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Identification of *Aspergillus* species in feed fed to caged birds using morphological characteristics in Zaria, Nigeria

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ABSTRACT

The growth of *Aspergillus* species on feed often causes, deterioration and mycotoxin production which in results economic losses in the poultry industry. The identification of *Aspergillus* species in feed fed to cage birds using morphological characteristics in Zaria, Nigeria was examined. Feed samples were cultured for fungi on potato dextrose agar to detect the presence of *Aspergillus* species. Out of the 250-poultry feed tested *Aspergillus* had a prevalence of 96.8% (242) (P < 0.0045). *Aspergillus* species isolated were: *A. niger*, *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. terreus*, *A. caelatus, A. nomius, A. nidulans,* and *A. tamarii* had the following isolation frequency 38.8, 30.1, 12.8, 9.5, 2.9, 2.1, 1.7, 1.2 and 0.8%, respectively. The isolation frequency of the type of feed was 91 (36.6%) commercial, 83 (34.4%) compounded feed and 68 (28.1%) concentrate respectively. The occurrence of some of the *Aspergillus* species may have health hazardous risk. Therefore, identification is very important. Nine different species of *Aspergillus* were macroscopically and microscopically identified from poultry feed in cages of bird in Zaria, Nigeria.

Keywords: *Aspergillus*, cages, feed, isolation, characterization, bird.

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INTRODUCTION

Mycotoxicoses of poultry are caused by ingestion of low concentration of feed contaminants over a long period. Poultry been the most sensitive farm animals to aflatoxin even small amounts are consumed (Mabett, 2005; Shuaib et al., 2010). Dose and exposure determines their adverse health effect, it can cause damage of vital organs, poor growth rate, reduced feed utilization, decline productivity and increase mortality (Akande et al., 2006).

Feed is essential to produce egg and meat for human consumption. Fungi are a continuous threat to livestock feeds of economic importance (Iheanacho et al., 2014), causing mechanical damage through feeding, or secreting mycotoxins such as aflatoxins in the case of aflatoxin producing fungi. Contamination of poultry feed with *Aspergillus* species producing aflatoxin causes aflatoxicosis, birds consuming aflatoxin-contaminated feed are characterized by poor performance, intestinal hemorrhage, liver damage, liver tumors, reduced feed intake, decreased weight gain, poor feed utilization weight gain, pigmentation, lower egg production and eggshell quality, immunotoxicity and productivity (Calnek et al., 1997; Bondy and Pestka, 2000; Tedesco et al., 2004; Bailey et al., 2006; Shi et al., 2006, 2009; Gowda et al., 2013). Several *Aspergillus* are endowed with toxic effects towards animals and human beings and are termed aflatoxins many species are *A. flavus, A. Parasiticus, A. nomius, A. arachidicola, A. bombycis, A. minisclerotigenes, A. parvisclerotigenus, A. pseudocelatus, A. pseudonomius and A. togoensis* (Varga et al., 2011). Among these species*, A. flavus, A. parasiticus*, and *A. tamarii* are widely distributed in West Africa and sub-Saharan Africa (Atehnkeng et al., 2008;

Donner et al., 2009; Diedhiou et al., 2011).

These metabolites are usually produced when feedstuffs such as maize and nuts which are active ingredient are subjected to mold attack, by the growth of the *A. flavus*, *A. parasitcus*, and *A. nominus* under favorable conditions of moisture, temperature and aeration (Goto et al., 1997; Dutta and Das, 2001; Bandyopadhyay et al., 2007). Their toxicity depends on factors such as concentration, the duration of exposure, the species, sex, age, and health status of animals (Jewers, 1990).

Feed contamination may occur during handling, processing, transportation, storage condition, high temperature, availability of water, oxygen, and carbon dioxide, insect and rodent's infestation, incidence of broken grains or nuts and cleaning of the product (William and McDonald, 1983; Diener et al., 1987; Dorner, 2008; Campbell et al., 2006; Molyneux et al., 2007). The presence of fungi in feed affects the nutritional values and could result in production of mycotoxins (Frisvad et al., 2006; Cegielska-Radziejewska et al., 2013). This affects essential nutrient such a lipids, proteins, and minerals responsible proper development and growth of farm animals (Greco et al., 2014). The contaminated animal feed is the major cause of exposure to these mycotoxins to animals and therefore ultimately to humans (Bryden, 2012).

Live bird markets in Nigeria are located in specific areas of general markets with very limited facilities, cages are wooden or porous metal materials which are difficult to clean and disinfect (FAOUN, 2008). Climate control is almost zero, ventilation is natural, and lighting is very poor. Bird seller rely on commercial feed, few use concentrate or mixed with a poor hygienic storage conditions. Birds receive feeds from containers, giving rise to considerable spillage of feed, which can lead to feed loss and contamination by birds walking and defecating on the feed, although dispersed feed may be an attraction for pests (FAOUN, 2008).

Zaria is situated in the tropical region of the country with a favorable environmental condition that facilitate *Aspergillus* growth. This *Aspergillus* contamination of poultry feed, leads to mycotoxin production. There is therefore, the need to clearly evaluate the potential risks of *Aspergillus* in the poultry feed for mitigation of aflatoxin contamination in the feed. This study was designed to isolate and characterized *Aspergillus* Species from poultry feed in cage birds in Zaria, Nigeria.

MATERIALS AND METHODS

Sampling

A total of 250 samples of different types of poultry feeds given to birds in cages were collected and analyzed from live bird markets. About 20 g feed sample each were collected following the standard sampling protocol of Candlish et al. (1998), to give a representative sample with sterilized spoon, polythene bag and sealed from the feeding troughs and feeder per stand in live bird markets, and taken to the laboratory of the Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University for analysis.

Fungal examination

A microbiological analytical procedure of Kaufman et al. (1968), with some modification, was used in this study and carried out under aseptic condition. Exactly 1 g of feed was added into 9 ml of sterile distilled water in a test tube and homogenized by vortexing (HGBTWT, USA). A suspension was inoculated into labeled plates, containing Potato dextrose agar (TM 344, TM media, India.) and incubated at 30°C for 5 to 7 days. Colonies were stained with lactophenol cotton blue and viewed microscopically. *Aspergillus* species were identified macro and microscopically as described by Klich and Pitt (1988) and Klich (2002). Colonies were examined macroscopically.

Data analysis

Data generated were analyzed using descriptive statistics (Snedecor and Cochran, 1989). Values of $P < 0.05$ were considered significant; they were analyzed using Graphpad prism version 5.0, Chi square was used to check for an association between location and feed samples.

RESULTS

The prevalence of *Aspergillus* was found to be 96.8% in this study with a P value 0.0045. A total of 242 out of 250 feed tested yielded growth of different species of *Aspergillus*. On the basis of the frequency of isolation *A. niger* 38.8% had the highest isolation frequency. This was followed by descending order of *A. flavus* 30.1%, *A. parasiticus* 12.8%, *A. fumigatus 9.5%, A. terreus 2.9%, A. caelatus* 2.1%, *A. nomius* 1.7%, *A. nidulans* 1.2% and *A. tamarii* 0.8% (Table 1). Based on the type of feed commercial feed had the highest isolation frequency of 91 (36.6%), followed by mixed feed 83 (34.4%) and concentrate had 68 (28.1%) respectively (Table 2). Microscopic and macroscopic characteristics of various *Aspergillus* species were showed (Figures 1 to 9).

DISCUSSION

Contamination of poultry feed with a high frequency of *Aspergillus* species could lead to the production of aflatoxin which poses a potential risk to both animals and human consumers. In this study, *A. flavus, A. parasiticus,* and *A. nomius* detected which aflatoxin producers a carcinogen (Diener et al., 1987). *A. niger* and *A. fumigatus* were also isolated in high frequency which are concerned Aspergiollosis (Noonimabc et al., 2009; Edwin et al., 2010; Gautam et al., 2012). This study agreed with various reports that have been published that used of morphological characteristics as a key factor for the identification of fungi (Bandh et al., 2012).

Table 1. Prevalence of *Aspergillus* species in cages of poultry feed.

	Market A (%)	Market B (%)	Market C (%)	Market D (%)	Market E (%)
A. flavus	22 (48.8)	13 (26.5)	11 (22.9)	17 (34.0)	10 (20.0)
A. parasiticus	7 (15.6)	5 (10.2)	2 (4.2)	10 (20.0)	7 (14.0)
A. niger	11 (24.4)	19 (38.8)	17 (35.4)	19 (38.0)	28 (56.0)
A. fumigatus	3 (6.6)	7 (14.3)	9 (18.7)	3 (6.0)	1 (2.0)
A. tamarii	0 (0)	1 (2.0)	0 (0)	1 (2.0)	0 (0)
A. terreus	0 (0)	0 (0)	4 (8.3)	0 (0)	3 (6)
A. nidulans	1 (2.2)	0 (0)	1 (2.1)	0 (0)	1 (2.0)
A. caelatus	0 (0)	2 (4.1)	3 (6.3)	0 (0)	0 (0)
A. nomius	1 (2.2)	2 (4.1)	1 (2.1)	0 (0)	0 (0)
Total	45 (99.9)	49 (99.9)	48 (100)	50 (100)	50 (100.0)

Prevalence = 96.8, P value= 0.0045, $\chi^2 = 56.76$, df = 32.

Table 2. Distribution of Aspergillus species based on type of feed consumed

Feed	*A. flavus*	*A. niger*	*A. nidulans*	*A. parasiticus*	*A. fumigatus*	*A. tamarii*	*A. caelatus*	*A. terreus*	*A. nomius*	Total (%)
Concentrate	21	23	1	7	9	0	3	3	1	68 (28.1)
Commercial	16	43	2	15	9	0	0	4	2	91 (37.6)
Mixed feed	36	28	0	9	5	2	2	0	1	83 (34.3)
Total	73 (30.1)	94 (38.8)	3 (1.2)	31 (12.8)	23 (9.5)	2 (0.8)	5 (2.1)	7 (2.9)	4 (1.7)	242 (100)

The features of *Aspergillus* species isolated in this study 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).

The result of this study showed that there was a high level of *Aspergillus* contamination (96.8%) in poultry feed, fed to cage birds, these could be as a result of the ingredient in the feed which agrees with Aly and Anwer (2009), that *Aspergillus* species are major contaminant of grain used for poultry feed. Feeding livestock with *Aspergillus* species contaminated feed could compromised the health of animals and humans (Frisvad et al., 2006).

The *Aspergillus* species observed in both commercial and mixed feed in this study may be due to the fact that the feed were compounded with ground nut, soya bean cake, maize and maize offal which agrees with Frisvad et al. (2006), that agricultural product use for the production of animal feed are exposed to various toxicogenic contaminants during pre harvest, post harvest and storage. This study agrees with Del Pilar Monge et al. (2012), that raw materials use in poultry feed are contaminated during processing, production, transportation and storage. Contamination of poultry feed with *Aspergillus* producing mycotoxins may induce sanitary disturbances and mortality among animals and secondary contamination of human consumers via eggs and meat (Nyamongo and Okioma, 2005).

The high contamination level of *Aspergillus* spp., in this present study might have being as a result of the study area which is located in tropical region of the country with high temperature and humidity, which is in accordance with Del Pilar Monge et al. (2012), that presences mycotoxin depend on temperature, humidity, geographical location and the year for fungi production.

Most of the live bird markets purchase their feeds from feed retail outlet where they store feed in storage facilities that are poorly ventilated. During

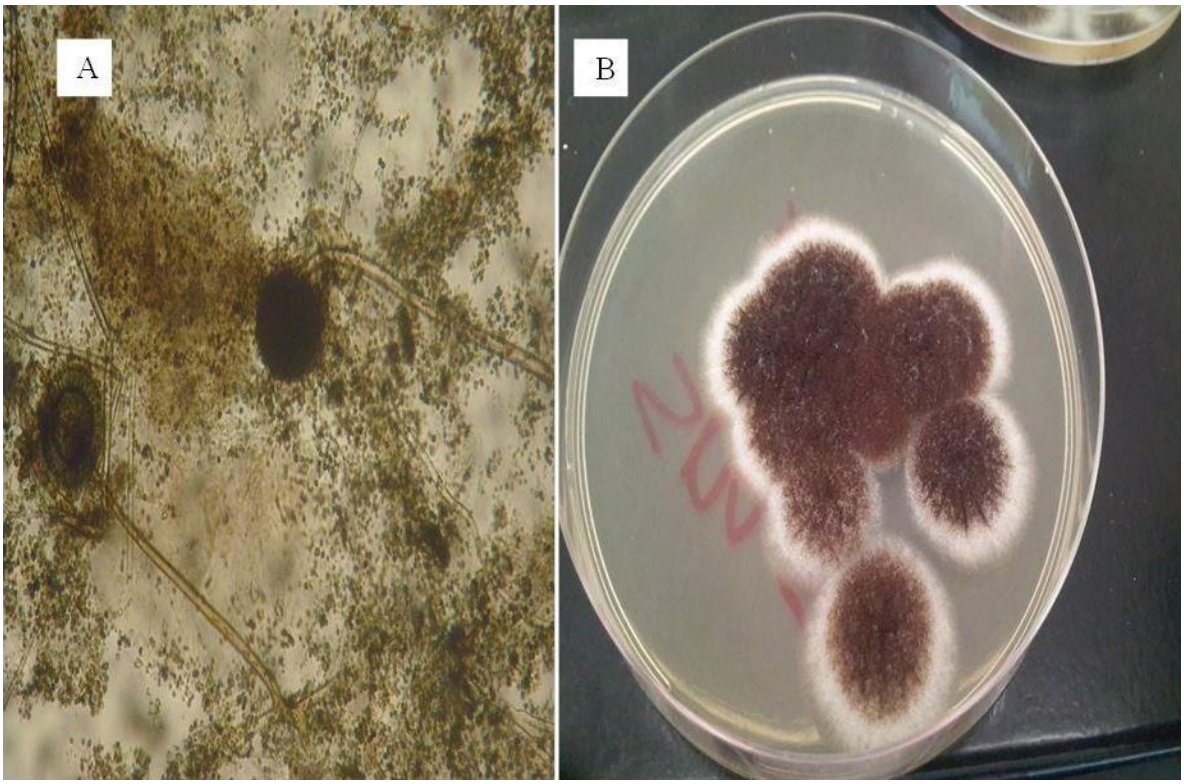

Figure 1. (*Aspergillus niger*): A: biseriate head with globose vesicle (Mag x40). B: Brown colony growth on potato dextrose agar at room temperature at 7th day.

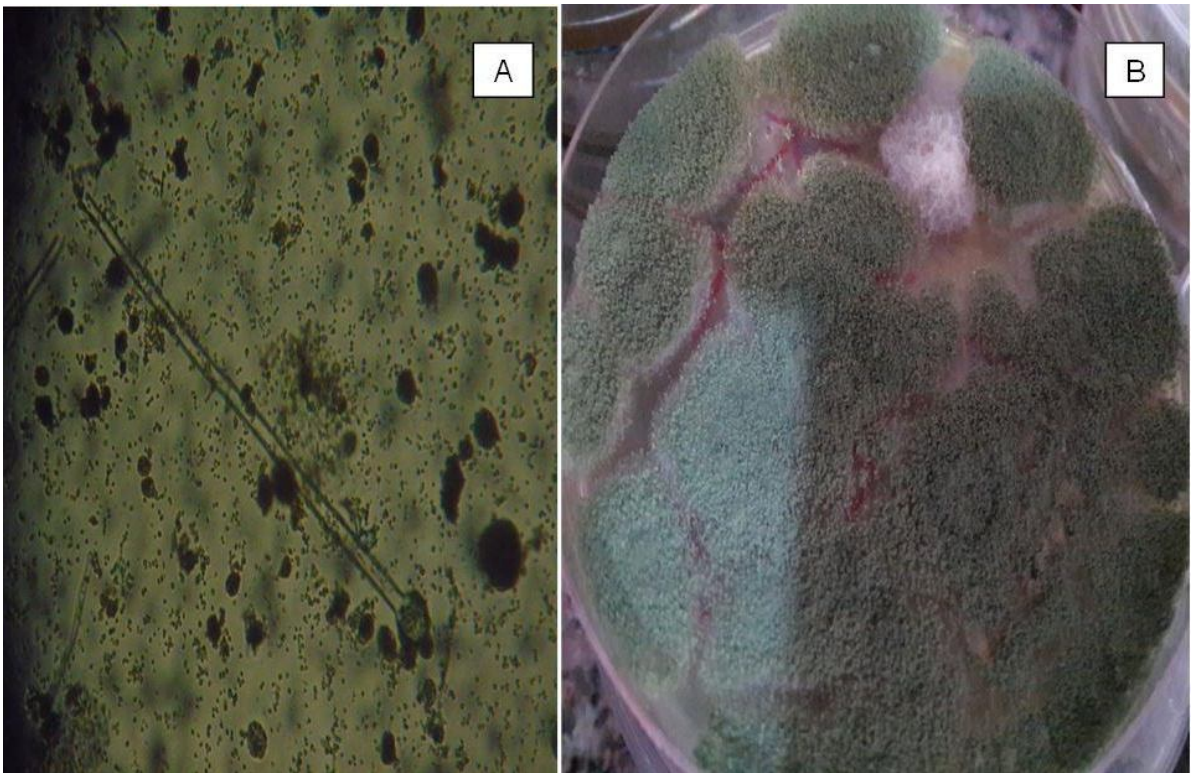

Figure 2. (*Aspergillus caelatus*); A: uni and biseriate head with subglose vesicle (Mag×40) B: green colony at 27°C after 7th day on potatoes dextrose agar.

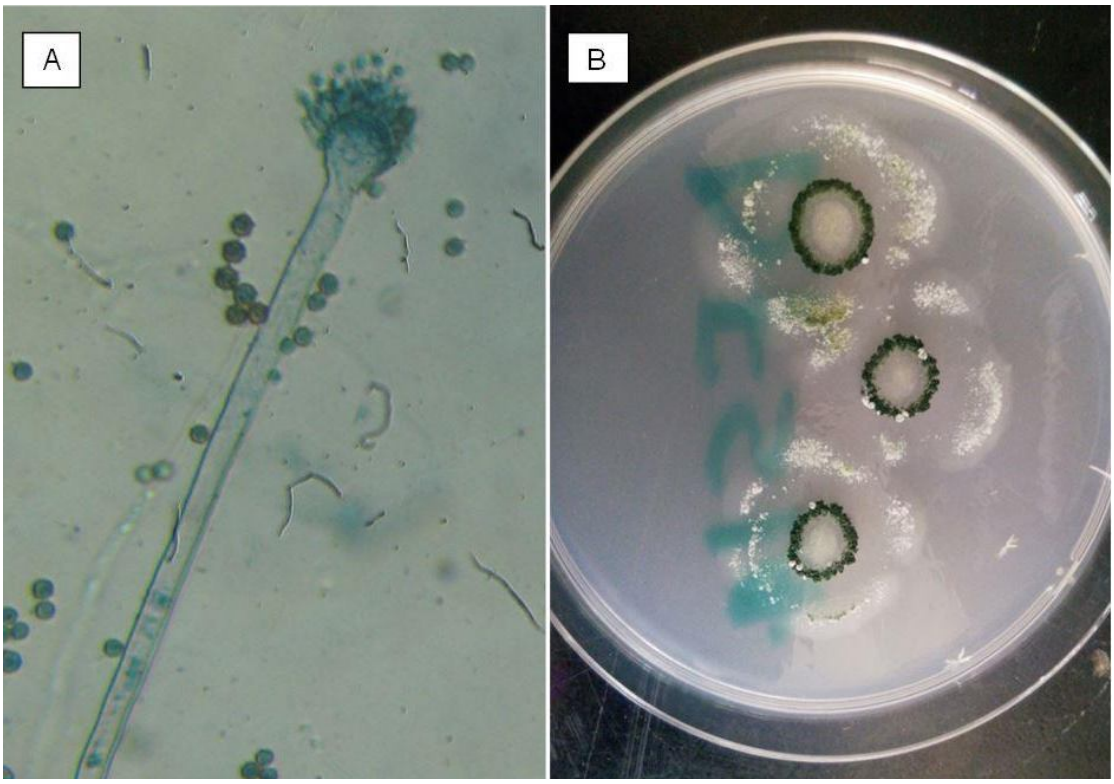

Figure 3. (*Aspergillus nidulans*); A: Short columnar and Biseriate head (Mag ×40). B: dark crees green colony at 27°C after 7th day on potatoes dextrose agar.

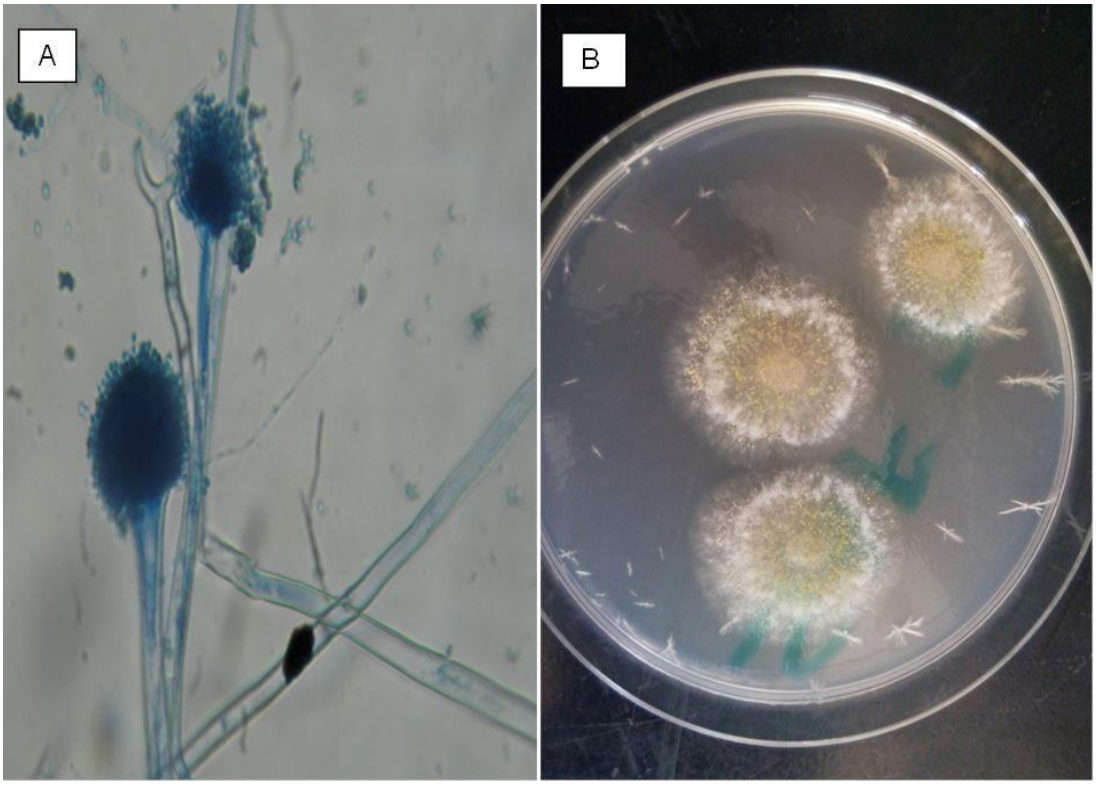

Figure 4. (*Aspergillus terreus*); A: Biseriate head with globose vesicle (Mag×40). B: pinkish cinnamon colony deeper with ages at 27°C after 7th day on potatoes dextrose agar.

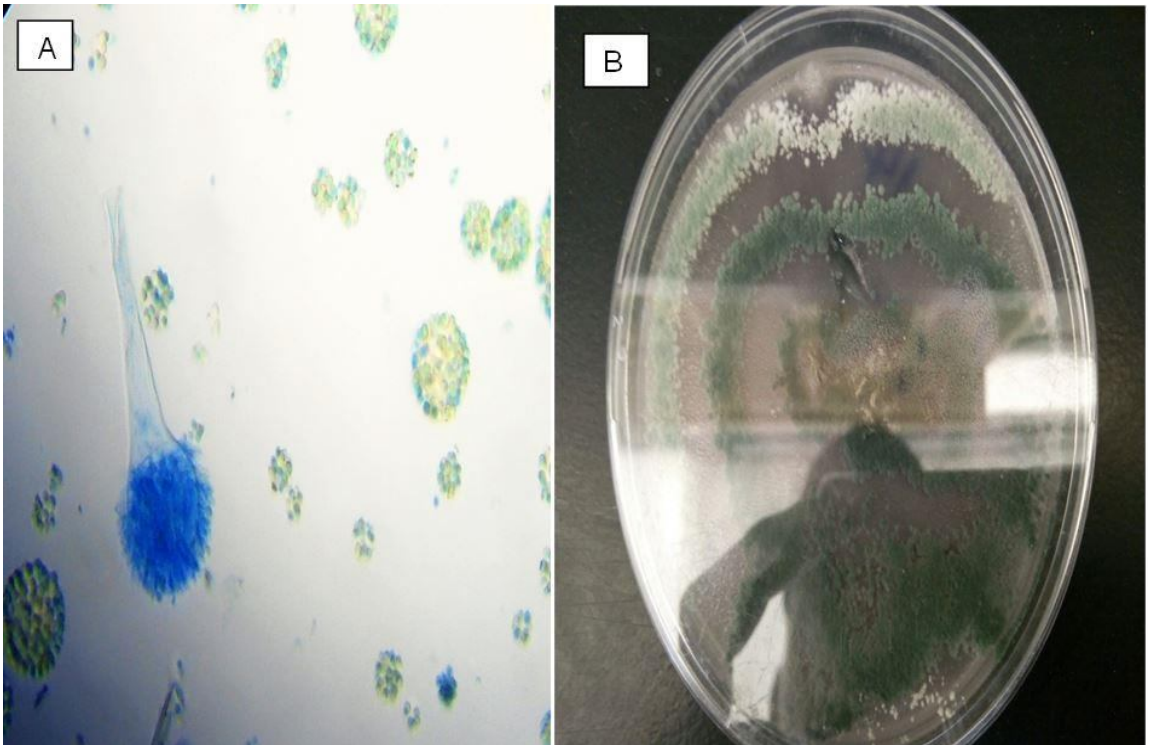

Figure 5. (*Aspergillus nomius*); A: Biseriate head with globose vesicle (Mag ×40). B: greenish colony deeper with ages at 27°C after 7th day on potatoes dextrose agar.

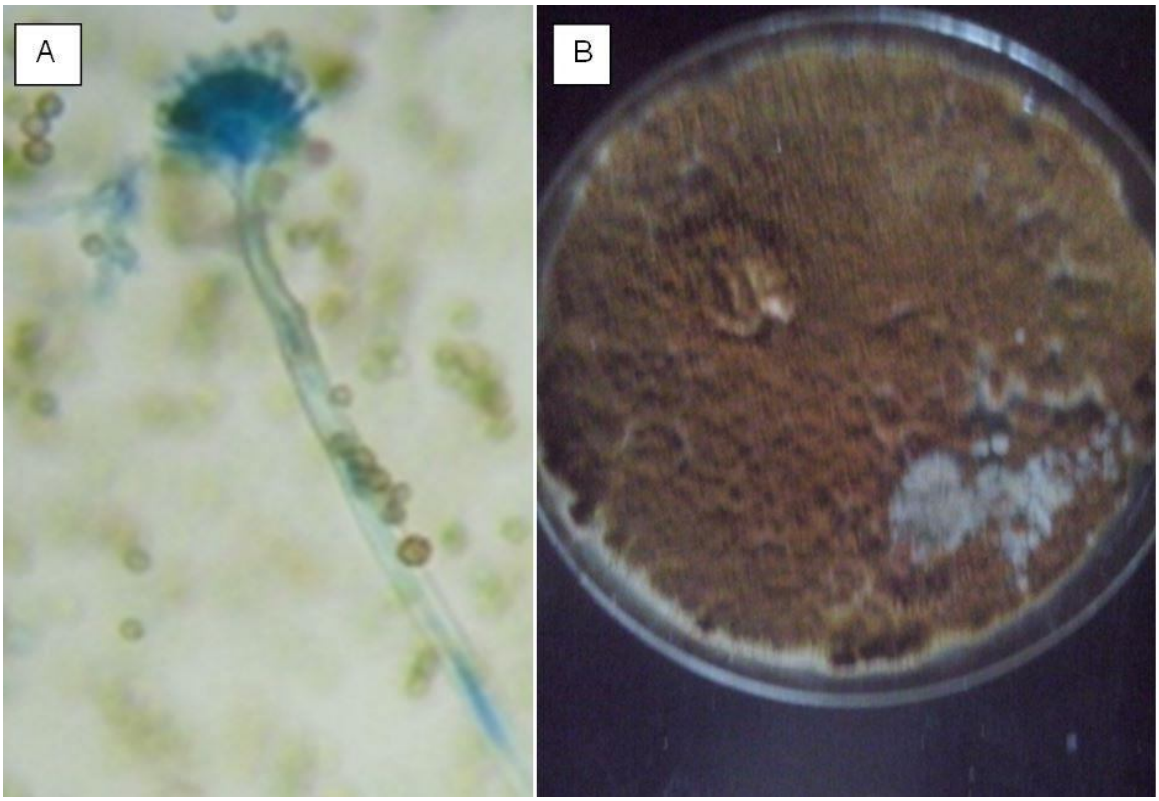

Figure 6. (*Aspergillus tamarii*); A: uniseriate and biseriate head with globose and subglobose with metulae covering entire vesicle, smooth conidia surface (Mag ×40). B: Sand brown colony of 27°C after 5th day on potatoes dextrose agar.

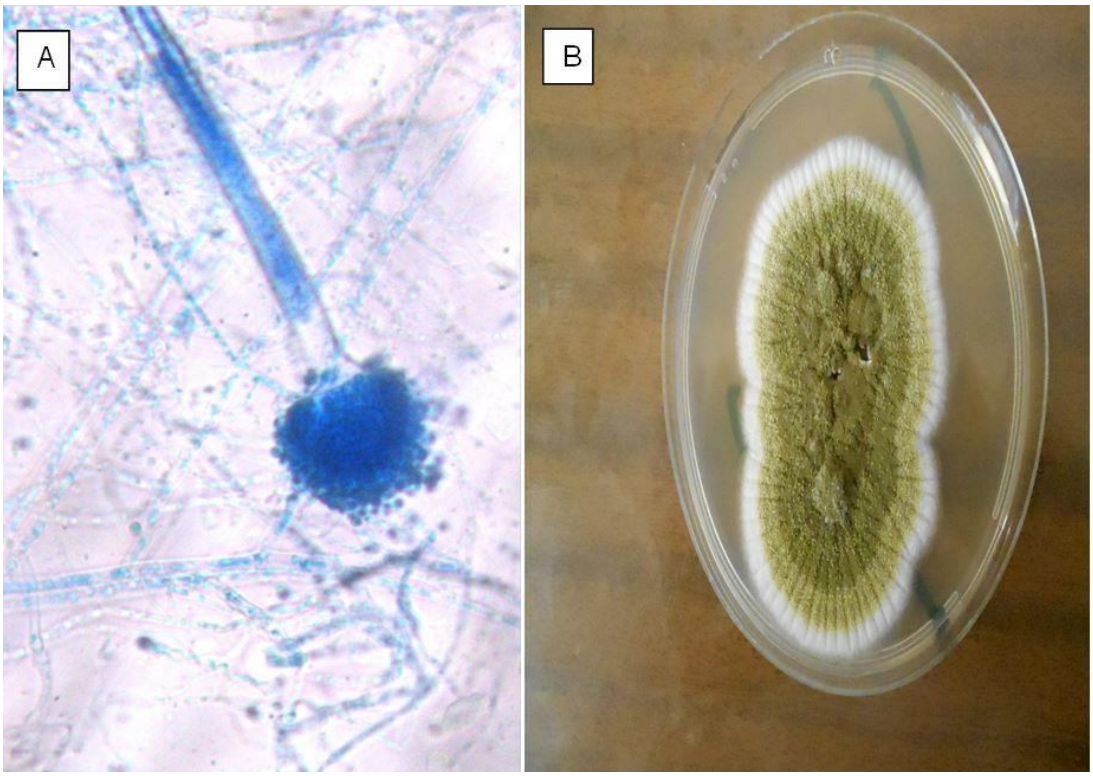

Figure 7. (*Aspergillus parasiticus*); A: uniseriate spherical head, globose vesicle, metulae cover half of vesicle with distinctly rough conidia surface (Mag ×40). B: Dark green colony at 27°C after 5th day on potatoes dextrose agar.

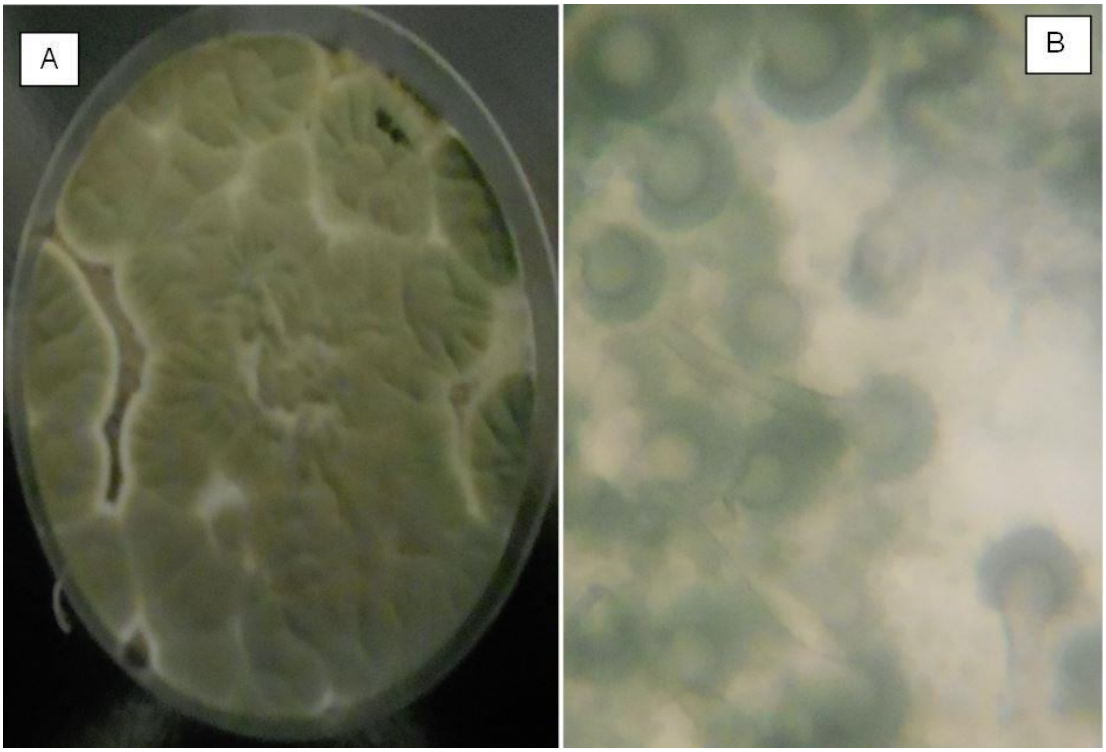

Figure 8. (*Aspergillus fumigatus*) A: Dark green colony after 5 days on potatoes dextrose agar at room temperature. B: smooth and uniseriate conidia head, globose vesicle, with metulae covers upper 2/3 of the vesicle head with ovate to flask shape vesicle fertile with ampulliform phialides. (Mag ×40).

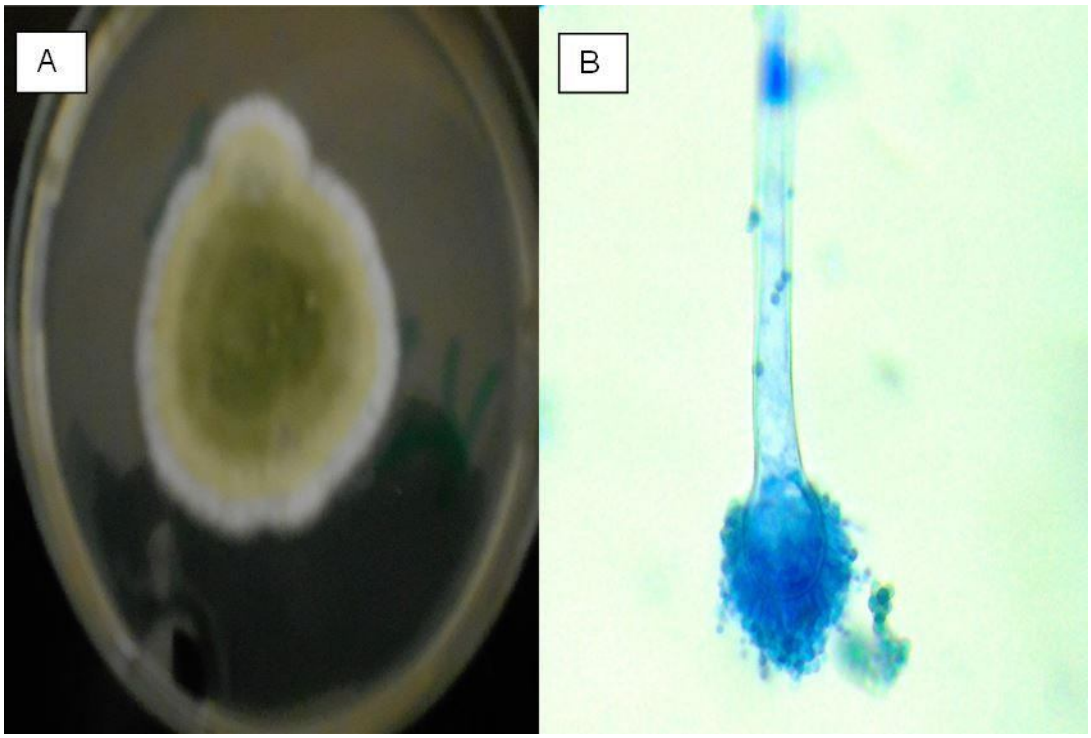

Figure 9. (*Aspergillus flavus);* A: yellowish/grayish green colony after 6th days on potatoes dextrose agar at room temperature. B: globose to ellipsoid with biseriate vesicle, smooth finely roughened conidia heads with roug and metulae cover ¾ of the vesicle (Mag ×40).

transportation, some of the feed could be contaminated either directly by rain drop and spillage of water by off-loaders. Most of the live bird markets are poorly ventilated where the temperature is high which can be enhanced fungi growth. While the compounded feed ingredients are sourced from feed millers and local market whose hazard analysis and critical control point are relatively poor. Sometimes the feed miller used a freshly harvested ingredient like maize or soya bean whose moisture content still high hence encourages mould growth.

Conclusion

Aspergillus section *flavor* and non-*Aspergillus* section *flavi* were isolated and identified from this study, *Aspergillus* section *flavis* is of worried in the food value chain. The use of anti-toxin binders should be encouraged to mitigate the production of aflatoxins.

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