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FUNGAL FLORA AND AFLATOXINS (AFTS) CONTAMINATION OF GARRI IN PARTS OF AKWA IBOM STATE, NIGERIA

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ABSTRACT

Garri, a pre-eminent staple diet in Nigeria, accounts for over 70% of the total calorie intake of more than 50% of Nigeria's population. This study investigated the fungal flora and the level of aflatoxin contamination of garri sold in two open markets (Uyo and Oron) in Akwa Ibom State, Nigeria. Samples randomly purchased from each market were aseptically transported to the laboratory and cultured for fungi. Identification of fungal cultures was done using morphology of the isolates. Three subsamples from each location were extracted, spotted and quantified for aflatoxins B1, B2, G1 and G2. Values were the means from 3 subsamples. Data were processed using T-test and means separated for statistical significance at $P<0.05$ using the Least Significant Difference (LSD). Fungi isolated from both markets were *Fusarium verticolloides, Cercospora* sp, *Aspergillus flavus*, *A. niger* and *A. parasiticus.* From the Uyo samples, the following additional fungi were found *Fusarium solanii* and *A. parasiticus*. Aflatoxin B1 (AFB1) was significantly higher ($P<0.05$) in samples from Oron (21.67 μg/kg) compared to Uyo (3.33 μg/kg). Aflatoxins B2, G1 and G2 were below detection limit in the samples. AFB1 in garri samples from these two markets in Akwa Ibom State is being reported, probably for the first time.

Keywords: Garri, Fungal flora, aflatoxins, Open market and Akwa Ibom state

INTRODUCTION

The diet of many indigenous African people is supplemented with cassava products such as garri, fufu, tapioca etc in their varied forms (Bottalico and Perrone, 2002). In Nigeria, garri, a pre-eminent staple food, accounts for over 70% of the total cassava consumption (Ezeh *et al*., 2012). If the growth and metabolism of microorganisms are not controlled, they are capable of altering the condition of any food, causing food spoilage (Liu *et al*, 2013) and poisoning. Food poisoning is an illness resulting from the consumption of food containing toxin(s) secreted into it by the contaminating microorganism(s) (Bottalico and Perrone, 2002).

In the processing of garri from cassava, the cassava is peeled, washed, grated and thereafter dehydrated under pressure, before being finally fried, packaged and stored preparatory for sales in the market (Hartley *et al*., 1963). Expectedly, garri is prone to microbial contamination if all of the above processes are not carried out in tandem with strict hygienic practices (Alum *et al*., 2016). Mycotoxin contamination of food materials in the open markets in Nigeria has been reported in previous studies (Ezeh *et al*., 2012 and Rubert *et al*.., 2013). Aflatoxins (AFTs) appear to be more wide spread than all the other mycotoxins (Osuret *et al*., 2016). AFTs are metabolites of the fungi, *Aspergillus flavus* and *Aspergillus parasiticus* (Adebayo-Tayo and Ekerete, 2010). Ingestion of AFT contaminated food is known to be of severe toxicological implication in humans and animals (Atarwodi *et al*., 1994) where they could cause chronic liver disease (Hutanasu *et al*., 2009). According to a 2018 report of the European Food Safety Authority (EFSA), AFTs in food are known carcinogenic and teratogenic agents. Other economic debacles associated with AFTs include low yields of food and fiber crops (Cheeke, 1998).

In view of the enormous economic and health implications of AFTs in food, this work set out to determine the types of fungal flora associated with garri and their toxic metabolites in parts of Akwa Ibom State and to identify and quantify the presence of AFTs contamination in these garri samples.

MATERIALS AND METHODS

Isolation and Identification of Fungal Flora from Garri Samples

About 50 g of garri samples were randomly purchased from five sellers from each of the following markets: Itam market, Uyo (5°03'32.1"N 7°53'22.1"E) and Oron main market, Oron (4° 50' 0" N, 8° 14' 0" E), both in Akwa Ibom State. These samples were aseptically transported in sterile containers and sent to the laboratory where each of them were bulked and thoroughly mixed under aseptic condition. From a total 250 g bulked sample from each of the market, exactly 10 g was weighed out and serial dilution up to 10^3 was done using the method of Nwachukwu (2000). From each of these, 1 ml was cultured in sterile plates containing freshly prepared Potato Dextrose Agar (PDA) containing antibiotic and lactic acid. This was thereafter incubated at 31 °C until fungal growth of the full diameter of each plate was obtained. There was repeated sub culturing of each of the plates until pure plates were achieved. Identification of each of the fungus was done based on the morphologic characters of each fungus. The plates were then compared with the descriptions given by Talbot (1971); Deacon (1980) and Bryce (1992) for identification.

Extraction and Quantification of Aflatoxins (AFTs) in Samples

These were carried out in the pathology laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan-Nigeria. The UK standard was adopted for the entire procedure. The method of Bankole and Adebanjo (2003) was used for the extraction, spotting and quantification of AFTs in samples using the High Performance Thin Layer Chromatography (HPTLC) Plate, U-V Light Scanner (ENF-260C/FE) and CAMAG TLC Scanner 3 (set at a detection limit of below 1 μg/kg). Recovery of toxin was >85%. Values reported for AFTs load were the means of three subsamples per sample from each location.

RESULTS

Mycoflora from Garri Samples

The mycoflora associated with garri samples from both locations were almost identical. These include *Fusarium* sp (Plate 1), *Cercospora* sp (Plate 2), *Aspergillus flavus* (Plate 3) and *Aspergillus niger* (Plate 4). From the Uyo samples however, *A. parasiticus* (Plate 5) was found in addition to all the others above.

Aflatoxins (AFTs) in Samples

The results as presented in table 1 show that aflatoxin B1 (AFB1) was present in the garri samples from both open markets. However, aflatoxins B2, G1 and G2 were below the detectable limit of 1 μg/kg in all the samples. AFB1 in Uyo samples had a mean value of 3.33 μg/kg, a value significantly lower ($p<0.05$) than the mean value of 21.67 μg/kg present in samples from the Oron open market.

Plate 1: *Fusarium verticolloides* × 0.5

Plate 2: *Cercospora* sp × 0.5

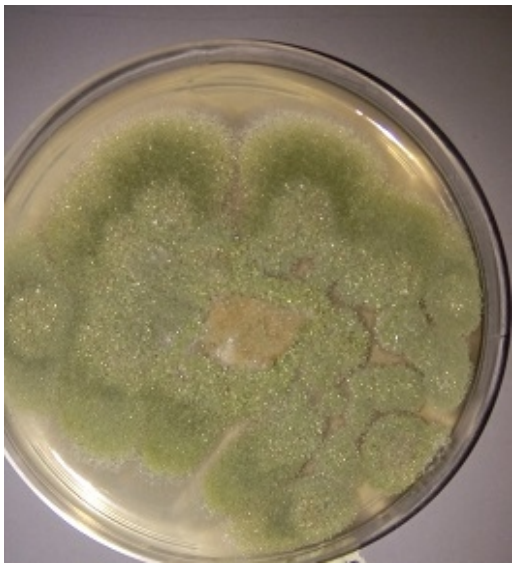

Plate 3: *Aspergillus flavus* × 0.5

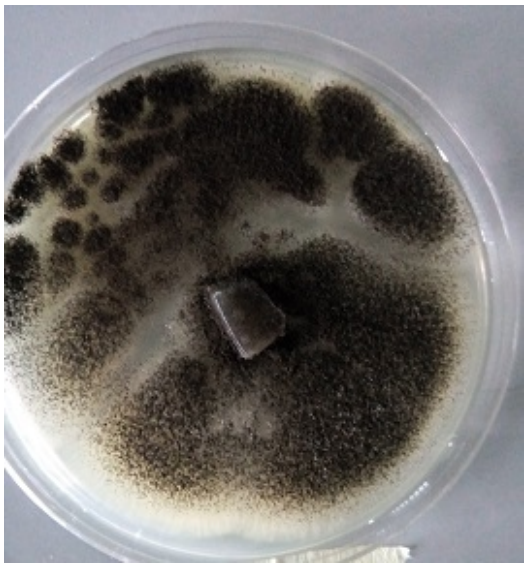

Plate 4: *Aspergillus niger* × 0.5

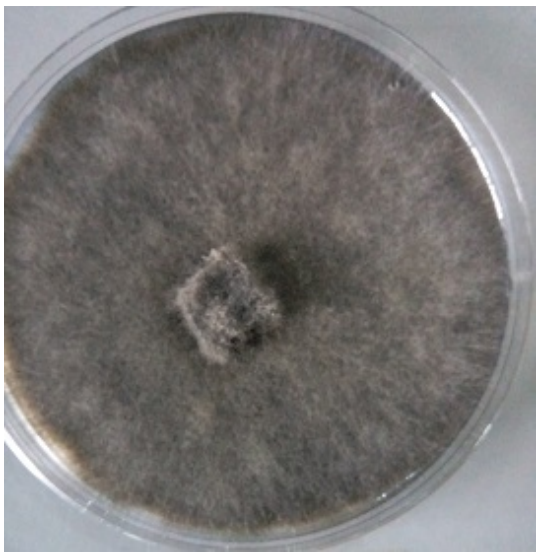

Plate 5: *Aspergillus parasiticus* × 0.5

Table 1: Aflatoxins Load (µg/kg) in Garri Samples from Uyo and Oron

Sample	Location	Mean±SEM AFB1	Mean±SEM AFB2	Mean±SEM AFG1	Mean±SEM AFG2
Garri	Uyo	3.33±2.50[a]	0	0	0
Garri	Oron	21.67±2.33[b]	0	0	0

Mean values carrying different superscripts are significantly different at $p < 0.05$.

DISCUSSION

Microbiological sources of food contamination are more preponderant and are therefore of greater concern than other sources of contamination such as chemical and physical sources because of the quantum of illnesses associated with it (Scallan *et al*., 2011). In food safety issues therefore, microbiological considerations are of paramount importance.

The most palpable concern associated with fungal contamination of food is in connection with the production and deposition of mycotoxins on the food material. The most important genera of mycotoxigenic fungi are *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium*, *Penicillium* and *Stachybotrys*. (Yaling *et al*., 2008 and Averkieva, 2009). The results obtained from the present study showed that the garri sold in our open markets in Akwa Ibom state are contaminated by two fungal genera (i.e *Aspergillus* and *Fusarium*) out of these five notorious fungal genera earlier mentioned.

AFTs and fumonisins have been reported as being the most common mycotoxin (Wu *et al*., 2014), affecting not less than 25% of the world´s agricultural food sources (Williams *et al.*, 2004; Yard *et al.*, 2013). Mycotoxins generally are produced by over 100 filamentous fungi containing some 400 secondary metabolites with toxigenic ability (Kabak *et al.*, 2006). On its own, it has been reported that over 4.5 billion individuals in developing countries are at a risk of exposure to AFTs poisoning in food (Williams *et al*., 2004). AFTs have been reported to be present in rates above the permissible level of 20 μg/kg or 20 ppb in staple foods such as cassava flour, groundnut and groundnut paste sold in some open markets in Kampala, Uganda (Osuret *et al*., 2016). Results from this study therefore leaves much to worry about as the mean value of 21.67 μg/kg of AFTB1 in garri samples from Oron market is above the permissible level in food. The far reaching implication of AFTB1 as a group 1 carcinogen in humans (Seo *et al*., 2011) as well as their hepatotoxic (Abdel-Wahhab *et al.*, 2007) and immunosuppressive nature (Mehrzad *et al.*, 2014) has been documented. By implication, apart from the obvious negative effects of AFTB1 poisoning in humans, it is also expected to have a colossal adverse effect on the socio-economic matrices of the society which include loss of human and animal life, increased cost in human health care and animal care, drop and or losses in livestock productivity, loss of forage plants and animal feeds, regulatory and research costs targeted at mitigating the effects of mycotoxin poisoning.

The fact that the other AFTs (B2, G1 and G2) were not detected in any of the samples does not necessarily indicate that they were altogether absent. Rather, it could be indicative of the fact that they were present below the detectable limit of 1μg/Kg. As such, an unwitting cumulative exposure to the sub lethal doses over a long time may eventually produce some undesirable effects on human health and well being.

In developed countries of Europe and the USA, stringent standards of evaluating and enforcing compliance to the permissible limits of mycotoxins in food have been well developed. In the developing countries of Africa however, there appears to be a lax and uncoordinated regulatory and enforcement regimes by the concerned agencies of government at ensuring compliance to the locally adopted permissible mycotoxin limits adopted by these countries.

The findings from this research may bear a subtle correlation with the increasing incidence of some debilitating cancer and organ failure experienced in Akwa Ibom state (Nwafor and Nwafor, 2018).

CONCLUSION

From available literature, this is probably a first report of AFTB1 contamination of garri samples sold in the open markets in Akwa Ibom state. This calls for the concern of all relevant stakeholders.

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