

Isolation and identification of *Aspergillus* species from poultry feeds in Kaduna State, Nigeria

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ABSTRACT

The majority of grain farmers reside in the northern part of Nigeria where storage facilities used are traditional types or locked up stores without proper ventilation. The aim of this study was to determine the presence of *Aspergillus* species in selected poultry feeds. A total of 180 samples of different feed types (20 broiler starter, 20 broiler super starter, 10 broiler finisher, 50 grower's (chick) mash, 80 layer finisher) were cultured for fungal isolates. In this investigation, Rose Bengal Chloramphenicol agar was used to culture the isolates. The growths were identified using standard mycological techniques. Of the 180 feed samples cultured, 178 (98.9%) yielded a variety of mould such as *A. fumigatus* 134 (75.1%), *A. parasiticus* 64 (35.56%), *A. flavus* 36 (20%), *A. niger* 5 (3%), *A. terreus* 3 (1.7 %). From the results obtained, all the feed samples were contaminated with a number of fungal species. There was no significant ($p > 0.05$) difference in aflatoxin concentration in feeds in the four LGAs in Kaduna metropolis. Conclusively, five different species of *Aspergillus* were isolated and identified from poultry feeds sampled in Kaduna metropolis.

Keywords: Fungi, chicken, feed millers, grains, toxin binders.

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INTRODUCTION

Food is the essence of life as well as its safety, yet the majority of people give little thought to ensure that food is safe to eat. When food safety issues are raised, it is normally the perceived problems of pesticides or other man-made chemicals. However, natural toxins produced by a range of microbes, are potent toxins and carcinogens and therefore of equal or a greater threat to food safety as manmade chemicals (Shepherd, 2008).

Food safety is an imperative in food production worldwide. Poultry meat, eggs, and poultry products derived from them are crucial in safe food chain. As far as safety is concerned, special attention is directed towards possible contamination of food and poultry feed with fungi and to the risk of mycotoxin contamination (Radmila et al., 2009).

From time to time families bring out their grains to clean and if the grains change colour or smell due to poor storage, they are sold to especially, toll poultry feed

millers either as whole grains or ground fine particles, at very cheap rates.

In poultry production, feed is the key vector for introducing mycotoxins into flocks. Other routes of exposure include litter.

Moulds are ubiquitous in nature and universally found where environmental conditions are conducive to mould growth. Because moulds are present in soil and plant debris, and are spread by wind currents, insects, and rain, they are frequently found in/on foods together with their associated mycotoxins (Chekowski and Vinsconti, 1992). Mycotoxins are toxic secondary metabolites produced by moulds, that is, metabolites not essential to the normal functioning of the cells. Mycotoxins are produced by a wide variety of diverse fungal species that generally are not aggressive pathogens. They are adapted for colonization and growth on substrates with a wide range of moisture availability and nutritional content.

Most of the mycotoxins that are considered to be important are produced primarily by three genera of fungi, namely, *Aspergillus*, *Penicillium* and *Fusarium*. *Claviceps* and *Stachybotrys* are also important producers of mycotoxins (CAST, 2003). The toxic effects of mycotoxins on animal and human health is referred to as mycotoxicoses, the severity of which depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed to (Negedu et al., 2011).

Aspergillus section *Flavi* includes *A. flavus*, *A. parasiticus* and *A. nomius* species that produce aflatoxins, potent carcinogenic compounds of concern in food safety. These toxic secondary metabolites are a group of structurally related difuranocoumarins. Aflatoxins detected as natural contaminants in foods are named B1, B2, G1 and G2 based on their fluorescence under UV light (Blue or Green) and relative chromatographic mobility. Aflatoxin B1 is the most potent natural hepatocarcinogen known and is usually the major aflatoxin produced by toxigenic strains. Aflatoxins can be produced by several species belonging to this section of the *Aspergillus* genus but the most important aflatoxin producers occurring naturally in food commodities are *A. flavus*, *A. parasiticus* and, to a lesser extent, *A. nomius* (Astoreca et al., 2011).

Fungi in *Aspergillus* section *Flavi* exist in complex communities composed of individuals that vary widely in their aflatoxin-producing ability. Toxigenicity profiles, the ability to produce type B and G aflatoxins and cyclopiazonic acid (CPA), have been used for identification purposes as criteria complementary to morphological and genetic characteristics of these closely related *Aspergillus* species (Rodrigues et al., 2009).

Fungal toxins produce a wide range of injurious effects in animals, in addition to posing foodborne hazards to humans. The economic impact of decreased productivity, subtle but chronic damage to vital organs and tissues, increased disease incidence because of immune suppression, and interference with reproductive capacity is many times greater than that of acute livestock death (CAST, 2003).

Mycotoxins cause illness and mortality in domestic animals fed mouldy feedstuffs. These acute intoxications can have devastating effects and are difficult to diagnose and treat because the suspect feed may be consumed before it can be tested (CAST, 2003). It has been estimated that more than five billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shepherd, 2005). The primary disease associated with aflatoxin intake is hepatocellular carcinoma (HCC, or liver cancer). The disease is the third-leading cause of cancer death globally according to WHO (2008), with about 550,000 to 600,000 new cases each year. Eighty-three percent of these deaths occur in East Asia and sub-Saharan Africa

(Parkin et al., 2002; Kirk et al., 2006). Liver cancer has an increasing incidence that parallels the rise in chronic hepatitis B (HBV) and hepatitis C (HCV) infection (Liu and Wu, 2010). Among the most potent hepatocarcinogenic agents known is aflatoxin.

Regrettably, many of the people in the developing countries are not even aware of the adverse and detrimental effects of consuming mouldy products. Due to the poor education levels and other socio-economic factors, even if steps are taken to make food products safe, the consumers will be unwilling to pay the extra costs, and will still prefer to buy cheap commodities (Bennett and Klich, 2003).

It is of concern that little or no effort has been made to study mycotoxins in food value chain in Nigeria, considering the public health implication. It is expected that this research will directly translate to a better understanding of the extent of contamination of commercial feeds with various species of mycotoxin producing fungi.

The objective of this study was to isolate and identify *Aspergillus* species in poultry feeds in Kaduna Metropolis, Nigeria.

MATERIALS AND METHODS

Sampling of feeds

A total of 180 samples of different types of poultry feeds (broiler finisher, broiler starter, broiler super starter, grower's mash, layers top mash) were collected and analysed from two Toll, two Custom and two Integrated feed millers. The sample size was determined using an online calculator (HyLown, USA) standard deviation of 0.5, confidence level of 95% and confidence interval (margin of error) of ± 7.3 %. Ten grams representative of each feed type were analysed twice a month for 3 months. Samples were collected randomly from feeding troughs, feeders and sacs and during milling biweekly for three months from farms, Custom, Toll and Integrated poultry feed millers. Sampling was performed manually from 8 points of bags of feed weighing 25 kg using a probing pattern (USDA, 1975) such that the product was collected from different parts of the sacs and different locations in the lot. The sample was pooled and mixed properly and then 10 g subsample was taken for further analysis.

Processing of feeds

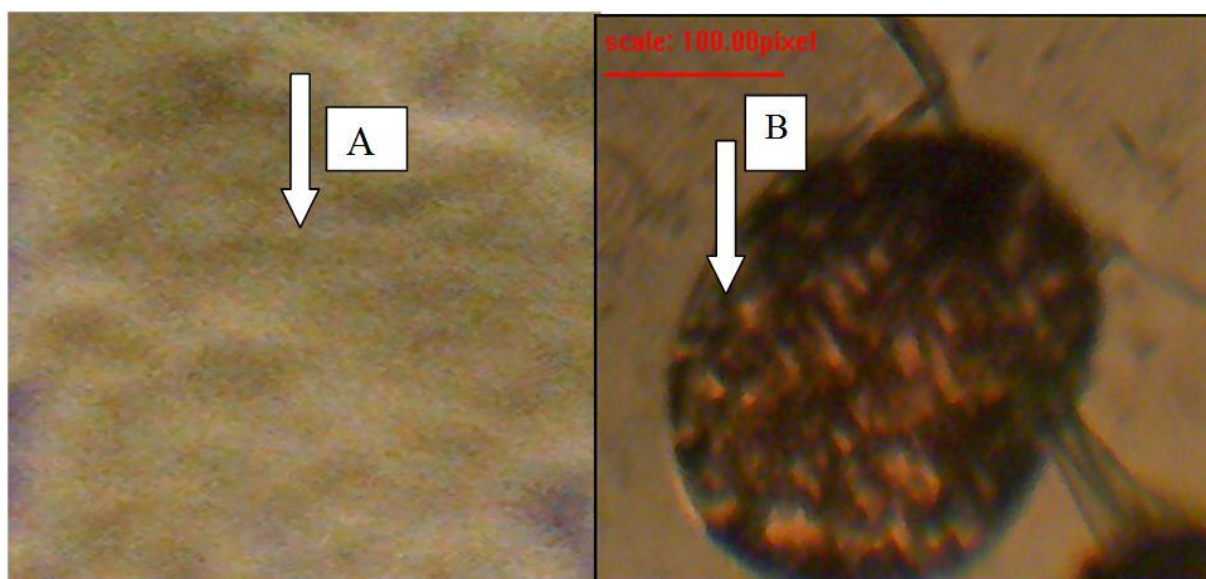
Exactly 10 g of feed were added into 90 ml of sterile distilled water and mixed thoroughly with stomacher (Stomacher® Bag, Seward, USA). One drop of each mixture was inoculated into labelled plates containing selective media for the growth of *Aspergillus* species (Rose Bengal chloramphenicol agar) and incubated at room temperature for 72 h. Thereafter, plates were examined macroscopically for characteristic colonies of *Aspergillus* species: colony and colour (Giorni et al., 2007). Colonies were then counted and pictures were taken.

Microscopic examination of cultures

One to two drops of lactophenol cotton blue was placed on a clean glass slide. Using a sterilized platinum inoculating pin, a bit of the

Table 1. Frequency of isolation of *Aspergillus* species in poultry feed in Kaduna Metropolis.

No. of poultry feed contaminated with moulds	% of poultry feed contaminated with moulds	Types of moulds identified
70	38.87	<i>A. fumigatus</i>
64	35.56	<i>A. parasiticus</i>
35	20.00	<i>A. flavus</i>
05	03.00	<i>A. niger</i>
03	01.70	<i>A. terreus</i>

**Figure 1.** Gross (arrow A) and microscopic (arrow B) appearance of *A. flavus*.

suspected fungal colonies was picked from the medium and placed in the stain on the slide. This was then teased out and covered with glass cover slip. This was pressed down slightly with the tip of the finger to expel any air bubble and further disintegrate the hyphal growth to enhance observation. The slides were observed under x 10 magnification of a light microscope. Microscopic characteristic of fungi such as hyphae, conidial heads and arrangements of conidia were observed.

Data analysis

Data generated were used to describe *Aspergillus* species from poultry feed. Summaries of data generated were presented in tables. Univariate Analysis of Variance (ANOVA) was used to compare the sampled feeds' aflatoxin concentration from the four different local governments.

RESULTS

Prevalence of *Aspergillus* from poultry feeds

The prevalence of *Aspergillus* was found to be 98.98% in the selected poultry feeds tested using selective media for the growth of *Aspergillus* species. A total of 178 of the 180 feeds tested yielded growth of different species of

Aspergillus. On the basis of frequency of isolation, *A. fumigatus* occurred most frequently 38.87%. This was followed by descending order by *A. parasiticus* 35.56%, *A. flavus* 20%, *A. niger* 3% and *A. terreus* 1.7% (Table 1). Figures 1 to 5 showed the macroscopic and microscopic features of *A. flavus*, *A. terreus*, *A. niger*, *A. parasiticus* and *A. fumigatus*, respectively. The results also revealed the isolation of other fungi such as Yeast, Mucor, and Rhizopus species.

DISCUSSION

The most common *Aspergillus* species observed in this study were *A. flavus*, *A. fumigatus*, *A. parasiticus* and *A. terreus*. The results of surveys conducted by various investigators on natural occurrence of aflatoxin in various feeds and feedstuff as compiled by Jelinek et al. (1989) also agreed with this study. The present study shows that *Aspergillus* is prevalent in poultry feed in Kaduna state but *A. fumigatus* is the most prevalent and *A. terreus* the least. *A. fumigatus* is ubiquitous and is found everywhere. It has been implicated in respiratory illnesses in animals and humans.

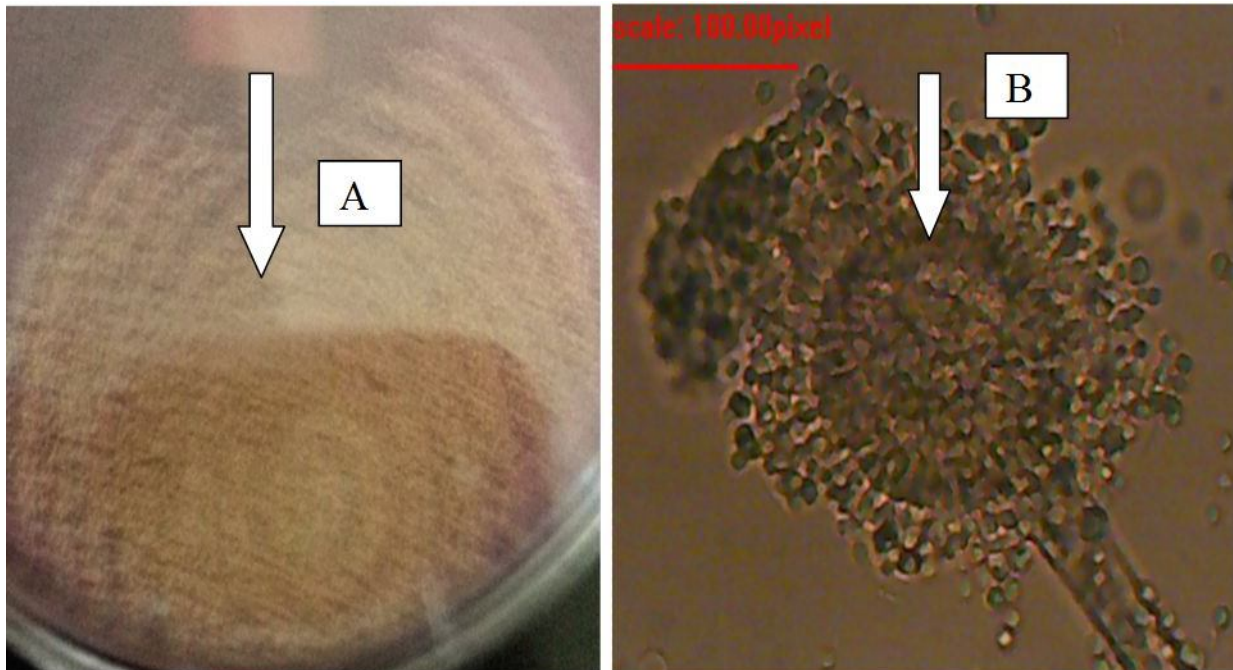


Figure 2. Gross (arrow A) and microscopic (arrow B) features of *A. terreus*.

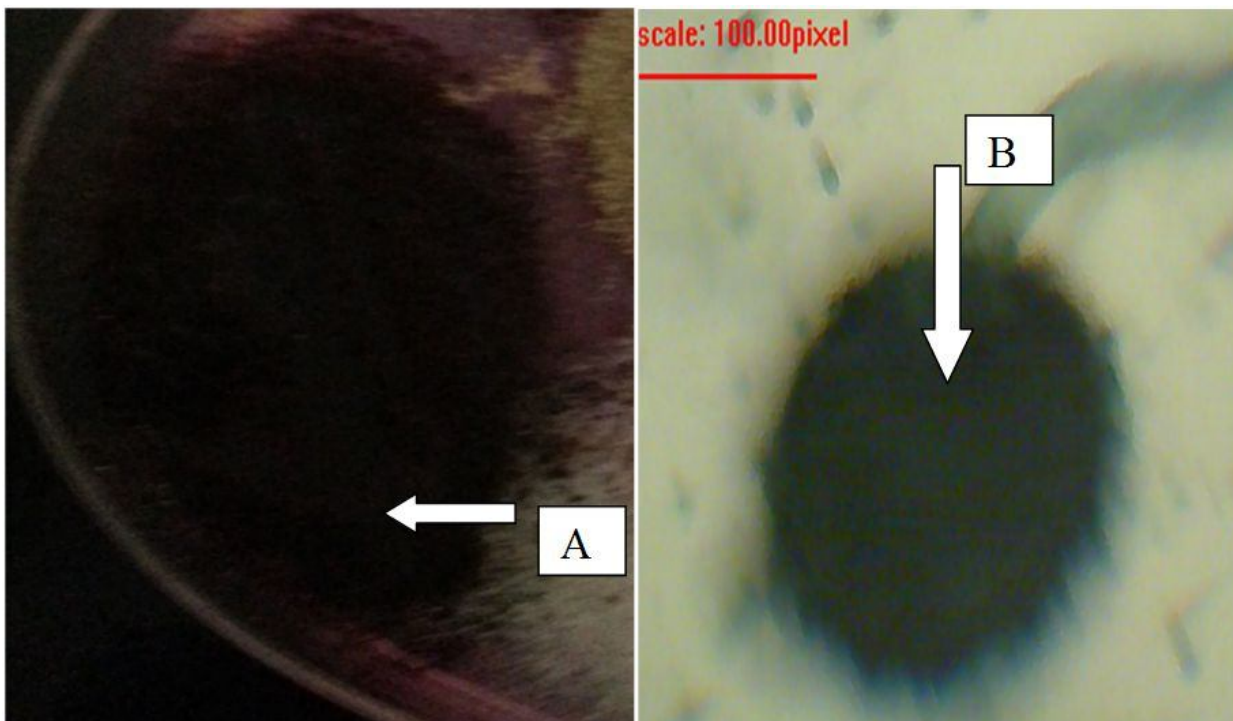


Figure 3. Gross dark colonies (arrow A) and microscopic (arrow B) features of *Aspergillus niger*.

The features of *Aspergillus* species isolated conform to different *Aspergillus* species enumerated in standard text (Raper and Fennell, 1965; Domsch et al., 1980, Samson and Pitt, 2000; McClenny, 2005; Diba et al., 2007; Gams

et al., 1985; Ellis et al., 2007).

Fungi could have emanated from the various activities along the food value chain. The most likely activity may be during drying of grains and oil seeds. Drying of grains

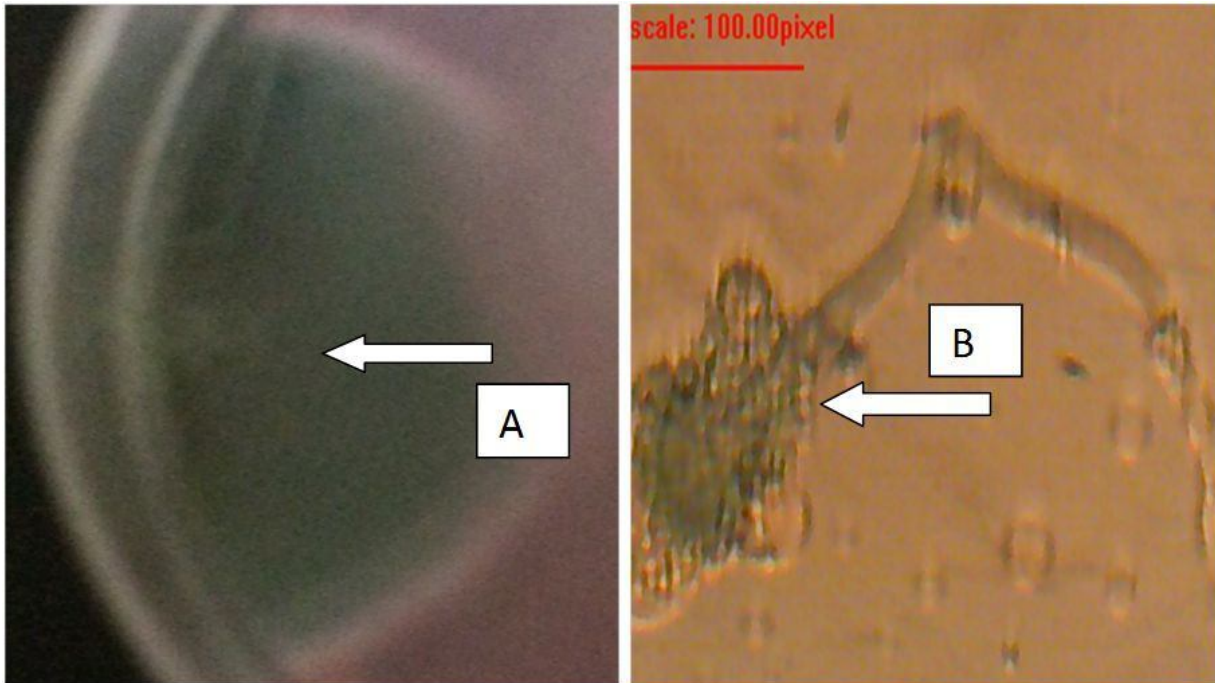


Figure 4. Gross (arrows A) and microscopic (arrows B) features of *A. parasiticus*.

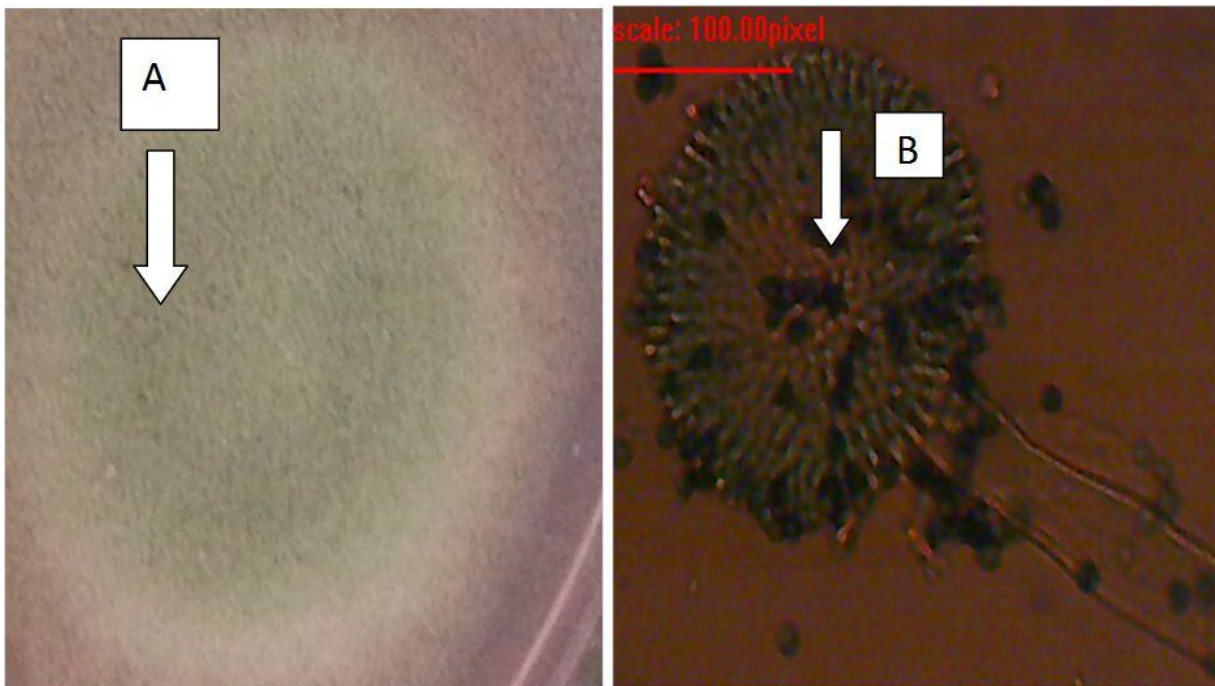


Figure 5. Gross (arrows A) and microscopic (arrows B) features of *A. fumigatus*.

by subsistent farmers was seen to be by the road side where all sorts of debris enter or on bare floor in their compounds where rodents and animals go to eat, defecate and urinate or in closed rooms without proper ventilation creating enabling environment for fungal

growth.

Broiler starter and layer mash are the most contaminated feeds. It could be because feed miller stored broiler starter for longer period due to less demand from farmers or level of biosecurity in the feed mills are

very poor.

Most of the chicken sellers feed the birds on grain offal or purchased small quantities of poultry feed from Toll feed millers. These grains offal were not properly dried in hygienic ways and may have been contaminated with aflatoxins and sometimes the birds were fed with fresh grain offal which may be left in the feeders in cages for days. They also have a habit of adding water to dry feed or dry grain offal they give to the birds. These practices may create conducive environment for mould growth and subsequent production of aflatoxins. These are probable sources of mouldy contamination but not confirmed. The birds are supplied to the market from different sources and each source (farm) has different management system and biosecurity practices from the others.

CONCLUSIONS

Different species of *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. terreus*, *A. niger* and *A. parasiticus*) were isolated and identified from poultry feeds in Kaduna Metropolis, and this is of concern because of the health hazards it presents to the value chain actors. The use of grains that are tolerant to fungal disease, properly dried grains and toxin binders for poultry feed production should be encouraged.

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