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Transmission of fungal profile and aflatoxin producing potential of some *Aspergillus Spp*. risk to animal and human environmental health

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ABSTRACT

Aflatoxins a group of heterocyclic metabolites produced by the fungi of the genus *Aspergillus*; particularly *Aspergillus flavus*, and *Aspergillus parasiticus*. This research aims to isolate aflatoxins-producing *Aspergillus spp*. from layers' feed sold within Sokoto metropolis. The sample of the layers' feeds, were analysed for aflatoxin using ELISA (Enzyme Linked Immunosorbent Assay). VOCs were analyzed using MS (Mass Spectrometer). Fungi was also isolated and identified. There is no higher concentration of aflatoxin found in a sample feed from Kofar Atiku, but were detected in other samples (> 20 ppb permissible in animal feed). The VOCs were identified as considerable, with highest abundance recorded at Masallacin Bafarawa (Cis-8-methyl-exo-tricylo [5.2.1.0(2.6), deconic acid = 4.49%), Mabera (2,6 Diethylnitrosobenzene = 4.44%), and Arkilla (1,2-Benzenedicarboxylic acid, diisooctyl ether = 4.06%). A total of 26 cultured of fungal species (100%) were identified, with *A. flavus* 10 (38.4%), *A.parasiticus* 8 (30.7%), *A. niger* 2 (7.7%), *A. fumigatus* 2(7.7%), *A. clavatus* 2(7.7%), and *A. oryzae* 2 (7.7%). 20 isolates being screened, with 7 isolates found to be positive for aflatoxin production, and 6 isolates also positive for their toxigenic capabilities. The study suggests that a proper treatment of poultry meats before eating is necessary. This will help minimize contamination, and ensure health security before consumption.

Keywords: Layers Feed, Fungi, Aflatoxins and Volatile Organic Compound

1. INTRODUCTION

Fungi are eukaryotic organism that comprised of both yeast and molds, and their infectious diseases are common in many kinds of poultry birds. Fungal diseases of poultry include Aspergillosis, Candidiasis, Dactylarosis, Torulopsis, Mucormycosis, Histoplasmosis, Cryptococcosis, Flavus and Rhodotorulopsis. Out of these, the first two, Aspergillosis and Candidiasis have much importance and impact, and the Histoplasmosis and Cryptococcosis have some zoonotic significance (Singh *et el.*, 2022). Fungi produce disease in two ways, viz. producing pathogenic signs and lesions of disease by invading, harming, and destroying the body tissues of the host (Dhama *et al.*, 2019). Likewise, it occurs by producing some toxins known as mycotoxins (Aflatoxins, Ochratoxins, Ergot, and Fusarium toxins) in food grains, and

feeds during crop production, harvesting and storage steps, the intake, consumption and subsequent intoxication of which produce disease, immunosuppressive conditions, and hamper production potential (Rai *et al.,* 2019). Sporadic infections are common in poultry birds, but sometimes they may take the form of outbreaks (Dhama *et al.,* 2019). However, fungal diseases are now more concerned in public health, because of the appropriate use of antibacterial, which eliminate the natural beneficial microflora which otherwise suppresses the growth of fungi (Singh *et al.,* 2022). The fungal pathogen affects the respiratory and nervous system of poultry, causing specific pathological changes in the host, which have been characterized by inflammation, lesions and sickness that leads to death (Singh *et al.,* 2022). Chronic exposure of fungal spores produces an allergic response that results in illness and decreased productivity in sensitized birds (McMillan and Petrak, 2009). The discovery of mycophages (fungal virus) has now been viewed as means of biologic control, and can be used to further understand the variation in antibiotic production and instability of fungal strains in poultry. This discovery describes the various fungal diseases of poultry with emphasis on their diagnosis, treatment, prevention and control.

According to Arne *et al.* (2023) the most pathogenic fungi affecting poultry, are caused by *Aspergillus spp* namely *A. flavus* and *A. parasiticus*, which lead to Aspergillosis (brooders pneumonia). Respiratory infection by *Aspergillus sp* has been reported in some of the poultry birds, viz. layers cockerels and broilers (Olias *et al.,* 2010). Other *Aspergillus spp* that may affect birds adversely are *A. terreus, A. glaucus, A. nidulans,* and *A. niger* (Beernert *et al.,* 2018; Dhama *et al.,* 2019). Poultry birds coming in contact with spores through contaminated feed or litter are affected after inhaling the spores (Arne *et al.,* 2023). The factors influencing the burning spore generation of dissemination in the air/environment include warm temperature, humidity, poor ventilation, and sanitation, along with long-term storage of feed (Khosravi *et al.,* 2020). Acute *Aspergillosis* (brooder pneumonia) occurs as a result of inhaling a high number of spores, wherein severe disease outbreaks in young birds are characteristically observed (McMillan and Petrak, 2009). Affected chickens may show gasping along with fever fetid diarrhea and rapid loss of condition with convulsions occurring sometimes (Arne *et al.,* 2023). Sub-acute form develops within 8-10 days in chickens up to 2 weeks acute signs often present in a milder form together with anemia, respiratory rattle as well as faces may become yellowish (Dhama *et al.,* 2019).

Mycotoxins are a heterogeneous group of secondary fungal metabolites. Their formation in food and feedstuff is influenced by many factors, including temperature, humidity, pH, and oxygen concentration, type of substrate, or presence of competitive microflora (Arne *et al.,* 2023). The ingestion of mycotoxins can produce both acute and chronic toxicities ranging from the systems to the alimentary track (Dhama *et al.,* 2019). Some mycotoxins are carcinogenic, mutagenic, teratogenic, and immunosuppressive (Kabak and Dobson, 2016). Mycotoxins have attracted worldwide attention over the last few decades. These are three main reasons, which are as follows: the effect of mycotoxins on human health, the loss of animal productivity, and due to the impact of mycotoxin contamination on international trade commodities. There are more than 300 known mycotoxins, and only few are relevant to consumer protection primarily because of their concentration in food, and toxic potential. These known mycotoxins include aflatoxins, ochratoxins and trichothecenes such as deoxynivalenal, zearalenone, and fumonisins (Etzel, 2012). Most of these mycotoxins, are produced primarily by three genera of fungi namely, Aspergillus, Penicillium and fusarium (Etzel, 2012). Thus, the toxic effect of mycotoxin on animal and human health is referred to as mycotoxicoses.

Aflatoxins are a group of heterocyclic metabolites produced by the fungi of the genus Aspergillus e.g. *Aspergillus flavus,* and *Aspergillus parasiticus* (Abrunhosa *et al.,* 2016). Aflatoxins are produced most common in most grains and nuts product stored under conditions that are favourable for growth of moulds. The occurrence of aflatoxin is highly dependent on favorable temperature of between 24 and 28^{0}c and substrate moisture content of at least (17.5%) (Dutton, 2014). Members of *Aspergillus spp,* the taxonomy which is complex and continuously evolving, are characterized by vesicle-bearing conidiospores. Although, the extent of aflatoxin toxicity varies depending on the food commodities involved, the Aspergillus are widespread in nature, and can cause contamination before harvest and during storage of food, (Lunyasunya *et al.,* 2016). Aflatoxin contaminates a wide range of agricultural food commodities, including groundnut, sorghum, millet, peas, cassava, and maize, with the latter being the most significantly contaminated (Morgan and Lee, 2018). Food contamination by aflatoxins poses a high risk to human health including acute aflatoxicosis, hepatocellular carcinoma, hepatitis B virus infection and growth impairment (Krishnamachari *et al.,* 2016). Commodities such as Brazil nuts, pistachio, copra and coconut are highly prone to contamination by aflatoxin (Bhat, 2011). Uncontrolled use of pesticides in crop production has been also linked to this contamination (Usman *et al.,* 2018; Usman *et al.,* 2024a).

Nigeria experienced high recorded aflatoxin exposure levels in humans and has also reported the highest estimated number of cases of Hepatocellular Carcinoma (HCC-liver cancer) attributable to aflatoxin in the world (Liu and Wu, 2010). According to Makun *et al.* (2010), the presence of aflatoxins in tiger nut (*cyperus escculentus*), millet, and rice, has been well established. Other food commodities identified with same problem also include solved bush mango seeds (Adebayo *et al.,* 2006), marketed pawpaw fruit (Baiyewu *et al.,* 2007), groundnut product (Williams *et al.,* 2004) and herbal drug plants stored for sale (Hartwell, 2002). Indeed, an Indian study

detected aflatoxin in a range of green leafy vegetables (Rao, 2017). Aflatoxin was detected in 5 of 9 cabbage samples, with concentration in the range 0.4-26.0μg/kg, and in 6 of 9 spinach samples with concentration in the range 0.9-15.3 μg/kg. Also, in one of the studies conducted in Turkey, aflatoxin was detected in 6 of 11 samples of dried vegetables (LOD=1 μg/kg) (Hertog *et al.,* 2013). Likewise, another study detected aflatoxin in 1 of 3 egg samples at a concentration of 0.73 μg/kg (Chu *et al.,* 2011). Aflatoxin was also detected in all of 15 samples of tea (LOD=1.7 μg/kg for total aflatoxin) from another study conducted in Istanbul, Turkey (Hertog *et al.,* 2013).

Toxigenic fungi and mycotoxins were reported in various foods and feedstuffs in many regions of Nigeria. Indeed, there are not less than forty five (45) fungal genera, and twenty (20) different mycotoxins, which have been detected in food commodities in Nigeria. It was reported that the animal mycotoxicosis involved pecking, ducklings, and horses, while the human mycotoxicosis involved primary school pupils more especially those consuming groundnut cake ("kulikuli'') as noted in Ibadan (Akano and Atanda, 2004). Osho *et al.* (2020) reported the isolation of *Aspergillus sp* and *Rhizopus sp.* in poultry feed in the southwestern region of Nigeria. Similarly, in a survey of fungal contamination in commercial poultry feed in the southern state of Nigeria, it was reported that 57-62% of the chick and broiler starter, grower, broiler finishers and layers rations were contaminated with aflatoxin (Akano and Atanda, 2004). In Oweri Imo State and Umuahia Abia State, pathogenic bacteria and fungi genera, were observed in the poultry feeds (Obi and Ozugbo, 2020). These studies entail that poultry feeds and food commodities must be assess to ensure food nutrition and health awareness to the public. This is because many of the infertile men in Nigeria are said to have high aflatoxin levels in their blood, and semen far above the permissible limits. Therefore, the production of poultry feeds for local and commercial farmers in Nigeria requires an average microbiological safety regulation to help ensure free microbial contamination (Obi and Ozugbo, 2020). This is important because of the high levels of aflatoxin B1 (AFB1; max: 6738μg.kg^{-1}), Fumonisin B1 (FB1; max: 10,447 μg.kg^{-1}), and Zearalenone (ZEN, max: 2044 μg.kg^{-1}), both indicated a contamination in stored grains of about 67%, 93%, and 17%, respectively (Adebayo *et al.,* 2006).

Aflatoxin exposure to humans and animals is through the consumption of fungal-contaminated foods and feeds, which poses serious hazards to humans and animals health (Uwaezuoke and Ogbulie, 2008). Acute or chronic aflatoxicosis in poultry birds result in decrease eggs production, immunosuppressant and hepatotoxicoses (Khan, 2016). Also, the ability of fungi to grow on dry surfaces can lead to serious threats to human, and animal health by causing various complications such as hepatotoxicity, immunotoxicity, and teratogenicity (Schnürer *et al.,* 2019). Chronic dietary exposure to aflatoxin is a major risk factor for hepatocellular carcinoma, especially areas where hepatitis B virus infestation is endemic. High level of aflatoxin exposure has been shown to cause acute aflatoxicosis which manifest as hepatotoxicity. A severe case of acute aflatoxicosis causes fulminant liver failure, kidney and heart (Makun *et al.,* 2010). The symptoms of acute aflatoxicosis include oedema, haemarrhagic necrosis of the liver and profound lethargy; however, the chronic effects include immunosuppression, growth retardation, and cancer. Human susceptibility to aflatoxicosis varies with age, health level, and duration of exposure. Children are particularly affected by aflatoxin exposure, which leads to stunted growth and delayed development (WHO, 2024).

Mycotoxins are fungal metabolites that can contaminate agricultural products and threaten food safety. Consumption of food contaminated with aflatoxin will produced pathogenic signs, which might lead to the damage of body tissue of host (WHO, 2024). The fungal pathogens mainly target the respiratory and nervous system of poultry and causes specific pathological changes in the host. Normally, the high incidences of aflatoxin contamination probably cause food spoilage, and biodeterioration that are capable of producing different mycotoxins in Nigeria. The consumption of food contaminated with aflatoxins, also caused chronic and acute aflatoxicoses that might result in loss of livestock (Khan, 2016). Fungal infections in crop production could lead to lower yields, nutrient losses, alteration of organoleptic properties, and decreased products shelf life. As such, severe disease outbreak is likely to occur in young birds because of inhaling high number of the spores in the affected crop farms. These fungal infections posses high risks to human health, and could lead to acute aflatoxicosis, hepatocellular carcinoma, hepatitis B virus infection, and growth impairment.

Chronic aflatoxicosis in poultry birds result in decrease eggs, meat production, immunosuppressant, and hepatoxicoses (Khan, 2016). Exposure of poultry birds to aflatoxin could results in several delibilitating effects that may extend to the overall well-being of the consumer because of the consumption of poultry eggs affected by aflatoxin. Aflatoxin in birds such as AFB1 could also leads to inhibition of transcription, and translation processes, which normally occur when attach with DNA, and RNA molecules through mutation (Evan *et al.,* 2015). However, as a result of consumption of contaminated feed with afaltoxin today Hepatocellular Carcinoma (HCC) is common and is the 6th most common cancer worldwide (Eaton, 2014). The histological studies, confirmed that the higher HCC cases, are common in different parts of Nigeria, and include 20.3% of liver biopsies in Jos, 33% in Lagos, and 27% in Kano (CAST, 2011). Moreover, fungi and their toxins cause obvious reduction in crop and animal livestock productions, and disease in human in different parts of northern Nigeria. For example, the groundnut case study in Sokoto, which became contaminated because of improper

harvesting, handling, transport, storage system and processing method (Reddy and Farid, 2010). Other cases were also reported in Kaduna where poultry feeds affected because the majority of grains used in feed production have been contaminated.

Poultry is an important economic business, and poultry meets are widely consumed by many people in Sokoto. However, the quality and health standard of the production sites/farms, required regular sanitation and routine management. This will help reduce contamination and ensure healthy eggs, and meet productions. Therefore, any study that will focus on the investigation of the level of aflatoxin, and contaminants is crucial. This will help ensure safe feeds for birds' consumption, and public that patronize poultry establishments through consumption of their product (Evan *et al.*, 2015). This has also entails the need for regular awareness among the feeds' producers, marketers and sellers (layers) in the region. This awareness, will guide the general public of the complex issues and health risks regarding the aflatoxins in food commodities, animal feeds and poultry products. Thus, detection of aflatoxins in poultry feeds as considered by this study is important because of the need to compare the levels of contamination and suggest some recommendations to feeds' producers, marketers and sellers in Nigeria. This study aims to determine the fungal profile, and aflatoxin producing potential of some *Aspergillus spp.* isolated from poultry feed (layers). The objectives are: (a) to determine the presence of aflatoxin in the feed samples, (b) isolate and identify fungal flora associated with the feed samples, (c) screen the fungal isolates for aflatoxin production, and (d) identify the volatile organic compound profile of the feed samples.

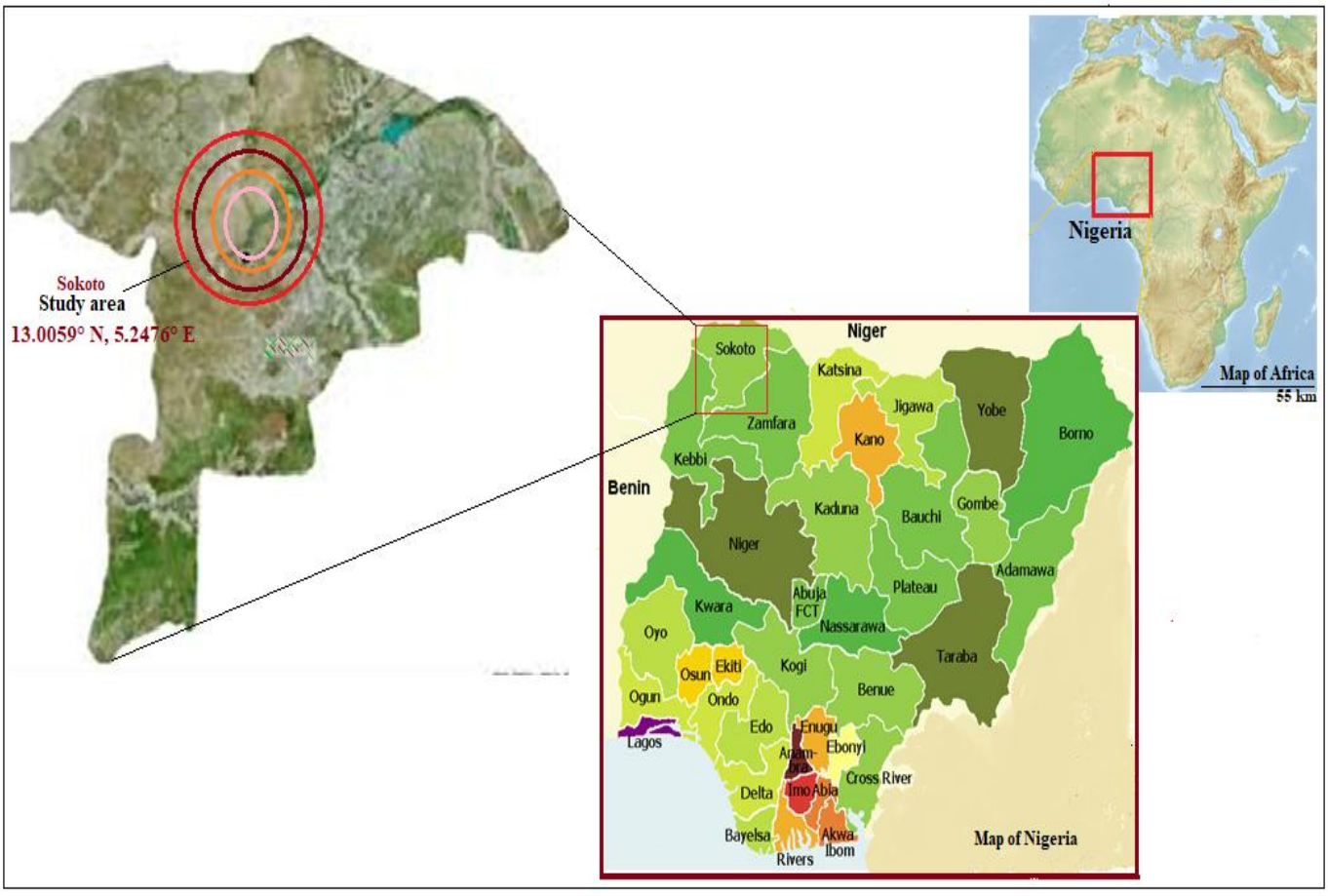

Figure 1: Map of the study area

2. MATERIALS AND METHODS

Study Area

Sokoto State is located in the northern part of Nigeria, dominated by Hausa and Fulani ethnic groups (Usman, 2007). The average annual rainfall is between 550-1300 mm, and a relative humidity of less than 20%. Temperature is between 30°C to 45°C (Salau *et al.*, 2016). Sokoto is geographical mapped on the coordinates of 13.005873 latitude and 5.247552 longitudes, located at near the Rima Rivers of northern Nigeria (Figure 1). Majority of the people living in the study location are Muslims farmers who greatly depend on

agriculture, business, and cattle rearing (Usman, 2007; Usman *et al.*, 2016). The two important agricultural lands in the state are dryland and fadama, which typically support varieties of crops under rainy and irrigation cropping systems (Usman, 2016; Usman *et al.*, 2024b; Usman and Jayeoba, 2025). Soil erosion and nutrient depletion are some of the key agricultural problems in the region (Usman *et al.*, 2017; Usman *et al.*, 2024c; Usman, 2026).

Sample Collection

Commercial poultry feeds (layers) were collected from four (4) different location within Sokoto metropolis: Masallacin Bafarawa, Mabera, Kofar Atiku and Arkilla. For each of the four sample locations, twenty grams (20g) of the feed were collected using sterile polythene bags. The samples were labelled indicating the sampling point, date of sample collection and sample weight. The samples were transported to the Usumanu Danfodio University Sokoto laboratory in an ice box for mycological analysis.

Analysis of Layers Feed Samples for Aflatoxin

Five (5) grams of the feed samples were weighed using an electric balance and transferred into separate 250ml conical flasks containing 70% ethanol. The mixture (sample + ethanol) was left to stand for 10 minutes, after which the samples filtered using Whatman filter paper (No 1). An aliquot (50µl) of the aflatoxin standard in separate dilution wells (green-bordered well), was dispense and mixed with 100µl of conjugate. Hunded microlitre (100µl) of the filtrate/standard-conjugate was mixed with antibody coated wells. The mixture was incubated at 25^{0}C for 15 minutes. The content of wells was discarded, and cleaned with distilled water/phosphate buffer saline. This was done 3 -4 times. Afterward, 100µl of substrate was added to each well, and incubated for 5 minutes to allow for color change (different shades of blue to colorless). However, stop solution (100µl) was then added. This converts the blue endpoint to yellow. The reading was then added at 450nm, using an ELISA plate reader. Also, the optical densities of standards (0ppb, 4ppb, 10ppb, 20ppb and 40ppb), were recorded. The actual aflatoxin concentration in (parts per billion) contained in a sample, the concentration obtained, was multiplied further, by the corresponding dilution factor. Total aflatoxin levels for finished feed should not exceed 20ppb.

Volatile Compounds Analysis of Layers Feed Using GC-MS

The GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with a flame ionization detector (FID). The injection was conducted in splitless mode at 250 °C for 3 minutes by using an inlet of 0.75 mm i.d to minimize peak broadening. Chromatography separations were performed using DB-WAX analytical column 30 m 0.25 mm, 0.25 mm (J&W scientific, Folsom, CA) with helium as carrier gas at a constant flow rate of 0.8ml/min. The oven temperature was programmed at 60 °C for 5 min, followed by an increase (held for 5 minutes), and finally at 10°C/min to 280 °C (held for 10 min). The temperature of the FID was set to 250 °C. The MS conditions (electron impact ionization mode) operated on a temperature of 200°C, ionization voltage of 70 eV, and mass scan range of m/z 23 to 450 at 2.76 scans/s (Ibrahim *et al.*, 2011).

Identification and Quantification of Volatile Compounds

The chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds, which are from the NIST library mass spectra database. This was done based on the basis of the criterion similarity (SI) >800 with the highest value of 1000. However, an approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software called Vienna, VA, and the data were presented as the peak area normalized (%).

Media Used

The Potato Dextrose Agar PDA (200g) and Potato Carrot Agar PCA (200g) are used for the isolation of fungi from layer feed samples. The Czapek Dox Agar CDA (27.05g) was considered for sub-culturing. Neutral Red Desiccated Coconut Agar NRDCA (100g), and the Phenol Red Desiccated Coconut Agar PRDCA (100g) for screening of aflatoxin production. In comparison, Yeast Extract Sucrose Agar YESA (9.4g) was used for detecting their toxigenic capabilities.

Isolation of Fungal species from Layers Feed Sample

Sample Processing: Ten grams (10g) of the layer feed was transferred into 90ml of Sterile Distilled Water (SDW) containing 0.5% Tween 80. The sample mixture was homogenized using a blender for 2 minutes, and the homogenate was transferred into a sterile 250ml conical flask and stored in the refrigerator for further analysis.

Serial Dilution: Serial dilution was made using twelve (12) test tubes. Three test-tubes containing 9ml of sterile distilled water were used for all the samples. However, 1ml from sample homogenate using sterile syringe/pipette was introduced into the 1st and 2nd test tube 10^{-1}, consequently. Likewise, another 1ml of sterile distilled water was transferred from the second test tube to the third 10^{-3}.

Plating: An aliquot of 0.1ml from 10^{-2} and 10^{-3} dilution was aseptically inoculated into duplicate plates of (PDA) and (PCA). A smear was done in all the inoculated plates using bent glass. Control plates containing both PDA and PCA were left un-inoculated and all plates incubated at a room temperature for 2-5 days. The fungal colonies observed on the plates were counted to determine the percentage frequency of occurrences of the isolates. The fungal isolates were subcultured into PDA slants and stored at 4^0C for further analysis.

Identification of Fungal Isolates

The fungal isolates were identified using Czapek Dox Agar (CDA) for macroscopic identification, while the microscopic features were identified using slide culture techniques. However, for the slide culture using sterile petridish, a sterile filter paper was placed in a sterile U-shaped glass rod, and placed on a sterile filter paper. Afterward, about 4mls of sterile water were poured on sterile filter paper to moisten it, completely. This was done using forceps sterile slide which placed on the upper end of U-shaped rod. Gently, a knife was flamed, and used to cut a 5mm square block of the medium from the plate of Potatoes Dextrose Agar medium, and the medium was picked up by inserting knife, and carefully the block was transferred to the centre of the slide. An inoculation was done on four sides of the medium with spores or mycelia fragments of the fungus to examined, and a loop before spores was flamed. Aseptically, a sterile cover glass was placed on the upper surface of the agar cube, and was pressed down slightly with the tip of the finger to expel any air bubble, and further disintegrate the hypal growth to enhance observation. The petri dish cover was placed and incubated at room temperature for 7 days. The slides were observed under X40 of a compound microscope every day. Microscopic characteristics of fungi such as hyphae, conidial heads and arrangements of conidia, were observed, accordingly (Atlas and Parks, 2011).

Screening of Fungal Isolates for Aflatoxin Production

The screening of fungal isolates for aflatoxin production potential was carried on duplicate plates of Neutral Red Desiccated Coconut Agar (NRDCA) and Phenol Red Desiccated Coconut Agar (PRDCA). The isolates have been screened for aflatoxin production out on (NRDCA) and (PRDCA) media. A single-spore from isolates were inoculated on Neutral Desiccated Coconut Agar (NRDCA) and Phenol Red Desiccated Agar (PRDCA), and incubated at temperature (31^0C), pH (4.8±2) was adjusted using pH meter. Aflatoxin-producing colonies have been detected under long-wave UV light (365mm) by blue fluorescence on the reverse side after 2 to 5 days of growth. Aflatoxin production was verified by chemical analysis by adding 2 to 4 drops of NH4OH using Yeast Extract Sucrose Agar (YESA) and the result was observed.

3. RESULTS

Aflatoxin Contents of the Feed Samples

Aflatoxin was detected in all the feed samples, except sample from Kofar Atiku which shows no presence of aflatoxin. The aflatoxin content of each of the samples in ascending order is as follows: Masallacin Bafarawa (275.0µg/kg) followed by Mabera (310.0µg/kg) and the highest was from Arkilla (400.0 µg/kg) (Table 1), and the result obtained is consistent with previous literature report of Rai *et al.* (2019).

Table 1. Aflatoxin content detected in layer feed sample

Sample labeled	Sample location	Total aflatoxin (µg/kg)
A	Masallacin Bafarawa	275.0
B	Mabera	310.0
C	Kofar Atiku	ND
D	Arkilla	400.0

ND = Not Detected

Volatile Organic Profile of the Feed Samples

The VOCs identified with Masallacin Bafarawa Cis-8-methyl-exo-tricylo [5.2.1.0(2.6) deconic acid (4.49%), Mabera; 2,6 Diethylnitrosobenzene (4.44%) and Arkilla 1,2-Benzenedicarboxylic acid, diisooctyl ether (4.06%) had the highest abundance, whereas Kofar Atiku Alpha.-D-Glucopyranoside 1,3,4,6-tetrakis had the least abundance of (3.46%), and these result is similar with previous report of Lavine et al. (2017).

Table 2. Containing volatile organic compound profile of layer feed sample (Masallacin Bafarawa) obtained following GC-MS analysis

RT^{-1} (min)	Volatile organic compounds	Abundance (%)
3.961	Tetrasiloxane, decamethyl	1.46
4.107	D-(-)-Lactic acid, trimethylsilyl ether, trimethyl	1.80
4.679	Tris(trimethylsilyl)amine	1.74
5.192	Pentasiloxane, dodecamethyl	1.74
5.400	Propanedioic acid, bis(trimethylsilyl) ester	0.64
5.741	Linalool, trimethyl ether	1.41
6.133	Xylulose tetrakis(trimethylsilyl)	1.66
6.451	Butanedioic acid, bis(trimethylsilyl) ester	1.41
8.448	Butanedioic acid, [(trimethylsilyl)oxyl]-, bis	1.90
8.832	Fructose oxime, hexakis(trimethylsilyl)	1.92
9.880	Diethyl Phthalate	3.03
10.626	Dodecanoic acid, trimethylsilyl ester	1.89
13.076	Tetradecanoic acid, trimethylsilyl ester	2.01
13.921	D-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)	1.97
14.252	n-Decanoic acid	1.51
14.363	Alpha.-D-Galactofuranose, 1,2,3,5,6-pentakis	2.62
14.452	Glucose oxime hexakis(trimethysilyl)	1.76
14.634	Palmitic acid, ethyl ester	1.53
14.869	D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)	1.71
15.187	Palmitic acid, trimethylsilyl ester	2.01
16.010	Octadec-9-enoic acid	2.16
16.303	Cis-8-methyl-exo-tricyclo[5.2.1.0(2.6)]decanoic acid	4.49
16.828	Oleic acid, trimethysilyl ester	2.19
17.048	Octadecanoic acid, trimethylsilyl ester	1.95
18.175	2-Methyl-6-hepten-3-o	2.58
18.545	Dodecanedioic acid, bis(trimethylsilyl) ester	3.12

Table 3. The table containing volatile organic compound profile of layer feed sample (Mabera) obtained following GC-MS analysis

RT^{-1} (min)	Volatile organic compounds	Abundance (%)
3.465	Glycerin 1,2,3-Propanetriol	2.57
4.034	Oxetane, 3,3-dimethyl- propane	1.22
4.219	Propanoic acid, 2 methyl-, anhydride	1.43
4.445	Furancarboxylic acid, 3-fluorophenyl ester	1.12
4.673	Silanamine, 1,1,1-trimethyl-N-N-bis[2-[(trimethylsilyl]	2.44
4.774	2,4-Azetidinedione, 3,3-diethyl	1.22
4.949	Butanoic acid, 2-ethyl-, butyl ester	2.55
5.059	Methylbutanoic anhydride	0.91
5.660	2-Furancarboxaldehyde, 5(hydroxymethyl)	1.37
5.720	Pentanoic acid, 2-(aminooxy)	1.43
6.134	Trimethysilyl ether of glycerol	1.45

6.442	Cyclohexane, 1-bromo-3-metthyl-	1.95
7.879	2,6-Diethylnitrosobenzene	4.44
8.227	Dodecanoic acid	2.29
9.150	N-Acetylethylenediamine	4.35
9.268	Nonanoic acid	1.49
9.873	1,3-Dioxane-4,6-dione, 5,5-dimethyl-2-(1-methylethylidene	3.58
11.940	Pentanoic acid, 2-hydroxy-, ethyl ester	1.10
13.326	Phthalic acid, cyclobutyl tridecyl ester	1.86
14.263	1-(+)-Ascorbic acid 2,6-dihexadecanoate	2.19
14.632	Heptanoic acid, 2-ethyl-	1.24
16.071	6-Octadecenoic acid	2.72
16.350	7-Tetradecyne	2.07
16.461	2-Octanol, 2,6-dimethyl-	2.18
18.085	9-Octadecenamide	2.48

Table 4. Containing volatile organic compound profile layer feed sample (Kofar Atiku) obtained following GC-MS analysis.

RT^{-1} (min)	**Volatile organic compounds**	**Abundance (%)**
4.461	Cyclotrisiloxane, hexamethyl	2.43
4.657	Tris(trimethylsilyl)amine $$	1.68
9.881	Diethyl Phthalate	3.06
13.326	4-Ethylformanilide	1.93
14.252	n-Capric acid	1.54
14.357	4-Ethylformanilide	1.91
16.005	Oleyl alcohol, trifluoracetate	2.02
16.301	3-Heptyne, 5-methyl	1.60
16.488	2-(4'-Trimethylsilyloxyphenyl)-2-(3'meth	3.20
16.822	Silane, trimethyl(1-methylbutoxyl)-$	1.55
18.508	Alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis	3.46

Table 5. Containing volatile organic compound profile of layer feed sample (Arkilla) obtained following GC-MS analysis

RT^{-1} (min)	**Volatile organic compounds**	**Abundance (%)**
4.675	Tris(trimethylsilyl)amine	2.27
6.132	Trimethylsilyl ether of glycerol	1.56
9.295	Dodecanoic acid	2.49
9.794	Diethyl Phthalates	4.04
11.955	Myristic acid $$	1.89
12.920	Cyclopropene, 3,3-diphenyl-1-trimethylsilyl	2.54
13.317	Phthalic acid, cyclobutyl isobutyl ester	1.89
13.798	SS)-or (RR)-2,3-hexanediol	1.65
13.918	D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)	1.76
14.296	1-(+)-Ascorbic acid 2,6-dihexadecanoate	3.55
14.451	Galactose oxime hexakis (trimethysilyl	1.65
14.631	Palmitic acid, ethyl ester	1.73
14.865	Alpha.-D-Galactopyranose, 1,2,3,4,6-pentakis	1.72
15.528	n-Nonadecanol	1.91
15.810	1,2-Benzenedicarboxylic acid, diisooctyl ether	4.06
16.130	Octadec-9-enoic acid	3.36
16.300	Butyl 9,12-octadecadienoate	3.02

16.356	Oleic acid, ethyl ester	2.53
17.509	Silacyclopent-3one, 3-methyl	1.54
17.669	2-Cyclopenten-1-, 3-methyl	2.72
17.858	Octanenitrile	3.23
18.054	Eicosanoic aci	3.33

Fungal Genera Isolated From the feed samples

The fungal genera isolated from each location are: Masallacin Bafarawa; *Aspergillus flavus*, Mabera; *Aspergillus flavus, Aspergillus parasiticus*, Kofar Atiku;*Aspergillus niger, Aspergillus fumigatus, Aspergillus clavatus* and *Aspergillus oryzae* and Arkilla; *Aspergillus parasiticus*. Of which the sample from Kofar Atiku had the highest genera of *Aspergillus spp*. followed by Mabera and the least was from Masallacin Bafarawa and Arkilla which had only one genera of *Aspergillus spp.*

Frequency and Percentage Occurrences of Fungal Isolate

The frequency of fungal isolate from each location were identified, Masallacin Bafarawa: *Aspergillus flavus* 7(70.0) had the highest frequency, followed by Arkilla: *Aspergillus parasiticus* 5(62.5%), Mabera: *Aspergillus parasiticus* 3 (37.5), *Aspergillus flavus* 3 (30.0), while Kofar Atiku: *Aspergillus niger* 2 (100), *Aspergillus fumigatus* 2 (100), *Aspergillus clavatus* 2 (100) *Aspergillus oryzae* 2(100), had the least frequency of isolation (Table 6) and the result is consistent with reference to Brown (2005).

Table 6. Frequency of isolation and percentage occurrences of *Aspergillus spp.* in layer feed sample

Location	*A. flavus*	*A. parasiticus*	*A. niger*	*A. fumigates*	*A. clavatus*	*A. oryzae*
Masallacin Bafarawa	7 (70.0)	0 (0.0)	0 (0.0)	0(0.0)	0(0.0)	0 0.0)
Mabera	3 (30.0)	3 (37.5)	0 (0.00)	0(0.0)	0(0.0)	0(0.0)
Kofar Atiku	0 (0.0)	0 (0.0)	2 (1.0)	2(1.0)	2(100)	2(100)
Arkilla	0 (0.0)	5 (62.5)	0 (0.00)	0(0.0)	0(0.0)	0(0.0)
Total	10 (100)	8 (100)	2 (100)	2 (100)	2 (100)	2 (100)

Screening of toxigenic strains of fungi

Using UV under long fluorescence (365nm) the *Aspergillus spp* were screened: *Aspergillus parasiticus* 8 (87.5%) had the highest percentage of positive strains, followed by *Aspergillus flavus* (50.0%), and *Aspergillus niger* 2 (50.0%), which had the least percentage of positive strains, while *Aspergillus fumigatus, Aspergillus clavatus* and *A. Oryza* shows ND (Not Detected), (Table 7). *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus niger* were found to be positive for aflatoxin production, while *Aspergillus fumigatus, Aspergillus clavatus* and *A. oryzae* were negative for aflatoxin production (Figure 2). The toxigenic capabilities of fungal isolate have been screened using NH_4OH, and shows that *Aspergillus flavus* changes from green to yellow, *Aspergillus parasiticus* from yellow-green to yellow, *Aspergillus oryzae* from green to pale-yellow, while *Aspergillus niger* from black to red (Table 8). However, the *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger,* and *Aspergillus oryzae* were positive, whereas *Aspergillus fumigates,* and *Aspergillus clavatus* were negative (Figures 3, 4, 5).

Table 7. Screening of toxigenic fungi in layer feed in Sokoto metropolis

Name	Number of strains	Number of toxins	Percentage of fungus screened producing strains positive strains
Aspergillus flavus	10	5	50.0
Aspergillus fumigatus	2	ND	---
Aspergillus parasiticus	8	7	87.5
Aspergillus niger	2	1	50.0
Aspergillus clavatus	2	ND	---
Aspergillus oryzae	2	ND	---
Total	26	13	187.5%

ND = Not Detected

Table 8. Screening of toxigenic capabilities of fungi in layer feed sample using (YESA)

Name of isolate	Surface color	Reverse color	Positive/ Negative results
A. flavus	Green	Yellow	+
A. fumigates	Suede	ND	_
A. flavus	Grey	Yellow	+
A. parasiticus	Green	Yellow	+
A. oryzae	Green	Pale yellow	+
A. niger	Black	Red	+
A. clavatus	Sandy	ND	_
A. parasiticus	Green	Yellow	+

+ = Positive, – = Negative

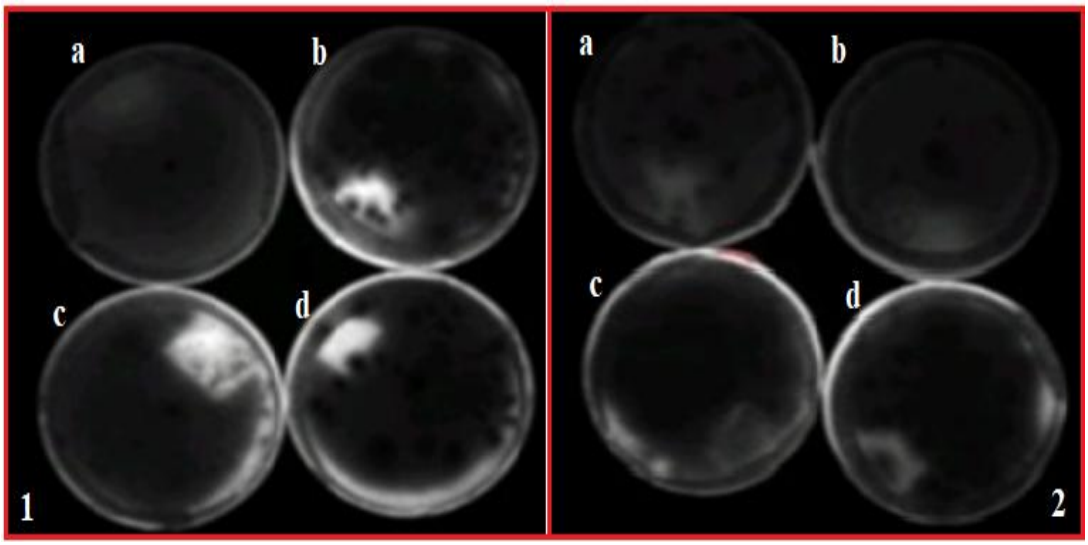

Figure 2. Screening of toxigenic potential of different fungal isolates from layers feed. **1.** (a) *A. fumigatus*, (b) *A. flavus*, (c) *A. niger*, (d) *A. parasiticus* and **2.** (a) *A. clavatus*, (b) *A. oryzae*, (c) A. flavus, (d) *A. parasiticus*

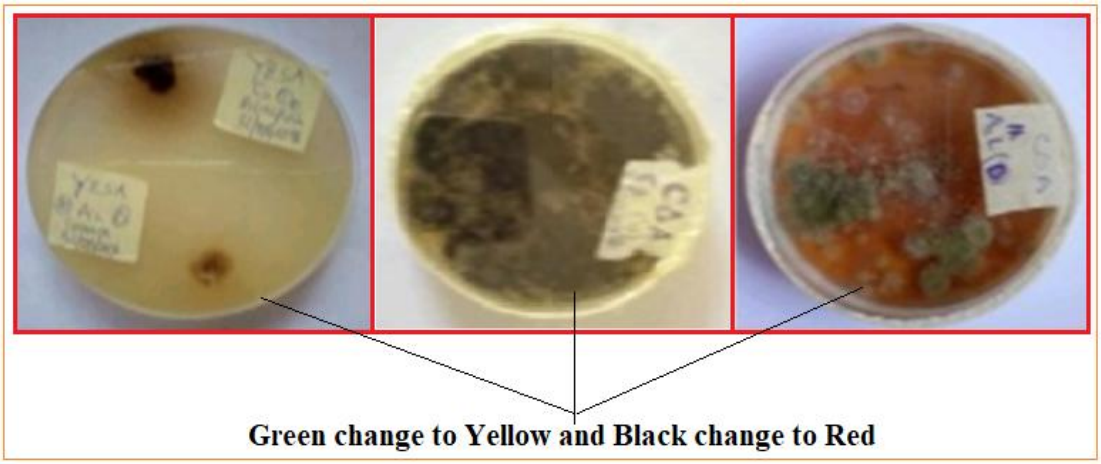

Figure 3. Screening of fungal isolates for their toxigenic capabilities: green change to yellow and black change to red

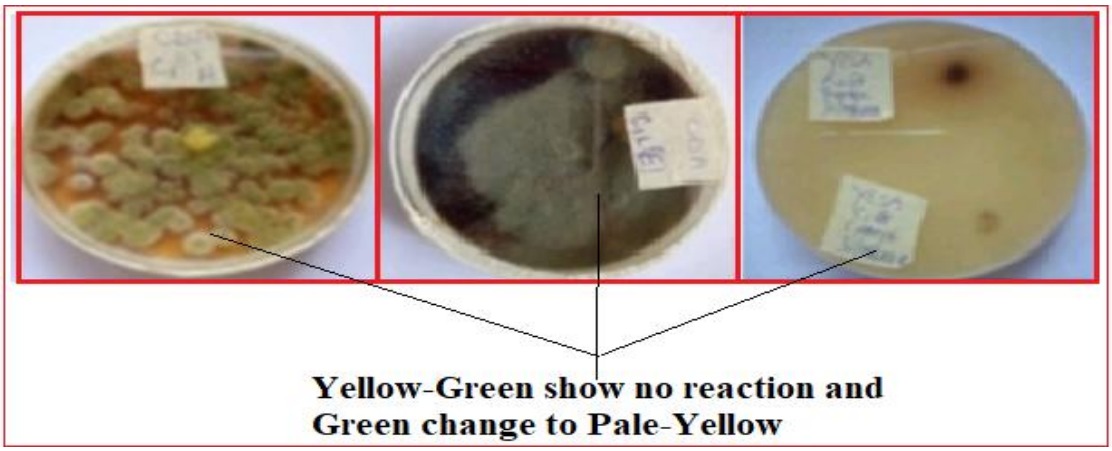

Figure 4. Screening of fungal isolates for their toxigenic capabilities: green fungal change to pale-yelow

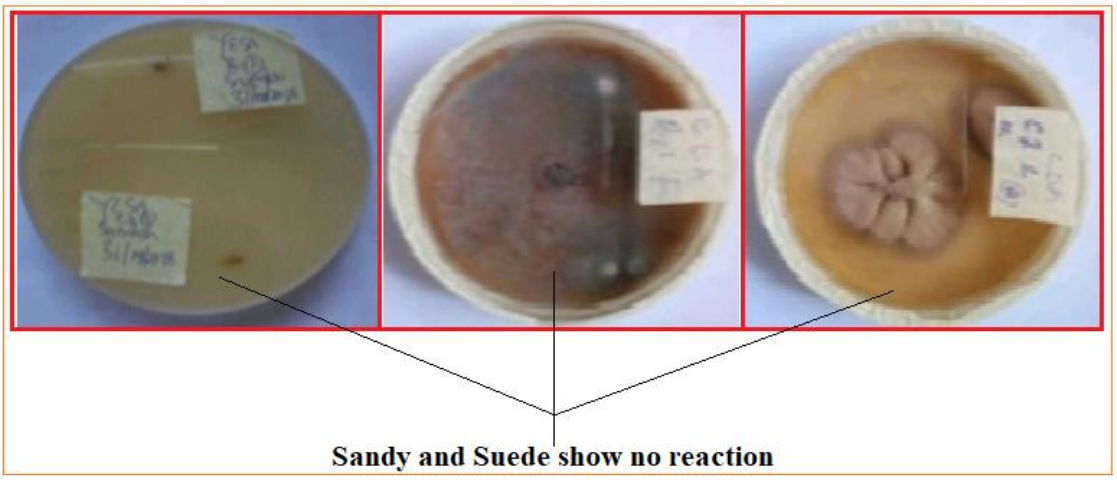

Figure 5. Screening of fungal isolates for their toxigenic capabilities: sandy and suede show no reaction

4. DISCUSSION

This study assessed four (4) feed samples collected from four different locations in Sokoto State Nigeria. The samples contained aflatoxin levels above maximum residue limit 20ppb, except the sample from Kofar Atiku which shows no aflatoxin concentration. The highest concentration was detected from Arkilla (400.0 µg/kg) followed by Mabera (310.0 µg/kg) and the least was from Masallacin Bafarawa (275.0 µg/kg). The findings accorded very well with the observation made by Ngindu *et al.* (2013) in his study of aflatoxin levels in maize and maize products in Kenya. Similar report by Lewis *et al.* (2005), also noted that 55% of maize products sampled during 2004 aflatoxicosis outbreak, had aflatoxins level greater than the Kenya regulatory limit of 20 ppb. According to Lunyasunya *et al.* (2016) out of the 342 maize samples studied in Makueni and Kitui County, a total of 182 (53.2%) had aflatoxin level above the recommended maximum limit of 20ppb. These studies tallied very well with the results recorded from Arkilla, Mabera and Masallacin Bafarawa areas of Sokoto State Nigeria (Table 8).

The groundnuts contained higher levels of aflatoxin than maize and rice as reported in this study, is in consistent with findings of Bankole and Eseigbe (2004) from other part of Nigeria. They analyzed 106 dry roasted groundnuts of 500g each for moisture content and aflatoxins, and noted that most samples were contaminated with aflatoxins. The high mean level of aflatoxin in feeds, and the poor storage practices among feed sellers in Sokoto, may be attributed to low level of awareness of aflatoxin contamination among feed sellers; although, one third of the sellers are aware of the contamination issues. Furthermore, out of 20 isolates, which have been screened under Ultra Violet (UV) fluorescence (365nm), 7 isolates were found to be positive for aflatoxin production (Table 8). This probably indicates that the storage systems among feed sellers are contrasted; and indeed, can be attributed to their level of awareness of aflatoxin contamination. Therefore, low level of awareness and poor storage practices may have been probably the major contributing factors to aflatoxin contamination among sellers and buyers in the study areas.

The production of aflatoxins is always associated with the production of other metabolites, some of which are volatile. Different VOCs (Volatile Organic Compounds) were identified from various locations as recorded (Tables 4. 5. 6). These volatile metabolites are produced during both primary and secondary metabolism, and are often collectively referred to as microbial volatile organic compounds (MVOCs) (Korpi *et al.,* 2009). MVOCs are widely investigated as the indicator of fungal growth (Ryan *et al.,* 2013), mycotoxins' production (Kuske *et al.,* 2005), and fungal taxonomy (Van lancker *et al.,* 2008). In recent years, metabolomics approaches have been widely used for the investigation of the metabolites of biological samples for identifying biomarkers, which correlate to a disease (Polizzi *et al.,* 2012), drug toxicity (Kaddurah-Daouk *et al.,* 2008; Wishart, 2008) or genetic, and environmental variation (Lin *et al.,* 2006). Fungal VOCs have been used to monitor good and bad food quality (Korpi *et al.,* 2009). As electronic nose technology improves, it is hoped that volatile compound "mapping" can be used to predict the levels of fungi found in agricultural products and perhaps to identify individual species (Schnürer *et al.,* 2009). Mold VOC profiles have also been studied in water-damaged buildings where compounds such as 2-methyl-1-proponol and 3-methyl-1-butanol have been suggested as useful indicators of mold growth (Korpi *et al.,* 2009). Fungus-like odors can be recognized by humans at concentrations greater than 0.035μg/m3. Generally, the background microbial VOC concentrations in mold-free buildings are similar to those found in outdoor air ranging from 2.2 to 8.8 μg/m3.Arabidopsis seeds responded differently to several biogenic C-8 compounds, which are similar in molecular size and masses. Exposure to 1-octen-3-one caused complete inhibition of germination, whereas 1-octanol caused radicles to stop growing as soon as they emerged.

The antagonistic activities of ketones, alcohols and aldehydes on seed germination and seedling formation as noted in this study, have been demonstrated previously by other reports (WHO, 2024). Their studies showed that out of 47 terpenoid compounds tested, 24 inhibited seedlings of about 140 formations in lettuce. In our current observations, once removed from the VOC testing conditions and placed into a fresh plant media, treated *A. thaliana* seeds, were able to complete germination and/or resume vegetative growth without application of a plant hormone. Studies found *M. albus* produced a mixture of volatile acids, alcohols, esters, ketones, and lipids, which individually had inhibitory, but not lethal effects against test species such as *Fusariumsolani*, *Pythium ultimum*, and *Rhizoctonia solani* (e.g. Maren, 2002; Korpi *et al.,* 2009).

The most common *Aspergillus* species observed in our findings, are *A. flavus*, *A. parasiticus*, *A. niger*, *A. fumigatus, A. clavatus,* and *A. oryzae*; these occured mostly because of the improper storage and lack of awareness on aflatoxin produced *Aspergillus spp.* Change of colour on feeds as noted in our findings, could be attributed to the presence of *A.f lavus* and *A. parasiticus* whose colonies on substrate appear yellow–green and yellow respectively. This is consistent with the studies by Maren (2002) who reported 68 conidial areas that A. *flavus* spread yellow-green colonies and *A. parasiticus* dark yellow-green.

The low awareness of sellers on the harmful effects of aflatoxins to human, and animal health, may be an indication of poor public awareness. Currently, few literatures have been published on awareness level of aflatoxins contamination among feed handler. However, much has been reported on its toxicity effects on human and animal health (e.g. Lunyasunya *et al.,* 2016). These studies noted that handling of mould infested feeds, hay stovers, silage and other feeds, may be harmful due to the presence of aflatoxin and actinomycetes, and are responsible for poisoning, and allergy diseases affecting man (farmers' lung disease). This is particularly serious in asthmatics and patients suffering from cystic fibrosis. This emphasis the need for an advanced awareness among farmers, producers, buyers, and general public on the contamination issues with regards to feeds and food items processed and marketing in the study area. This also needs to be considered across the Nigerian regions. Primarily, because Nigeria produces bumper harvests in the farms, but loses a quantum of them during storage or transportation. These bumper harvests have higher tendency of contamination by fungi-infected, and loaded with aflatoxins in the cooking pot and dining table. Also, in many road-side eating places, soups and stews are made from rotten peppers, which the Yoruba call *ata-esa*. Many people buy and eat "injured" or "wounded" banana, but unknown to them, the rot areas on the bananas, are the handiwork of a fungus or fungi.

Toxigenic potential of different fungi isolated, and screamed from layer feed is also important to public health and awareness. Out of 20 screened isolates of *Aspergillus spp.* 7 isolates were found to be positive for aflatoxin production, and 6 were positive for their toxigenic capabilities (Table 8). According to Yunus *et al.* (2011) and Andretta *et al.* (2011), feeds produced must be assessed to reduce the concentration level and improve the feed quality. Therefore, this study entails that the public health and food/feeds quality, remain an important issue which need to be considered for present, and future agricultural development in study area, Nigeria, and sub-Saharan Africa at large.

5. CONCLUSION

The feeds were found to have high mean level of aflatoxin as recommended level by FDA and KEBS (20ppb), except sample from Kofar Atiku which shows negative level of aflatoxin. These feeds are not safe for poultry consumption because the level of aflatoxin is serious, and probably effect poultry health and production. The finding shows that there might be some problems, which could leads to serious human health issues in the study area. This study emphasize that efforts should be made to maintain the level of aflatoxin, which are safe for consumers' consumption. People should avoid eating poultry without a proper treatment so as to be safe from ailments and/ contamination before consumption. There is need for feed processors to maintain food safety in order to minimized contamination by microorganism. This study provides the following recommendation:

a) There is need for better feed storage facilities within markets. The federal and state government should improve the existing building structures within the markets, and give proper guidelines to storage systems.
b) There is need to campaign for seminars and workshops by stockholders such as public health, and ministry of Agriculture. This will help create awareness among feeds' handlers against poor practices that contribute to aflatoxin contamination.
c) There is need for mechanisms of assessing the feeds before getting access to the market and/ public.

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Author Contributions

Samaila Usman designed the study, collected the date, and presented the results. Suleiman Usman reviewed the work, edited and improves the technical quality. Also, Yusuf, AB improved the literature aspect and managed the laboratory analyses.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethical approval & declaration

This work is mainly on soil and soil resources and does not involve any information on humans or animals; thus, an ethical statement is not applicable to the context of the manuscript.

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Data and materials availability

Data was obtained from field and laboratory assessment conducted at Usumanu Danfodiyo University Sokoto. All data are available in the Department of Microbiology, Faculty of Science, Usumanu Danfodiyo University Sokoto, Nigeria. All data associated with this study will be available based on the reasonable request to corresponding author.

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