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AFLATOXIN B1 CONTAMINATION IN WHEAT GRAINS FROM SELECTED GRAINS MARKETS IN KANO STATE, NIGERIA

Abdullahi, A.M.[1], Shamsuddeen, U.[1] and Rabiu, I.[2]
[1]Department of Microbiology, Faculty of Life Science, College of Natural and Pharmaceutical Sciences Bayero University, PMB: 3011, Kano, Nigeria.
[2]Department of Microbiology, School of Science and Information Technology, Skyline University Nigeria. No. 2, Zaria Road, Kano, Nigeria
*kingameenu@gmail.com +2348063338086

ABSTRACT
Consumption of wheat grains and other whole-wheat products has grown in recent years in Nigeria due to the policies and empowerments by the government in Agriculture. These foods are considered more nutritious and have been associated with a reduced risk of some major chronic diseases such as diabetes. On the other hand, studies have found different groups of fungal toxins, called mycotoxins, contaminating these wheat products. Among these mycotoxins, are the aflatoxins, a group of genotoxic and carcinogenic compounds produced by Aspergillus flavus and Aspergillus parasiticus. This study aimed to determine the levels of aflatoxins B1 in samples of wheat grains intended for human consumption from some markets in Kano State, Nigeria. Composite samples of wheat marketed in Dawanau, Wudil and Chiromawa were obtained. Macroscopic and microscopic examinations of the fungal isolates led to the identification of the following genera; Aspergillus flavus 92%, Alternaria spp. 66%, Aspergillus niger 50%, Penicillium spp 50%, Mucor 50%, Trichoderma spp. 50%, Rhizoctonia spp. 50%, Aspergillus fumigatus 33%, Curvularia spp. 33%, Aspergillus terreus 25% and Fusarium spp. 25%. Aflatoxin B1 was also extracted and quantified using Enzyme Linked Immunosorbent Assay (ELISA). Out of the samples analyzed, 75% were positive for aflatoxin B1. Two samples (from Dawanau and Wudil) showed aflatoxin B1 levels (4.3 µg/kg) higher than the limit established by EU legislation (4µg/kg) but none of the samples exceeded the level set by Standard Organization of Nigeria (10 µg/kg).
Keywords: mycotoxins, aflatoxins, ELISA, Grains, Contamination

INTRODUCTION

Cereal crops provide essential nutrients and energy in the everyday human diet through direct human consumption and also via meat production since they comprise a major livestock feed. According to the Food and Agriculture Organization, total crop production in 2016 reached 2577.85 million tons, whereas the production of coarse grains (cereal grains other than wheat and rice used primarily for animal feed or brewing) reached 1330.02 million tons (FAO-Agricultural Market Information System, 2017). The term "cereals" refers to members of the Gramineae family, which includes nine species: wheat (*Triticum*), rye (*Secale*), barley (*Hordeum*), oat (*Avena*), rice (*Oryza*), millet (*Pennisetum*), corn (*Zea*), sorghum (*Sorghum*), and triticale, which is a hybrid of wheat and rye. The top cereals produced in the world in 2014, ranked on the basis of tonnage (in million tons), are corn (1253.6), rice (paddy, 949.7), wheat (854.9), barley (146.3), oat (23.2), and rye (15.8) (Food and Agricultural Organization Statistics, 2017).

Wheat has been cultivated in Nigeria for centuries. Ample evidence shows that wheat has been cultivated in Nigeria as early as 200BC. However, Nigeria's domestic wheat production has remained at a very low level in spite of the ever – rising demand for the crop. The limitations that comes with the cultivation of wheat in many wheat growing areas in Nigeria include climatic requirements, inappropriate agronomic practices and preference for the cultivation of vegetables (Oyewole, 2016).

When grains are colonized by moulds, there is a serious risk of contamination with mycotoxins, which are toxic chemical products, formed as secondary metabolites by fungi. Many species of *Fusarium, Aspergillus, Penicillium, and Alternaria* are recognized as sources of important mycotoxins of concern in relation to animal and human health (Sadhasivam *et al.*, 2017).

Mycotoxins are low molecular weight, secondary metabolites produced mostly by species of the phyla Ascomycota and Basidiomycota. The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxin-producing fungi. Faced with this threat, a legal framework is gradually being set up at the global level to set standards defining the maximum acceptable level of mycotoxin in food stuffs, and enforcing such standards requires methods to detect and quantify mycotoxins (Sokefun *et al.,* 2018). Therefore, in view of this warning, this study aims to assess the Aflatoxin B1 levels in wheat from selected grains markets in Kano State.

MATERIAL AND METHODS

Areas of study are three markets in Kano State, Dawanau, Wudil and Chiromawa, located in Dawakin Tofa, Wudil and Kura local Government areas of Kano State, Nigeria. Kano has a population of 9,383,682 (estimated to be around 13 million by 2022) with a landmass that approximately covers an area of 21,000 km^2. It is located between latitude 12° N and longitude 8° E. It is some 900 km from the edge of the Sahara desert and some 1,140 km away from the Atlantic Ocean (Barau, 2007).

The sampling protocol of the European Commission regulation for the methods of sampling and analysis for the official control of Mycotoxins in food stuffs was followed (European Commission, 2014).

Incremental raw wheat samples were collected from three markets in Kano State, namely, Dawanau from Kano South, Chiromawa from Kano Central and Wudil from Kano South. Five (5) incremental samples of 120g were collected from different vendors (each vendor was classified as a lot) in each of the markets. This procedure was repeated 3 times (March, April and May, 2021). The incremental samples were mixed to form 1 aggregate sample of 1.2 kg making a total of 12 aggregate samples, the aggregate samples were thoroughly homogenized prior to division procedure intended to obtain the laboratory sample and the retention/reference sample. The samples were coned on a clean table, fattened and divided into four equal parts of 300g each, where 300g was carried to the laboratory and the rest were kept as retention/reference samples (EUROPEAN COMMISSION, 2014)

Isolation and Identification of Fungi

The study of the mycobiota present in the samples was carried out by determination of internal fungi. For isolation of the internal mycobiota, a subsample of 200 kernels of each sample was surface disinfested in a 3% aqueous solution of sodium hypochlorite for 2 minutes, and rinsed twice with sterile distilled water. The kernels were aseptically plated on Potato dextrose agar (PDA) (5 kernels/plate) (Ennouri *et al.,* 2018).

The plates were incubated at room temperature and examined daily for growth and sporulation (Sekar *et al*., 2008), after 5 days of incubation, the different fungal colonies observed were transferred into fresh plates. The fungi isolated were identified by a drop of distilled water placed on a clean glass slide and a loopful of the fungal colony was taken and placed on the slide. With the help of sterile needles, the fungal mycelia were teased gently and a cover slip was placed over the drop of distilled water. The slide was then observed under the microscope (x40) and identified based on morphological characteristics (Sekar *et al*., 2008).

Determination of Aflatoxin B1 using Enzyme Linked Immunosorbent Assay

Aflatoxin B1 extraction and quantification was carried out at the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University Zaria using Elisa kit from ICRISAT.

Extraction

Fifty grams of wheat samples was weighed and ground into fine powder, 20g of the grounded sample was put in 250 ml conical flask and 100 ml of 70% methanol was added. The mixture was vortexed for 30 minutes in an orbit shaker at 150 rpm then filtered using Whatmans No 1 filter paper and the filterate was kept at low temperature for Aflatoxin analysis.

Preparation of Aflatoxin B_1 standards

Aflatoxin B_1 - Bovine Serum Albumin conjugate was prepared in carbonate coating buffer at 100ng/ml concentration. 150 µl of the diluted toxin BSA was dispensed into each well of ELISA plate and incubated at 37°C for 1 hour. The plates were washed 3 times after incubation using Phosphate Buffer Saline -Tween allowing 3 minutes for each wash, a 0.2% BSA was prepared in PBS-Tween and 150 µl was added to each well and incubated for 30 minutes at 37°C. A 1:6 dilution of antiserum was prepared in PBS-Tween containing 0.2% BSA and incubated for 30 min at 37°C. The blocked plates were washed 3 times using washing buffer allowing 3 minutes for each wash.

Aflatoxin B_1 standards were diluted (using 1:10 diluted wheat extract) at concentrations ranging from 25 ng to 10 picogram/ml in 100 µl volume, 50 µl of antiserum was added to each dilution of Aflatoxin standards and wheat extract (100 µl) intended for analysis and incubated for 1 hour at 37°C to facilitate reaction between the toxin present in the sample and the antibody. The process was done in the ELISA plate.

A 1:4 of goat anti rabbit IgG (Enzyme conjugate) was prepared in PBS-Tween containing 0.2% BSA and 150µl was added to each well and incubated for 1 hour at 37°C. The plates were washed 3 times using PBS- Tween allowing 3 minutes for each wash.

A 150µl substrate solution (p-nitrophenyl phosphate prepared in 10% diethanolamine buffer, pH 9.8) was added to the wells and incubated for 1 hour at room temperature. Absorbance was measured at 450nm in an Elisa reader. Using the values obtained for aflatoxin B_1 standards,

Aflatoxin B1 quantification

Aflatoxin B1 was calculated using the following formula.

AFB_1 (µg/kg) = $\frac{\frac{A \times D \times E}{G}}{\frac{A \times E}{C \times G}}$ or ..... (1)

Where

A = AFB_1 concentration in diluted or concentrated sample extract (ng/ml)

D = Times dilution with buffer

C = Times concentration after cleanup

E = Extraction solvent volume used (ml)

G = Sample weight (g)

Data Analysis

Mean, standard deviation and analysis of variance of data generated were calculated using SPSS (version 2.0) software

RESULTS AND DISCUSSION

The result of isolation and identification of fungi associated with wheat samples from Dawanau, Wudil and Chiromawa grains Market in Kano State led to the identification of both field and storage fungi with different morphological characteristics, Macroscopic and microscopic examinations of the isolates showed different fungal genera with *Aspergillus flavus* 92%, having the highest percentage frequency of occurrence followed by *Alternaria spp 66%, Aspergillus niger 50%, Penicillium spp 50%, Mucor spp 50%, Tichoderma spp 50%, Rhizoctonia spp 50%,Curvularia spp 33%,* and *Fusarium spp* 25% having the least percentage frequency of occurrence. The result is presented in Table 2

Out of the 12 samples analyzed, 9 yielded positive for AFB1 while AFB1 was not detected in the remaining 3. The lowest Aflatoxin B1 concentration was recorded from Chiromawa 0.2µg/kg, in April and May while the highest was recorded at Dawanau and Wudil 4.3µg/kg, in April and May respectively. The mean of the 3 markets showed Dawanau has the highest concentration of 1.96µg/kg followed by Wudil 1.8µg/kg, and Chiromawa 0.2µg/kg. Foreign wheat from Dawanau was also found to be contaminated with AFB1 at a mean concentration of 0.8µg/kg.

Table 1: Frequency of Fungi isolated from Dawanau, Wudil and Chiromawa markets in Kano State

Fungi	Occurrence	Percentage (%)
Aspergillus flavus	11	92
Aspergilus niger	6	50
Aspergilus fumigates	4	33
Aspergillus terreus	3	25
Alternaria spp	8	67
Curvularia spp	4	33
Penicillium spp	6	50
Mucor spp	6	50
Trichoderma spp	6	50
Fusarium spp	3	25
Rhizochtonia spp	6	50

Table 2. Mean AFB1 concentration (µg/kg) of wheat collected from Dawanau, Wudil and Chiromawa Markets in Kano State

Month of collection	Market			
	DW1	DW2	WD	CR
March	1.1±0.0	0.3±0.0	ND	0.5±0.0
April	4.3±0.0	0.2±0.0	4.3±0.0	0.2±0.0
May	ND	ND	0.8±0.0	0.2±0.0
Average	1.8	0.17	1.7	0.3

Key: DW1= Dawanau local Wheat, DW2= Dawanau Foreign Wheat, WD = Wudil, CR = Chiromawa ND= Not Detected

DISCUSSION

According to the results on wheat fungal biodiversity in this study, it can be seen that both field and storage molds are present. *Aspergillus flavus* has the highest occurrence, appearing in 11 samples, followed by *Alternaria Spp* appearing in 8 samples. The least was *Fusarium spp* occurring in only 3 samples. The high occurrence of *Aspergillus flavus* might be because *Aspergillus* can be present both in the field and during storage.

The findings in this study are similar to the work of Nesrine *et al.* (2013) where mycological analysis of wheat grains showed a predominance of *Alternaria spp, Penicillium spp, Fusarium spp, Aspergillus spp, Rhizopus spp,* and *Mucor spp* genera. The matching result may be due to the similarities of climate conditions between the two locations as well as variety of wheat. Other similar studies have found the same fungal genera predominance pattern in Algerian wheat (Ennouri *et al.,* 2018).

While diseases caused by most of the pathogenic organisms are acute in nature, reverse seems to be the case with Aflatoxicosis caused by aflatoxins. In the latter case, manifestation always appears to be on chronic basis (Garba *et al.,* 2017). The levels of aflatoxin B1 (µg kg) in the wheat samples showed that, out of total samples analyzed, 75% were positive for Aflatoxin B1 while 25% were negative. The results revealed low levels of aflatoxin B1 ND – 4.3 µg kg in all the samples examined. Two samples from Dawanau and Wudil showed Aflatoxin B1 levels 4.3µg/kg higher than the limit established by EU legislation 4µg/kg but none of the samples exceeds the level set by Standard Organization of Nigeria 10 µg/kg (European Commission, 2014)

Aflatoxin-contaminated foods and feeds are associated with health risk for human beings and animals. Aflatoxins have been shown to have adverse health effects such as hepatotoxicity, mutagenesis, carcinogenesis, immunosuppression, neurotoxicity, epigenetic effects, reproductive dysfunctions and stunted growth (Nazhaand *et al.*, 2020).

Environmental factors (such as temperature, relative humidity and amount of rainfall), insect infestation, pre and post-harvest handing influence the production of Aflatoxin in the field and during storage. Studies done on the effect of environmental conditions on Aflatoxin contamination of corn showed that, when the conditions were favourable, the occurrence of Aflatoxin B1 was highly related to these factors (Viquez *et al.*, 2006).

In a study by Muga *et al.* (2019), the storage temperature significantly *($p \leq 0.05$)* affected Aflatoxin production in maize at 60% and 90% Relative Humidity. Aflatoxin levels were greater at 30°C than at 20°C, particularly at 90% RH, Aflatoxin production was also significantly *($p < 0.05$)* affected by relative humidity (RH). Aflatoxin levels were higher at 90% RH than at 60% RH. At 60% RH, 30°C still resulted in higher levels of aflatoxin than 20°C. Therefore, the presence of Aflatoxin B1 in wheat grains could be attributed to climatic conditions of the study area which is located in west Africa and has an all year round ambient temperature, ranging from 21^{o}C in December/January and over 35^{o}C in hottest months (April/May) (Barau, 2007). This high temperature as well as relative humidity, provides optimal conditions for the growth of molds and subsequent production of aflatoxins (Makun *et al.,* 2010)

Grains in Northern Nigeria, are handpicked at harvest, left to dry for weeks at a threshing ground, threshed, packaged and transported in sacks to markets where they are sold in open containers. The complex effects of relative humidity, temperature, precipitation and insect and rodent infestation and their daily variation may interplay to provide conditions conducive for fungal growth and aflatoxin contamination on such exposed and poorly stored grains (Makun *et al.,* 2010). These methods of harvesting, storage and transportation may be responsible for the detection of aflatoxin B1 in the samples albeit at low concentrations.

The presence of Aflatoxin B1 in the grains despite the low moisture content of less than 13% suggests that contamination may have occurred in the field where moisture content is high along with hot humid days and cold dry nights.

The result also showed that the locations where the samples were collected as well as time of collection had no significant effect on Aflatoxin B1 levels ($p<0.05$). This may be due to the same climatic conditions of the markets where samples were collected as well as similarities in drying techniques. The result also revealed that Aflatoxin B1 concentration between local and foreign wheat did not vary significantly ($p<0.05$).

Aflatoxin B1 concentration reported in this study differs with the results of Odoemelam and Osu (2009) who reported a mean concentration of 19.00 µg/kg on wheat from the Niger Delta region of Nigeria, the difference might be due to the difference in soil characteristics between Niger Delta and Northern Nigeria as well as high rainfall and relative humidity in Southern Nigeria (Atanda *et al.*, 2013) which aids in fungal

growth and consequently Aflatoxin B1 production. The results of Aflatoxin B1 in this study are similar to that of Felipe *et al.* (2014) who reported a mean value of 4.2µg/kg in wheat grains from different markets of Rio de Janeiro region of Brazil.

CONCLUSION

It can be concluded that Wheat grains were contaminated with either or both of field and storage molds, with *Aspergillus flavus* having the highest occurrence with *92%* and *Fusarium spp* having the lowest with 25% which is in correlation with other findings (Nazhaand *et al.*, 2020), (Ennouri *et al.,* 2018). Out of the samples analyzed, 75% were contaminated with Aflatoxin B1 with Dawanau market having the highest concentration of 4.3 µg/kg and Chiromawa having the lowest with 0.2 µg/kg. Furthermore, 16.6% of the samples were found to be higher than the limit set by EU 4.0µg/kg, while none of the samples exceeded the level set by Standards Organisation of Nigeria 10µg/kg.

RECOMMENDATIONS

Assessment of local processing and unit operations of wheat prior to eating should be carried out to ascertain their effects on Aflatoxin B1 concentration.

REFERENCES

Atanda, O., Makun, H. A., Ogara, I. M., Edema, M., Idahor, K. O., Eshiett, M. E., & Oluwabamiwo, B. F. (2013). Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds. *Mycotoxin and Food Safety in Developing Countries*. doi:http://dx.doi.org/10.5772/55664

Barau, A. S. (2007). *The Great Attractions of Kano.* Kano: Researchgate. doi:DOI: 10.13140/RG.2.1.3958.9922

Ennouri, A., Sanchis, V., Rahouti, M., & Zinedine, A. (2018). Isolation and molecular identification of mycotoxin producing fungi in durum wheat from morocco. *Journal of Materials and*, 1470-1479. https://doi.org/10.26872/jmes.2018.9.5.161

FAO-AMIS. (2017). Retrieved from http://statistics.amis-outlook.org/data/index.html#DOWNLOAD_STANDARD.

FAOSTAT. (2014). Retrieved from http://Faostat.fao.org

FAOSTAT. (2017). Retrieved from http://www.fao.org/faostat/en/#data/QC.

Felipe, M. T., Douglas, d. Á., Yuri, D. P., Santos, T. B., Glória, M. D., Marcelo, E. F., & Tatiana, S. (2014). Determination of Aflatoxins in Wheat and Wheat by-products Intended for Human Consumption, Marketed in Rio de Janeiro, Brazil. *Journal of Food and Nutrition Research*, 671-674.

Garba, M. H., Makun, H. A., Jigam, A. A., Hadiza, L. M., Patrick, B. N., & Kabiru, A. Y. (2017). Viability of Fungal Spores Isolated from Sorghum Grains Sampled from the Field, Market and Different Storage Facilities in the Six Agro-ecological Zones of Nigeria. *Microbiology Research Journal International*, 1-11.

Makun, H. A., Anjorin, S. T., B, M., Adejo, F. O., Afolabi, O. A., & Fagbayibo, G. (2010). Fungal and aflatoxin contamination of some human. *African Journal of Food Science*, 127-135.

Nazhand, A., Durazzo, A., Lucarini, M., Souto, E. B., & Santini, A. (2020). Characteristics, Occurance, Detection and Detoxification of Aflatoxins in Foods and Feeds. *Foods*, 644 doi:10.3390/foods9050644

Nesrine, B.-H., Semmar, N., Isabelle, P.-G., Guesmi, A., Marwa, C., Cherif, I., . . . Sevastianos, R. (2013). Spatio-temporal analysis of post-harvest moulds genera distribution on stored durum wheat cultivated in Tunisia. *Journal of Stored Products Research*, 116-123.

Odoemelam, S. A., & Osu, C. I. (2009). Aflatoxin B1 Contamination of Some Edible Grains Marketed in Nigeria. *E-Journal of Chemistry, 6*(2), 308-314.

Oyewole, C. I. (2016). *The Wheat Crop.* Researchgate. doi:DOI: 10.13140/RG.2.2.13776.92164

Sadhasivam, S., Britzi, M., Zakin, V., Kostyukovsky, M., Trostanetsky, A., Quinn, E., & Sionov, E. (2017). Rapid Detection and Identification of Mycotoxigenic Fungi and Mycotoxins in Stored Wheat Grain. *Toxins*, 1-17.

Sokefun, E., Ayepola, O. O., & Olasehinde, I. G. (2018). Mycotoxins: Food Production and Exportation in Nigeria. *Earth and Environmental Science 210*. doi:10.1088/1755-1315/210/1/012018