UJMR, Volume 2 Number 1 June, 2017 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.1721.016
Received: 18th Jan, 2017 **Accepted:** 5th Feb, 2017

Evaluation of Aflatoxin Contamination in *Zea mays* (Maize) Sold in Katsina Central Market, Nigeria

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Abstract
This study was carried out to isolate moulds and detect Aflatoxin in maize sold in Katsina Central Market. A total of ten (10) samples of maize were collected from five different vendors in the market and subjected to mould isolation, Aflatoxin extraction and characterization of the toxin type using Thin Layer Chromatography (TLC). Four fungal genera namely *Aspergillus, Penicillium, Fusarium* and *Rhizopus* species were isolated from 7(70%), 1(10%), 3(30%) and 7(70%) of the samples respectively. *Aspergillus* species were the predominant species isolated with *Aspergillus flavus* occurring in 7(70) and *Aspergillus niger* 6(60) of the total samples. Extraction of the toxin was carried out using methanol and water while type of the toxin was determined using thin layer chromatography (TLC), in which blue fluorescence on the TLC plates in 7(70%) indicated the presence of aflatoxin B in the samples. Retention factor (RF) value with a range of 0.40-0.60 was calculated using the standard formulae. The research suggest proper harvesting, drying, storage and public enlightenment to avoid contamination and consumption of maize grains infested by the number one carcinogenic toxin.
Keywords: *Zea mays* (Maize); Moulds; Aflatoxins; Thin Layer Chromatography, UV light.

INTRODUCTION

Maize (*Zea mays*) is a cereal crop grown throughout the world, it is native to America and widely grown in Nigeria, and plays an important role in the diet of millions of African people due to its high yield per hectare (Sule *et al*., 2014). In Nigeria Between 1992 and 1996 total area of maize production was more than doubled from 1.8 million to 4.0 million/hectare (Manyoung *et al.,* 1996).

Huge quantities of food are wasted every year because they are invaded by toxigenic fungi and get contaminated by fungal metabolic products, such spoilage is prominent in tropical countries like Nigeria where problem of storage exists (Sule *et al*., 2014). Contamination of such products by fungi not only render grains unfit for consumption by discoloration and reduction of nutritional value, but can lead to production of harmful substances called mycotoxins (Sule *et al.*,2014). The Food and Agriculture Organization (FAO) estimated that about 25% of the world's agricultural produce is contaminated with mycotoxins which cause huge losses for farmers (Wu, 2007).

Mycotoxins that develop from *Aspergillus flavus*, a common post-harvest fungi in maize are called aflatoxins. These toxins are hazardous to animals and human health, and constitute a factor in economic losses in food production in the world (Lubulwa and Davis, 1994).

Aflatoxins, ochratoxins, trichothecenes, Zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. Often more than one mycotoxin is found on a contaminated substrate (Ashiq *et al*., 2014).

Aflatoxins occur in maize due to contamination by the toxin producing fungi (e.g. *Aspergillus spp*), in the field or during storage making the product unfit for consumption. Aflatoxins are a group of toxic and carcinogenic secondary metabolites of fungal origin. They are produced by strains of *Aspergillus flavus, A. parasiticus* and, in rare cases, *A. nominus* and *A. pseudotamarii* (Mazaheri, 2009). They are very powerful hepatocarcinogens, and naturally occurring mixtures of aflatoxins have been classified as a class 1 human carcinogen (IARC, 1993). The naturally occurring aflatoxins designated as aflatoxin B1, B2, G1, and G2. B and G forms are so referred to because they emit blue or green fluorescence respectively upon exposure to ultraviolet light (Murphy *et al*., 2006). They cause aflatoxicosis upon ingestion of aflatoxin in contaminated food or feed (Ellis *et al*., 1991).

Occurrence of aflatoxins in maize has been reported in Nigeria, by Shamsuddeen and Kabir, (2015). The aim of this study is to carry out the isolate of moulds and detect Aflatoxin in maize sold in Katsina Central Market.

MATERIALS AND METHODS

Study Area and Sample Collection

A total of ten (10) samples were collected randomly from five (5) different vendors in Katsina central market. Two (2) samples were obtained (white and Yellow maize) from each of the five vendors, the samples were labelled and transported to the Department of Microbiology laboratory at Umaru Musa Yar'adua University, Katsina in a sterile polythene bag for analysis (Pearce *et al.,* 2004; Sule *et al.,* 2014).

Isolation and Identification of Moulds from Maize Grains

Potato Dextrose Agar (PDA) was prepared according to manufacturer's instructions and sterilized by autoclaving at 121^{o}C pressure for 15 minutes. Five seeds of each maize grain was randomly chosen using sterile forceps and placed on prepared petridishes. The cultures were covered, labeled and incubated at room temperature for two to three days. Mixed growth was sub-cultured into a freshly prepared medium (PDA) to obtain pure cultures (Mukhtar *et al.*, 2010). The moulds were identified according to Lina (2013) based on colonial appearance on culture plates.

Extraction of Aflatoxin from Maize Grains

Thirty (30g) of the samples each was ground using pestle and mortar. Twenty (20g) of each of the ground samples was measured into a clean jar with seal. A 100ml of 70:30(v/v) methanol-water solution was added and the jar sealed. The mixture was vigorously shaken for three (3) minutes, allowed to settled and then filtered through a filter paper and the filtrate was obtained, the residue was discarded and the filtrate was concentrated using a rotary evaporating machine. The concentrated extracts were poured in well bijou bottles, labelled and kept in a refrigerator for further analysis (Tijjani *et al.*, 2013).

Detection of Aflatoxin Using Thin Layer Chromatography (TLC)

The chromatography plates were coated with silica gel. Thirty grams (30g) of silica gel was mixed with 75ml of distilled water, this was used to coat the chromatographic plates and allowed to dry for 30minutes.The coated plates were activated by heating in an oven at 100^{o}C for 30minutes, then, the extract from extraction were spotted on the plates using capillary tubes. Chloroform: methanol mixture of 97:3ml was used as the chromatographic solvent, it was run into the chromatogram or chromatographic tank to serve as the mobile phase of the chromatography (Audi, 2010). The spotted plates were dipped into the tank containing the solvent without allowing the solvent to touch the areas spotted with the extract. The solvent was allowed to move the substance (extracts) until the solvent stop moving. The distance moved by the solvent and that moved by the substance were marked immediately after removing from the solvent and measured for calculating the retention factor using the relation;

$$R_F = \frac{\text{Distance Moved by Substance (DMS)}}{\text{Distance Moved by Solvent (SF)}}$$

The presence of aflatoxins was detected by illuminating the plates with ultra-violet light (UV) were blue or green fluorescence indicate aflatoxin B and G respectively (Shamsuddeen and Kabir, 2015).

RESULTS

Mould Isolation from Maize Grains

Four fungal genera namely *Aspergillus, Fusarium, Rhizopus* and *Penicillium* species were isolated from the ten samples analysed. *Aspergillus* and *Rhizopus* spp has the highest number of occurrence followed by *Fusarium* spp. with *Penicillium* spp. occurring in only one out of the ten samples analysed. Aspergillus spp. present in 7(70%), *Fusarium* spp. in 3(30%), *Rhizopus* spp. in 7(70%) and *Penicillium* spp. in 1(10%) of all the samples analysed (Table 1).

Table 1: Moulds Isolated from Maize Grains

Sample(s)	*Aspergillus spp*	*Fusarium spp*	*Rhizopus spp.*	*Penicillium spp.*
WM_1	–	–	+	–
WM_2	+	+	+	–
WM_3	+	–	+	–
WM_4	–	+	+	–
WM_5	+	+	–	–
YM_1	+	–	+	–
YM_2	+	–	+	–
YM_3	+	–	–	–
YM_4	–	–	+	–
YM_5	+	–	–	+
Total	7(70%)	3(30%)	7(70%)	1(10%)

KEY: WM = White maize. YM = Yellow maize. += Present. - = Absent

Aspergillus species isolated from Maize Grains
The results revealed that *Aspergillus flavus* has the highest number of occurrence and *Aspergillus flavus* was found to be present in 7(70%) while *Aspergillus niger* was present in 6(60%) of the ten samples analysed as seen in Table 2.

Table 2: *Aspergillus Species* Identified

Sample(s)	*Aspergillus flavus*	*Aspergillus niger*
WM_1	–	–
WM_2	+	+
WM_3	+	–
WM_4	–	–
WM_5	+	+
YM_1	+	+
YM_2	+	+
YM_3	+	+
YM_4	–	–
YM_5	+	+
Total	**7(70%)**	**6(60%)**

KEY: WM = White maize. YM = Yellow maize. += Present. - = Absent

Nature and Colour of Extracts obtained from Maize samples
Table 3 showed the extracts to have colour that ranged from amber to pale yellow while texturally some were oily and some watery

Table 3: Nature and Colour of Extracts obtained from Maize samples

Sample(s)	Colour of extract	Texture of the extract
WM_1	Amber	Oily
WM_2	Amber	Watery with oil suspensions
WM_3	Amber	Watery with oil suspensions
WM_4	Amber	Watery with oil suspensions
WM_5	Amber	Watery with oil suspensions
YM_1	Amber	Oily
YM_2	Pale yellow	Oily
YM_3	Pale yellow	Oily
YM_4	Pale yellow	Watery with oil suspensions
YM_5	Yellow	Oily

KEY: WM = White Maize YM = Yellow Maize

Thin Layer Chromatography, retention factor obtained and type of toxin detected
Findings revealed that (Table 4) aflatoxin is present in seven (7) samples and absent in three (3) samples. The retention factor after separation of individual components using thin layer chromatography (TLC) has a range of 0.42 to 0.57 while illumination under UV light shows a blue fluorescent spot in seven samples indicating aflatoxin B.

Table 4: Retention Factor and Fluorescence under UV Light for Maize Grains

Sample(s)	DMS(cm)	SF(cm)	RF	Fluorescence
WM_1	8.2	14.2	0.57	ND
WM_2	6.5	14.2	0.46	Blue
WM_3	6.4	14.2	0.45	Blue
WM_4	6.3	14.2	0.44	ND
WM_5	6.6	14.2	0.46	Blue
YM_1	6.1	14.2	0.43	Blue
YM_2	6.0	14.2	0.42	Blue
YM_3	6.0	14.2	0.42	Blue
YM_4	6.1	14.2	0.43	ND
YM_5	6.2	14.2	0.44	Blue

KEY: WM = White Maize YM = Yellow Maize ND = Not Detected Blue= Aflatoxin B

DISCUSSION

A total of four fungal genera namely, *Aspergillus* species, *Fusarium* species, *Rhizopus* species *and Penicillium* species were isolated from the samples, these results agrees with those of Mukhtar *et al* (2010); Hassan *et al*. (2014); Sule *et al*. (2014), Shamsuddeen and Kabir (2015) were the presence of the same fungal species were reported in their research on maize. *Aspergillus* genera (*Aspergillus flavus* and *Aspergillus niger*) has the highest number of occurrence which was also reported by Richard *et al*. (2007) and Hassan *et al* (2014) in some maize samples analysed. The high occurrence of *Aspergillus* spp might be due to careless handling of grains during shelling which consequently exposes the cotyledon which contain a lot of nutritious food substances to fungal pathogens (Mukhtar *et al*., 2010).

From analysis of the extract using thin layer chromatography, a range of 0.40-0.57 retention factor (RF) value was obtained which agrees to the result obtained in maize samples from Dawanau grains market in Kano (Shamsuddeen and Kabir, 2015). It's also clear that all extracts of maize from which *Aspergillus* species were isolated produces a blue fluorescence after illumination with ultraviolent light which is an indication of the presence of aflatoxin in the sample. Similar result was obtained by Sule *et al* (2014) where they reported fungal isolates to have produced aflatoxin in maize and maize products. while production of blue fluorescence on TLC plates after illumination with UV light was a similar result to that obtained by Shamsuddeen and Magashi (2004).

The contamination by these fungi and their toxic metabolites has been associated with several human and animal diseases including liver and oesophageal cancer, particularly in Africa (Marasa, 2001), they also play a considerable role in causing liver damage, liver cirrhosis, induction of tumor in both man and animals (Shamsuddeen and Magashi, 2004), infertility in men (Uriah *et al*., 2001), reduction in salivatory secretory IgA levels (Turner *et al*., 2003).

Aflatoxin contamination has also been shown to reduce feed intake, increase liver and kidney weights of farm animals, as well as induce immunosuppression and hepatitis in them, all of which contribute to increased mortality in farm animals (Hussein and Brussel, 2001) as well as reduction in quality of milk produced by farm animals (Zain, 2011).

CONCLUSION

The maize samples were found to be contaminated with *Aspergillus* species, *Fusarium* species, *Rhizopus* species *and Penicillium* species. The study showed that maize grains were contaminated with aflatoxins

RECOMMENDATIONS

- Adequate drying of the grain after harvesting by reducing the grain moisture to less than 15% within 48hours before storage should be adopted.
- Storage facilities should be monitored regularly to detect grain toxigenic mould development.
- Damage of all kind (i.e. mechanical and insect) should be avoided during and after harvesting of the grains.
- Seed dressing and use of fungicides before planting should be encouraged. Consumption of visibly spoiled grains should be avoided to minimize human and animal contamination.
- Time to time checks on level of aflatoxin and degree of fungal contamination should be adopted by designated authorities.
- Public enlightments should be encouraged and supported by the government to minimize contamination risk.

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