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Assessment of Fungal and Mycotoxin Contamination of Maize Grains Collected from Senatorial Zones of Benue State

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Abstract

Purpose: Assessment of fungal and mycotoxin contamination of maize grains collected from Senatorial Zones of Benue State was carried out in this study.

Methodology: Maize samples were collected in sterile polythene bags, labelled according to the sample locations and taken to the laboratory for analysis. Isolation and identification of the fungi was carried out using dilution method and standard mycological procedures. Samples were plated on Potato Dextrose Agar (PDA) supplemented with 60ug ml^{-1} chloramphenicol as a bacteriostat and incubated at room temperature for mycological identification and biomass. Quantifications of aflatoxin in the maize samples was carried out using Specific ELISA kit.

Findings: Results showed that there was a high level of fungal contamination found in maize grains in Benue State. Many (40%) of Zone A maize grains had a high level ($\geq 3.1 \times 10^7$ cfu/g) of fungal contamination, 40% of Zone B maize grains also had ($\geq 3.1 \times 10^7$ cfu/g) of fungal contamination. While 26.7% of grains purchased from Zone C had ($\geq 3.1 \times 10^7$ cfu/g) of fungal contamination. It was also observed that none (0%) of Zone A maize grains had aflatoxin level above 6.57ppb, while 10% of Zone B seeds produced aflatoxin levels above 6.57ppb and 25% of maize seeds from Zone C had aflatoxin levels above 6.57ppb. It was also observed that Zone B maize grains had the highest aflatoxin level of 9.50ppb, followed by Zone C with 9.20ppb, while Zone A had the lowest aflatoxin level of 5.10ppb. High aflatoxin levels above the 5.0ppb recommended by Standard Organization of Nigeria (SON) as tolerance limit for maize grains were observed in many of the locations studied.

Unique Contributions to Theory, Policy and Practice: Therefore, maize grains should be dried properly and stored in less humid environment to avoid fungal growth and aflatoxin production, so as to prevent public health issues among consumers.

Keywords: *Fungi, Aflatoxin, Contamination, Maize Grains, Senatorial Zones, Benue State*

BACKGROUND

Mycotoxin are secondary metabolites produced by fungi (commonly *Aspergillus Flavus* and *Aspergillus Paraciticus*) which grow on food crops (maize, wheat, groundnut, etc.) and also on commodities of animal origin (meat products, sausages) (Milicevic *et al*., 2010). These fungi grow on agricultural commodities on the field and during storage, under different climate and weather conditions (Mady, 2001). Presence of these fungi on foods is grossly significant in food safety (Mady, 2001). Aflatoxins and other mycotoxins are responsible for contaminating many food supply worldwide (Dohlman, 2003). According to report by FAO (1991) mycotoxin contaminates 25% of foodstuff worldwide causing huge losses in food trade (Otsuki *et al*., 2001) and severe illnesses and death in humans and farm animals (Williams *et al*., 2004; Liu and Wu, 2010).

In many developing countries of Africa, maize is a staple food but it's frequently contaminated with aflatoxins because of its high colonization by aflatoxin producing fungi (Klich, 2002; Bandyopadhyay and Cotty, 2011). There are more than 300 mycotoxins known, but scientific interest has focused on few which are known to be dangerous to human and animal health (Wu *et al*., 2011). They are aflatoxins, ochratoxins, fumonisins, trichothecenes, deoxynivalenol (DON), and zearalenone (ZEA). (Moss, 1996; Brera *et al*., 2008). Among these mycotoxins, aflatoxin has been given more attention because of its high carcinogenic toxicity and potency (FAO, 1979). The international agency for research on cancer (IARC) (1993), reported that aflatoxins (AFB1) are the most potent natural carcinogenic substances and are being linked to severe illnesses and also increase the risk of liver cancer in humans. This study investigates fungal and aflatoxin contamination of maize grains collected from Senatorial Zones of Benue State.

MATERIALS AND METHODS

Study area

Benue State consists of twenty-three (23) Local Government Areas (LGA) and three (3) Senatorial Districts or Zones which includes: (1) Zone A is Benue North East: Katsina Ala, Konshisha, Kwande, Ushongo, Logo, Ukum, Vandeikya. (2) Zone B is Benue North West: Buruku, Gboko, Guma, Gwer-East, Gwer-West, Makurdi, Tarka. (3) Zone C is Benue South: Ado, Agatu, Apa, Obi, Ogbadibo, Ohimini, Oju, Okpokwu, Otukpo. Two LGAs were randomly selected from each Senatorial Zones for the purpose of this research.

Sample collection

Maize samples were collected in sterile polythene bags, packaged and labelled according to the sample locations. After collection of the maize samples, they were immediately transported to Microbiology Laboratory University of Agriculture Makurdi for mycological identification and aflatoxin detection.

Mycological analysis

Samples collected from each location was analyzed for mycological contamination under aseptic conditions. Potato Dextrose Agar (PDA) medium was prepared according to manufacturer's instruction. Potato Dextrose Agar powder (19.50 g) was weighed using a weighing balance and poured into a conical flask. Distilled water (500 ml) was added to the PDA and stirred vigorously (3.9 g of PDA for each 100 ml of distilled water). Chloramphenicol (500 g/L) was added to prevent bacterial contamination. The mixture was then heated on a hot plate to obtain a homogeneous mixture and was autoclaved (121^{o}C; 15lb; 15 min) (Sani and Kasim, 2019).

Determination of fungal load

Maize samples collected from the markets were examined for fungal contamination. Each sample (100 g) was pulverized using a Philip blender. Nine test tubes containing 9 ml of sterile distilled water were placed on a rack on the bench and 1 g of the pulverized sample was mixed with 9 ml of sterile distilled water. One-ml was taken from the solution and was pipetted aseptically into the first test tube and mixed. This was repeated up to the last tube (10^{-9}). One millilitre from each dilution was placed onto Petri-dish and was overlaid with molten PDA medium (Postagate, 1992; Sani and Kasim, 2019 & Akoma *et al.*, 2019). The PDA mixture was allowed to solidify and incubated (30 °C, 5-7 days). The plates were examined visually for fungi growth and identification using macroscopic and microscopic characteristics such as colony colours, colony texture, reverse colour, soluble pigment (Pitt and Hocking, 1997; Elbashiti *et al.*, 2010), nature of spore, conidiophores, sporangiophore and vessicles using identification keys by Davise (2002), Klich (2002) and Robert *et al.*, (2004).

Identification of fungi

Identification of the fungal isolates based on microscopic characteristics was done. A small part of the fungal specimen was picked with a sterile inoculating needle and placed on a glass slide. A drop of cotton blue lactophenol was placed on the glass slide and cover slip was used to cover the specimen. Overflow edges of the glass slide was cleaned with cotton wool and the glass slide was placed on the microscope stage for examination and observed at x 40 objectives. (Raper and Fennel, 1965; Nelson *et al.* (1983); Rechard (1996), and Klich, 2002).

Detection, identification and quantification of aflatoxin

Specific ELISA kit was used for the detection, identification and quantification of the aflatoxin in the maize samples. The samples preparation, extraction and purification were done according to the instruction given by the company (RIDASCREEN®Aflatoxin B1, Germany). ELISA reader was employed for the quantification of aflatoxin B1.

STATISTICAL ANALYSIS

Data was subjected to Analysis of Variance (ANOVA) and Chi square test using GENSTAT statistical software (17^{th} edition). Statistically significant means was separated using F-LSD at 5% level of probability. Descriptive statistics was also used.

RESULTS

Table 1: Number of Fungal contamination in Maize Grains across Senatorial Zones of Benue State

Senatorial Zone	No. of colony forming units (cfu) per gram of seeds		Total (%)
	$\leq 3.0 \times 10^7$	$\geq 3.1 \times 10^7$	
Zone A	16 (59.25%)	11 (40.7%)	27 (100.00%)
Zone B	27 (60.0%)	18 (40.0%)	45(100.00%)
Zone C	33 (73.3%)	12 (26.7%)	45 (100.00%)
Total	76 (65.0%)	41 (35.0%)	117 (100.00%)

P=0.172 (P>0.05)

Table 2: Aflatoxin levels (ppb-parts per billion) of Maize grains according to Senatorial Zones

Senatorial	Number of seeds	Aflatoxin Levels (ppb-part per billion)		
		(0.70 - 3.62)	(3.63 - 6.56)	(> 6.57)
	tested (%)			
Zone A	12 (100)	8 (66.7)	4 (33.3)	0 (0)
Zone B	20 (100)	15 (75.0)	3 (15.0)	2 (10.0)
Zone C	20 (100)	10 (50.0)	5 (25.0)	5 (25.0)
Total	52 (100)	33 (63.5)	12 (23.1)	7 (13.5)

(Chi-square 5.926; df = 4; P = 0.205).

Table 3: Meteorological Data obtained from Sampling Areas (grain markets of LGAs) of Benue State.

Local Government Areas	Mean Temperature (o C)	Mean Humidity (%)	of Latitude	Longitude
Gwer-west	30	63	N7^o 34.940^o	E8^o 000
Agatu	32	85	N7^o 77.4513^o	E9^o 25.22
Buruku	28	94	N7^o 27.479^o	E9^o 11.958
Oju	31	82	N7^o 07.5343^o	E9^o 33.395
Katsina Ala	30	52	N7^o 10.803^o	E9^o 17.395
Logo	32	86	N7^o 49.0340^o	E9^o 14.672

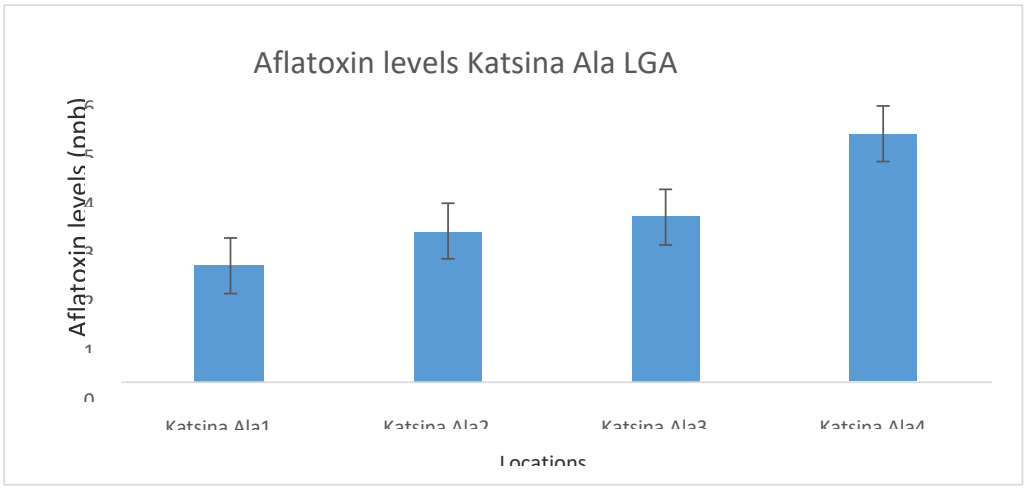

Figure 1: Aflatoxin levels for Katsina Ala Local Government Area (Zone A)

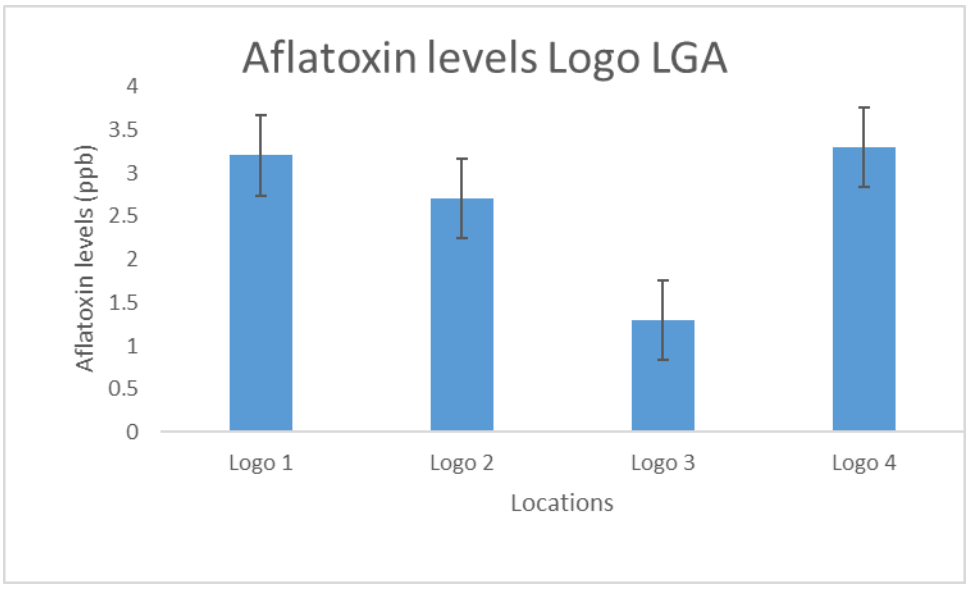

Figure 2: Aflatoxin levels for Logo Local Government Area (Zone A)

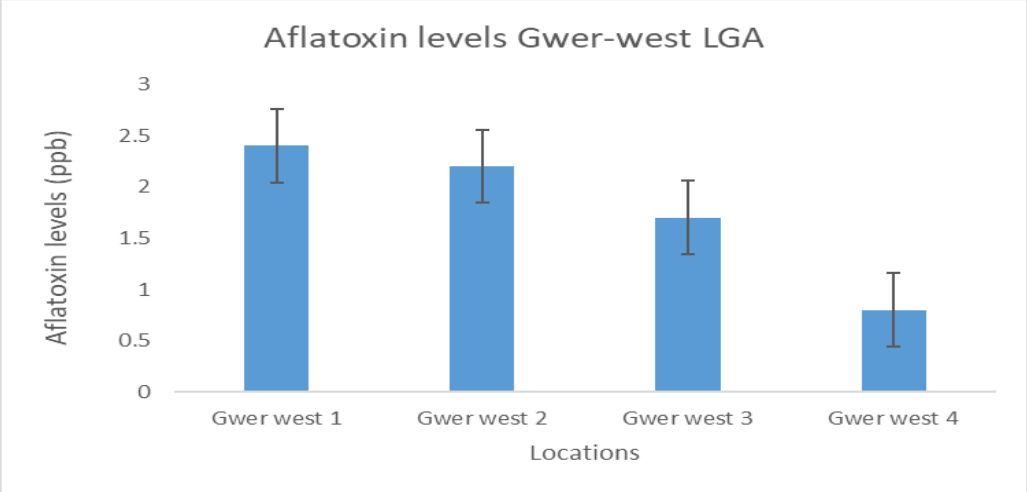

Figure 3: Aflatoxin levels for Gwer-west Local Government Area (Zone B)

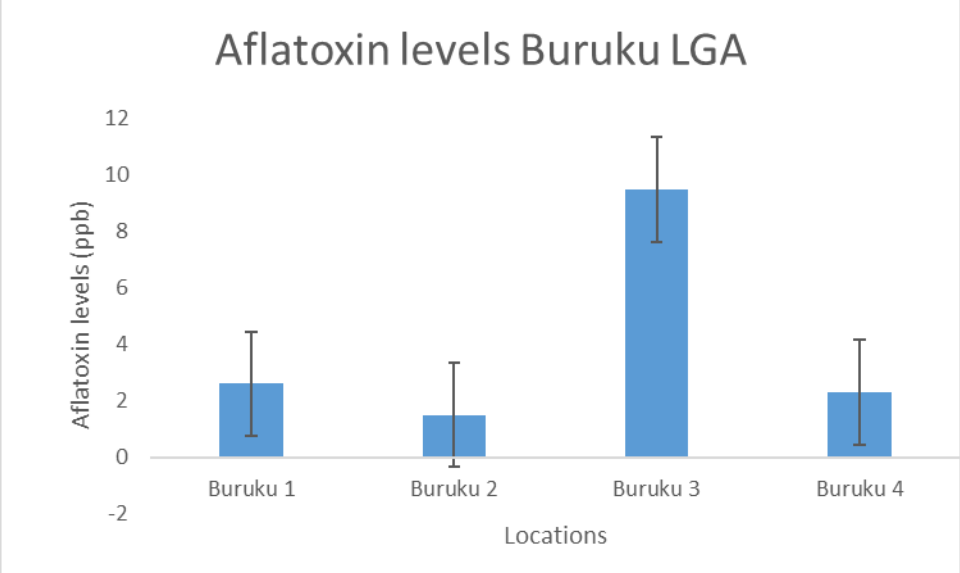

Figure 4: Aflatoxin levels for Buruku LGA (Zone B)

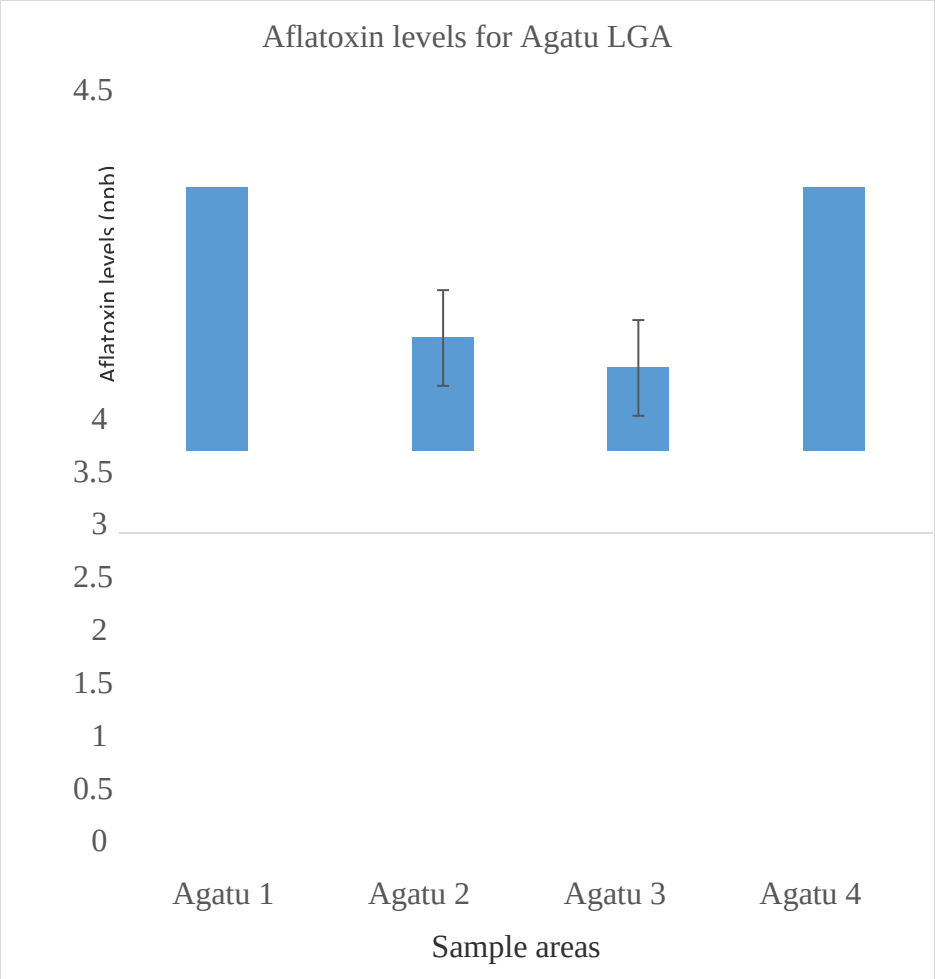

Figure 5: Aflatoxin levels for Agatu LGA (Zone C)

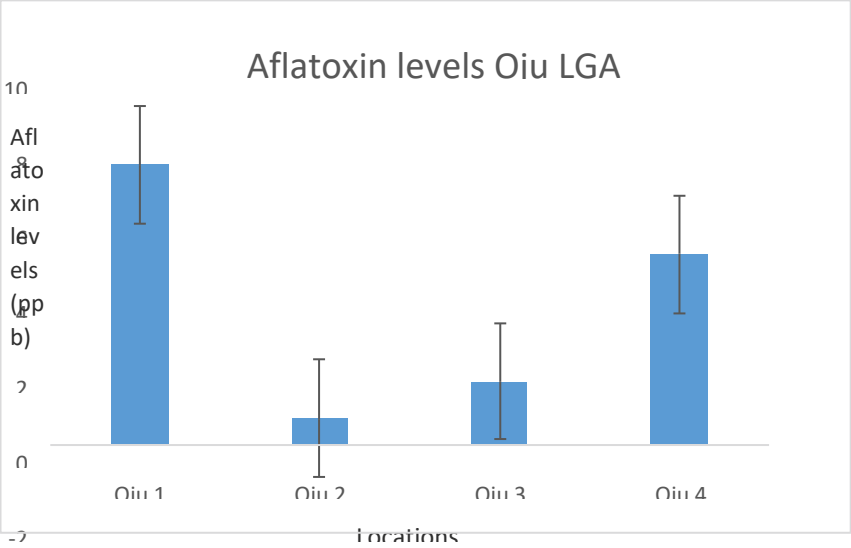

Figure 6: Aflatoxin levels for Oju LGA (Zone C)

DISCUSSION

Determination of fungal and aflatoxin contamination of maize grains carried out in this study showed that there was a high level of fungal contamination found in maize grains in Benue State.

Exactly 40% of Zone A maize grains had high level ($\geq 3.1 \times 10^7$ cfu/g) (Table 1) of fungal contamination, 40% of Zone B and 26.7% of Zone C maize grains also had high level ($\geq 3.1 \times 10^7$ cfu/g) of fungal contamination. The high susceptibility of maize grain to fungal growth observed in this survey had also been observed in different locations by Jelinek *et al*. (1989) and Okoye (1992). Similarly, Alptekin *et al.* (2009) and Krnjaja *et al*. (2017), had reported higher fungal loads in their studies. Alptekin *et al*. (2009) carried out analysis on maize samples and got a high fungal count. Gonzalel *et al*. (2012) also investigated maize

grain samples intended for animal feed and found total fungal count ranging from 0 to 2.10 x 10^8 cfu/g. This variation in contamination levels could be attributed to different environmental conditions. Environmental factors, in particular temperature and water activity (a_w), play a fundamental role in determining fungal species prevalence. (Magan and Medina, 2016).

Literatures had previously reported that aflatoxigenic fungi is able to survive in diverse temperature ranges, but the temperature range for its optimal growth is 28 ^{o}C to 37 ^{o}C at a high humidity above 80% (Klueken *et al*., 2009; Hell and Mutegi, 2011; Yu, 2012). According to the meteorological data collected from the sample areas, majority falls within these temperature and humidity ranges. This could be responsible for the high level of fungal contamination observed in these locations.

It was also discovered that majority (66.7%) of Zone A (Table 2) maize seeds had aflatoxin levels ranging from (0.70-3.62 ppb), while only 33.3% had aflatoxin levels from 3.63 to 6.56 ppb and none (0%) of Zone A maize grains had aflatoxin level above 6.57ppb. Exactly 10% of Zone B grains produced aflatoxin levels above 6.57ppb, while (25%) of maize grains from Zone C had aflatoxin levels above 6.57ppb. (P=0.205; P>0.05). Similarly Setamou *et al*., (1998) had reported higher aflatoxin levels. In a similar study by Aristil and Spada (2020), aflatoxin was present in more than half of the samples of maize kernels, maize meal, moringa seeds and peanut samples. This also agrees with reports of Udoh *et al.* (2000), they observed that high number of maize samples from different ecological zones of Nigeria were contaminated with aflatoxin.

Figure 1 showed that Katsina Ala Local Government Area (LGA) had a high aflatoxin level above 5ppb while Logo LGA (Figure 2) had very low aflatoxin level of less than 3.5ppb (Zone A). According to Zone B (Figure 3), Gwer-west also had low aflatoxin contamination, while in figure 4 (Buruku LGA) had a very high level (9.50ppb) of aflatoxin contamination. Agatu and Oju LGAs both from Zone C (Figures 5 and 6) recorded high aflatoxin levels (8.80ppb and 7.50ppb respectively). Meteorological data collected from these locations provides an insight into the reason for the variation in contamination levels observed in these locations. High temperature and humidity were recorded for Buruku and Oju LGAs, while Gwer-west had low temperature and humidity. Previous studies have also proposed that the occurrence of fungi and aflatoxins in food products is mainly influenced by favourable conditions such as high moisture content and temperature (Wu *et al*. 2011).

In Kenya, Lewis *et al*. (2005) had earlier reported the presence of high levels of AFB1 in maize. Futhermore, a study by Williams *et al*. (2004) about aflatoxin contamination of market samples of foods showed that aflatoxin level of maize consumed in Nigeria was very high. High aflatoxin levels were observed in maize grains in Ethiopia and were attributed to the exposure of the

grain to favourable temperature and rain (Yilma *et al*., 2019). Similar findings were reported by Chauhan *et al*. (2008), that the extent of contamination of aflatoxins also varies with different geographic location, agricultural and agronomic practices, storage condition of crops and more importantly processing of food materials under favourable temperature and humidity conditions. This explains the reason for variation in contamination levels of the maize grains from different locations observed in this study.

CONCLUSION

There was a high level of fungal and aflatoxin contamination of maize grains in Benue State. Climatic conditions in Benue State was observed to play a role in the growth of these fungi and contamination of aflatoxin. High temperature and humidity was noted to be responsible for the high level of fungal and aflatoxin contamination found in this study.

RECOMMENDATION

Government should enforce enlightenment programs to educate the citizens about food safety. Training programmes on appropriate techniques for harvesting, handling and storage should also be developed for farmers and marketers.

Budgetary allocations for aflatoxin detection and control should be increased by the government.

DECLARATIONS

ETHICS APPROVAL AND CONSENT: Not applicable

CONSENT FOR PUBLICATION: Not applicable

AVAILABILITY OF DATA AND MATERIALS: All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS: The authors declare that they have no competing interests

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AUTHORS CONTRIBUTION: All the authors contributed to this work.

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