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Aflatoxin research in southeast Nigeria: current status and possible risks in the era of climate change.

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ABSTRACT

Aflatoxins are *Aspergillus* secondary metabolites found in various crops and agricultural products. The contamination of food by aflatoxins can occur during crop production, processing, storage, transportation or marketing of the food products. High temperature, moisture content and water activity are among the predisposing factors that facilitate mould growth and the production of aflatoxins in food. Aflatoxins are considered the major mycotoxins produced in crops and food. In Nigeria and beyond, aflatoxin contamination is considered to be a major problem with implications that affect human and animal health as well as the economic potentials of crops. The available, updated information on the incidence of aflatoxin contamination, decontamination and its public health importance in south eastern Nigeria is lacking. The aim of this review is to highlight, update and discuss the available information on the incidence of aflatoxins in south eastern states of Nigeria. The public health implications, risk factors and the recommended strategies for control of aflatoxins in food and agricultural crops are also deliberated.

Key words: Aflatoxins, food contamination, crops and products, health hazards, Southeast Nigeria.

Aflatoxins are highly toxic metabolism products of mainly *Aspergillus flavus* and *A. parasiticus*. They are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *A. flavus* and *A. parasiticus* on a variety of food products. Aflatoxin has been classified as a human carcinogen and is the subject of regulation world-wide. Aflatoxin contamination in food grains is now well recognized as a public health hazard. They are the strongest known naturally-occurring carcinogens composed of a group of compounds and structurally related toxic secondary metabolites. Therefore the monitoring of aflatoxins in agricultural products should continue to receive high priority (FAO, 2004; Strosnider *et al.*, 2006). Aflatoxins are a far greater problem in the tropics than in temperate zones of the world. However, because of the movement of agricultural commodities around the world, no region is free of aflatoxins. *Aspergillus flavus* is predominant in Asia and Africa while *A. parasiticus* is mostly found in the Americas. These fungi produce aflatoxins which is a potent natural

carcinogen and cyclopiazonic acid (CPA). *A. flavus*, in particular, is a common contaminant in agricultural products. *A. flavus* is favoured by hot dry conditions. *A. flavus* occurs in the soil, decaying vegetation, hay and grains undergoing microbiological deterioration. The optimum temperature for its growth is between 36 to 38°C while optimum temperature for toxin production occurs is 25 to 27°C (Gordon, 2005). These fungi infect crops and produce the toxin in the field and in stores. Contamination of crops is possible without visible signs of the fungal growth on the crop. The fungus also has ability to infect seeds produced both above and below the ground (Yu *et al.*, 2005). Studies on occurrence of toxigenic fungi on herbal drugs showed that *A. flavus*, in particular, was the main contaminant of different herbal and spice samples which therefore stand a potential risk of aflatoxins contamination (Adriana *et al.*, 2006). There are eighteen different types of aflatoxin identified. However, studies revealed that there are four major classes of aflatoxins (Reddy and Waliyar, 2005); based on their fluorescence under

UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography. These include: B₁, B₂, G₁, and G₂; plus two additional metabolites, M₁ and M₂ mostly found in the milk of animals fed with aflatoxin contaminated feed (Bennett and Klich, 2003; Reddy and Waliyar, 2005). Aflatoxin M₁ and M₂ as documented by (Donner and Wuppertal, 2009) were first isolated from milk of lactating animals fed aflatoxin contaminated feed, hence the M designation (Chemical Book, 2010). Aflatoxin formations in crops occur before or immediately after harvest and that during storage of commodities or food (Cotty and Jaime-Garcia, 2007). Wounding of developing crops by birds, mammals, insects, rats, mechanically or drought, terminal water stress prior to harvest and elevated temperatures promote *the fungi* infections and aflatoxin contamination (Kumar *et al.*, 2000; Guo *et al.*, 2002, Melvin, 2012). In postharvest contamination the fungi continue to grow in grains infected from the field and produce aflatoxins under high moisture and warm temperatures. In melon, ogbono, cashew nut, and some other crops, aflatoxin contamination usually occurs at the postharvest stage. *Aspergillus* spp have not been reported as a field disease of these crops in Nigeria. Crop processing, transportation and storage in a warm climatic condition favour *Aspergillus* growth and enhance the production of aflatoxin. Postharvest contamination is also enhanced by delayed drying. Aflatoxin contamination of crops is so hazardous that the effects are felt in different sectors of the economy. It reduces export potential of crops which amounts to a loss of several millions of dollars, retards growth in children fed with contaminated grains. Children with kwashiorkor in Nigeria were found to have high levels of aflatoxin (N'dede *et al.*, 2012). Specifically, they cause cell membrane damage, necrosis, gastritis, kwashiorkor and certain occupational respiratory diseases, liver damage, liver necrosis, liver cirrhosis, fever, progressive jaundice, limb swelling, pain, vomiting and enlarged liver and mutagenesis in the affected cells (Stronsnider *et al.*, 2006; Mohamed *et al.*, 2009, Chemical Book, 2010; Darwin *et al.*, 2014). Generally, prolong exposure to aflatoxins results in under-development of the population, liver cancer, impaired development in children and immune system suppression that is associated with reduced

resistance to diseases and reduced success of vaccination programs, teratogenicity in animals and reduced life-expectancy (Bandyopadhyay *et al.*, 2007; Hernandez-Vargas *et al.*, 2015; Williams *et al.*, 2004). Aflatoxins are problematic when they occur in food products above the levels established for human and animal consumption (Kumar *et al.*, 2000; Uriah *et al.*, 2001). Reports abound of the symptoms of aflatoxin exposure in factory workers handling contaminated animal feed stuff (IARC, 2002; Nuntharatanapong *et al.*, 2001). Aflatoxins is a subject of regulation world-wide and have received considerable research attention (Bandyopadhyay *et al.*, 2016). Regulatory controls are totally nonexistent in eastern Nigeria and largely ineffective in Nigeria as well as in Africa (FAO, 2004). In this paper, the importance of aflatoxins in south east Nigeria, Crops prone to infection from field to storage, their production, storage and consumption patterns, export potentials and aflatoxin status and risk factors in the face of climate change were reviewed .

AFLATOXIN IN SOUTHEAST NIGERIA

Aspergillus flavus and *A. parasiticus* are the major producers of aflatoxins. *A. parasiticus* produces four major aflatoxins: B₁, B₂, G₁ and G₂, while AFB₁ is the most toxic in the group and the toxicity is in the order of B₁ > G₁ > B₂ > G₂ (Dorner, 2004; Abbas *et al.*, 2005). *A. flavus* produces two major aflatoxins: B₁ and B₂. Even though much report is not available with regards to *A. flavus* frequency in most crops in south eastern Nigeria, reports abound in literature that it is frequently isolated in most agricultural samples (N'dede *et al.*, 2012; Perrone *et al.*, 2014). Hot and humid climates existing in eastern Nigeria have been found to be quite conducive for mould growth and toxin production. Mycotoxigenic fungi are very abundant in this region and are major food spoilage agents (Atanda *et al.*, 2013). The optimum temperature for the growth of *Aspergillus* species is between 24°C to 28° which is approximate the ambient climatic conditions in this part of the country and hence can predispose the crops to high prevalence of the aflatoxin contamination. Furthermore, toxigenic fungi contamination of crops is higher during the rainy season than the dry season, therefore, there is a greater risk of aflatoxin contamination of crops in this region since they

have more rainy months. The reports of Makun *et al.* (2009a and b) affirmed this fact.

CROPS PRONE TO INFECTION FROM FIELD TO STORAGE IN SOUTHEAST NIGERIA

South eastern Nigeria is replete with herbs, spices, medicinal plants, dried vegetables which are eaten with relish in different forms. These food items have been reported to be infected with moulds and contaminated with aflatoxin in Egypt (Darwish *et al.*, 2014). A positive correlation was found between the addition of spices to fresh meats and AF contamination. Being prone to heavy and prolonged months of rainfall particularly during harvesting of most crops grown, south eastern Nigeria stands a great risk of aflatoxin contamination of its crops and agricultural products. This is so because these crops require primarily drying during as part of the processing prior to storage. Although different agricultural products have varying degree they tolerate mould growth; many food products have been reported to be contaminated with mycotoxins (Atanda *et al.*, 2013). Cashew nuts, ogbono, cassava chips (abacha), rice, plantain chips, cocoa yam chips, gari, maize and a myriad of other dried vegetables, herbs and spices are produced in abundance in eastern Nigeria and are likely to be contaminated with aflatoxins as reported elsewhere within and outside (Table 1) Nigeria.

CROP HARVESTING, PROCESSING AND STORAGE PATTERN AND THEIR INFLUENCE ON AFLATOXIN CONTAMINATION

The period of crop harvesting plays a key role especially in post-harvest aflatoxin contamination of crops. Grains harvested after a session of dry weather and adequately dried to safe moisture content, sorted to remove infected and insect damaged grains before storage will have little or no chance of aflatoxin accumulation during storage compared to those harvested during or after a spell of wet weather. This is not always possible due to prolonged months of rains especially during the harvesting periods, hence inappropriate harvest time is a risk factor in Nigeria (Atanda *et al.*, 2013). Timely harvesting of crops also helps to limit aflatoxin formation. Aflatoxin contamination

increases with delayed harvesting of crops. Furthermore, crops harvested early require timely and proper harvesting to prevent fungal growth and subsequent aflatoxin formation (Kaaya *et al.*, 2006; Atanda *et al.*, 2013). Proper processing helps to keep crop products longer. Improper processing can increase aflatoxin contamination in several folds. To effectively control post-harvest aflatoxins contamination moisture content should be kept very low during storage, transportation and marketing to hinder biological activity of storage fungi. Adequate storage condition will ensure that activities of storage fungi particularly *Aspergillus* species and insect activity are prevented. Also storing produce in clean containers and sacks free from debris from previous harvest, sorting out damaged and bad grains which could be discoloured, deformed can considerably reduce aflatoxin levels in stored crop products (Makun *et al.*, 2007; Makun *et al.* 2009; Atanda *et al.*, 2013).

AFLATOXIN AND EXPORT POTENTIALS OF CROPS

Many crops produced in abundance in this part of the country have potentials to be exported to other countries where they can be sold to foreigners or to African emigrants. However, aflatoxin contamination of these crops will make it impossible for these crops to make it across Nigerian borders. Hence, aflatoxin contaminated crops cannot be marketed internationally were they will attract more income for the producers and/or traders. Such crops are either destroyed or most of the times forced back into the local markets where aflatoxin monitoring is grossly ineffective or non-existing at all.

AFLATOXIN CONTAMINATION LEVELS IN SOUTHEASTERN NIGERIA CROPS

Information on aflatoxin levels in crops in eastern Nigeria is almost not existing even though mycotoxins particularly aflatoxin research is a subject that have raised so much concern due to the hazardous nature of these toxins. Aflatoxin data available from the eastern part of Nigeria is only that analysed from dried okra and pepper and left over beans and gari from restaurant dishes in 1992 which ranged from 31.20-268.32 µg/kg (Atanda *et al.*, 2013). This level was quite very high in samples

Table 1. Aflatoxin status of crops in south east and other parts of Nigeria

Crops/products	Aflatoxin concentration (µg/kg)	Aflatoxin analysed elsewhere in Nigeria or other country?	Aflatoxin analysed in eastern Nigeria?
Spices	6.0	Yes	No
Ground red pepper	Not detected	Yes	No
Okra	Not detected	Yes	Yes
Stored Groundnut	100-2000	Yes	No
Palm wine	Not detected	Yes	No
Bitter leaf	>90		No
Rice	150-300	Yes	No
Maize	>500	Yes	No
Acha	<1.00	Yes	No
Melon (egusi)	>1000	Yes	No
Palm kernel	Not detected	Yes	No
Cowpea		Yes	No
Hot chili	<1	Yes	No
Dried pepper	Not detected	Yes	Yes
Cassava flour	<1.00	Yes	No
Yam	<1.00	Yes	No
Gari	<1.00	Yes	No
Cocoa beans	-	No	No
Cocoa yam	-	No	No
Kola nut	-	No	No
Spices	>1.00	Yes	No
Cereal grains	>3.0	Yes	No
Nuts and seeds	>3.0	Yes	No
Medicinal plants	>3.0	Yes	No
Abacha	-	No	No
Ogbono	-	No	No
Cashew nut	-	No	No
Dried plantain	-	No	No

Sourced from Makun *et al.*, 2011; Atanda *et al.*, 2013, Darwish *et al.*, 2014.

which are not known to have high affinity for *Aspergillus* growth. This is a pointer that aflatoxin contamination of crops in this region may quite be at alarming rates when analysed like done in other regions of the country.

AFLATOXIN RESEARCH IN SOUTHEAST NIGERIA: CURRENT STATUS

Available literature and reports on aflatoxin contamination analysis reveals that crops and agricultural products from other regions have been analyzed for aflatoxin from as far back as the 1960s yet only in Nsukka a town in one of the states from

the eastern region that aflatoxin was analysed from dried okra and pepper and left over beans and gari from restaurant dishes in 1992 according to Atanda *et al.* (2013). From then till date, there is a complete dearth of information on aflatoxin contamination of crops in this area. Yet, there are many crops and agricultural products with potential risk of aflatoxin contamination in the region. Hence, there could be utter ignorance of the existence and harmful effects of aflatoxins and other mycotoxins even among most of the well-read the society.

RISK FACTORS

(i). LIMITED PROCESSING FACILITIES

Environmental conditions, poor processing, and lack of proper storage facilities is linked to aflatoxin contamination in foods (Farombi, 2006). Storage and processing levels along the marketing chain is the most critical period in aflatoxin contamination especially when crops are not properly handled. Although most contaminated crops are discoloured, some contaminated crops show no sign of discolourations or deformity. Hence, they cannot be sorted away during processing. In such situations only, only laboratory analysis can detect presence of aflatoxin. Processing of crops and products are still largely dependent on primitive methods. Drying of produce is still 100% dependent on sunshine which is only sufficient during the dry season when crop produce requiring drying is almost not available anymore. Farmers and traders are forced to store their produce when not properly dried which predisposes them to mould infection. Many crops are being dried on bare tared road sides without placing them on nylon sheets; this is another unhealthy practice that predispose crops to mould infection during processing for storage.

(ii). LIMITED SUNSHINE AND LACK OF DRYING FACILITIES

In south eastern Nigeria, sunshine which is the main source heat for drying farm produce requiring drying is limited by heavy rains experienced in this region during the peak of harvesting of most crops. Also, there is no availability of artificial drying facilities that can serve as relief to insufficient sunlight during the drying periods. This will predispose most crops to growth of moulds and subsequent formation of

toxins in them. Aflatoxin contamination is usually most recurrent and severe at the storage and processing levels along the crops value chain.

(iii).OUTRIGHT IGNORANCE OF AFLATOXIN EXISTENCE

High incidence of aflatoxin in Nigeria is mainly caused majorly by dearth of awareness of the hazardous effects of aflatoxin contamination of crops/produce. Most farmers, producers and food crop handlers and/or processors have little or no knowledge of the effects of aflatoxigenic fungi or mould growth on crops. Moulded farm products are either dusted, washed or worst still processed for consumption or sales and/or consumption. The knowledge of the existence of mycotoxins would have led to the disposal of moulded crops and products.

CLIMATE CHANGE AND AFLATOXIN CONTAMINATION POSSIBILITIES IN SOUTH EASTERN NIGERIA

There is an array of reports that shows evidence that the ability of fungi to thrive and effectively produce mycotoxins in crops is greatly influenced by the prevailing ambient temperature and relative humidity in the crop growing, processing and storage area; as well as insect damage on crops either before or after harvesting. Climate change influence these factors and leads to variation of temperature and rainfalls which will enhance mould growth and hence greater chances of aflatoxin contamination. Food safety is a subject of worldwide concern and the possible upshot of long term change in weather patterns on the yields and quality of food crops produced, particularly for aflatoxins contaminations, have drawn the attention of some food crop production and processing experts in the past years, specifically from the stand point of risk analysis. With the increasing average temperatures, a shift in the incidence of mycotoxins especially aflatoxins is expected. Aflatoxin contaminations in the field are more prevalent during periods of high heat and drought stress on the host plant which favours *A. flavus* growth. As the effects of climate change are becoming more evident, regions that as of now are relatively unaffected are expected to increase as a result of hotter, dryer conditions (Battilani *et al.*, 2016). Such climatic condition will support the occurrence and

domination of highly toxigenic *Aspergillus* decent which will culminate to greater aflatoxin contamination events (Bandyopadhyay *et al.*, 2016). Aflatoxin contaminations are likely to occur in crops planted during extended cropping season that resulted in crop exposure to hot, dry temperatures. However, aflatoxin control technologies are not available to farmers in this region and this could result in high aflatoxin concentrations that will be faced in no distant time (Bandyopadhyay *et al.*, 2016).

AFLATOXIN MONITORING IN NIGERIA: CURRENT STATUS

Local and informal markets are mainly the form markets existing in Nigeria which makes aflatoxin concentrations in commodities offered in these markets unknown. There is high human aflatoxin exposure due to frequent occurrence of aflatoxins in crops and inadequate monitoring capacity (Bandyopadhyay *et al.*, 2016). However, some aflatoxin-conscious market segments are beginning to emerge in Nigeria and sub-Saharan Africa. Regrettably, the aflatoxin monitoring efforts are more or less targeted to crops/produce meant for export purposes or those to be sold to industries using these crops as raw materials. Crops meant for local consumption or sales still receive little or no substantial monitoring attention in Nigeria as a nation and no attention at all in the eastern region of Nigeria.

CURRENT EFFORTS TO MITIGATE AFLATOXIN IN NIGERIA

Aflatoxin control effort in Nigeria has received considerable attention in recent years. Highly toxigenic *Aspergillus* lineages have been successfully managed with biocontrol agents in both arid and semi-arid Nigeria, (Atehnkeng *et al.*, 2014; Bandyopadhyay *et al.*, 2016). However, this management effort is mostly targeted towards maize and groundnut fields in northern Nigeria. Aflatoxin contaminations in crops other than maize and groundnut have received little or no attention in Nigeria and still remain a major source of concern. More research to identify effective aflatoxin control technologies in other crops especially those where aflatoxin contamination occurs at post-harvest levels is very necessary for a holistic aflatoxin management in crops and products.

CONCLUSION

Review of available literature on aflatoxin studies reveals that crops and other agricultural products in south eastern Nigeria have not been analysed for aflatoxins and other mycotoxins. This calls for a serious concern to all and sundry. There is an urgent need to conduct such studies in south eastern region to ascertain associated aflatoxin and other mycotoxins contaminating south eastern consumer goods and agricultural products because there is a huge problem of mycotoxin contamination in other parts of Nigeria where there still exists a dearth of scientific information and data on the extent of the problem. The combination of insufficient drying and humid atmospheric conditions encourage *Aspergillus* growth and proliferation which results in unacceptable levels of aflatoxins in crops and other agricultural produce. Several studies in Nigeria have reported toxin in food and agricultural products in many regions of Nigeria with almost no report of such studies in the eastern region. The problem and effects of aflatoxin contamination cuts across many disciplines and hence multidisciplinary teams involvement is needed for effective study, recording, monitoring, evaluation and control of aflatoxins in the south east region and Nigeria at large. Also research on aflatoxin status of this region will help in the construction of the mycotoxin map of Nigeria by capturing all the regions of the country. The control of aflatoxins is an encompassing management approach that includes prevention of fungi growth in crops, crop products and feedstuffs, detoxification contaminated foods as an additional control strategy and continuous investigation of agricultural crops for aflatoxins accumulation, as well as in animal feedstuffs and foods for human consumption. Furthermore, good Agricultural practices ensuring early planting and use of recommended crop production practices, early harvesting, prevention of crop damage during harvesting, processing and storage, adequate drying of crops to very low moisture content and proper storage clean and dry environment will help to mitigate aflatoxin contamination of crops.

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