Determination of Deoxynivalenol and Total Aflatoxin Levels in Commonly Traded Grains in The Commercial City of Katsina-Ala North-Central Nigeria

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***Abstract*: This study determined the total aflatoxin and deoxynivalenol levels in grains traded in the commercial city of Katsina-Ala, North-Central Nigeria, from 300 samples obtained from 30 composites, of 10 different locations, using the direct competitive Enzyme Linked Immunosorbent Assay (ELISA). Moisture content, total aerobic microbial count, mould count, coliform and *Escherichia coli* were determined using standard analytical methods. Mycotoxins were detected in all samples across the study locations; total aflatoxin ranged from 2.30 to 8.4 ppb, which were within the maximum limits for aflatoxins, deoxynivalenol (DON) concentration ranged from 2,900.00 to 8,400.00 ppb that was above the maximum limits. Moisture was high in all samples with content ranging from 9.0279 to 12.3851 %, microbial contamination was observed on the grains. It was also observed that, these grains were more predisposed to DON than aflatoxin, thus vulnerability of the grains to DON was in the order: sorghum > maize > millet, whereas total aflatoxin level was in the order: sorghum > millet > maize respectively. Pearson's 2-tailed correlation at 0.05 level of significance revealed strong correlation of deoxynivalenol with moisture content across locations, while ANOVA at 5 % level of significance established the presence of mycotoxins and their precursors across the locations. This fundamentally, showed microbial tainting of these cereals in the study location indicating that farmers and marketers handle these grains in an unhygienic manner.**

***Keywords*: Total aflatoxins, deoxynivalenol, maize, sorghum, and millets.**

I. INTRODUCTION

Grains such as millet, sorghum and maize and processed food substances made from composite flours of these grains such as bread, cakes, doughnuts, popcorn, biscuits, *kunu*, *burukutu* (local brew) etc., highly consumed as fast foods in public places are often exposed to moisture and attack by pathogenic organisms such as moulds, fungi, bacteria and certain protozoa (CAST, 2003). Moulds produce secondary metabolites known as mycotoxins that have attracted much concern lately, because of their pathogenic roles (CAST, 2003; IFPRI. 2013).

Cereals are one of the most commonly consumed globally but their poor handling during storage and processing has made them prone to mycotoxin contamination and this poses a serious challenge for their safety and quality (CDC, 2004; Ayejuyo *et al.*, 2008). Cereals are often susceptible to moulds plague on the field, during storage (if not properly dried) and during processing and packaging as an outcome of which they are accountable to contagion by mycotoxins (e.g. trichothecenes and aflatoxins (Bankole and Adebanjo 2003; Wang and Liu, 2007; Kabak, 2010). Research have shown that poor handling processes can heighten the levels of mycotoxins contamination along the marketing value chain (Gordon, 2003; Adejumo *et al.*, 2007; Ubwa *et al.*, 2014). Pest attacks are also known to accelerate mould invasion of several stored products (Igor *et al.*, 2008; Ennouari *et al.*, 2013).

The Food and Agricultural Organization (FAO, 1995a) submitted that up to 25% of foodstuffs available worldwide are contaminated with mycotoxins, commonly trichothecenes especially, deoxynivalenol and aflatoxins being identified as the most toxic of these mycotoxins. Studies have revealed that foodstuffs such as; cereals, fruits, nuts and tea which are exposed to fungal attack and are often contaminated with deoxynivalenol and aflatoxins (Miller, 1995; IFPRI., 2013; Tor *et al.*, 2020).

Trichothecenes are a large group of chemically related mycotoxins mainly produced by the fungi of the *Fusarium* genus (Hell *et al.*, 2000). They are one of the most commonly occurring contaminants in the food chain. These mycotoxins are commonly found in cereals, particularly in wheat, barley, oats and maize, sorghum, millet (Reddy *et al.*, 2008; EFSA, 2013). Trichothecenes can be divided into four types (A-D) according to characteristic functional groups. Members of type A do not contain carbonyl on C-8. The examples of these are represented by T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS) (Sudakin, 2003; Igor *et al.*, 2008).

Deoxynivalenol and aflatoxins are tremendously persistent under most circumstances of storage, handling and processing of foods. It is, thus, impossible to remove them once the foodstuffs are contaminated (Gordon, 2003; Kabak, 2010). Grain contaminants; deoxynivalenol and aflatoxin are not affected by temperature, pH and remain active even at 160 °C (Bankole and Adebanjo, 2003).

Aflatoxins comprises four major types; B_1, B_2, G_1, G_2 and two other metabolic products; M_1 and M_2; which are significant in their contamination of foodstuffs and feeds.

Aflatoxins AFM_1 and AFM_2 have M-designation, as they were first isolated from milk of lactating animals fed with aflatoxin preparations (CAST, 2003).

The AFB_1 and AFB_2 were labelled B because they emit blue fluorescence under UV-light while the AFG_1 and AFG_2 designation refers to those that give yellow-green fluorescence under UV-light (Ubwa *et al.*, 2014; Tor *et al.*, 2020). AFB_1 is most commonly found in plant materials such as grains, and shows the extreme toxigenic potential and hence, has been categorised as a class 1 human carcinogen (CDC, 2004; IFPRI, 2013).

Foods contaminated with fungal growth expose humans to mycotoxins. Such exposures are not easy to avoid since it is difficult to regulate fungal growth in foods (Wagacha and Muthomi, 2008). It was also concluded that even though, deeply contaminated food supplies are not permitted in the market place in several countries, concern still remains for the possible adverse effects resulting from long-term exposure to low levels of mycotoxin (especially trichothecenes and aflatoxins) in the food supply (CAST, 2003; Wagacha and Muthomi, 2008). Acute mycotoxicosis in humans has been reported in many third world countries, like India, Taiwan and Uganda (Sudakin, 2003; Ennouari *et al.*, 2013).

Aspergillus flavus, the main producer of aflatoxin, only grows in grains when the moisture content surpasses 12.5 – 14 % and has peak growth temperature conditions of between 25 °C and 30 °C (Kabak, 2010). The warm and damp environmental conditions in Africa are ideal for the growth of *Aspergillus flavus* making aflatoxin contamination of food, including cereals and other agricultural produce, a pervasive problem across the continent (Adejumo *et al.*, 2007; Igor *et al.*, 2008).

Sorghum, maize and millet in particular, as well as their products are the most traded by food vendors in Katsina-Ala town in North central Nigeria and are extensively consumed in all seasons by majority of the inhabitants (including animals). The abundance and unhygienic handling of maize, sorghum, millet and rice, among other food substances in the study area makes the grains prone to contamination by mycotoxins (commonly deoxynivalenol and aflatoxins) because of the critical conditions of high temperature and humidity that are known to favour the growth of mycotoxin-producing moulds. This study was therefore, conducted with the objective of determining the levels of deoxynivalenol (DON) and the total aflatoxin in sorghum, maize and millet traded in the commercial city of Katsina-Ala.

II. MATERIALS AND METHODS

2.1 Study area

Katsina-Ala is among the major commercial cities in Benue state, North-central geopolitical zone of Nigeria (James, 2001; Hope, 2003). It is a cosmopolitan settlement on the Northern Bank of river Katsina-Ala from which the town takes its name. The city was used extensively in the colonial period by the Royal Niger Company as a produce buying and evacuation centre for agricultural produce (Mokhtar, 1981; NIPOST, 2012; IAB, 2021). It has an area of 2,402 km^2 (927 sq miles) and a population of 224,718 inhabitants as at the year 2005 (NPC, 2005). A vast majority of the dwellers are the native Tiv speaking ethnic group and are predominantly agrarian (Hope, 2003). It has a guinea savannah vegetation with undulating hills and shrubs and lies on latitudes 7º 10' N and 9º 77' N; longitudes 9º 17' E and 4º 74' E; an altitude of 144.58 m above sea level, barometric pressure of 100 KPa with about 29 °C average temperature, wind speed at 10 km/h and 40 % humidity (James, 2001). The town has an annual rainfall range between 158 mm to 185 mm (NIMET, 2016).

The area experiences two seasonal climatic conditions of dry and rainy seasons characterised by high temperature and humidity. The climatic conditions as well as poor handling and storage system favour production and proliferation of mycotoxin-producing moulds in grains and other foodstuffs (Bankole and Adebanjo, 2003; Adejumo *et al.*, 2007; Kubo, 2012).

2.2 Methods

2.2.1 Sampling

Grains from the study area were sampled, using the random sampling method. The samples were further prepared and analysed for mycotoxins using instrumental and immunochemical assays, microbial counts, moisture contents determination. Data obtained were analysed using the general linear model procedures of statistical package for social sciences (SPSS).

2.2.2 Determination of moisture content

Moisture content analyser, Sartorius, M 100 certified conferring to ISO 9001 was used. A measured quantity (5.0 g) of each the typical sample was macerated using a Romer series II Miller. Then, 3.0 g of each pulverised sample was weighed into a clean aluminium moisture plate and placed on the tray after which the lid was closed. Running the analysis started when the button “Run analysis” was depressed, and after waiting a while, alarm triggered, signifying the end of the analysis. The analysis was accomplished at the temperature of 105 °C and the results displayed on the digital screen in percentage (%) (Adejumo *et al.*, 2007: EFSA, 2013).

2.2.3 Sample preparation for total aflatoxin and deoxynivalenol analysis

From each composite, thirty grams (30.0 g) of sample was crushed using a Romer series II Miller and sieved through a 20-mesh screen. Then, 20.0 g of the sieved sample was weighed into a pre-cleaned jar followed by the addition of 100 mL of 70/30 (v/v) methanol-water extraction solution and the jar was sealed. The sample extraction solution was mixed and shaken vigorously for 3 minutes and then allowed to settle. Thereafter, the supernatant was filtered through a Whatman No. 1 filter paper and the filtrate collected (Afla-Guard, 2005; Julie *et al.,* 2011; Ubwa *et al.,* 2014; Tor *et al.,* 2020).

2.2.4 Determination of total aflatoxin by AgraQuant method

0.10 mL of the filtrate and of enzyme-conjugated aflatoxins was mixed and added into the antibody-coated micro-well (ACM). This allowed the aflatoxins in the sample to compete with the enzyme-conjugated aflatoxins for the antibody binding sites (Gordon, 2003; Kabak, 2010). After a washing step with 50.0 mL of deionised water, 2.0 mL aflatoxin enzyme substrate was added and blue colour was seen. The concentration of aflatoxin in the sample was determined by observing the intensity of the colour (intensity of the colour is inversely proportional to the concentration of aflatoxin in the sample or standard) (Kabak, 2010). 2.0 mL "stop solution" was added which changed the colour from blue to yellow.

The micro-wells were measured optically using a micro-well reader with an absorbance filter of optical density, 450 nm (OD_{450}), and a differential filter of 630 nm. The optical densities (ODs) of the samples were compared to the ODs of the standards and an interpretative result was determined from a system of coordinates on semilogarithmic graph against the total aflatoxin concentration (ppb) (Wagacha and Muthomi, 2008).

The OD values were expressed as a percentage of the OD of the zero (0.0) standard and then a dose-response curve was constructed using the five (0.0, 2.0, 4.0, 10.0 and 20.0 ppb) standards. Since the amount of aflatoxin in each standard was known, the unknown was measured by interpolation from this standard curve. Results were further calculated using the Romer Log/Logit spreadsheet and the Log/Logit regression model was used for the results interpretation; the linearity coefficient (r^2) of the calibration curve was not less than 0.985 (Miller, 1995).

2.2.5 Determination of deoxynivalenol (DON) by Ridascreen® DON method

The basis of the tests were the antigen-antibody reaction. The 96-microtiter wells were coated with capture antibodies directed against anti-deoxynivalenol antibodies. Then 0.10 mL each of deoxynivalenol standards (as reference) and sample solutions, deoxynivalenol enzyme conjugate and anti-deoxynivalenol antibodies were added. Free deoxynivalenol and deoxynivalenol enzyme conjugate competed for the deoxynivalenol antibody-binding sites (competitive enzyme immunoassay). At the same time, the deoxynivalenol antibodies were also bound by the immobilized capture antibodies (Ennouari *et al.,* 2013). The unbound enzyme conjugate were then removed in a washing step with 50.0 mL distil water.

Then 1.0 mL each of substrate (sample) and chromogen (1.0 M tetramethylbenzidine) were added into the antibody-coated micro-wells (ACMW), bound enzyme conjugate which converted the chromogen into a blue product. The addition of 2.0 mL stop solution (mixture of 1 N sulphuric acid and washing buffer salt, buffered at pH 7.4) led to a colour change from blue to yellow. The measurements were made photometrically at 450 nm using these standards (0.0, 3.7, 11.1, 33.3 and 100.0 ppb). The absorbance was inversely proportional to the deoxynivalenol concentrations in the sample.

2.2.6 Isolation of moulds and microbial counts

The procedure described below was followed for the isolation of moulds from each sample. Four pre-cleaned bottles were labelled with arbitrary letters **(j** to **m)** and the solutions they contained were respectively identified by the labels. Then 5.0 g of pulverised sample was transferred into the bottle, '**a**' containing 45.0 mL of peptone water and shaken thoroughly to mix (solution j). With a sterile syringe, 2.0 mL of solution **j** was transferred into another bottle, '**b**' containing 18.0 mL peptone water and again shaken properly to mix (solution k). A 2.0 mL of solution '**k'** was transferred into another bottle, '**c**', also containing 18.0 mL peptone water and mixed (solution '**l**') after which 1.0 mL of the solution '**l**' was transferred into a set of duplicate petri dishes labelled (1**d** and 1**d**').

Then, 2.0 mL of solution '**l**' was transferred into another bottle, '**d**' containing 18.0 mL peptone water, mixed (solution '**m**') and 1.0 mL of solution '**m'** was transferred into another set of duplicate petri dishes (1**e** and 1**e**'). Then, 50.0 mL Durhan tubes were filled with 40.0 mL MacConkey Broth (MCB) by gently tilting; ensuring that no air bubble was trapped in the broth. When the molten Sabourand Dextrose Agar (SDA) cooled to 54 °C, 10.0 mL of the molten Agar was transferred into each of the petri dishes and gently swirled to mix. Another 10.0 mL of the agar was taken into a control petri dish (D), and allowed to set/gel. Then, the petri dishes were incubated at 37 °C for 48 hours after which the microbial growth were examined microscopically using Lacto-phenol Cotton Blue (LPCB) stain and classified by reporting the culture physiognomies at the face and reverse side of the inoculated Petri dishes (CDC, 2004; Bankole and Adebanjo, 2003). The results were determined in colony-forming unit per millilitre (CFU/mL) (Hell *et al.,* 2000; Bankole and Adebanjo, 2003).

2.3 Analytical method validation

In the method validation on the immunochemical methods used, the detection limit of was 18.5 ppm for

(cereals, malt, feed). The recovery rate of the analytical method was 85 - 110 % for cereals, malt, feed, beer and wort. The specificity of the RIDASCREEN® DON/total aflatoxin test was determined by analysing the cross-reactivities to corresponding mycotoxins was 100 % each for total aflatoxin and deoxynivalenol. The limit of detection was 1.0 ppb for corn. Limit of quantification was 1.0 ppb (which was the lowest concentration on the calibration curve that the test can reliably detect aflatoxin and deoxynivalenol). Range of quantification was 1.0 – 20.0 ppb (for quantitation of samples above 20.0 ppb, they were diluted to fall within the range 2.0 – 20.0 ppb, while including the dilution factor in the result).

III. RESULTS AND DISCUSSION

3.1 Results

The results of the analysis for the white and red sorghum, white and yellow maize as well as grey and brown millets from the various sampling stations are as shown in the tables below;

3.1.1 Moisture content

Results of moisture content of the grains are presented in Table 1.

Table 1: Moisture content of samples across the stations

S/N	Sampling station	Moisture content (%)					
		Sorghum		Maize		Millet	
		White	Red	White	Yellow	Grey	Brown
1	Katsina-Ala Main Market	10.3702	9.0279	12.2547	10.3702	9.7830	10.1307
2	Abaji-Kpav Market	10.0603	12.2742	9.6390	11.2547	10.3569	12.0445
3	Amaafu Market	11.8355	12.3911	9.6390	12.3851	9.0279	10.1307
4	Gbor Market	10.0356	11.9365	12.3062	10.3546	10.3702	9.1932
5	Tor-Donga Market	12.3001	12.0094	10.3951	10.3702	12.2547	9.3946
	Range	2.2645	3.2783	2.6672	2.0305	3.2268	2.8513

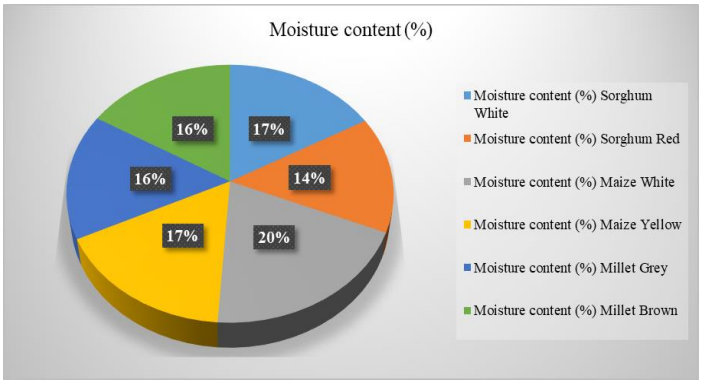

Figure 1: Pie chart of the percentage (%) moisture content in the samples across stations

3.1.2 Microbial analysis

The results of the microbial and fungal counts (%) of grains are presented in Table 2.

Table 2: Total microbial count in samples across the stations

S/N	Location	Sample	Sample type	Microbial analysis (cfu/g)			
				1	2	3	4
1	Katsina-Ala Main Market	Sorghum	Red	20×10^1	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Maize	Yellow	10×10^1	0	1	10×10^1
			White	15×10^1	0	0	10×10^1
		Millet	Grey	20×10^1	1	1	10×10^1
			Brown	15×10^1	3	1	10×10^1
2	Abaji-Kpav Market	Sorghum	Red	15×10^1	0	0	10×10^1

			White	10×10^1	3	0	10×10^0
		Maize	Yellow	20×10^1	0	0	10×10^1
			White	10×10^1	0	0	10×10^1
		Millet	Grey	20×10^1	0	3	5×10^1
			Brown	30×10^1	3	1	15×10^1
3	Amaafu Market	Sorghum	Red	20×10^1	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Maize	Yellow	20×10^1	3	1	1×10^1
			White	15×10^1	3	1	15×10^1
		Millet	Grey	20×10^1	3	0	10×10^1
			Brown	10×10^1	0	0	10×10^1
4	Gbor Market	Sorghum	Red	15×10^1	3	0	30×10^1
			White	20×10^1	3	1	10×10^1
		Maize	Yellow	20×10^1	1	1	1×10^1
			White	15×10^1	3	1	15×10^1
		Millet	Grey	20×10^1	3	0	10×10^1
			Brown	20×10^1	0	1	1×10^1
5	Tor-Donga Market	Sorghum	Red	15×10^1	3	1	15×10^1
			White	20×10^1	3	0	10×10^1
		Maize	Yellow	10×10^1	0	0	10×10^1
			White	20×10^1	3	1	1×10^1
		Millet	Grey	15×10^1	3	1	15×10^1
			Brown	20×10^1	3	0	10×10^1

Key: 1. Total aerobic microbial, 2. Coliform, 3. Escherichia Coli, and 4. Mould counts (cfu/g)

3.1.3 Mycotoxins

Results of mycotoxins in the samples are presented in Table 3.

Table 3: Concentration of total aflatoxin and deoxynivalenol in the samples across the stations

S/N	Sampling station	Mycotoxins											
		Total aflatoxin (ppb)						Deoxynivalenol (DON) (ppb)					
		Sorghum		Maize		Millet		Sorghum		Maize		Millet	
		White	Red	White	Yellow	Grey	Brown	White	Red	White	Yellow	Grey	Brown
1	Katsina-Ala Main Market	3.20	3.60	3.40	2.90	3.90	3.80	4200.00	3600.00	4400.00	5900.00	4500.00	3800.00
2	Abaji-Kpav Market	5.30	5.20	7.40	4.80	4.60	4.00	5500.00	5000.00	7200.00	4600.00	5600.00	4400.00
3	Amaafu Market	5.80	5.50	4.70	4.10	4.9	7.3	5700.00	5300.00	4600.00	4300.00	4800.00	7300.00
4	Gbor Market	5.40	6.20	3.70	5.6	5.9	5.7	5400.00	6200.00	4800.00	5600.00	5300.00	5800.00
5	Tor-Donga Market	4.60	7.50	2.30	5.7	8.4	6.4	4900.00	7500.00	2900.00	5300.00	8400.00	6200.00
	Range	2.60	3.90	5.10	2.8	4.5	3.5	1500.00	3900.00	4300.00	1600.00	3900.00	3500.00

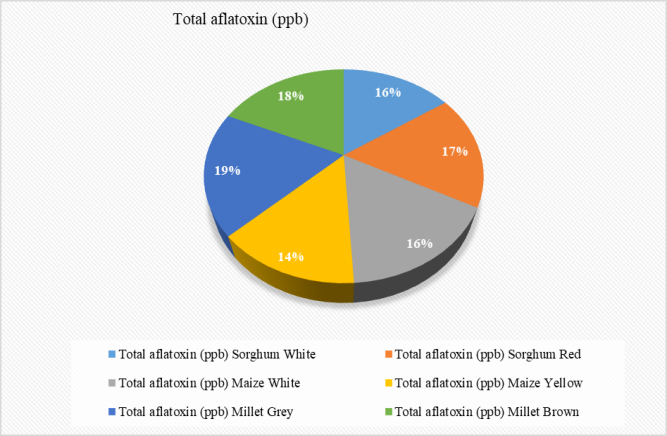

Figure 2: Pie chart of the concentration of total aflatoxins in the samples across the stations

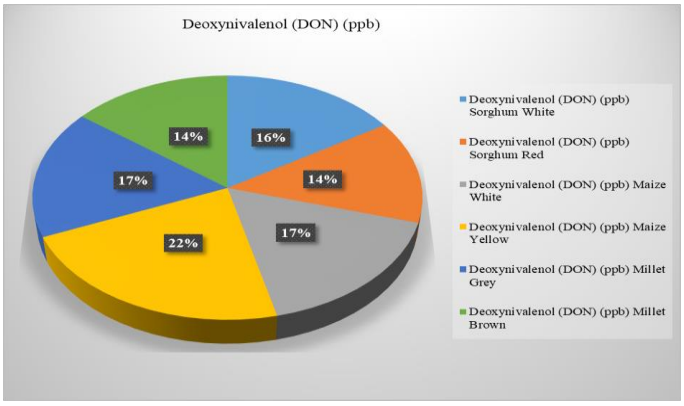

Figure 3: Pie chart of the concentration of deoxynivalenol in the samples across the stations

3.2 Discussion

Results (Table 1) showed that, percentage (%) moisture contents varied in white sorghum from 10.03 to 12.30, red sorghum varied from 9.03 to 12.39, white maize varied from 9.64 to 12.30 and yellow maize between 10.35 and 12.38, grey millet varied from 9.03 to 12.25 while brown millet varied from 9.19 to 12.04. Across the study area, moisture content indicated a narrow range of 2.2645 % (white sorghum) to 3.3768 % (brown millet). The moisture contents showed a direct proportionality of deoxynivalenol and aflatoxin contamination to the samples studied. Results revealed that % moisture content of samples were within limits of regulatory bodies (Tor *et al.*, 2020; CODEX and 15 % by NAFDAC).

Results of total aerobic microbial counts of the grains (Table 2) showed that the white sorghum varied between 10×10^1 cfu/g and 20×10^1 cfu/g, red sorghum 15×10^1 cfu/g to 20×10^1 cfu/g, white maize varied from 10×10^1 cfu/g to 20×10^1 cfu/g and yellow maize from 10×10^1 cfu/g to 20×10^1 cfu/g; grey millet from 15×10^1 cfu/g to 20×10^1 cfu/g, while brown millet varied from 10×10^1 cfu/g to 30×10^1 cfu/g. This specifies that, there is microbial contagion on these cereals studied. This also may be a reason why there is significant contamination of both aflatoxins and DON on these cereals/grains examined.

Results of coliform counts (Table 2) showed that the white sorghum varied across the sampling points between 1 cfu/g and < 3 cfu/g, red sorghum between 0 cfu/g and < 3 cfu/g, white maize varied from 0 cfu/g to < 3 cfu/g and yellow maize from 0 cfu/g to < 3 cfu/g; grey millet from 0 cfu/g to < 3 cfu/g, while brown millet varied from 0 cfu/g to < 3 cfu/g. This indicates microbial contagion on these grains studied, accusing farmers and marketers of poor hygienic handling and processing of the grains.

Results of *Escherichia coli* (*E. coli*) counts (Table 2) showed that the white sorghum varied from 0 cfu/g to 1 cfu/g, red sorghum 0 cfu/g to 1 cfu/g, white maize was constant at 0 cfu/g and yellow maize 0 cfu/g to 1 cfu/g, grey millet from 0 cfu/g to <3 cfu/g, while brown millet varied from 0 cfu/g to 1 cfu/g. Significant number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage of the food substance. This is a strong proof pointing to the mycotoxin (aflatoxins and DON) on the grains studied as well as the probable poor hygiene conditions of the grains.

Results of mould counts (Table 2) showed that the white sorghum varied between 10×10^0 cfu/g and 20×10^1 cfu/g, red sorghum varied from 10×10^1 cfu/g to 30×10^1 cfu/g, white maize varied between $<10 \times 10^0$ and 15×10^1 cfu/g and yellow maize varied from 1×10^1 cfu/g to 10×10^1 cfu/g, grey millet from 5×10^1 cfu/g to 15×10^1 cfu/g; while brown millet varied from 1×10^1 cfu/g to 15×10^1 cfu/g. Over-all, the mould counts were detected on all the grains studied, inferring widespread of aflatoxins and deoxynivalenol.

Results of the total aflatoxin concentration (ppb) (Table 3 and Figure 2), revealed that the total aflatoxin levels of the white sorghum varied between 3.20 and 5.80, red sorghum varied from 3.60 to 7.50, white maize from 2.30 to 7.40 and yellow maize from 2.90 to 5.70 and grey millet from 3.90 to 8.40 while brown millet varied from 3.80 to 7.30. Aflatoxins were detected in all samples examined, though within maximum permissible limits (MPLs) of 10.00 ppb set by EU, NAFDAC and CODEX.

Results of deoxynivalenol (DON) (ppb) (Table 3 and Figure 3) revealed that the deoxynivalenol (DON) levels of the white sorghum varied between 4200.00 and 5700.00, red sorghum from 3600.00 to 7500.00, white maize from 2900.00 to 7200.00 and yellow maize from 4300.00 to 5900.00 and grey millet from 4500.00 to 8400.00 while brown millet varied from 3800.00 to 7300.00. These result showed that, all the cereals contaminated with DON at levels above the maximum permissible limits (1,000.00 ppb) set by NAFDAC, EU and CODEX.

3.2.1 Correlation analysis of research data

The Pearson's 2- tailed correlation analysis on deoxynivalenol with moisture content of grains across the locations (Table 4) revealed that there is significant

correlation of moisture content with total aerobic microbial count, mould count, coliform count as well as *E. coli* count at 0.05 level of significance. The moisture content in the grains greatly supports the proliferation of mycotoxin producing moulds (Bankole and Adebanjo, 2003; Tor *et al.*, 2020).

In addition, the Pearson's 2-tailed correlation analysis on total aflatoxins, deoxynivalenol and moisture content with samples (grains) across and within locations (Table 5) revealed that there is significant correlation of total aflatoxins, deoxynivalenol and moisture content with samples (grains) across the locations at 0.05 level of significance. This implies that total aflatoxins, deoxynivalenol and moisture content in the grains, which enhances the production of mycotoxin producing moulds, were found on the grains in all the study locations at significant amounts.

3.2.2 Statistical treatment of research data using ANOVA

ANOVA results for multiple comparisons of moisture content between locations (Tables 6-9) revealed that, there was insignificant difference between the locations. Although, by mere observations, there were significant interactions, but not large enough to be statistically significant across these locations. The thresholds of moisture has a significant contribution to the level of microbial infections as well as mycotoxin (aflatoxin and DON) contaminations on the grains in the study areas. Therefore, the moisture content highly favoured the proliferation of aflatoxins and deoxynivalenol (DON) on the grains in the areas of research.

ANOVA results each of microbial analysis, total aflatoxins and deoxynivalenol (DON) at 5 % confidence limit of grains by sampling stations showed insignificant difference. This implied that the precursor effect of mycotoxin proliferation is the same across as well as their concentration on the grains in the location.

3.3 Conclusion

This study revealed that, grains contained high percentage (%) moisture content, supporting microbial and fungal growth. Millet contaminated most with microbes, which grey millet contaminated most with *E.coli*, while white maize was resistant, white sorghum was equally resistant to microbial contamination than red sorghum. Pearson's analysis revealed a linear/positive correlation of microbial count with mycotoxin growth. In general, data indicated that the contamination by mycotoxin (especially DON and aflatoxin) and its precursors on the studied grains was statistically significant at 95% confidence level ($p < 0.05$). Similarly, ANOVA at 5.0 % confidence limit revealed no significant variance of the study variables between locations.

Deoxynivalenol levels exceeded MPLs of the regulatory bodies, although total aflatoxin was recorded in all samples but its levels were within MPLs of the regulatory bodies. It was observed that these grains were more susceptible to DON than aflatoxin, thus susceptibility of the grains to DON was in the order; sorghum > maize > millet. Whereas total aflatoxin level was in the order; maize > millet > sorghum respectively.

The implication of this is that, the sale and consumption of these grains could be detrimental to health. Therefore, recommended that urgent steps should be taken in reducing the contamination of foodstuff especially grains by mycotoxins.

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List of appendices

The appendix extends from tables tables 4 to 9

Table 4: Statistical correlation of levels of total aflatoxin, deoxynivalenol, moisture content and microbial count with samples

Control variables		Moisture content (%)	Total aerobic microbial count	Coliform count	E. coli count	Mould count	Total aflatoxins	Deoxynivalenol
Moisture content (%)	Correlation	1.000	.176	.193	-.229	.155	.182	-.274
	Significance (2-tailed)	.	.389	.344	.260	.448	.374	.175
	df	0	24	24	24	24	24	24
Total aerobic microbial count	Correlation	.176	1.000	-.726	.018	.600	-.721	-.007
	Significance (2-tailed)	.389	.	.000	.929	.001	.000	.974
	df	24	0	24	24	24	24	24
Coliform count	Correlation	.193	-.726	1.000	-.199	-.246	.903	-.145
	Significance (2-tailed)	.344	.000	.	.329	.227	.000	.479
	df	24	24	0	24	24	24	24
E. coli count	Correlation	-.229	.018	-.199	1.000	-.141	-.258	.000
	Significance (2-tailed)	.260	.929	.329	.	.491	.202	.999
	df	24	24	24	0	24	24	24
Mould count	Correlation	.155	.600	-.246	-.141	1.000	-.228	.085
	Significance (2-tailed)	.448	.001	.227	.491	.	.262	.679
	df	24	24	24	24	0	24	24
Total aflatoxins	Correlation	.182	-.721	.903	-.258	-.228	1.000	-.130
	Significance (2-tailed)	.374	.000	.000	.202	.262	.	.525
	df	24	24	24	24	24	0	24
Deoxynivalenol	Correlation	-.274	-.007	-.145	.000	.085	-.130	1.000
	Significance (2-tailed)	.175	.974	.479	.999	.679	.525	.
	df	24	24	24	24	24	24	0

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5: Statistical correlation of the mycotoxins with other parameters analysed across the samples.

Control variables		Deoxynivalenol	Total aflatoxin	Moisture content	Microbial count	Sorghum	Maize	Millet
Deoxynivalenol	Pearson Correlation	1	-.107	.082	.007	-.097	-.018	.039
	Sig. (2-tailed)		.596	.683	.972	.631	.930	.846
	N	27	27	27	27	27	27	27
Total aflatoxins	Pearson Correlation	-.107	1	-.245	-.093	.905**	-.778**	-.647**
	Sig. (2-tailed)	.596		.218	.644	.000	.000	.000
	N	27	27	27	27	27	27	27
Moisture content	Pearson Correlation	.082	-.245	1	-.162	-.248	.569**	-.038
	Sig. (2-tailed)	.683	.218		.420	.212	.002	.851
	N	27	27	27	27	27	27	27
Microbial count	Pearson Correlation	.007	-.093	-.162	1	.040	-.126	.037
	Sig. (2-tailed)	.972	.644	.420		.842	.531	.855
	N	27	27	27	27	27	27	27
Sorghum	Pearson Correlation	-.097	.905**	-.248	.040	1	-.796**	-.800**
	Sig. (2-tailed)	.631	.000	.212	.842		.000	.000
	N	27	27	27	27	27	27	27
Maize	Pearson Correlation	-.018	-.778**	.569**	-.126	-.796**	1	.425*
	Sig. (2-tailed)	.930	.000	.002	.531	.000		.027
	N	27	27	27	27	27	27	27
Millet	Pearson Correlation	.039	-.647**	-.038	.037	-.800**	.425*	1
	Sig. (2-tailed)	.846	.000	.851	.855	.000	.027	
	N	27	27	27	27	27	27	27
**. Correlation is significant at the 0.01 level (2-tailed).								
*. Correlation is significant at the 0.05 level (2-tailed).								

Table 6: One-way ANOVA results for multiple comparisons of moisture on all the study variables.

Variables		Sum of Squares	df	Mean Square	F	Sig.
Katsina-ala market	Between Groups	192.698	22	8.759	1.281	.450
	Within Groups	27.343	4	6.836		
	Total	220.040	26			
Abaji-kpav market	Between Groups	345.968	22	15.726	.654	.772
	Within Groups	96.239	4	24.060		
	Total	442.207	26			
Amaafu market	Between Groups	361.442	22	16.429	.730	.723
	Within Groups	89.992	4	22.498		
	Total	451.434	26			
Tor-Donga market	Between Groups	228.794	22	10.400	2.459	.198
	Within Groups	16.915	4	4.229		
	Total	245.709	26			
Gbor market	Between Groups	361.137	22	16.415	.836	.660
	Within Groups	78.519	4	19.630		
	Total	439.656	26			

White sorghum	Between Groups	159435.038	49	3253.776	55.487	.000
	Within Groups	1700.556	29	58.640		
	Total	161135.594	78			
Red sorghum	Between Groups	97344.843	49	1986.629	11.424	.000
	Within Groups	5043.296	29	173.907		
	Total	102388.139	78			
Yellow maize	Between Groups	63248.457	49	1290.785	5.582	.000
	Within Groups	6705.383	29	231.220		
	Total	69953.839	78			
Grey millet	Between Groups	99972.760	49	2040.260	7.456	.000
	Within Groups	7935.928	29	273.653		
	Total	107908.687	78			
Total aerobic microbial count	Between Groups	302.149	22	13.734	.596	.809
	Within Groups	92.236	4	23.059		
	Total	394.386	26			
Coliform count	Between Groups	166580.056	22	7571.821	1.105	.522
	Within Groups	27403.089	4	6850.772		
	Total	193983.144	26			
E. Coli count	Between Groups	11.241	22	.511	1.363	.421
	Within Groups	1.500	4	.375		
	Total	12.741	26			
Mould count	Between Groups	9.500	22	.432	.691	.748
	Within Groups	2.500	4	.625		
	Total	12.000	26			
Total aflatoxins	Between Groups	220.641	22	10.029	2.938	.152
	Within Groups	13.656	4	3.414		
	Total	234.297	26			
	Within Groups	31.672	4	7.918		
	Total	229.013	26			
Deoxynivalenol	Between Groups	55.509	22	2.523	1.070	.538
	Within Groups	9.430	4	2.357		
	Total	64.939	26			
Brown millet	Between Groups	79538.938	49	1623.244	36.818	.000
	Within Groups	1278.558	29	44.088		
	Total	80817.496	78			

If p>0.05, there is no significant difference, however there is significant difference of moisture between locations

Table 7: One-way ANOVA on microbial analysis of the grains by sampling stations

Variables		Sum of Squares	df	Mean Square	F	Sig.
Katsina-Ala market	Between Groups	192.698	22	8.759	1.281	.450
	Within Groups	27.343	4	6.836		
	Total	220.040	26			
Abaji-Kpav market	Between Groups	345.968	22	15.726	.654	.772
	Within Groups	96.239	4	24.060		
	Total	442.207	26			

Amaafu market	Between Groups	361.442	22	16.429	.730	.723
	Within Groups	89.992	4	22.498		
	Total	451.434	26			
Tor-Donga market	Between Groups	228.794	22	10.400	2.459	.198
	Within Groups	16.915	4	4.229		
	Total	245.709	26			
Gbor market	Between Groups	361.137	22	16.415	.836	.660
	Within Groups	78.519	4	19.630		
	Total	439.656	26			

If p>0.05, there is no significant difference, however there is significant difference of microbial analysis between locations

Table 8: One-way ANOVA on total aflatoxins of grains by sampling stations

Variables		Sum of Squares	df	Mean Square	F	Sig.
Total Aflatoxins (ppb) (White Sorghum)	Between Groups	.769	3	.256	.145	.932
	Within Groups	30.109	17	1.771		
	Total	30.878	20			
Total Aflatoxins (ppb) (Red Sorghum)	Between Groups	2.044	3	.681	.271	.845
	Within Groups	42.708	17	2.512		
	Total	44.752	20			
Total Aflatoxins (ppb) (White Maize)	Between Groups	6.569	3	2.190	1.173	.349
	Within Groups	31.729	17	1.866		
	Total	38.298	20			
Total Aflatoxins (ppb) (Red Maize)	Between Groups	2.380	3	.793	.473	.705
	Within Groups	28.509	17	1.677		
	Total	30.890	20			
Total Aflatoxins (ppb) (Grey Millet)	Between Groups	4.647	3	1.549	.641	.599
	Within Groups	41.065	17	2.416		
	Total	45.712	20			
Total Aflatoxins (ppb) (Brown Millet)	Between Groups	6.197	3	2.066	1.526	.244
	Within Groups	23.012	17	1.354		
	Total	29.210	20			

If p>0.05, there is no significant difference, however there is significant difference of total aflatoxins between locations

Table 9: One-way ANOVA on deoxynivalenol (DON) of grains by sampling stations

		Sum of Squares	df	Mean Square	F	Sig.
Deoxynivalenol (DON) (ppm) (White Sorghum)	Between Groups	3.021	3	1.007	.514	.678
	Within Groups	33.288	17	1.958		
	Total	36.310	20			
Deoxynivalenol (DON) (ppm) (Red Sorghum)	Between Groups	2.598	3	.866	.344	.794
	Within Groups	42.732	17	2.514		
	Total	45.330	20			
Deoxynivalenol (DON) (ppm) (White Maize)	Between Groups	3.899	3	1.300	.904	.459
	Within Groups	24.424	17	1.437		
	Total	28.323	20			
Deoxynivalenol (DON) (ppm) (Red Maize)	Between Groups	1.352	3	.451	.282	.837
	Within Groups	27.137	17	1.596		

	Total	28.490	20			
Deoxynivalenol (DON) (ppm) (Grey Millet)	Between Groups	5.238	3	1.746	.900	.462
	Within Groups	32.980	17	1.940		
	Total	38.218	20			
Deoxynivalenol (DON) (ppm) (Brown Millet)	Between Groups	8.459	3	2.820	2.396	.104
	Within Groups	20.004	17	1.177		
	Total	28.463	20			

If p>0.05, there is no significant difference, however there is significant difference of deoxynivalenol (DON) between locations