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STUDY ON AFLATOXIN CONTENTS OF MAIZE SAMPLED FROM DAWANAU GRAINS MARKET IN KANO.

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ABSTRACT

A study was carried out on the Aflatoxin content of maize sourced from Dawanau grains market in Kano. A total of 25 maize samples were collected and subjected to laboratory analysis to determine moisture content, moulds isolation as well as aflatoxin extraction and then followed by the analysis of the toxin. In 25 maize samples 9 (36%), 6 (24%), 19 (76%), 7 (28%), 4 (16%), 8 (32%), 2 (8%) and 4 (8%) which yielded *A. flavus*, *A. parasiticus*, *Fusarium*, *A. niger*, *Penicillium*, *Mucor*, *Nigrospora* and *Trichoderma* respectively. Analysis of the extracts using thin layer chromatography revealed blue and blue green fluorescence on the TLC plate after U.V light illumination with Rf values which ranged between 0.40 - 0.65. Analysis of the extracts using ELISA kit showed the aflatoxin content of the maize to be in the range of 22.05 - 44.56. The study stressed the need for public enlightenment on the dangers and potential hazards associated with the consumption of mycotoxins.

INTRODUCTION

Maize is a very versatile cereal and is used in the production of food, feed, adhesive, oil, syrups, flakes, alcoholic and non-alcoholic drinks, starch and ethanol among others while locally it is used to make pap, *nakia*, *tuwo*, pop corn etc. In addition Nigeria is the 10th largest producer of maize globally; producing about 98.8% of its domestic maize needs locally (El-Imam *et. al.*, 2012).

Aflatoxins are biologically active secondary metabolites produced by

certain strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *A. nominus*. However *A. flavus* is the most common producer. The Aflatoxins producing fungi are widely distributed in nature and can grow over a wide range of environmental conditions (Alposy, 2010). Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis.

There is enough evidence from epidemiological studies regarding the hazardous nature of Aflatoxins as potent carcinogens, in connection to incidence of liver cancer in populations exposed to the toxin from contaminated foods (Rustom, 1997). Human exposure to aflatoxins can result directly from ingestion of contaminated foods, or indirectly from consumption of foods from animals previously exposed to aflatoxins in feeds (Rustom 1997).

The incidence of aflatoxins in foods is relatively high in tropical and subtropical regions where warm humid weather provides optimal growth conditions for the molds. Grains of various varieties in all geographical locations are liable to contamination with mycotoxins in the field, before and after harvest, during curing and storage. Presence of mycotoxins is a major concern which has received worldwide attention due to their deleterious effect on human and animal health as well as their importance in international food trade (Al-Rahmah *et al.*, 2003).

There is enough evidence from epidemiological studies regarding the hazardous nature of aflatoxins as potent carcinogens in connection with the incidence of liver cancer in populations exposed to the toxins from contaminated foods (Rustom 1996). The aim of this work is therefore to assess the level of contamination of maize grains with the fungal metabolite, the aflatoxin.

METHODOLOGY

Sample Collection

A total of twenty five samples of white maize grains were collected from different bags at Dawanau grains market Kano. The collected grains were stored in paper bags to avoid moisture build up which could result in increased fungal growth and mycotoxin production (El-Imam *et al.*, 2012). The samples were taken to Microbiology Laboratory, Bayero University Kano.

Moisture Content Determination

This was carried out according to (ISTA, 1996). Oven was pre heated to a temperature of 130°C for 30mins before the samples were placed in it to ensure uniform heat distribution. The sample was placed in a dry blender and grounded to powder. Ten grams of each ground sample was weighed, this was done in duplicate for each sample. The weighed samples were placed in a crucible and placed in a pre-heated oven. The samples were heated until a constant weight was obtained. The moisture content of the samples was obtained by the following relation.

$$\text{Moisture content} = X \times 100$$

Fungal Isolation

The maize grains were thoroughly mixed to obtain homogeneity. Twenty (20) maize seeds, Per sample were randomly selected and surface sterilized using 5.25% sodium hypochlorite solution.

The seeds were aseptically rinsed five times with sterile distilled water (Makun *et al.*, 2009), then dried between layers of sterile filter paper whatman No 1 (Alrahmar *et al.*, 2011).

Five (5) seeds per plate were plated on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). Plating was done in duplicate for each medium.

Chloramphenicol (0.5mg/ml) was added to the culture media before autoclaving to inhibit bacterial growth (Aljouraifani, 2011).

The plates were incubated at room temperature for 5-7 days. Following incubation, pure culture of the fungi was obtained by repeated subcultures, which were preserved on PDA slants till identified (Al-Rahmah *et al.*, 2011).

Extraction of Aflatoxins

Ten grams (10g) of ground maize sample was macerated with 10ml of water and 100ml of chloroform in a blender for 5mins. The extract was filtered with Whatman No 1 filter paper. The filtrate was concentrated and kept at 4°C for further analysis (Jones, 1972).

Thin layer Chromatography (TLC)

This was achieved by mixing 100g of silica gel (powder) with 220ml of distilled water. The mixture was used to coat chromatoplates. The plates were air dried, then activated by heating in an oven at 100°C for one hour. Equal volumes of extracts were spotted on the plates in a line 2cm from the bottom of the chromatoplate and at least 2cm from either sides. The plate was developed by standing it in a chromatographic tank containing Diethyl ether over a 12cm path length. After drying the plate was further developed in chloroform. Methanol (97:3) v/v until the solvent ran 10cm from baseline (Jones, 1972).

The developed chromatogram was air dried then was illuminated with UV light

of wavelength 365nm. The plate was observed for the presence or absence of blue or green fluorescent spots (Jones, 1972). Quantification and determination of mycotoxins was achieved by calculation of the resolution/retention factor.

$$R_f = (\text{Mahmoud } et al., 1994).$$

Quantitative Evaluation/Estimation of the Aflatoxin using ELISA based Kits

Elisa kits were purchased from Shenzhen Lvshiyuan Biotechnology Ltd China. The samples were prepared and analyses were carried out according to the manufacturers' instruction. Sample extract solution was prepared by dissolving 3 parts of methanol in two parts of deionized water. Sample redissolving solution was obtained by dissolving one part of provided 2x concentrated redissolving solution with one part of deionized water. One gram of pulverized maize sample was dispensed in 7ml plain vacutener bottles 5ml sample extract solution was added, this was shaken thoroughly for 30secs after which it was placed in a centrifuge at 4000r/min for 10mins at a temperature of 15°C.

Hundred (100) µl of supernatant was pipetted to which 500µl of sample redissolving solution was added. From the above solution 50µl was taken and dispensed into the micro wells.

The micro wells were labeled appropriately and all samples were analyzed in duplicates alongside standards.

Using a micropipette 50µl of enzyme conjugate per well was dispensed, then 50µl/well of antibody working solution was added, this was mixed gently. The micro plate was sealed and incubated at room temperature for 30mins.

Following incubation the solution was discarded and the micro wells washed 5 times with 250µl/well of washing buffer. After washing 50µl of substrate A was dispensed into each well, this was followed by the addition of 50µl/well of substrate B. this was mixed and incubated in the dark for 15mins at room temperature for coloration.

After incubation 50µl/well of solution was added into each well. The microplate was shaken gently to ensure mixing of the solutions. The plate was placed in a microplate reader and read at a wavelength of 450nm to obtain the optical density (OD) value of each microwell.

Result

The result of the moisture content of the maize samples is presented in table 1. The

moisture content range from 1% to 6% with sample 25 having the highest value of 6%.

Table 1 Moisture contents of maize samples,(%).

Sample	Moisture (%)
1.	4
2.	3
3.	6
4.	5
5.	3
6.	1
7.	2
8.	3
9.	4
10.	3
11.	4
12.	3
13.	2
14.	1
15.	4
16.	3
17.	2
18.	3
19.	5
20.	4
21.	5
22.	4
23.	5
24.	2
25.	6

The various maize samples yielded a variety of fungal species which included *A. flavus* in 9 samples which corresponds to 36%, *A. parasiticus* 6 (24%), *Fusarium* 19 (76%), *A. niger* 7 (28%), *Penicillium* 4 (16%), *Mucor* 8 (32%), *Nigrospora* 2 (8%) and *Trichoderma* 2 (8%) as presented in Table 2.

Table 2: Percentage of Occurrence of Fungi

S/No	Fungal Specie	Frequency of Occurrence out of 25 samples	% Occurrence Out of 25
1.	<i>A. flavus</i>	9	36
2.	<i>A. parasiticus</i>	6	24
3.	<i>Fusarium spp</i>	19	76
4.	<i>A. niger</i>	7	28
5.	<i>Penicillium spp</i>	4	16
6.	<i>Mucor</i>	8	32
7.	<i>Nigrospora</i>	2	8
8.	<i>Trichoderma</i>	2	8
Total		57	99.9%

Table 3 shows the R_f values of the various extracts on TLC plates as shown below.

Table 3: R_f values of grains using TLC

Sample	DMS (cm)	SF (cm)	RF
1.	5.4	10	0.54
2.	4.4	10	0.44
3.	4.5	10	0.45
4.	4.6	10	0.46
5.	4.7	10	0.47
6.	4.2	10	0.42
7.	4.0	10	0.40
8.	4.0	10	0.40
9.	4.5	10	0.45
10.	4.8	10	0.48
11.	5.0	10	0.50
12.	4.1	10	0.41
13.	6.1	10	0.61
14.	4.3	10	0.43
15.	6.0	10	0.60
16.	5.6	10	0.56
17.	6.3	10	0.63
18.	6.5	10	0.65
19.	4.8	10	0.48
20.	5.0	10	0.50
21.	4.5	10	0.45
22.	4.9	10	0.49
23.	4.3	10	0.43
24.	5.0	10	0.50
25.	4.7	10	0.47

Key:

DMS = Distance moved by sample, SF = Solvent front

R_f = Retention factor or relative frontal mobility, TLC = Thin layer chromatography

The result of the aflatoxin (ppb) of the samples is presented in table 4. The mean aflatoxin is 34.67.

Table 4: Estimated Aflatoxin Content

Samples	Aflatoxin (ppb)
1	34.71
2	39.40
3	39.40
4	41.74
5	41.74
6	22.05
7	29.55
8	29.55
9	31.89
10	41.74
11	41.74
12	29.55
13	27.20
14	29.55
15	31.89
16	31.89
17	37.05
18	22.05
19	39.40
20	31.89
21	37.05
22	34.71
23	44.56
24	39.40
25	37.05
Mean	34.67166979

Discussion

A total of 25 maize grain samples were collected from Dawanau grains market, Kano. From Table 1 the moisture content of all samples was discovered to ranged from 1-6%. Samples 14 and 6 had the lowest moisture content value of 1%. While the highest moisture content value of 6% was obtained in samples 3 and 25. The low moisture content value of the grains which ranged from 1-6% shows that the maize grains were well dried to the appropriate moisture content level of

below 12% before storage. However these grains appear dried at storage, it does not give an indication or insight about the procedure, process and period of drying of the grains.

During the process of drying the grains were stirred manually until the product was dried,, in this process there is continuous mixing of moist and dry grains. This method of drying is a suitable means for propagation of fungal spores and subsequent infestation of the grains.

The fungal flora isolated included; *Fusarium* spp, *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *Penicillium* spp, *Mucor* spp., *Trichoderma* and *Nigrospora* spp., Sources of fungal contamination of cereals are many but all are traceable to the environment in which these grains are grown, handled and processed. Other factors that influence fungal contamination of grains include; rainfall, drought, temperature and humidity, soil conditions and wind; others are harvesting equipment, storage and handling as well as moisture control (Sauer et al., 1992).

The high incidence of *A. flavus* and *Fusarium* spp is in line with the findings of Mohana et al.(2014) which found 25 maize samples showing high incidence of both *A. flavus* and *F. verticillioides* out of 45 maize samples collected from different regions of southern Karnataka India.

Thin layer chromatographic (TLC) analysis for Aflatoxin yielded R_f values between 0.40 – 0.65. Blue and blue green fluorescence were observed on the TLC plates upon illumination with UV light. According to Jones (1970), R_f value range between 0.50 – 0.55 with blue fluorescence following illumination with UV light indicates the presence of Aflatoxin B while R_f value between 0.45 – 0.50 with blue green fluorescence following illumination with UV light indicates the presence of Aflatoxin G. Magashi and Shamsudeen (2004) reported R_f values of extracts from dehulled and undehulled maize which ranged from 0.40 – 0.50.

Aflatoxin producing molds could be found both as field and storage fungi. The production of Aflatoxin by *A. flavus* and *A. parasiticus* in the grains are influenced by a number of factors which include; moisture content of the grains, relative

humidity of surroundings, temperature, physical damage of the Kernels, drought, plant stress as well as the storage conditions of the maize grains (Ismail, 1997).

Pkhakamile et al. (2007) detected aflatoxin in seventeen out of twenty three samples of raw ingredients (wheat, maize sun flower seeds, cotton seeds, gluten, bagasse, wheaten bran) used to manufacture pet feed in South Africa. Levels of Aflatoxins were in the range of 0.8 ± 0.2 and $156 \pm 8 \mu\text{g}/\text{kg}$

In a study carried out by Mohana et al (2014) in Karnatka India out of 25 samples of maize analysed Aflatoxin B_1 (AFB $_1$) was detected in 19 samples at concentrations ranging from 20.6 to 402.4 $\mu\text{g}/\text{kg}$ and FB $_1$ was detected in 12 samples at concentrations ranging from 49.6 to 650.0 $\mu\text{g}/\text{kg}$.

All 25 samples have aflatoxin levels greater than 20ppb. This value is higher than the value recommended by United States Food and Drug Administration (FDA), which recommends a limit of 20ppb for corn products intended for human consumption while a limit of 100ppb for corn products intended for animal feeds. The European Union (EU) has a limit of 4ppb for cereals intended for direct consumption. In Nigeria National Agency for Food Drug Administration and Control (NAFDAC) has set a limit of 10ppb for food that will be further processed and a limit of 4ppb for ready to eat foods (FDA, 2000).

Conclusion

In conclusion the maize grains were found to be contaminated with either one or more molds. All samples were contaminated with Aflatoxin. All samples had Aflatoxin levels greater than the benchmark figures (FDA/Eu/NAFDAC Limits). which recommends a limit

of 20ppb for corn products intended for human consumption while a limit of 100ppb for corn products intended for animal feeds.

Recommendation

Mass awareness and enlightenment campaign to educate the farmers, about good agricultural practices, storage, harvest and handling of the grains to reduce the level of mycotoxin

contamination. It is recommended that grains should be dried immediately after harvest within 24 – 48hrs to below 14% moisture content. Then grains should be further dried to a moisture content of below 12% before storage. Development of affordable and easy to use kits for testing and quantification of mycotoxins in African countries.

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