

Isolation and Identification of Aflatoxin Producing Fungi from Different Foodstuffs at Shuwarin Market, Jigawa State, Nigeria

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Abstract

Aflatoxin is a word derived from *Aspergillus spp* toxin which is naturally occurring mycotoxins that is produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi. The aim of this study was to isolate and identify aflatoxin producing fungi from four different grains (maize, rice, cowpea, and groundnut). During the research, four (4) different grain samples were obtained from Shuwarin market (Kiyawa L.G.A), Jigawa state. The samples were analyzed for microbial examination through serial dilution, inoculation, incubation, subculture and microscopy. Six (6), fungal species were isolated which included *Aspergillus flavus*, *Rhizopus sp*, *Penicillium sp*, *Fusarium sp*, *Cladosporium sp* and *Aspergillus niger*, where *A. niger* has the highest number of isolates (16; 40%), *Rhizopus sp* with 12 (30%) isolates, and *A. flavus* having 8 isolates (20%), *Penicillium sp*, 2 (5%), *Cladosporium sp* has and *Fusarium sp* has 1(2.5%) having 1 (2.5%), each, having the lowest number of isolate. Furthermore, the samples were also used to detect the presence of aflatoxin using thin layer chromatography technique (TLC). The contamination levels found ranged from the relative factor value (Rf) for maize, the Rf 0.94 registering the highest level of aflatoxin content and groundnut with Rf value of 0.08 registering the lowest level of aflatoxin content. This is due to improper harvesting, handling, processing, storage, and poor hygiene of the farmers and vendors. Therefore, the farmers and vendors have to be trained and enlightened on the method of harvesting, storage, handling, processing, transportation and selling of cereals and legumes so as to reduce the risk of causing health problems to the consumers.

Keyword: Aflatoxin, Fungi, *Aspergillus flavus*, *Rhizopus spp*, *Penicillium spp*

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INTRODUCTION

Aflatoxin is a highly toxic secondary metabolite that contaminates a number of crops causing a great economic loss (Cary *et al.*, 2009; CAST 2002). Several other moulds and fungal species also produce aflatoxin and among them include *A. nomius*, *A. pseudotamarii* and *A. bombycis* (Peterson *et al.*, 2001). The biosynthetic pathway of aflatoxin in *A. flavus* and *A. parasiticus* are similar and characterized well by Cary *et al.*, (2009) and Yu *et al.*, (2004).

Aflatoxin is produced in certain foods and animal feeds and without doubt its the best known and most intensively researched mycotoxins in the world. It is responsible for various diseases, such as aflatoxicosis in livestock, domestic animals and humans throughout the world (Bhatnagar *et al.*, 2003). The occurrence of aflatoxin is influenced by certain environmental factors, hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of crop commodities to fungal invasion during pre-harvest, storage or processing periods (Bhatnagar *et al.*, 2003).

To avert this situation, an increase in the productivity of agricultural food crops such as groundnut, maize, rice millet and cowpea are not only necessary being the part of the staple foods and the main cash crops of most of these countries but must go hand-in-hand with better farming practices and post-harvest handling. These agricultural produce are very prone to aflatoxin contamination, particularly during growth periods, harvesting, threshing, and drying (Siriacha *et al.*, 1989). Contamination can also occur when grains are poorly stored which can accelerate growth rates of *Aspergillus* and other fungal species (WHO, 2006).

Aflatoxin-producing species of *Aspergillus* are common and widespread in nature. These crops (maize, rice, cowpea, and groundnut) are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high-humid environment, or damage from stressful conditions (Udoh, 2000).

Mycotoxins affect nutritional and economic value of staple foods and cash crops especially in developing countries including those in Africa. Of the many mycotoxins, aflatoxin is of major concern especially in countries where agricultural practices are not strictly controlled, human and animal exposure to mycotoxins is very high (Wagacha *et al.*, 2008).

Attention is only paid to meet export criteria while the effects of aflatoxin on health of the local consumers is not prioritized. The contamination of foods with aflatoxin has in recent times created a great alarm on food security in Africa (Leslie, 2005). It has caused massive economic losses on export and import markets and diseases such as impaired immune system, cancer and stunted growth in infants (Williams *et al.*, 2004).

In Nigeria, groundnut, maize and other cereals and legumes are sold in the open market with less or no regulation of quality. Most of the contaminated foods find their way into households and restaurants and patronized by unsuspecting consumers. The assessment of the levels of aflatoxin in food crops and the identification of fungi responsible for their contamination will inform policy makers to improve upon proper handling to reduce the toxin in foods.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

Raw samples of groundnut, maize, cowpea and rice were collected from different sales points in Shuwarin Market, Kiyawa L.G.A, Jigawa State, Nigeria. Contaminated samples of groundnut, maize, rice and cowpea were collected from the different sales points (the maximum time limit for the sales consignment to be completely sold). Maximum of four samples were collected in a clean polythene bags to give a representative sample and transported to the Microbiology Laboratory, Federal University Dutse. The samples were ground to powder using mortar and pestle thereafter, sub-samples were made into different portions for thin layer chromatography and microbiological culture. (Jallow, 2015).

MICROBIAL CULTURE PROCEDURE

A microbiological analytical culture procedure was carried out under aseptic conditions. A 10 mL of normal saline was pipetted into a sterile test tube 1, and 9 mL into another test tube 2, test tube 3, test tube 4 and test tube 5. i.e. (dilution factor at 10^{-4}). Then 1g of each of the samples (groundnut, maize, beans and rice) were weighed, using weighing balance into the respective labelled test tube 1 and shaken vigorously to suspend the sample. Thereafter, 1mL of the suspend was serially diluted in test tube 2, test tube 3, test tube 4 and test tube 5. Finally, 0.1 mL from each dilution factor was cultured by spread plate technique on the various agars (Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), containing 250 mg/l of chloramphenicol to suppress the growth of bacteria both incubated at room temperature for appropriate days. Fungal growth was observed within the appropriate days in plate colonies. (Jallow, 2015).

ISOLATION AND IDENTIFICATION

After the incubation period, distinct colonies were sub-cultured to the appropriate media (potato dextrose agar) and further incubated to obtained pure cultures of each fungal species. After obtaining colonies of pure isolates, the colonies were further observed phenotypically and verified microscopically by examining colony colour, size, appearance and microscopic morphology (Hogg, 2005).

AFLATOXIN TLC PROCEDURE

The milled samples of maize, rice, cowpea and groundnut each were weigh to carry out the following procedures for Aflatoxin extraction, 10 g of each samples was placed into a 250mL capacity of wide mouth conical flasks, a cleaned glass rod was used to stirr the mixture of the samples, this was followed by the addition of 100mL chloroform.

The toxin was finally extracted by shaking using glass rod for 30minutes and allowed to settle for two days. The extracts was filtered, using filter paper and the extracts were placed into bottles and kept in a refrigerator till further analyses.

ESTIMATION OF AFLATOXIN CONTENT EXTRACTS BY QUANTITATIVE THINLAYER CHROMOTOGRAPHY METHOD (QTLC).

One hundred grams (100g) of powdered silica gel was mixed with 220mL of water and allowed for 20 minutes to saturate, the saturated silica gel was applied to chromatoplates with a suitable apparatus (spatula). Then the plates were coated to a depth of 50 cm³ and allowed to dry for 1 hour; the plates were activated by heating in an oven at 100°C for 30 minutes.

The extracts containing the materials to be analyzed were then spotted on the plates by measuring the distance of the spotting point of the extract material. This was followed by

running the chromatogram with chloroform: methanol 97:3(mL) as the solvents system. After running the chromatography for ten centimeter (10 cm), the plates were then removed from the chromatography tank and left for sometimes for the solvent to evaporate, the presence of Aflatoxin was detected by illuminating the plates with ultra violet light (UV), the area where the blue florescence appeared and the area where the extracts were spotted was marked for the relative fraction values (Rf value).

RESULTS AND DISCUSSION

Macroscopic and Microscopic identification of the isolates.

Table 1: shows the macroscopic and microscopic identification of the isolates, the individual isolates where macroscopically identified base on the features such as color, shapes and size. Microscopically the isolates were identified following the simple staining and accordingly observed under X40 objective lens.

Table 1: colony appearance and microscopic characteristics of fungal contaminants associated with contamination of cereals and legume

Colony appearance	Microscopic examination	Fungi isolated
Grow rapidly and the colonies are cotton like, usually white turning to brown at the center.	Micro conidia were produce from phialides on branched conidiophore were formed on short simple conidiophores with non saptate hyphae	<i>Fusarium sp</i>
Colonies with loose white mycelium rapidly become black	The conidiophore are large with septate hyphae	<i>Aspergillus sp</i>
Colonies are yellow-green to velvet flat with a white margin at the edge	The appears as a uniform septate hyphae with dichotomous branching	<i>Aspergillus sp</i>
Green fluffy mycelia with some white sporangiospore	Septate hyphae with filamentous structure	<i>Penicillium sp</i>
Ash and slow grower With a flat a slightly raise center and a velvety gray or green-black	The hyphae are dark septate and have branches. The conidiophore are elongated and produce chains of ellipsoid	<i>Cladosporium sp</i>
fluffy and cottony with a mixture of white and gray color and filling the culture plates with a dense mycelium	mycelium aseptate with many hyphal branches connecting group of unbranched sporangiophore	<i>Rhizopus sp</i>

Frequency distribution of individual isolates on the examined samples.

The distribution of individual isolate is shown in table two (2), it was found out that the *A. niger* have high degree of occurrence among all the samples examined with *F. oxysporum* and *Cladosporium carrionii* being the least, each with the percentage distribution of 40% and 2.5% respectively among the total number of 40 isolates.

Table 2: Distribution of the isolates on the samples examined

Fungal isolates	maize	Rice	cowpea	groundnut	Total in %
<i>P. oxalicum</i>	0	0	0	22	(5%)
<i>A. flavus</i> 4	0	4	0	8	(20%)
<i>A. niger</i>	4	2	6	416	(40%)
<i>F. oxysporum</i> 0	0	0	1	1	(2.5%)
<i>Rhizopus oligosporus</i> 4	4	4	0	12	(30%)
<i>Cladosporium carrionii</i>	0	0	0	11	(2.5%)
Total number of the isolates				495	40% (100%)

$$\text{Frequency} = \frac{\text{Number of isolates}}{\text{Total number of isolates}} \times 100\%$$

Table 3 shows the Rf value for the chromatographically qualitative determination of the aflatoxin. It was found out that the Isolate grown on maize yielded a high level of Aflatoxin with groundnut grown isolates being the least each with the Rf value 0.94 and 0.08 respectively.

Table 3: The results of Rf value for different samples

Samples	Dist. Move by the extract(cm)	Dist. move by the solvent(cm)	Rf
Groundnut	1.2	15	0.08
Cowpea	12.5	15	0.83
Rice	13	15	0.86
Maize	14.2	15	0.94

Six (6) fungal species were found to be associated with contamination of the selected cereals and legumes. These include *Penicillium sp.*, *Rhizopus sp.*, *Aspergillus sp.*, *Cladosporium sp.*, *Fusarium sp.*, *Aspergillus sp.* This concurs with reports of Jallow (2015) who reported that these organisms were also isolated from his research with different frequency of occurrence from the result presented in table 4. It is clearly shown that *Aspergillus sp* has the highest frequency of 16(40%), *Rhizopus sp* 12(30%), *Aspergillus sp* 8(20%), while *Penicillium sp*, 2(5%), *Fusarium sp* 1(2.5%), and *Cladosporium sp* 1(2.5%) are known to have the least frequency of occurrence .

However, *Aspergillus sp.* was found to have the highest frequency, which may be due to environmental factors such as bad agronomical practice, pre-harvest storage, could account for the contamination of those samples as reported by (Wu *et al.*, 2010). While *Penicillium sp.*, *Fusarium sp* and *Cladosporium sp* which has the least frequency. This occur as a result of proper heat drying and good agricultural practice can effectively limit the spread of harmful fungi that produce different mycotoxins especially aflatoxin as stated by (Hell *et al.*, 2000)

Results of the thin layer chromatography showed that all the extracts produce blue fluorescent except for groundnut which has the lowest Rf value of 0.08 while maize with the Rf value of 0.94 show the highest level of aflatoxin. This is because fungal species are more susceptible to carbohydrate sources than fat and oil sources as reported by (Jone, 1972, and Čolović et al.,2019).

CONCLUSION

It was clearly observed that *Aspergillus sp* is the major contaminants of maize, groundnut, cowpea and rice since it was detected from all the samples. Aflatoxins were also detected in three samples except groundnut which show low level of aflatoxin. This shows that it is difficult to produce consumer maize, rice, cowpea and groundnut that are free of fungal contamination.

Therefore, the improvement in storage, handling and processing of these cereals and legumes can minimize the mold growth and so reduce the risk of Aflatoxins contaminations in the cereals and legumes. The potential role of *A.flavus* in causing different diseases in man and animals including bird as well as affecting crops in the field or in storages should be a sufficient incentive for further investigation on it and the Aflatoxin. It produces particularly in Africa where occurrence and significant of Mycotoxins has not yet been fully assessed.

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