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ASSESSMENT OF FIVE MAJOR MYCOTOXINS IN MILLET AND SORGHUM IN BENUE SOUTH SENATORIAL DISTRICT, BENUE STATE, NIGERIA.

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Abstract

Millet and sorghum grain are not consumed soon after harvest but often stored for many months to be sold or consumed later.Extremely high and low temperatures, humidity, improper handling and improper storage have been contributory to mycotoxin production by filamentous fungi in most food crops either on the field, in storage or during transportation. This study considered the assessment of mycotoxin in indigenous millet and sorghum produced by farmers in Benue South Senatorial District of Benue state. Mycotoxin Extraction and Evaluation of Deoxynivalenol (DON) by HPLC were carried out. The result obtained from the different analysis showed mycotoxin occurrence in both food samples analysed at varying concentrations. Incidences of all five mycotoxins were determined in both food samples up to 94.9%. Data obtained from the analysis (Tables 2 and 3) showed the occurrence of aflatoxins and ochratoxin A in both millet and sorghum samples in comparison to the other three mycotoxins. From the result above the percentage occurrence of various fungi species are reported thus; Aspergillus spp (41.67%), Fusarium spp. (27.08%), Mucor spp. (12.5%), Penicillium spp. (10.42%) and Rhizopus spp. (8.33%). Aspergillus spp occurred in all three zones with higher frequency and are known producers of mycotoxins such as aflatoxin, sterigmatocystin and ochratoxin. The high incidence of both DON and ZEA in the food commodities analysed is a cause for concern and reason formore research into other mycotoxins contamination of Nigerian food commodities as well as control strategies in order to combat mycotoxin contamination.

Key words: Mycotoxins, Ochratoxin, Millet, Sorghum and Aflatoxins.

1.0 Introduction

1.1 Background of the Study

Cereal grains (rice, corn, wheat etc) are staple foods that provide more food energy worldwide than any other type of crop (Okpiaifo *et al*., 2020). Cereal grains, maize and rice are some of the staple foods consumed in most parts of sub-Saharan Africa and the world over. Maize is the third most important cereal crop in the world (Zain, 2013), and one of the most staple foods in the Northern Nigeria. Nigeria is one of the largest maize producing countries in Africa. The nutritional components which include carbohydrates, potassium, vitamins, minerals and fibers can be compared to those of sorghum, rice, cassava, yam, potato etc. This crop serves a vital link in the human food chain in Nigeria and most parts of the world. Maize grains are presently used in the food industries as an important component in weaning foods for infants and it is equally valued by adults (Akuma *et al.*, 2019).

Millet and sorghum grain are not consumed soon after harvest but often stored for many months to be sold or consumed later. It has been reported by several researchers that fungal infestation in maize results in color change, decreases in nutritional values, and reduction of overall quality and quantity of the maize. Fungi are agents of food contamination and many species are saprobes, found in a variety of habitats and are ubiquitous agents of decay. Several of these fungal species have been found associated with production of mycotoxins, which are of public health importance (Amadi and Adeniyi, 2009).

In Nigeria, especially in the rural areas most of the food commodities consumed are staple food with less consumption of processed food. Millet and sorghum are some of the food crops widely consumed across the country. These grains are cultivated in the country as well as imported in other to meet the high demand for grains in the country. It was reported that up to 32.9 kg of rice was consumed per person on a yearly basis between 2007 and 2010 in the country (Amadi and Adeniyi, 2009), with an assumption that this value must have increased between 2010 and now. Sorghum on the other hand, is one food crop that is consumed in different forms in the country (Awopetu *et al.*, 2017). It can be cooked, roasted or processed into a porridge meal (“ogi”) that is at most times used as a weaning food for infants. Most of these food crops are cultivated by subsistence farmers who do not implore high tech farming techniques. Also, because food crops are cultivated in seasons they are usually stored in proper and improper storage houses for availability throughout the year (Zain, 2013).

Mycotoxin production by filamentous fungi mainly *Aspergillus*, *Fusarium* and *Penicillium* species is usually triggered by specific conditions which range from environmental and climatic to human (Di Stefano *et al*., 2014). Extremely high and low temperatures, humidity, improper handling and improper storage have been contributory to mycotoxin production by filamentous fungi in most food crops either on the field, in storage or during transportation (Awopetu *et al.*, 2017). There are two major seasons in Nigeria: the rainy and dry (harmattan) seasons, with high temperatures almost all through the year. These climatic conditions alongside other conditions are some of the challenges faced by farmers in this part of the world which has prompted the need for research into mycotoxin contamination of a variety of food crops cultivated in the country (Di Stefano *et al*., 2014).

Major fungi associated with grain storage, including millet and sorghum, are *Aspergillus flavus, Fusarium* sp, and others. Fungal load in millet and sorghum presents a major risk for humans and animals, through production of mycotoxins (especially Aflatoxins) (Apeh *et al.*, 2015). While in storage, grains are mostly susceptible to infection by species of fungi. Infected grains by fungi result in reduced germination, visible mould discoloration; chemical

and nutritional changes, increased its mustiness, production of carcinogenic toxins and finally leading to spoilage of grains in many ways (Amadi and Adeniyi, 2009).

Food spoilage is a worldwide concern, arising mainly from chemicals, environmental factors, cross-contamination during processing, from food packaging materials and through natural toxins (especially those produced by fungi). It usually poses a health concern, leading to strict regulations in food products by national and international governments (Di Stefano *et al*., 2014). Favourable climatic and environmental factors usually promote fungi prevalence. Indeed, fungi are found growing on crops and foodstuffs (cereals, nuts, spices, dried fruits, apples and coffee beans). The health effect of fungal infection due to mycotoxin contamination depends on the degree of contamination (Freire and Da Rhocha, 2016).

Exposure to mycotoxins may lead to carcinogenic, immunosuppressant and estrogenic effects due to their toxicity (Pradeep *et al.,* 2017). Aflatoxins which are also mycotoxins are known to infect corn, corn silage, all cereal grains, sorghum, peanuts, and other oilseeds etc. According to Najeeb and Farag (2019), aflatoxin B1 is commonly found in rice. Reddy and Muralidharan (2009), observed that 67.8% of rice cultivated in India are contaminated with aflatoxin B1 (AFB1) with concentrations ranging from 0.1 to 308.0 µg/kg. Similarly, Amin *et al*., (2015) reported that hepatocellular carcinoma (HCC) incidence being reported in some countries is linked to consumption of AFB1 in food staples.

Fungal growth in maize is facilitated by hot and humid conditions (Makun *et al.*, 2009). In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains. Subsequently, the maize is stored while still relatively moist and warm; both warmth and high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains (Ehrilic, 2007). Maize, like other stored products is hygroscopic in nature and tends to absorb or release moisture. Even if properly dried after harvest, exposure to moist and humid conditions during storage will cause the (kernel) to absorb water from the surroundings, leading to increased millet moisture contents, which result in enhanced deterioration (EC, 2005). To maintain high quality maize during storage, millet should be protected from weather (including relative humidity and temperature), growth of microorganisms, and insects (Ehrilic, 2007). Also, poor harvesting practices, unsuitable storage conditions, improper transportation, marketing, and processing also contribute to fungal growth. These environmental conditions as well as the food production chains are characteristic in most parts of Benue South where these staple foods are susceptible to toxigenic fungi and obviously their mycotoxin contaminants. Fungal presence and growth in these grains therefore present a major risk for humans and animals, through production of mycotoxins (Ben-chando, *et al.,* 2017).

Owing to the significance most families attached to rice as a priority meal, the need to define the toxic status of locally produce maize cannot be underplayed.

It is in this light that this study considered the assessment of mycotoxin in indigenous millet and sorghum produced by farmers Benue South Senatorial District of Benue state.

2.0 Materials and Methods

2.1 Study Area

This study was carried out in Benue South Senatorial District. The sample for the study was collected from various millet and sorghumselling points including homes, on the field, in the

open, jute or polypropylene bags, conical structures, raised platforms, clay structures, and baskets; randomly selected from the study area. These locations were selected because they are well known for millet and sorghum cultivation. The reason for using indigenous millet and sorghum crops from these areas was to identify any underlying climatic factor to the problem and practices prevalent in the area.

2.2 Sample Collections

Samples of Sorghumand millet grains were collected from various zones in the study area. Precautions were taken to obtain random samples and placed in sterile paper bags, and labeled samples were then transported to the laboratory for evaluation.

2.3 Isolation of Storage Fungi

2.3.1 Media preparation

39 g of potato dextrose agar was weighed using a weighing balance and poured into a conical flask and dissolved in 1litre of distilled water, boil while mixing to dissolve well. Autoclave for 15 min at 121ºC, 0.2 g of chloramphenicol was also added to the prepared medium, this medium was allowed to cool before it was used.

2.3.2 Serial dilution

Six test tubes containing 9ml of sterile distilled water were placed on a rack on the bench, 1 ml from the sample solution was pipetted aseptically into the first test tube and mixed and was repeated up to the last tube (10-6). 1 ml of 10-3, and 10-6 dilutions was inoculated using spread plate technique on potato dextrose agar (PDA).

2.3.3 Culturing

Seed samples were blended using a Philip blender, six test tubes containing 9 ml of sterile distilled water were placed on a rack on the bench, 1 ml from the sample solution containing 1 g of the blended maize was pipetted aseptically into the first test tube and mixed and was repeated up to the last tube (10-6). 1 ml of 10-3 and 10-6 dilutions was inoculated using spread plate technique on potato dextrose agar (PDA) [4].

The plates were incubated at 27±2ºC in an incubator for 5 to 7 days after which the plates were examined visually for fungal growth and the numbers of fungi colonies developed was recorded (Akuma *et al.*, 2019).

2.3.4 Sub-culturing

This was carried out to separate different colonies of fungi to obtain pure colonies; the fungi that grew from the serial diluted maize were separately sub-cultured into fresh PDA media using sterile inoculating needle.

The sub-cultured plates (in two replicates) were incubated at 27±2ºC for another 5 to 7 days and the growth observed and resulting fungi identified (El tawila *et al*., 2013).

2.4 Identification of Fungi

All the materials needed for the test were well checked and cleaned before use, especially the glass slides, small part of the specimen was picked with the use of sterile inoculating needle, and placed on the glass slide. A drop of cotton blue lactophenol was placed on the glass side, cover slip was used to cover the specimen and tissue paper/ cotton wool was used to clean the over flow at the edges of the slide, then placed on the microscope stage for examination using low objective lens, and change to higher power for further examination of morphological structures. Fungal colonies were identified to species level were possible under the

microscope using conidial and/ or spore structures and mycelia characteristics (Akuma *et al.*, 2019).

2.5 Mycotoxin Extraction and Analysis

The sample was subsequently prepared for extraction and evaluation for detectable level of any associated mycotoxin. The mycotoxin evaluation was limited to *Fusarium* toxin, deoxynivalenol. 10g of millet and sorghum samples were weighed; 40 ml (50:50 v/v) of acetonitrile: water was added, and 10 g of MgSO4 and 3 g of NaSO4 were also added. The solution was centrifuged for five minutes. 10 ml of supernatant was loaded on solid phase extraction column conditioned with 20 ml ethyl acetate for elution and the eluent was evaporated and dissolved to dryness with mobile phase before HPLC analysis (Akuma *et al.*, 2019).

2.5.1 Evaluation of Deoxynivalenol (DON) by HPLC

The extracts were injected into the HPLC machine and the determination was carried out using HPLC instrument: HPLC MODEL 1100 Series- with waters 501 components (Germany HPLC).

3.0 Results and Discussion

3.1 Result

Result obtained from the different analysis showed mycotoxin occurrence in both food samples analysed at varying concentrations. Results showed some of the food samples being contaminated by all five mycotoxins investigated.

Table 1. Analyses of Mycotoxins in Millet Samples as Determined by High Performance Liquid Chromatography.

Mycotoxins	% incidence	Range(µg/kg)	Mean	Std. Dev.
AFs	87.8	**0.01-6.50**	**0.76**	**3.10**
OTA	92.7	**0.7-180.9**	**18.2**	**112.3**
FB1	90.2	**0.9-59.6**	**18.8**	**112.6**
FB2	n.d	**n.d**	**n.d**	**n.d**
ZEA	80.5	**0.7-570.6**	**203.9**	**440.0**
DON	70.7	**0.1-0.7**	**0.3**	**1.6**

Mean- mean of positive samples. % incidence- no. of positive samples in percentage. Std. Dev.- standarddeviation*

Table 2. Analyses of Mycotoxins in Sorghum Samples as Determined by High Performance Liquid Chromatography

Mycotoxins	% Incidence	Range(µg/kg)	Mean	Std. Dev.
AFs	94.9	0.07-109.78	10.24	24.92
OTA	92.3	0.6-79.0	14.8	16.7
FB1	94.9	10.0-3644.0	645.2	919.1
FB2	66.6	15.2-1123.9	137.8	525.4
ZEA	64.1	1.83-652.3	139.0	155.8
DON	69.2	0.1-0.7	0.3	0.2

Mean- mean of positive samples. % incidence- no. of positive samples in percentage. Std. Dev.- standarddeviation*

Incidences of all five mycotoxins were determined in both food samples up to 94.9%. Data obtained from the analysis (Tables 1 and 2) showed the occurrence of aflatoxins and ochratoxin A in both millet and sorghum samples in comparison to the other three mycotoxins.

Table 3. Fungal Isolates and Percentage Occurrence

Isolate	Frequency	Fungal CFU/ml	Percentage (%)
Rhizopus spp.	4	$4x10^3$	8.33
Aspergillus spp.	20	$20x10^3$	41.67
Mucor spp.	6	$6x10^3$	12.5
Penicillium spp.	5	$5x10^3$	10.42
Fusarium spp	13	$13x10^3$	27.08
Total	**48**		**100**

From the result above the percentage occurrence of various fungi species are reported thus; *Aspergillus* spp (41.67%), *Fusarium* spp. (27.08%), *Mucor* spp. (12.5%), *Penicillium* spp.(10.42%) and *Rhizopus* spp. (8.33%). *Aspergillus* spp occurred in all three zones with higherfrequency and are known producers of mycotoxins such as aflatoxin, sterigmatocystin and ochratoxin. *Fusarium* toxins which equally occurred in all the zones are produced by over 50 species of Fusarium especially, *F. graminearium* and *F.culmorum*, and have a history of infecting the grain of developing cereals such as wheat andmaize. They include a range of mycotoxins, such as: the fumonisins, which affect the nervoussystems of horses and may cause cancer in rodents; the trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and zearalenone, which is not correlated to any fatal toxic effects in animals or humans (Egbuta *et al.*, 2020). DON is a tricothecene produced by either *F. graminearium* or *F. culmorum*. In this work, 27% of the fungalisolates were members of the *Fusarium* family and this accounted for the large part of the mycotoxin (DON) contamination in the study area.

3.2 Discussion

The concentration of the different mycotoxins detected in the food samples via HPLC were based on the chromatograms of AFB1, AFB2, AFG1, AFG2, OTA, FB1, FB2, ZEA and DON standards used as well as calibration curves plotted from the areas of peaks of standards and concentration of standards (Zain, 2013).

Food contamination by mycotoxins is a major problem in the tropics and sub-tropics due to climatic conditions and other conditions that favour the growth of fungi and subsequent production of mycotoxins by these fungi (Akuma *et al.*, 2019).

Nigeria is not left out of this situation because of its adverse weather conditions, farming practices, storage conditions and mode of transportation of food crops. Out of over 20 commonly occurring mycotoxins in food and agricultural products (Feka *et al.*, 2020), the aflatoxins, ochratoxins, fumonisins, zearalenone and thetrichothecenes have been reported to be major mycotoxins occurring in food.

Milletgrain is a highly suitable substrate for the production of the five major occurring mycotoxins mentioned as these mycotoxins have been reported to occur in millet grains at different stages of production worldwide. Mycotoxin contamination of rice often occurs in the field prior to harvest, while post-harvest contamination can occur if drying was delayed and during storage, if moisture content is allowed to exceed critical values for mould growth (Georgievski *et al.*, 2016). Results obtained from the mycotoxin screening of millet samples

also agree with this views about mycotoxin contamination of millet crops. Although lower incidences were obtained when samples were analysed using TLC which could be attributed to the detection limit of thin layer chromatographic techniques (Chilaka *et al.*, 2016), very highincidences were determined through HPLCanalysis. The occurrence of these mycotoxinsAFs, OTA, FBs, DON and ZEA evaluated in thisfood sample could be attributed to contaminationof the samples by mycotoxigenic fungi *Aspergillus*, *Fusarium* and *Penicillium* (Zain, 2013).

Maize is also another food commodity that act asa good substrate for growth of toxigenic fungi producing mycotoxins. One major mycotoxinassociated with maize is fumonisin which is produced mainly by *F. verticillioides* and *F. proliferatum* (Chilaka *et al.*, 2016). Other mycotoxins reportedto be associated with contamination of maizeinclude OTA, ZEA, AFs and the trichothecenes (Batagarawa, *et al.*, 2015).

Conclusion

In this study, mycotoxins were found to co-occur at different concentrations above the set limits which are a cause for concern in terms of mycotoxin control strategies in Nigeria. As was mentioned earlier much emphasis has been given to aflatoxins, ochratoxin A and fumonisins in Nigerian foods with little attention given to other less common mycotoxins which also have negative health effects. The high incidence of both DON and ZEA in the food commodities analysed is a cause for concern and reason for more research into other mycotoxins contamination of Nigerian food commodities as well as control strategies in order to combat mycotoxin contamination.

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