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Assessment of mycotoxin presence and distribution in maize grains across North central states of Nigeria

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Abstract

This research determined the fungal species prevalent and the concentration and distribution of mycotoxins in maize grains consumed in the North-central states of Nigeria. Six hundred composite samples were collected and screened for fungal contamination. The concentrations of fumonisins, aflatoxins, ochratoxin, trichothecenes, and zearalenone were quantified in the samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Kogi State had the highest concentration of fumonisin B1 (FB1) (755.7±56.7 µg/kg), Deoxynivalenol (DON) (1211 µg/kg), zearalenone (ZEA) (313 µg/kg) and aflatoxin B1 (AFB1) (7.5±1.6 µg/kg) and B2 (1.6±0.3 µg/kg), respectively. Ochratoxin A (OTA) value in Benue State (3.2±0.7 µg/kg) exceeded the European Union (EU) recommended amount of 3 µg/kg, while AFB1 concentrations in Kogi State (7.5±1.6 µg/kg) and Benue State (6.8±1.4 µg/kg) exceeded the EU and Standard Organisation of Nigeria (SON) recommended amount of 2 µg/kg and 4 µg/kg, respectively. Trichothecene concentrations were all low compared to the EU-recommended amount in maize grains meant for the table.

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1. Introduction

The continued contamination of agricultural produce and crops in Africa is a challenge requiring new mitigation approaches from the regional state governments [1]. Mycotoxins' contamination of staple food sources leads to mycotoxicosis and other health challenges in humans and animals [2]. Mycotoxins also reduce the yield and quality of crops and destroy plant development in the field [3]. As a result of factors like high humidity and temperature that encourage fungal growth, multiple contaminations of crops with different mycotoxins have been reported [4].

Maize, a staple food consumed in the tropical countries of Africa, is a grain whose demand increases with the increasing human population. It is cheap and mostly produced through subsistence agricultural farming methods, where it promotes economic growth, increases farmers' income, and reduces poverty. It grows easily on most soils, yielding grains that could be consumed fresh or processed into other forms to extend its shelf life, improve taste, and make food available over long distances and time, thereby providing food security. Maize is composed of carbohydrates, fats, vitamins and some amount of protein, making it a good substrate

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for fungi proliferation. Fungi invade the crop on the field and in storage, reducing the yield, quality, volume and aesthetic value over time. Factors such as late harvesting, poor agricultural practices, poor storage, and drying on the floor all encourage fungal infection and pest infestation. Prolonged storage under a warm and humid atmosphere in the tropics encourages the production of secondary metabolites known as mycotoxins.

Mycotoxins are naturally synthesised by fungi as secondary metabolic products with lethal and toxic effects in humans and animals. Apart from antibiotics, they are the second most active metabolites of microbial origin. The causal moulds are filamentous fungi which are widespread globally [5]. Filamentous fungi, namely *Aspergillus, Fusarium, Talaromyces* and *Penicillium,* are the major genera decimating yield and producing toxins and other secondary metabolites in crops [6]. They colonise plants and crops and multiply in warm and humid environments where poor agricultural management practices and a changing climate promote fungal proliferation. Fumonisins, trichothecenes, and zearalenone are produced by Fusarium species, ochratoxins by *Penicillium* and *Aspergillus* species, while aflatoxins are mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*.

Mycotoxins produce chronic and acute mycotoxicosis depending on the type and nature of the toxin in question. *Fusarium* species produce over 100 toxins divided into trichothecenes, zearalenone and fumonisins with different structures which determine their mode of action and effects on human and animal hosts. Trichothecenes are cyclic sesquiterpenes that potently inhibit protein synthesis and cause chronic intoxication. Mycotoxins are varied, and over 300 types and their analogues have been documented. These chemicals are produced in minute quantities when conditions are optimal for their synthesis. While some have been reported to have debilitating health effects, others do not. They are mostly thermostable and stable under most cooking types. These toxins cause cancer, abortion, mutation, and mycotoxicosis, among others, apart from the burden associated with such diseases [7]. Mycotoxins' contamination of agricultural produce impacts food security adversely, challenging food safety and production by at least 25% or higher [8]. Other associated negative impacts include rejection and subsequent destruction of food crops, loss of foreign exchange, livestock production impacts, an increase in the supply of contaminated food crops, and loss of income to the farmers.

The demand for maize seeds in Nigeria is estimated to be 50% above what is produced, and meeting the deficit supply is further challenged by eroding desertification from the Sahel savanna, human activities, pests and diseases [8]. The provision of safe, adequate and stable food to meet the growing population necessitates this study as the research provides evidence regarding the safe nature of the maize supply chain, identifies pathogenic agents responsible for the contamination of seeds, and contributes new information and data to food managers in the North-central states. With accurate exposure data on mycotoxins provided, assessing food quality and the development of control strategies for ensuring food safety, which are important inputs in risk assessment and management, can be assured in the region. The study aims to determine the concentration of mycotoxins in maize consumed in the North Central Nigerian States. The study will create awareness and guide governments and individuals on the need to take proactive measures in curtailing the menace of food crop contamination, as climate change and increasing temperature are promoting resistance in microorganisms. Nigeria's unique climate, characterised by high temperatures and humidity, fosters the growth of toxic fungi and the contamination of staple foods such as cereals and grains, which are widely consumed by the population. A thorough understanding of the concurrent contamination of multiple mycotoxins in grains due to extreme weather conditions caused by climate change is necessary to establish a foundation for further toxicological investigations, as noted by Shi *et al.* [4].

2. Materials and methods

2.1. The study area and sample collection

The study was carried out in six states, composed of North-central Nigeria, including Nasarawa, Benue, Kogi, Plateau, Kwara and Niger respectively. These states produce over 60 per cent of the food consumed in Nigeria. The states have hectares of arable land, which is good for crop production and livestock farming and forestry. Maize grains were collected for analysis in the study. Maize that had been stored for a minimum of 42 days (six weeks) was collected for six months, and the storage conditions were recorded. Six hundred maize samples (1 kg each) composed of sixty composite samples made up of ten sub-samples from ten Local Government Areas per State were randomly collected. A grain sampling spear (25 mm diameter) was used to collect maize seeds from the top, middle and bottom of maize bags at varied distances until 1 kg of the grain was collected from each store. Samples from ten different stores were mixed for each local government to obtain a composite sample, which is heterogeneously representative for each 60 local government areas where samples were collected. The representative samples from each local government were carefully mixed to obtain 60 composite samples (10 per State).

2.2. Isolation of toxigenic fungi from maize grains

The direct plating technique was used to isolate fungi on Potato Dextrose agar (PDA) from maize grains. The grains were placed at equal distances from each other on the plate and incubated at 25°C for 3-7 days, adopting the protocols of the International Commission on Microbiological Safety of Foods (ICSMF). Fungal colonies were sub-cultured and identified using the Methuen Handbook of Colour [9]. Confirmation of fungal identity and capability of suspected isolates to produce toxin was determined using the 18SrRNA genes amplified using primers *Euk1a* (F- 5' CTGGTTGATCCTGCCAG-3') and *Euk1a* (R- 5'- ACCAGACTTGCCCTCC-3').

2.3. Molecular identification of toxigenic fungi

2.3.1. DNA extraction from toxigenic fungi

The manufacturer's protocol (Zymo Research USA) was adhered to for the extraction of fungal DNA. Fungal cells (15 mg) were suspended in 50 μL Phosphate Buffered Saline - PBS, and ZR Bashingbeads were added to a tube, and 400 μL of BashingBead™ Buffer was added. The mixture was centrifuged for 5 min at ≥ 3,000 x g. The supernatant (250 μL) was transferred into another tube, and 750 μL of Genomic Lysis Buffer was added, and mixed for 2 min by vortexing. The centrifugation was repeated for another 5 min, after which 500 μL extract was transferred into another tube and centrifuged again for 5 min at 3,000 x g. The flow-through was discarded, and the process was repeated. Next, add 200 μL of DNA Pre-Wash Buffer. Centrifuge for 5 min at a minimum of 3,000 x g. Add 500 μL of g-DNA Wash Buffer to it and centrifuge for 5 min at 3,000 x g. Add 100 μL of DNA Elution Buffer to the matrices and centrifuge for 5 min at a minimum of 3,000 x g to obtain ultra-pure DNA. A nanodrop (ND1000) spectrophotometer was used to quantify extracted DNA.

For the determination of the presence of mycotoxin production capacity, the Applied Biosystems thermal cycler, ABI 9700, was utilised to conduct a multiplex polymerase chain reaction (mPCR), with a total volume of 35 μL. The reaction mixture consisted of 2.5 μL of $MgCl_2$ (50 mM), 2.5 μL of 10 × buffer, 0.9 μL of each primer (2.7 μM), 8 mM of dNTPs, 0.5 μL of Taq DNA polymerase and 5 μL of DNA template. The amplification followed a specific program: a 5 min initial denaturation step at 95°C, 35 denaturation cycles at 94°C for 1 min, annealing at 65°C for 30 s, and extension at 68°C for 30 s, ending with a final extension step at 68°C for 7 min.

2.3.2. Chemicals and reagents

Methanol, acetonitrile, acetic acid, and ethyl acetate used were HPLC grade and purchased from Merck (Darmstadt, Germany). Anhydrous magnesium sulfate and sodium chloride were of analytical grade. AFB1, AFB2, FUM 1, FUM 2, OTA, ZEA, and DON standards (purity ≥98%) were from Novakits (Nantes, France). A stock solution (1 mg of mycotoxin standard dissolved in 10 mL of acetonitrile) was used for the preparation of working standard solutions for calibration curves and recoveries. The working standard solutions are 100 μg/L for AFB1, FUM1, and OTA, 450 μg/L for AFB2, and 500 μg/L for DON. They were prepared by diluting stock solutions with mixed acetonitrile/water (30/70, v/v) containing 5 mmol/L ammonium acetate. Cellulose syringe filters (15 mm, 0.2 μm) were purchased from ALBET.

2.3.3. Extraction of mycotoxins from maize grains

Fumonisins were extracted from the maize matrix by grinding the maize seeds, and 10 g of the powder was weighed and added to 50 mL acetonitrile:water (80:20). The mixture was blended for 3 min, and the supernatant was filtered and adjusted to pH 6–9. It was mixed with 3 mL extract with 8 mL of 3:1 methanol:water. The column was conditioned by applying 5 mL of methanol followed by 5 mL of 3:1 methanol:water. Eleven millilitres of the diluted sample extract was applied to condition the column. It was then washed with 8 mL 3:1 methanol:water and 3 mL of methanol. Elution of the MultiSep 211 Fum was with 10 mL 99:1 methanol:acetic acid and allowed to evaporate to dryness. The elution was re-dissolved in the mobile phase and injected in 1 mL acetonitrile:water. The MultiSep 211 Fum showed >80% recovery rate. The LC-MS system used was LTQ Velos Scientific. Extraction of Type B trichothecenes (Deoxynivalenol, 3-Acetyl-DON (3ADON), and 15-Acetyl-DON (15ADON)) and zearalenone was done using the Bond Elut Mycotoxin Method. Finely homogenised powder of maize sample (10 g) was extracted with 50 mL acetonitrile:water (80:20; v/v) and blended for 3 min. The filtrate (4 mL) was thereafter passed through a Bond Elut Mycotoxin column, and the resulting extract (200 μL) was diluted with 800 μL water and injected into the LC-MS.

After dissolution in aqueous acidic media and purification on diatomaceous earth, OTA was extracted from corn samples using a mixture of ethyl acetate, methanol, and acetic acid (95:5:0.5, v/v/v). Aflatoxins were extracted following the method of Sirhan *et al.* [10] with modifications. Corn kernels (25 g) were ground and homogenised in 75 mL of methanol-acetonitrile solution (60:40, v/v) and vortexed for 20 min. Approximately 6.25 g NaCl and 33 g anhydrous $MgSO_4$ were added and shaken for 5 min to achieve phase separation. The mixture was then centrifuged at 4000 rpm for 10 min. The extraction time was 60 min to allow complete recovery of mycotoxins. To complete the procedure, 0.5 mL of the organic phase was passed through a 0.2 μm cellulose syringe filter, and the sample analysis was repeated three times. The supernatants were stored until injection into LC-MS/MS for mycotoxin quantification.

2.4. LC-MS measurement of mycotoxin

The chromatographic separation was performed on a Zorbax SB-C18 column with gradient elution using a mobile phase of acetonitrile and 0.1% (v/v) acetic acid in water at 48 °C with a flow rate of 1 mL/min. Ochratoxin A was detected in MRM mode using an ESI source in negative mode. The monitored transition was m/z 402 → (358 + 359 + 360). The run-time of chromatographic analysis was 18 min, and the retention time of OTA was 13 min. For zearalenone, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol, the LC conditions were: Column: Kinetex 2.6 μm XB-C18 100A, 150 x 4.6 mm; Eluent: B: Water/acetonitrile (95/5), C: acetonitrile; Flow: 0.5 mL/min. Chromatographic column in Agilent EclipseXDB-C8 column (150 mm × 4.6 mm, 5 μm i.d., Agilent, Santa Clara, CA, USA).

The quantification of aflatoxins was conducted using a Scientific Surveyor HPLC system (Thermo Scientific, CA, USA) connected to a TSQ Quantum Access tandem mass spectrometer. The HPLC system consists of a Surveyor MS Pump Plus, an online

Table 1. Distribution of fungal species isolated from maize grains in the different States.

Isolated fungi species	Benue	Kogi	Kwara	Nasarawa	Niger	Plateau	Total species isolated
Number of isolates (%)	(N = 390)						
Aspergillus wentii	3 (5.0%)	1 (1.8%)	1 (1.3%)	0	1 (1.6%)	1 (1.2%)	7 (1.8%)
Aspergillus fumigatus	22 (36.6%)	0	0	0	10 (16.1%)	0	32 (8.2%)
Aspergillus niger	0	7 (12.5%)	16 (21.1%)	6 (10.9%)	0	10 (12.3%)	39 (10.0%)
Aspergillus ochraceus	2 (3.3%)	1 (1.8%)	0	0	0	0	3 (0.8%)
Aspergillus flavus	18 (30.0%)	25 (44.6%)	11 (14.5%)	18 (32.7%)	18 (29.0%)	6 (7.4%)	96 (24.6%)
Aspergillus tubingensis	1 (1.7%)	0	1 (1.3%)	0	2 (3.2%)	0	4 (1.0%)
Aspergillus welwitschiae	1 (1.7%)	0	0	0	0	0	1 (0.3%)
Fusarium oxysporum	0	2 (3.6%)	7 (9.2%)	4 (7.3%)	1 (1.6%)	13 (16.0%)	27 (6.9%)
Fusarium subglutinans	0	0	2 (2.6%)	0	1 (1.6%)	2 (2.5%)	5 (1.3%)
Fusarium proliferatum	0	1 (1.8%)	0	0	0	2 (2.5%)	3 (0.8%)
Fusarium venenatum	0	0	2 (2.6%)	1 (1.8%)	0	1 (1.2%)	4 (1.0%)
Fusarium langsethiae	0	0	2 (2.6%)	1 (1.8%)	0	1 (1.2%)	4 (1.0%)
Talaromyces variabilis	0	1 (1.8%)	0	1 (1.8%)	0	0	2 (0.5%)
Mucor sp.	10 (16.7%)	0	34 (44.7%)	3 (5.5%)	28 (45.2%)	38 (46.9%)	113 (28.9%)
Trichophyton sp.	0	18 (32.1%)	0	21 (38.2%)	0	0	39 (10.0%)
Penicillium sp.	0	0	0	0	0	5 (6.2%)	5 (1.3%)
Penicillium notatum	0	0	0	0	0	2 (2.5%)	2 (0.5%)
Penicillium oxalicum	3 (5.0%)	0	0	0	1 (1.6%)	0	4 (1.0%)
Individual Total (N=390)	60 (15.4%)	56 (14.4%)	76 (19.5%)	55 (14.1%)	62 (15.9%)	81 (20.8%)	390 (100%)
Taxa_S	8	8	9	8	8	11	
Dominance_D	0.2463	0.3084	0.2667	0.2513	0.3051	0.2636	
Simpson_1-D	0.7537	0.6916	0.7333	0.7487	0.6949	0.7364	

degasser, and a Surveyor Autosampler Plus, while the spectrometer is equipped with an electrospray ionisation source from Thermo Scientific, San Jose, CA, USA. An analytical column of Hypersil GOLD (100 mm × 2.1 mm, 5 μm) (Thermo Fisher Scientific, San Jose, CA, USA) was utilised. The solvents used for gradient elution were acetonitrile (A) and a mixture of water, ammonium acetate (5 mM), and formic acid (0.1%) (B). The gradient elution sequence was: at 0 min, 30% A; at 3.0 min, 90% A; at 5 min, 90% A; from 5 min to 8 min, 30% A. The column was operated at a flow rate of 0.25 mL/min, with an injection volume of 5 μL. The entire analytical process using the MS/MS system took approximately 10 min.

To generate the calibration curves using a solvent, aliquots of the intermediate mixed solution measuring 5, 10, 15, 20, and 25 μL were obtained. These aliquots were then diluted with acetonitrile/water (50:50, v/v) to a final volume of 1 mL. This resulted in five different concentration levels: 0.25, 0.5, 0.75, 1, and 1.25 ng/mL for both AFB1 and AFB2; 0.235 for MS/MS detection. The ESI was operated in both positive and negative modes. For quantitation, the multiple reaction monitoring (MRM) mode was utilised, simultaneously scanning in both the positive mode (ESI^+) and negative mode (ESI^-). MS/MS parameters include capillary temperature, 350 °C; ion spray voltage: 4.5 kV; collision energy: 1.5 eV; column offset: 118 V; collision pressure: 1.5 mTorr. The mass spectrometer was operated using product ion scanning and selected reaction monitoring mode (SRM). For each mycotoxin, two selected precursor ion productions were monitored in SRM mode, and one of the productions was used for quantification. The MRM ion transition for each mycotoxin was optimised using single MS full scan mode, followed by product ion scan mode after injection of the intermediate solution to obtain one precursor ion and two product ions for each compound. One ion was used for quantification and one for confirmation. The response was calculated as the peak area for all compounds in the MRM chromatogram and used to determine the concentration of each compound in the sample.

3. Results and discussion

A total of 390 fungal isolates were identified in the study. The fungal species obtained were mostly *Aspergillus*, *Fusarium* and *Penicillium* species (Table 1). Plateau State had 81(21.3%) incidences of fungal species, and Benue State had the fewest 51(13.4%). *Mucor* species 113(29.7%) was the most isolated, while only one *Aspergillus welwitschiae* was isolated from Benue in the whole study. Plateau State had the highest number of species (11), while Benue, Kogi and Niger States had eight respectively. Simpson Diversity Index range between 0.6916 and 0.7537.

The Kogi state in Figure 1 had the highest concentration of AFB1 (7.5±1.6 μg/kg), followed by the Benue state with 6.8±1.4 μg/kg, while AFB2 had the highest amount (1.6±0.3 μg/kg) in Kogi State. AFB2 was not detected in Niger and Kwara States, respectively. Ochratoxin A in the States ranges from 1.4±0.5 μg/kg to 3.2±0.7 μg/kg.

Fumonisin B1 concentration (Table 2) was highest in Kogi State (755.7 μk/kg), followed by Benue State (267.5 μk/kg), while Niger had the least concentration of 9.2 μk/kg. The concentration of FB2 in this study was low compared with the EU Standard.

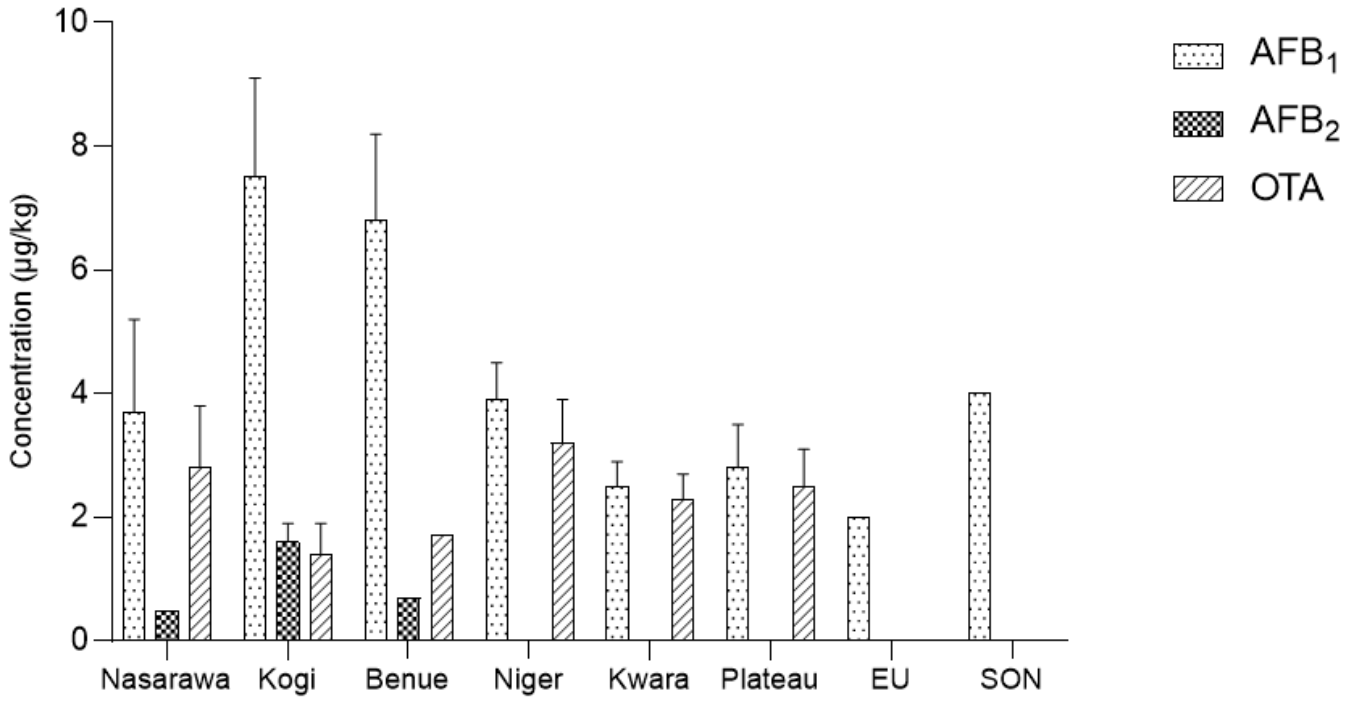

Figure 1. Mean concentration of aflatoxins and ochratoxin A in maize samples from North Central Nigeria (AFB_1 - Aflatoxin B1, AFB_2 – Aflatoxin B2; OTA – Ochratoxin A; Error bars indicate standard error of mean (n=10)).

Table 2. Mean concentration of Fumonisins B1 and B2 in maize samples from North central Nigeria.

SN	State	FB1	FB2	DON	ZEA
		Amount in µg/kg (Mean ± SD)			
1	Nasarawa	17.5 ± 3.2^{a}	3.5 ± 0.4^{a}	968 ± 18.9^{d}	15 ± 3.5^{b}
2	Kogi	755.7 ± 56.7^{c}	202.6 ± 18.8^{c}	1211 ± 25.4^{e}	313 ± 11.5^{e}
3	Benue	267.5 ± 28.9^{b}	69.7 ± 11.4^{b}	360 ± 12.4^{a}	62 ± 3.1^{d}
4	Niger	9.2 ± 2.4^{a}	0.0	503 ± 22.0^{b}	38 ± 2.0^{c}
5	Kwara	12.5 ± 4.0^{a}	3.1 ± 0.4^{b}	772 ± 8.5^{c}	35.9 ± 2.3^{c}
6	Plateau	21.8 ± 3.4^{a}	2.7 ± 0.8^{a}	485 ± 17.6^{b}	6 ± 0.7^{a}
	EU Standard	4000	4000	1750	100

Means with different letters are significantly different at $p = 0.05$ (Tukey HSD all-pairwise comparisons test).

The concentrations obtained from state to state were statistically significant at $P < 0.001$. There was no significant difference in means values among the groups in Plateau, Kwara and Niger States, respectively, at the 0.05 level of significance, while between the states, significance values were obtained at F values of 9951.322, 1565.221 and 3908.336, respectively, for ZEA, DON and FUM at p=0.000 significance level.

DON concentrations from the six states were below the EU value of 1750 µg/kg (Table 3). Nasarawa state had the highest 967.6 µg/kg, while the lowest amount of 360.4 µg/kg came from Benue State. Zearalenone value was high in Kogi State (313 µg/kg) and lowest in Plateau State (6.1 µg/kg).

There is a strong positive correlation between FB1 and ZEA, FB1 and DON, and DON and ZEA (Pearson correlation coefficient of 0.969, 0.563 and 0.671, respectively, at the p-value of 0.001 at 1% level of significance) (Figure 2).

3.1. Discussion

Maize is an important food source for animal feed, fuel and alcohol production. Maize in the sub-Saharan region has provided food security, employment, and income for the poor populace, while its demand increases as the population in the sub-region increases. The fungal species isolated were mostly similar from state to state. The states in the North Central region of Nigeria have similar climatic factors of high temperature and rainfall, and cereals undergoing similar storage conditions, made up of the

Table 3. Mean concentration of deoxynivalenol and zearalenone in maize from North Central Nigeria.

State	Amount in µg/kg	
	3-A-DON	15-A-DON
Nasarawa	234	13
Kogi	218	28
Benue	128	5
Niger	88	7
Kwara	158	4
Plateau	116	7
EU Standard	1250–1750	1250–1750

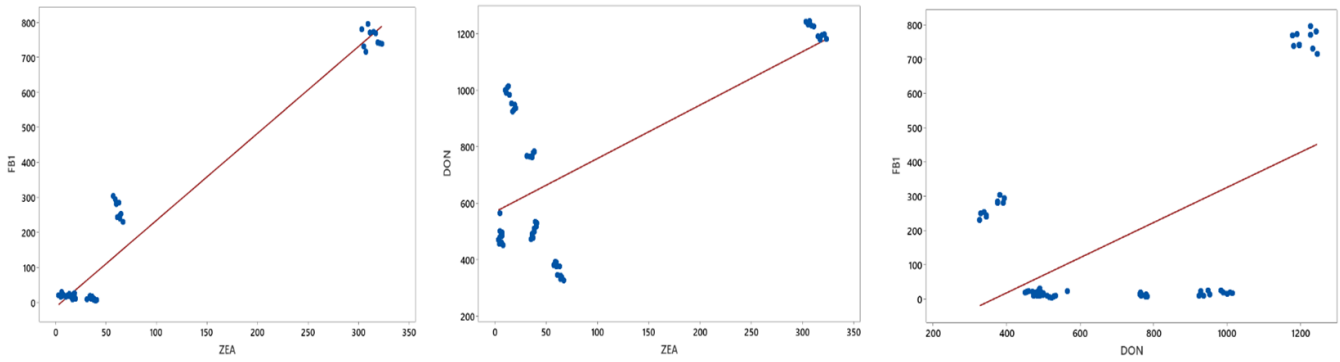

Figure 2. Correlation between FB1 and Zearalenone, FB1 and DON, and DON and ZEA.

unclean environment, high humidity and poor aeration of the storage stores, which might be responsible for the development. The isolates obtained were similar to those of Orole *et al.* [11] from Nasarawa State and Muhammad *et al.* [12] from Niger State, who isolated *A. niger, A. ochraceous, A. flavus, A. fumigatus, Mucor* sp., *Fusarium* sp., *Penicillium* and *Rhizopus* sp., respectively. The predominant fungi isolated were *Aspergillus* species (49.8%), which aligns with findings by Akande *et al.* [13], who had an 85.7% prevalence of the fungi from Osun State. Some *Aspergillus* and *Fusarium* species colonise maize parts at all stages of development, provided conditions are favourable from the field to the table [14], while others, such as *Aspergillus flavus* and *Aspergillus parasiticus,* are part-specific [15]. The Simpson diversity was greater than 0.5, showing the fungal species were from different taxa. Kwara State had the highest taxa richness in the study. The richness of the fungal communities and evenness of such communities determine the dominance diversity. Most of the fungi from the different states have highly dominated communities. It shows that most of the fungal species belong to two or more taxa (*Mucor, A. flavus* and *A. niger*), with few species belonging to the other taxa, as seen in Table 1.

The concentration of AFB1 was recorded to be at its highest in Kogi state, with Benue state following closely. These were the two states with concentrations above the EU tolerable value of 2 µg/kg, and the Standard Organisation of Nigeria recommended 4 µg/kg. Maize was reported by Batagarawa *et al.* [16] as the most susceptible to mycotoxin contamination of all cereal crops. Batagarawa *et al.* (2015) and Egbuta *et al.* [17] in their studies got AFB1 values ranging between 30.9 µg/kg – 507 µg/kg (Katsina and Zaria) and 0.07–109 µg/kg (South-East Nigeria), respectively, which disagreed with the 2.5 µg/kg – 7.5 µg/kg obtained in the study, while Neji *et al.* [18] got 1.73–1.78 µg/kg (Calabar, Cross River State) that agreed with the study. Aflatoxin is a known carcinogen that causes liver damage and suppresses immune functions. It can lead to weight loss, decreased milk production, and death in animals. Bandyopadhyay *et al.* [19] advise on the imperative to establish facilities that can provide farmers with testing and training opportunities, and awareness of effective preventive strategies. Fumonisins concentrations in the states were lower than the recommended EU value of 1000 µg/kg, though Nigeria has no regulatory standard for FB1. Kogi and Benue states had very high concentrations when compared with the other states sampled. The data presented here contradicts the findings of Liverpool-Tasie *et al.* [20], who cited higher concentration and incidence (13%) of fumonisin in Southwest, Nigeria. The results obtained confirmed possible improvements in the subsistence agricultural practices employed by maize farmers. Of the *Fusarium* species isolated, only two species, *F. subglutinans* and *F. proliferatum* are fumonisin producers. FB1 is carcinogenic to humans, hepatotoxic and nephrotoxic in animals, and causes birth defects in livestock [21]. It is a potent chemical that is phytotoxic to plants.

The mean concentration of DON (233.7 µg/kg) did not exceed the EU tolerable value of 1750 µg/kg, which agrees with the reports of Joshi *et al.* [22] who got between 110–520 µg/kg in Nepalese maize. The low concentration obtained in the study does not eliminate the adverse effects (such as weight loss) associated with the consumption of DON. Akoma *et al.* [23] conducted a separate investigation in Kogi State, Nigeria, where it was discovered that stored maize contained DON at levels ranging from 1.34 to 9.25 µg/kg. Astonishingly, 65% of these samples exceeded the Joint Expert Committee for Food Additives (JECFA) provisional

tolerable maximum daily intake (PTMDI) of 1 μg/kg for human consumption. Despite the incidence of zearalenone, DON, and other trichothecenes in most samples collected, the main fungi *Fusarium graminearum* and *F. verticillioides,* responsible for the production of most of the mycotoxins, were not detected. The fungus *F. graminearum* is introduced to maize at the pre-harvest stage and causes *Fusarium* head blight disease. Findings in other studies reported the production of new mycotoxic metabolites by previously known fungi in their effort to better adapt to the changing environment [24, 25]. This could be attributed to environmental triggers or climate change, genetic variation, or epigenetic modifications, which could be responsible for the presence of DON and absence of known producers of the metabolites in the study. The identification of the new metabolites in those studies was because of improved analytical methods adopted for such studies, which were limited in our study. Other reasons include the introduction of resistant maize cultivars, which have drastically reduced the incidence of fungus in agricultural produce [26]. Hyperestrogenism, early embryonic death, small litters, and reduced conception are the common symptoms associated with mycotoxins.

This study reported low levels of OTA, though with a high incidence in the states sampled, which agrees with Ekwomadu *et al.* [27], who had an incidence of 93.0% in South Africa. The positive correlation recorded between fumonisin B1 and zearalenone, though the *Fusarium* species responsible for the production of the two mycotoxins are different, reveals the interdependent relationship, showing a connection between the toxigenic fungi. The mycotoxin producing fungi (*Fusarium* species) are pre-harvest pathogens. The fungi produce an array of metabolites with different characteristics and toxicities. These pathogens utilise the same substrate from the environment to maintain their metabolic processes and, in the process, can compete for such a limiting resource in the community. The study observed that increasing concentration of fumonisin B1 correlates to increasing concentration of zearalenone across the states. While it is known that production of metabolites is determined by different pathways, available substrate and stress factors, the need to survive in a resource-limited environment can bring about alterations in the genetic makeup of the fungi, the use of new substrate, or the modification of available resources in an unfriendly environment. Microorganisms coexist to overcome unfriendly environments.

Mycotoxin quantified was mostly not regulated in Nigeria, except for AFB1. The development calls for concern because the other mycotoxins have correlated with human and animal disease conditions. The presence of multiple mycotoxins was reported in some of the samples collected. Alassane-Kpembi *et al.* [28] in their study reported that co-contamination of different toxins increases toxicity resulting from the synergistic action of the mycotoxin. Co-exposure to aflatoxin B1 and fumonisin initiates hepatocarcinogenicity, which results from decreased excretion of AFB1 [29, 30]. The mycotoxin concentrations in the study varied, which might be attributable to the maize variety, cropping location and the colonising fungal agents [31]. The farming conditions in the states were generally the same (rainfall volume of 1190 mm to 1590 mm, dry spell, humidity 57.9%, temperature range 34 °C, tillage and cropping systems, and pesticide application and timing), which might also allude to obtaining similar fungi and mycotoxins concentrations.

4. Conclusion

The study confirmed the presence of filamentous fungi of the *Aspergillus, Fusarium* and *Penicillium* genera with the potential to produce secondary metabolites that have been variously designated toxic and lethal to humans, animals and plants. The five major toxins (OTA, FUM, ZEA, DON, and AFB1) were prevalent in the sampled maize grains, with varying concentrations. The study observed that contamination levels were mostly below the European Union's recommended levels, indicating that the grains are relatively safe for consumption. It is recommended that regular screening of maize grains and other cereal crops be routinely monitored, and farmers and other stakeholders along the food chain be trained on newer methods of keeping grains safe for consumption.

Data availability

Data will be made available upon reasonable request from the corresponding author.

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