

Effects of Farmers' Practices on Maize (*Zea mays*) Contamination by Potential Aflatoxigenic Fungi and Aflatoxin in Benue State, Nigeria

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Abstract

Aflatoxin associated with a number of cereals and legumes is estimated to increase the chances of developing primary liver cancer in 152.7 and 61.1 cancer/year/100,000 population of infants and children, respectively in Nigeria. This study was carried out to assess the implications of some agronomic practices on the infestation of aflatoxigenic fungi and total aflatoxin in maize produce in Benue State. Two maize sample types (Pre-harvest and Post-harvest) collected from 3 locations in Benue State were cultured on Sabouraud dextrose agar for fungi isolation and identification. Moisture content and total aflatoxin concentration were determined (ELISA method) in both pre-harvest oven dried and farmer's post-harvest sun dried samples. Information on agronomic practices was obtained with the aid of questionnaire. T-test and analysis of variance (ANOVA) were used to analyze the data with confident levels set at 95%. Overall, 5 mould genera were identified: *Aspergillus* (44.0%), *Fusarium* (24.44%), *Botryodiplodia* (17.78%), *Rhizopus* (15.56%) and *Penicillium* (4.44%). Moisture content of oven dried samples was significantly less than that of farmers sun dried samples ($t = 10.45$, $P < 0.001$). Similarly, a significant difference in total aflatoxin concentration was recorded between farmers sun dried and oven dried samples ($t = 2.37$, $P = 0.03$). Half of the farmer's sun dried samples had aflatoxin concentration above the recommended EU (4 ug/Kg) limit, but none of the pre-harvest oven dried samples exceeded EU limits. Maize samples from fertilized farms were more likely than non-fertilized farms to have higher aflatoxin concentration ($P = 0.002$). Similarly, Maize seeds purchase from the open market were more likely than seeds from previous harvest to be contaminated with aflatoxin ($P = 0.003$). The study advocates rapid drying of timely harvested maize so as to reduce or

stop the action of heavy field fungi contaminants as well as aflatoxin accumulation.

Keywords

Aflatoxigenic Fungi, Aflatoxin, Farmers Practices, Maize, Benue State

1. Introduction

Maize (*Zea mays*) is an important staple crop which is consumed by people with varying food preferences and socio-economic background [1]. It is reportedly adaptable to different weather and agro-ecological zones [2], and has become the most widely grown cereal crop in the world [3]. In Nigeria, maize was ranked the second most cultivated crop (5.8 million hectares after Cassava with 7.1 million hectares), making Nigeria the second largest maize producer in Africa with 10.79 metric tonnes in 2014 [4].

However, mycotoxins associated with fungi infestation of maize have been reported [5]. Of great importance is the aflatoxins which are secondary metabolites produced by certain species of fungi, notably *Aspergillus flavus* and *Aspergillus parasiticus* [6]. Different types of aflatoxins have been identified: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) [7]. They are reportedly heat stable (except at 360°C); can withstand fermentation, pasteurization, cooking, frying and boiling [8].

Aflatoxin is reported to be carcinogenic, mutagenic, teratogenic, and hepatocarcinogenic [8]. A risk assessment of aflatoxin in maize grains in Nigeria [9], shows high exposure levels among infants and children with a national mean Probable Daily Intake (PDI) of 1909.1 and 763.6 ng·kg⁻¹ bw·day⁻¹ respectively, as compared to adults with a mean national PDI of 318.2 ng·kg⁻¹ bw·day⁻¹. While the risk of developing liver cancer among Infants and Young Children were estimated at 978.2 and 391.3 cancer/year/100,000 populations respectively, as compared to those of adults at 163 cancer/year/100,000 populations [9]. Detection of high AFB1 levels in blood samples of feed mill workers in Nigeria suggests inhalation as another mode of exposure in addition to ingestion [10].

Evidence has shown that contamination of produce with aflatoxigenic fungi takes its roots from the farms as well as during storage and when in transit [11]. Consequently, control has been suggested to include pre-harvest management, post-harvest management and detoxification [12]. Though, a number of researches have been carried out on the isolation of aflatoxigenic fungi as well as detection of aflatoxin in a variety of food substances, none directly involve the producers. For example, a good number of these researches investigated edible grains sold in open market and stores from different regions in Nigeria [5] [13]-[18]. Others investigated cereal products [19] [20]. To effectively fight to reduce mycotoxin contamination of our local produce so as to improve their marketability, there

must be a deliberate effort to involve our subsistence farmers that constitute over 80% of grain producers. Hence, the study was carried out to assess the implications of some agronomic practices on the infestation of aflatoxigenic fungi and total aflatoxin of maize in Benue State.

2. Materials and Methods

2.1. Study Area

The study was conducted in 3 locations (Adikpo, Makurdi and Oju) from three senatorial zones of Benue State; Benue North East, Benue North West and Benue South senatorial districts. Benue State lies between latitude 6° 15' and 7° 65' North, and longitude 7° 30' and 09° 30' (**Figure 1**). The climate of Benue State is tropical and is characterized by two distinct seasons: the wet and dry seasons. The mean temperatures are between 32°C and 33°C, while the mean annual rainfall ranges from 800 to 1500 mm [21]. Benue is an agrarian society with more than 70% of the population engaged in agriculture. The climate supports the production of a variety of crops which include yam, soybean, sesame, millet, sweet potato, rice, maize, sorghum, cassava, watermelon, mango, etc., hence the slogan, “Food Basket of the Nation”. Crop production is mainly sustained by rainfall with little vegetable farming supported through irrigation.

2.2. Sample Collection

A total of 24 consented maize farmers were selected for the study after talks delivered by agricultural extension workers. Maize samples were collected from the selected farmers in two batches. First batch (August, 2018), physiologically mature maize cobs were collected from 10 - 20 locations on each of the farmers' farms (depending on the size of the farm). Maize cobs were transported in dry polyethylene bags to Microbiology laboratory of Benue State University Makurdi and dehusked. Maize cobs were randomly selected from each of the farms, seeds were removed from the cobs for determination of moisture content and cultured for isolation of the pre-harvest fungal contaminants. The remaining sampled maize cobs from each farm were dried mechanically in the oven at 46°C for 4 - 5 days, shelled and the seeds were again cultured for fungi isolation and analyzed for total aflatoxin concentration.

During the second batch (October, 2018), maize samples were collected from the same farmers when the grains are harvested, dried and ready for storage and were assessed for moisture content, post-harvest fungi infestation and aflatoxin contamination. Information on Agronomic practices of the farmers was obtained using semi structured questionnaire.

2.3. Determination of Moisture Content

Two crucibles were properly washed and allowed to dry in an oven at 108°C for 30 minutes, cooled in desiccators for 30 min, labeled and weighed (W_1).

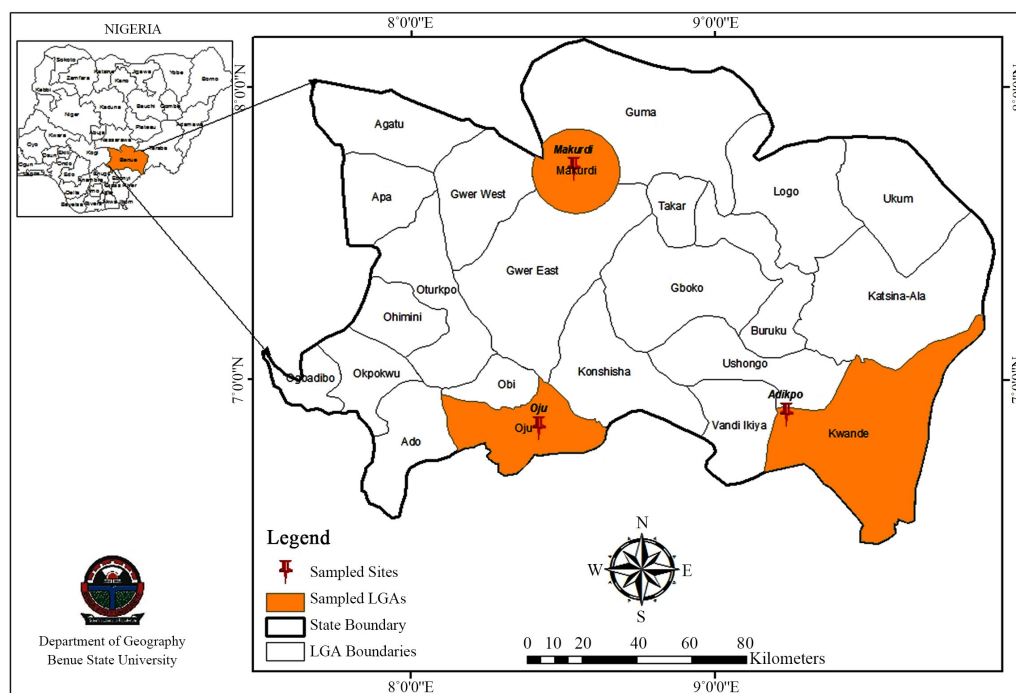


Figure 1. Map of Benue State showing sampled sites.

Equal quantity of each maize sample was added to the labeled crucibles and re-weighed (W_2). The crucibles containing the samples were placed in an oven maintained at 108°C until constant weight was obtained (W_3). The percentage moisture was calculated as follows:

$$\% \text{ moisture} = (W_2 - W_3 / W_2 - W_1) \times 100$$

where, W_1 = initial weight of empty porcelain crucible;

W_2 = weight of porcelain crucible + sample before drying;

W_3 = weight of porcelain crucible + sample after drying [22].

2.4. Sample Preparation and Cultivation

Culture media was Sabouraud dextrose agar (SDA). Samples were surface sterilized for 30 seconds in 2.5% Sodium hypochlorite solution, rinsed in three changes of sterile distilled water and blotted with sterile filter paper. The sterilized grains were inoculated onto SDA medium at the rate of three seeds per plate. Two replicates were made for each sample and the inoculated plates were incubated at 36°C . After 3 - 5 days, fungi growth was sub-cultured to obtain pure cultures. Identification of isolates was done on the basis of their macro and micro morphological characteristics.

2.5. Sample Preparation and Analysis for Aflatoxin

Detection and quantification of Total aflatoxin was carried out using Enzyme linked immunosorbent assay (ELISA) kits (Green Spring inc., China). Before preparing sample for analysis, some solutions were prepared according to in-

structions by manufacturers of the kit. For example, sample re-dissolving solution was prepared by mixing 20x concentrated re-dissolving solution (Chemical name not provided) with freshly prepared distilled water in the ratio of 1:19. The sample extract solution was prepared by mixing methanol (99.8%, JHD; Lot; 20100408) in freshly prepared distilled water in the ratio of 7:3. The washing buffer solution was prepared by mixing the provided 20x concentrated washing buffer solution (Chemical name not provide) with distilled water in the ratio of 1:19.

Samples were grounded using a sterilized hand Miller (Corona) and mixed well. A weigh of 1 g of the grounded sample was introduced into a 15 mL centrifuge tube. A volume of 5 mL sample extract solution was added to each tube containing the samples and the tubes were shake vigorously for about 3 minutes and centrifuged for 10 minutes at 4000 revolutions per minute (rpm) at ambient temperature. The Supernatant (100 μ L) was added to 700 μ L of sample redissolving solution for total aflatoxin analysis.

For the analysis proper, 50 μ L each of the prepared sample solutions and the provided total aflatoxin standard solutions were pipette accordingly into their respective microwells. This was followed by 50 μ L each of Enzyme conjugate and antibody working solutions. The microwells were covered and incubated at 25°C for 30 minutes after which the contents were washed 5 times using microplate washer (Stat fax-2600, Awareness Technology USA). After washing, 50 μ L of substrates labelled “A” and another labelled “B” were added, incubation was repeated for 15 mins and 50 μ L of stop solution was added to each well. The optical density value (OD value) of each well was determined at 450 nm and 630 nm wave length using a micro plate reader (Stat fax-2200, Awareness Tech. Inc., USA). In order to calculate the total aflatoxin concentration of samples, the OD values of both standards and samples were converted to percentages using the formula below:

$$\%A.V. = B/B_o \times 100.$$

where: B = the average OD value of standard or the sample;

B_o = the average OD of the 0ppb standard.

The percentages were converted into Log and plotted against standard total aflatoxin concentration on a semi-log graph. The aflatoxin concentration of each sample was read from the graph.

2.6. Statistical Analysis

Student T test was used to compare aflatoxin concentration and moisture content values of pre-harvest maize samples, oven dried and sun dried samples.

Analysis of variance (ANOVA) was used to compare moisture content and aflatoxin concentration from the 3 sampled locations as well as agronomic practices of the farmers.

Confidence limit was set at 95% level of probability and probability values (P values) of equal or less than 0.05 were considered significant.

3. Results

Of all the samples cultured (Pre-harvest fresh samples, pre-harvest oven dried samples and post-harvest farmers' sun dried samples), 5 mould genera were isolated: *Aspergillus* (44.0%), *Fusarium* (24.44%), *Botryodiplodia* (17.78%), *Rhizopus* (15.56%) and *Penicillium* (4.44%). There were more species diversity on fresh pre-harvest samples than the oven dried and farmers sun dried samples (**Table 1**).

Total aflatoxin concentration of farmers' sun dried samples ranges from 0 - 80 ppb while aflatoxin quantification in oven dried samples ranges from 0 - 1.80 ppb. Comparison shows significantly ($t = 2.37$, $P = 0.03$) higher total aflatoxin concentration in farmers sun dried samples than the oven dried samples. Similarly, moisture content of farmers' sun dried maize samples ranges from 10.20% - 18.83% while that of oven dried maize samples ranges from 5.09% - 10.80%. There was also a significant difference ($t = 10.45$, $P < 0.001$) between the moisture content of sun dried and oven dried samples (**Table 2**).

Mean moisture content of samples collected from Adikpo was significantly higher compared with samples from Makurdi ($P < 0.001$) and Oju ($P < 0.001$). But mean aflatoxin concentration of samples from Adikpo was higher compared with those of Makurdi ($P = 0.04$) but not Oju (**Table 3**).

Table 1. Frequency of isolation of fungi species in relation to natural of sample treatment.

Organism	Fresh Samples (%)	Oven Dried Samples (%)	Sun Dried Samples (%)	Total (%)
<i>A. flavus</i>	2 (4.44)	2 (4.44)	5 (11.11)	9 (20.00)
<i>A. niger</i>	2 (4.44)	1 (2.22)	3 (6.67)	6 (13.33)
<i>A. terreus</i>	1 (2.22)	-	-	1 (2.22)
<i>A. fumigatus</i>	1 (2.22)	-	-	1 (2.22)
<i>Rhizopus</i> spp	3 (6.67)	-	4 (8.89)	7 (15.56)
<i>Fusarium</i> spp	4 (8.89)	1 (2.22)	6 (13.33)	11 (24.44)
<i>Botryodiplodia</i> spp	4 (8.89)	-	4 (8.89)	8 (17.78)
<i>Penicillium</i> spp	-	-	2 (4.44)	2 (4.44)
Total	17 (37.78)	4 (8.89)	24 (53.33)	45 (100)

Table 2. Comparison of moisture content and total aflatoxin concentration between farmers sun dried and oven dried maize samples in Benue State, Nigeria.

	Sample	No. of samples	Mean	Mean Difference	S.D	Std Error of mean	P-value
Aflatoxin concentration	Sun Dried	20	13.31				
	Oven Dried	20	0.46	12.85	24.23	5.41	0.03*
Moisture content	Sun Dried	22	13.51				
	Oven Dried	22	7.20	6.31	2.83	0.60	0.00**

Table 3. Mean moisture content and aflatoxin concentration of sun dried samples according to sampled locations in Benue State, Nigeria.

Location	Moisture Content (%)	Aflatoxin Concentration (ppb)
Makurdi	11.91 ^a	2.05 ^a
Adikpo	17.14 ^b	29.07 ^b
Oju	12.55 ^a	12.58 ^{ba}
Total Mean	13.51	13.31

Means with different superscript letters are significantly different ($P < 0.05$).

Comparison of questionnaire responses to agronomic practices among the studied farmers with mean aflatoxin concentration of the respective samples (Table 4), recorded significantly higher aflatoxin concentration with samples from fertilized farm than with samples from non-fertilized farms ($P = 0.002$). Aflatoxin concentration was significantly lower in samples from farms that source their seeds from previous harvest ($P = 0.003$) compared with samples whose seeds were from the open market and Agricultural centres ($P = 0.028$). None of the farmers in this study treated their seeds before planting and weeding of all the sampled farms was done manually. Most of the maize farms (66.7%) preferably planted yellow corn variety rather than the white variety and mean total aflatoxin concentration was also found higher in the yellow corn variety (27.4 ppb) as against 7.3 ppb recorded with the white maize variety. Samples from farms that residues of previous harvest was ploughed into the soil before planting new seeds were less contaminated with aflatoxin when compared with samples from farms that previous residues were burned ($P > 0.05$).

4. Discussion

It is evidently clear from the findings of this study that contamination of maize from the studied farms starts from the field. Hence, efforts at minimizing infestation by aflatoxin and other mycotoxin producing fungi should start from the field. Most of the fungal isolates from this study have been reported as the fungal isolates of maize by different authors in Nigeria. For example, the occurrence of *Aspergillus*, *Rhizopus*, *Fusarium*, and *Penicillium* species in maize and maize products have been reported in Kaduna [16]. Similar species of *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, have been isolated from maize in Niger and Kogi States [23].

The absence of *Aspergillus flavus* and *Aspergillus parasiticus* (widely reported aflatoxin producing species) from some samples in this study may not necessarily exclude the presence of other mycotoxins as some of the fungal species isolated are known mycotoxin producers in food. For example, *Aspergillus niger* have been reported to produce fumonisins in raisins under favourable conditions [24]. *Fusarium* species isolated from banana have been reported to produce fumonisins, zearalenone, deoxynivalenol, among other toxins [25].

Table 4. Questionnaire responses to agronomic practices and mean aflatoxin concentration for each response group.

Agronomic Practice	Number of Response (n)	Frequency (%)	Mean Aflatoxin Concentration (ppb)
Time of planting			
April	6	25	17.3
May	16	66.7	13.7
June	2	8.3	2.4
Seed treatment			
Yes	0		
No	24	100	
Variety Planted			
Yellow	16	66.7	27.4
White	8	33.3	7.3
Source of planting materials			
Previous harvest	12	50	2.5
Market	4	16.7	42.4**
Agric center	8	33.3	11.9
Disposal of previous residues			
Ploughed into soil	14	58.3	7.2
Gathered and burned	10	41.7	19.4
Maize intercropped with other crops			
Yes	22	91.7	14.6
No	2	8.33	1.4
Fertilizer application			
Yes	8	33.3	36.3**
No	16	66.7	3.5
Weeding method			
Manual	24	100	
Chemical	0	0	
Frequency of Weeding			
Once	8	33.3	11.9
Twice	16	66.7	13.9

**Means are significant different ($P < 0.05$).

Higher diversity of fungal species observed in the fresh maize samples in this study may be as a result of higher moisture content of the fresh samples as well as the relative humidity of the cob environment. The husk covering of the cobs, combined with the high moisture content of maize cobs may have provided a humid and warm environment favourable for the growth of different fungal species. For example, nitrogen fertilizer has been shown to increase the severity of *Fusarium* head blight in wheat, and it has been suggested that this might be the result of a nitrogen-induced increase in canopy size, leading to an altered microclimate [26]. Notwithstanding, reducing the moisture content through sun-drying as compared to the fresh samples resulted in lower fungal diversity. Further drying of the samples to even lower moisture as observed with the mechanically dried/oven dried samples impact a greater reduction on the fungal flora. This buttresses an earlier reported need for rapid drying of timely harvested maize to

low moisture content [27] to reduce fungal/microbial contamination which may lead to the production of aflatoxin, and other mycotoxins in the grains. Harvesting maize when moisture levels reaches 28 to 30 percent reportedly reduces field exposure compared to when corn is allowed to dry in the field to 15 percent or less [28].

Though the range of total aflatoxin concentrations of 0 - 80 ppb was observed in this study, only four samples were above the National agency for Food and Drug Administration and Control (NAFDAC) recommended limit of 20 µg/kg for Nigeria [29]. However, going by the EU standard of 4 µg/kg, more samples (about 50%) of the analyzed sundried samples were above the acceptable limit. The level of aflatoxin contamination reported in this study is at variance with an earlier report [15] of 0 µg/kg of AFB1 for all maize samples studied in Benue State. This may be as a result of the method of analysis used. Enzyme Linked Immunosorbent Assay (ELISA) method used in this study detects aflatoxin concentration of 0.02 µg/kg compared to 3.125 µg/kg detection limit by Thin Layer Chromatography (TLC) used by the previous study [15]. In addition, total aflatoxin quantified in the present study comprises AFB1, AFB2, AFG1, AFG2, and AFM1, but Ubwa *et al.* [15] only reported AFB1. Hence, this study has a higher chance of reporting higher concentration of aflatoxin. In agreement to the findings of this study however, Tersoo-Abiem [20], in the same study environment, using ELISA method, reported aflatoxin concentration of 9.20 - 35.35 ppb in maize kernels purchased from markets in Makurdi.

Moisture content of produce is reportedly an important factor which influences fungal contamination and subsequent aflatoxin production in products [30]. Samples in this study with higher moisture content also had higher aflatoxin levels. For example, samples from Adikpo, with higher moisture content (15.12 - 18.83) than those from Makurdi and Oju (10.80 - 13.52 and 11.59 - 13.70 respectively), also recorded higher level of contamination (2.40 - 80 ppb). This is in accordance with a Ugandan report [31], where about 48% of maize samples having moisture higher than 14% were contaminated with aflatoxin levels ranging from 0 - 50 ppb.

The moisture content of the farmers sun-dried samples obtained from this study was similar to the finding in Ghana [32] where moisture content of maize ranging from 12.5% - 16.6% was recorded. But an earlier study of maize stored for several months in Benue State [15] reported lower moisture content. Maize kernels are likely to lose more moisture with the length of storage, but this depends also on the storage environment as well as the presence or absence of insect attacks [33].

The relationship between moisture content and aflatoxin deposition is further buttressed in this study considering less moisture content (5.0% - 10.8%) of the oven dried sample as against equivalent very low aflatoxin concentration (0.0 - 1.08 ppb). On the other hand, higher moisture content of the sundried samples also yielded higher aflatoxin concentration (0.0 - 80.0 ppb). This explains why

the oven dried samples exceeded the 4 ug/Kg acceptable limit set by the European Union (EU) total aflatoxin contamination of produce.

Significant difference between the mean values of aflatoxin concentration of oven-dried and sun-dried samples in this study shows that mechanical drying of harvested maize is effective towards the control of the development of aflatoxin causing fungi on harvested produce. Higher aflatoxin concentration of sun-dried samples compared to oven-dried samples, may be associated with noticeable difference in the level of *A. flavus* distribution between sun-dried and oven-dried samples. The difference could be attributed to the moisture content to which the grains were dried. While all the mechanically dried (oven dried) samples had moisture percentage lower than 14%, which is recommended [31] for reduced fungal contamination of maize for storage, many of the sundried samples had moisture percentages higher than this number. This may have favored more *A. flavus* contamination and hence, higher level of aflatoxin.

Treatment of seeds with fungicides reportedly reduces *Aspergillus* species contamination of groundnut [34]. Responses on agronomic practices of farmers in this study show that none of the farmers treated their seeds before planting contrary to farmers studied in the Southwestern part of Nigeria [35] where maize farmers of Aiyere regularly accessed treated maize seed for planting. Findings of this study further reveals that sourcing of planted seeds from the open market can significantly influenced aflatoxin concentration of produce.

Higher concentration of aflatoxin recorded with samples from fertilized farms was not unexpected. It is commonly thought that application of fertilizer (nitrogen fertilizer) can increase disease severity via effects on crop canopy development. Thus, large canopies with high shoot densities may be more conducive to spore transfer and pathogen infection than sparse canopies [26]. Furthermore, oral interview of the sampled farmers who applied fertilizer revealed that fertilizer was applied simply by scooping and applying to the soil a nonspecific desired quantity. This may have increased the susceptibility of the plants similar to findings of a study [36] in Tanzania which shows that maize crops that received sub-optimal level of nitrogen and phosphorus fertilizers (60 and 30 kg/ha respectively) suffers more aflatoxin than those which received the recommended quantity (120 and 60 kg/ha). Another reason for higher aflatoxin in maize from fertilized farms may be due to drought and heat stress. Reports have shown that plants exposed to drought, coupled with heat stress in the field suffers increased susceptibility to fungi infection culminating in aflatoxin production in crops [37]. This may be applicable to our studied farms as the rainfall data of the sampled locations showed a short break of rainfall during the period the maize were in the field. Maize when planted, take between three to four months to attain physiological maturity. Majority of maize in this study having been planted around May were still have been in the fields when a brief period of drought was recorded in July and so crops from fertilized farms, being less accustomed to stress, unlike those without fertilizer, may have felt the effect of drought more than

crops which did not receive fertilizer. This corroborates findings of a study [38] carried out in Aberystwyth University, Wales, where plants grown in nutrient-rich compost were more affected by drought than those grown in less nutritive compost.

The practice of ploughing back into the soil cereal crop residues as reported in this study have a positive reductive effect on aflatoxin contamination, similar to report which attributes 50% - 90% *A. flavus* reduction to utilization of farm yard manure and cereal crop residue as soil amendment [11].

5. Conclusion

Pre-harvested maize samples obtained from the field in this study were heavily contaminated with a variety of fungi species. However, rapidly drying of timely harvested maize cobs using mechanical method to a moisture content of less than 10% was effective in reducing the fungal population as well as aflatoxin accumulation below safe health permissible levels of 4 µg/Kg for EU and 20 µg/Kg stipulated by National Agency for Food and Drug Administration and Control of Nigeria. Hence, the study advocates rapid drying of timely harvested maize so as to reduce or stop the action of heavy field fungi contaminants as well as aflatoxin accumulation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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