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MYCOFLORA AND AFLATOXIN CONTAMINATION OF SOME FOODSTUFFS

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ABSTRACT

Analysis was made of the mycoflora and aflatoxin contamination of Rice (*Oryza sativa)*, Beans (*Phaseolus vulgaris),* Corn (*Zea mays),* and Groundnut (*Arachis hypogaea)* sold in four different markets in Sango-Ota, Ogun state, Nigeria. Sixty four samples comprising of four samples of each foodstuff from four food vendors in four different markets was assayed. The samples were contaminated with different species of fungi to include *Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, Rhizopus nigricans, Rhizopus oryzae, Saccharomyces cerevisiae, Aspergillus parasiticus, Fusarium moniliforme, Fusarium verticilliodes, Aspergillus ochraceus, Cladosporium cladosporioide, Mucor spp, Trichodema spp, Rhizopus arrhizus* and *Aspergillus fumigates. Aspergillus flavus and Fusarium spp* had the highest rate of occurrence among the isolated fungi. Aflatoxins B1, B2, G1 and G2 were found associated with the samples at concentration ranging from 9 - 25 ppb, 8 - 12 ppb, 6 - 21 ppb, 4 - 8 ppb respectively. The fungal counts were between 6.3 x 10^2 to 7.0 x 10^3 cfu/g. The moisture content and the pH of samples were between 10.9 to 28.0% and 6.20 to 6.66 respectively. Effective storage and adherence to HACCP principles will help prevent contamination of foodstuffs with aflatoxigenic fungi.

Keywords: Aflatoxins, Aflatoxigenic Fungi; Foodstuffs; Food Storage, HACCP

INTRODUCTION

The growth of some fungal spp in food and under conducive environmental conditions results in the production of mycotoxin(s). Some mycotoxins or mycotoxin derivatives have found use as antibiotics, growth promotants, and other kinds of drugs; still others have been implicated in disease and death in humans and other animals and as chemical warfare agents **[1, 2, 3]**.

The most important mycotoxins associated with human and veterinary diseases, include: aflatoxin, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone. Others are

alternaria toxins, penicillic acid, sterigmatocystin and sambutoxin.

Mycotoxins are not only hard to define, they are also challenging to classify due to their diverse chemical structures and biosynthetic origins, their myriad biological effects, and their production by a wide number of different fungal species. Based on their biological effects, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, teratogens, mutagens, carcinogens, and allergens **[1, 2, 4, 5]**.

The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the Aspergillus fungi specifically *Aspergillus flavus* and *Aspergillus parasiticus*. Under favourable conditions of temperature and humidity, these fungi grow on certain foodstuffs and feeds, resulting in the production of aflatoxins, which can enter into the human food chain directly through foods of plant origin (cereal grains, nuts), indirectly through foods of animal origin (kidney, liver, milk, eggs) **[6-9]**.

The most pronounced contamination has been encountered in tree nuts, peanuts and oilseeds, including corn and cottonseed. The major aflatoxins of concern are designated B1, B2, G1 and G2. These toxins are usually found together in various foods and feeds in various proportions **[7, 8, 10, 11, 12]**. However, aflatoxin B1 is usually predominant and is the most toxic. Aflatoxin M, a major metabolic product of aflatoxin B1 in animals and is usually excreted in the milk and urine of dairy cattle and other mammalian species that have consumed aflatoxin-contaminated food **[2, 13, 14, 15]**.

These poisons are completely heat stable, so neither cooking nor freezing destroys the toxin. They remain on the food indefinitely. Aflatoxins are produced on grains and legumes mostly during storage, so the grains and legumes must be stored correctly to limit this problem **[16, 17]**.

Aflatoxins produce acute necrosis, cirrhosis and carcinoma of the liver in a number of animal species, no animal species is resistant to the acute toxic effects of aflatoxins. Hence it is logical to assume that humans may be similarly affected. Aflatoxin B1 is a very potent carcinogen in many species, including non human primates, birds, fish and rodents. In each species, the liver is the primary target organ of acute injury **[14, 18-21]**.

Ascorbic acid and *Nigella sativa* a spicy potent oil belonging to ranunculacea seeds oil could reduce aflatoxin induced liver cancer **[22, 23]**.

Fix in Toxin is a kind of pentonite (clay) consisting of sodium calcium alumina silicate, a non toxic agent and absorbent for

a wide variety of toxic agents [24]. It acts as an enterosorbant that rapidly and preferentially binds aflatoxin uptake and bioavailability [25].

In view of the fact that foods from corn, rice, groundnut, beans are consumed in a high rate in Nigeria, It is important to ensure that foods consumed is of premium quality of zero or minimal levels of aflatoxin and other contaminants. This is because people are as healthy as the food they eat. In addition to this, Food has added to the economical income of man but unacceptable aflatoxin levels could pose a threat to this opportunity.

This work was to assay for the mycoflora and aflatoxins contamination associated with some foodstuffs sold in Sango-Ota with a view to enlightening the people on need for proper foodstuff storage.

MATERIALS AND METHODS

Sample Collection and Treatment

Sixty four samples comprising of four samples of each foodstuff from four vendors in four different markets was assayed. Approximate 200g samples of Rice (*Oryza sativa)*, Beans (*Phaseolus vulgaris),* Corn (*Zea mays),* Groundnut (*Arachis hypogaea)* were randomly purchased from the vendors and placed in sterile specimen containers that were appropriately labelled. Samples were transported to the laboratory for analysis within one hour of collection. The samples were ground to powder using sterile Warring blender and stored in sterile, dry specimen bottles at room temperature for further use.

Isolation of Fungi

Standard microbiological procedure was adopted, Saboraud Dextrose Agar (SDA) (Oxoid, England) to which Penicillin and Streptomycin had been incorporated and using spread plate technique. The medium was prepared based on manufacturer's instructions and allowed to solidify. Ten grams of the samples was diluted to 10^{-5}. One millilitre of each dilution was dispensed in duplicate on media and spread uniformly.

The plates were allowed to set and incubated at 28±2°C for 3-5 days. Colonies were counted using the colony counter (Gallenkamp, England). Counts were expressed as colony forming unit per ml of sample homogenate (cfu/ml). Discrete colonies were isolated and purified by repeated sub-culturing. Pure cultures were stored on slants at 4^0C for further characterization. Identification of isolates was based on their macroscopic and microscopic characteristics with reference to standard identification keys and atlas [26, 27].

Determination of Moisture Content and pH of Samples

The moisture content was determined by standard analytical method [28, 29].

Samples were oven dried at 105°C for 2 hours to constant weight. For pH determination, ten grams each of the samples were weighed and 90ml of distilled water was added and mixed thoroughly to make slurry. The pH readings were taken using digital pH meter (digital thermo pH meter mod B-E105). Triplicate readings were recorded and the mean value was obtained.

Assay for Aflatoxins in Samples

The Thin Layer Chromatography (TLC) technique was employed **[30, 31]**. Ten grams (10 g) of each powdered food samples were extracted separately with 50 ml of methanol/water (60:40 v/v) and later with 50 ml chloroform. The resultant solution was filtered through anhydrous sodium sulphate. The final extract was evaporated to dryness under liquid nitrogen and the residue was dissolved in 2ml chloroform .These were transferred into a screw cap tubes and kept at 4°C inside the refrigerator. From the extract, 5, 10 and 15µl were spotted on three different points on silica gel DG 254; along side with the same volumes of the aflatoxin standards near the previous sample extract spotted points **[32]**. The plates were developed in the TLC tank using 100 ml chloroform/acetone mixture (of ratio 96:4 v/v solvent system). Aflatoxins were identified and quantified by the intensity of the colour fluorescence under UV-light at wavelength of 365nm of colour with that of aflatoxin standards **[30]**.

RESULTS

The mean fungal count, moisture content and pH of samples is presented in **Table 1**. It shows that O*ryza sativa* and *Zea mays* has higher levels of fungal contaminations with counts of 7.0 x 10^3 and 3.7 x 10^3 cfu/ml. Similarly, *Zea mays, Arachis hypogaea* and *Oryza sativa* has 28.0, 23.0 and 22.0% moisture contents respectively. All the samples had pH above 6.0.

Table 2 reveals the fungal isolates from the samples. All the samples were contaminated with mixed mycoflora. *Aspergillus flavus* and/or *Aspergillus parasiticus* and fusarium spp are common place to all the samples. Different species of fungi were isolated to include *Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, Rhizopus nigricans, Rhizopus oryzae, Saccharomyces cerevisiae, Aspergillus parasiticus, Fusarium moniliforme, Fusarium verticilliodes, Aspergillus ochraceus, Cladosporium cladosporioide, Mucor spp, Trichodema spp, Rhizopus arrhizus* and *Aspergillus fumigates.*

The concentrations of aflatoxins are shown in **Figure 1**. Corn and groundnut contain 22ppb and 25ppb of aflatoxin B1 (AFB1). The **Figure 1** also reveals that beans contain aflatoxin G1 (AFG1) of 21ppb. Rice had the least concentration of aflatoxins.

Table 1: Mean Fungal Count (cfu/ml), Moisture Contents and pH of Samples

Samples	Fungi Count (cfu/ml)	Moisture Content (%)	pH
Oryza sativa	7.0 x 10^3	22.0	6.48
Phaseolus vulgaris	7.5 x 10^2	10.9	6.66
Zea mays	3.7 x 10^3	28.0	6.59
Arachis hypogaea	6.3 x 10^2	23.0	6.20

Table 2: Fungal Isolates from Foodstuff Samples

Samples	Fungi Isolated
Oryza sativa	*Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, Rhizopus nigricans, Rhizopus oryzae, Saccharomyces cerevisiae, Fusarium spp.*
Phaseolus vulgaris	*Aspergillus niger, Aspergillus parasiticus, Fusarium spp, Cladosporium spp, Trichodema spp.*
Zea mays	*Fusarium moniliforme, Fusarium verticilliodes, Aspergillus flavus, Aspergillus ochraceus.*
Arachis hypogaea	*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Mucor spp, Fusarium spp, Rhizopus arrhizus, Cladosporium cladosporioide.*

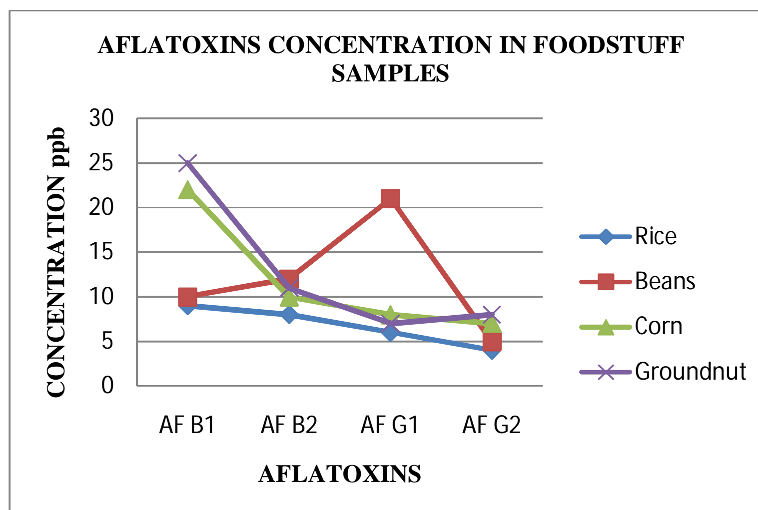

Figure 1: Aflatoxins Concentration in the Foodstuff Samples

DISCUSSION

The mean fungal count of 6.3 x 10^2 - 7.0 x 10^3 cfu/ml, is within acceptable limit for microbial contaminations in foods **[33]**. The presence of *A. flavus, A. parasiticus* and Mucor and Aspergillus spp however, call for concern as these moulds are known to produce aflatoxins and have been implicated in mycoses. The higher counts recorded for *Oryza sativa* and *Zea mays* could be attributed to the higher moisture contents.

High water activity is known to encourage growth and proliferation of microorganisms. The presence of varied mycoflora of *Aspergillus spp, Rhizopus spp, Saccharomyces spp, Fusarium spp, Mucor spp,Trichodema spp, Cladosporium spp* make its consumption hazardous to health considering the fact that apart from aflatoxins, several other deleterious fungal metabolites that were not assayed for in this research could be produced in the foodstuffs. The microbial contaminants in food and food products have been reported to be a direct reflection of the environment through which the product has passed. Similarly the extent of microbial contamination of foods have been reported to depends on the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce **[34-37]**. Microorganisms form part of the natural community of plants and animal products and many will be present at the time of consumption **[35, 37]**.

The environment in which foodstuffs are displayed in the markets is not always hygienic and this is an avenue for contamination. Very often, food vendors display the food samples in an open tray or bowl beside gutters or refuse heaps, this encourages fungi attack and subsequent production of toxins.

Aflatoxins were detected in samples of the four foodstuffs analysed. The US FDA action levels for aflatoxins, stipulates 20ng/g as limits for all products except milk, designated for humans. A concentration of 21; 22; and 25ppb detected in *Phaseolus vulgaris, Zea mays* and *Arachis hypogaea* calls for concern as these levels are higher than stipulated limits for products designated for humans **[7, 10, 38, 39]**. Aflatoxins are highly carcinogenic, causing hepatoma (cancer of the liver) and have also been associated with acute hepatitis in man, mostly the developing world **[7, 10, 12, 40]**. Aflatoxin have been reported in grapes and musts **[41]**, edible nuts and nut products, milk and milk products, bush mango seeds **[42]**.

CONCLUSION

In Nigeria rice, beans, corn and groundnut are staple foods; it could mean that most consumers would have been consuming high doses of aflatoxins and other fungal metabolites. Therefore, complicated aflatoxin analysis procedure should be replaced with commercial kits that are easy to run and health regulatory bodies such as IPAN, NAFDAC and SON should carry this out so that the toxin can easily be detected and samples containing them discarded. Improper storage of food may lead to insect infestation, fungal attack and mycotoxins production.

Some foodstuffs stored for sale in Sango-Ota were contaminated with aflatoxigenic fungi and contain aflatoxins above limits stipulated for humans, they are therefore not recommended for human consumption, prolonged intake may constitute a health hazard. Since most of the moulds isolated are probably contaminants rather than originating in the food samples, better methods of storage and effective HACCP application will reduce their incidence or eliminate them.

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