Original Research Article

Mycotoxin Content of Fungi-Contaminated Sorghum Sold in Selected Markets in Ibadan, Nigeria

ABSTRACT

Aim: This study sought to evaluate the mycoflora associated with sorghum retailed in some selected markets in Ibadan metropolis and assessing the risks associated with their consumption.
Methodology: Sorghum samples were obtained from *Sango*, *Oja Oba*, *Ojoo*, *Bodija* and *Orita* markets in Ibadan, Nigeria. Associated fungi were isolated by direct plating of the sample on Potato Dextrose Agar while they were identified by morphological and microscopic methods. Proximate and aflatoxin content of the samples were determined using standard methods.
Results: The presence of *Aspergillus flavus, A. niger, Mucor sp*, *Penicillium sp*, *Trichotheciumsp*, *Curvularia sp.*, *Fusarium monoliforme, Alternaria alternata*, and *P. roquefort*iwere observed in the samples. Among these, *A*. *flavus* and *A. niger* were the most predominant with occurrence of 34.92% and 23.81% respectively. The aflatoxin analysis showed a concentration ranging from 46 – 102ppb and was highest in samples collected from *Ojoo* market. Aflatoxin contamination was not detected in sixty percent of the samples collected while samples from*Oja Oba* and *Bodija* markets had the most samples contaminated by aflatoxins.
Conclusion: The presence of fungi and aflatoxin was confirmed in some of the samples collected from major markets in Ibadan. This suggests the need for continuous surveillance and the recommendation of measures that will prevent fungal infestation.

Keywords: Sorghum, mycotoxin, fungi, Aspergillus flavus

1. INTRODUCTION

Cereals and their products constitute an important component of the staple diet of human and animal populations. Therefore, they form a major ingredient in farm animal diets while the co- and by-products obtained from them are important in animal feed formulas [1]. Nutritionally, cereals are good sources of energy that are rich in carbohydrate, fat and fibre; and provides many of the vitamins and essential minerals required for nutrient and metabolism.

Sorghum bicolor is a grass species mainly cultivated for its grain, and like other cereals, serves as food for humans, incorporated in animal feed formulation, and for ethanol production through a fermentation process. Sorghum is an annual crop that is commercially cultivated in tropical and subtropical regions [2]. Next to rice, wheat, maize, and barley, sorghum is consideredthe fifth-most important cereal crop in the world, with 59.34 million metric tons of annual global production in 2018. Nigeria is considered a major producer of sorghum in the world with a predominant farming of the crop in Northern Nigeria.

Sorghum grains are often contaminated by moulds [3], posing a serious biotic limitation in sorghum production areas. Several of these fungi are either facultative parasites or saprophytic fungi, contributing to pre- and post-harvest deterioration of grains [4]. Spoilage fungi species thrive at 15–19% moisture content, thus, significantly increasing respiratory activity which brings about an elevated temperature through fungal colonization and succession [5]. Some grain moulds are capable of producing mycotoxins which are harmful to both humans and animals [6]. The epidemic of

mycotoxin proliferation in sorghum and other cereals globally, has hampered production of the crop, which is responsible, in turn, for food shortage, economic crunch of exporting nations, poor quality of life of farmers who cultivate sorghum for either commercial or subsistent purposes, amongst many other setbacks. These losses are mediated by species that are associated with mycotoxin production on contaminated sorghum grains which include *Aspergillus* sp. *Fusarium* sp. and *Penicillium sp*. [6]. The study therefore aimed at evaluating the mycoflora associated with sorghum retailed in selected markets in Ibadan metropolis and assessing the risks associated with their consumption.

2. MATERIAL AND METHODS

2.1 SAMPLE COLLECTION AND PROCESSING

A total of twenty- five (25) samples were obtained from the following markets: *Ojaoba, Ojoo, Orita Challenge*, *Bodija* and *Sango* markets in Ibadan, Oyo state, Nigeria. The samples were collected and sealed in Ziploc bags. In the laboratory, the samples were pulverized using an electric blender to obtain a fine powdery texture.

2.2 ISOLATION

One gram (1g) of the powder sample was directly plated on Potato Dextrose Agar (PDA) plates which was supplemented with Streptomycin antibiotic and incubated at 28±2°C for 72 hours. The isolated fungi were characterized by cultural features and morphological characteristics by microscopic examination of wet mounts of the isolate in lactophenol cotton blue stain. The features of the isolates obtained were compared with those described in standard manual of fungi identification [7].

2.3 AFLATOXIN DETERMINATION

2.3.1 Sample Preparation

The test sample (5g) was weighed into a clear conical flask and 25 ml of 70% methanol solution was added. The flask was vigorously shaken and allowed to stand for 15 minutes. The mixture was filtered and the filtrate (100µl) obtained was added to 900µl of 70% methanol solution to give one-tenth dilution.

2.3.2ELISA Procedure

Fifty microliters (50µl) of each standard (0ppb, 4ppb, 10ppb, 20ppb, 40ppb) was added into the first 5 dilution wells and 50µl of test sample (from one tenth dilution) to the remaining well(s); these were properly mixed. One hundred microlitres(100µl) from dilution wells were transferred into antibody coated wells and incubated at room temperature for 15 minutes; these were washed three times with distilled water and tap dried. Exactly 100µl of substrate solution was transferred into the antibody coated wells and incubated at room temperature for 5 minutes. The stock solution (100µl) was dispensed into the antibody coated wells and the absorbance of each well was observed at 450nm with a differential filter of 630nm [8].

3. RESULTS AND DISCUSSION

The results of the characterization of the fungi isolates (Table 1) showed the presence of the following fungi: *Aspergillusflavus, A. niger, Mucor*, *Penicillium*, *Trichothecium*, *Curvularia*, *F. monoliforme, Alternaria alternata*and *P. roqueforti*. Samples sourced from *Sango* market were found to contain *A*. *flavus, Mucor* and *Penicillium*. Fungi isolates including *Trichothecium*and *A*. *flavus* were present in samples obtained from Oja-oba market while *Curvularia*, *A*. *niger*and *A*. *flavus* are present in samples obtained from *Bodija* market. Samples from *Orita* market were contaminated with *F. monoliforme, P. roqueforti,* and *Mucor.* The results reveal that samples obtained from all locations were contaminated with *Aspergillus spp.,* except those obtained from Orita market.

Table 1: Percentage Frequency of Occurrence of isolated fungi in the various locations

Isolate	Frequency of Occurrence By Location (%)					Total	%
	Sango	*Oja-Oba*	*Ojoo*	*Bodija*	*Orita*		
A, flavus	3.5	3	-	4.5	-	11	34.92
A. niger	-	1	3	3.5	-	7.5	23.81
Mucor	1	-	-	-	1	2	6.34
Penicillium	1.5	-	-	-	-	1.5	4.80
Trichothecium	-	1	-	-	-	1	3.17
Curvularia	-	-	1.5	1.5	-	3	9.52
F. moniliforme	-	-	-	2	-	2	6.34
A. alternata	-	-	1.5	-	-	1.5	4.80
P. roqueforti	-	-	-	-	2	2	6.34
Total (%)	6(19.05)	5(15.87)	6(19.05)	11.5(36.51)	3(9.52)	31.5(100)	100

The mean frequency of occurrence (%) of the isolates obtained is shown in Table 1 and Figure 1. *A. flavus* occurred with the highest frequency (34.92%). This was followed by *A. niger*(23.81%) and *Curvularia* (9.52%). *Trichothecium*had the least percentage occurrence of 3.17%. Samples collected from *Bodija* market had the highest occurrence of microorganisms (36.51%) while the samples from *Orita* market had 9.52% of all the isolates obtained.

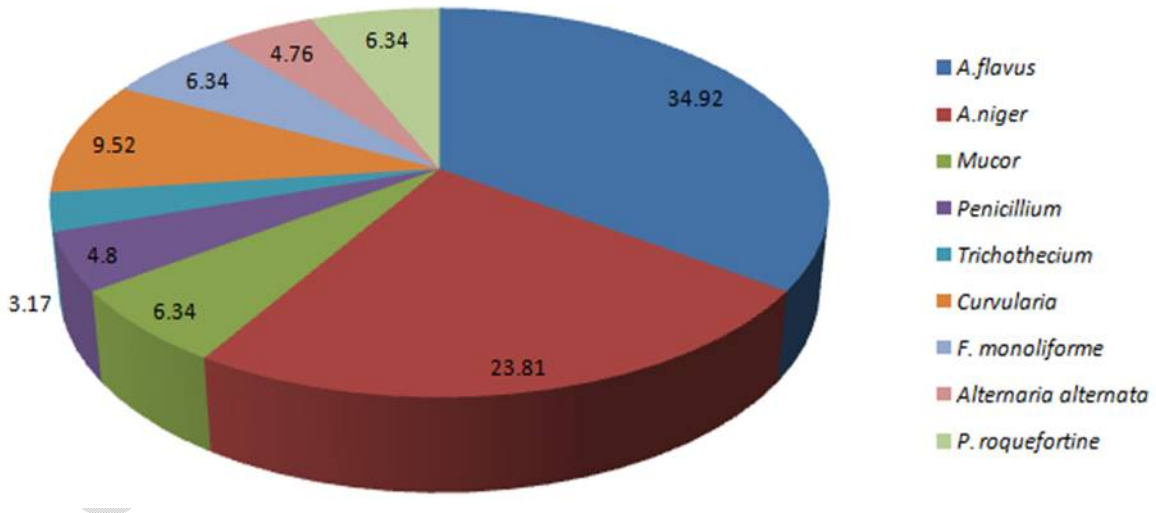

Figure 1: Percentage Mean Occurrence of fungi isolated

Table 2 shows the aflatoxin concentration (ppb) for selected sorghum grain samples obtained from different locations. Fifteen of the twenty five samples collected had either infinitesimal content or aflatoxin was absent in such samples, hence they were not reported here. The other ten samples have aflatoxin content ranging between 46-102 ppb. Three samples from each of *Bodija* and *Oja Oba* markets have high aflatoxin contents ranging between 78-80ppb and 66-72ppb

respectively. The samples (2) from *Ojoo* market recorded the highest aflatoxin content (83 ppb and 102ppb) while the only sample in this category from *Orita* market, was recorded to have the least aflatoxin content (46ppb).

Table 2: Aflatoxin concentration of sorghum samples

Sample	**Total Aflatoxin Concentration (ppb)**
Sango 5	68
Oja Oba 3	72
Bodija 1	78
Orita 2	46
Bodija 3	78
Ojoo 5	83
Ojoo 3	102
Bodija 4	80
Oja Oba 5	71
Oja Oba 4	66

Microbial culture from the samples collected led to the isolation of fungi from seven different genera; *Aspergillus,Mucor*, *Penicillium*, *Trichothecium*, *Curvularia*, *Fusarium* and *Alternaria*. Representatives from these genera are commonly associated with grain infestation especially in storage. The research of [9] found similar fungal population with the exception of *Trichothecium*. According to their research, the grains had been infected from the field, and this continued to storage with further proliferation. As observed in our research also, the most predominant fungus was *A. flavus* and this was followed by another fungus from the same genus, which agrees with the findings of [10]. Being natural residents of the soil, they infect the plant early on in the field and, in the presence of suitable conditions, are able to proliferate remarkably. The presence of species of *Aspergillus, Fusarium, Penicillium, Curvularia, Mucor* and *Alternaria* have been previously reported in sorghum samples collected in Nigeria. Researches carried out in other regions have discovered a similar trend. For instance, the same group of isolates stated above were isolated from sorghum grains collected in Eastern Ethiopia while proposing that variation in fungal population may be caused by a complex interaction of factors available during sorghum growth on the field, during harvest, and storage practices used [11].

Where aflatoxin was present, the concentration ranged between 46-102ppb which is above the maximum limit of 4µg/kg set by the EU and the limit of 20 µg/kg set for aflatoxin (B1) in Nigeria. The contamination of sorghum by mycotoxins has been previously reported in Nigeria and some of these contaminated grains were found to contain toxin levels beyond the prescribed levels. Some of the isolates obtained including *Aspergillus*, *Penicillium* and *Fusarium* are known to be mycotoxin-producing fungi. Furthermore, the storage conditions of the grain, including the nutrients present in the grains are favourable for fungal growth and mycotoxin production [12]. This is in addition to other environmental conditions including high temperature, moisture content, poor pre-harvest and postharvest conditions [13].

4. CONCLUSION

Samples obtained at the sampling sites were found to be infected with fungi including the toxigenic strains, hence a likely chance of predisposing consumers to severe health hazards. While considering the factors that predispose sorghum to fungal infestation and the eventual production of aflatoxins, it is necessary to establish mitigation techniques against these factors at all stages of production and retail of sorghum which is a very important raw material for many food products that are used for infants, adults and livestock.

CONSENT

Not Applicable

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