol, zearalenone and T-2 toxin were produced by *F. chlamydosporum* and other *Fusarium* spp. Garba, (2017) will subsequently prove that aflatoxins B_1, B_2, G_1 and G_2 are elaborated by *A. flavus* and *A. parasiticus*, ochratoxins by *A. ochraceus, A. niger, A. carbonarius, A. oryzae, Neosartorya fischeri, Sterocleista ornate, Emericella quadrilineate* and *Pencillium verrucosum,* fumonisin B_1 by *F. oxysporum, F. verticillioides, F. proliferatum, F.*

chlamydosporum, F. poae, F.acuminatum, zearalenone by *F. oxysporum, F. verticillioides, F. proliferatum,* and *F. graminearum* and deoxynivalenol by *F. verticillioides, F. graminearum* and *F. poae.*

In summary, the hazards we identified in Nigerian foods and feeds are fungi some of which secrete toxins that are lethal to mice, and the five agriculturally significant mycotoxins, aflatoxins, fumonisins, ochratoxins, zearalenones and deoxynivalenol are major toxins secreted by these fungi in our foods as shown in below. 91 fungi species most of which were lethal to mice and produce aflatoxins, ochratoxins, fumonisins, zearalenone and deoxynivalenol were isolated from 2133 samples of Nigerian food crops and south African animal feeds. Conservatively, only 411 (19.3%) samples were safe for human and animal consumption. Simultaneous occurrence of mycotoxins in same sample will further reduce the number of safe samples.

Hazard Characterization

The next logical step in risk assessment is to evaluate the dose response nature of the adverse effects of the fungi and their mycotoxins. Therefore 13 of the very toxic fungi isolated from guinea corn and rice (Makun 2009a) were further subjected to acute toxicity testing at doses of 40, 160, 640, 2560 mg/kg body weight. At 40 mg/kg body weight four isolates (*A.niger, Trichoderma spp, Fusarium verticillioides* and *Penicillium verrucosum*) killed a mouse each out of the three used for the test. Four fungal isolates (*A.niger, A.parasiticus, F.verticillioides* and *Penicillium verrucosum*) caused death at 160 mg/kg body weight. All the thirteen fungal isolates except *Helminthosporium* and *Trichoderma spp* were lethal to mice at 640 mg/kg body weight. Except for *A. parasiticus* and *Helminthosporium spp,* all other extracts caused 100% mortality at 2560 mg/kg body weight. From this result, *A. niger* and *F. verticillioides* caused the highest lethality in mice even at low concentration and therefore were the two most toxic fungi found in guinea corn and rice. *F. verticillioides* was selected as the novel most toxic fungi contaminating guinea corn and rice in Niger State because less information about its toxicity is available in literature as compared to *A. niger.*

The culture material of *Fusarium verticillioides* was therefore subjected to acute toxicity studies in chicks and mice (Makun *et al*. 2010a). Oral administration of the fungal extract to mice and chicks caused mortality at 833.33mg/kg and 2500mg/kg body weight respectively. The

intraperitoneal LD_{50} values of the extract in both animals were between 45.40–87.90 mg/kg body weight with the mice being more susceptible. The total fumonisin content of the fungal residue as analyzed using veratox competitive direct enzyme linked immunosorbent assay (CD-ELISA) was 8.233ppm. The crude extract invariably fumonisins are harmful to the liver, kidney and gastrointestinal tract. Haemorrhage and degenerative necrosis of the liver cell, mucous layer of the digestive tract and the digestive tract in its entirety were histopathological changes observed in the chicks. Kidney of the mice show wide spread intra renal tubular necrosis with micro thrombi formation while there was wide spread fatty degeneration of mice liver as evidence by empty clear vacuoles and broad fibrosis appearing as septae signifying early cirrhosis (plates 5, 6, 7 and 8).

Using more accurate equipment, flow cytometry, TLC and HPLC, the culture material of same *Fusarium verticillioides* was tested for its *in vitro* cytoxic effect to human lymphocytes in comparison with those of aflatoxin B_1, fumonisin B_1 and ochratoxin A (Makun *et al*. 2011a). The mycotoxin profile of the extract was elucidated using TLC, column chromatography and HPLC. Figure 2 show a dose-dependent cytotoxic effects of the toxins to human lymphocytes. At concentrations of 25, 50 and 100 µg/ml, OTA was more toxic than AFB_1 followed by the extract which was comparatively as toxic as FB_1. Cytotoxicity data also revealed that, apoptosis and necrosis were the major form of cell death induced by the tested mycotoxins and extract. The extract was found to contain fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) at concentrations of 16.302, 6.423 and 2.456 ppm, respectively. The two studies therefore reveal that *F. verticillioides* produces the three major fumonisins and that these toxins can damage the liver, kidney and digestive tracts of animals and possibly human beings and elicit immunodeficiency in human beings. Aflatoxins and ochratoxin were also shown to be immunotoxic to man in the studies.

Iheanacho *et al.* (2014a) would also prove the cytotoxicity of aflatoxin B_1 to human lymphocytes using the same methyl tetrazolium bromide (MTT) method. The AFB_1 standard (80 µl/ml) used as a point of reference exhibited the greatest cytotoxic effect in causing cell mortality (73% cell viability recorded after 24 hrs of exposure), which increased over time (59% cell viability recorded after 72 hrs of exposure). Garba (2017), also tested the cytotoxic potentials of aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol on human mononuclear lymphocytes at 24 and 72 hours respectively in various concentrations of 2, 4 and 8µl and found that these toxins

at concentrations detected in our sorghum and sorghum based foods reduce the viability of human immune cells by between 50 and 97%.

The conclusion here is that culture material of *Fusarium verticillioides* from rice which contains fumonisins are lethal to chicks and mice causing damage to the liver, kidney and gastrointestinal tract. We have also shown that the five major mycotoxins found in our foods kill human lymphocytes.

Exposure Assessment

The question to answer at this point is, are we exposed and ingest aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol at unsafe levels? The maximum tolerable limits for aflatoxins in human foods is 4 µg/kg while for animal feeds is 20 ppb with infant foods having the least regulated levels (0 – 4 µg/kg). The lowest maximum allowable concentrations by countries that legislate against mycotoxin as recorded by CAST, (2003) are 5 µg/kg for OTA, 100 µg/kg for ZEA, 1000 µg/kg for FB and 500 µg/kg for DON. The regulated limit for aflatoxin M_1 in milk and milk products for infants is 0.05µg/l. Considering the mean values obtained during our screening of foods and feeds for the five mycotoxins in animal feeds, bean, groundnut, garri, maize, meat, millet, milk and milk products, sesame, sorghum, bitter leaf, red pepper and wheat and *burukutu*, Nigerians are exposed to mycotoxins especially aflatoxins and ochratoxins at very unsafe levels. However, the aflatoxin levels found in South African animal feeds were all below legislated levels and therefore safe for consumption. Similarly, baobab (kuka), okra, pumpkin, spinach, tomatoes, dairy products from commercial farms, powdered milk and to lesser extent Fonio (*acha*) and millet are safe foods with regard to mycotoxin contamination.

Estimation of the amount of toxins ingested from our processed food per kilogram body weight per day, week, month or year was carried out after obtaining the levels of mycotoxins in three commonly consumed sorghum based foods, weight of processed food taken daily and body weight of four categories of Nigerians subsisting on sorghum in Northern Nigeria (Garba, 2017). We calculated the dietary intake of the five mycotoxins in infants (0-3 years), children (4-17years), adults (18-49years) and 50 and above years for sorghum as follows total aflatoxins (0.59-3.33, 0.35-2.06, 0.29-1.29 and 0.28-1.62), fumonisins (2.18-11.15, 1.24-5.80, 0.79-5.47 and 1.08-5.03),

ochratoxin A (0.08-0.85, 0.14-5.01, 0.04-0.42, 0.04-1.12), zearalenone (10.45-19.12, 6.58-11.29, 5.51-9.60, 2.29-8.09) and deoxynivalenol (4.62-17.26, 2.73-10.47, 2.32-8.86 and 2.29-8.15) µg/kg respectively. In a separate work, aflatoxin B_1 and total aflatoxin exposure from sorghum in Niger State was also calculated to be 1.75 and 2.04µg/kg bw/day (Apeh, 2014). Additionally, he estimated the daily intake of B_1 from traditional alcoholic beverage marketed in Niger State to be 3.42µg/L. Bandyopadhyay *et al*. (2007) had earlier reported an average daily aflatoxin exposure per person from sorghum in Nigeria as 3.3µg.

On Codex scale, provisional maximum tolerably daily intake-PMTDI is to be as low as reasonably possible for aflatoxins because they carcinogenic (formerly 0.0004µg/kg bw/day), 2µg/kg for fumonisins, 0.1µg/kg for ochratoxin A, 0.2µg/kg for zearalenone and 1µg/kg for deoxynivalenol. It is obvious that all the determined dietary daily intake levels for aflatoxins, zearalenone and deoxynivalenol from sorghum food products in Northern Nigeria have exceeded the allowable limits. Except for very few persons, the intake levels of fumonisins and ochratoxin A have generally exceeded permitted levels also. Considering that sorghum is resistant to mycotoxin contamination than most crops, it is very reasonable to conclude that Nigerians ingest the five major mycotoxins at alarming levels daily from their meals. Only about 19.3% (411) of the total samples of Nigerian foods and feeds tested (2133) in all our works were found to be fit for human consumption.

Risk Characterization

Based on the above three risk evaluation findings, it is possible to estimate the severity or otherwise of the health and economic implications of the studied mycotoxins in Nigerian populace. The unacceptable levels of mycotoxin contamination and consequently intake from our foods and feeds have grievous public health and economic implications.

Aflatoxins are potent carcinogens of the liver which we have shown in our works to also be immunotoxic to human beings. If at 350 µg/kg of aflatoxins death can result (Azziz-Baumgartner *et al.,* 2005), the toxin likely accounts for the deaths of some primary school children in Ibadan, Nigeria who ingested incriminating levels of AF in groundnut cake '*kulikuli'* in 1988 (Fapohunda, 2011) and 125 death of persons who consumed maize 355µg/kg of the toxin in Kenya. Apart from

death, chronic ingestion of aflatoxins above 0.001µg/kg bw/day by persons with hepatitis B virus exacerbate liver cancer incidence. This level has been exceeded in most of our foods. Based on the exposure level determined and the 13.2% prevence of Hepatitis B virus in Nigerian populace, using the model of Liu and Wu, (2010), we estimated 33,453 liver cancer cases due to aflatoxin B1 in sorghum in the Nigeria annually which culminates to a financial health loss of $1,637 million. No wonder aflatoxin ingestion from maize and groundnut only is the cause of 7761 liver cancer cases in Nigeria annually and the monetized total aflatoxin liver cancer burden from these two crops is between $380 and $3, 174 million (Meridian Institute, 2013). Its immunosuppressive ability as shown in our works aggravates malaria and HIV/AIDS, kwashiorkor, growth stunting in children, genetic defects at neonatal stages and other liver diseases (Makun *et al*., 2012).

Ochratoxins are potent nephrotoxins, immunosuppressants, teratogens and carcinogens that cause kidney and liver impairments in man and animals especially pigs. It is the causative agent of endemic nephropathy in 20,000 people in Croatia, Bosnia and Herzegovina, Yugoslavia, Bulgaria, and Romania, and urothelial tumours of pelvis and ureter in Egypt, Croatia, Bulgaria and Yugoslavia and chronic interstitial nephropathy in Tunisia (Peraica *et al*. 1999). The presence of the toxin which are within the lower limits of OTA concentrations (200–1,000 µg/kg) that caused mycotoxic porcine nephropathy in Bulgaria (Stoev *et al*., 2002), could with other factors, such as malaria, hypertension and diabetes, cause the rising incidences of chronic renal diseases experienced presently in Nigeria as well as animal nephropathy. Chronic renal failure (CRF) accounts for about 10% of medical admissions in Nigeria and an extrapolation of this puts the frequency figure between 200 and 300 patients per million of population (NAN, 2008)

Fumonisins have been classified by the International Agency for Research on Cancer (IARC, 1993) as possible human carcinogens in category IIB. They are associated with increased incidence of human oesphageal cancer in parts of South Africa, North Eastern Iran and China, upper gastrointestinal tract cancer in Northern Italy and neural tube defects in human babes, and leukoencephalomalacia in equine and pulmonary oedema in pigs (Marasas, 2001). Zearalenone, on the other hand is an oestrogenic toxin causes infertility in animals and is associated with outbreaks of precocious pubertal changes in children in Puerto Rico and has been suggested to

have a possible involvement in human cervical cancer (JECFA, 2000). Deoxynivalenol is RNA, DNA and protein inhibitor with consequent immunosuppressive effects, causing severe damage to the digestive tract and death due to intestinal haemorrhage. We are therefore vulnerable to these diseases as the toxins are present in foods.

Simultaneous occurrence of the five studied mycotoxins in twos, threes, fours and fives in same sample was a common observation on the course of our works. The interactions of these toxins could be synergistic, additive or antagonistic (Miller, 1995). Aflatoxin B_1 and fumonisin B_1 synergistic interaction in exacerbating liver cancer in human population in China and experimental animals (Uena *et al*., 1997) has drawn global attention. While aflatoxin aggravates the nephrotoxigenicity of ochratoxin with increased growth inhibition and mortality of chicks, ochratoxin A also aggravates the mutagenicity of aflatoxins in some other experimental animals (Speijer and Speijer, 2004). Other combinations reviewed by same authors that exhibit synergistic interactions include AFB_1 and the trichothecenes, FB_1 and OTA, and FB_1 and ZEA. Synergistic and additive growth depression effects of DON and FB_1 in pigs and broiler chicks respectively.
Economic losses due to mycotoxins arise from reduction in crop and livestock production, and human health. FAO statistics estimates that 25% of world's food crops are lost to mycotoxin yearly and a substantial part of the wastage is in Africa. African countries including Nigeria loss $670 million annually in order to meet European Union regulation on aflatoxins (Otuki *et al*. 2001). National Agency for Food and Drug Administration Control destroyed aflatoxin-contaminated food worth more than US$200,000 (SFI, 2005). Between 2007 and 2016 there were rejections of Nigerian produces at EU borders due to aflatoxin level which culminated to the imposition on import ban restricting export of five major agricultural produce from Nigeria to any European Union member country. This ban caused *a decline of ₦671.1 billion or 34.6%* non-crude component of trade including processed and unprocessed food items (National Bureau of Statistics). Africa loses 40% labour productivity in Africa due to diseases and deaths exacerbated by AFs (Miller, 1995). But how does one assess the economic losses following increased pre-five mortality rates, and the death of the primary school pupils in Ibadan, and people in an Indian village and two districts in Kenya after eating moulded food contaminated with aflatoxin?

The presence of these toxins at unacceptable levels particularly in simultaneous occurrence in our foods are not only associated to the increased severity and incidence of liver, kidney, malnutrition, infertility, HIV/AIDS, cancer and other infectious diseases but have adverse implications on international trade and consequently our nation's economy which necessitates their elimination from our food and trade systems.

Prevention and Control

With regards to mycotoxin intervention strategies, we have established the reducing effects of local processing methods on mycotoxin levels, derived botanicals with fungicidal effects, obtained indigenous atoxigenic fungi that bioexcluded and suppress mycotoxin production by the toxigenic ones of same species and provided code of practice for the prevention and reduction of aflatoxins and ochratoxin A in sorghum and sorghum based products.

Deriving New Analytical Methods

It is essential for analytical methods to accurately generate valid results that can be used in assessing risks in order to apply appropriate intervention strategies. This is particularly important when dealing with health related hazards like fungi and mycotoxins in foods and feeds. The biggest challenges in the field of mycology is to differentiate fungal species from same genus and determine concentrations of mycotoxins which occur most times at picogram levels. A false result could lead to fatality of people and animals. In addressing these difficulties, we derived a simple easy to use and rapid molecular method for analysis of morphological form of species of *Aspergillus*. It was employed in accurate identification and differentiation of *Aspergillus flavus* and *A. parasiticus* (Iheneacho *et al*. 2014). The DNA was extracted and amplified using commercial kits but the gel electrophoresis used which was a modified method of Saghai- Maroof *et al*. (1984) allowed for the easy, rapid separation and visualization of DNA fragments of *A flavus* and *A. parasiticus* for routine use (Figure 1).

An attempt to correlate expression of Nor~1 (aflD) gene which is the main factor responsible for AFs production with levels of AFs in South African compound feeds to obtain a predictive model for aflatoxins in compound foods was carried out (Iheneacho *et al*. 2014a). To achieve this, compound feeds (n = 30) were analyzed for Nor~1 gene using real time polymerase chain reaction

(RT-PCR), while AFs levels in same samples were analyzed using high-performance liquid chromatography (HPLC) after an immune-affinity clean-up extraction procedure. Results indicated that AFs levels in positive samples ranged from 0.7 to 33.0 ppb. These levels generally did not statistically correlate (R2 = 0.093) with those of Nor~1 gene in similar samples, the reason being that even if the gene is present it may or may not have been expressed to produce aflatoxins. Consequently, Nor~1 gene levels established via RT-PCR cannot be used as a predicting model for AFs in compound feeds.

Effects of Processing

Gbodi *et al.* (2001) examined the effects of local processing methods on aflatoxin levels of some common Nigerian maize, rice and sorghum based foods. Of all the procedure tested, cooking of rice with oil, salt, pepper and other seasonings to prepare jollof rice and the preparation of fried rice by frying and boiling with salts and other seasonings eliminated aflatoxins from the foods. Other cooking methods reduced aflatoxins by between 49.8 and 98.1% (Table 3). Processing contaminated guinea corn to boiled corn starch (pap) has no effect on aflatoxin content. Wet cooking, use of salt and frying with oil were most effective in aflatoxin reduction. Moist heat opens the lactone ring of aflatoxin forming carboxylic acid which then undergoes decarboxylation, and salts also destroys aflatoxins.

Garba (2017) has also evaluated the effectiveness of seven of our traditional sorghum food processing methods which end products are f*ura*, dough (Tuwo), alcoholic beverage (*pito*), waina, Chichion (*Dambu*), pap (*Kamu*) and *ogi*. He proved that the processing methods involved significantly reduce the levels of aflatoxin B_1 (59.4-95.2%), ochratoxin A (74.0-96.1%), fumonisin B_1 (73.5-94.8%), zearalenone (39.4-82.3%) and deoxynivalenol (60.8-95.3%) in the end products. Dehulling, grinding, boiling in water into thick paste, being the procedure for preparation of tuwo was the most effective in reduction of aflatoxin B_1, fumonisin B_1, ochratoxin A and deoxynivalenol while processing to waina was most effective for reduction of zearalenone. In all the samples, only a tuwo sample from Southern Guinea Savannah showed 272.3% increase in ZEA concentration while there was 66.7% increase in the concentration of OTA in Masa/ Waina sample from the

Sahel Savannah. Such increase could be because of concentration or introduction of toxin during processing.

Phytofungicides

Application of synthetic seed dressing fungicides are effective measure of control of seed borne fungi and consequently mycotoxin contamination. However, the toxicity of synthetic fungicide to animals and human beings has led to the quest for non toxic, environment friendly phytofungicides. Accordingly, after showing that *Fusarium verticillioides* and its metabolites are harmful to germinating maize seedling, we proved that application of the ethanolic extract of neem reversed the adverse effect of the fungus and its metabolities by improving on germination percentage and seedling vigour with a concomitant reduction in rot index (Anjorin *et al.*, 2008). Similarly, the ethanolic extract of the leaf of *Lippia multiflora* (L) Modenke Family Verbanaceae (commonly called lemon-scented verbena) did not only improve on the germination rate and seedling vigour of sorghum seed but reversed the damage caused by *Aspergillus flavus* and its metabolites on infected sorghum seed (Anjorin *et al*., 2008a). The *in-vitro* and *in-vivo* investigation of the antifungal properties of *Jatropha curcas* and *Ricinus cumunis* seed extracts in the control of mycelia growth and rot development of yam caused by *Fusarium verticilliodes* and *Aspergillus flavus* by Makun *et al*., 2011b) indicates the promising potentials of *J. curcas* and *R. cumunis* seeds in management of plant fungal diseases caused by the studied fungi. Makun *et al*. (2012b) also subsequently demonstrated the *in-vitro* fungistatic efficacy of crude leaf extracts of *Azadirachta indica*, *Blumea perotitiana* and *Lippia multiflora* against *A. niger* and *F. verticilloides* which were the predominant fungi contaminating cowpea (bean) marketed in Minna. While the neem leaf extract had the least efficacy against the fungi, combination of *Lippa* and *Blumea* inhibited the mycelial growth of the fungi at 65% and 48.75% respectively.

Berkheya setifera and *Carissa bispinosa* are promising candidates for phytofungicides. Garba (2017), using two methods, food poisoning and Agar well diffusion method has demonstrated the effective antifungal properties of these plants against 18 common toxic fungal contaminants of food and feed namely *Aspergillus. versicolor, A. carbonaris, A. flavus, A. parasiticus, A. ochraceus, A. niger, A. fumigatus, Penicillium verrucosum, Fusarium verticillioides, F. solani, F. oxysporum, F. chamydosporum, F. subglittinans, F. acuminatum, F. avenaecem, F. poae, F.*

proliferatum and *F. graminearum*. Using the food poisoning method, the extract of *Berkheya setifera* when aseptically diluted into 20ml of molten agar media, at different concentrations showed "Regular" (60 – 69%) and "Good" (70 – 79%) antifungal activity. The unique feature of the extract at all concentration is that, there is no significant difference (P> 0.05) between the 80, 100, 120, 140 and 160µg/20ml of the extract. Employing same technique, the extract, of *Carissa bispinosa* at concentrations of 80, 100, 120, 140 and 160µg/20ml demonstrated a good antifungal activity. All the concentrations tested showed test scores of "Regular" (60 – 69%) and "Good" (70 – 79%) and on two occasions "Very good" (80 – 90%) antifungal activity. The unique feature of the extract at all concentration is that, there is no significant difference (P> 0.05) between the levels of the extract tested.

Berkheya setifera extract using Agar well diffusion method clearly showed a low activity even at high concentration. The effective inhibition limit of **6.00mm** was attained in few cases and a significant difference (P = 0.05) existed between the standard drug (AmphotericinB) and the extract in all concentrations with regards to the zone of inhibition. The antifungal activity of *Carissa bispinosa* extract using Agar well diffusion method clearly revealed a higher activity when compared with the *Berkheya setifera* extract. The effective inhibition limit of **6.00mm** has been attained in most cases and across all the concentrations. There is no significant difference (P = 0.05) between the standard drug (Amphotericin B) and the extract in all concentrations with regards to the zone of inhibition. Microscopic examination (plates 1, 2, 3, 4) showed dose dependent disintegration of fungal mycelium, vesicles, verticillates and macronidia of treated fungi. Thin layer chromatography of the extracts of the morphologically transformed fungi reveal suppression of ability to elaborate aflatoxin and ochratoxin A. Further purification of the extracts of the two plants will yield higher efficacy at lower dose with regards to inhibition of fungal growth and mycotoxin production.

Biocontrol

One of the newest most successful preharvest biocontrol method against aflatoxin contamination deployed in USA and Africa is the use of non toxigenic *Aspergillus flavus* and *A. parasiticus* to competitively exclude the aflatoxin producing ones of same species from maize farms. The technology has been developed into products referred to as *aflaguard* and *aflasafe* in USA and

Africa respectively. In line with such technology, we have obtained indigenous atoxigenic strains of *Aspergillus flavus, A. parasiticus, A. carbonarius* and *A. niger* that suppress both the growth and, aflatoxin and ochratoxin A producing potentials of fungal isolates of same species found in Nigerian sorghum. These are bio resources that can progress into an industry that will control mycotoxins in sorghum and sorghum based products.

Participation in Prevention and Control of Mycotoxins by Codex Alimentarius Commission

Codex Alimentarius Commission (CAC) is the world body that proposes, promote coordination of all works on food standards, determine priorities and initiates and guide the preparation of draft standards, finalize standards and publish them, and amend published standards as appropriate in the light of development. The Food and Agriculture Organization and World Health Organization consults CAC in matters pertaining to implementation of Joint FAO/WHO food standards programme. The Committee that provides CAC with technical advice is the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Standards protect the health of consumers and ensures fair practices in the food trade. We prepared the first official document on sorghum for CAC in 2012 (**CX/CF 12/6/14)** after Algeria, Tunisia and Sudan had failed in the previous years to produce a document with sufficient data to initiate discussion towards setting mycotoxin standards for sorghum. This was the first assignment Nigeria did for CAC. The discussion paper on sorghum led WHO to conduct extensive multi-mycotoxin screening of sorghum in Burkina Faso, Mali, Ethiopia and Sudan where it was discovered that sterigmatocystin and diacetoxyscirpenol occurred more than the dread aflatoxins.

Under our expert advice and leadership, in 2013 Nigeria subsequently prepared the proposed draft annex for the prevention and reduction of aflatoxins and ochratoxin A contamination in sorghum (code of practice for the prevention and reduction of mycotoxin contamination in cereals (CAC/RCP 51-2003). The document (**CX/CF 13/7/8**) provides good agricultural, hygienic, and manufacturing practices that prevent and control aflatoxins and ochratoxin A in guinea corn. It gives the codes on when, how, and what techniques to employ in planting, harvesting, transport, storage, processing, packaging and marketing of sorghum to ensure grains and products meet the international and safe limits of 4µg/kg and 5µg/kg for aflatoxin and ochratoxin A.

Following the outcome of the WHO sorghum survey, the expert advice of JECFA was sought on sterigmatocystin and diacetoxyscirpenol. Being one of two experts representing Nigeria on JECFA since 2012, I wrote on the prevention and control of sterigmatocystin and diacetoxyscirpenol for the 83rd meeting of JECFA held in November 2016 in Rome (**WHO Technical Report Series; No 1002, 2017**). We observed that diacetoxyscirpenol has reproductive and developmental toxicity and therefore recommend the development of new sensitive analytical techniques and markers for its analysis and the modified forms and encourage screening of food commodities for the toxin. Same recommendations were given for sterigmatocystin.

Another indirect contribution in the work of CAC that I participate in, is as a member of the African Union Codex Expert Committee on Contaminants in Food. The Strategic Plan for the FAO/WHO Coordinating Committee for Africa (CCAFRICA) was developed with the overall objective to strengthen the role and enhance the participation and effectiveness of CCAFRICA within the Codex Alimentarius Commission and the Codex African region. In support of this objective, the African Union's Interafrican Bureau for Animal Resources (AU/IBAR) has been organizing Expert meetings since 2009. Since then AU/IBAR has convened the meeting of experts to develop science based regional positions on various Codex issues of relevance to the region before sessions of Codex Committees and Task Forces. Expert meetings have been organized in preparation for sessions of various Codex Committees. I am one of the four African Union experts taking the science based decisions on contaminants in foods for Africa since 2011 which has greatly improved Africa's participations in CAC meetings.

Providing Resource information on Mycotoxins

In addressing paucity of information on mycotoxins particularly for students, educators, researchers, regulatory officers and policy makers in the African continent, we have shared our mycotoxin research experience in three books and two book chapters that are open access and are used by scientists all over the world as specialized references and textbooks. The first book chapter, Njobeh *et al.* (2010) provides incidence data, exposure, health impact and control of toxic fungi and mycotoxins, the second which is on aflatoxin contamination in foods and feeds in Africa (Makun *et al.* 2012) has cumulative download of 7000 as at 23rd May 2017. The compendium of abstracts of Mycotoxicology in West Africa: 1980-2015, captures 295 research efforts in various

aspects of mycotoxin research in the region (Edema *et al*. 2015). The second book dwells on the epidemiology, prevention and control of food related diseases, cholera, staphylococcal food poisoning and *Toxoplasma gondii* and prion diseases in the food value chain (Makun, 2016). The book which has 10 chapters has contributors from Brazil, Mexico, Saudi Arabia, South Africa, Spain, Turkey and USA. It had a cumulative download of 5000 as at 24th May 2017. The third which was the first book I edited had a download of 45000 as at 24th July 2017 and is titled "Mycotoxin and food safety in developing countries" (Makun 2013). It captures the mycotoxin research experience of scientists from Africa, Asia and Middle East

RECOMMENDATIONS

1. Cereals dependent diets system should be abolished. Diversifying our dietary system to include mycotoxin resistant crops like *acha*, millet, fresh root and tuber and vegetables to form a substantial part of our meals is recommended.

2. Good agricultural, hygienic and manufacturing practices as enshrined in the Code of practice for the prevention and reduction of mycotoxin contamination in cereals (CAC/RCP 51-2003) be adhered to by our farmers.

3. Federal Ministry of Agriculture and Rural Development whose mandate it is to regulate contaminants in food commodities within the country needs to establish regional laboratories that will conduct annual surveillance to identify contaminants to regulate, communicate risks of mycotoxin contamination and train farmers on how to eliminate them before enforcing legislated limits.

4. Standards Organization of Nigeria to conduct national toxicological and risk analysis of mycotoxins and other contaminants in Nigerian dietary system and use the generated data to set national standards that fit our agricultural and cultural systems instead of just adopting international standards.

5. Mycotoxicology Society of Nigeria is advised to formulate a national mycotoxin policy on mycotoxin management for inclusion in the national food policy which is expected to give

policy directions to government, food regulatory agencies and stakeholders in the food value chain.

6. Practitioners in the public health, agriculture and food sectors are expected to be very knowledgably in the impact and control of carcinogenic food toxins, mycotoxins, and therefore we recommend the training on mycotoxins as taught courses to undergraduate and postgraduate students of medicine, veterinary medicine, agriculture and food science and other related fields.

7. One of the major challenges faced in mycotoxin research in Nigerian universities is the lack of analytical equipment. In most cases, the equipment is available but cannot be operated or maintained. The solution to this is contracting out the maintenance of these costly, sophisticated machines to qualified technical companies along global practice. The initial rise in cost of analysis will come down with increased patronage guaranteed by efficient and endurable service.

8. Mycotoxin research like in other laboratory based sciences is capital intensive that requires research grants. This undergoes the need for the Federal Government to invigorate the national research and innovation system by implementing the National Research and Innovation Council (Establishment etc.) Bill of 2016 sponsored by Senator David Umaru of Niger East Senatorial District. It is also a call for TETFund to resuscitate the National Research Grant.

9. The fate of Nigeria lies in the hands of the academic and military institutions. While the soldiers keep it united, the academics are expected to innovate and convert it a wealthy knowledge based nation. These institutions and other instruments of governance cannot achieve these goals without merit being their guiding principle and so must be insulated from obnoxious partisan politics, ethnic and religious influence.

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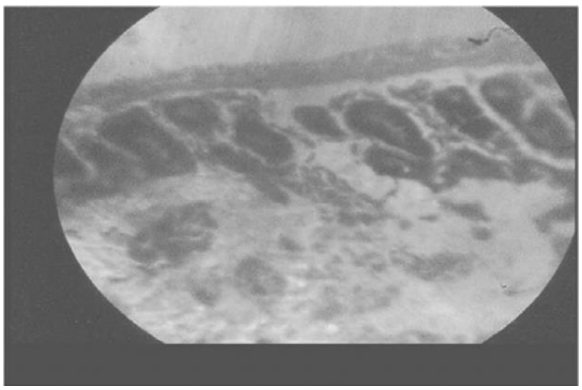


Plate 1: Micrograph of chick intestine after oral administration of 5000 mg/kg body weight of extract. (x 400 H & E). Mucosal denudation of intestine

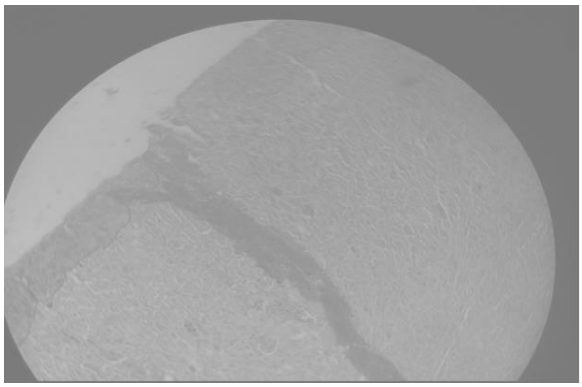


Plate 2: Micrograph of mice kidney at X 100 H & E magnification after dosing interperitoneally with 66.36mg/kg body weight of extract of *F. verticillioides.* Showing wide spread intra renal tubular necrosis with micro thrombi formation

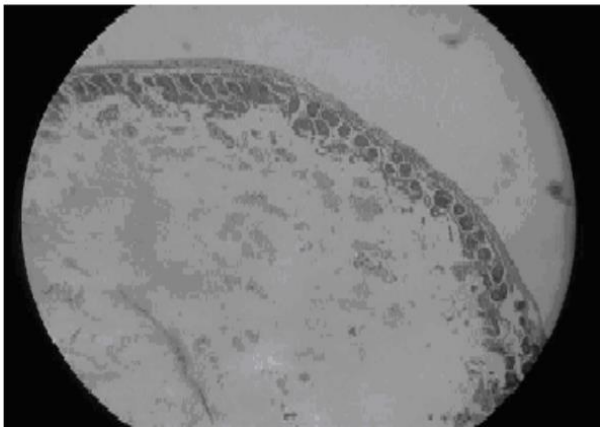


Plate 3 Micrograph of mice liver at X 100 H % Emagnification after administeration of a single oral dose (1438.80 mg/kg body weight) of extract
Showing massive eosinophillic leucocytes infilteration and aggregation in the mucosa. This implies eosinopillic graniloma resulting from intense inflammation caused by injury.

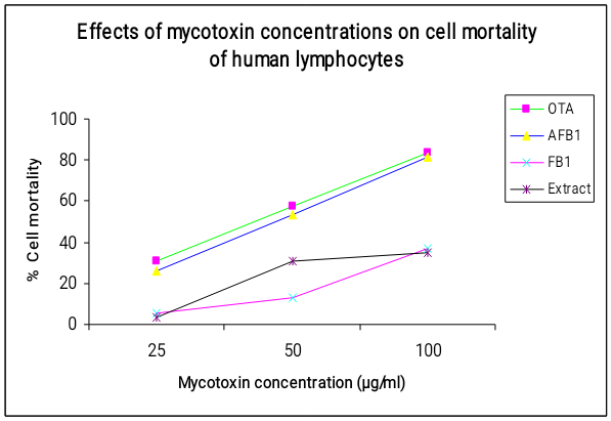
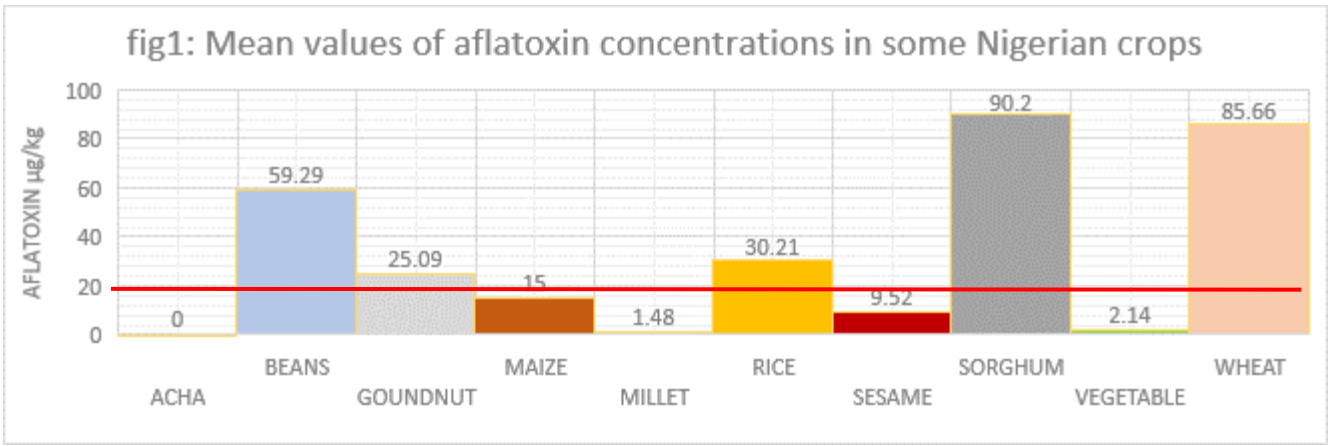


Figure 1: Effects of mycotoxin concentration on cell mortality of human lymphocytes



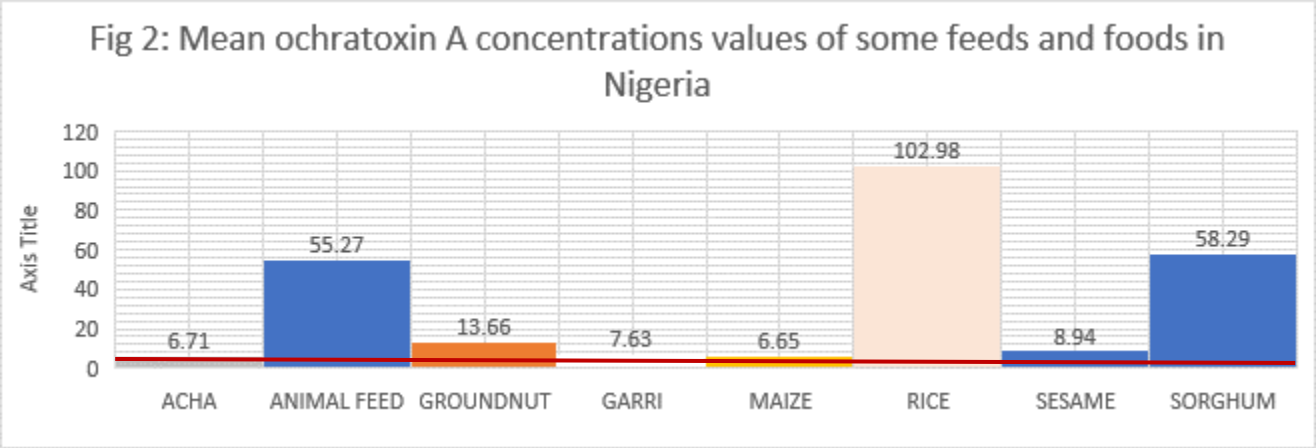


Fig 2: Mean ochratoxin A concentrations values of some feeds and foods in Nigeria
Axis Title
120
100
80
60
40
20
0
6.71
55.27
13.66
7.63
6.65
102.98
8.94
58.29
ACHA
ANIMAL FEED
GROUNDNUT
GARRI
MAIZE
RICE
SESAME
SORGHUM

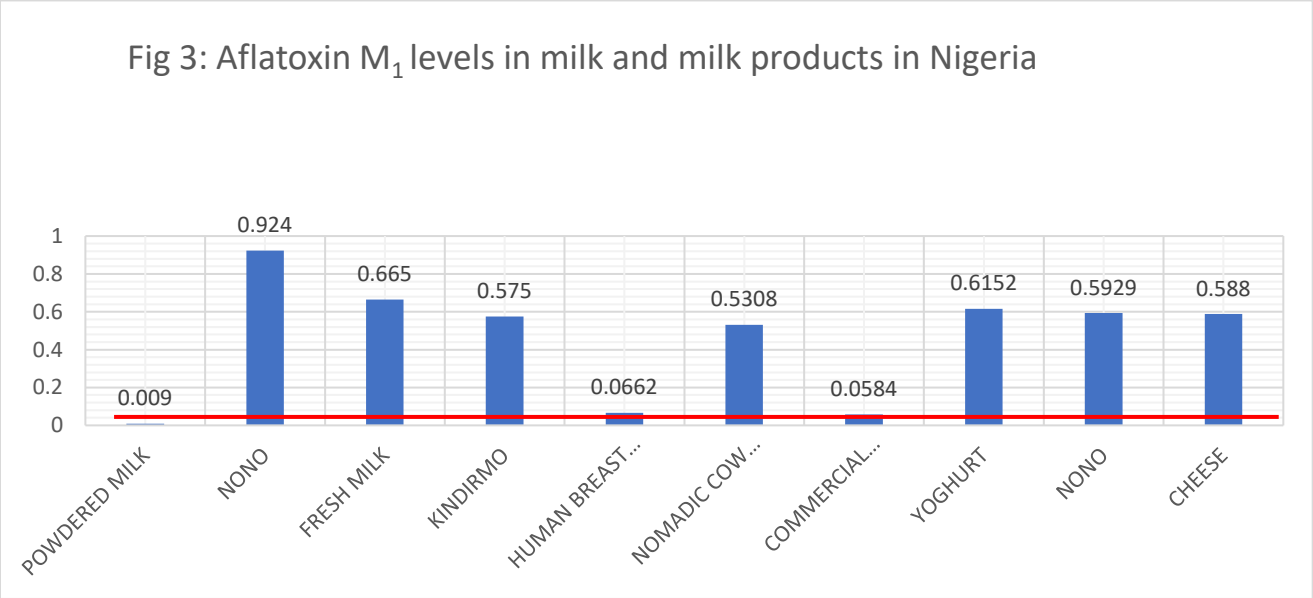


Fig 3: Aflatoxin M_1 levels in milk and milk products in Nigeria
1
0.8
0.6
0.4
0.2
0
0.009
0.924
0.665
0.575
0.0662
0.5308
0.0584
0.6152
0.5929
0.588
POWDERED MILK
NONO
FRESH MILK
KINDIRMO
HUMAN BREAST...
NOMADIC COW...
COMMERCIAL...
YOGHURT
NONO
CHEESE

Fig 4: Mean concentrations of Fumonisin, zearalenone and deoxynivalenol in Nigerian grown rice and sorghum

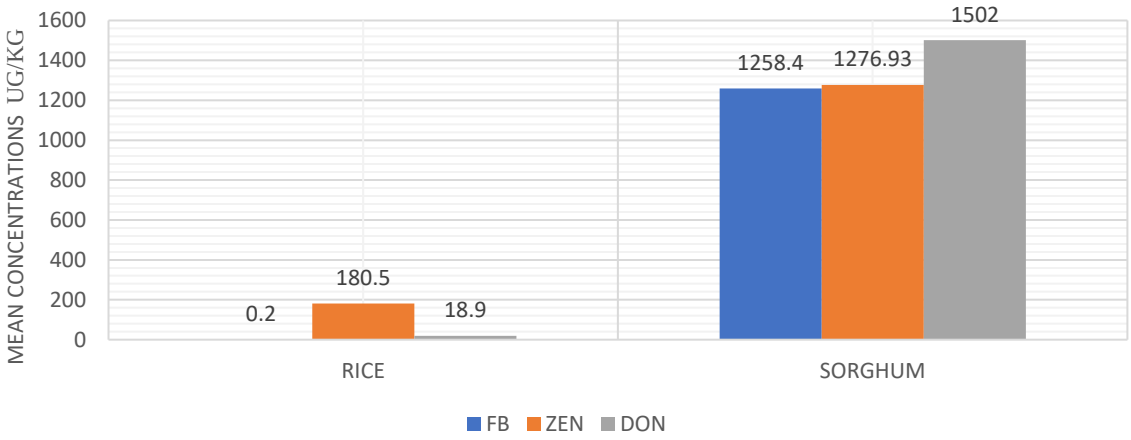


Table 1: **FUNGI CONTAMINATING FOODS AND FEEDS IN NIGERIA AND SOUTH AFRICA ESTABLISHED BY MAKUN *et al*. FROM 2007-2017**

Animal feed	Groundnut	Maize	Milk products	Millet	Rice
Alternaria spp *A flavus* *A.fumigatus* *A.malleus* *A. nidulans* *A. niger* *A.ochraceus* *A. parasiticus* *A. flaviceps* *Cladosporum spp* *Curvularia spp* *Fusarium spp* *F. oxysporum* *F.semitectum* *F.solani* *Mucor spp* *Penicillium spp* *P. citrinum* *P. notatum* *P. rubrum* *P.verrucosum* *Rhizopus spp* *Torula spp* *Yeast*	*A. niger* *A. flayus* *A. fumigates* *A. ochraeus* *A.parasiticus* *Fusarium* *Mucor spp.* *Penicillium* *Rhizopus spp.*	*Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus glaucus* *Aspergillus nidulan* *Aspergillus niger* *Aspergillus parasiticus* *Aspergillus terreus* *Aspergillus versicolor* *Fusarium* spp *Mucor* spp *Rhizopus* spp *Syncephalastrum* spp *Penicillium* spp *Fusarium oxysporum* *Fusanum* spp *Penicillium notatum* *Pithomyces chartanum* *A.ochraceus* *Yeast spp*	*Fusarium spp.* *Aspergillus flavus* *Mucor spp.* *Penicillium spp.* *Rhizopus spp.* *Aspergillus niger*	*Aspergillus flavus* *Aspergillus nidulans* *Aspergillus niger* *Aspergillus glaucus* *Aspergillus parasiticus* *Aspergillus versicolor* *Arthroconidia* spp. *Cladosporium* spp. *Fusarium nwale* *Penicillium* spp. *Mucor* spp. *Penicillium rubrum* *Rhizopus* spp. *Phoma* spp. *Syncephalastrum* spp. *Fusarium verticillioides* *Fusarium* spp *Aspergillus fumigatus* *Fusarium equiseti* *Fusarium spp* *Fusarium trincintum* *Helminthosporium spp* *Penicillium verrucosum* *Rhizopus stolonifer* *Syncephalastrum spp* *Aspergillus tamari* *Cercospora spp* *Coryospora sp* *Curvularia spp*	*Aspergillus. aculeatus* *A. candidus* *A. flavus* *A .fumigatus* *A. niger* *A. niveus* *A. ochraceus* *A. oryzae* *A. parasiticus* *A. penicillioides* *A. sclerotiorum* *A. terreus* *A. tubingensis* *A. unguis* *Eurotium amstelodami* *Penicillium .oxalicum* *Fusarium .chlamydosporum* *F. proliferatum* *F. pseudonygamai* *F.verticillioides* *Fusaruim spp.* *Pseudofusarium purpureum* *Acremonium* sp *Alternaria azukiae* *Alternaria* sp *Ascomycota*. sp *Botryosphaeria.dothidea* *Curvularia affinis* *Curvularia* sp. *Sarocladium attenuatum* *Sarocladium oryzae*

				Macrophomena spp	

Table 2: FUNGI CONTAMINATING FOODS IN NIGERIA AND SOUTH AFRICA ESTABLISHED BY MAKUN *et al*. FROM 2007-2017

Sesame	Sorghum and sorghum products		Vegetables	Yam flour
Aspergillus flavus, *A. parasiticus,* *Aspergillus niger,* *A. tamari,* *Alternaria spp,* *Fusarium spp,* *Cercospora spp* *Coryospora spp* *Macrophomena spp* *Penicillium spp* *Phoma spp* *Rhizopus spp,*	*Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus carbonarius* *Aspergillus parasiticus* *Aspergillus oryzae* *Aspergillus unguis* *Aspergillus niger* *Apergillus ustus* *Aspergillus versicolor* *Neosartorya fischeri* *Aspergillus melleus* *Aspergillus ochraceus* *Emericella nidulans* *Aspergillus japonicum* *Sclerocleista ornata* *Aspergillus paradoxus* *Emericella quadrilineata* *Penicillium citreonigrum* *Penicillium restrictum* *Penicillium crustosum* *Penicillium implicatum* *Penicillium malodoratum* *Penicillium rogulosum* *Penicillium expansum* *Penicillium janczewski* *Penicillium fellatum* *Penicillium paxillii* *Penicillium aurentiogresum* *Penicillium glabrum* *Penicillium nalgiovense* *Paecilomyces variotii* *Penicillium decumbens*	*Fusarium moniliforme* *Fusarium poae* *Fusarium acuminatum* *Fusarium hlamydosporum* *Fusarium proliferatum* *Fusarium subglutinans* *Fusarium avenaceum* *Fusarium sambucinum* *Fusarium trincinctum* *Fusarium equiseti* *Fusarium decemcellulare* *Fusarium dimerium* *Fusarium longipes* *Fusarium lateritium* *Alternaria alternata* *Alternaria infectoria* *Curvularia lunata* *Curvularia pallescens* *Endomyces fibuliger* *Phoma sorghina* *Absidia cocorymbifera* *Rhizomucor pussillus* *Rhizomucor stolonifer* *Candida krusei* *Schizosaccharomyces pombe* *Rhodontonila mucilaginosa* *Rhizomucor vuil* *A. tamari,* *Cercospora spp* *Coryospora spp* *Macrophomena spp* *Phoma spp* *Rhizopus spp,*	*Aspergillus flavus,* *Aspergillus parasiticus,* *Aspergillus niger,* *Mucor spp* *Penicillium. brevicopactum* *Fusarium culmorum* *Penicillium Brevicopactum* *Penicillum chrysogenium*	*Fusarium spp* *Aspergillus spp* *Aspergillus niger* *Penicillium spp* *Mucor spp* *Geotrichum candidum*

	Fusarium verticilloides *Fusarium solani* *Fusarium oxysporum*			