Original Research Article

LEVELS OF DEOXYNIVALENOL AND AFLATOXIN MYCOTOXINS IN GRAINS IN SOME MARKETS OF MAKURDI METROPOLIS - NIGERIA

Abstract

This studyaimed to estimated ~~assessed~~ deoxynivalenol (DON) and total aflatoxin in two varieties ~~each~~ of millet, sorghum and maize, in some markets of Makurdi metropolis, North-Central, Nigeria using direct competitive Enzyme-linked Immunosorbent Assay (ELISA) method. Moisture content, total aerobic microbial count, mould count, ~~coliform~~ Coliform and *Escherichia coli* were determined using standard analytical methods. Moisture content profile of 9.5032 to 12.9940 % (sorghum), 9.5932 to 12.3604 % (maize) and 9.0279 to 12.4108 % (millet) was considered high?(up to 13% was high). Proliferation of total aerobic microbial count was observed in the samples across the stations (what do you mean~~stations~~?. Mycotoxins under study were detected in all samples across the stations(which stations)?. Whereas total aflatoxins were within permissible limits, DON exceeded thresholds. It was also observed that, these grains were more susceptible to the fungus sp producer of DON than the producer of aflatoxin, thus susceptibility of the grains to fungus producer of DON was in the order: sorghum > maize > millet, whereas the susceptibility against the fungus producer or producers of total aflatoxin level was in the order: sorghum > millet > maize respectively. This in general, indicated microbial contamination of these cereals in the study location indicting farmers and marketers of poor hygienic handling and processing of the grains. Pearson's 2-tailed correlation at 0.05 level of significance revealed strong correlation of deoxynivalenol with moisture content of grains across locations.(poor English)

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***Keywords*:** Makurdi, millet, sorghum, maize, total aflatoxins, deoxynivalenol (DON).

1 Introduction

Sorghum, millet and maize are among the most traded grains throughout the season in Makurdi and the business is favoured by the high population and commercial activities of the town. Grains (millet, sorghum and maize) are among the commonest cereals that are consumed in Benue State. These grains are packaged in polyethylene bags of 50 - 100 kg and are sold in whole bags or in discrete measures of '*basins* or *mudus*'. These are consumed in processed form in households majorly as '*fufu*', '*kunu*', 'local brew'('*burukutu*'), '*ibier*', '*mumu*', *flour*,popcorn?(popcorn) is one of six major types of corn, which includes dent corn, flint

corn, pod corn, flour corn, and sweet corn or zea mays variety everta. Are you utilise this specie in your study ~~popcorn~~ ? '*akamu'*, fed to animals and general carbohydrate bases for most recipes. The grains as well as their products are widely consumed in all seasons and it is a known fact that people consume grains without regard to their safety.

Moulds are ~~microscopic~~among , fungi ~~plant-like organisms~~,imperfecti named as filamentous fungi which composed of long filaments called *hyphae*. Mould *hyphae* grow over the surface and inside of nearly all substances of plant or animal origin. Because of their filamentous construction and consistent lack of chlorophyll, they are considered by most biologists as being separate from the plant kingdom and members of the kingdom of fungi (Speijers and Speijers, 2004)Not correct Basidiomycetes only and few of Oomycetes . They are widely distributed and found wherever moisture is present and very readily to produce toxins during metabolic processes (Sweeney and Dobson, 1998; Bennett and Klich, 2003; Marin *et al.,* 2013). Fungi that produce toxins in food are therefore classified into field fungi and storage fungi based on their ecological requirements for growth (Christensen,1955 not IARC, 2021(2012). Although there are many species of toxigenic moulds, only a few (not correct) You must read litteratures enough(Mycotoxins are found where there is mold; however, **not all molds produce (dangerous) mycotoxins** but certain species produce more than others, with both indoor and outdoor molds having the ability to do mycotoxins, particularly those affecting cereals (maize, wheat, sorghum, barley, millet, oats and rice) and groundnuts are considered to be of significant harm to humans (CFDA, 2017).

Fungi are major causes of food and feedstuff spoilage (Boevre *et al.,* 2012). The proliferation of various fungi in agricultural products leads to reduction in yield(you speek about agricultural products, so use the word yield with the harvested

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grains) and quality with significant economic losses. They produce secondary metabolites, which are referred to as mycotoxins, which have been found to be present in most food substances (Robens and Cardwell 2003; Boevre *et al.,* 2012).

Mycotoxins are a group of secondary metabolites which includes aflatoxins and deoxynivalenol (DON) produced by filamentous fungi?please Mentioned their genera, which may contaminate foods, feeds or the raw materials used to produce them (Bennett and Klich, 2003). The genera of mycotoxigenic fungi are mainly represented by *Aspergillus, Penicillium* and *Fusarium,* but *Trichoderma(not correct),* *Trichothecium(is not among mycotoxigenic fungi)* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Sweeney and Dobson 1998; Bennett and Klich, 2003). Mycotoxins differed in their structures, which ~~clarifies~~ explained their great disparity of symptoms.Mycotoxins are classified according to their symptoms. They also induce mycotoxicosis in humans and animals. Toxicity of mycotoxins can be acute ~~and~~ , chronic and can lead to death. In acute cases, the effects of the toxin will appear after a short exposure time (seconds, minutes or hours), (EC, 2006). Usually, acute toxicity is the result of exposure to high doses and is characterized by the presence of easily recognizable severe symptoms. Chronic toxicity is characterized by weaker symptoms that might only occur after an initial period of exposure (Ahmad and Jae-Hyuk, 2017). Chronic toxicity can occur with long-term exposure to low doses of mycotoxins. A chronic effect of some mycotoxins (e.g. aflatoxin) is the induction of cancer, especially of the liver.

Aflatoxins are naturally occurring mycotoxins that are formed by many species of the fungus *Aspergillus*, notable ones being *Aspergillus flavus* and *Aspergillus parasiticus*. Their names are derived from the initial work that revealed ***A**spergillus **Fla**vus **toxins*** (Sweeney and Dobson, 1998; Bennett and Klich, 2003; Robens and

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Cardwell, 2003; Marin *et al.,* 2013). These toxins are named from the fungus producing them, e.g. "A" from the genus name *Aspergillus*, "fla" from the species name *flavus* added to toxin to give the name "aflatoxin". There are several different ~~toxins in the~~types of aflatoxins ~~aflatoxin group~~. Aflatoxins are toxic and are amid the most carcinogenic substances known (Sweeney and Dobson, 1998; Pitt, 2000). After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to the less harmful aflatoxin moeity (Sweeney and Dobson, 1998; Bennett and Klich, 2003). Aflatoxins (AFTs) and ochratoxin A (OTA) pose a significant threat to human health (Chu and Li, 1994; Bhat and Vasanthi, 2003). Aflatoxins are potent carcinogens and in association with hepatitis B virus are responsible for many thousands of human deaths per annum, mostly in non-industrialized tropical countries (Pereira *et al.,* 2014; CFDA, 2017). AFTs are secondary metabolites which belong to the difuranocoumarins, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, commonly found in food and feeds and have been associated with various diseases such as aflatoxicosis in livestock, domestic animals and humans throughout the world (Robens and Cardwell, 2003; Ayejuyo *et al.,* 2008; Igor *et al.,* 2008).

The occurrence of deoxynivalenol (DON) in grains may be due to contamination of the grains by the toxigenic strains of *Fusarium* species? Mentioned the species please. Deoxynivalenol contamination occurs when mycotoxigenic species of *Fusarium* group successfully colonize the sample, grow in it and produce toxins such as deoxynivalenol, zearalenone as secondary metabolites (Speijers and Speijers, 2004; Marin *et al.,* 2013; Pereira *et al.,* 2014). The occurrence of deoxynivalenol in other food products such as rice, wheat and maize have also been reported (Bankole and Adebanjo, 2003; Boevre *et al.,* 2012).

The production of mycotoxins is stimulated by environmental factors such as humidity, high temperature and wind speed akin to what is foundlike in Makurdi. Therefore, the extent of contamination will differ according to ~~with~~ geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing (EC, 2006; Iqbal *et al.,* 2016). This study was therefore, conducted with the objective of determining the total aflatoxin and deoxynivalenol levels of maize (white and yellow), sorghum (white and red), and millets (grey and brown) traded in the commercial city of Makurdi, North Central Nigeria.(where is the markets) there is a one place.

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2 Materials and methods

2.1 *Study area*

Makurdi is located on latitudes 7º 73' N and 7º 44' N; longitudes 8º 54' E and 8º 32' E, and on altitude of 94 m above sea level, barometric pressure of 100 KPa ~~with a population of about 292,645 (NPC, 2005) and 437,877 inhabitants by world population review prediction in 2022 (WPR, 2022).~~ Makurdi lies in the tropical guinea savannah zone of the North Central Nigeria and has a typical climate with two distinct seasons: the dry season (from November to March) and rainy season (April to October Our study was carried out during the----season). The area has a mean annual rainfall of 1,290 mm. Temperature is however, generally high (fluctuates between a minimum of 27 ºC to 28 ºC and a maximum of 30 ºC to 34 ºC) throughout the year, with February and March as the hottest months (Seibert, 2007; Hell *et al.,* 2000; NIMET, 2022).

2.2 Sample collection

Ten (10) samples of each ~~of the~~ grain type -----grams each~~s~~ were collected ~~at~~ randomly from the following markets in Makurdi; High-Level, Railway, Wurukum, Wadata, North-Bank, Modern Market. From each market, ten (10) samples were randomly collected from sellers based on high concentrations of human traffic and sellers of these grains/cereals (millets, sorghum and maize). Three hundred and sixty (360) samples were collected for ~~each of~~ the tested grains across the sampling points in Makurdi. The grains were sorghum (white and red), maize (white and yellow) and millet (grey and brown). These were pooled into thirty six (36?) representative composites? What do you mean are you mix it?.You take 10 samples for white maize,10 for yellow.etc so you have 60 samples if you triplicated 180 samples

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2. 3 *Determination of moisture content*

Moisture content detection was carried out by using an analyser, Sartorius, M 100 certified according to ISO 9001 ~~was used~~. ~~A measured quantity, (5.0 g) of~~ sample(5.0g) was macerated using a Romer series II Miller. Then, 3.0 g of pulverised sample was weighed into aluminium moisture plate and placed on the tray after which the lid was closed and analysis run as specified at 105 °C and the resulted moisture contents ~~displayed on the digital screen in~~ percentage (%) was recorded (Araujo, 2009; Afla-Guard, 2005).

2. 4 *Determination of deoxynivalenol and total aflatoxin levels*

2. 4. 1 *Sample preparation*

Thirty grams (30.0 g) of composite? 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, shaken

vigorously for three minutes ~~and~~then wait for another three minutes to ~~then~~ allow particles ~~ed~~ to settle. Thereafter, the supernatant was filtered through a Whatman No. 1 filter paper then~~and~~ the filtratewas collected (Afla-Guard, 2005; Julie *et al.,* 2011; Ubwa *et al.,* 2014; Tor *et al.,* 2020).

2. 4. 2 Assay of the AgraQuant total aflatoxin method

The sixteen blue/green–bordered dilution strips were positioned in a micro-well strip holder. Separate dilution wells were used for each standard (0.0, 2.0, 4.0, 10.0 and 20.0 ppb). Then, an equal numbers of Antibody Coated Micro-well (ACM) strips were placed in the holder. Using an 8-channel pipettor, 2.0 mL of conjugate was dispensed into each blue/green-bordered dilution well. Further, using separate single channel pipettor, 0.10 mL (100.0 µL) each of the analyte or standard were respectively dispensed into the corresponding micro-well strip containing the conjugate. A fresh 8-channel pipettor was used to mix the sample ~~by~~ carefully pipetting it up and down three times and immediately, 0.10 mL (100.0 µL) of the mixture was transferred into the antibody coated micro-well strips and incubated for 15 minutes at room temperature. The antibody coated micro-well strips were then emptied and washed five times with deionised water. Maximum care was taken to prevent ~~not to extricate~~ the strips to extricate from the holder during the washing process (Afla-Guard, 2005; Julie *et al.,* 2011).

Then, for each micro-well strip, absorbent paper towels were folded into several layers, laid on a flat surface and the strip tapped onto the towels to absorb as much residual water as possible before drying the bottom with a dry-towel. Using the 8-channel pipettor, 1 mL/strip portion of the substrate was put into the micro-well strips and incubated for 5 minutes. Then, using a fresh 8- channel pipettor, 0.10 mL (100.0 µL) of “stop solution” was pipetted into each micro-well strip and the colour

changed from blue to yellow. Thereafter, the strips were read with a micro-well reader using a 450 nm filter and a differential filter of 630 nm. The optical density (OD) readings were recorded for each micro-well (Afla-Guard, 2005).

The OD values were ~~each~~ expressed as a percentage of the OD of the zero (0.0) standard and then a dose-response curve was constructed using the five 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 (Afla-Guard, 2005).

2.4.3 Assay for deoxynivalenol (DON)

The basis of the tests were the antigen-antibody reaction. Ninety - six (96) micro-titre wells were coated with capture antibodies directed against anti-deoxynivalenol antibodies. Then 10.0 mL each of deoxynivalenol standards (as reference) and sample solution, 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. Any unbound enzyme conjugate was then removed in a washing step with 50 mL distil water.

Then, 1.0 mL each of substrate (sample) and chromogen were added to the antibody coated micro-wells (ACMW), bound enzyme conjugate converted the chromogen into a blue product. The addition of 10 mL stop solution led to a colour change from blue to yellow. The measurements were made photometrically at 450

nm. The absorbance was inversely proportional to the deoxynivalenol concentrations in the sample.

2. 5 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 u to x and the solutions they contained were respectively identified by the labels. Then, 5.0 g of each sample was pulverised and transferred into the bottle, 't' containing 45 mL of peptone water and shaken thoroughly to mix (solution 'u'). With a sterile syringe, 2.0 mL of solution 'u' was transferred into another bottle, 's' containing 18.0 mL peptone water and again shaken properly to mix (solution 'v'). 2.0 mL of solution 'v' was transferred into another bottle, 'w', also containing 18.0 mL peptone water and mixed (solution 'y') after which 2.0 mL of the solution 'y' was transferred into a set of duplicate petri dishes labelled (1a, 1a').

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Then, 2.0 mL of solution 'y' was transferred into another bottle, 'z' containing 18.0 mL peptone water, mixed (solution 'z') and 2.0 mL of solution 'z' was transferred into another set of duplicate petri dishes (1b, 1b'). Then, 50.0 mL ~~Durhan~~Durham tubes were filled with 40 mL MacConkey Broth (MCB) by gently tilting; ensuring that no air bubble was trapped in the broth. When the molten ~~Sabourand~~ Sabouraud 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. The petri dishes were incubated at 37 °C for 48 hours and 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

238 petri dishes (Iqbal *et al.,* 2016). The results were determined in colony-forming unit
239 per millilitre (cfu/mL) (Adejumo *et al.,* 2007; Araujo, 2009).

240 **2.6 Analytical method validation**

241 Detection limit for cereals, malt and feed (18.5 ppm) used for
242 immunochemical analysis was validated. The recovery rate of the analytical method
243 for cereals, malt, feed, beer and wort (85 – 110 %) was adopted. The specificity of
244 the RIDASCREEN® DON/total aflatoxin test was determined by analysing the cross-
245 reactivities to corresponding mycotoxins and both total aflatoxin and deoxynivalenol
246 set at 100 %.

247 **3 Results and discussion**

248 **3. 1 Moisture content**

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

250 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	High-Level Market	9.7032	11.9365	12.3604	9.7032	9.5219	10.7412
2	Railway Market	10.0356	11.9365	12.2547	12.0445	10.1307	10.3702
3	Wurukum Market	9.8872	12.0047	10.3102	10.6414	12.4047	10.0032
4	Wadata Market	12.3944	12.9940	12.3941	10.1569	9.0279	9.7032
5	North Bank Market	12.3112	11.3921	9.5932	10.3941	10.3682	12.4108
6	Modern Market	12.0445	9.5032	10.1307	10.0032	11.9365	10.5412
	Range	2.6921	3.4908	3.3478	2.3413	3.3768	2.7076

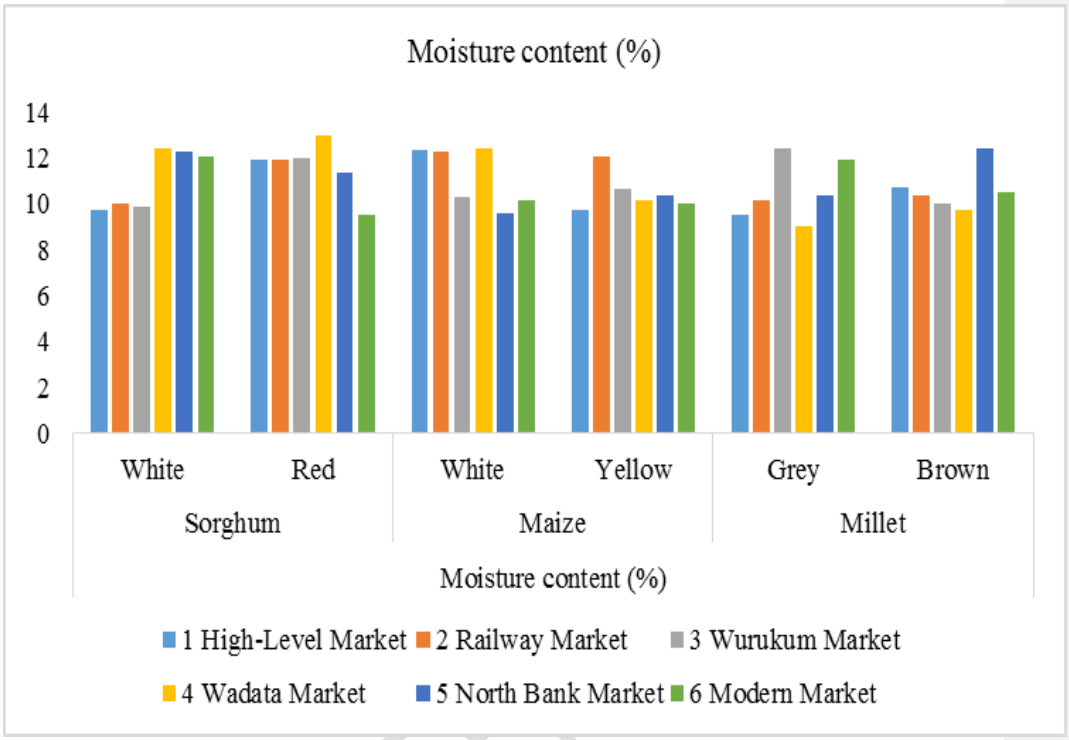

Figure 1: Bar chart of the percentage (%) moisture content in the samples across stations

3.2 Microbial analysis

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

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276
277

278 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	High-Level Market	Sorghum	Red	20×10^1	3	1	1×10^1
			White	15×10^1	3	1	15×10^1
		Maize	Yellow	20×10^1	3	0	10×10^1
			White	10×10^1	0	0	10×10^1
		Millet	Grey	15×10^1	3	0	30×10^1
			Brown	20×10^1	3	1	10×10^1
2	Railway 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	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Millet	Grey	10×10^1	0	1	10×10^1
			Brown	15×10^1	0	0	10×10^1
3	Wurukum Market	Sorghum	Red	20×10^1	1	1	10×10^1
			White	15×10^1	3	1	10×10^1
		Maize	Yellow	20×10^1	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Millet	Grey	10×10^1	0	1	10×10^1
			Brown	15×10^1	0	0	10×10^1
4	Wadata Market	Sorghum	Red	20×10^1	1	1	10×10^1
			White	15×10^1	3	1	10×10^1
		Maize	Yellow	20×10^1	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Millet	Grey	10×10^1	0	1	10×10^1
			Brown	15×10^1	0	0	10×10^1
5	North Bank Market	Sorghum	Red	20×10^1	1	1	10×10^1
			White	15×10^1	3	1	10×10^1
		Maize	Yellow	20×10^1	1	1	15×10^1
			White	10×10^1	1	0	20×10^1
		Millet	Grey	10×10^1	0	1	10×10^1
			Brown	15×10^1	0	0	10×10^1
6	Modern Market	Sorghum	Red	20×10^1	1	1	10×10^1
			White	15×10^1	3	1	10×10^1
		Maize	Yellow	15×10^1	0	0	10×10^1
			White	10×10^1	3	0	10×10^0
		Millet	Grey	20×10^1	0	0	10×10^1
			Brown	10×10^1	0	0	10×10^1

279 *Key: 1. Total aerobic microbial, 2. Coliform, 3. Escherichia Coli,* and *4. Mould counts*
280 *(cfu/g)*
281
282
283 **3.3 Aflatoxin analysis**

284 Results of aflatoxin concentration (ppb) in the samples are presented in Table
285 3.

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

S/N	Sampling station	Total aflatoxin (ppb)					
		Sorghum		Maize		Millet	
		White	Red	White	Yellow	Grey	Brown
1	High-Level Market	3.10	3.70	5.30	3.90	3.80	3.50
2	Railway Market	4.60	7.60	3.60	3.80	3.90	5.30
3	Wurukum Market	4.80	4.60	6.60	5.30	4.00	5.70
4	Wadata Market	5.10	4.30	5.80	2.90	5.30	5.60
5	North Bank Market	5.90	8.00	6.30	5.40	6.80	4.70
6	Modern Market	4.90	5.30	5.80	3.70	4.60	2.90
	Range	2.80	4.30	3.00	2.40	3.00	2.80

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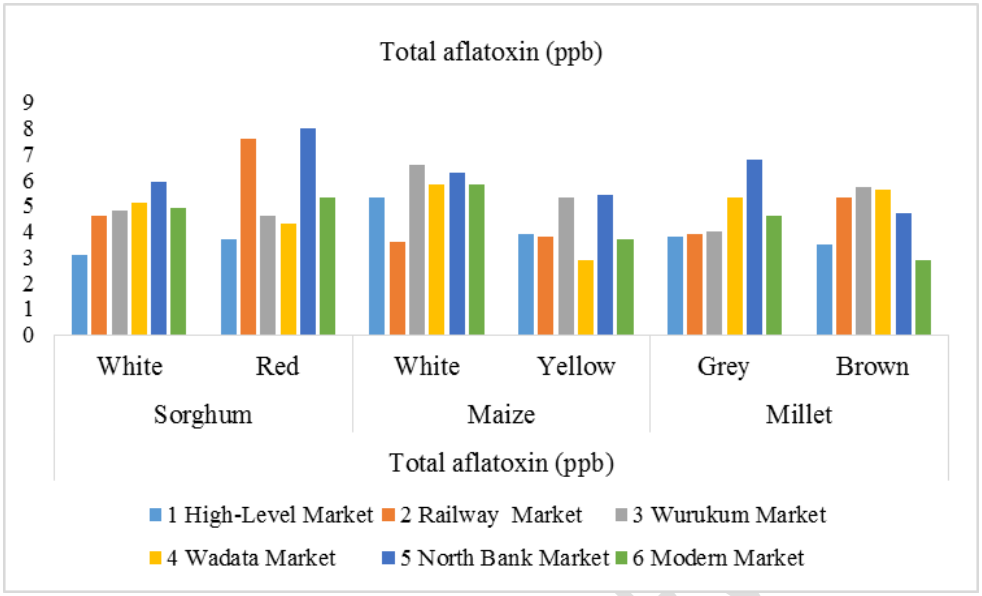

Figure 2: Bar chart of the concentration of total aflatoxins in the samples across the stations.

3.4 Deoxynivalenol analysis

Results of the deoxynivalenol (DON) concentration (ppb) in samples are presented in Table 4.

Table 4: Concentration of the deoxynivalenol (DON) in the samples across the stations

S/N	Sampling station	Deoxynivalenol (DON) (ppb)					
		Sorghum		Maize		Millet	
		White	Red	White	Yellow	Grey	Brown
1	High-Level Market	4100.00	3500.00	6300.00	4900.00	3200.00	3800.00
2	Railway Market	6600.00	8100.00	3900.00	3500.00	4900.00	5400.00
3	Wurukum Market	4700.00	4500.00	6800.00	5400.00	4600.00	5200.00
4	Wadata Market	8100.00	6300.00	5800.00	7900.00	5400.00	5800.00
5	North Bank Market	5400.00	8200.00	6400.00	5600.00	6200.00	4300.00

6	Modern Market	4200.00	5400.00	5700.00	4700.00	4600.00	2500.00
	Range	4000.00	4700.00	2900.00	4400.00	3000.00	3300.00

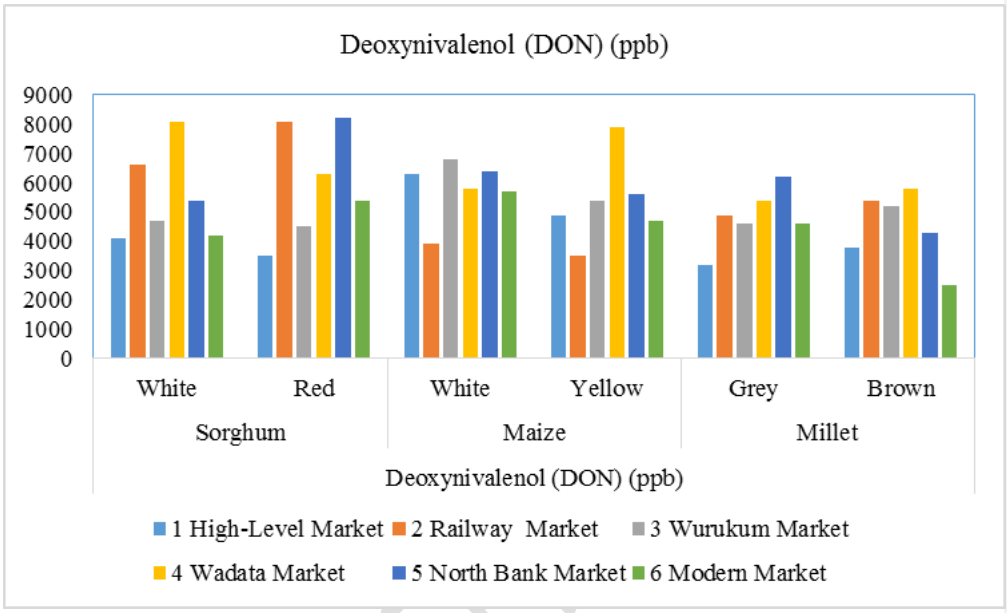

Figure 3: Bar chart of the concentration of deoxynivalenol in the samples across the stations.

Table 5: Statistical correlation of levels of total aflatoxin, deoxynivalenol, moisture content and microbial count with samples.

		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).

303 The statiscal analysis is not complete .

304 Why you detect the coliform total count .Please mentioned the method of counting

305 E.coli and its reference

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311 Table 6: Statistical correlation of the mycotoxins with other parameters analysed
312 across the 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 aflatoxin	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).

313

314 **3.6 Discussion**

315 Results (Table 1) revealed that, percentage (%) moisture content varied in

316 white sorghum from 9.70 to 12.39, red sorghum from 9.50 to 12.99, white maize 9.59

317 to 12.39 and yellow maize 9.70 to 12.04, grey millet from 9.03 to 12.40 while brown
318 millet varied from 9.70 to 12.40. Across the study area, moisture content indicated a
319 narrow range of 2.6921 (white sorghum) to 3.3768 %. Results revealed that %
320 moisture content of samples were within limits of regulatory bodies (Tor *et al.,* 2020;
321 CODEX and 15 % by NAFDAC).

322 The results of total aerobic microbial counts of the white and red sorghum,
323 white and yellow maize as well as grey and brown millets (Table 2) showed that the
324 white sorghum varied between 10×10^1 cfu/g to 15×10^1 cfu/g, red sorghum $15 \times$
325 10^1 cfu/g to 20×10^1 cfu/g, white maize was constant at 10×10^1 cfu/g and yellow
326 maize 15×10^1 cfu/g to 20×10^1 cfu/g, grey millet from 10×10^1 cfu/g to 20×10^1
327 cfu/g, while brown millet varied 10×10^1 cfu/g to 20×10^1 cfu/g. This indicates that
328 there is microbial contamination on these cereals in the study location. This also may
329 be a reason why there is significant contamination of both aflatoxins and
330 trichothecenes (DON) on these cereals/grains studied.

331 Results of coliform counts of white and red sorghum, white and yellow maize
332 as well as grey and brown millets (Table 2) showed that the white sorghum remained
333 constant across the sampling points at (< 3 cfu/g), red sorghum (0 cfu/g to < 3
334 cfu/g), white maize (0 cfu/g to < 3 cfu/g) and yellow maize (0 cfu/g to < 3 cfu/g),
335 grey millet (0 cfu/g to < 3 cfu/g) while brown millet (0 cfu/g to < 3 cfu/g). This
336 indicates microbial contamination on these cereals in the study location indicting
337 farmers and marketers of poor hygienic handling and processing of the grains.

338 Results of *Escherichia coli* (*E. coli*) counts of the grains (Table 2) showed that
339 the white sorghum varied between 0 cfu/g and 1 cfu/g red sorghum 0 cfu/g and 1
340 cfu/g, white maize was constant at 0 cfu/g and yellow maize 0 cfu/g and 1 cfu/g,
341 grey millet 0 cfu/g and 1 cfu/g, while brown millet 0 cfu/g and 1 cfu/g. Substantial

number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage of the food substance.

Results of mould counts of the grains (Table 2) showed that white sorghum varied from (10×10^0 cfu/g to 15×10^1 cfu/g), red sorghum (10×10^1 cfu/g to 15×10^1 cfu/g), white maize ($<10 \times 10^0$ to 20×10^1 cfu/g) and yellow maize (10×10^1 cfu/g to 15×10^1 cfu/g), grey millet ($<10 \times 10^1$ cfu/g to 30×10^1 cfu/g), while brown millet ($<10 \times 10^1$ cfu/g to 10×10^1 cfu/g). In general, the mould counts were detected across all the sampling points, lending credence to ubiquitous presence of aflatoxins and deoxynivalenol.

Table 3 revealed that the total aflatoxin levels of the white sorghum varied between 3.10 to 5.90, red sorghum 3.70 to 8.00, white maize 3.60 to 6.60 and yellow maize 2.90 to 5.40, grey millet from 3.80 to 6.80 while brown millet varied from 2.90 to 5.70 ppb. The results also documented high levels of total aflatoxin (8.00 ppb) for red sorghum though within maximum permissible limits (MPLs) at North bank sampling station and low 2.90 ppb in brown millet at Modern Market and so it was detected in all samples collected at the other locations. The detected levels were below the maximum permissible limits (MPLs) of 10.00 ppb set by EU, NAFDAC and CODEX.

Results for deoxynivalenol (DON) (ppb) in the grains in (Table 4) revealed that the deoxynivalenol (DON) levels of the white sorghum varied between 4.10 and 8.10, red sorghum 3.50 and 8.20, white maize 3.90 and 6.80 and yellow maize 3.50 and 7.90, grey millet 3.20 and 6.20, while brown millet varied from 2.50 to 5.80 ppb. These values revealed that, all the cereals are burdened with DON at levels above the maximum permissible limits (1,000.00 ppbnot correct) set by NAFDAC, EU and CODEX.

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3.6.1 Correlation analysis of research data

Pearson's 2-tailed correlation analysis on deoxynivalenol with moisture content of grains across locations (Table 5) 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. Supporting assertion that moisture content as well as water activity level in the grains greatly support the proliferation of mycotoxin producing moulds (Bennett and Klich, 2003).

Pearson's 2- tailed correlation analysis on sampling stations with moisture content of white sorghum, red sorghum, white maize red maize, grey millet and brown millet across and within locations (Table 6) 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, implying a linear relationship of hygienic environment with mycotoxin producing moulds.

Similarly, Pearson's 2-tailed correlation analysis on total aflatoxins, deoxynivalenol and moisture content with samples across locations, revealed a significant correlation of total aflatoxins, deoxynivalenol and moisture content with samples across locations at 0.05 level of significance. This implies that total aflatoxins, deoxynivalenol and moisture content as well as water activity level in the grains; enhance the production of mycotoxin producing moulds.

3.7 Conclusion

This study revealed that, samples contained high percentage (%) moisture content, favouring microbial and fungal growth. Yellow maize contaminated most with *E.coli*, while white maize was resistant, brown millet was equally resistant to microbial contamination than grey. 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 ($p < 0.05$). 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; sorghum > millet > maize respectively.

The implication of this is that, 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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